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Chapter 14

Gastrointestinal Function

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I. INTRODUCTION

The digestive system is composed of the gastrointestinal (GI) tract or the alimentary canal, salivary glands, the liver, and the exocrine pancreas. The principal functions of the gastrointestinal tract are to digest and absorb ingested nutrients and to excrete waste products of digestion. Most nutrients are ingested in a form that is either too complex for absorption or insoluble and therefore indigestible or incapable of being digested. Within the GI tract, much of these substances are solubilized and further degraded enzymatically to simple molecules, sufficiently small in size and in a form that permits absorption across the mucosal epithelium. This chapter describes the normal biochemical processes of intestinal secretion, digestion, and absorption. Once these issues have been put in perspective, the chapter explores the pathogenesis of the important gastrointestinal diseases of domestic animals and the biochemical basis for their diagnosis and treatment.

II. SALIVARY SECRETIONS

A. Mechanisms of Secretion

Saliva is produced by three major pairs of salivary glands and by small glands distributed throughout the buccal mucosa and submucosa. Two types of secretory cells are found in the acinar portions of the salivary glands: (1) the mucous cells, which contain droplets of mucus, and (2) the serous cells, which contain multiple secretory granules.

In those species that produce salivary amylase (e.g., pig and human), the secretory granules are the zymogen precursors of this enzyme. A third cell type is found lining the striated ducts. The striations along the basal borders of these cells are caused by vertical infoldings of the cell membrane, a characteristic of epithelial cells involved in rapid movement of water and electrolytes. The primary secretion of the acinar cells is modified by active transport processes of the ductal epithelium.

The distribution of the different types of secretory cells in the salivary glands varies among species. The parotid glands of most animals are serous glands, which produce a secretion of low-specific gravity and osmolality containing electrolytes and proteins including certain hydrolytic enzymes. The mandibular (submaxillary) and sublingual glands are mixed salivary glands that contain both mucous and serous types of cells and produce a more viscous secretion that contains large amounts of mucus (Dukes, 1955).

B. Composition of Saliva

1. *Mucus*

Mucus is an aqueous mixture of proteoglycans and glycoproteins. One of the most completely studied glycoproteins is mucin. Salivary mucins are O-glycosylated and consist of peptides with many oligosaccharides linked covalently to the hydroxyamino acid serine or threonine. The carbohydrate portion of submaxillary mucin from sheep is a disaccharide of N-acetylneuraminic acid (sialic acid) and N-acetylgalactosamine (Carlson *et al.,* 1973). The enzymes that link protein with hexosamine have been purified from the mandibular glands of sheep (Carlson *et al.,* 1973) and swine (Schachter *et al.,* 1971).

The physiological functions of mucin are related to its high viscosity. N-acetylneuraminic acid is the component responsible for the formation of viscous aqueous solutions and, at physiological pH, causes expansion and stiffening of the mucin molecule. The resistance of mucin to enzymatic breakdown is also due to the presence of disaccharide residues. Removal of terminal N-acetylneuraminic acid residues by action of neuraminidase significantly increases the susceptibility of peptide bonds to trypsin.

2. *Electrolytes*

The principal inorganic constituents of saliva are sodium, potassium, chloride, and bicarbonate, which, with the exception of bicarbonate, originate directly from the plasma. Rates of salivary flow vary depending on stimulation, and there are wide variations in electrolyte concentration. Saliva is formed by a process that initially requires uptake of sodium and other electrolytes from the interstitium of the terminal structural unit of the salivary gland, the acinus or end piece. Water flows passively. This primary or precursor fluid has a sodium concentration similar to plasma, and the potassium concentration is similar to or slightly higher than plasma. As the primary fluid passes from the acinus along the duct system, the concentration of sodium, potassium, and other electrolytes changes. In most species, there is net sodium absorption and potassium secretion. Wide variations in electrolyte composition may occur depending on the flow rate (Young and Schneyer, 1981), the salivary gland of origin, and the species (Table 14-1).

3. *Amylase*

The saliva of rodents contains the α-amylase, ptyalin, but this enzyme activity is absent in the saliva of dogs, cats, horses, cattle, and sheep (Dukes, 1955; Young and Schneyer, 1981). Salivary amylase splits the α1,4-glucosidic bonds of various polysaccharides. Salivary amylase is similar in major respects to pancreatic α-amylase, which is described in Section V.B. Salivary amylase initiates digestion of starch and glycogen in the mouths of those species that secrete the enzyme. The optimal pH for amylase activity is approximately 7, so that this activity ceases when the enzyme mixes with acidic gastric contents.

TABLE 14-1 Electrolyte Concentration of Mandibular Gland and Parotid Gland Saliva Observed during Maximum Rates of Secretion (mmol/l)

	Mandibular Gland			Parotid Gland		
	Na^+	K^+	HCO_3^-	Na^+	K^+	HCO_3^-
Sheep	20	7	23	160–175	9–10	113–140
Dog	70–100	12–15	10–30	80–110	6–14	50
Cat	40–51	9–10	26	—	—	—
Rabbit	50–100	10–40	25	110–140	10	12–30

4. *Lipase*

Lingual lipase is secreted by Von Ebner's gland of the tongue and is important in the digestive processes of the human newborn, rats, and preruminant calves (Cook *et al.,*1994; Plucinski *et al.,* 1979).

C. Functions of Saliva

Saliva continuously bathes the oral cavity, which protects the surface epithelium. Ingested food is moistened and lubricated by saliva, thereby facilitating mastication and swallowing. Saliva also protects teeth from decay by washing food particles from the surfaces of the teeth and using its buffering capacity to neutralize the organic acids produced by bacteria normally present in the mouth. Saliva is necessary for vocalization, and, in some species that groom themselves, saliva promotes cooling as it evaporates. Additionally, it may be a source of pheromones. Salivary glands contain large numbers of growth factors, vasoactive serine proteases, and regulatory peptides (Cook *et al.,* 1994). There is reason to believe that these glandular constituents affect a wide range of biological functions not necessarily limited to the alimentary system.

Ruminants produce much greater quantities of saliva than do simple-stomached animals, and their saliva has a higher pH and bicarbonate ion concentration. In ruminants, saliva serves several unique functions (Phillipson, 1977). It is required for maintenance of the fluid composition of the contents of the rumen. The great buffering capacity of ruminant saliva is necessary to neutralize the large amounts of organic acids that are end products of rumen fermentation.

Rumen bacteria for protein synthesis can utilize the urea in saliva. Protein synthesized in the rumen is then used to meet dietary protein requirements. In this way, urea nitrogen can be "recycled" through the amino acid pool of the body, and in ruminants it need not be considered an end stage in protein catabolism. The ability to reutilize urea has also been demonstrated in the horse, and this may be of particular benefit during periods of protein deficiency (Houpt and Houpt, 1971).

III. GASTRIC SECRETIONS

The stomach is divided into two main regions on the basis of secretory function. The oxyntic gland area corresponds approximately to the body of the stomach in most species of domestic animals and also to the fundus in the dog and cat. The oxyntic glands contain (1) oxyntic or parietal cells that produce hydrochloric (HCl) acid, (2) peptic (zymogenic, chief) cells that produce pepsinogen, and (3) mucous cells. The pyloric gland area contains mucus-producing pyloric glands whose secretion is slightly alkaline. This area also contains the G cells, which produce the polypeptide hormone, gastrin.

A. Composition of Gastric Secretions

1. *Basal versus Stimulated Secretion*

There are two components of gastric secretion. The surface epithelial cells and other mucus-producing cells continuously secrete the basal component. This component is neutral or slightly alkaline pH. The electrolyte composition is similar to that of an ultrafiltrate of plasma (Table 14-2). The basal secretion contains large amounts of mucus, which has a cytoprotective effect on the epithelium. The secretory component produced by the oxyntic gland cells in response to stimulation contains free HCl and pepsinogen, the principal enzyme of gastric digestion.

The composition of gastric juice depends on the relative amounts of the basal and secretory components in the juice and, in turn, is a function of the flow rate of each. In the dog, gastric juice is produced in the resting state at a rate of approximately 5 ml/h. The composition is similar to that of the basal component, containing practically no peptic activity or HCl. When the flow of gastric juice is stimulated maximally, the dog may produce 80 ml or more per hour of a secretion containing large amounts of peptic activity and HCl. Na^+, the principal cation in the basal secretion, is replaced to a large extent by H^+ ion. The concentration of K^+ is similar in both basal and stimulated secretions and, therefore, remains relatively constant at the various rates of flow.

HCl and pepsinogen are secreted by separate mechanisms, but their production appears closely linked under physiological conditions. Stimulation of the vagus nerve or intravenous injection of gastrin increases pepsinogen and HCl levels together. Other stimuli may affect the two processes differently; for example, in the dog histamine infusion stimulates HCl production maximally but appears to inhibit pepsinogen secretion (Emas and Grossman, 1967).

TABLE 14-2 Composition of Parietal and Nonparietal Secretions of Canine Gastric Mucos[a]

Component	Parietal Secretion[a] (mmol/liter)	Nonparietal Secretion[a] (mmol/liter)	Nonparietal Secretion[b] (mmol/liter)
Na^+	—	155.0	138.0
H^+	159.0	—	—
K^+	7.4	7.4	4.0
Ca^{2+}	—	3.7	5.0
Cl^-	166.0	133.0	117.0
pH	<1.0	7.54[c]	7.42

[a] *Determined* in vivo *using dogs with gastric fistulas* (Gray and Bucher, 1941).
[b] *Determined* in vitro *with isolated gastric mucosa* (Altamirano, 1963).
[c] *Calculated from bicarbonate concentration assuming* pCO_2 *of* 40 Torr.

2. *Pepsin*

Pepsinogen is the zymogen, or inactive precursor, of pepsin, the principal proteolytic enzyme of gastric juice. Pepsinogen was first crystallized from the gastric mucosa of swine, and several pepsinogens have now been separated. The porcine pepsinogen has a molecular weight of approximately 43 kd and is composed of the pepsin molecule and several smaller peptides. One of these peptides has a molecular weight of 3.2 kd and is an inhibitor of peptic activity. Activation of pepsin from pepsinogen occurs by selective cleavage of this small basic peptide from the parent pepsinogen (Neurath and Walsh, 1976). Autocatalytic conversion begins below pH 6. At pH 5.4, the inhibitor peptide dissociates from the parent molecule, and at pH 3.5 to 4, the inhibitor is completely digested by pepsin.

Pepsin has a very acidic isoelectric point and is stable in acidic solution below pH 6, but it is irreversibly denatured at pH 7 or above. In contrast, pepsinogen is stable in neutral or slightly alkaline solution. The optimal pH for peptic activity is generally between 1.6 and 2.5, but the effect of pH may vary with the substrate. Pepsin is capable of hydrolyzing peptide bonds of most proteins, mucin being one important exception. Pepsin splits bonds involving phenylalanine, tyrosine, and leucine most readily but can hydrolyze almost all other peptide bonds.

3. *Gastric Lipase*

In canines, gastric lipase is secreted in response to pentagastrin, histamine, prostaglandin E_2, and secretin (Simpson, 2005). It parallels the secretion of gastric mucosa and plays a role in fat digestion. Unlike pepsin, it is not dependent on an acid pH, remains active in the small intestine, and constitutes up to 30% of the total lipase secreted over a 3-hour period. Gastric lipase as well as pepsin are not essential in fat digestion, but resulting fatty acids and peptides help coordinate gastric emptying and pancreatic secretion.

4. *Rennin*

Rennin is another proteolytic enzyme produced by the gastric mucosa and has characteristics that are similar to those of pepsin. It has been separated from pepsin in preparations from the stomachs of newborn calves. Rennin splits a mucopeptide from casein to form paracasein, which then reacts with calcium ion to form an insoluble coagulum. The coagulated milk protein probably delays gastric emptying and increases the efficiency of protein digestion in young calves.

5. HCL

The oxyntic cells produce HCl. When the normal mucosa is stimulated, both Cl^- and H^+ are secreted together, but current evidence suggests that H^+ and Cl^- are secreted by separate, closely coupled mechanisms. Unstimulated oxyntic cells continuously secrete small amounts of Cl^- in the absence of H^+ secretion, and this mechanism is responsible for the negative charge of the resting mucosal surface of the stomach relative to the serosa. For every H^+ secreted, an electron is removed that ultimately is accepted by oxygen to form OH^-, which is neutralized within the cell by H^+ from H_2CO_3. The HCO_3^- then enters the venous blood by means of a Cl^-/HCO_3^- exchange ("alkaline tide"), and during HCl secretion, the pH of gastric venous blood frequently is greater than that of arterial blood (Davenport, 1966).

The membrane-bound enzyme responsible for transport of H^+ by the oxyntic cell is a K^+-stimulated ATPase (Sachs *et al.,* 1976; Wallmark *et al.,* 1980) that serves as an H^+/K^+ exchange pump. At the time of oxyntic cell stimulation, the secretory membrane is altered to provide augmented K^+ and Cl^- conductances (Wolosin, 1985). KCl leaves the apical cell membrane passively, and net production of HCl results from the electroneutral exchange of K^+ for H^+ (Fig. 14-1).

B. Control of Gastric Secretion

1. *General*

A variety of stimuli can initiate gastric secretion. The sight or smell of food or the presence of food within the mouth causes gastric secretion by a reflex mechanism involving the vagus nerve. The presence of certain foods within the stomach or distension of the stomach alone can also initiate both intrinsic and vagal nerve reflexes, which cause secretion of gastric fluid. In addition to neural reflexes, these stimuli also cause the release of the gastrin from the pyloric gland area, which enters the bloodstream, stimulating gastric secretion. The release of gastrin from G cells is inhibited by excess H^+, and this negative feedback mechanism is important in the control of HCl production.

2. *Gastrin*

Gastrin has been isolated in pure form from the antral mucosa of swine (Gregory *et al.,* 1964). When administered intravenously, the purified hormone causes the secretion of HCl and pepsin and stimulates gastrointestinal motility and pancreatic secretion. Two separate peptides have been obtained from porcine gastric mucosa and have been designated gastrin I and gastrin II. Gastrin is a heptadecapeptide amide, with a pyroglutamyl N-terminal residue and with the amide of phenylalanine as the C-terminal residue (Fig. 14-2). In the center of the molecule is a sequence of five glutamyl residues, which give the molecule its acidic properties. Gastrin II differs from gastrin I only in the presence of a sulfate ester group linked to the single tyrosyl residue. The C-terminal tetrapeptide amide,

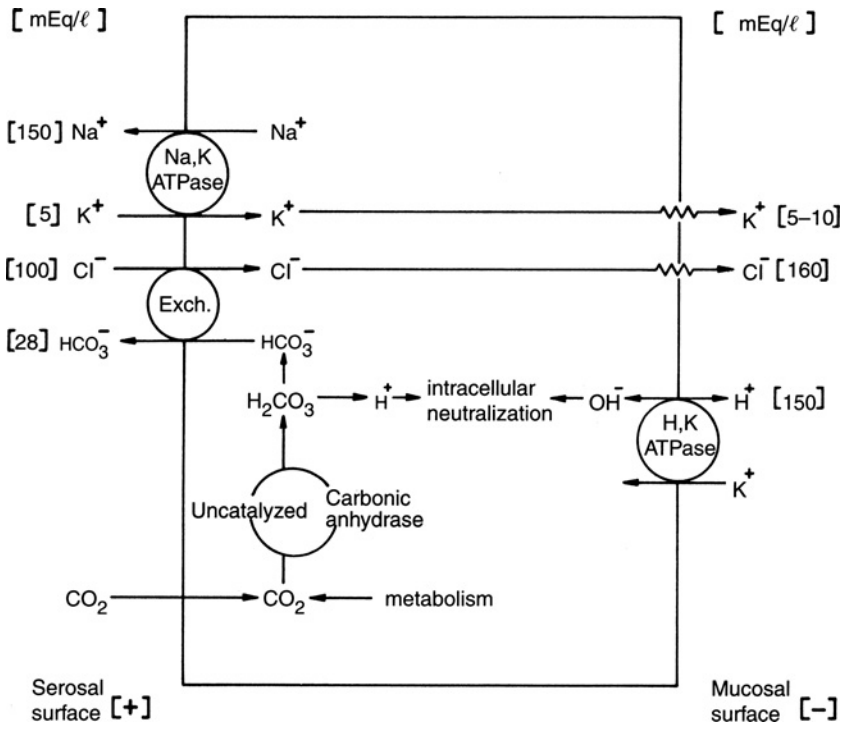

FIGURE 14-1 Movement of ions across the mucosal (apical) and serosal (basal) cell membranes of the parietal during HCl secretion.

FIGURE 14-2 Amino acid sequence of porcine gastrin I (Gregory, 1966). Gastrin II differs from gastrin I by the presence of a sulfate ester group on the single tyrosyl residue.

Trp-Met-Asp-Phe-NH_2, is identical in all species so far studied (Gregory, 1967). The tetrapeptide has all of the activities of the natural hormone. It is not as potent as the parent molecule, but lengthening of the peptide chain can increase activity.

Gastrin-releasing peptide—as well as luminal peptides, digested protein, and acetylcholine—stimulates gastrin secretion from G cells and affects histamine release from enterochromaffin-like cells (Simpson, 2005). Gastrin is the only hormone known to simulate HCl (Walsh and Grossman, 1975).

3. H*istamine*

Histamine secreted locally within the mucosa has a major effect on the function of oxyntic cells (Soll and Grossman, 1978). For many years, histamine has been recognized as a potent stimulant of HCl production, but this effect was not inhibited by traditional antihistaminic drugs (H_1 antagonists), and until the demonstration by Black *et al.* (1972) of H_2 receptors in the stomach (the atrium and uterus), the physiological role of histamine in HCl secretion was controversial. Specific H_2 antagonists (cimetidine) now have been shown to inhibit the secretory response not only to histamine but to other secretory stimuli as well (Grossman and Konturek, 1974).

The complex of oxyntic cell receptors involved in the control of oxyntic cell function is shown in Figure 14-3. When the H_2 receptor of the oxyntic cell is occupied by histamine, basal lateral adenylate cyclase is activated, resulting in increased cellular cyclic AMP (cAMP) and in a sustained secretory response. The secretogogue action of cAMP is mediated by the activity of cAMP-dependent protein kinases (Chew, 1985).

Cholinergic stimulation of the oxyntic cell involves type I muscarinic receptors and a calcium activation pathway. Calmodulin inhibitors such as trifluoroperazine inhibit H^+ secretion (Raphael *et al.,* 1984). The Ca-calmodulin system may influence the rate of cAMP synthesis, and a more distal site of action has been suggested by the identification of a Ca-dependent protein kinase activity in a membrane fraction prepared from oxyntic cells that was rich in H^+, K^+-ATPase (Schlatz *et al.,* 1981). A specific receptor for gastrin has been demonstrated on oxyntic cells and a specific gastrin antagonist, proglutamide, inhibits H^+ production. Gastrin appears to act synergistically with histamine and acetylcholine (AcCh) to regulate H^+ production but the actual mechanism of action of gastrin is unknown.

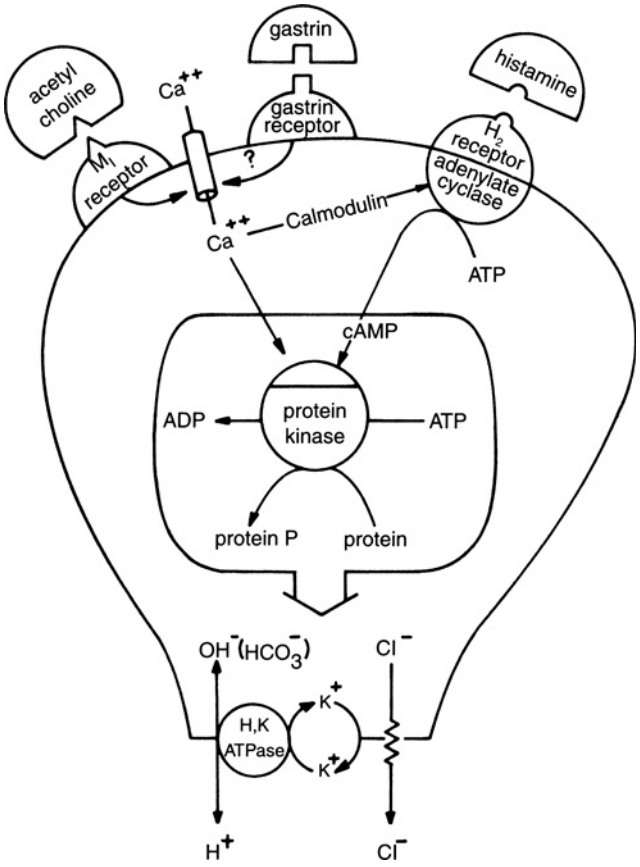

FIGURE 14-3 Pathways of secretagogue action on the parietal cell. Stimulation by gastrin and acetylcholine is mediated by entry of Ca^{2+} onto the cell. Histamine activates adenylate cyclase with production of cAMP, the action of which is mediated by protein kinase.

TABLE 14-3 Inhibitors of Oxyntic Cell Function

A. Inhibitors of H^+, K^+-ATPase: omeprazole, verapamil, vanadate
B. Inhibitors of carbonic anhydrase: acetazolamide
C. Inhibitors of cell activation or response 1. Calcium channel antagonists: verapamil, lanthanum 2. Prostaglandin E_2
D. Receptor antagonists 1. H_2-receptor antagonists: cimetidine, ranitidine 2. Gastrin antagonists: proglumide, benzotript 3. Anticholinergic agents: atropine
E. Inhibitors of calmodulin: trifluoroperazine

4. *Prostaglandins*

Prostaglandins, in addition to inhibiting HCl secretion, also act on a mucosal cell population that is distinct from oxyntic cells, which secrete cytoprotective substances (mucin, glycosaminoglycans). The ulcerogenic effects of inhibitors of prostaglandin synthesis (indomethacin, aspirin) apparently are the result of inhibition of the protective effect of endogenous prostaglandins.

Knowledge of the molecular aspects of receptor function of HCl secretion by oxyntic cells now provides the opportunity for specific pharmacological intervention for the control and treatment of ulcerative diseases of the upper gastrointestinal tract that appear to be the result of HCl-induced mucosal injury (Aclund *et al.,* 1983; Becht and Byars, 1986; Campbell-Thompson and Merritt, 1987). Potential therapeutic target sites are listed in Table 14-3. Famotidine is commonly administered to dogs and cats. Injectable ranitidine is administered to foals and horses during critical stages of gastrointestinal ulceration before switching to oral administration of omeprazole.

IV. BILIARY SECRETIONS

A. Composition of Bile

The hepatocytes continuously secrete bile into the bile canaliculi; it is transported through a system of ducts to the gallbladder, where it is modified, concentrated, and stored. During digestion, bile is discharged into the lumen of the duodenum, where it aids in emulsification, hydrolysis, and solubilization of dietary lipids. The digestive functions of bile are accomplished almost exclusively by the detergent action of its major components, the bile salts and phospholipids.

B. Properties of Bile

The carboxyl group of the bile acids is completely ionized at the pH of bile and is neutralized by Na^+ resulting in the formation of bile salts. These bile salts are effective detergents. They are amphipathic molecules that have both hydrophobic and hydrophilic regions. In low concentrations, bile salts form molecular or ideal solutions, but when their concentration increases above a certain critical level, they form polymolecular aggregates known as micelles. The concentration at which these molecules aggregate is called the critical micellar concentration (CMC).

Bile salt micelles are spherical and consist of a central nonpolar core and an external polar region. Fatty acids, monoglycerides, and other lipids are solubilized when they enter the central core of the micelle and are covered by the outside polar coat. Solubilization occurs only when the CMC is reached. For the bile salt-monoglyceride-fatty acid-water system present during normal fat digestion, the CMC is approximately 2 mM, which normally is exceeded both in bile and in the contents of the upper small intestine (Hofmann, 1963, 1967). Phospholipids, principally lecithin, are also major components of bile. In the lumen of

the small intestine, pancreatic phospholipase catalyzes the hydrolysis of lecithin, forming free fatty acid and lysolecithin. The latter compound also is a potent detergent, which acts with the bile salts to disperse and solubilize lipids in the aqueous micellar phase of the intestinal contents.

C. Synthesis of Bile Acids

The primary bile acids (BA) are C-24 carboxylic acids synthesized by the liver from cholesterol. BA synthesis is the major end-stage pathway for cholesterol metabolism (Danielsson, 1963). Cholic acid (3α,7α,12α-trihydroxy-5β-cholanoic acid) (CA) and chenodeoxycholic acid (3α-,7α-dihydroxy-5β-cholanoic acid) (CDCA) are the primary BA synthesized by most species of domestic animals. In swine, CDCA is hydroxylated at the 6α position by the liver to yield hyocholic acid (HCA), which is a major primary BA in this species.

BA are secreted as amino acid conjugates of either glycine or taurine. Taurine conjugates predominate in the dog, cat, and rat. In the rabbit, the conjugating enzyme system appears to be almost completely specific for glycine (Bremer, 1956). Both taurine and glycine conjugates are present in ruminants. In the newborn lamb, 90% of the bile acids are conjugated with taurine. As the lamb matures, glycine conjugates increase to reach one-third of the total BA in mature sheep (Peric-Golia and Socic, 1968).

Under normal conditions, only conjugated BA are present in the bile and in the contents of the proximal small intestine. In the large intestine, the conjugated BA are hydrolyzed rapidly by bacterial enzymes so that in the contents of the large intestine and in the feces, free or unconjugated BA predominate. Several genera of intestinal bacteria, including clostridium, enterococcus, bacteroides, and lactobacillus, are capable of splitting the amide bonds of conjugated BA.

Intestinal bacteria also modify the basic structure of the BA. One such reaction is the removal of the α-hydroxyl group at the 7 position of CA or CDCA. These bacterial reactions yield the secondary BA, deoxycholic acid (DCA), and lithocholic acid (LCA) (Gustafsson *et al.,* 1957). LCA is relatively insoluble and is not reabsorbed to any great extent (Gustafsson and Norman, 1962). DCA is reabsorbed from the large intestine in significant quantities and is either rehydroxylated by the liver to CA and secreted (Lindstedt and Samuelsson, 1959) or secreted as the conjugated DCA. The extent to which bacteria transform the primary BA depends on the nature of the diet, the composition of the intestinal microflora, and the influences of these and other factors on intestinal motility (Gustafsson, 1969; Gustafsson *et al.,* 1966; Gustafsson and Norman, 1969).

D. Enterohepatic Circulation of Bile Acids

The enterohepatic circulation begins as conjugated BA enter the duodenum and mix with the intestinal contents, forming emulsions and micellar solutions. The BA are not absorbed in significant amounts from the lumen of the proximal small intestine. Absorption occurs primarily in the ileum (Lack and Weiner, 1961, 1966; Weiner and Lack, 1962) where an active transport process has been demonstrated (Dietschy *et al.,* 1966). The absorbed conjugated BA pass unaltered into the portal circulation (Playoust and Isselbacher, 1964) and return to the liver, where the cycle begins again. This arrangement provides for optimal concentrations of BA in the proximal small intestine where fat digestion occurs and then for efficient absorption after these functions have been accomplished. Absorption of unconjugated BA from the large intestine accounts for 3% to 15% of the total enterohepatic circulation (Weiner and Lack, 1968).

In dogs, the total BA pool was estimated to be 1.1 to 1.2 g. The half-life of the bile acids in the pool ranged between 1.3 and 2.3 days, and the rate of hepatic synthesis was 0.3 to 0.7 g/day. Because the daily requirement for bile acids greatly exceeds the normal synthetic rate, the repeated reutilization of the BA is facilitated by the enterohepatic circulation. Under steady-state conditions, the total BA pool passes through the enterohepatic circulation approximately 10 times each day.

The size of the BA pool depends on the diet, the rate of hepatic synthesis, and the efficiency of the enterohepatic circulation. Surgical removal of the ileum in dogs interrupts the enterohepatic circulation, thereby increasing the BA turnover and reducing the size of the BA pool (Playoust *et al.,* 1965). In diseases of the ileum, there may be defective BA reabsorption and a bile salt deficiency. If the deficiency is severe, the utilization of dietary fat may be impaired, resulting in steatorrhea and impaired absorption of the fat-soluble vitamins.

V. EXOCRINE PANCREATIC SECRETIONS

The exocrine pancreas is an acinus gland with a general structure that is similar to the salivary glands. The cytoplasm of the secretory cells contains numerous zymogen granules, which vary in size and number depending on the activity of the gland. These granules contain the precursors of the hydrolytic enzymes responsible for digestion of the major components of the diet. Cells of the terminal ducts appear to secrete the HCO^{3-} responsible for neutralizing the HCl, which enters the duodenum from the stomach.

A. Composition of Pancreatic Juice

1. *Electrolyte Composition*

The cation content of pancreatic secretion is similar to that of plasma, Na^+ being the predominant cation and the concentrations of K^+ and Ca^{2+} being much lower. A unique

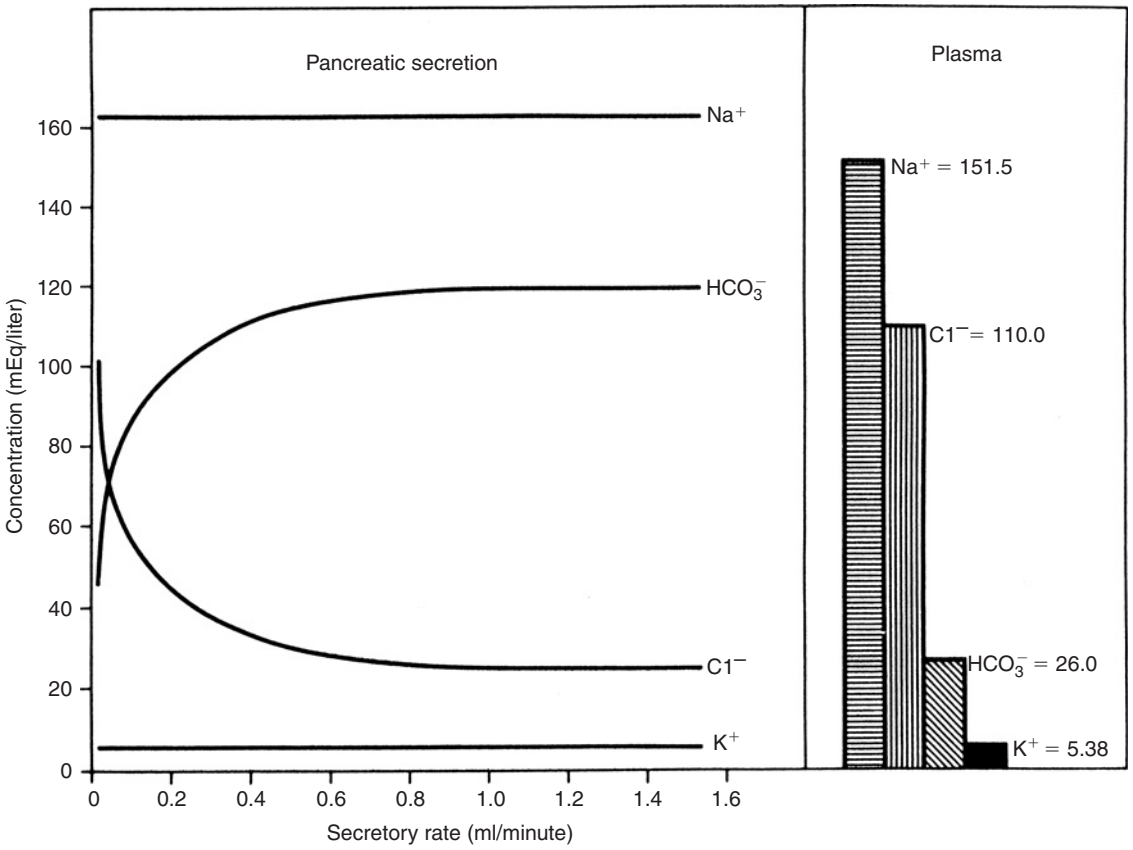

FIGURE 14-4 Influence of secretory rate on the electrolyte composition of canine pancreatic juice. From Bro-Rasmussen *et al.* (1956).

characteristic of pancreatic fluid is its high HCO_3^- concentration and alkaline pH. In the dog, the pH ranges from 7.4 to 8.3, depending on HCO_3^- content. The volume of pancreatic secretion is directly related to its HCO_3^- content, and the pH increases and Cl^- concentration decreases as the rate of flow increases. The Na^+ and K^+ concentrations and osmolality appear to be independent of the secretory rate (Fig. 14-4).

2. α-Amylase

The amylase produced by the pancreas catalyzes the specific hydrolysis of α-1,4-glucosidic bonds, which are present in starch and glycogen (α-1,4-glycan-4-glycan hydrolase). Pancreatic amylase appears to be essentially identical to the amylase of saliva. It is a calcium-containing metalloenzyme. Removal of calcium by dialysis inactivates the enzyme and markedly reduces the stability of the apoenzyme. Pancreatic amylase has an optimal pH for activity of 6.7 to 7.2 and is activated by Cl^-.

After synthesis of pancreatic α-amylase in the ribosomes, the enzyme is transferred from the endoplasmic reticulum to cytoplasmic zymogen granules for storage. It is secreted in active form upon stimulation of the acinar cells. Newborn calves and pigs secrete amylase at a significantly lower rate than mature animals. The rate of synthesis is also influenced by diet. Animals fed a high-carbohydrate diet synthesize amylase at several times the rate of animals on a high-protein diet.

Unbranched α-1,4-glucosidic chains, such as those found in starch, are hydrolyzed in two steps. The first is rapid and results in formation of the maltose and maltotriose. The second step is slower and involves hydrolysis of maltotriose into glucose and maltose. Polysaccharides such as amylopectin and glycogen contain branched chains with both α-1,4- and α-1,6-glucosidic linkages. When α-amylase attacks these compounds, the principal products are maltose (α-1,4-glycosidic bond), isomaltose (α-1,6-glucosidic bond), and small amounts of glucose. Final hydrolysis of the maltose and isomaltose occurs at the surface of the mucosal cell, where the enzymes maltase and isomaltase are integral parts of the microvillous membrane.

3. Proteolytic Enzymes

The proteolytic enzymes of the pancreas are responsible for the major portion of protein hydrolysis, which occurs within the lumen of the gastrointestinal tract. The pancreas secretes two types of peptidases. Trypsin, chymotrypsin, and elastase are endopeptidases that attack peptide bonds along the polypeptide chain to produce smaller peptides.

TABLE 14-4 Relationships among the Activities of Pancreatic Endopeptidases and Exopeptidases

Enzyme	Type	Activity
Trypsin	Endopeptidase	Produces peptides with C-terminal basic amino acids
Carboxypeptidase B	Exopeptidase	Removes C-terminal basic amino acids
Chymotrypsin	Endopeptidase	Produces peptides with C-terminal aromatic amino acids
Elastase	Endopeptidase	Produces peptides with C-terminal nonpolar amino acids
Carboxypeptidase A	Exopeptidase	Removes C-terminal aromatic and nonpolar amino acids

The exopeptidases attack either the carboxy-terminal or amino-terminal peptide bonds, releasing single amino acids. The principal exopeptidases secreted by the pancreas are carboxypeptidases A and B. The endopeptidases and exopeptidases act in complementary fashion (Table 14-4), ultimately producing free amino acids or very small peptides. The free amino acids are absorbed directly, and the small peptides are further hydrolyzed by the aminopeptidases of the intestinal mucosa.

The pancreatic peptidases are secreted as the inactive proenzymes (zymogens), trypsinogen, chymotrypsinogen, and the procarboxypeptidases A and B. Trypsinogen is converted to active trypsin in two ways. At alkaline pH, trypsinogen can be converted autocatalytically to trypsin. The activated enzyme is then capable of converting more zymogen to active enzyme. Trypsinogen also can be activated by the enzyme enterokinase, which is produced by duodenal mucosa. The latter reaction is highly specific in that enterokinase will activate trypsinogen but not chymotrypsinogen. Chymotrypsinogen, proelastase, and the procarboxypeptidases A and B are converted to active enzymes by the action of trypsin.

The amino acid sequences and other structural characteristics of bovine trypsinogen and chymotrypsinogen have been determined (Brown and Hartley, 1966; Hartley *et al.,* 1965; Hartley and Kauffman, 1966). The polypeptide chain of trypsinogen contains 229 amino acid residues. Activation of trypsinogen occurs with hydrolysis of a single peptide bond located in the 6 position between lysine and isoleucine. As the C-terminal hexapeptide is released, enzyme activity appears along with a helical structure of the parent molecule. Chymotrypsinogen A is composed of 245 amino acid residues and has numerous structural similarities to trypsinogen. Activation of the chymotrypsinogen also occurs with cleavage of a single peptide bond.

4. *Lipase*

The pancreas produces several lipolytic enzymes with different substrate specificities. The most important of these from a nutritional viewpoint is the lipase responsible for hydrolysis of dietary triglyceride. This enzyme has the unique property of requiring an oil-water interface for activity so that only emulsions can be effectively attacked. The principal products of lipolysis are glycerol, monoglycerides, and fatty acids. The monoglycerides and fatty acids accumulate at the oil-water interface and can inhibit lipase activity. Transfer of these products from the interface to the aqueous phase is favored by HCO_3^- secreted by the pancreas and by the bile salts.

Two other carboxylic ester hydrolases have been characterized in pancreatic secretion. Both enzymes have an absolute requirement for bile salts, in contrast to glycerol ester hydrolase, which is actually inhibited by bile salts at pH 8. One of the enzymes requiring bile salts is a sterol ester hydrolase responsible for hydrolysis of cholesterol esters, and the other enzyme hydrolyzes various water-soluble esters. The pancreas also secretes phospholipase A, which in the presence of bile converts lecithin to lysolecithin, an effective detergent that contributes to the emulsification of dietary fat.

B. Control of Pancreatic Secretions

1. *Hormonal Control*

Pancreatic secretion is controlled and coordinated by both neural and endocrine mechanisms. When ingesta or HCl enters the duodenum, the hormone secretin, which is produced by the duodenal mucosa, is released into the circulation. Secretin increases the volume, pH, and HCO_3^- concentration of the pancreatic secretion.

Secretin is a polypeptide hormone containing 27 amino acid residues, and all 27 amino acids are required to maintain the helical structure of the molecule and its activity (Bodanszky *et al.,* 1969). The C-terminal amide of secretin is a property shared with other polypeptide hormones such as gastrin and vasopressin, which act on the flow of water in biological systems (Mutt and Jorpes, 1967). In addition to its effects on the pancreas, secretin also increases the rate of bile formation.

The secretin-stimulated pancreatic juice has a large volume, high HCO_3^- concentration but a low enzyme activity.

TABLE 14-5 Gastrointestinal Peptide Hormones

Hormone	Source	Action
Gastrin	G cells of pyloric antrum	Gastric acid secretion
Secretin	S cells of duodenum and jejunum	Pancreatic fluid and HCO_3^- secretion, bile secretion
Cholecystokinin (pancreozymin)	Duodenal and jejunal mucosa; myenteric plexus	Pancreatic enzyme secretion, gallbladder contraction, and sphincter of Oddi relaxation
Somatostatin	D cells of pancreas, CNS, gastric and intestinal mucosa	Inhibits effect of gastrin on gastric secretion, inhibits pancreatic enzyme secretion, stimulates ileal water and NaCl
Enteroglucagon	L cells of small intestine, canine stomach	Control of intestinal cell growth
Gastric inhibitory polypeptide	Duodenal and jejunal mucosa	Inhibits gastric secretion and stimulates intestinal secretion
Motilin	Upper small intestinal mucosa	Stimulates gastrointestinal motility

Stimulation of the vagus nerve causes a significant rise in pancreatic enzyme concentration. This type of response also is produced by cholecystokinin (pancreozymin), another polypeptide hormone produced by the duodenal mucosa, which also causes contraction of the gallbladder. The C-terminal pentapeptide of cholecystokinin-pancreozymin is exactly the same as that of gastrin. This fascinating relationship suggests that gastrin and cholecystokinin-pancreozymin participate in some integrated yet poorly understood system of digestive control.

Several molecular forms of cholecystokinin (CCK) exist (Ward and Washabau, 2005). CCK-33, CCK-39, and CCK-59 are the predominant forms that account for most of the gastrointestinal hormone responses. Endocrine cells in the duodenum and jejunum secrete CCK in response to intraduodenal fatty acids, amino acids, and H^+ ion. CCK-8 is particularly important in cats. Intraluminal distension will activate CCK-8 containing neurons, resulting in acetylcholine release from the myenteric plexus and subsequent peristaltic reflexes in the ileum and colon. CCK-8 neurons in the brain are involved in mediating the satiety response following eating.

VI. OTHER GASTROINTESTINAL HORMONES

A large number of polypeptides have been isolated from the gastrointestinal mucosa and have been classified as gut hormones (Table 14-5). Some of these substances have not yet met all the rigid physiological requirements of true hormones. Some may have paracrine rather than endocrine activities—that is, their actions are on cells and tissues in the immediate vicinity of the cells of origin rather than being released into the vascular system.

A. Motilin

Motilin is a polypeptide containing 22 amino acids that was originally isolated from porcine duodenal mucosa (Brown *et al.,* 1971). The amino acid composition and sequence have been described (Brown *et al.,* 1972, 1973). Immunoreactive motilin has been found in the enterochromaffin cells of the duodenum and jejunum of several species (Polak *et al.,* 1975), and, by means of radioimmunoassay, motilin has been identified in the plasma of dogs (Dryburgh and Brown, 1975). Motilin has been shown to stimulate pepsin output and motor activity of the stomach (Brown *et al.,* 1971) and to induce lower esophageal sphincter contractions (Jennewein *et al.,* 1975). Studies by Itoh *et al.* (1978) suggest that motilin plays an important role in initiating interdigestive gastrointestinal contractions, which are referred to as the interdigestive motility complex or the migrating motility complex (MMC).

The cyclic release of motilin from the intestinal mucosa coordinates gastric, pancreatic, and biliary secretions with phase III of the MMC (Ward and Washabau, 2005). Erythromycin has been shown to induce an MMC similar to motilin and, along with other macrolide-like antibiotics, might be useful in selected cases with motility disorders.

B. Somatostatin

Somatostatin, which is named for its activity of inhibitory release of growth hormone from the pituitary gland, has been purified from ovine and bovine hypothalamus. The hypothalamic hormone is composed of 14 amino acids. Somatostatin also has been demonstrated in the stomach, pancreas, and intestinal mucosa in concentrations higher than in the brain (Pearse *et al.,* 1977). Somatostatin from porcine intestine has been isolated and sequenced, and it

contains 28 amino acids and apparently is a prohormone (Pradayrol *et al.,* 1980). Somatostatin is a potent inhibitor of insulin and glucagon release. It also inhibits gastrin release and gastric acid secretion (Barros D'Sa *et al.,* 1975; Bloom *et al.,* 1974), apparently acting independently on parietal cells and on G cells. These and a variety of other physiological effects suggest that somatostatin has important gastrointestinal regulatory functions.

C. Enteroglucagon

Enteroglucagon is the hyperglycemic, glycogenolytic factor isolated from the intestinal mucosa. It occurs in two forms, one a 3.5 kd form and another somewhat larger (Valverde *et al.,* 1970). Enteroglucagon differs from pancreatic glucagon biochemically, immunologically, and in its mode of release. The physiological function of enteroglucagon is not known, but its release from the mucosa following a meal and the associated increase in circulating blood levels have suggested a regulatory role on bowel function (Pearse *et al.,* 1977). Enteroglucagon also differs significantly from the glucagon produced by the A cells of the gastric mucosa of the dog (Sasaki *et al.,* 1975). Canine gastric glucagon is biologically and immunochemically identical to pancreatic glucagon. Gastric glucagon appears to be unique to the dog, similar activity not being observed in the stomach of the pig or the abomasum of cattle and sheep.

VII. DIGESTION AND ABSORPTION

A. Water and Electrolytes

1. *Mechanisms of Mucosal Transport*

The microvillous membrane of the intestinal mucosa, because of its lipid composition, acts as a barrier to water and water-soluble substances. Water and polar solutes penetrate the mucosa in one of three ways. They may pass through aqueous pores or channels that connect the luminal surface of the cell with the apical cytoplasm, they may attach to membrane carriers that facilitate passage through the lipid phase of the mucosal cell membrane, or they may pass paracellularly through tight junctions (shunt pathway). Transport of water and water-soluble compounds is influenced by the permeability characteristics of the limiting membrane and by the nature of the driving forces that provide energy for transport. Passive movement occurs either by simple diffusion or as a result of concentration gradients (activity), pH, osmotic pressure, or electrical potential that may exist across the membrane. The movement of an ion in the direction of an electrochemical gradient is considered passive in nature. Active transport is said to occur when a substance moves in a direction opposite that of an established electrochemical gradient.

Most water-soluble compounds, such as monosaccharides and amino acids, cannot diffuse across the intestinal mucosal membrane at rates that are adequate to meet nutritional requirements. The transport of these nutrients requires membrane carriers, which are integral parts of the membrane and their binding is highly specific. Carrier-mediated transport systems can be saturated and competitively inhibited by related compounds.

Three types of carrier transport mechanisms are recognized (Curran and Schultz, 1968). (1) *Active transport*, as stated previously, involves movement of electrolytes against an electrochemical gradient. In the case of nonelectrolytes such as glucose, active transport is defined as movement against a concentration gradient. Active transport requires metabolic energy and is inhibited by various metabolic blocking agents or by low temperature. (2) *Facilitated diffusion* occurs when the passive movement of a substance is more rapid than can be accounted for by simple diffusion. Facilitated diffusion systems may increase the rate of movement across the membrane by two or three orders of magnitude. The responsible carrier mechanism is similar to that involved in active transport in that it displays saturation kinetics, may be inhibited competitively, and is temperature dependent. However, transport does not occur against concentration or electrochemical gradients, and direct expenditure of energy is not required. (3) *Exchange diffusion* is a transfer mechanism similar to facilitated diffusion and was postulated originally to explain the rapid transfer of radioactive Na^+ across epithelial cell membranes *in vitro*. The mechanism involves the exchange of one ion for another of like charge (e.g., Na^+ and H^+ or Cl^- and HCO_3^-), not giving rise to net transport but contributing in a major way to unidirectional flux rate.

In the intestine, net water absorption is the result of bulk flow through pores. Diffusion in the usual sense plays no important role in water movement. When bulk flow of water occurs, it is possible for solutes to move across the membrane in the direction of flow by a phenomenon called *solvent drag*. The effect of solvent drag on the transport of a given solute depends on the rate of volume flow and on the reflection coefficient, an expression of the relationship between the radius of membrane pores and the radius of the solute molecule being transported. By means of solvent drag, it is possible for a solute such as urea to be transported by the intestine against a concentration gradient (Hakim and Lifson, 1964).

2. *Sodium and Chloride Absorption*

Na^+ and Cl^- are the major ions in the fluid that are transported by the intestine during absorption or secretion, and under most conditions, transport of these two ions is coupled. The transport of water and electrolytes by the intestinal mucosa is a dynamic process, with rapid unidirectional fluxes of both occurring continuously. Net absorption occurs when the flow from lumen to plasma exceeds that from plasma to lumen. Active transport of Na^+ can occur along

the entire length of the intestine, but the rate and net absorption is greatest in the ileum and colon. Na^+ transport is by an energy-requiring "sodium pump" mechanism that is intimately associated with the Na^+-K^+-ATPase located within the basolateral cell membrane of the absorptive epithelial cell. Three mechanisms exist for the entry of Na^+ at the brush border: (1) electrodiffusion down a concentration gradient, (2) cotransport of electrolytes that either enter (Cl^-) or exit (H^+) the cell as Na^+ enters, and (3) Na^+ entry coupled with organic nonelectrolytes (glucose, amino acids). Current evidence suggests that in the absence of the absorption of nonelectrolytes, electroneutral uptake accounts for most NaCl absorption. At the brush border, Na^+ enters down a concentration gradient but exits at the basolateral cell surface against a substantial gradient. Maintenance of the transmembrane Na^+ gradient by the Na pump requires continual metabolism and generation of ATP. The Na^+-K^+-ATPase can be inhibited by cardiac glycosides such as ouabain, which are effective inhibitors of Na^+ transport. The Na^+ gradient ultimately serves as an energy source for transport of other solutes (Schultz and Curran, 1970).

In the jejunum, net absorption of sodium occurs slowly unless nonelectrolytes, such as glucose or amino acids, are absorbed simultaneously. In the ileum, Na^+ absorption is independent of glucose absorption. Net water absorption in the jejunum is almost entirely dependent on the absorption of glucose and other nonelectrolytes, whereas absorption from the ileum is unaffected by glucose. The differential effect of glucose on absorption from the jejunum and ileum is the result of fundamental differences in electrolyte transport mechanisms in these two regions of the intestine.

As Na^+ is transported across the mucosa, an equivalent amount of anion must be transported to maintain electrical neutrality. A major fraction of Cl^- absorption can be accounted for by passive cotransport with Na^+. Under certain circumstances, Cl^- enters the cell in exchange for HCO_3^-.

3. Potassium Absorption

Dietary K^+ is absorbed almost entirely in the proximal small intestine. Absorption across the intestinal mucosa occurs down a concentration gradient (high luminal concentration to a low concentration in plasma). The intestinal fluid reaching the ileum from the jejunum has a K^+ concentration and a Na^+/K^+ ratio that are similar to plasma. In the ileum and colon, the rate of Na^+ absorption is much greater than that of K^+ so that, under normal conditions, the Na^+/K^+ ratio in the feces is much lower than that of plasma, approaching a ratio of 1.

4. Water Absorption

The absorption of water has been one of the most extensively studied aspects of intestinal transport. Water movement is the result of bulk flow through membranous pores, and simple diffusion plays only a minor role. The question of whether water is actively or passively transported has been the subject of considerable controversy, and the controversy itself points to the fundamental difficulties that arise in trying to establish a definition of active transport. Hypertonic saline solutions can be absorbed from canine intestine *in vivo* and from canine and rat intestine *in vitro*. These observations indicate that water absorption can occur against an activity gradient and that the process is dependent on metabolic energy. This suggests that an active transport process is involved, but Curran (1965) presented an alternate interpretation, which is now generally accepted. This view is that water transport occurs secondarily to active solute transport and is the result of local gradients established within the mucosal membrane. Water transport is then coupled to the energy-dependent processes responsible for solute transport but is one step removed from it.

In the dog and probably other carnivores, the ileum is the main site of net Na^+ and water absorption. In the dog, the colon accounts for no more than perhaps 20% of the total. In herbivorous animals that have a well-developed large intestine, there may actually be a net secretion of water within the small intestine during digestion. For example, in the guinea pig (Powell *et al.,* 1968) and horse (Argenzio, 1975), all net absorption of water takes place in the cecum and colon.

Vasoactive intestinal polypeptide (VIP) and acetylcholine have an important role in fluid and electrolyte balance (Hall and German, 2005). As mediators of secretion, they increase intracellular calcium and cyclic adenosine monophosphate (cAMP), inhibit neutral sodium and chloride absorption, and facilitate transcellular chloride efflux. Some bacterial infections result in diarrhea because of an increase in cAMP; functional tumors of VIP-producing cells can also produce diarrhea. Noradrenaline, somatostatin, and opioids, which are the important regulators of absorption, lower intracellular cAMP and calcium concentrations and stimulate neutral NaCl absorption. For these reasons, they can have antidiarrheal effects.

B. Carbohydrate Digestion and Absorption

1. Polysaccharide Digestion

a. Starch and Glycogen

Carbohydrate is present in the diet primarily in the form of polysaccharides. The most common polysaccharides are starch, glycogen, and cellulose. Starch and glycogen are composed of long chains of glucose molecules linked together by repeating α-1,4-glucosidic bonds. Branch points of the chains are linked by α-1,6-glucosidic bonds. In those species that secrete salivary amylase, digestion of starch and glycogen begins in the mouth when this enzyme mixes with food. The action of salivary amylase is interrupted in the stomach, however, because of the low pH of the gastric secretion.

Starch digestion begins again in the proximal small intestine with the highly specific action of pancreatic amylase

TABLE 14-6 Enzymes of the Intestinal Brush Border

Enzyme	Substrate	Product	Reference
Lactase	Lactose	Glucose, galactose	Alpers (1969), Forstner *et al.* (1968)
Sucrase	Sucrose; 1,4% dextrins	Glucose, fructose; residual 1,6-oligosaccharides	Gray *et al.* (1979)
Isomaltase	1,6% Dextrins	Glucose	Gray *et al.* (1979), Rodriguez *et al.* (1984)
%-Limit dextrinase	1,6% Dextrins	Glucose	Taraval *et al.* (1983)
Trehalase	Trehalose	Glucose	Eichholtz (1967), Nakano *et al.* (1977)
Enterokinase	Trypsinogen	Trypsin	Grant and Herman-Taylor (1976)
Aminopeptidase A	Acidic amino-terminal amino acids	Acidic amino acids	Benajiba and Maroux (1980)
Aminopeptidase N	Neutral amino-terminal amino acids	Neutral amino acids	Kim and Brophy (1976), Erickson *et al.* (1983)
(-Glutamyl transferase)	Peptides with (-glutamyl bonds)	(-Glutamyl amino acids)	Benajaba and Maroux (1980), Hughey and Curthoys (1976)
Alkaline phosphatase	Phosphate esters	Inorganic phosphate	Eichholz (1967), Forstner *et al.* (1968)

on α-1,4-glucosidic bonds. This enzyme catalyzes a series of stepwise hydrolytic reactions, resulting in formation of the principal end products of starch digestion, the disaccharides maltose and isomaltose, and small amounts of glucose. Glucose is absorbed directly by the intestinal mucosa and transported to the portal vein. Enzymes of the intestinal cell brush border hydrolyze the disaccharides further.

b. Cellulose

Cellulose, like starch, is a polysaccharide of glucose but differs from starch in that the glucose molecules are linked by β-1,4-glucosidic bonds. All species can utilize starch, but only animals that have extensive bacterial fermentation within the gastrointestinal tract utilize cellulose indirectly as a significant source of energy. Ruminant species digest cellulose most efficiently, but other animals in which the large intestine is well developed (e.g., the horse) also utilize cellulose as an important energy source.

In ruminants, hydrolysis of cellulose is accomplished by cellulitic bacteria, which are part of the complex rumen microflora. The primary end products of cellulose fermentation are short-chain fatty acids: acetic, propionic, and butyric acids. These are absorbed directly from the rumen and serve as the major source of energy for ruminants. Propionic acid is the major precursor for carbohydrate synthesis in mature ruminants.

2. *Disaccharide Digestion*

Maltose and isomaltose are the disaccharides (glucose-glucose) produced as end products of starch digestion. The diet also may contain lactose (galactose-glucose) and sucrose (fructose-glucose). There is general agreement that disaccharide digestion is completed at the surface of the cell by disaccharidases (Gray, 1975), which are components of the brush border (Table 14-6).

The disaccharidases have been solubilized from the brush border and partially purified. Sucrase and isomaltase have been purified together as a two-enzyme complex (Gray *et al.,* 1979; Kolinska and Semenza, 1967), and this enzyme complex accounts for the total hydrolysis of the products of amylase digestion (Gray *et al.,* 1979; Rodriguez *et al.,* 1984). The mutual mucosa contains two enzymes with lactase activity. One of these is a nonspecific β-galactosidase that hydrolyzes synthetic β-galactosides effectively but hydrolyzes lactose at a slow rate. This enzyme has an optimal pH of 3 and is associated with the lysosomal fraction of the cell. The other lactase hydrolyzes lactose readily, is associated with the brush border fraction of the cell, and is the enzyme of primary importance in the digestive process (Alpers, 1969).

Maltase, isomaltase, and sucrase are almost completely absent from the intestine in newborn pigs (Dahlqvist, 1961) and calves. The activity of these disaccharidases increases after birth and reaches adult levels during the first months of life. Lactase activity is highest at birth and decreases gradually during the neonatal period. The relatively high lactase activity may be an advantage to the newborn in utilizing the large quantities of lactose present in their diets. Bywater and Penhale (1969) demonstrated lactase deficiency following acute enteric infections and suggested that lactose utilization may be decreased in such cases.

3. *Monosaccharide Transport*

a. Specificity of Monosaccharide Transport

Regardless of whether monosaccharides originate in the lumen of the intestine or are formed at the surface of the mucosal cell, transport across the mucosa involves processes that have a high degree of chemical specificity. Glucose and galactose are absorbed from the intestine more rapidly than other monosaccharides. Fructose is absorbed at approximately half the rate of glucose, and mannose is absorbed at less than one-tenth the rate of glucose (Kohn *et al.,* 1965).

Glucose and galactose can be absorbed against a concentration gradient. The monosaccharides that are transported most efficiently against gradients have common structural characteristics: (1) the presence of a pyranose ring, (2) a carbon atom attached to C-5, and (3) a hydroxyl group at C-2 with the same stereoconfiguration as D-glucose, but these features are not absolute requirements. Both D-xylose, which has no substituted carbon atom at C-5, and D-mannose, which lacks the appropriate hydroxyl configuration at C-2, can be transported against concentration gradients under specific experimental conditions (Alvarado, 1966b).

Glucose transport is competitively inhibited by galactose (Fisher and Parsons, 1953) and by a variety of substituted hexoses that compete with glucose for carrier binding sites. The glucoside phlorizin is a potent inhibitor (Alvarado and Crane, 1962; Parsons *et al.,* 1958). Phlorizin also competes for binding sites but has a much higher affinity for these sites than does glucose.

The absorptive surface of the mucosal cell is the microvillous membrane, or brush border. It is through this part of the plasma membrane that glucose must pass during the initial phase of mucosal transport. Techniques have been developed for isolating highly purified preparations of microvillous membranes from mucosal homogenates (Forstner *et al.,* 1968). Faust *et al.* (1967) studied the binding of various sugars to these isolated membrane fractions. They found that D-glucose was bound by the membrane preferentially to L-glucose or to D-mannose and that glucose binding was completely inhibited by 0.1mM phlorizin. The specificity of their observations suggested that binding represented an initial step in glucose transport, namely, attachment to a membrane carrier.

b. Sodium Requirement

The absorption of glucose and other monosaccharides is influenced significantly by Na^+ (Kimmich, 1973; Schultz and Curran, 1970). When Na^+ is present in the solution bathing the intestinal mucosa, glucose is absorbed rapidly, but when Na^+ is removed and replaced by equimolar amounts of other cations, glucose absorption virtually stops (Bihler and Crane, 1962; Bihler *et al.,* 1962; Csaky, 1961; Riklis and Quastel, 1958). Glucose absorption is inhibited by ouabain, digitalis, and other cardiac glycosides that are also inhibitors of Na^+-K^+-ATPase activity and Na^+ transport

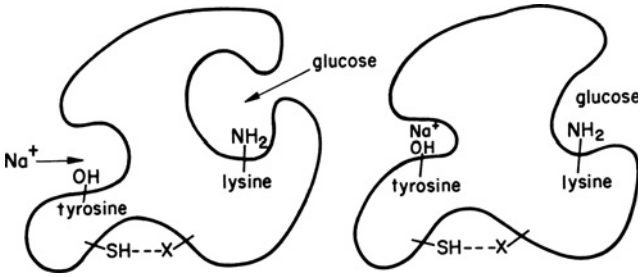

FIGURE 14-5 Model of a Na^+-activated glucose carrier of the intestinal brush border. (From Wright and Peerce, 1985).

(Csaky and Hara, 1965; Schultz and Zalusky, 1964). These observations demonstrate the close relationship between the transport of glucose and Na^+.

c. Characteristics of the Na^+-Glucose Transporter (Carrier)

The concentrative step in the active transport of glucose occurs at the brush border membrane, and energy for this process is derived from an electrochemical Na^+ gradient (Schultz, 1977; Schultz and Curran, 1970). Under conditions of net influx, Na^+ and glucose enter in a ratio of 1:1 (Goldner *et al.,* 1969; Hopfer and Groseclose, 1980). Cotransport of glucose and Na^+ involves a membrane transporter or carrier that is believed to be a 75-kd polypeptide (Wright and Peerce, 1985). Na^+ activates glucose transport primarily by increasing the affinity of the carrier for glucose. A model showing two hypothetical forms of the glucose carrier is presented in Figure 14-5. A galent channel or pore mechanism has been proposed in which the glucose binding site is located within the membrane. The translocation of glucose in this model is believed to be the result of a Na^+-induced conformational change in the transporter (Semenza *et al.,* 1984).

C. Proteins

1. *Enzymatic Hydrolysis*

The initial step in protein digestion is the enzymatic hydrolysis of peptide bonds by proteases with formation of smaller peptides and amino acids. The endopeptidases hydrolyze peptide bonds within the protein molecule and also hydrolyze certain model peptides. Exopeptidases hydrolyze either the carboxy-terminal (carboxypeptidase) or the amino-terminal (aminopeptidase) amino acids of peptides and certain proteins. Thus, a mixture of exopeptidases and endopeptidases cleaves long chain polypeptides from the ends as well as within the length of the chain resulting in sequentially shorter and shorter polypeptide chains and amino acids.

Dietary proteins first come in contact with proteolytic enzymes in the stomach. The best known of the gastric proteases is the family of pepsins (Samloff, 1971), which

hydrolyze most proteins with the exception of keratins, protamines, and mucins. Pepsins are relatively nonselective and hydrolyze peptide bonds involving many amino acids, the most readily hydrolyzed of which involve leucine, phenylalanine, tyrosine, and glutamic acid.

The extent of proteolysis in the stomach depends on the nature of the dietary protein and the duration of time the protein remains in the stomach. The food bolus mixed with saliva has a neutral or slightly alkaline pH as it enters the stomach, and a period of time is required for it to mix with gastric secretions and become acidified. Proteolytic digestion begins when the pH of the gastric contents approaches 4 and occurs optimally in two pH ranges, 1.6 to 2.4 and 3.3 to 4 (Taylor, 1959a, 1959b). Because of the relative lack of specificity of the pepsins, some peptide bonds of almost all dietary proteins are split during passage through the stomach. The gastric phase of protein digestion may have a minor and possibly dispensable role in overall protein assimilation (Freeman and Kim, 1978), but the reservoir function of the stomach contributes to the gradual release of nutrients, ensuring more efficient utilization in the small intestine.

Partially digested peptides pass from the stomach to the duodenum, where the acidic contents are neutralized by sodium bicarbonate present in bile and pancreatic juice. Peptic activity persists in the duodenum only during the period required to raise the pH above 4. The major peptidases that are active within the lumen of the small intestine are the pancreatic enzymes trypsin, chymotrypsin, elastase, and carboxypeptidases A and B. The action of these enzymes is integrated so that the endopeptidases produce peptides with C-terminal amino acids, which then become substrates for the exopeptidases. Trypsin produces peptides with basic C-terminal amino acids that are particularly suited for the action of carboxypeptidase B. Chymotrypsin produces peptides with aromatic amino acids in the C-terminal position, and elastase produces peptides with C-terminal amino acids that are nonpolar. Carboxypeptidase A hydrolyzes both types of C terminal peptide bonds (Table 14-4).

The final steps in peptide digestion are associated with mucosal epithelial cells. Almost all of the aminopeptidase activity is associated with the mucosa, and very little activity is present in luminal contents. Mucosal aminopeptidase activity is located both in the cytosol and in the brush border membrane fractions of the epithelial cell (Heizer and Laster, 1969; Kim *et al.,* 1972). These physically separate enzymes have remarkably different substrate specificities (Kim *et al.,* 1974). The brush border enzyme has more than 50% of the activity for tripeptides yet less than 10% of the total activity for dipeptides relative to the cytosolic enzyme(s) (Kim *et al.,* 1972; Peters, 1970). Almost all activity for tetrapeptides is present in the brush border (Freeman and Kim, 1978). Proline-containing peptides are hydrolyzed almost exclusively by cytosolic peptidases, whereas leucine aminopeptidase activity is located primarily in the brush border. The brush border peptidases appear to have digestive functions similar to the disaccharidases and oligosaccharidases of the brush border. Endopeptidase activity of the intestinal mucosa is associated primarily within the lysosomal fraction of the cell.

2. *Absorption of Proteolytic Products*

Despite the long interest in the subject of this section, the relative amounts of the various protein digestion products (i.e., peptides versus amino acids) that are actually absorbed by intestinal mucosal cells during normal digestion remain problematic. It is a difficult process to investigate because the products of proteolysis are absorbed rapidly after they are formed and, therefore, studies of luminal contents give only an estimate of the overall rate of protein digestion. Equally important, dietary protein is continually mixed with endogenous protein in the form of digestive secretions and extruded mucosal cells. Most endogenous proteins are hydrolyzed and the amino acids absorbed in a manner similar to that of dietary protein, and the two processes occur simultaneously. Endogenous protein accounts for a significant part of the amino acids of the intestinal contents. Even when dietary protein is labeled with a radioactive tracer, there is such rapid utilization that the tracer soon reenters the lumen in the form of endogenous protein secretion.

In adult mammals, protein is not absorbed from the intestine in quantities of nutritional significance without previous hydrolysis. Most neonatal animals absorb significant amounts of immunoglobulin and other colostral proteins, but this capacity is lost soon after birth. The intestinal mucosa, however, is not totally impermeable to large polypeptide molecules. The absorption of insulin (MW 5700; Danforth and Moore, 1959; Laskowski *et al.,* 1958), ribonuclease (MW 13,700; Alpers and Isselbacher, 1967), ferritin, and horseradish peroxidase (Warshaw *et al.,* 1971) has been demonstrated.

During the digestion of protein, the amino acid content of portal blood increases rapidly, but attempts to demonstrate parallel increases in peptides in the portal blood have not been uniformly successful. This has been regarded as evidence that only amino acids can be absorbed by the intestinal mucosa and that the absorption of peptides does not occur. Although it seems clear that most dietary protein is absorbed by the mucosal epithelium in the form of free amino acids, peptides also may be taken up by the mucosal cell in quantitatively significant amounts. Peptides so absorbed may be hydrolyzed either at the cell surface or intracellularly, and individual amino acids finally enter the portal circulation via the basolateral cell membrane.

Small peptides, under certain circumstances, may cross the intestinal epithelium intact and enter the portal circulation. Webb (1986) suggested that intact peptide absorption accounted for more than half of luminal amino acid nitrogen in the calf. The amount of peptide nitrogen entering the portal circulation in other species characteristically has

been lower and variable depending on the source of protein and on the digestibility of the peptide being investigated (Gardner, 1984).

3. *Transport of Amino Acids*

Amino acids, like glucose and certain other monosaccharides, are absorbed and transferred to the portal circulation by active transport processes. The same type of saturation kinetics observed in studies of monosaccharide absorption are observed with amino acids, which suggests the presence of carrier transport mechanisms. Certain monosaccharides inhibit amino acid transport (Newey and Smyth, 1964; Saunders and Isselbacher, 1965), and whereas inhibition generally has been of the noncompetitive type, competitive inhibition between galactose and cycloleucine has been demonstrated (Alvarado, 1966a), which suggests that a common carrier may be involved.

Most amino acids are transported against concentration and electrochemical gradients, and the overall transport process requires metabolic energy. The chemical specificity of these transport mechanisms is shown by the fact that the natural l-forms of various amino acids are absorbed more rapidly than the corresponding d-forms and that only the l-amino acids appear to be actively transported. For most transport systems, Na^+ is necessary for absorption of amino acids as it is for a variety of other nonelectrolyte substances (Gray and Cooper, 1971; Schultz and Curran, 1970).

Separate transport systems exist for different groups of amino acids. Each member of a group inhibits the transport of other members competitively, suggesting that they share the same carrier. There is demonstrable overlap between groups, indicating that the overall transport process is complex (Christensen, 1984, 1985; Stevens *et al.,* 1984). The following is a summary of the designations and substrates of the recognized amino acid transport systems of the intestinal brush border (Stevens *et al.,* 1984):

1. The neutral brush border (NBB) pathway is responsible for monoaminomonocarboxylic (neutral) amino acids and histidine. Na^+ is required, and these amino acids show mutual competition for transport.
2. The monoaminodicarboxylic acids (aspartic and glutamic acid) pathway (XGA) requires Na^+. Aspartic and glutamic acids are not transported against concentration gradients. Following uptake, they are transaminated by the intestinal mucosa and under physiological conditions enter the portal vein as alanine.
3. Imino acids (IMINO), proline, hydroxyproline, methylaminoisobutyric acid, and N-substituted glycine derivatives sarcosine (N-methyl glycine) and betaine (N-dimethylglycine). This pathway also has a Na^+ requirement.
4. Dibasic amino acids (Y^+), including lysine, arginine, ornithine, and the neutral amino acid cystine.
5. Phenylalanine and methionine share the PHE amino acid transport system.

The ϑ-glutamyl cycle has been proposed as a possible transport system for amino acids (Meister and Tate, 1976). ϑ-Glutamyltransferase (GGT) is a membrane-bound enzyme that is present in a number of mammalian tissues and catalyzes the initial step in glutathione degradation. The 0-glutamyl moiety of glutathione is transferred to amino acid (or peptide) receptors with the production of cysteinylglycine:

$$\text{glutathione} + \text{amino acid} \xrightarrow{\text{GGT}} \vartheta\text{-glutamyl-amino acid} + \text{Cys-Gly}$$

The highest GGT activity is present in tissues that are known to transport amino acids actively (e.g., the jejunal villus, the proximal convoluted tubule of the kidney and liver). Meister and his colleagues (1976) have suggested that GGT may function in translocation by interaction with extracellular amino acids and with intracellular glutathione. The hypothetical mechanism involves the noncovalent binding of extracellular amino acids to the plasma membrane, whereas intracellular glutathione interacts with GGT to yield a ϑ-glutamyl-enzyme complex. When the ϑ-glutamyl moiety is transferred to the membrane-bound amino acid, a ϑ-glutamyl-amino acid complex is formed, which, when released from the membrane binding site, moves into the cell. The ϑ-glutamyl-amino acid complex is split by the action of ϑ-glutamyl cyclotransferase, an enzyme appropriately located in the cytosol. Glutathione is regenerated by means of the ϑ-glutamyl cycle, which is a good substrate for GGT (Thompson and Meister, 1975).

The ϑ-glutamyl cycle does not require sodium, and the cycle would not explain the previously demonstrated sodium dependence for amino acid transport. The cycle is not considered to be the only amino acid transport system, and its quantitative significance in individual tissues is unknown.

4. *Neonatal Absorption of Immunoglobulin*

At birth, most domestic species, including the calf, foal, lamb, pig, kitten, and pup, absorb significant quantities of colostral protein from the small intestine. Immune globulin (Ig) either is absent in the serum of domestic species at birth or the level is low. Within a few hours after ingestion of colostrum, the serum Ig levels rise. This represents the principal mechanism by which the young of most domestic animal species acquire maternal immunity. Under normal environmental conditions, ingestion of colostrum is an absolute requirement for health during the neonatal period. The rabbit is the exception in that maternal Ig is received primarily *in utero* by transplacental transfer.

Protein enters the neonatal absorptive cell by pinocytosis and passes through the cell to the lymphatics. The process

is not selective because many proteins other than Ig can be absorbed (Payne and Marsh, 1962). The ability to absorb intact protein is lost by domestic species soon after birth. In the piglet, "closure" occurs within 1 to 2 days (Leary and Lecce, 1978; Westrom *et al.,* 1984) beginning in the duodenum and occurring last in the ileum. In rodents, protein absorption normally continues for approximately 3 weeks. The mechanism of intestinal "closure" was studied, and researchers found that complete starvation of pigs lengthened the period of protein absorption to 4 to 5 days, whereas early feeding shortened the period (Lecce, 1965; Lecce and Morgan, 1962; Leece *et al.,* 1964). Feeding different fractions of colostrum including lactose and galactose resulted in loss of protein absorptive capacity. The route of feeding may not be the critical factor, however. Calves that are prevented from eating but that receive nutrients parenterally lose the ability to absorb protein at the same time as control calves (Deutsch and Smith, 1957).

In the neonatal calf, Ig deficiency resulting from a failure of colostral Ig absorption plays a role in the pathogenesis of Gram-negative septicemia (Gay, 1965; Smith, 1962). Most calves deprived of colostrum develop septicemia early in life and may develop acute diarrhea before death (Smith, 1962; Tennant *et al.,* 1975; Wood, 1955). Hypogammaglobulinemia is almost always demonstrable in calves dying of Gram-negative septicemia and is the result either of insufficient Ig intake or of insufficient intestinal absorption. The Ig fraction is the essential factor in colostrum that protects against systemic infections (Penhale *et al.,* 1971).

Serum immunoglobulin values of neonatal calves vary, and a 10% incidence of hypogammaglobulinemia may occur in clinically normal calves (Braun *et al.,* 1973; House and Baker, 1968; Smith *et al.,* 1967; Tennant *et al.,* 1969; Thornton *et al.,* 1972). Most such individuals probably have insufficient colostrum intake. Even when calves were given the opportunity to ingest colostrum, however, a surprising number were hypogammaglobulinemic. Some of the reasons for varying gammaglobulinemia values are recognized, but the relative importance of each is not known. The concentration of lactoglobulin, the volume consumed (Selman *et al.,* 1971), the time elapsed from birth to ingestion of colostrum (Selman *et al.,* 1971), and the method of ingestion (natural suckling versus bucket feeding) may have an important influence on the serum IgG (McBeath *et al.,* 1971; Smith *et al.,* 1967). Calves that suckle their dams usually attain serum IgG concentrations that are higher than those attained by calves given colostrum from a bucket. The frequency of hypogammaglobulinemia may be influenced by season (Gay *et al.,* 1965b; McEwan *et al.,* 1970a), although this relationship is not consistent (Smith *et al.,* 1967; Thornton *et al.,* 1972). Familial factors also may influence development of hypogammaglobulinemia (Tennant *et al.,* 1969).

Regardless of cause, the mortality of hypogammaglobulinemic calves is higher than that of calves with normal serum IgG levels (Boyd, 1972; Gay, 1965; House and Baker, 1968; McEwan *et al.,* 1970a; Naylor *et al.,* 1977; Thornton *et al.,* 1972). In addition to having more septicemic infections (Gay, 1965; McEwan *et al.,* 1970a; Roberts *et al.,* 1954; Smith, 1962; Wood, 1955), hypogammaglobulinemic calves have a greater prevalence of acute diarrheal disease (Boyd, 1972; Gay *et al.,* 1965a; Naylor *et al.,* 1977; Penhale *et al.,* 1970), which indicates that the local protective effects of Ig in the intestine are important (Fisher *et al.,* 1975; Logan and Penhale, 1971).

The prevalence of hypogammaglobulinemia and the high mortality associated with it has led to the development of several rapid tests for identification of hypogammaglobulinemic calves (Aschaffenburg, 1949; Fisher and McEwan, 1967b; McBeath *et al.,* 1971; Patterson, 1967; Stone and Gitter, 1969). The zinc sulfate turbidity test (Kunkel, 1947) was the first to be used to determine the serum immunoglobulin concentrations of neonatal calves (McEwan *et al.,* 1970b). A close correlation has been established between test results and the amount of serum IgG and IgM (Fisher and McEwan, 1967b, 1967b; McEwan *et al.,* 1970b).

The sodium sulfite turbidity test is similar to the zinc sulfate test and also has been used to identify hypogammaglobulinemic calves (Pfeiffer and McGuire, 1977; Stone and Gitter, 1969). Failure of turbidity to develop when serum is added to a saturated solution of sodium sulfite indicates immunoglobulin deficiency. A semiquantitative assessment of the Ig concentration is made by grading the degree of turbidity (Stone and Gitter, 1969).

The refractometer is used as a rapid test for Ig deficiency (Boyd, 1972; McBeath *et al.,* 1971). There is a close relationship between the concentration of IgG and total serum protein (TSP) in neonatal calves (Tennant *et al.,* 1969), and the wide variations in TSP were due to variations in IgG. Direct linear correlation between the refractive index (RI) and the Ig concentration has also been observed (McBeath *et al.,* 1971). The regression line for this relationship was independently confirmed (Tennant *et al.,* 1978). The Y intercepts in these studies were identical (4 g/dl). The refractometer has a value as a rapid field instrument for the assessment of Ig status, but in cases of hemoconcentration it has limitations (Boyd, 1972).

The glutaraldehyde coagulation test was used originally in cattle to detect hypergammaglobulinemia in samples of whole blood (Sandholm, 1974). Glutaraldehyde has also been used in a semiquantitative test to evaluate IgG in canine (Sandholm and Kivisto, 1975) and human serum (Sandholm, 1976). This procedure has been modified to detect hypogammaglobulinemic calves. Calves with a negative test result (serum IgG #0.4 g/dl) had markedly higher mortality than calves with positive results (Table 14-7) (Tennant *et al.,* 1979), which is similar to results obtained by using the zinc sulfate turbidity test (Gay *et al.,* 1965a; McEwan *et al.,* 1970a) or other estimates of circulating IgG. Many tests can be initiated quickly using the glutaraldehyde coagulation test, and results can be evaluated rapidly without instrumentation.

TABLE 14-7 Relationship between Results of the Glutaraldehyde Coagulation Test, Serum (-Globulin) Concentration, and Death Rate

Source of Calves	No.	Glutaraldehyde Reaction	Serum (-Globulin (/dl))		Death Rate (%)
			Mean (±SD)	Extremes	
Calves before ingestion of colostrums	10	Negative	0.18 (±0.06)	0.1–0.25	—[a]
Calves from production unit	60	Negative	0.35 (±0.13)	0.11–0.63	16.7[b]
	13	Incomplete	0.60 (±0.13)	0.42–0.85	7.7
	208	Positive	1.46 (±0.63)	0.42–4.4	3.4

[a] *Samples of serum were obtained at birth, but no follow-up of calves was made.*
[b] *The death rate of calves that were test-negative was significantly* ($p < 0.01$) *greater than that of test-positive calves, using t-test for significance of differences between two percentages.*

D. Lipids

1. *Absorption of Fats*

a. Luminal Phase

The fat in the diet is primarily in the form of triglycerides or long-chain fatty acids. In the dog, gastric lipase plays a credible role in fat digestion, leading to the formation of fatty acids, which help coordinate gastric emptying and pancreatic secretions. In other species, the initial step in utilization of triglycerides occurs in the lumen of the proximal small intestine, where hydrolysis is catalyzed by pancreatic lipase. The pancreas secretes lipase in active form. The enzyme requires an oil-water interface for activity, so only emulsions of fat can be hydrolyzed. Enzyme activity is directly related to the surface area of the emulsion, so the smaller the emulsion particle, the greater the total surface area of a given quantity of triglyceride and the greater the rate of hydrolysis (Benzonana and Desnuelle, 1965). Bile salts are not an absolute requirement but favor hydrolysis by their detergent action, which causes formation of emulsions with small particle sizes and by stimulating lipase activity within the physiological pH range of the duodenum. A colipase is present in the pancreatic secretion, which facilitates the interaction of lipase with its triglyceride substrate and protects lipase from inactivation (Borgstrom and Erlanson, 1971).

Pancreatic lipase splits the ester bonds of triglycerides preferentially at the 1 and 3 positions so that the major end products of hydrolysis are 2-monoglycerides and free fatty acids. Both compounds are relatively insoluble in water but are brought rapidly into micellar solution by the detergent action of bile salts. The mixed micelles so formed have a diameter of approximately 2nm and are believed to be the form in which the products of fat digestion are actually taken up by the mucosal cell (Hofmann and Small, 1967). The intraluminal events that occur in fat absorption are schematically summarized in Figure 14-6.

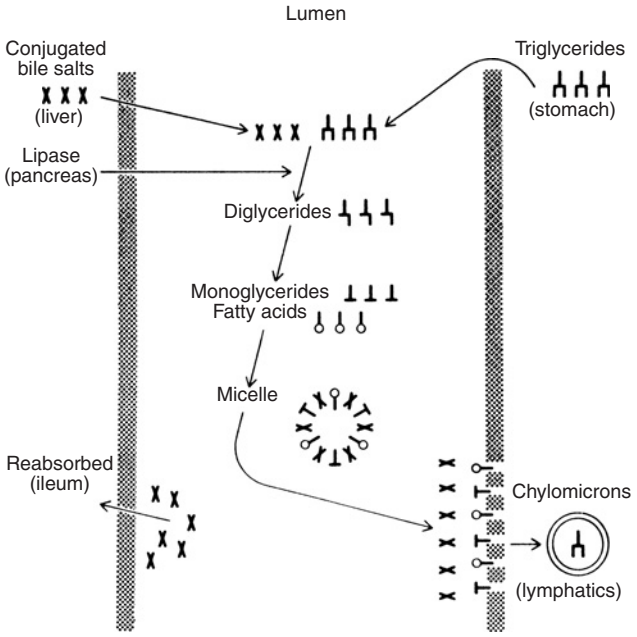

FIGURE 14-6 Intraluminal events during fat absorption. From Isselbacher (1967).

b. Mucosal Phase

The initial step in intestinal transport of fat is the uptake of fatty acids and monoglycerides by the mucosal cell from micellar solution. The precise mechanism is yet unclear, but present evidence suggests that the lipid contents of the micelle are somehow discharged at the cell surface and enter the mucosal cell in molecular rather than micellar form (Isselbacher, 1967). The net effect is the absorption of the end products of lipolysis and the exclusion of bile salts, which are absorbed farther down the intestine, primarily in the ileum. Uptake of fatty acids appears to be a passive process having no requirement for metabolic energy.

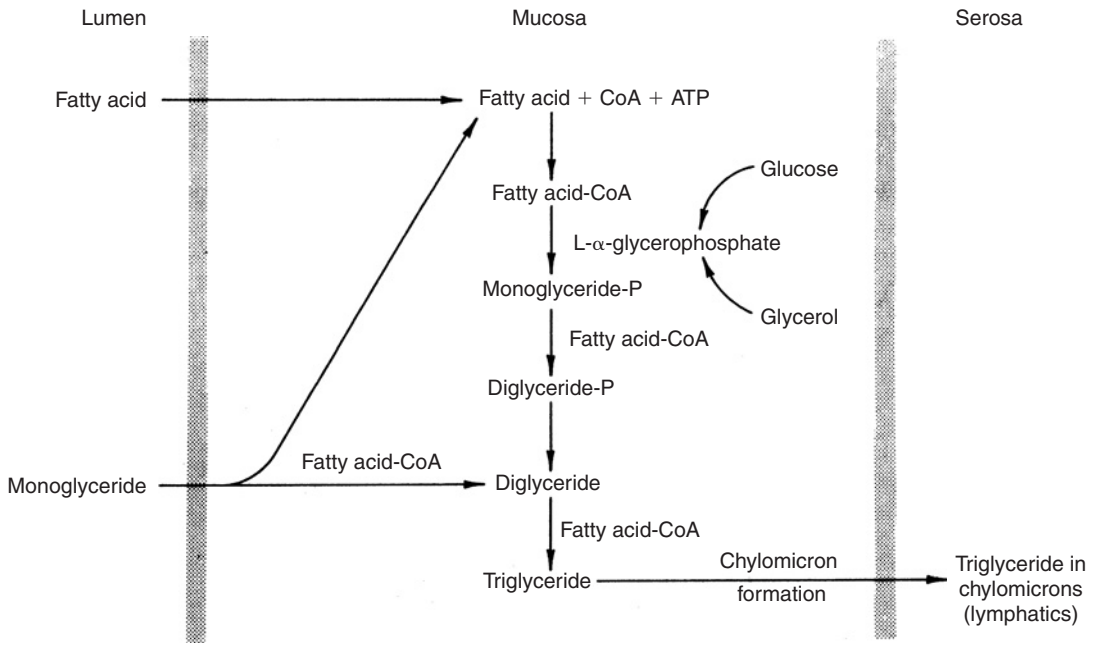

FIGURE 14-7 Biochemical reactions involved in intestinal transport of long chain fatty acids and monoglycerides. From Isselbacher (1966).

Within the mucosal cell, the fatty acids are transported by a soluble binding protein to the endoplasmic reticulum, where the fatty acids and monoglycerides are rapidly reesterified to triglyceride (Ockner and Isselbacher, 1974; Ockner and Manning, 1974). The two biochemical pathways for triglyceride biosynthesis in the intestine are summarized in Figure 14-7. Direct acylation of monoglyceride occurs in the intestine and is the major pathway for lipogenesis in the intestine during normal fat absorption. The initial step in this series of reactions involves activation of fatty acids by acyl-CoA synthetase, a reaction that requires Mg^{2+}, ATP, and CoA and that has a marked specificity for long-chain fatty acids. This specificity explains the observation by Bloom *et al.* (1951) that medium- and short-chain fatty acids are not incorporated into triglycerides during intestinal transport but enter the portal circulation as nonesterified fatty acids. The activated fatty acids then react sequentially with mono- and diglycerides to form triglycerides in steps catalyzed by mono- and diglyceride transacylases. The enzymes responsible for this series of reactions are present in the microsomal fraction of the cell (Rao and Johnston, 1966). These enzymes occur together in the endoplasmic reticulum as a "triglyceride-synthetase" complex.

An alternate route that is available for fatty acid esterification involves L-α-glycerophosphate derived either from glucose or from dietary glycerol by the action of intestinal glycerokinase. Activated fatty acid CoA derivatives react with L-α-glycerophosphate to form lysophosphatidic acid (monoglyceride phosphate), which by a second acylation forms phosphatidic acid (diglyceride phosphate). Phosphatidic acid phosphatase then hydrolyzes the phosphate ester bond, forming diglyceride, and by means of a transacylase step similar to that described previously, triglyceride is formed. Although this pathway appears to be of minor importance for triglyceride synthesis in the intestine, intermediates in this sequence of reactions are important in the synthesis of phospholipids, which are essential for stabilization of the chylomicron.

The next step in fat transport is formation of chylomicrons within the endoplasmic reticulum. The chylomicron is composed primarily of triglyceride and has an outer membranous coating of cholesterol, phospholipid, and protein (Zilversmit, 1965). The β-lipoprotein component of the chylomicron is synthesized by the intestinal mucosal cell. Inhibition of protein synthesis by puromycin or acetoxycycloheximide interferes with chylomicron formation and significantly reduces fat transport (Sabesin and Isselbacher, 1965).

The final step in fat absorption is extrusion of the chylomicra into the intercellular space opposite the basal lateral portion of the absorptive cell by reverse pinocytosis. From the intercellular space, the chylomicra pass through the basement membrane and enter the lacteal. The chylomicra then pass from the lacteals into lymph ducts and into the general circulation, thereby completely bypassing the liver during the initial phase of absorption.

2. *Absorption of Other Lipids*

a. Cholesterol

Dietary cholesterol is present in both free and esterified forms, but only nonesterified cholesterol is absorbed.

Cholesterol esters are hydrolyzed within the lumen of the intestine by sterol esterases secreted by the pancreas. Bile salts are required both for the action of this enzyme and for the absorption of nonesterified cholesterol. In the mucosal cell, cholesterol is reesterified and transferred by way of the lymph to the general circulation. The type of triglyceride present in the diet significantly affects the absorption of cholesterol and its distribution in lymph lipids (Ockner *et al.,* 1969).

b. Vitamin A

The diet contains vitamin A activity in two principal forms: (1) as esters of preformed vitamin A alcohol (retinol) and fatty acids and (2) as provitamin A, primarily β-carotene. Vitamin A ester is hydrolyzed by a pancreatic esterase within the lumen (Murthy and Ganguly, 1962), and the free alcohol is absorbed in the upper small intestine. Vitamin A alcohol is reesterified in the mucosa primarily with palmitic acid. The vitamin A ester is absorbed by way of the lymph, and after reaching the general circulation, it is rapidly cleared from the plasma and stored in the liver. In the postabsorptive state, vitamin A circulates as the free alcohol, the form released as needed from the liver by the action of hepatic retinylpalmitase esterase. The blood level of vitamin A is independent of liver reserves, and, as long as a small amount of vitamin A is present in the liver, the blood level remains normal (Dowling and Wald, 1958).

In diets that lack animal fat, the carotenes, primarily β-carotene, serve as the major precursor of vitamin A. The intestinal mucosa has a primary role in conversion of provitamin A to the active vitamin, although conversion can occur to a limited degree in other tissues. The mechanism involves central cleavage of β-carotene into two active vitamin A alcohol molecules that are subsequently esterified and absorbed by the lymphatics as with preformed vitamin A.

Bile salts are required for the mucosal uptake of β-carotene and for the conversion of β-carotene to vitamin A. Uptake of carotene and release of vitamin A ester into the lymph are rate-limiting steps. Cattle absorb substantial amounts of β-carotene without prior conversion to vitamin A, and these pigments are responsible for much of the yellow color of the plasma. Most other species have no β-carotene in the plasma, and extraintestinal conversion is thought to be more efficient in these species than in cattle (Ganguly and Murthy, 1967).

c. Vitamin D

Vitamin D, like cholesterol, is a sterol that is absorbed by the intestine and transported via the lymph (Schachter *et al.,* 1964). Intestinal absorption differs, however, in that vitamin D is transported to the lymph in nonesterified form. The uptake of vitamin D by the mucosal cell is favored by the presence of bile salts. Simultaneous absorption of fat from micellar solutions increases transport of vitamin D out of the cell into the lymph, the limiting step.

One of the major actions of vitamin D is to enhance the intestinal absorption of calcium. Wasserman and coworkers (1968) (Wasserman and Taylor, 1966, 1968) have described the mechanism of action of vitamin D. They have shown that vitamin D causes synthesis of a calcium-binding protein that plays a central role in the transport of calcium.

E. Cobalamin

Following ingestion, cobalamin is released from food in the stomach (Batt and Morgan, 1982; Simpson *et al.,* 2001). It is then bound to a nonspecific cobalamin-binding protein of salivary and gastric origin called haptocorrin. Intrinsic factor (IF), a cobalamin-binding protein that promotes cobalamin absorption in the ileum, is produced by parietal cells and cells at the base of antral glands in the dog but not the cat; IF is produced in the pancreas of cats. The affinity of cobalamin for haptocorrin is higher at acid pH than for IF, so most is bound to haptocorrin in the stomach. Upon entering the duodenum, haptocorrin is degraded by pancreatic proteases, and cobalamin is transferred from haptocorrin to IF, a process facilitated by the high affinity of IF for cobalamin at neutral pH. Cobalamin-IF complexes traverse the intestine until they bind to specific receptors (previously called IFCR, but recently dubbed cubilin) located in the microvillous pits of the apical brush border membrane of ileal enterocytes. Cobalamin is then transcytosed to the portal bloodstream and binds to a protein called transcobalamin 2(TC II), which mediates cobalamin absorption by target cells. A portion of cobalamin taken up by hepatocytes is rapidly (within an hour in the dog) reexcreted in bile bound to haptocorrin. Cobalamin of hepatobiliary origin, in common with dietary derived cobalamin, undergoes transfer to IF and receptor mediated absorption, thus establishing enterohepatic recirculation of the vitamin.

Low serum cobalamin concentrations in dogs have been associated with exocrine pancreatic insufficiency (EPI), severe intestinal disease, IF-Cbl receptor abnormalities, and conditions associated with the proliferation of enteric bacteria (e.g., stagnant loops). Cobalamin deficiency in cats and dogs results in a significant metabolic disorder, which can be ameliorated by treatment or correction of the underlying cause.

Dietary folate polyglutamate is deconjugated by folate deconjugase to folate monoglutamate, which is absorbed by specific carriers in the proximal small intestine. Folate deconjugase is a jejunal brush border enzyme. Folic acid, which is produced by microorganisms in the small intestine, is also absorbed and can increase existing serum levels of folate. Serum levels of folate are expected to decrease when the absorptive capacity of the proximal intestine is severely compromised, as might occur with infiltrative bowel disease.

VIII. DISTURBANCES OF GASTROINTESTINAL FUNCTION

A. Vomition

Vomiting is a coordinated reflex act that results in rapid, forceful expulsion of gastric contents through the mouth. The reflex may be initiated by local gastric irritation caused by a variety of toxic irritants, infectious agents, foreign bodies, gastric tumors, obstructions of the pyloric canal or the small intestine, or by drugs such as apomorphine or other toxic substances that act centrally on the "vomiting center" of the medulla.

Severe vomiting produces loss of large quantities of water and of $H^+$and $C1^-$ ions. These losses cause dehydration, metabolic alkalosis with increased plasma HCO_3^-, and hypochloremia. Chronic vomiting may also be associated with the loss of significant tissue K^+ and with hypokalemia. The K^+ deficit is caused primarily by increased urinary excretion resulting from alkalosis (Leaf and Santos, 1961). Gastric secretions contain significant quantities of K^+, and losses in the vomitus also contribute to the K^+ deficiency. K^+ deficiency, which develops initially because of the alkalosis, perpetuates the alkalotic state by interfering with the ability of the kidney to conserve H^+ (Brazeau *et al.,*1956; Darrow, 1964). Both K^+ and the hypovolemia caused by dehydration may result in renal tubular damage and in renal failure.

Vomiting occurs frequently in the dog, cat, and pig but is an unusual sign in the horse, which has anatomical restrictions of the esophagus that interfere with expulsion of gastric contents. In cattle, sheep, and goats, the physiological process of rumination utilizes neuromuscular mechanisms similar to those involved in vomiting. Uncontrolled expulsion of ruminal contents is an uncommon sign, most frequently occurring after ingestion of toxic materials or associated with traumatic reticulitis and resulting "vagal indigestion." The contents of the abomasum are not expelled directly even when the pyloric canal is obstructed. Pyloric outflow obstruction does occur in cattle, which is similar metabolically to that observed in nonruminants. This obstruction may be observed in right-sided displacement of the abomasum with or without torsion, occasionally with left-sided displacement of the abomasum, in cows with functional pyloric obstruction as a result of reticuloperitonitis and from "vagal indigestion." When the pylorus is obstructed, abomasal contents are retained, causing distension of the abomasum, which in turn stimulates further secretion and retention. Retained abomasal contents may be regurgitated into the large reservoir of the rumen and sequestered there from other fluid compartments of the body. The net result is loss of H^+ and Cl^- ions and development of metabolic alkalosis, hypochloremia, and hypokalemia. This metabolic syndrome often is associated with fluid distension of the rumen related to pyloric outflow obstruction. Similar distension of the rumen in the absence of hypochloremic, hypokalemic metabolic alkalosis suggests more proximal obstruction of rumen outflow, namely the omasum.

Brachycephalic, middle-aged, small breed dogs (e.g., Shih Tzus) seem predisposed to hypertrophy of the pyloric mucosa or muscularis (Simpson, 2005); this syndrome, as well as other causes of pyloric outflow obstruction, can result in vomiting, metabolic alkalemia, and paradoxic aciduria. Chronic hypertrophic gastritis, which resembles Menetrier's disease in humans, has been demonstrated in the dog (Happe and van der Gagg, 1977; Kippins, 1978; van der Gagg *et al.,* 1976; Van Kruiningen, 1977). Van Kruiningen's series of cases were basenjis that had concomitant lymphocytic-plasmocytic enteritis. The primary disease, however, has been observed in other breeds without intestinal lesions. Signs of illness usually involve chronic vomiting, weight loss, and occasionally diarrhea. Hypoalbuminemia occurs in most cases. In humans, hyperchlorhydria or achlorhydria can occur. The morphological changes in the stomach wall (hypertrophic rugae) and some of the clinical features help to differentiate this disease from gastric neoplasia.

Functional gastrinomas have been rarely diagnosed in the dog and have been compared to the Zollinger-Ellison syndrome in humans (English *et al.,* 1988; Straus *et al.,* 1977; van der Gagg and Happe, 1978). Clinical disease is associated with hypergastrinemia, hyperchlorhydria, hypertrophic gastritis, peptic esophagitis, and duodenal ulcers. A more recent overview by Simpson (2000) revealed a wide variety of breeds with a mean age of 9 years; no sex bias was identified. The diagnostic workup usually centered around the problems of vomiting, weight loss and anorexia, and the pursuit of localizing findings of melena, hematemesis, and abdominal pain. Some of these dogs had signs associated with gastrointestinal preformation/peritonitis. Surgical treatment or medical management, to include omeprazole, famotidine, sucralfate, or octreotide, is indicated. Because metastasis is frequently present, the prognosis for recovery is poor.

B. Gastric Dilatation-Volvulus

Gastric dilatation-volvulus (GDV) is an acute gastrointestinal disorder associated with high mortality (Leib and Blass, 1984; Morgan, 1982). It typically occurs in large deep-chested dogs but has been reported in smaller dogs, the cat, and other species. Gastric dilatation precedes development of volvulus and is the result of the accumulation of gas and fluid in the stomach as a result either of mechanical or functional disturbances in pyloric outflow. As the stomach distends and rotates about the distal esophagus, displacement and occlusion of the pylorus and duodenum occur. Necrosis and perforation of the stomach wall and peritonitis are common causes of death.

Distension and displacement of the stomach cause obstruction of the caudal vena cava and portal vein resulting

in venous stasis and sequestration of blood in splanchnic, renal, and posterior muscular capillary beds. This decrease in circulating blood volume (venous return) and subsequent decrease in cardiac output, arterial blood pressure, and tissue perfusion culminate in hypovolemic shock. Endotoxemia, a consequence of portal vein occlusion, contributes to the shock syndrome. The release of myocardial depressant factors from ischemic pancreatic tissue impairs the clearance of endotoxins by the reticuloendothelial system as well as causing direct cardiodepressant effects. Altered microvascular perfusion with hypoxemia and endotoxemia favors development of disseminated intravascular coagulopathy (DIC) (Lees *et al.,* 1977).

Increased plasma gastrin immunoreactivity has been reported in dogs with GDV (Leib *et al.,* 1984). Preexisting conditions of relative hypergastrinemia may predispose to GDV. Gastrin can increase caudal esophageal sphincter pressure, delay gastric emptying, and predispose to pyloric outflow obstruction by causing gastric mucosal and pyloric muscular hypertrophy.

Experimental gastric dilatation and dilatation with torsion have been studied in the dog (Wingfield *et al.,* 1974). Hyperkalemia and hyperphosphatemia were consistent findings in dogs with gastric dilatation and torsion (Wingfield *et al.,* 1974). This was the result of hypovolemia, decreased renal perfusion, and renal insufficiency on the one hand and the loss of intracellular K^+ from damaged tissue on the other. Increased blood urea nitrogen (BUN) and serum creatinine (Cr) levels persisted after decompression of the stomach. Hemoconcentration and increased TSP were attributed to fluid shifts from the vascular compartment into the lumen of the alimentary tract, wall of the stomach, and peritoneal cavity. Increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were most apparent following decompression of the stomach and were attributed to alteration of hepatocytes and smooth muscle of the stomach and spleen. Increased levels of creatine kinase (CK) resulted from the effects of tissue hypoxia on striated muscle. Metabolic acidosis was attributed in part to increased production of lactic acid caused by tissue hypoxia.

A wide range of acid-base and electrolyte disturbances has been reported in clinical patients with GDV (Kagan and Schaer, 1983; Muir, 1982; Wingfield *et al.,* 1982). Dogs presenting with GDV may have normal acid-base status. Metabolic acidosis and hypokalemia commonly occur. Metabolic alkalemia and respiratory alkalosis also have been observed. Hyperkalemia is unusual. The absence of an increase in anion gap in one study indicated that the production of volatile fatty acids and lactic acid was not excessive (Wingfield *et al.,* 1982).

C. Ischemia-Reperfusion Injury

Ischemia-reperfusion injury is a contributing cause of death in horses with strangulating intestinal obstruction (Moore *et al.,* 1995) and in dogs with GDV. Together with luminal occlusion of the alimentary tract, functional constriction or mechanical obstruction of intestinal vasculature occurs. Depending on the duration and severity of ischemia, oxygenation of tissue is compromised, and there is a subsequent attenuation of oxidative phosphorylation and a decrease in ATP. Anaerobic glycolysis ensues, leading to intracellular acidosis and increased intracellular concentrations of Ca^{2+}. Unless timely restoration of blood flow and oxygenation occurs, these metabolic derangements eventually contribute to cellular edema, lysosomal release of degradative enzymes, autolytic destruction of cellular organelles, and cell death.

When intestinal obstruction is relieved and tissue perfusion is reestablished, reoxygenation of tissue can result in a cascade of biochemical events that can aggravate ischemic-induced tissue injury. The resulting reperfusion injury is caused in part by oxygen-free radicals (OFR), particularly superoxide (O_2^-) and hydroxyl-free radicals (OH.), and is characterized by increased microvascular and mucosal permeability and mucosal necrosis (Moore *et al.,* 1995). The formation of OFRs is preceded by accumulation of hypoxanthine in endothelial cells and intestinal mucosal cells during ischemia. The conversion of xanthine dehydrogenase to xanthine oxidase also occurs during ischemia, a reaction that is facilitated by high intracellular levels of calcium ions and the protease, calpain. When reperfusion occurs, xanthine oxidase converts hypoxanthine to uric acid and superoxide radicals. O_2^- and hydrogen peroxide (H_2O_2), a product of superoxide dismutase (SOD) reduction, are converted to highly reactive OH. in the presence of an iron catalyst. OH. initiates structural and functional cellular membrane damage via lipid peroxidation. The release of inflammatory mediators attending lipoperoxidation contributes to tissue injury.

Malondialdehyde (MDA) is a stable by-product of lipoperoxidation, and investigators can utilize its detection as an indicator of ischemia-reperfusion injury (Moore *et al.,* 1995).

Neutrophils are recruited into ischemic and reperfused tissue by xanthine oxidase-derived OFRs and chemoattractants released from cellular membranes during lipid peroxidation. Increased cytosolic calcium concentrations during ischemia and subsequent lipoperoxidation activate phospholipase A2, which in turn causes the release of platelet-activating factor (PAF), metabolites of arachidonic acid (leukotrienes and prostaglandins), and lysophosphatidylcholine. Leukotriene B4, thromboxane A2, and PAF are the primary products of phospholipid metabolism that promote infiltration and degranulation of neutrophils in affected tissue.

When neutrophils attach to endothelium, they release elastase and lactoferrin, which promotes extravasation (Moore *et al.,* 1995). The conversion of oxygen to O_2^- within neutrophils is facilitated by the NADPH oxidase

system. These O_2^- are metabolized to H_2O_2, and the latter reacts with Cl^- to form hypochlorous acid. Myeloperoxidase (MPO), an enzyme contained in neutrophils, catalyzes this reaction. MPO activity in intestinal mucosa correlates well with the degree of neutrophil infiltration and mucosal injury.

Serine proteases are believed to play a contributing role in ischemia-reperfusion injury (Moore *et al.,* 1995). The pancreas is an important source of endoproteases (trypsin, chymotrypsin, and elastase), which can cause mucosal injury, particularly in the small intestine. Proteases produced by granulocytes, as well as lysosomes, are more important in mucosal injury of the large bowel, elastase, neutral proteases, and cathepsin G are released from granulocytes during phagocytosis. Cathepsin B is a lysosomal protease that has trypsin-like activity.

Several pharmacological agents have been used in experimental and clinical studies of ischemia-reperfusion injury (Moore *et al.,* 1995). The mechanistic rationale for many of these agents is comparable to the role of endogenous antioxidants. Examples of commonly used agents include xanthine oxidase inhibitors (allopurinol), deferoxamine, 21-aminosteroids, inhibitors of PLA2, cyclooxygenase, and lipoxygenase. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (Gpx) are free radical scavenging enzymes. Mannitol, albumin, dimethyl sulfoxide (DMSO), dimethyl thiourea, and manganese chloride represent nonenzymatic free radical scavengers. Other agents that have been studied include nitric acid, protease inhibitors, hydroxyethyl starch, and neutrophil-directed agents. Although there has been demonstrable efficacy of the aforementioned agents in some studies, there are many inconsistencies. From a clinical perspective, success has been limited with single agents, and there is more interest in combination or multimodal therapy.

D. Acute Diarrheas

The term *diarrhea* is used to generically describe the passage of abnormally fluid feces with increased frequency, increased volume, or both. The significance of diarrhea depends primarily on the underlying cause and on the secondary nutritional and metabolic disturbances that are caused by excessive fecal losses.

There are theoretically three factors that can act independently or in combination to produce diarrhea. An increase in the rate of intestinal transit is one factor believed important in functional disorders of the gastrointestinal tract in which "hypermotility" has been considered to be the primary cause. Although increased intestinal motility may be a factor in certain types of diarrheal disease, when motility patterns have been investigated, diarrheal disease has actually been associated with decreased motility (Christensen *et al.,* 1972). A second factor in the pathogenesis of diarrhea is decreased intestinal assimilation of nutrients that may result either from decreased intraluminal hydrolysis of nutrients (e.g., *maldigestion* resulting from pancreatic exocrine insufficiency), bile salt deficiency, or defective mucosal transport of nutrients (i.e., *malabsorption*) that results from various types of inflammatory bowel disease, villus atrophy, intestinal lymphoma, or intrinsic biochemical defects in the mucosal cell that interfere with digestion or absorption. Finally, increased intestinal secretion of water and electrolytes is a major factor in the pathogenesis of certain types of acute diarrhea.

Enteropathogenic strains of *Escherichia coli* produce soluble enterotoxins (Kohler, 1968; Moon, 1978; Smith and Halls, 1967), which alter bidirectional Na^+ and water flux (Fig. 14-8). The most extensively studied enterotoxin is that produced by *Vibrio cholerae.* This bacterium produces a large-molecular-weight, heat-labile toxin (CT), one subunit of which has properties similar to those of the heat-labile (LT) enterotoxin produced by certain strains of *E. coli* (Richards and Douglas, 1978). The mechanism of action of CT is believed to involve the activation of adenylate cyclase. This membrane-bound enzyme converts ATP to cAMP, which through the action of protein kinase is responsible for the greatly increased secretion of water and electrolytes by the intestinal mucosa. Although species differences have been observed (Forsyth *et al.,* 1978), this mechanism appears to be important in the mode of action of LT of *E. coli* as well (Richards and Douglas, 1978).

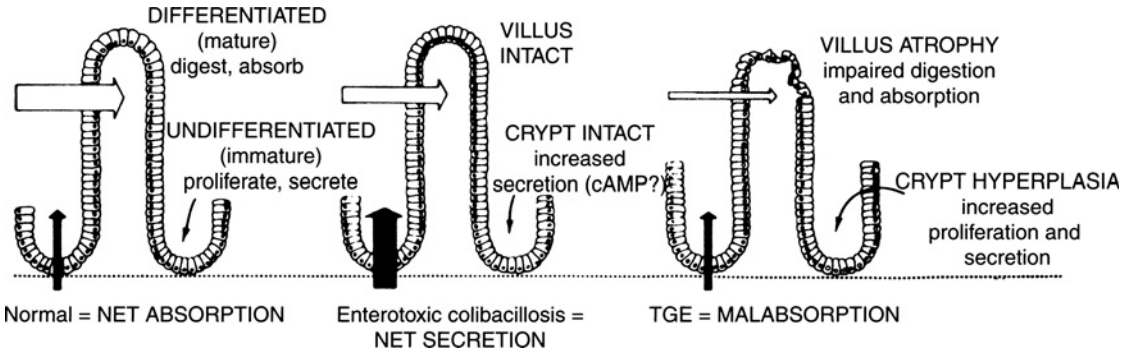

FIGURE 14-8 Pathogenesis of diarrhea caused by *E. coli* enterotoxin and by coronaviruses. From Moon (1978).

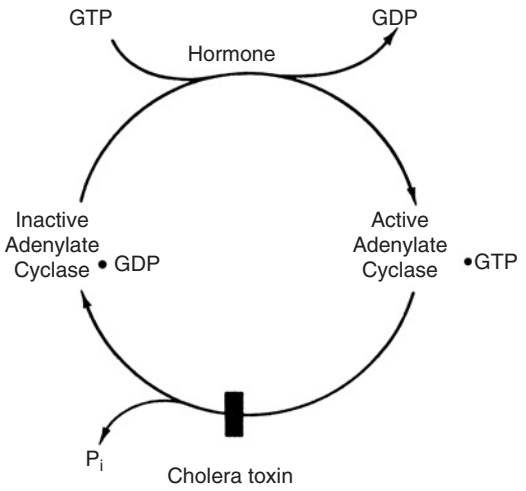

FIGURE 14-9 Mechanism of action of cholera toxin, which inhibits hydrolysis of GTP, thereby increasing adenylate cyclase activity. From Cassell and Selinger (1978).

Additional extensive studies have centered on the molecular mechanism of action of CT. Under physiological conditions, adenylate cyclase is activated by the binding of guanosine triphosphate to the inactive enzyme. An associated GTPase inactivates the enzyme by converting enzyme-bound GTP to GDP and inorganic phosphate. This GTP-GDP system plays a critical role in the physiological regulation of adenylate cyclase. Cholera toxin is believed to bind to the adenyl cyclase in a way that inhibits hydrolysis of GTP, thereby maintaining the enzyme in an activated state (Cassel and Pfeuffer, 1978; Johnson *et al.,* 1978; Levinson and Blume, 1977) (Fig. 14-9).

Certain enteropathogenic strains of *E. coli* produce a low-molecular-weight, heat-stable toxin (ST) alone or in addition to LT (Moon, 1978; Richards and Douglas, 1978). In epidemiological studies of neonatal diarrheal diseases of calves, most isolated strains of *E. coli* produce only ST (Braaten and Myers, 1977; Lariviere *et al.,* 1979; Moon *et al.,* 1976). In contrast to LT and CT, which induce intestinal Na^+ and water secretion only after a lag phase of several hours, ST induces intestinal secretion immediately. ST induces intestinal secretion by activating guanylate cyclase and the mediator of intestinal secretion induced by ST is cyclic 3′,5′-guanosine monophosphate (Field *et al.,* 1978; Hughes *et al.,* 1978).

Enterotoxin-induced intestinal secretion may be blocked by cycloheximide, an inhibitor of protein synthesis (Serebro *et al.,* 1969). The lack of specificity and the toxicity of cycloheximide precluded its clinical use, but acetazolamide has been shown to inhibit intestinal fluid secretion (Moore *et al.,* 1971; Norris *et al.,* 1969). Ethacrynic acid, another potent diuretic, has been shown to inhibit enterotoxin-induced fluid secretion (Carpenter *et al.,* 1969). Unfortunately, the diuretic effects of these drugs preclude their clinical use but similar drugs with "intestinal specificity" would have significant therapeutic potential. Adenosine analogues also have been shown to inhibit cholera toxin-stimulated intestinal adenylate cyclase.

Prostaglandin E_1 (PgE_1) and CT have similar effects on electrolyte transport in rabbit ileum. Application of either to the mucosa inhibits NaCl absorption and stimulates Cl^+ secretion. Both indomethacin (Gots *et al.,* 1974) and aspirin (Farris *et al.,* 1976) inhibit enterotoxin-induced intestinal secretion in laboratory animal models, and the prostaglandins do not function as mediators in the pathogenesis of cholera (Schwartz *et al.,* 1975). However, Jones *et al.* (1977) demonstrated a positive therapeutic response to a new prostaglandin inhibitor in calves with acute enteritis.

The effects of the *E. coli* ST can be inhibited *in vitro* by the calcium channel blockers diltiazem and lodoxamide tromethamine and the prostaglandin synthesis inhibitors indomethacin and quinacrine (Knoop and Abbey, 1981; Thomas and Knoop, 1983). Neither class of drug blocks the effect of cGMP, suggesting that calcium and prostaglandin influence the earliest step(s) in ST response: either its brush border binding or the activation of guanylate cyclase.

The autonomic nervous system has important effects on intestinal ion transport and water absorption (Tapper *et al.,* 1978). Catecholamines stimulate formation of cAMP in a variety of mammalian cells (Schultz *et al.,* 1975), apparently by activating the GTP-GDP system described above (Cassel and Selinger, 1978). Adrenergic blocking agents, such as chlorpromazine (Holmgren *et al.,* 1978) and propranolol (Donowitz and Charney, 1979), have significant inhibitory effects on enterotoxin-induced intestinal secretion. Although the mechanism of action of these two adrenergic blockers is not known, they represent still another class of drugs that may be of therapeutic benefit.

The intestinal "adsorbent" drug, Pepto Bismol, containing bismuth subsalicylate, and attapulgite, a heat-treated silicate, have antienterotoxic effects (Drucker *et al.,* 1977; Ericsson *et al.,* 1977; Gyles and Zigler, 1978). Therapeutic trials with bismuth subsalicylate have significant therapeutic benefit in certain large-volume diarrheal diseases of humans, which are enterotoxigenic in origin (DuPont, 1978; DuPont *et al.,* 1977; Portnoy *et al.,* 1976). The mechanism of the intestinal secretion inhibition is not known, but the chemical relation of bismuth subsalicylate to other known prostaglandin inhibitors is known. It is possible that such drugs, by decreasing endogenous production of prostaglandin, decrease the basal level of cyclic nucleotides, which in turn causes an increase in the threshold of response to enterotoxin. Salicylates also may stimulate sodium chloride absorption (Powell *et al.,* 1979). Collectively, these observations suggest that new, innovative methods for therapy and control of acute clinical diarrheal disease may be developed.

Acute diarrhea represents the leading cause of morbidity and mortality in neonatal calves and pigs. The pathogenesis

of the neonatal enteric infection is complex, often involving nutritional or environmental factors as well as infectious agents, such as enteropathogenic strains of *E. coli*, the transmissible gastroenteritis virus (TGE), rotaviruses, and other bacterial and viral pathogens. The severe clinical signs and frequently fatal outcome of acute diarrheal disease are often directly related to dehydration and to associated H^+ and electrolyte disturbances (Dalton *et al.,* 1965; Fisher and McEwan, 1967a; Tennant *et al.,* 1972, 1978).

In acute diarrhea with large-volume, watery stools, the fecal fluid originates primarily from the small intestine. The electrolyte composition of the stool in such cases is similar to that of the fluid found normally in the lumen of the small intestine, which in turn is similar to that of an ultrafiltrate of the plasma. The rapid dehydration that accompanies acute enteritis in the newborn soon produces hemoconcentration and leads to hypovolemic shock. These cases are characterized by metabolic acidosis (Dalton *et al.,* 1965) caused by (1) decreased excretion of H^+ resulting from decreased renal perfusion and (2) increased production of organic acids, the result of which is observed characteristically in young, severely dehydrated animals. Hyperkalemia in such cases is the result of increased movement of cellular K^+ into the extracellular fluid and to decreased renal excretion. Cardiac irregularities caused by hyperkalemia can be demonstrated with the electrocardiogram, and cardiac arrest related to hyperkalemia is a direct cause of death in calves with acute diarrhea (Fisher, 1965; Fisher and McEwan, 1967b). Marked hypoglycemia also has been observed occasionally before death in calves with acute enteric infections. Hypoglycemia is believed to be due to decreased gluconeogenesis and increased anaerobic glycolysis, the result of hypovolemic shock (Tennant *et al.,* 1968). The sequence of metabolic changes that occur during acute neonatal diarrhea is summarized in Figure 14-10.

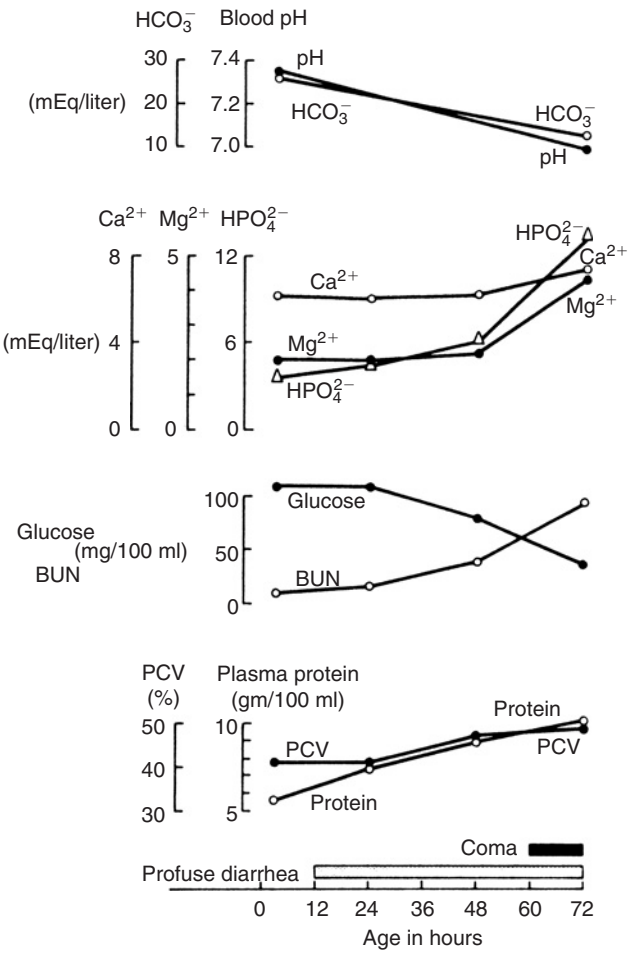

FIGURE 14-10 Metabolic alterations during the course of fatal enteric infection in a neonatal calf. From Tennant *et al*. (1972).

In chronic forms of diarrheal disease, excessive fecal losses of electrolyte and fluid are compensated in part by renal conservation mechanisms and in part by ingestion. If water is consumed without adequate ingestion of electrolytes, hyponatremia and hypokalemia may develop (Patterson *et al.,* 1968). In such cases, the osmolality of the plasma is significantly decreased and hypotonic dehydration occurs. In longer-standing cases of chronic diarrhea, the plasma K^+ concentration may become dangerously low. It is imperative, in this case, that intravenous fluids contain sufficient K^+ to prevent further reduction in concentration and to avoid additional cardiac irregularities or cardiac arrest.

E. Malabsorption

Decreased absorption of nutrients may occur either as a result of defective intraluminal digestion (maldigestion) associated with pancreatic insufficiency (juvenile pancreatic atrophy, chronic pancreatitis) or because of defects in mucosal transport (malabsorption). Intestinal malabsorption is associated with several types of intestinal disease including chronic inflammatory diseases (lymphocytic-plasmacytic enteropathy, eosinophilic enteritis), granulomatous diseases (Johne's disease, intestinal parasitism), and lymphoma. The cardinal clinical signs of malabsorption include persistent or recurrent diarrhea, steatorrhea, and weight loss. In the horse, small intestinal malabsorption such as that associated with granulomatous enteritis may be associated with weight loss, but diarrhea may not be present because of the compensatory capacity of the uninvolved cecum and colon.

The initial reports of primary or idiopathic intestinal malabsorption in dogs (Kaneko *et al.,* 1965; Vernon, 1962) were compared to nontropical sprue (adult celiac disease, gluten-induced enteropathy) of humans, but association with gluten sensitivity was not demonstrated. Wheat-sensitive enteropathy has been described in the Irish setter breed (Batt *et al.,* 1984). Most of the dogs were seen between 7 months to 2 years of age and had poor weight gain, weight loss, inappetence, or hyperphagia. Diarrhea was not a consistent observation. The most consistent morphological abnormality in peroral jejunal biopsies was partial villus atrophy.

Enzymatic changes included decreased mucosal alkaline phosphatase and peptidases, whereas disaccharidases and GGT activities were unaffected. Recovery of morphological and biochemical abnormalities occurred in affected dogs that received cereal-free diets but recurred when wheat flour was added to the ration. A variety of other causes of intestinal malabsorption have been reported in the dog (Anderson, 1975, 1977; Burrows *et al.,* 1979; Ewing, 1971; Hill, 1972; Hill and Kelly, 1974; Schall, 1974; Van Kruiningen, 1968; Van Kruiningen and Hayden, 1973). An enteropathy said to resemble tropical sprue in humans has been described in German shepherds (Batt *et al.,* 1983, 1984). Affected dogs were 5 years of age or older and had diarrhea and weight loss for at least 4 months before the diagnosis was made. Peroral jejunal biopsies revealed partial villus atrophy and variable infiltrations of lymphocytes and plasma cells in the lamina propria. Subcellar biochemical studies of jejunal enterocytes revealed decreased activity of many brush border enzymes and increased lysosomal enzymes.

Enteropathy associated with bacterial overgrowth of the small intestine also has been observed in German shepherds (Batt and McLean, 1987). The dogs were 2 years of age or younger, and all had chronic histories of intermittent diarrhea with or without weight loss. Bacterial counts of greater than 10^6 colonies per milliliter were observed in duodenal fluid. *Enterococci*, *E. coli*, and *Clostridium* spp. were identified in cultures. Peroral jejunal biopsies revealed no characteristic histopathological changes. A deficiency of the immunoglobulin, IgA, may explain the vulnerability of these German shepherds to intestinal bacterial overgrowth (Whitbread *et al.,* 1984).

Early reports of intestinal malabsorption in the cat (Wilkinson, 1969) gave the impression that this condition was more common in dogs. This has changed since inflammatory bowel disease or intestinal infiltrations of small cell lymphoma have been diagnosed more often in cats. Malabsorption syndromes similar to those recognized in dogs are being recognized with increased frequency in farm animals (Blood *et al.,* 1979). Cimprich (1974), Merritt *et al.* (1976), and Meuten *et al.* (1978), have reported malabsorption in the horse secondary to chronic granulomatous enteritis, and specific amino acid malabsorption has been reported in Johne's disease (Patterson and Berrett, 1969).

Steatorrhea, the presence of excessive amounts of fat in the feces, is a prominent sign of intestinal malabsorption in dogs. The stools are bulky, gray or tan, and, grossly, may have an oily appearance. The normal dog excretes 3 to 5 g of fat in the stool each day. This level of fecal fat is quite constant and is independent of dietary fat intake over a wide range of 15 to 48g/day. In intestinal malabsorption, the ability to absorb fat is decreased and fecal fat excretion increases significantly. Under these conditions, the amount of fecal fat excreted becomes proportional to dietary intake.

Merritt *et al.* (1979) reported that body weight is an important factor in fat output. In small dogs (i.e., less than 10 to 15 kg body weight) with intestinal malabsorption, the abnormality in fecal fat output was quantitatively less severe than in larger dogs. Fecal fat excretion for normal dogs was 0.24 ± 0.01 g/kg body weight per day.

Steatorrhea can be documented qualitatively by staining the fresh stool with a lipophilic stain, such as Sudan III, and observing increased numbers of oil droplets under the light microscope. In experienced hands, this method is a reliable diagnostic procedure (Drummey *et al.,* 1961). The following methods can be used to demonstrate neutral and split fats. For neutral fat, two drops of water are added to a stool sample on a glass slide and mixed. Two drops of 95% ethanol are then added and mixed followed by several drops of a saturated solution of Sudan III in 95% ethanol. A coverslip is applied to the mixture, which is then examined for yellow or pale orange refractile globules of fat, particularly at the edges of the coverslip. Normally, two or three fat droplets per high-power field are present. A large number of neutral fat droplets suggest a lack of pancreatic lipase activity (i.e., exocrine pancreatic insufficiency).

For free fatty acids (split fats), several drops of 36% acetic acid are added to a stool sample on a glass slide and mixed. Several drops of Sudan III solution are then added and mixed. A coverslip is applied, and the slide is gently heated over an alcohol burner until it begins to boil. The slide is air-cooled and then quickly heated again; this procedure is repeated two or three times. The warm slide is examined for stained free fatty acid droplets, which, when warm, appear as deep orange fat droplets from which spicules and soaps, resembling the pinna of the ear, form as the preparation cools. Normal stools may contain many tiny droplets of fatty acids (up to 100 per high-power field). With increasing amounts of split fats, the droplets become larger and more numerous, which suggests an abnormality in fat absorption.

Quantitation of fecal fat is the most accurate method of assessing steatorrhea (Burrows *et al.,* 1979) with dietary fat balance being determined for a period of 48 to 72 h. Fecal fat is analyzed using a modification of the technique of van de Kamer *et al.* (1949), which employs ether extraction of fecal lipid and titration of fatty acids. The results are expressed as grams of neutral fat excreted per 24 h. Merritt *et al.* (1979) have suggested that dogs be fed 50 g fat per kilogram per day for 2 to 3 days before fecal collection. Analysis of a 24-hour collection of stool when this is done is believed to be as accurate as a 72-hour stool collection. Results are expressed as fat excretion in grams per kilogram body weight.

In addition to malabsorption of fat, the canine malabsorption syndrome is associated with decreased absorption of other nutrients. These defects in absorption are responsible for the progressive malnutrition that is a cardinal feature of the disease. There may be malabsorption of vitamin D or calcium that results in osteomalacia. Anemia may result from malabsorption of iron or of the B vitamins required for normal erythropoiesis. Malabsorption of vitamin K can result in hypoprothrombinemia and delayed

clotting of blood. Glucose malabsorption has been documented by Kaneko *et al.* (1965) and it is likely that amino acids are similarly malabsorbed at the small intestinal level. Carbohydrate and fat malabsorption unquestionably contribute to the energy deficit that results in weight loss. Amino acid malabsorption may contribute to the development of hypoproteinemia, although increased intestinal loss of plasma proteins is believed to be more important.

The diagnosis of idiopathic canine malabsorption is made only after ruling out other primary inflammatory, neoplastic, or parasitic diseases of the intestine and diseases of the pancreas, liver, or stomach that result in defective intraluminal digestion. The presence of parasitic infection is established by examining the feces for parasite cysts or ova. Other inflammatory or neoplastic diseases of the intestine may be suggested on the basis of clinical or radiological examination, but a definitive diagnosis usually depends on histopathological examination of an intestinal biopsy specimen.

Both idiopathic and secondary intestinal malabsorption must be differentiated from those diseases in which there is decreased intraluminal hydrolysis of nutrients. The latter are due most frequently to pancreatic exocrine insufficiency as a result of chronic pancreatitis or juvenile atrophy. In these diseases, hydrolysis of the major dietary constituents is reduced because of the lack of pancreatic enzymes. Intraluminal hydrolysis of fat may also be decreased because of a deficiency of bile salts caused either by decreased hepatic secretion or by bile duct obstruction. Experimentally, however, complete diversion of bile flow in the dog actually has a quantitatively small effect on fat absorption (Hill and Kidder, 1972a).

A radioimmunoassay for trypsin-like immunoreactivity (TLI) is currently widely used to identify dogs with pancreatic exocrine insufficiency (Williams *et al.,* 1987) and is useful in differentiating maldigestion from primary malabsorption. The TLI in normal dog serum is trypsinogen. The route of entry of trypsinogen into the systemic circulation is believed to be the pancreatic venous or lymphatic vessels. Trypsinogen release in inflammatory pancreatic disease (i.e., acute or chronic pancreatitis) may increase TLI values. Increased TLI values have been reported in a dog with confirmed pancreatic exocrine insufficiency that had normal PABA values and fecal proteolytic activity (Williams and Batt, 1986). In most cases of pancreatic exocrine insufficiency, TLI is remarkably reduced compared to normal dogs or dogs with intestinal malabsorption (Williams and Batt, 1988).

An indirect method to detect chymotrypsin activity has been described as a means to differentiate dogs with pancreatic exocrine insufficiency from those with intestinal malabsorption (Batt *et al.,* 1979; Batt and Mann, 1981; Imondi *et al.,* 1972; Strombeck, 1978; Strombeck and Harrold, 1982; Zimmer and Todd, 1985). The synthetic peptide N-benzoyltyrosine-P-aminobenzoic acid (bentiromide) is orally administered to dogs. If chymotrypsin is present in the duodenum, hydrolysis of the bentiromide occurs, and P-aminobenzoic acid (PABA) is released, which is subsequently absorbed and then excreted in the urine within 6 h. The urine or plasma is analyzed for PABA. Less than 43% PABA excretion identifies dogs with suspected pancreatic exocrine insufficiency (Strombeck, 1978). Thirty- or 60-min blood levels of PABA are used to detect dogs with pancreatic exocrine disease (Zimmer and Todd, 1985), but this method did not identify dogs with exocrine pancreatic insufficiency as consistently as the 6 h urinary excretion (Strombeck and Harrold, 1982). Factors that may influence results of the bentiromide test include the rate of gastric emptying, intestinal absorption of the PABA, and the peptide cleavage by other peptidases (Batt *et al.,* 1979).

F. Tests of Malabsorption

1. *Cobalamin and Folate Absorption*

The measurement of circulating serum concentrations of cobalamin and folate (Batt and Morgan, 1982; Waters and Mollin, 1961) may give an indication of the site of intestinal dysfunction in dogs and cats, but it does not define the existing lesion or etiology. The use of cobalamin and folate concentrations as an indirect indicator of intestinal and pancreatic disease has been reported less frequently in the cat; the authors have documented cobalamin deficiency in cats with severe inflammatory bowel disease and intestinal lymphosarcoma. Low concentrations have also been encountered in cats with pancreatitis and exocrine pancreatic insufficiency. Low cobalamin levels are associated with increased levels of methylmalonic acid (Ruarux *et al.,* 2005; Simpson *et al.,* 2001).

Plasma concentrations of cobalamin and folate are labile and reflect the balance among dietary intake, bacterial utilization and production, intestinal absorption, and body losses. The interpretation of plasma concentrations of cobalamin and folate concentrations with regard to small intestinal disease is only valid if exocrine pancreatic insufficiency, oral supplementation, and parenteral administration have been excluded and attention is paid to dietary vitamin content. Plasma cobalamin and folate concentrations may also be affected by certain medications (e.g., sulfasalazine). Although serum folate levels can decrease markedly within several days, the folate concentration within erythrocytes decreases much more slowly, so low erythrocyte folate values may be a more accurate indicator of a chronic disorder.

Low serum cobalamin concentrations have been observed in dogs with EPI, severe intestinal disease, and apparent idiopathic small intestinal bacterial overgrowth (SIBO). Absolute cobalamin deficiency has been recognized in giant schnauzers with inappetence and failure to thrive with laboratory findings of anemia, leukopenia, and methylmalonyl aciduria. This deficiency appears to be a consequence of the

defective synthesis of the ileal cobalamin-intrinsic factor receptor and signs are completely reversed by the parenteral administration of cobalamin. Some shar-peis also appear to have a deficiency of cobalamin. The physiological significance of the low cobalamin concentrations detected in other gastrointestinal diseases has not been reported.

Low serum folate concentrations have been observed in dogs with severe jejunal disease and some Irish setters with a gluten-sensitive enteropathy. High folate concentrations have been reported in experimentally induced SIBO (blind loops), EPI, German shepherds with antibiotic-responsive enteropathy, and other Irish setters with gluten-sensitive enteropathy.

In the authors' experience, the finding of a low folate or low cobalamin concentration is useful in supporting the presence of an intestinal problem. Where low cobalamin is detected and EPI and intestinal abnormalities of the GI tract (blind loops) have been excluded, localization of the problem to the ileum can be inferred. Serum cobalamin and folate are inadequate markers of predicting response to antibiotics. Concomitant increases in folate and cobalamin are consistent with high intake or supplementation. Finally, normal serum concentrations of cobalamin and folate neither exclude nor support a diagnosis of intestinal disease.

2. *Glucose Absorption*

The absorption of glucose can be evaluated by the oral glucose tolerance test (OGTT) where an oral test dose of glucose is given and the blood glucose levels are measured at half-hour intervals for 3 to 4h. In canine malabsorption, the OGTT curve of blood glucose is diminished or flat (Kaneko *et al.,* 1965). The test also has been used in the horse for evaluation of small intestinal malabsorption (Roberts and Hill, 1973). Dogs with pancreatic exocrine deficiency may have "prediabetic" or high OGTT curves (Hill and Kidder, 1972b). The major disadvantage of this test is that it does not differentiate between decreased intestinal absorption and increased tissue uptake after absorption. This problem can be alleviated by comparing results of the OGTT with those of the intravenous glucose tolerance test (IVGTT). Hill and Kidder (1972b) reported that normal dogs on low-carbohydrate/high protein diets can have "diabetic" tolerance curves so that test dogs should be on a high-carbohydrate diet for 3 to 5 days before testing.

3. D-X*ylose Absorption*

D-xylose is used clinically to evaluate intestinal absorption (Craig and Atkinson, 1988). The body does not metabolize D-xylose to any significant degree, and the problems of evaluating tissue utilization that occur with glucose are avoided. Because large amounts of D-xylose must be used, the rate of absorption is proportional to luminal concentration and independent of active transport processes.

Van Kruiningen (1968) has described a D-xylose absorption test for dogs. In this procedure, a standard 25-g dose of D-xylose is administered by stomach tube. During the 5-h period after administration, the patient is confined in a metabolism cage and urine is collected. At the end of the 5-h test period, the urine remaining in the bladder is removed by catheter and the total quantity excreted in 5 h is determined. Normal dogs excreted an average of 12.2 g of the 25-g dose during the test period, with a range of 9.1 to 16.5 g. Because this test is dependent on the rate of intestinal absorption as well as the rate of renal excretion, it is necessary to establish that kidney function is normal.

A modified D-xylose tolerance test is now most widely used clinically (Hayden and Van Kruiningen, 1973; Hill *et al.,* 1970). Dogs are fasted overnight, a baseline blood sample is taken, and D-xylose is administered by stomach tube at the rate of 0.5 g/kg. A control test is performed on a normal dog simultaneously. Blood samples are taken at 0.05, 1, 2, 3, 4, and 5 h after administration. The D-xylose concentration in the blood is determined by the phloroglucinol microassay (Merritt and Duelly, 1983). The phloroglucinol procedure is more economical, requires less plasma, and is technically easier than the orcinol-ferric chloride procedure of Roe and Rice (Merritt and Duelly, 1983). Maximal blood levels of D-xylose are normally reached at 1h after administration. A D-xylose level of at least 45mg/dl within 60 to 90 min is expected in normal dogs (Hill *et al.,* 1970).

The D-xylose absorption test is also used for differential diagnosis of equine diarrheal diseases (Roberts, 1974). Bolton *et al.* (1976) reported that a dosage of 0.5-g D-xylose/kg bw was useful in detecting horses with intestinal malabsorption. The peak plasma concentration in normal horses is less than one-third that seen in normal dogs given D-xylose at comparable doses.

4. *Oleic Acid and Triolein Absorption*

Several tests have been developed for the clinical evaluation of intestinal absorptive capacity. The absorption of ^{131}I-labeled oleic acid and ^{131}I-labeled triolein has been studied extensively in normal dogs (Michaelson *et al.,* 1960; Turner, 1958), and Kaneko *et al.* (1965) used this test to study dogs with intestinal malabsorption. The day before administration of the ^{131}I-labeled compound, a small amount of Lugol's iodine solution is administered to block thyroidal uptake of the isotope. Tracer amounts of the test substances are mixed with nonradioactive carrier and are administered orally. Absorption is determined by measuring the radioactivity of the plasma at intervals following administration and calculating the percentage of the dose absorbed on the basis of plasma volume.

The ^{131}I-oleic acid and ^{131}I-triolein tests performed in sequence are used to differentiate steatorrhea caused by pancreatic enzyme deficiency from that caused by a primary defect in absorption (Kallfelz *et al.,* 1968). If steatorrhea is

caused by a lack of pancreatic lipase, oleic acid absorption will be normal, whereas that of triolein, which requires lipolysis for absorption, will be significantly reduced. The absorption of both compounds is reduced in intestinal malabsorption.

5. *Vitamin A Absorption*

The vitamin A absorption test measures intestinal lipid absorption (Hayden and Van Kruiningen, 1976). Normal absorption of vitamin A requires secretion of bile and pancreatic enzymes. After oral administration of 200,000 units of vitamin A in normal dogs, serum vitamin A concentrations reach their peak at 6 to 8h, with values ranging between three and five times fasting serum levels. There are small differences in vitamin A absorption between breeds and delayed gastric emptying will also alter results.

6. *Other Tests for Assessment of Intestinal Function*

Simultaneous evaluation of pancreatic exocrine function and intestinal absorptive function is used in dogs (Rogers *et al.,* 1980; Stradley *et al.,* 1979) and cats (Hawkins, *et al.,* 1986; Sherding *et al.,* 1982). The combined bentiromide and D-xylose absorption tests have proved to be useful diagnostically in dogs. Blood is normally taken at 0, {½}, 1, 1{½}, 2, 2{½}, and 3 h after oral administration of the test solution but a single blood sample taken at 1{½}h was adequate for differential diagnostic purposes (Stradley *et al.,* 1979). The combined bentiromide/D-xylose absorption test was of limited usefulness in cats because of marked individual variations. Peak blood PABA levels (60 to 120 min) and peak blood D-xylose levels (30 to 120 min) in healthy cats were less than those of normal dogs, and blood D-xylose levels in cats with infiltrative small bowel disease were not abnormal (Hawkins *et al.,* 1986).

The content of exhaled hydrogen gas has been evaluated as an indicator of carbohydrate malassimilation in the dog (Washabau *et al.,* 1986), cat (Muir *et al.,* 1991), calves (Holland *et al.,* 1986), and humans (Perman, 1991). Unabsorbed carbohydrate is fermented by bacteria in the colon to H_2 and organic acids. Ten to 14% of the H_2 is absorbed and excreted by the lungs (Washabau *et al.,* 1986). Increases in pulmonary H_2 excretion can occur in normal dogs fed rations containing wheat or corn flour. Increased H_2 excretion normally occurs in most species receiving lactulose. Mild increases in H_2 excretion occur in normal humans and dogs receiving xylose but not in the cat.

Breath H_2 excretion has diagnostic value in determining mouth-to-cecum transit time and for identifying small intestinal bacterial overgrowth (Muir *et al.,* 1991). False-negative H_2 breath tests have been seen in humans receiving antibiotics. Diet as well as variations in bacterial flora can also cause false-positive test results.

The nitrosonaphthol test qualitatively measures urinary excretion of 4-hydroxyphenylacetic acid and related compounds, which are intestinal bacterial degradation products of tyrosine. The test has been used to differentiate pancreatic or small intestinal diarrheal diseases from those associated primarily with large bowel disease (Burrows and Jezyk, 1983). The test was positive in 77% of the dogs with pancreatic and small intestinal disease and in only 9.5% of those dogs with large bowel disease. Positive tests were associated with bacterial overgrowth of the small intestine and became negative during antibiotic treatment that resulted in clinical improvement. The test may be useful in dogs to select patients with small intestinal bacterial overgrowth, which might respond to antibiotic therapy.

G. Bacterial Overgrowth

The bacterial flora of the canine intestine increases in number from the duodenum to colon. Factors maintaining this aboral gradient are luminal patency, intestinal motility, limited substrate availability, various bacteriostatic/cidal secretions, and an intact ileocecocolic valve. Abnormalities of these control mechanisms facilitate small intestinal bacterial overgrowth (SIBO). SIBO is usually secondary to another disease process, but it has been reported as a primary idiopathic form. Many clinicians prefer to use the term *antibiotic-responsive diarrhea* instead of *idiopathic SIBO* (German *et al.,* 2003). Regardless, bacterial overgrowth can interfere with the absorption of nutrients and fluid by reducing microvillar enzyme activity, increasing cellular or intercellular permeability, deconjugating bile acids, and hydroxylating fatty acids.

A number of diseases (Rutgers *et al.,* 1988, 1993, 1995; Simpson *et al*., 1990; Williams *et al*., 1987) need to be ruled out before making a diagnosis of idiopathic SIBO. This includes exocrine pancreatic insufficiency (EPI), partial or complete intestinal obstruction, intestinal stasis, resection of the ileocecocolic valve, and intestinal mucosal diseases that cause malabsorption (e.g., moderate to severe inflammatory bowel disease), lymphoma, and lymphangiectasia. Dogs and cats with partial intestinal obstruction often have a history of chronic diarrhea and weight loss, which responds to antibiotics (Batt *et al.,* 1988). Much of the literature pertains to German shepherd dogs (GSD) with subnormal levels of IgA (Delles *et al*., 1993, 1994; Willard *et al*., 1994a, 1994b). SIBO has also been reported in beagles with normal IgA levels (Batt *et al*., 1992).

In the dog, total bacterial counts exceeding 10^5 colony-forming units per milliliter (cfu/ml) of proximal jejunal or duodenal fluid and anaerobic bacterial counts exceeding $\geq 10^5$ cfu/ml have been reported (Burrows *et al*., 1994). Culture of duodenal juice has been regarded as the gold standard for detecting bacterial counts $\geq 10^5$ cfu/ml; this assumption has been questioned because of the variability

of counts in other reports, which detail duodenal bacterial counts ranging from $\leq 10^2$ to 10^7 cfu/ml; counts of $\geq$10cfu/ml in clinically healthy GSD, beagles, and greyhounds.

Healthy cats have higher numbers of bacterial flora in their small intestine than do other species (Johnston *et al*., 1993), in numbers that approximate those for SIBO in dogs and humans. Bacterial counts $\geq 10^5$ cfu/ml are being increasingly documented in other breeds with clinical signs of weight loss, chronic diarrhea, borborygmi, intestinal cramping, or vomiting, with no evidence of intestinal obstruction, EPI, or severe mucosal infiltrates; clinical signs are often responsive to antibiotic therapy.

A comprehensive review of the literature, to include the aforementioned studies, by Johnston (1999) refuted the existing criterion for defining bacterial overgrowth. She concluded that defining SIBO as greater that 10 fifth cfu/ml in the duodenum or proximal jejunum was not appropriate for dogs and cats. Furthermore, she believed future investigations need to separate patients who have SIBO from those who have other antibiotic-responsive enteropathies.

Dogs with idiopathic SIBO may be subclinical or have chronic gastrointestinal signs. Chronic SIBO can cause inflammatory bowel disease. The lesions consist of villus atrophy and infiltrations of lymphocytes and plasmacytes in the lamina propria. There is substantial but reversible biochemical injury to enterocytes of the brush border membrane (Batt and McLean, 1987). Aerobic bacteria, such as enterococci and *Escherichia coli*, cause a selective loss of brush border alkaline phosphatase activity and peroxisomal catalase, as well as changes that are consistent with mitochondrial disruption. There are exceptions but aerobic overgrowth is typical for the dog, in contrast to anaerobic overgrowth in humans. The high floral counts in the intestine of cats are thought to predispose them to certain nutritional deficiencies, such as taurine deficiency, and intestinal disturbances attributed to deconjugated bile salts (Johnston *et al*., 1993).

The culturing and quantitation of intestinal bacterial flora (Simpson *et al*., 1990) are the definitive means of diagnosing SIBO. Less invasive diagnostic methods include determination of serum cobalamin/folate levels, the measurement of breath hydrogen or intestinal permeability, and the nitrosonaphthol test on urine (Burrows *et al*., 1995; Simpson, 2005). Deconjugation of bile acids by intestinal flora, with subsequent disproportionate increase in unconjugated bile acids in the circulation, is seen in humans with bacterial overgrowth (Einarsson *et al*., 1992).

The treatment of SIBO is directed at correcting the underlying structural abnormalities, treating EPI, and controlling the abnormal flora with antibiotics. Patients with IBD often require treatment of SIBO and the mucosal infiltrate. In dogs with suspected idiopathic SIBO, antibiotic therapy is usually given for 28 days. Suitable antibiotics include oxytetracycline (10 to 20 mg/kg TID PO), tylosin (10 mg/kg TID PO), or metronidazole (15 mg/kg BID PO). Dietary supplementation with fructooligosaccharide in IgA-deficient German shepherds resulted in decreased bacterial counts in luminal fluid and intestinal mucosa tissue (Willard *et al*., 1994a, 1994b). Plasma-cell infiltrations in jejunal villi were decreased by feeding different protein sources (Edwards *et al*., 1995). There is also anecdotal evidence that supports the use of highly digestible, low-fat diets, which may less be likely to metabolize to hydroxy fatty acids and stimulate colonic secretions.

The overall prognosis for idiopathic SIBO is guarded and the prognosis for secondary SIBO depends on the underlying disease. Many animals with suspected idiopathic SIBO relapse when antibiotics are stopped and require further courses or long-term maintenance.

H. Helicobacteria

Helicobacter pylori have been associated with chronic gastritis, atrophic gastritis, peptic ulcers, and gastric adenocarcinoma and lymphosarcoma in humans (Handt *et al.,* 1994; Isaacson 1994; Paarsonnett *et al.,* 1991). Studies in other animals have led to the discovery of *H. mustelae* in ferrets with gastritis and peptic ulcers, *H. acinonychis* in cheetahs with severe gastritis, and *H. Heilmannii* in pigs with gastric ulcers. The presence of gastric *Helicobacter*-like organisms (HLO) in the stomachs of dogs and cats has been known for many years, but the relationship of these organisms to gastric disease remains controversial. *H. felis*, *"H. heilmannii," H.bizzozeronii*, and *H. pametensis* have been detected in gastric mucosa of pet cats. *H. bizzozeronii*, *H. heilmannii*, *H. felii*, *H. salomonis*, *H. rappini*, and *H. bilis* have been isolated from dogs. Simultaneous colonization of the stomach with multiple species of *Helicobacter* has been observed in the dog and cat.

Helicobacter pylori, which is the predominant pathogen in humans, can cause infection in other animals (anthroponosis). Although it has not been isolated from the stomachs of pet dogs and cats, experimental infections have been produced in the nonhuman primates, cats, dogs, and pigs. Studies of the pathogenicity of *H. felis*, as well as *H. pylori*, in laboratory cats have demonstrated gastritis, lymphoid follicular hyperplasia, and seroconversion. Many of these cats did not exhibit clinical signs of illness. "*H. heilmannii*," the predominant species in pet cats and 20% to 40% pet dogs, is also found in the mucosa of 0.4% to 4.0% of people. The zoonotic risk posed by dogs and cats was regarded as small because their 16s rDNA sequences were not consistent with *H. heilmanii type I*, which is the principal subtype in people; subtypes in dogs and cats were predominantly types II and IV.

Heliocobacter spp. isolated from the stomachs of dogs and cats are spiral-shaped or curved or sometimes coccoid Gram-negative bacteria that inhabit the glands, parietal cells, and mucus of the stomach. They are morphologically indistinguishable by light microscopy and are classified into several

Helicobacter spp. on the basis of 16S rRNA sequencing, DNA hybridization, and electron microscopic appearance. The majority of studies in cats and dogs with naturally acquired *Helicobacter* infections demonstrates that the fundus and cardia are more densely colonized than the pylorus. Large HLO colonize the superficial mucus and gastric glands and may also be observed intracellularly. Degeneration of the gastric glands, with vacuolation, pyknosis, and necrosis of parietal cells, is more common in infected than uninfected dogs and cats. Inflammatory infiltrates in the gastric mucosa of infected animals are generally mononuclear and range from mild to moderate in severity.

Analysis of gastric juice and biopsies from kittens in an *H. pylori*-infected cat colony, using rapid urease tests, *UreB* PCR patterns, and histopathology demonstrated *H. pylori* in nine of 17 kittens by 8 weeks and in 16 by 14 weeks of age. *UreB* PCR patterns and sequences of PCR products from gastric mucosa were identical in mothers and kittens. Bacterial densities were similar in the stomach and the presence of circulating anti-*Helicobacter* IgG antibodies and histopathological findings were consistent with infection.

Studies have been done in cats chronically infected with *H. pylori* to measure the development of inflammatory and immune responses, and their relationship to the putative bacterial virulence factors *cag* pathogenicity island (*cag*PAI), *vacA* allele, and *oipA* in combination with bacterial colonization density. Infecting *H. pylori* strains were positive for *vacAsI* but lacked the *cag*PAI and an active *oipA* gene. Colonization density was uniform throughout the stomach. Up-regulation of IFN-gamma, IL-1a, IL-B, IL-8, and increased severity of infiltrates and fibrosis were observed in infected cats. The median number and total area of lymphoid aggregates were five and ten times greater, respectively, in the stomachs of infected cats than uninfected cats. Secondary lymphoid follicles were frequent and positive for BLA.36, CD79a, and CD3 but negative for B220. Cats with *H. pylori* can also develop antigastric antibodies that cross-react with *Helicobacter* antigens, as well as changes in gastric acid secretion and serum gastrin levels.

The evaluation of cytokines in cats with naturally acquired *Helicobacter* spp. seems to complement histopathological changes in the stomach. Compared to uninfected cats, infected cats have up-regulation of IL-8 and IL-1beta, but not IFN-gamma or IL-10 and gastric lymphoid follicle hyperplasia is more common and extensive. Circulating anti-*Helicobacter* IgG has been detected in sera of naturally infected cats. To date, there has been no association made between infection and gastrointestinal ulcers or gastric neoplasia in cats. Spontaneous gastritis in the dog is typically consistent with lymphoplasmacytic infiltrations and the expression of IL-10 and IFN-gamma. *Helicobacter* spp. infection is associated with increased expression of TGF-β and fibrosis.

HLO have been observed in gastric biopsies from 41% to 100% clinically healthy cats, 67% to 100% healthy dogs, 57% to 100% of vomiting cats, 74% to 90% vomiting dogs, and 100% laboratory beagles. The prevalence of individual *Helicobacter* spp. has not been thoroughly investigated because of specialized techniques. The high prevalence of gastric colonization with HLO in healthy and sick dogs and cats indicates that there is no simple "infection = disease" relationship. An uncontrolled treatment trial of pets with gastritis and *Helicobacter* infection showed that clinical signs in 90% of 63 dogs and cats responded to treatment, with a combination of metronidazole, amoxicillin, and famotidine, and that 74% of 19 animals reendoscoped had no evidence of *Helicobacter* in gastric biopsies. However, controlled studies in asymptomatic cats suggest that it is difficult to eradicate gastric organisms with a variety of therapeutic agents.

I. Intestinal Permeability

Changes in intestinal mucosal permeability can be a factor in the pathogenesis of mucosal injury and subsequent gastrointestinal disease (Burrows *et al.,* 1995; Sanderson and Walker 1993). Whether as a primary or secondary disorder, increased permeability predisposes to the passage of intraluminal macromolecules across the intestinal mucosa. Depending on the noxious or antigenic characteristics of these macromolecules, pathological features of toxic or immune-mediated injury may occur. A primary mucosal permeability defect is suspected in humans and Irish setters with gluten-induced enteropathy (Hall and Batt, 1991a, 1991b). Enhanced mucosal permeability resulting from small intestinal bacterial overgrowth has been reported in clinically healthy beagles (Batt *et al.,* 1992). Secondary permeability disorders have been resolved by appropriate treatment of giardiasis and bacterial overgrowth in dogs (Hall and Batt, 1990).

Clinicopathological evaluation of intestinal permeability is based on the oral administration of simple, nondigestible molecules (probes) and their recovery in urine (Elwood *et al.,* 1993; Papasouliotis *et al.,* 1993). Inappropriate levels of these probes in urine indicate abnormal macromolecular permeation through transcellular or paracellular pathways. Polyethylene glycols, ^{51}CR-labeled ethylenediaminetetraacetate (^{51}EDTA) (Batt *et al.,* 1992; Hall *et al.,* 1989; Hall and Batt 1991a, 1991b), and nonhydrolyzable sugars have been used in permeability tests. The disaccharides, cellobiose and lactulose, and the monosaccharides, mannitol and L-rhamnose, are unable to penetrate healthy enterocytes (Papasouliatis *et al.,* 1993). In the presence of abnormal mucosal permeability, the disaccharides diffuse passively through the mucosa via paracellular pathways, and the monosaccharides passively diffuse transcellularly.

Differential sugar absorption and calculated disaccharide-to-monosaccharide excretion ratio are preferred over single sugar measurements. The use of lactulose and

mannitol in the evaluation of intestinal permeability has been reported in healthy cats (Papasouliotis *et al.,* 1993). The cellobiose-to-mannitol urinary excretion ratio was increased in Irish setters with gluten-sensitive enteropathy (Hall and Batt, 1991a, 1991b). Simultaneous quantification of rhamnose, lactulose, 3-O-methyl-D-glucose, and xylose in urine by a unique chromatographic technique has been reported to assess both intestinal function and permeability (Sorensen *et al.,* 1993).

J. Protein-Losing Enteropathy

Albumin, IgG, and other plasma proteins are present in low concentration in normal gastrointestinal secretions. Because protein usually undergoes complete degradation within the intestinal lumen, it has been suggested that the gastrointestinal tract must have a physiological role in the catabolism of plasma proteins. The relative significance of this pathway, however, has been the subject of considerable controversy. Some investigators have concluded, for example, that as much as 50% or more of the normal catabolism of albumin (Campbell *et al.,* 1961; Glenert *et al.,* 1961, 1962; Wetterfors, 1964, 1965; Wetterfors *et al.,* 1965) and ϑ-globulin (Andersen *et al.,* 1963) may occur in the gastrointestinal tract. Others believe that the physiological role of the intestine in plasma protein catabolism is far less significant, accounting for about 10% of the total catabolism (Franks *et al.,* 1963a, 1963b; Katz *et al.,* 1961; Waldmann *et al.,* 1967, 1969).

Regardless of questions concerning the physiological significance of the gastrointestinal tract in plasma protein catabolism, it is well established that normal intestinal losses are substantially increased in a variety of gastrointestinal diseases, collectively referred to as the protein-losing enteropathies (PLE). The increased loss causes hypoproteinemia (especially hypoalbuminemia), which may be observed in various types of chronic enteric diseases. The excessive losses are the result of ulcerations or other mucosal changes that alter permeability or obstruct lymphatic drainage from the intestine. If severe, hypoalbuminemia may result in retention of fluid with development of ascites and subcutaneous edema of pendant areas.

Excessive plasma protein loss has been seen in swine with chronic ileitis (Nielsen, 1966), in calves with acute enteric infections (Marsh *et al.,* 1969), in cattle with parasitic or other inflammatory abomasal disease (Halliday *et al.,* 1968; Murray, 1969; Nielsen and Nansen, 1967), and in Johne's disease (Patterson *et al.,* 1967; Patterson and Berrett, 1969). In addition to the classic mucosal and submucosal lesions of Johne's disease, secondary intestinal lymphangiectasia can occur. Meuten *et al.* (1978) observed PLE associated with granulomatous enteritis in two horses.

PLE is seen with some frequency in the dog (Campbell *et al.,* 1968; Farrow and Penny, 1969; Finco *et al.,* 1973; Hayden and Van Kruiningen, 1973; Hill, 1972; Hill and Kelly, 1974; Mattheeuws *et al.,* 1974; Milstein and Sanford, 1977; Olson and Zimmer, 1978). The most common cause appears to be lymphocytic-plasmacytic enteropathy. Intestinal lymphangiectasia also has been reported as a cause of increased intestinal protein loss; it is most commonly seen in small terrier breeds (e.g., Yorkshire, Maltese) and the Norwegian Lundehund, suggesting a genetic predisposition (Simpson, 2005). Increased plasma protein loss from the stomach has been seen in dogs with hypertrophic gastritis.

Familial protein-losing enteropathy (PLE) and protein-losing nephropathy (PLN) have been described in soft-coated wheaten terriers (Littman *et al.,* 2000). Dogs with PLE were diagnosed earlier than dogs with PLN or with both diseases. Clinical signs included vomiting, diarrhea, weight loss, pleural and peritoneal effusions, and thromboembolic disease. Panhypoproteinemia and hypocholesterolemia were consistent findings and intestinal lesions included inflammatory bowel disease, dilated lymphatics, and lipogranulomatous lymphangitis. In another study, food hypersensitivities were identified in six affected dogs (Vaden *et al.,* 2000), but the presence of preexisting inflammatory disease made it impossible to determine if food allergies were the cause or result of enteric disease.

Increased intestinal protein loss is the most likely cause of the hypoalbuminemia associated with certain other enteric diseases including lymphoma and malabsorptive syndromes. Munro (1974) demonstrated that protein loss in dogs with experimentally induced protein-losing gastropathy occurs by an intercellular route. Isotope-labeled polyvinylpyrrolidone (^{131}I-PVP), ^{51}Cr-labeled ceruloplasmin, and ^{51}Cr-labeled albumin have been used to evaluate enteric protein loss in the dog (Finco *et al.,* 1973; Hill and Kelly, 1974; Olson and Zimmer, 1978; van der Gagg *et al.,* 1976).

Fecal alpha 1-proteinase inhibitor (α1-PI) is minimally degraded as it passes down the gastrointestinal tract. In conditions where there is excessive loss of plasma protein into the gut, there is an increase in fecal α1-PI (Williams *et al.,* 1990). The value of this test has been reported in dogs with chronic gastrointestinal disease (Murphy *et al.,* 2003; Ruaux *et al.,* 2004) and in cats with inflammatory bowel disease or gastrointestinal neoplasia (Fetz *et al.,* 2006a, 2006b).

Murphy *et al.* (2003) reported that fecal α1-PI concentrations in dogs with gastrointestinal diseases associated with histological abnormalities (median 60.6μg/g, range 7.4–201.7μg/g) were higher than dogs with gastrointestinal disease and normal histology (median 3.8, 0.7–74) and control dogs (9.9, 0.0–32.1). Although there was no direct correlation with serum albumin levels, the fecal α1-PI was believed to be a useful test in identifying early stages of PLE before decreased levels of serum albumin occurred. Moreover, the test was useful in justifying gastrointestinal biopsies in some cases. Ruaux *et al.* (2004) reported that

increased fecal loss of α1-proteinase inhibitor in dogs with PLE is associated with a significant decrease in fecal proteolytic activity and may result in a false-positive diagnosis of exocrine pancreatic insufficiency.

The studies performed by Fetz *et al.* (2006b) proved that cats with chronic gastrointestinal disease can be associated with gastrointestinal protein loss. The upper limit of the reference range for mean fecal α1-PI concentrations in healthy cats was 1.6μg/g; the concentrations in eight of the nine study cats ranged from 2.2 to 180.77. Fetz *et al.* (2006a) reported that increased fecal α1-PI concentrations in association with low serum albumin and total protein levels are common findings in cats with inflammatory bowel disease (IBD) and gastrointestinal neoplasia. Furthermore, fecal α1-PI concentrations tend to be higher in cats with severe IBD or neoplasia when compared to cats with mild to moderate IBD.

K. Ulcerative Colitis

Canine ulcerative colitis, including granulomatous colitis of boxer dogs, has been reported (Ewing and Gomez, 1973; Gomez *et al.,* 1977; Kennedy and Cello, 1966; Koch and Skelley, 1967; Russell *et al.,* 1971; Sander and Langham, 1968; Van Kruininger *et al.,* 1965). In boxer dogs, the disease is characterized by intractable diarrhea that is often hemorrhagic. Histopathologically, there is a granulomatous or histiocytic submucosal infiltrate and the macrophages are laden with periodic-acid-Schiff-positive material. Immunopathological studies describe an increase in IgG3 and IgG4 plasma cells, PAS positive macrophages and CD3-T cells, L1- and MHC11-positive cells, with pathological lesions similar to human ulcerative colitis (German *et al.,* 2003). Electron photomicrography demonstrated bacteria in the macrophages (Russell *et al.,* 1971), and, in some instances, these organisms resembled *Chlamydia. Mycoplasma* has been cultured from the colon and regional lymph nodes in four boxers, but attempts to reproduce granulomatous colitis with *Mycoplasma* have been unsuccessful. Until recently, this failure to identify or isolate an infectious agent has led to the belief that granulomatous colitis of boxers is an immune-mediated disease.

The development of culture-independent techniques utilizing PCR probes has renewed suspicion that granulomatous colitis in normal dogs is an infectious disease (Simpson *et al.,* 2006). Colonic biopsies from affected dogs (13 boxers with colitis) and 38 control dogs were examined by fluorescent *in situ* hybridization (FISH) with a eubacterial 16s rRna probe. Culture, 16s rDNA sequencing, and histochemistry were used to define invasive flora and guide subsequent FISH. Intramucosal bacteria, predominantly Gram-negative coccobacilli, were present in the affected boxers, but none of the controls. Culture and 16s rDNA sequencing yielded mostly *Enterobacteriaceae* and invasive bacteria hybridized with FISH probes to *E. coli.* These findings complement the observation that affected boxers often respond to enrofloxacin alone or in combination with amoxicillin and metronidazole.

Cases of ulcerative colitis in dogs have also been attributed to trichuriasis, balantidiasis, protothecosis, histoplasmosis, eosinophilic ulcerative colitis, or neoplasia (Lorenz, 1975). Severe ulcerative colitis has also been reported in cats and in some the feline leukemia virus (FeLV) is demonstrated. Feline panleukopenia can also cause colonic lesions.

Biochemical manifestations of ulcerative colitis depend on the duration and severity of illness, the degree of colorectal involvement, and the presence of systemic complications. In severe cases of long duration with extensive colorectal involvement, hypoalbuminemia and hypergammaglobulinemia are often observed. Hypoalbuminemia is attributed to increased loss of plasma through the denuded and inflamed colorectal mucosa and hypergammaglobulinemia is the response to continuing chronic inflammation.

L. Equine Hyperammonemia

Hyperammonemia and subsequent neurological signs have been documented in horses with liver disease (Divers *et al.,* 2006), adult horses with gastrointestinal disease and presumed bacterial overgrowth (Desrochers *et al.,* 2003; Peek *et al.,* 1997; Sharkey *et al.*, 2006), nursing foals with portosystemic shunts (Fortier *et al.,* 1996), and weanling Morgan foals with a genetic abnormality in hepatic ammonia metabolism (McCornico *et al.,* 1997). The shunt foals and Morgan weanlings are discussed elsewhere.

Pertinent to this chapter is the incidence of hyperammonemia in horses with gastrointestinal dysfunction (i.e., colic and diarrhea) that do not have hepatic disease. There is an increased absorption of ammonia across inflamed intestinal mucosa, or massive overproduction of ammonia within the lumen of the gut, or a combination of both. Under normal circumstances, ammonia is delivered to liver via portal circulation and is metabolized by the Krebs-Hensleit cycle to urea and glutamine. Systemic hyperammonemia results when this cycle is overwhelmed. Ammonia readily crosses the blood-brain barrier and, in high concentrations, has a toxic effect on neuronal cell membranes. Resulting encephalopathic signs may be reversible if the underlying intestinal lesion or ammonia-producing bacteria are treated in an appropriate manner.

A diagnosis of idiopathic hyperammonemia of intestinal origin is based on the absence of infectious, toxic, or developmental causes. In some of the reported cases, recent travel or suspected changes in feeding or husbandry preceded the acute onset of gastrointestinal signs. Increased blood ammonia levels and subsequent encephalopathy developed within a matter of hours. Hyperglycemia and metabolic acidemia were also unique to the cases described by Peek *et al.*

(1997). Although Gram-negative bacilli such as *Escherichia coli*, *Klebsiella*, *Proteus*, and *Pseudomonas* spp. are known to be potent ammonia producers, reports pertaining to the role of specific organisms in affected horses has been limited to *Clostridium sordelli* (Desrochers *et al.,* 2003).

Urea toxicity and ammonia intoxication, as well as ingestion of high protein feeds, were ruled out as causes of hyperammonemia in horses described by Peek *et al.* (1997). Horses would have to ingest a large amount of urea to become toxic for a couple of reasons: (1) most of the urea is absorbed in the small intestine before reaching the caecum, which is the predominant site of urease activity; (2) urease activity in the horse's caecum is much less than that in the cow's rumen. Although horses are much more susceptible to ammonia salts than urea, none of the horses were on pastures that had been fertilized with ammonia salts.

M. Clostridial-Associated Diseases in Horses and Cows

Clostridium difficile has been reported in several sources as a cause of colitis in horses and various small bowel disorders in horses, foals, and ponies. The potential role of this organism in causing duodenitis-proximal jejunitis (DPJ) in horses was proposed by Arroyo *et al.* (2006). DPJ was previously thought to be an idiopathic condition characterized by inflammation and edema of the duodenum and jejunum. Affected horses acutely developed signs of colic, depression, ileus, fluid accumulation in the small intestine and stomach, and endotoxemia. In the study reported by Arroyo and his coinvestigator, toxigenic strains of *C. difficile* were isolated from 10/10 horses with DPJ, and only 1 of 16 horses with other causes of nasogastric reflux. *C. perfrigens* or *Salmonella* spp. were ruled out as causes of DPF in affected horses.

Horses with proximal enteritis are predisposed to hepatic injury (Davis *et al.,* 2003). When compared to horses with small intestinal strangulation obstruction (SISO), horses with proximal enteritis had significantly higher serum gamma-glutamyltransferase (GGT), aspartate aminotransferase, and alkaline phosphatase activities. Horses with proximal enteritis were 12.1 times more likely to have high GGT activities than were horses with SISO. Suspected mechanisms for hepatic injury were ascending infection from the common bile duct, absorption of endotoxin or inflammatory mediators from the portal circulation, or hepatic hypoxia resulting from systemic inflammation and endotoxemic shock.

Hemorrhagic bowel syndrome (HBS) or jejunal hemorrhage syndrome is an acute sporadic enteric disease recognized most frequently in dairy cattle (Berghaus *et al.,* 2005). Affected cows develop clinical signs consistent with intestinal obstruction, which is attributed to segmental necrohemorrhagic enteritis and large intraluminal blood clots. *Clostridium perfringens* and *Aspergillus fumigatus* have received the most attention as possible etiological agents. The implementation of management practices to achieve high mild production, as well as increased consumption of high-energy rations, seems to be predisposing a risk factor. The fatality risk is very high, and seldom does medical and surgical intervention change the outcome.

IX. DISTURBANCES OF RUMEN FUNCTION

The digestive process of ruminants differs from that of other animals because rumen microbial digestion occurs before other normal digestive processes. The short-chain fatty acids (acetic, propionic, and butyric acids) are the primary end products of rumen fermentation and are the chief sources of energy available to ruminants from the diet. Cellulose, which undergoes only limited digestion in most simple-stomached animals, is readily digested because of the cellulitic bacteria in the rumen. Ruminal bacteria can also use significant quantities of nonprotein nitrogen (NPN) for protein synthesis, and this bacterial protein subsequently can be utilized to meet the protein requirements of the animal. Under experimental conditions, ruminants may grow and reproduce while receiving diets containing only NPN (e.g., urea) sources of nitrogen. Bacterial production of vitamins can also meet essentially all the requirements of ruminants.

Although nutritionally essential, bacterial fermentation within the rumen presents certain unusual hazards for ruminants. For example, when rapid changes in diet occur, the products of fermentation can be released more rapidly than they can be removed or utilized. Acute rumen tympany, acute indigestion or D-lactic acidosis, and urea poisoning are diseases that result from such abrupt changes in diet (Hungate, 1966, 1968).

A. Acute Rumen Indigestion (Rumen Overload, Lactic Acidosis)

Acute rumen indigestion occurs in sheep or cattle consuming high-roughage diets when they inadvertently are allowed access to large amounts of readily fermentable carbohydrate (e.g., grain or apples) (Dunlop, 1972). *Streptococcus bovis* is the rumen microorganism believed to be chiefly responsible for rapid fermentation and for production of large quantities of lactic acid (Hungate *et al.,* 1952; Krogh, 1963a, 1963b).

When lactic acid accumulates more rapidly than it is absorbed, rumen pH falls and rumen atony develops. Rumen bacteria produce a racemic mixture of lactic acid. Some L-lactate is absorbed and metabolized by the liver and other tissues, but D-lactate cannot be utilized and contributes significantly to the acid load of the body. The excessive lactic acid production results in metabolic acidosis

characterized by reduced blood pH and HCO_3^- concentration and by a fall in urine pH from a normal alkaline value to as low as pH 5. Fluid accumulates in the rumen because of the increased osmolality of the rumen fluid. This accumulation of fluid into the rumen causes hemoconcentration, which in turn may lead to hypovolemic shock and death. If affected animals survive the initial period of rapid fermentation, chemical rumenitis induced by the hyperosmolality of the rumen fluid and by the excess lactic acid may develop. Secondary mycotic rumenitis may then follow, which in severe cases can be fatal. In surviving cattle, metastatic hepatic abscesses may also occur.

B. Acute Rumen Tympany (Bloat)

The rumen of mature cattle can produce 1.2 to 2 liters of gas per minute (Hungate *et al.,* 1955, 1961). The gas is the product of rumen fermentation and is composed primarily of carbon dioxide (CO_2) and methane. CO_2 is also released when salivary HCO_3^- comes in contact with the organic acids in the rumen. Under normal conditions, these large amounts of gas are continually removed by eructation.

Any factor that interferes with eructation can produce acute tympany of the rumen (bloat) leading to rapid death. Interruption of the normal eructation reflex or mechanical obstruction of the esophagus typically results in free gas bloat. The most important form of bloat, however, is seen in cattle consuming large quantities of legumes or in feedlot cattle on high-concentrate diets. The primary factor in these more common forms of bloat is a change in the ruminal contents to a foamy or frothy character because of altered surface tension. Gas becomes trapped in small bubbles within the rumen and cannot be eliminated by eructation (Clarke and Reid, 1974).

The chemical changes that cause foam to form within the rumen are not fully understood. Some reports (Nichols, 1966; Nichols and Deese, 1966) suggest that plant pectin and pectin methyl esterase, a plant enzyme, are critical factors. The enzyme acts on pectin to release pectic and galacturonic acids, which greatly increase the viscosity of the rumen fluid, resulting in formation of a highly stable foam. A soluble legume protein fraction with ribulose diphosphate carboxylase activity has been suggested as another important dietary factor in the pathogenesis of bloat (Howarth, 1975). Slime-producing bacteria also have been incriminated in the pathogenesis of frothy bloat. These microorganisms produce an extracellular polysaccharide that results in stable foam formation.

Effective medical treatment and control are directed toward decreasing or preventing foam formation. This has been accomplished with certain nonionic detergents with surfactant properties that break up or prevent formation of foam within the rumen (Bartley, 1965). Another approach has been the prophylactic administration of sodium alkyl sulfonate, which inhibits pectin methyl esterase activity and prevents foam formation by eliminating the products of this enzyme reaction (Nichols, 1963). Antifoaming agents such as poloxalene administered before ingestion of bloat-producing diets have been shown to be effective prophylactically (Howarth, 1975). Silicone antifoaming agents also have been used for this purpose (Clark and Reid, 1974). Genetic selection of cattle that are less susceptible to rumen tympany has also been pursued (Howarth, 1975).

C. Urea Poisoning

Unlike monogastric animals, ruminants, via their microbial flora, can effectively use nonprotein nitrogen (NPN) to meet some of their dietary protein requirements. Urea, biuret (Oltjen *et al.,* 1969), and ammonium salts (Webb *et al.,* 1972) can serve as dietary NPN sources. Urea, which is the most frequently used, is hydrolyzed by ruminal bacterial urease into CO_2 and NH_3. The free NH_3 is incorporated into amino acids and protein by the rumen microorganisms. The bacterial protein is digested and absorbed in the abomasum and small intestine along with dietary protein.

Signs of urea poisoning typically develop within minutes after consumption of food containing toxic amounts of urea. Clinical manifestations reflect the encephalotoxic effects of excess absorbed NH_3 (Word *et al.,* 1969). Tolerance to urea may be significantly increased by increasing the amount of urea in the diet gradually or by adding readily fermentable carbohydrate to the diet. Ruminants can actually adapt and thrive on a diet in which urea is the sole source of dietary nitrogen. However, if urea is fed at more than 3% in the diet in unadapted animals, toxic effects are likely to occur.

Urea poisoning may occur accidentally when animals engorge on large amounts of urea-containing dietary supplement, when there has been an error in formulation of bulk feed, or when the urea-containing additive is incompletely mixed. Oral administration of acetic acid has been shown to reduce acute urea toxicity, apparently by decreasing absorption of free NH_3 from the rumen. Normally, NH_3 is in equilibrium:

$$NH_3 + H^+ \rightarrow NH_4^+$$

with only 1% in the free form. Acidification shifts the equilibrium farther to the right, thereby reducing the amount of the NH_3. Because only the free form crosses cell membranes, the net effect is a reduction of absorption of NH_3 by the cell. Acetic acid is used as a treatment for urea poisoning, but it is of more value as a prophylactic agent (Word *et al.,* 1969).

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