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8

Gastrointestinal Function

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I. INTRODUCTION

The digestive system is composed of the gastrointestinal tract or alimentary canal, salivary glands, liver, and exocrine pancreas. The principal functions of the gastrointestinal tract are assimilation of nutrients and excretion of the waste products of digestion. Most nutrients are ingested in a form which is either too complex or insoluble for absorption. Within the gastrointestinal tract, these substances are solubilized and degraded enzymatically to simple molecules, sufficiently small in size and in a form which permits absorption across the mucosal epithelium. In the following section, the normal biochemi-

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cal processes of intestinal secretion, digestion, and absorption are described. With these in perspective, we then discuss the mechanisms involved in the pathogenesis of the most important gastrointestinal diseases and the biochemical basis for diagnosis and treatment.

II. GASTROINTESTINAL SECRETION

A. Saliva

1. Mechanism 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 which produce salivary amylase, 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 osmolarity, containing electrolytes and proteins including certain hydrolytic enzymes. The mandibular (submaxillary) and sublingual glands are mixed salivary glands containing both mucous and serous types of cells and produce a viscous secretion which contains large amounts of mucus (Dukes, 1955).

2. Composition

a. Mucus. Mucus is an aqueous mixture of protein–polysaccharide complexes and glycoproteins (Gottschalk, 1972), which have relatively large amounts of carbohydrate bound to protein. The protein–polysaccharide complexes have long polysaccharide chains containing repeating units bound to a protein core. The glycoproteins contain numerous oligosaccharide residues distributed along the polypeptide chain.

One of the most completely studied glycoproteins is mucin from the submaxillary glands of ruminants. The carbohydrate portion is a disaccharide of *N*-acetylneuraminic acid (a sialic acid) and *N*-acetylgalactosamine. Approximately 800 such disaccharide molecules are present per molecule of mucin (Bhavanandan *et al.*, 1964; Bertolini and Pigman, 1967). An enzyme capable of linking protein with hexosamine was demonstrated in sheep submaxillary glands (McGuire and Roseman, 1967).

The physiological functions of mucin are closely related to its high viscosity. *N*-Acetylneuraminic acid is the component responsible for the formation of viscous aqueous solutions. At physiological pH, it causes expansion and stiffening of the mucin molecule (Gottschalk and Thomas, 1961). The resistance of mucin to enzymatic breakdown is also due to the presence of disaccharide residues. Removal of the terminal *N*-acetylneuraminic acid residues by neuraminidase significantly increases the susceptibility of peptide bonds to trypsin (Gottschalk and Fazekas de St. Groth, 1960).

b. Amylase. The saliva of most species contains the α-amylase *ptyalin*. This enzyme is said to be absent, however, in the saliva of dogs, cats, and horses (Dukes, 1955). Salivary amylase splits the α-1,4-glucosidic bonds of various polysaccharides. The salivary enzyme is similar in all major respects to pancreatic α-amylase, which is described below (Section II,D). Salivary amylase initiates digestion of starch and glycogen in the mouth of those species which secrete the enzyme. The optimal pH for amylase activity is approximately 7, and activity therefore terminates when the enzyme mixes with acidic gastric contents.

3. *Functions of Saliva*

Saliva bathes the oral cavity continuously, serving to protect the surface epithelium. Ingested food is moistened and lubricated by saliva, facilitating mastication and swallowing. The teeth also are protected from decay by saliva, which washes food particles from the surfaces of the teeth and, because of its buffering capacity, neutralizes the organic acids produced by bacteria normally present in the mouth.

Ruminants produce much greater quantities of saliva than simple-stomached animals, and the 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 composition of the contents of the rumen. The great buffering capacity is necessary to neutralize the large amounts of short-chain fatty acids which are the major end products of rumen fermentation. The urea in saliva can be utilized by rumen bacteria for protein synthesis. 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 need not be considered an end stage in protein catabolism. The ability to reutilize urea has also been demonstrated in the horse and may be of particular benefit during periods of protein deficiency (Houpt and Houpt, 1971; Prior *et al.*, 1974).

B. Gastric Secretion

The stomach is divided into two main regions on the basis of secretory function (Grossman, 1958). 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,* which are responsible for hydrochloric acid production, (b) *peptic* (zymogenic, chief) *cells,* which produce pepsinogen, and (c) *mucous cells*. The *pyloric gland area* contains the pyloric glands, which are slightly alkaline, and, in addition to mucus, contains the polypeptide hormone gastrin.

1. *Control of Gastric Secretion*

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 distention of the stomach alone also can initiate both intrinsic and vagal nerve reflexes which cause secretion of gastric juice. In addition to neural reflexes, these stimuli cause release of the polypeptide hormone *gastrin* from the pyloric gland area, which enters the bloodstream, stimulating gastric secretion. The release of gastrin from the specific G cells responsible for synthesis is inhibited by excess hydrogen ion, and this negative feedback mechanism is

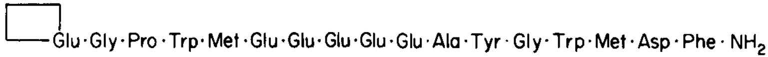


Fig. 1. Amino acid sequence of porcine gastrin I (Gregory, 1966). Gastrin II differs from gastrin I by the presence of a sulfate ester group on the single tyrosyl residue.

believed to be of physiological importance in the control of hydrochloric acid production.

Gastrin has been isolated in pure form from the antral mucosa of swine (Gregory *et al.*, 1964; Gregory and Tracy, 1964; Tracy and Gregory, 1964). When administered intravenously, the purified hormone causes the secretion of hydrochloric acid and pepsin. It also stimulates gastrointestinal motility and causes pancreatic secretion.

Two separate peptides have been obtained from porcine gastric mucosa and have been designated gastrin I and gastrin II. The structure of gastrin has been determined (Gregory *et al.*, 1964) and has been confirmed by synthesis (Anderson *et al.*, 1964). It is a heptadecapeptide amide, with a pyroglutamyl N-terminal residue and the amide of phenylalanine as the C-terminal residue (Fig. 1). 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, 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 activity can be increased by lengthening the peptide chain.

Gastrin is the only hormone known to stimulate HCl secretion (Walsh and Grossman, 1975). As indicated above, gastrin is released in response to vagal stimulation by distention of the pyloric antrum and by direct luminal contact with food, particularly partially hydrolyzed protein (Walsh and Grossman, 1975). The exact mechanism of action is not known, but studies using isolated preparations of isolated parietal cells suggest that the effects of gastrin are not mediated by cyclic AMP (Soll, 1977).

Some of the other factors which are important in regulation of HCl secretion are summarized in Fig. 2 after Dousa and Dozois (1977). There is little doubt that histamine secreted locally within the mucosa has a major effect on the function of parietal cells (Soll

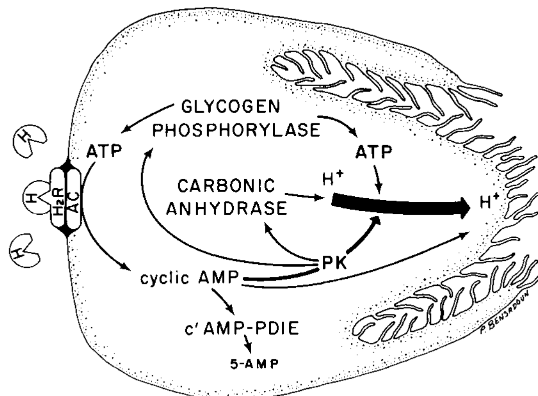


Fig. 2. Schematic representation of a parietal cell and proposed role of histamine in HCl secretion. Abbreviations: H_2R, H_2-receptor; H, histamine molecule; AC, adenylate cyclase; PK, protein kinase; cAMP–PDIE, cyclic AMP phosphodiesterase. (After Dousa and Dozois, 1977.)

and Grossman, 1978). Histamine has been recognized as a potent stimulant of HCl production for many years (Code, 1965). This effect, however, was not inhibited by traditional antihistaminic drugs (H_1 antagonists), and, until the demonstration of H_2 receptors in the stomach (the atrium and uterus) by Black *et al.* (1972), the physiological role of histamine in HCl secretion was controversial. Specific H_2 antagonists (burimamide, cimetidine, metiamide) now have been shown to inhibit the secretory response not only to histamine, but also to gastrin, to cholinergic stimuli, and to food (Grossman and Konturek, 1974).

Although there has been significant conflict in the published literature, current evidence suggests that histamine activates the adenylate cyclase of parietal cells (Dousa and Dozois, 1977), resulting in synthesis of cyclic AMP and ultimately in HCl secretion (Fig. 2). The controversy with regard to the role of cyclic AMP as a mediator of histamine action has come from observations that prostaglandins and secretin, both potent inhibitors of gastric HCl secretion, also stimulate adenylate cyclase (Thompson *et al.*, 1977). It is now believed that prostaglandins, in addition to inhibiting HCl secretion, act on a mucosal cell population which is different from parietal cells and that these cells secrete cytoprotective substances (mucin, glycosaminoglycans). The ulcerogenic effects of prostaglandin inhibitors (indomethacin, acetylsalicylic acid) apparently result from inhibition of this protective effect of endogenous prostaglandins.

2. Composition of Gastric Secretion

a. Basal versus Stimulated Secretion. Gastric juice is composed of two components. One is secreted continuously by the surface epithelial cells and other mucus-producing cells. The other component is produced by the oxyntic glands in response to various stimuli. The basal component is neutral or slightly alkaline. The electrolyte composition is similar to that of an ultrafiltrate of plasma (Table I) and contains large amounts of mucus, which protects the epithelium. The secretory component produced by the oxyntic glands in response to stimulation contains free hydrochloric acid and pepsinogen, the principal enzyme of gastric digestion.

The composition of gastric juice depends on the relative amounts of the two secretory components present, which in turn is a function of flow rate. In the dog, gastric juice is

TABLE I

Composition of Parietal and Nonparietal Secretions of Canine Gastric Mucosa

Component	Parietal secretion[a] (mEq/liter)	Nonparietal secretion[a] (mEq/liter)	Nonparietal secretion[b] (mEq/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 CO_2 of 40 mm Hg.

produced in the resting state at a rate of approximately 5 ml/hour (Gray and Bucher, 1941), and the composition is similar to that of the basal component, containing practically no peptic activity or hydrochloric acid. When the flow of gastric juice is stimulated maximally, the dog may produce 80 ml or more per hour (Gray and Bucher, 1941), and this secretion contains large amounts of peptic activity and hydrochloric acid. Sodium, which is the principal cation in the basal secretion, is replaced to a large extent by hydrogen ion. The concentration of potassium is similar in both basal and stimulated secretions and therefore remains relatively constant at various rates of flow.

Hydrochloric acid and pepsinogen are secreted by separate mechanisms, but these appear to be closely linked under physiological conditions. Stimulation of the vagus nerve (Bachrach, 1953; Hirschowitz and Sachs, 1965) or intravenous injection of gastrin (Hirschowitz, 1966) increases pepsinogen and hydrochloric acid levels together. Other stimuli may affect the two processes differently. In the dog, for example, histamine infusion stimulates hydrochloric acid production maximally but inhibits pepsinogen secretion (Abrams and Brooks, 1960; Hirschowitz, 1966; Emas and Grossman, 1967).

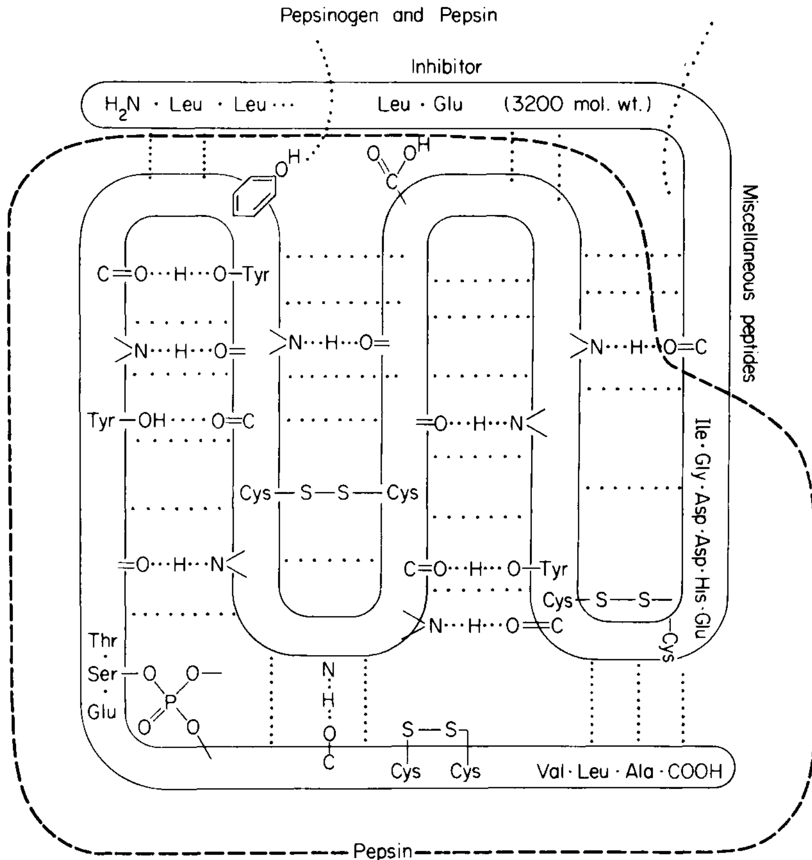


Fig. 3. Structure of pepsinogen-pepsin molecule showing position of inhibitor polypeptide, as proposed by Herriott (1962).

b. 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 (Herriott, 1938), and several pepsinogens have been separated by Ryle (1965), Ryle and Porter (1959), and Ryle and Hamilton (1966). Porcine pepsinogen has a molecular weight of approximately 43,000 and is composed of the pepsin molecule and several smaller peptides (Fig. 3).

One of these peptides has a molecular weight of 3200 and is an inhibitor of peptic activity (Herriott, 1962). 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 6.0. At pH 5.4, the inhibitor peptide dissociates from the parent molecule, and, at pH 3.5–4.0, the inhibitor is completely digested by pepsin (Taylor, 1968).

Pepsin has a very acidic isoelectric point, being stable in acidic solution below pH 6.0 but irreversibly denatured at pH 7.0 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.

c. Rennin. Rennin is another proteolytic enzyme produced by the gastric mucosa and has some characteristics which 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.

d. Hydrochloric Acid. Hydrochloric acid is produced by the oxyntic cells. When the normal mucosa is stimulated, both chloride and hydrogen ions are secreted together, but current evidence suggests that H^+ and Cl^- are secreted by separate, closely coupled pump mechanisms. Small amounts of Cl^- are secreted continuously by the unstimulated parietal cells in the absence of H^+ secretion, and this mechanism is responsible for the relative negative charge of the resting mucosal surface. Hydrogen ion and Cl^- secretory systems may also be differentiated *in vitro* by the demonstration of hydrogen ion secretion in the absence of Cl^-. A scheme for the secretion of hydrochloric acid is presented in Fig. 4. For every H^+ secreted, an electron is removed. The electron ultimately is accepted by oxygen to form OH^-, which is neutralized within the cell by H^+ from carbonic acid. The bicarbonate ion produced enters the venous blood, and this explains why the pH of gastric venous blood frequently is greater than that of arterial blood during hydrochloric acid secretion (Davenport, 1966).

Conversion of carbon dioxide and water to carbonic acid is catalyzed by carbonic anhydrase, which is present in high concentration within parietal cells. When the rate of acid secretion is high, this enzyme contributes to the secretory mechanism by maintaining normal intracellular pH. Carbonic anhydrase inhibitors, such as acetazolamide, interfere with hydrochloric acid production in high concentrations and when the rate of acid secretion is high (Janowitz *et al.*, 1952).

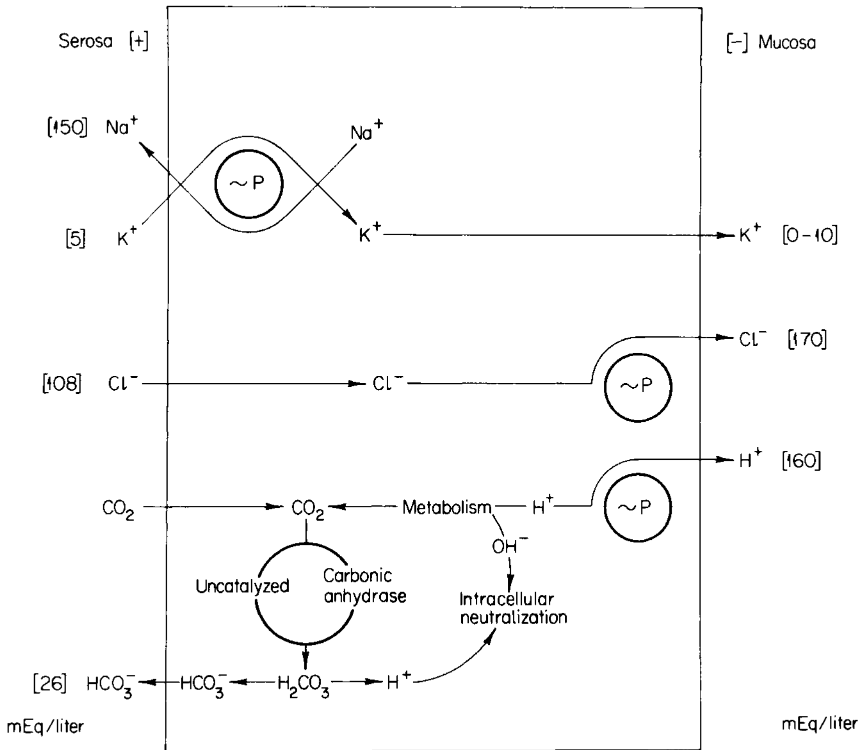


Fig. 4. Movement of ions in the parietal cell during secretion of hydrochloric acid. [Modified from Davenport (1966) and based on the data of Harris and Edelman (1964).]

C. Bile

Bile is secreted continuously by the hepatocytes into the bile canaliculi and 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.

1. Synthesis of Bile Acids

The primary bile acids are C^{24} carboxylic acids synthesized by the liver from cholesterol. Bile acid formation represents the major pathway for cholesterol metabolism (Danielsson, 1963). Cholic acid ($3\alpha,7\alpha,12\alpha$-trihydroxy-5β-cholanoic acid) and chenodeoxycholic acid ($3\alpha,7\alpha$-dihydroxy-5β-cholanoic acid) are the primary bile acids formed by most species of domestic animals. In swine, chenodeoxycholic acid is hydroxylated at the 6α position by the liver to yield hyocholic acid, which is a major primary bile acid in this species (Haslewood, 1964).

Bile acids 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, accounting for one-third of the total in mature sheep (Peric-Golia and Socic, 1968).

Under normal conditions, only conjugated bile acids are present in the bile and in the contents of the proximal small intestine. In the large intestine, the conjugated bile acids are hydrolyzed rapidly by bacterial enzymes so that, in the contents of the large intestine and in the feces, free or unconjugated bile acids predominate. Several genera of intestinal bacteria, including *Clostridium, Enterococcus, Bacteroides,* and *Lactobacillus* (Midtvedt and Norman, 1967), are capable of splitting the amide bonds of conjugated bile acids.

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

2. Detergent Properties of Bile

The carboxyl group of the bile acids is completely ionized at the pH of bile and is neutralized by sodium ion, resulting in the formation of *bile salts.* The bile salts are effective detergents. They are amphipathic molecules, which 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 m*M*, which is ordinarily exceeded both in bile and in the contents of the upper small intestine (Hofmann, 1963). 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.

3. Enterohepatic Circulation of Bile Acids

The enterohepatic circulation begins as conjugated bile acids near the duodenum and mix with the intestinal contents, forming emulsions and micellar solutions. The bile acids 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 conjugated bile acids pass unaltered into the portal circulation (Playoust and

Isselbacher, 1964) and return to the liver, where the cycle begins again. This arrangement provides optimal concentrations of bile acids in the proximal small intestine, where fat digestion and absorption occur, and then efficient absorption after these functions have been accomplished. Absorption of unconjugated bile acids from the large intestine accounts for 3–15% of the total enterohepatic circulation (Weiner and Lack, 1968).

In dogs, the total bile acid pool was estimated to be 1.1–1.2 gm. 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 0.3-0.7 gm/day (Wollenweber *et al.*, 1965). The daily requirement for bile acids greatly exceeds the normal synthetic rate. This necessitates repeated reutilization of the bile acids, which is accomplished by means of the enterohepatic circulation. Under steady-state conditions, the entire bile acid pool passes through the enterohepatic circulation approximately ten times each day (Hofmann, 1966).

The size of the bile acid pool is dependent upon diet, the rate of hepatic synthesis, and the efficiency of the enterohepatic circulation. Surgical removal of the ileum in dogs interrupts the enterohepatic circulation, causing an increase in bile acid turnover rate and a reduction in the size of the bile acid pool (Playoust *et al.*, 1965). In diseases of the ileum, there may be defective bile salt absorption and bile salt deficiency. If severe, impaired utilization of dietary fat may occur, resulting in steatorrhea and impaired absorption of the fat-soluble vitamins.

D. Secretion of the Exocrine Pancreas

The exocrine pancreas is an acinous gland with the same general structure as 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 dietary components. The cells of the terminal ducts probably secrete the bicarbonate ion responsible for neutralizing hydrochloric acid which enters the duodenum from the stomach.

1. Composition

a. Electrolyte Composition. The cation content of pancreatic secretion is similar to that of plasma. Sodium is the predominant cation, with smaller concentrations of potassium and calcium being present. A unique characteristic of pancreatic juice is its high bicarbonate ion 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 juice is directly related to HCO_3^- content and pH increase and the Cl^- concentration decreases. The sodium and potassium ion concentrations and osmolarity appear to be independent of secretory rate (Fig. 5).

b. 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 (Vallee *et al.*, 1959). 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 6.7–7.2 and is activated by chloride ion.

Synthesis of pancreatic α-amylase occurs in the ribosomes. The enzyme is transferred

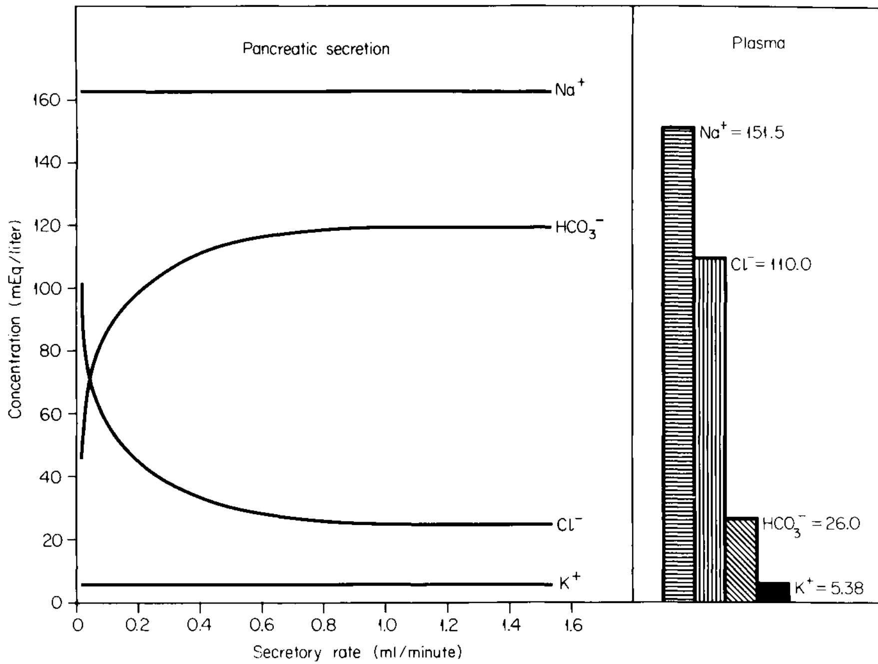


Fig. 5. Influence of secretory rate on the electrolyte composition of canine pancreatic juice. (From Bro-Rasmussen *et al.*, 1956.)

from the endoplasmic reticulum to cytoplasmic zymogen granules for storage (Redman *et al.*, 1966). It is secreted in active form upon stimulation of the acinar cells. Newborn calves (Huber *et al.*, 1961) and pigs (Walker, 1959) 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 (Ben Abdeljlil and Desnuelle, 1974).

Unbranched α-1,4-glucosidic chains, such as those found in amylase, are hydrolyzed in two steps. The first is rapid and results in formation of the disaccaride maltose and maltotriose. The second step is slower and involves hydrolysis of maltotriose with formation of 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.

c. 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. Two types of peptidases are secreted by the pancreas. Trypsin, chymotrypsin, and elastase are *endopeptidases,* which attack peptide bonds along the polypeptide chain, producing smaller peptides. 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 exopep-

TABLE II

Relationship between 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

tidases act in complementary fashion (Table II), producing free amino acids, which are absorbed directly, or small peptides, which are further hydrolyzed by the aminopeptidases of the intestinal mucosa (see Section III,C).

The pancreatic peptidases are secreted as inactive proenzymes or zymogens termed trypsinogen, chymotrypsinogen, and procarboxypeptidase 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 converting more zymogen to active enzyme. Trypsinogen can also be activated by the enzyme *enterokinase,* which is produced by the duodenal mucosa. The latter reaction appears to be highly specific in that enterokinase will not activate 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 (Hartley *et al.,* 1965; Hartley and Kauffman, 1966; Brown and Hartley, 1966). The polypeptide chain of trypsinogen contains 229 amino acid residues. Activation of the proenzyme occurs with hydrolysis of a single peptide bond located in the 6 position between lysine and isoleucine. The C-terminal hexapeptide is released as enzyme activity appears. There is also substantial change in the helical structure of the parent molecule (Davie and Neurath, 1955; Neurath *et al.,* 1956). 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. For a complete discussion of this subject, see the review by Keller (1968).

d. 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 (Sarda and Desnuelle, 1958). 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 enzyme activity. Their transfer from the interface to the aqueous phase is favored by the presence of sodium bicarbonate also secreted by the pancreas and by bile salts.

Mattson and Volpenhein (1966) described two other carboxylic ester hydrolases in pancreatic juice. 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 these

enzymes is a sterol ester hydrolase responsible for hydrolysis of cholesterol esters. The other enzyme hydrolyzes various water-soluble esters. The two enzyme activities have been differentiated on the basis of stability and optimal pH.

The pancreas secretes a third lipolytic enzyme which hydrolyzes phospholids. Phospholipase A converts lecithin which is present in bile to lysolecithin, an effective detergent which aids in emulsification of dietary fat.

2. *Control of Secretion*

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

Secretin is a polypeptide hormone which contains 27 amino acid residues. 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 is a property of 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 increases the rate of bile formation (Wheeler and Mancusi-Ungaro, 1966).

The pancreatic juice which results from stimulation by secretin is large in volume and has high bicarbonate concentration but is low in enzyme activity. Stimulation of the vagus nerve causes a significant rise in enzyme concentration. This type of response also is produced by *pancreozymin,* another polypeptide hormone secreted by the duodenal mucosa. Pancreozymin is now believed to be identical to cholecystokinin, an intestinal hormone which causes contraction of the gallbladder (Thompson, 1969). The C-terminal pentapeptide of pancreozymin–cholecystokinin is exactly the same as that of gastrin. This fascinating relationship suggests that gastrin and pancreozymin–cholecystokinin may participate in some unified but as yet poorly understood system of digestive control (Thompson, 1969).

E. Other Gastrointestinal Polypeptide Hormones

During the past several years, a large number of papers have been published on the endocrine function of the gastrointestinal mucosa and on several new polypeptides which are being classified as gut hormones (Table III). Many of these new substances have not met the rigid physiological requirements for true hormone status, including (1) biological action in very small concentration, (2) release into the bloodstream, and (3) normal serum levels comparable to those provided experimentally by exogenous administration. These criteria probably will be modified, particularly with regard to requirements for transport in the vascular system. A large class of peptides are under investigation which have paracrine rather than endocrine activities; that is, their actions are on cells and tissues in the immediate vicinity of the cells of origin.

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

TABLE III

Gastrointestinal Polypeptide Hormones[a]

Hormone	Source
Motilin	Small intestine (enterochromaffin cell); gastric fundus (enterochromaffin-like cell)
Glucagon	Gastric fundus, canine (A cells)
Enteroglucagon	Distal small intestine; colon
Vasoactive intestinal polypeptide	Intestine
Gastric inhibitory polypeptide	Small intestine
Somatostatin	Pyloric antrum; upper small intestine
Secretin	Small intestine (S cells)
Cholecystokinin-pancreozymin	Duodenum; jejunum

[a] From Pearse *et al.* (1977).

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.

Somatostatin, which is named for its growth hormone release-inhibiting activity, was first purified from bovine hypothalamus (Brazlua and Guilleman, 1974). Somatostatin also has been demonstrated in the stomach, pancreas, and intestinal mucosa in concentrations higher than in the brain (Pearse *et al.*, 1977). 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 (Pearse *et al.*, 1977).

Enteroglucagon is the hyperglycemic, glycogenolytic factor isolated from the intestinal mucosa. It occurs primarily in the distal small intestine and colon in at least two forms, one with a molecular mass of 3500 daltons and the other 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 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 (Sutherland and de Duve, 1948).

III. DIGESTION AND ABSORPTION

A. Water and Electrolyte Absorption

1. Mechanisms of Mucosal Transport

The microvillous membrane of the intestinal mucosa, like other cell membranes, is a lipid structure which acts as a barrier to water and water-soluble substances. Water and polar solutes penetrate in one of two ways. (1) They may pass through *pores* in the membrane, which are believed to be aqueous channels connecting the luminal surface of

the cell with the apical cytoplasm. The "effective" diameter of jejunal pores has been estimated to be approximately 0.4 nm (Lindemann and Solomon, 1962). (2) They may attach to *membrane carriers,* which facilitate passage through the lipid phase of the membrane.

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 which provide energy for transport. Passive movement occurs either by simple diffusion or as a result of gradients in concentration (activity), pH, osmotic pressure, or electrical potential which may be present across the membrane. The passive movement of an ion in the direction of an electrochemical gradient is referred to as *single-file* diffusion (Hladky, 1965). When a substance moves in a direction opposite that of an established electrochemical gradient, an *active transport* process is said to be responsible.

Most water-soluble compounds, such as monosaccharides and amino acids, cannot diffuse across the intestinal mucosal membrane at rates which are adequate to meet nutritional requirements. Transport of these substances is believed to be by means of membrane carriers. The nature of thse carriers is not well understood, but they are believed to be an integral part of the membrane and responsible for binding the transported substance in a rather specific way. Their existence is based primarily on kinetic evidence. Carrier-mediated transport systems can be saturated and are 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 then can be accounted for by simple diffusion. Facilitated diffusion systems can increase the rate of movement across the membrane by two or three orders of magnitude. The 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. It was postulated originally by Ussing (1947) to explain the rapid transfer of radioactive Na^+ across cell membranes. The mechanism does not give rise to net transport but contributes in a major way to unidirectional flux rates, which are measured with isotopic tracers.

In the intestine, net water absorption is the result of bulk flow through pores in the membrane. Diffusion in the usual sense plays no important role in a water movement (Section III,A,4). When bulk flow 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 upon the *reflection* coefficient, which is an expression of the relationship between the pore radius and the radius of solute molecule being transported. A solute such as urea can be transported by the intestine against a concentration gradient by means of solvent drag (Hakim and Lifson, 1964).

2. Sodium Chloride Absorption

Studies with isotopic tracers have shown that transport of water and electrolytes by the intestinal mucosa is a dynamic process, with rapid unidirectional fluxes of the substances

occurring continuously in both directions. Net absorption occurs when the flow from lumen to plasma exceeds that in the opposite direction (Code *et al.*, 1960; Berger *et al.*, 1959; Hindle and Code, 1962).

Active transport of Na^+ can occur along the entire length of the intestine, but the rate of absorption is greatest in the ileum and colon, where most net sodium and water absorption occurs. Sodium transport is believed to be accomplished by an energy-requiring "sodium pump." The characteristics of this pump are not completely understood, but Skou (1965) presented evidence that the pump is intimately related to the activity of a Na^+-K^+-dependent adenosine triphosphatase located within the cell membrane. This enzyme is inhibited by cardiac glycosides, such as oubain, which also are effective inhibitors of Na^+ transport, and it has been suggested that this enzyme system may actually be the pump. In the jejunum, net absorption of sodium occurs slowly unless nonelectrolytes, such as glucose or amino acids, are absorbed simultaneously. In *in vivo* studies by Fordtran *et al.* (1968), jejunal absorption of sodium appeared to be explained, in part, by solvent drag which was associated with active glucose transport. In the ileum, Na^+ absorption was independent of glucose absorption. Water absorption in the jejunum also appears to be almost entirely dependent upon the absorption of glucose, while absorption from the ileum is unaffected by glucose (Barry *et al.*, 1961). The differential effect of glucose on absorption from the jejunum and ileum appears to be the result of fundamental metabolic differences between these two areas of the intestine (Curran, 1960; Gilman and Koelle, 1960).

As sodium is transported across the mucosal membrane, an equivalent amount of anion must be transported simultaneously to maintain electrical neutrality. A significant amount of chloride ion absorption can be accounted for on this basis. It is generally agreed that chloride transport in the intestine is a passive process (Clarkson *et al.*, 1961), although active secretion by the gastric mucosa seems well established. The intestinal mucosa can, under certain circumstances, absorb Cl^- independently of cation absorption and maintain electrical neutrality by exchange secretion of bicarbonate into the lumen (Ingraham and Visscher, 1936).

3. *Potassium Absorption*

Dietary potassium is absorbed almost entirely in the proximal small intestine. Absorption appears to be a passive process since movement across the mucosa occurs down a concentration gradient (high luminal concentration to a low concentration in plasma). The fluid which reaches the ileum from the jejunum has a potassium concentration and a sodium/potassium ratio which is similar to that of plasma. In the ileum and colon, the rate of sodium absorption is much greater than that of potassium so that, under normal conditions, the sodium/potassium 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. It is now generally agreed that water movement is the result of bulk flow through membranous pores and that 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 which arise in trying to establish a definition of active transport. Hypertonic saline solutions can be absorbed from canine intestine *in vivo* (Grim, 1962) and from canine

(Hakim *et al.*, 1963) and rat (Parsons and Wingate, 1961) intestine *in vitro*. These observations indicate that water absorption can occur against an activity gradient and that the process is dependent upon metabolic energy. This would suggest that an active transport process is involved. Curran (1965), however, presents 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 process 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 sodium and water absorption. The colon accounts for no more than perhaps 20% of the total. In the case of herbivorous animals in which the large intestine is developed extensively, net secretion of water may occur in the ileum so that all net absorption of water must take place in the cecum and colon (Powell *et al.*, 1968; Argenzio, 1975).

B. Carbohydrate Digestion and Absorption

1. Polysaccharide Digestion

a. Starch and Glycogen. Carbohydrate is present in the diet primarily in the form of polysaccharides of glucose. 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. Branching chains are linked by α-1,6-glucosidic bonds. In those species which 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 action of pancreatic amylase. This enzyme catalyzes a series of stepwise hydrolytic reactions, resulting in formation of the principle 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. The disaccharides are broken down further by hydrolytic enzymes of the brush border.

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. Starch can be utilized by all species, but cellulose is utilized as a source of energy only by animals which have extensive bacterial fermentation within the gastrointestinal tract. Ruminant species digest cellulose most efficiently, but other animals in which the large intestine is well developed also can utilized cellulose to some degree.

In ruminants, hydrolysis of cellulose is accomplished by cellulytic bacteria, which are part of the complex rumen microflora. The 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 synthesis of carbohydrate.

2. Disaccharide Digestion

Maltose and isomaltose are the disaccharides (glucose–glucose) produced as end products of starch digestion. The diet also contains lactose (galactose–glucose) and sucrose (fructose–glucose). It once was believed that disaccharides were hydrolyzed within the

TABLE IV

Enzymes of the Microvillous Membrane

Enzyme	Reference
Lactase	Alpers (1969), Forstner *et al.* (1968)
Sucrase	Eichholz (1967), Forstner *et al.* (1968)
Maltase	Eichholz (1967), Forstner *et al.* (1968)
Isomaltase	Eichholz (1967), Forstner *et al.* (1968)
Trehalase	Eichholz (1967), Forstner *et al.* (1968)
Cellobiase	Forstner *et al.* (1968)
Leucylnapthylamidase	Rhodes *et al.* (1967), Eichholz (1968)
Leucylglycine hydrolase	Rhodes *et al.* (1967), Eichholz (1968)
Cholesterol ester hydrolase	Malathi (1967)
Retinyl ester hydrolase	Malathi (1967)
Alkaline phosphatase	Eichholz (1967), Forstner *et al.* (1968)
ATPase	Eichholz (1967), Forstner *et al.* (1968)

lumen of the intestine by enzymes secreted by the mucosa. There is now general agreement, however, that disaccharide digestion is completed at the surface of the cell by disaccharidases (Gray, 1975), which are components of the brush border (Table IV). This is considered a form of intracellular digestion (Ugolev, 1965).

The disaccharidases have been solubilized from the brush border and partially purified. Two separate maltases have been isolated (Auricchio *et al.*, 1965). Isomaltase and sucrase have been separated and purified together as a two-enzyme complex (Kolinská and Semenza, 1967). The mucosa also contains two enzymes with lactase activity. One of these is a nonspecific β-galactosidase which hydrolyzes synthetic β-galactosides effectively but which hydrolyzes lactose at a slow rate. This enzyme has an optimal pH of 3 and is associated with the lysozomal fraction of the cell. The other lactase hydrolyzes lactose readily. It is associated with the brush border fraction of the cell and is the enzyme which is important in the digestive process (Alpers, 1969).

Maltase, isomaltase, and sucrase are almost completely absent from the intestine in newborn pigs (Hartman *et al.*, 1961; Dahlqvist, 1961) and calves (Huber *et al.*, 1961). 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 lactose activity seems to be an advantage to the newborn in utilizing the large quantities of lactose present in the diet. 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 which 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 one-half of 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 concentration gradients and are said, by definition, to be actively transported. Active absorption requires metabolic energy and can be inhibited by a variety of substances which block oxidative phosphorylation.

The monosaccharides that are transported most efficiently against concentration gradients have certain common structural characteristics, which were summarized by Wilson (1962). These include (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. These features once were believed to be necessary for active monosaccharide transport, but recent observations suggest that they 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 proper experimental conditions (Csáky and Lassen, 1964; Csáky and Ho, 1966; Alvarado, 1966b).

b. Characteristics of the Membrane Carrier. Most current concepts imply that, during the initial phase of monosaccharide absorption, the monosaccharide molecule attaches to a mobile carrier located within the cell membrane (Crane, 1965). The evidence for such membrane carriers comes from kinetic studies of the overall transport process. The rate of glucose absorption is independent of luminal concentration over a rather wide range, but a maximal rate of absorption can be demonstrated at very high concentrations. This limitation of transport is believed to be due to saturation of binding sites on the membrane carrier.

Glucose transport is competitively inhibited by galactose (Cori, 1925; Fisher and Parsons, 1953) and by a variety of substituted hexoses, which compete with glucose for carrier binding sites. The glucoside phlorizin is a very potent inhibitor (Parsons *et al.*, 1958; Alvarado and Crane, 1962). 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 (Figs. 6a and 6b). 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 (Eichholz and Crane, 1965; 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 0.1 m*M* phlorizin. The specificity of their observations suggested that binding represented an initial step in glucose transport, namely, attachment to a membrane carrier.

c. Sodium Requirement. The absorption of glucose and other monosaccharides is influenced significantly by sodium ion (Schultz and Curran, 1970; Kimmich, 1973). When sodium is present in the solution bathing the intestinal mucosa, glucose is absorbed rapidly, but, when sodium is removed and replaced by equimolar amounts of other cations, glucose absorption virtually stops (Riklis and Quastel, 1958; Csáky, 1961; Bihler and Crane, 1962; Bihler *et al.*, 1962). Glucose abosrption is inhibited by oubain, digitalis, and other cardiac glycosides which are also inhibitors of Na–K-dependent adenosine

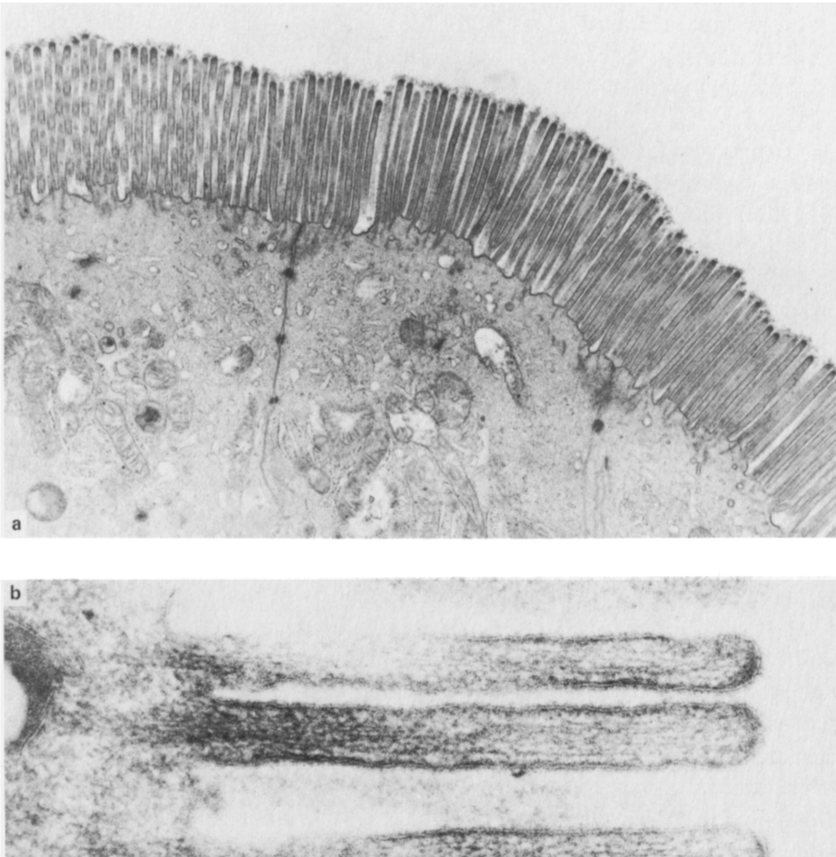


Fig. 6. (a) Absorptive surface of the mucosal epithelium showing the microvillous membrane and, below it, the apical cytoplasm (×8500). (b) Electron photomicrograph of microvilli (×51,500). (Courtesy of S. Lui and K. J. Isselbacher, Massachusetts General Hospital, Boston, Massachusetts.)

triphosphatase activity and sodium transport (Csáky and Hara, 1965; Schultz and Zalusky, 1964). These observations suggest a close relationship between the transport of glucose and sodium. On the basis of their own observations, Crane and co-workers (1965) suggested that sodium ion acts directly upon the membrane carrier to increase affinity of the carrier for glucose. Csáky (1963) interprets the apparent coupling of sodium transport to the transport of various nonelectrolytes as being due to the need to maintain a critical intracellular sodium concentration, which, in turn, is essential for conversion of metabolic energy (ATP, etc.) to energy for transport.

C. Protein Digestion and Absorption

1. Enzymatic Hydrolysis

The initial step in protein digestion is enzymatic hydrolysis of peptide bonds with formation of smaller peptides and amino acids. The *endopeptidases* (proteases) 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.

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 attack most proteins with the exception of keratins, protamines, and mucins. Pepsins are relatively nonspecific endopeptidases and split peptide bonds involving many amino acids. The most readily hydrolyzed peptide bonds are those of leucine, phenylalanine, tyrosine, and glutamic acid (Ryle, 1965; Ryle and Hamilton, 1966; Meyer and Kelly, 1977).

The extent of proteolysis in the stomach depends on the nature of the dietary protein and the length of time spent in the stomach. The food bolus mixed with saliva has a neutral or slightly alkaline pH as it enters the stomach, and a certain period of time is necessary 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, 1.6–2.4 and 3.3–4.0 (Taylor, 1959a,b). 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 appears to have only a minor and probably dispensable role in overall protein assimilation (Freeman and Kim, 1978). The reservoir function of the stomach, however, contributes to the gradual release of nutrients, insuring more efficient utilization in the small intestine.

Partially digested protein passes from the stomach to the duodenum, where the acidic contents are neutralized by sodium bicarbonate secreted in the bile and pancreatic juices. Peptic activity persists in the duodenum only during the period required to raise the pH above 4.0. The major peptidase activity in the lumen of the small intestine comes from 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 are appropriate substrates for the exopeptidases. Trypsin produces peptides with basic C-terminal amino acids which 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 which are nonpolar. Carboxypeptidase A hydrolyze both types of C-terminal peptide bonds (Table II).

The intestinal mucosa contains a broad range of aminopeptidases which complete the process of protein digestion (Heizer and Laster, 1969). Most of the aminopeptidase activity is found in the soluble fraction of the cell (Newey and Smyth, 1960), but a small fraction is tightly bound to the microvillous membrane and appears to serve a digestive function at the cell surface similar to that described for the disaccaridases (Rhodes *et al.*, 1967). An endopeptidase from the intestinal mucosa was studied by Hsu and Tappel (1965) using hemoglobin as substrate. Over 95% of the activity was located in the particulate fraction of the cell. The association with other acid hydrolases suggests that this is a lysozomal enzyme, and its relationship to the normal process of protein digestion is not known.

2. *Form in Which Products of Protein Hydrolysis Are Absorbed*

Despite the long interest in and controversy regarding the subject of this section, the relative amounts of the various types of protein digestion products, i.e., peptides and amino acids, which are actually absorbed by intestinal mucosal cells during normal digestion are still not known. It is a difficult process to investigate from a kinetic

standpoint because the products of proteolysis are absorbed rapidly after they are formed. Studies of luminal contents, therefore, give only an estimate of the overall rate of protein digestion. In addition, dietary protein is continually mixed with endogenous protein in the form of digestive secretions and extruded mucosal cells. Endogenous protein is 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 (Nasset and Ju, 1961). Even when the 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 immunoglubin and other colostral protein, but this capacity is lost soon after birth (see Section III,A,4 below). The intestinal mucosa is not totally impermeable to large polypeptide molecules, however. The absorption of insulin (MW 5700) (Laskowski *et al.*, 1958; Danforth and Moore, 1959), ribonuclease (MW 13,700) (Alpers and Isselbacher, 1967), territin (Bockman and Winborn, 1966), and horseradish peroxidase (Cornell *et al.*, 1971) has been demonstrated. The intestine produces a part of the plasma β-globulin. This is believed to be the result of *de novo* synthesis of protein, however, presumably from individual amino acid precursors.

During the digestion of protein, the amino acid content of the portal blood increases rapidly. Attempts to demonstrate parallel increases in the level of peptides in the portal blood have not been successful (Levenson *et al.*, 1959). This has sometimes been taken as evidence that only amino acids can be absorbed by the intestinal mucosa and that the absorption of peptides does not occur. While it seems clear that a significant part of the dietary protein is absorbed in the form of free amino acids, peptides also may be taken up by the mucosal cell.

Evidence of the mucosal uptake of peptides came originally from experiments with isolated loops of intestine (Wiggans and Johnston, 1959; Newey and Smyth, 1959). Various peptides were placed in solutions bathing the mucosa and analyses made subsequently of the serosal fluid. With the exception of small amounts of glycylglycine, peptides were never found on the serosal side, but free amino acids were found in significant quantities.

The final steps to peptide digestion appear to be 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 (Lindberg, 1966). As described above, mucosal aminopeptidase activity is located 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) (Peters, 1970; Kim *et al.*, 1972). 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.

From these studies, it appears that, in the intact animal, peptides are absorbed in physiologically important quantities by intestinal mucosal cells and hydrolyzed either at

the cell surface or intracellularly to constituent amino acids. The individual amino acids then are transported to the apical part of the cell and finally enter the portal circulation.

3. *Transport of Amino Acids*

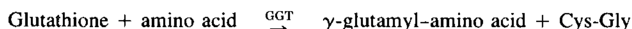
Amino acids, like glucose and certain other monosaccharides, are absorbed and transferred to the portal circulation by active transport processes. The same type of saturation kinetics observed in studies of monosaccharide absorption are observed with amino acids, suggesting carrier transport mechanisms. Certain monosaccharides inhibit amino acid transport (Saunders and Isselbacher, 1965; Newey and Smyth, 1964). Inhibition generally has been of the noncompetitive type, but Alvarado (1966a) demonstrated competitive inhibition between galactose and cycloleucine, suggesting that some form of 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 demonstrated by the observation that the natural *l* forms of various amino acids are absorbed more rapidly than the corresponding *d* forms, and only the *l*-amino acids appear to be actively transported. Sodium ion is necessary for absorption of amino acids as it is for a variety of other nonelectrolyte substances (Schultz and Curran, 1970; Gray and Cooper, 1971).

Separate transport systems appear to 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 binding site. There is some overlap between groups, indicating that, in the overall transport process, certain steps may be common to all amino acids and other steps more specific (Saunders and Isselbacher, 1966; Matthews and Laster, 1965; Wiseman, 1968). These groups are the following:

1. Monoaminomonocarboxylic (neutral) amino acids, including histidine. These amino acids show mutual competition for transport and have the greatest requirement for Na^+.
2. Monoaminodicarboxylic amino acids. Aspartic and glutamin acids are not transported against concentration gradients. Following uptake, they are transaminated, and, under physiological conditions, almost all of the aspartic and glutamic acid enters the portal blood as alanine.
3. Dibasic amino acids, including lysine, arginine, ornithine, and the neutral amino acid cystine. These amino acids are apparently transported by the same transport system.
4. Proline, hydroxyproline, the *N*-substituted glycine derivatives *N*-methylglycine (sarcosine), and *N*-dimethylglycine, and betaine. Proline and hydroxyproline also can be transported by the first mechanism but the affinity of both amino acids for the Na-dependent pathway is low.

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



$$\text{Glutathione} + \text{amino acid} \xrightarrow{\text{GGT}} \gamma\text{-glutamyl-amino acid} + \text{Cys-Gly}$$

The highest GGT activity is present in tissues which are known to transport amino acids

actively, e.g., the jejunal villus and the proximal convoluted tubule of the kidney. 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, while intracellular glutathione interacts with GGT to yield a γ-glutamyl *enzyme*. When the γ-glutamyl moiety is transferred to the membrane-bound amino acid, a γ-glutamyl–amino acid complex is formed and, when released from the membrane binding site, moves into the cell. The γ-glutamyl–amino acid complex is split by the action of γ-glutamylcyclotransferase, an enzyme appropriately located in the cytosol. Glutathione is regenerated by means of the γ-glutamyl cycle, which are good substrates for GGT (Thompson and Meister, 1975).

The γ-glutamyl cycle does not require sodium, and the previously demonstrated sodium dependence for amino acid transport would not be explained by the cycle. The cycle is not considered to be the only amino acid transport system, and its quantitative significance in individual tissues is unknown. Certain nutrient cell types which are deficient in GGT have been shown to transport amino acids normally.

4. Neonatal Absorption of Immunoglobulin

At birth most domestic species, including the calf, foal, lamb, pig, kitten, pup, and infant, absorb significant quantities of colostral protein from the small intestine (Brambell, 1958; Walker and Isselbacher, 1974). γ-Globulin either is absent in the serum of these species at birth, or is at a low level. Within a few hours after ingestion of colostrum, the serum γ-globulin level rises. This is the principal mechanism by which the young of the above-listed species acquire maternal immunity. Under normal environmental conditions, ingestion of colostrum is an absolute requirement for the health of these species during the neonatal period (Fig. 7).

In the neonatal calf, immunoglobulin deficiency has a role in the pathogenesis of gram-negative bacterial septicemia (Smith, 1962; Gay, 1965; Roberts *et al.*, 1954). Most calves deprived of colostrum develop septicemia early in life but may develop diarrhea before death (Smith, 1962; Roberts *et al.*, 1954; Wood, 1955; Tennant *et al.*, 1975). Hypogammaglobulinemia is almost always demonstrable in calves dying of gram-negative bacterial septicemia (Fey, 1971), and hypogammaglobulinemia is believed to be due to insufficient immunoglobulin intake or to insufficient intestinal absorption. The factor in colostrum that protects against systemic infections is the IgM fraction (Penhale *et al.*, 1971).

Serum immunoglobulin values of neonatal calves vary, and a 10% incidence of hypogammaglobulinemia may occur in clinically normal calves (Tennant *et al.*, 1969a; House and Baker, 1968; Smith *et al.*, 1967; Thornton *et al.*, 1972; Braun *et al.*, 1973). Most hypogammaglobulinemic individuals probably had insufficient colostrum intake. Even when calves were given the opportunity to ingest colostrum, a surprising number were hypogammaglobulinemic. Some of the reasons for varying gammaglobulinemia values are recognized, but the relative importance of each reason is not known. The concentration of lactoglobulin, the volume consumed (Bush *et al.*, 1971; 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 γ-globulin (Smith *et al.*, 1967; McBeath *et al.*, 1971). Calves that suckle their dams usually attain serum γ-globulin concentrations that are higher than those

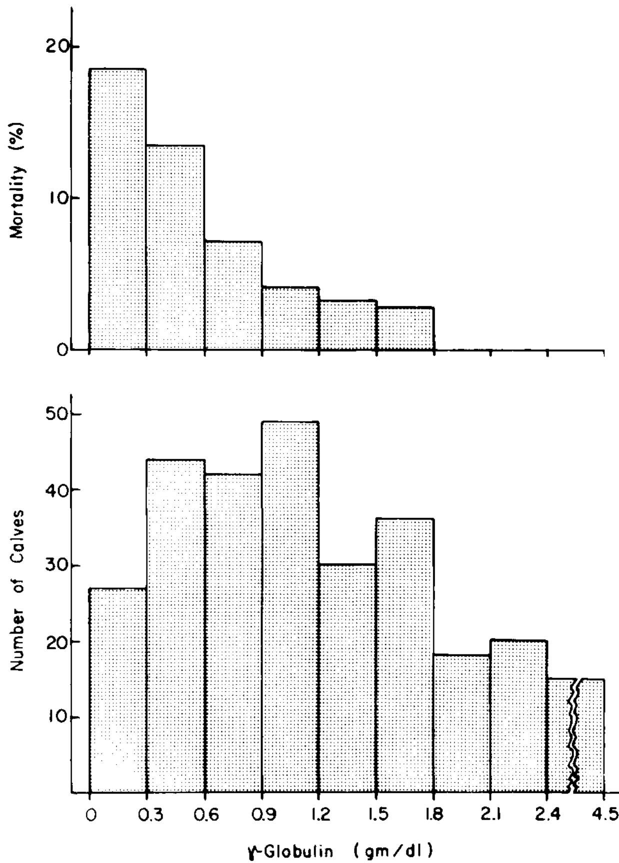


Fig. 7. Histogram showing distribution of serum γ-globulin levels in neonatal market calves and the relation of hypogammaglobulinemia to mortality (From Braun and Tennat, 1979).

attained by calves given colostrum from a bucket. The frequency of hypogammaglobulinemia may be influenced by seasons (Gay *et al.*, 1965b; McEwan *et al.*, 1970a), although this relationship has not always been observed (Smith *et al.*, 1967; Thornton *et al.*, 1972). Familial factors also influence hypogammaglobulinemia (Tennant *et al.*, 1969a).

Regardless of cause, the mortality of hypogammaglobulinemic calves is higher than that of calves with normal serum γ-globulin values (Gay, 1965; House and Baker, 1968; Thornton *et al.*, 1972; McEwan *et al.*, 1970a; Boyd, 1972; Naylor *et al.*, 1977). In addition to having more septicemic infections (Smith, 1962; Gay, 1965a; Roberts *et al.*, 1954; Wood, 1955; Fey, 1971; McEwan *et al.*, 1970a), hypogammaglobulinemic calves have a greater prevalence of acute diarrheal disease (Boyd, 1972; Naylor *et al.*, 1977; Penhale *et al.*, 1970; Gay *et al.*, 1965); the local protective effects of immunoglobulin in the intestine apparently 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 (McBeath *et al.*, 1971; Aschaffenburg, 1949; Fisher and McEwan, 1967a; Patterson, 1967; Stone and Gitter, 1969). The zinc sulfate turbidity test (Kunkel,

1947) was the first to be used for determination of serum immunoglobulin concentrations of neonatal calves (McEwan *et al.*, 1970). A close correlation has been established between test results and the amount of serum IgG and IgM (Fisher and McEwan, 1967a,b; McEwan *et al.*, 1970b; Penhale *et al.*, 1967).

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

The refractometer is used as a rapid test for immunoglobulin deficiency (McBeath *et al.*, 1971; Boyd, 1972). The close relationship between the concentration of γ-globulin and that of total serum protein in neonatal calves was described previously (Tennant *et al.*, 1969a), and the wide variation in total protein concentration was due to differences in γ-globulin concentration. Direct linear correlation between the serum protein concentration (refractive index) and the immunoglobulin concentration also has been described (McBeath *et al.*, 1971). The equation for the regression line in that report was virtually identical to that observed recently (Tennant, *et al.*, 1978). The *Y* intercepts in our study and in that previously reported were identical (4 gm/dl). The refractometer has value as a rapid field instrument for the assessment of immunoglobulin status, but in cases of hemoconcentration it has limitations (Boyd, 1972).

The glutaraldehyde coagulation test was used originally for the detection of hypergammaglobulinemia in cattle, using whole blood (Sandholm, 1974). Glutaraldehyde reagent also has been used in a semiquantitative test to evaluate γ-globulin in canine (Sandholm and Kivisto, 1975) and human serum (Sandholm, 1976). We modified this procedure to detect hypogammaglobulinemic calves (Table V). Calves that had a negative test result (serum γ-globulin $\leq$0.4 gm/dl) had markedly higher mortality than did calves with positive results (Table VI) (Tennant, *et al.*, 1979), findings similar to those obtained by using the zinc sulfate turbidity test (Gay *et al.*, 1965a; McEwan *et al.*, 1970a). Many tests can be initiated at one time using the glutaraldehyde coagulation test, and all results can be evaluated rapidly without instrumentation (Tables V and VI).

Protein enters the absorptive cell by pinocytosis and passes across the cell to the lymphatics. The process is not selective because many proteins other than the immune globulins can be absorbed (Payne and Marsh, 1962a,b). The ability to absorb intact protein is lost by domestic species within 1 or 2 days following birth. In rodents, protein absorption normally continues for approximately 3 weeks. The mechanism of intestinal "closure" was studied by Lecce and co-workers (1964; Lecce, 1966; Lecce and Morgan,

TABLE V

Procedure for Detection of Hypogammaglobulinemia

Reagent	10% glutaraldehyde: 4 ml 25% glutaraldehyde 6 ml distilled water
Procedure	Mix 1 part reagent (50 μl), 10 parts serum (0.5 ml) Observe coagulation time by tilting tube; samples that do not coagulate within 1 hour can be classified as hypogammaglobulinemia

TABLE VI

Relationship between Results of the Glutaraldehyde Coagulation Test, Serum γ-Globulin Concentration, and Death Rate

Source of calves	No.	Glutaraldehyde reaction	Serum γ-globulin (gm/dl)		Death rate (%)
			Mean (±SD)	Extremes	
Calves before ingestion of colostrum	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.

1962). They found that complete starvation of pigs lengthened the period of protein absorption to 4-5 days, whereas early feeding shortened the period. 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 prevented from eating but which receive nutrients parenterally lose the ability to absorb protein at the same time as control calves (Deutsch and Smith, 1957).

D. Lipid Digestion and Absorption

1. Absorption of Fats

a. Luminal Phase. The fat present in the diet is primarily in the form of triglycerides of long-chain fatty acids. The initial step in utilization of triglycerides occurs in the lumen of the proximal small intestine, where hydrolysis is catalyzed by *pancreatic lipase*. This enzyme, which is secreted in active form, requires an oil-water interface for activity so that only emulsions are attacked (Sarda and Desnuelle, 1958). Enzyme activity is directly related to the surface area of the emulsion. 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 they favor hydrolysis (1) by their detergent action, which causes formation of emulsions with small particle sizes, and (2) by stimulating lipase activity within the physiological pH range of the duodenum (Borgström, 1954, 1964a).

A colipase is present in the pancreatic secretion which facilitates the interaction of lipase with its triglyceride substrate and protects lipase from inactivation (Borgström and Erlanson, 1971).

Pancreatic lipase splits the ester bonds of triglycerides preferentially at the 1 and 3 positions (Sari *et al.*, 1966), so that the major end products of hydrolysis are 2-monoglycerides and nonesterified fatty acids (Mattson *et al.*, 1952; Mattson and Volpenhein, 1962, 1964). 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 2.0 nm (Borgström, 1964b; Laurent and Persson, 1965) and are believed to be the form in which the products of fat digestion are

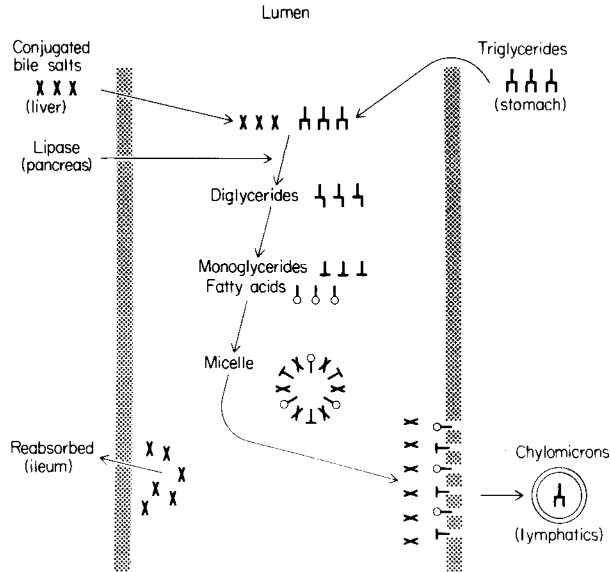


Fig. 8. Intraluminal events which occur during fat absorption. (From Isselbacher, 1967.)

actually taken up by the mucosal cell (Hofmann and Small, 1967). The intraluminal events which occur in fat absorption are schematically summarized in Fig. 8.

b. Mucosal Phase. The initial step in fat transport is the uptake of fatty acids and monoglycerides by the mucosal cell from micellar solution. Just how this occurs is not completely clear, but present evidence suggests that the lipid contents of the micelle are somehow discharged at the cell surface so that they enter the cell in molecular rather than micellar form (Isselbacher, 1967). The net effect is the absorption of the end products of lipolysis with the exclusion of bile salts, which are absorbed farther down the intestine, primarily in the ileum (Lack and Weiner, 1963). Uptake of fatty acids appears to be a passive process having no requirement for metabolic energy (Johnston and Borgström, 1964; Strauss, 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 Manning, 1974; Ockner and Isselbacher, 1974). The two biochemical pathways for triglyceride biosynthesis in the intestine are summarized in Fig. 9. Direct acylation of monoglyceride occurs in the intestine (Senior and Isselbacher, 1962) and probably is the major pathway for lipogenesis in the intestine during normal fat absorption (Kern and Borgström, 1965; Mattson and Volpenhein, 1964). The initial step in this series of reactions involves activation of fatty acids by acyl-CoA synthetase, a reaction which requires Mg^{2+}, ATP, and CoA (Dawson and Isselbacher, 1960; Clark and Hübscher, 1960, 1961; Brindley and Hübscher, 1965) and which has a marked specificity for long-chain fatty acids (Dawson and Isselbacher, 1960; Brindley and Hübscher, 1965). This specificity appears to explain 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

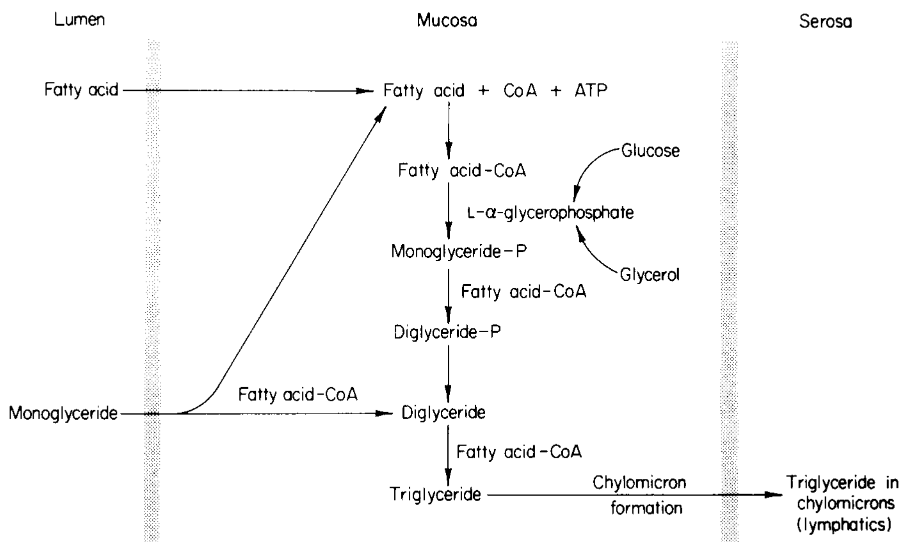


Fig. 9. Biochemical reactions involved in intestinal transport of long-chain fatty acids and monoglycerides. (From Isselbacher, 1966.)

then react sequentially with mono- and diglycerides to form triglycerides in steps catalyzed by mono- and diglyceride transacylases (Ailhaud *et al.*, 1964). The enzymes responsible for this series of reactions were partially purified by Rao and Johnston (1966) from the microsomal fraction of the cell. They observed that purification of the separate enzyme activities occurred simultaneously, suggesting that these enzymes occur together in the endoplasmic reticulum as a "triglyceride-synthetase" complex.

An alternate route which is available for fatty acid esterification involves L-α-glycerophosphate, which may be derived from glucose or from dietary glycerol by the action of intestinal glycerokinase (Haessler and Isselbacher, 1963; Clark and Hübscher, 1962). 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 in the previous paragraph, triglyceride can then be formed. Although this pathway appears to be one of minor importance for triglyceride synthesis in the intestine, Johnston (1968) pointed out the importance of certain of the intermediates in this sequence of reactions in the synthesis of phospholipids which are necessary 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 (Isselbacher and Budz, 1963; Hatch *et al.*, 1966; Windmueller and Levy, 1968). 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 chylomicron into the intercellular space opposite the basal lateral portion of the absorptive cell. This is accomplished by a

process which is essentially the reverse of pinocytosis (Palay and Karlin, 1959). From the intercellular space the chylomicron passes through the basement membrane and enters the lacteals through small pores. The chylomicron passes from the lacteal into lymph ducts and ultimately reaches the general circulation, having bypassed the liver completely 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 (Vahouny and Treadwell, 1964). Cholesterol esters are hydrolyzed within the lumen of the intestine by sterol esterase secreted by the pancreas. Bile salts are required both for the action of this enzyme (Vahouny *et al.*, 1965) 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 in the form of 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 by a process which apparently requires metabolic energy (Skála and Hrubá, 1964). Vitamin A alcohol is reesterified in the mucosa utilizing primarily palmitic acid (Mahadevan *et al.*, 1963). The vitamin A ester is absorbed by way of the lymph. 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. This is also the form released from the liver as needed by the action of a specific hepatic retinylpalmitase esterase (Mahadevan *et al.*, 1966). The blood level of vitamin A is independent of the liver reserve, 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 which lack animal fat, the carotenes, mainly β-carotene, serve as the major vitamin A precursors. The intestinal mucosa plays the primary role in conversion of provitamin A to the active vitamin, although conversion can occur to a limited degree in other tissues (Bieri and Pollard, 1954; Zachman and Olson, 1963). The exact mechanism involved in the conversion of β-carotene to vitamin A is not completely established, but studies by Olson (1961) suggest that there is central cleavage of β-carotene into two active vitamin A alcohol molecules, which are subsequently esterified and transported by the lymphatics as with the preformed vitamin.

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 appear to be rate-limiting steps. Cattle also absorb substantial amounts of 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 carotene in the plasma, and it has been suggested that extraintestinal conversion may be more efficient in these species than in cattle (Ganguly and Murthy, 1967).

c. Vitamin D. Vitamin D, like cholesterol, is a sterol which is absorbed from the intestine by way of the lymph (Schachter *et al.*, 1964). Intestinal absorption differs,

however, in that vitamin D is transported to the lymph in nonesterified form (Bell, 1966). 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 out of the cell into the lymph, a step which appears to be rate limiting (Thompson *et al.,* 1969).

One of the major actions of vitamin D is to enhance the intestinal absorption of calcium ion. The mechanism of action of vitamin D has been described by Wasserman and co-workers (1968; Wasserman and Taylor, 1966, 1968). They have shown that vitamin D causes synthesis of a calcium-binding protein present in the soluble fraction of the intestinal mucosal cell. They have accumulated a substantial amount of evidence which suggests that this protein plays a central role in the active transport of calcium.

IV. DISTURBANCES OF GASTROINTESTINAL FUNCTION

A. Vomiting

Vomiting is a coordinated reflex act which results in rapid, forceful expulsion of gastric contents through the mouth. The reflex may be initiated by (1) local gastric irritation caused by a variety of toxic irritants or infectious agents, (2) foreign bodies, (3) gastric tumors, (4) obstruction of the pyloric canal or of the small intestine, or (5) drugs, such as apomorphine, or other toxic substances which act centrally on the "vomiting center" located in the medulla.

Severe vomiting produces loss of large quantities of water and of H^+ and Cl^- ions. These losses cause *dehydration, metabolic alkalosis* with elevated plasma bicarbonate concentration, and *hypochloremia.* Chronic vomiting may also be associated with loss of tissue K^+ and hypokalemia. The K^+ deficit is caused primarily by increased urinary excretion, which is the result of the existing alkalosis (Leaf and Santos, 1961). Gastric secretions contain significant quantities of K^+ (Section II,B), and losses in the vomitus also contribute to the K^+ deficiency. Potassium deficiency, which develops initially because of alkalosis, ultimately may perpetuate the alkalotic state by interfering with the ability of the kidney to conserve H^+ (Koch *et al.,* 1956; Darrow, 1964). Both potassium deficiency and the hypovolemia caused by dehydration may result in renal tubular damage and ultimately in renal failure (Haden and Orr, 1923, 1924).

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, however, an uncommon sign, occurring most frequently after ingestion of toxic materials. The contents of the abomasum are not expelled directly even when the pyloric canal is obstructed. A syndrome does occur in cattle with pyloric obstruction, however, which is similar metabolically to that observed in nonruminants. The syndrome has been observed in right-sided displacement of the abomasum with torsion (Espersen, 1961; Boucher and Abt, 1968). We have also observed the syndrome in cows with functional pyloric obstruction, the result of reticuloperitonitis (a variety of "vagal indigestion"). When the pylorus is obstructed, abomasal contents are retained, causing distention 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 there are sequestered 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 (Espersen and Simesen, 1961; Svendsen, 1969).

Chronic hypertrophic gastritis has been demonstrated in the dog (van der Gagg *et al.*, 1976; Van Kruiningen, 1977; Happe and van der Gagg, 1977; Kipnis, 1978) which resembles Menetrier's disease in man. Van Kruiningen's series of cases were Basenjis which had concomitant lymphocytic-plasmocytic enteritis. Three unpublished cases were studied at the New York State College of Veterinary Medicine. Signs of illness usually involved chronic vomiting, weight loss, and occasionally diarrhea. Hypoalbuminemia was documented in most of these cases. In man, hyperchlorhydria or achlorhydria can occur. The morphological changes in the stomach wall (hypertrophic rugae) as well as some of the clinical features help to differentiate this disease from gastric neoplasia and canine Zollinger-Ellison syndrome.

Canine Zollinger-Ellison syndrome was reported in four dogs (Straus *et al.*, 1977; van der Gagg *et al.*, 1978). Vomiting, diarrhea, inappetance, and weight loss were reported. All of the dogs had pancreatic non-β islet cell tumors, resulting in hypergastrinemia, hyperchlorhydria, hypertrophic gastritis, peptic esophagitis, and duodenal ulcers.

B. Diarrhea

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

There are theoretically three factors which could act independently or in combination to produce diarrhea: (1) increased rate of intestinal transit, (2) decreased intestinal absorptive capacity, and (3) increased secretion into the intestinal lumen. An increase in the rate of intestinal transit has been considered to be important in various functional disorders of the gastrointestinal tract in which "hypermotility" has been considered the primary cause. Although increased intestinal motility may be a factor in certain types of diarrheal disease when the direction of motility has been investigated, diarrhea has actually been associated with decreased motility (Christiansen, 1972).

Decreased intestinal assimilation of nutrients may result from either (1) decreased intraluminal hydrolysis of nutrients, e.g., *maldigestion* (Kalser, 1964), due to pancreatic exocrine insufficiency or to bile salt deficiency or (2) defective mucosal transport of nutrients, *malabsorption,* which may be the result of various types of inflammatory bowel disease, intestinal lymphoma, or intrinsic biochemical defects in the mucosal cell which interfere with normal digestion and absorption. The role of increased intestinal secretion in the pathogenesis of certain types of acute diarrhea is now recognized.

Enteropathogenic strains of *Escherichia coli* have been shown to produce soluble enterotoxins (Smith and Halls, 1967; Kohler, 1968; Moon, 1978), which alter bidirectional sodium and water flux (Fig. 10). Rapid advances in understanding the pathogenesis of enterotoxin-induced diarrhea and the molecular basis of enterotoxin action have been made. 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 heat-labile (LT) enterotoxin produced by certain strains of *E. coli* (Richards and Douglas, 1978). The mechanism of action of CT is believed to

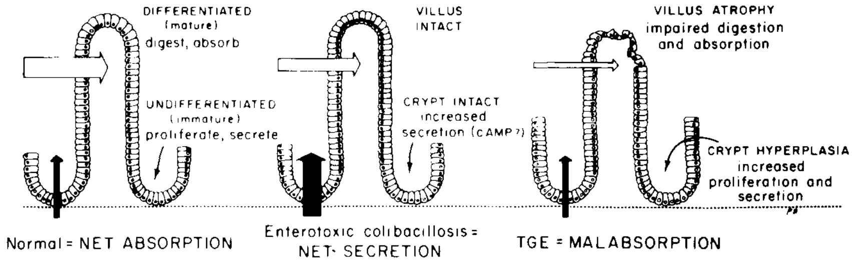


Fig. 10. Proposed pathogenesis of diarrhea caused by *E. coli* enterotoxin and by coronavirus. (After Moon, 1978.)

involve the activation of adenylate cyclase. This membrane-bound enzyme converts ATP to cyclic 3′,5′-adenosine monophosphate (cAMP), which is then responsible for the greatly increased secretion of water and electrolytes by the intestinal mucosa (Moon, 1978). Although species differences have been observed (Hamilton *et al.*, 1978a,b; Forsyth *et al.*, 1978), this mechanism appears to be important in the mode of action of *E. coli* LT as well (Richards and Douglas, 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 apparently plays a critical role in the regulation of adenylate cyclase. Cholera toxin is believed to bind to the adenyl cyclase in a way which inhibits hydrolysis of GTP, thereby maintaining the enzyme in an activated state (Levinson and Blume, 1977; Johnson *et al.*, 1978; Cassel and Pfeuffer, 1978) (Fig. 11).

Certain enteropathogenic strains of *E. coli* produce a low molecular weight heat-stable toxin (ST) alone or in addition to LT (Richards and Douglas, 1978; Moon, 1978; Hamilton *et al.*, 1978a). In most epidemiological studies of neonatal diarrheal diseases of calves, isolated strains of *E. coli* produce only ST (Moon *et al.*, 1976; Braaten and Myer, 1977; Larivier *et al.*, 1979). In contrast to LT and CT, which induce intestinal sodium and

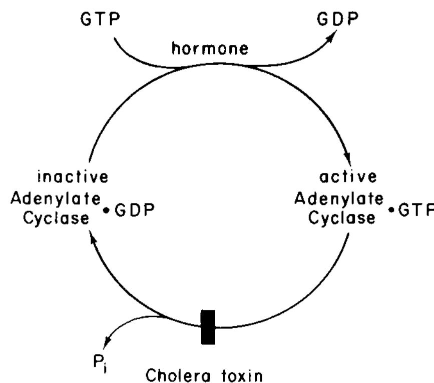


Fig. 11. Proposed mechanism of action of cholera toxin, which inhibits hydrolysis of GTP, thereby increasing adenylate cyclase activity. (After Cassel and Selinger, 1978.)

water secretion only after a lag phase of several hours, ST increases intestinal secretion at once. Recent evidence suggests that ST induces intestinal secretion by activating guanylate cyclase and that the mediator of intestinal secretion induced by ST is cyclic 3′,5′-guanosine monophosphate (Hughes *et al.*, 1978; Field *et al.*, 1978).

Such advances in our fundamental knowledge of the pathogenesis of enterotoxin-induced diarrheal disease have opened several avenues of investigation which may lead to pharmacological modification of intestinal secretion as a mode of therapy or prophylaxis. Enterotoxin-induced intestinal secretion has been shown to be effectively blocked by cycloheximide, 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 (Norris *et al.*, 1969; Moore *et al.*, 1971), and 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 an "intestinal-specific" derivative would have significant therapeutic potential. Adenosine analogues also have been shown in preliminary studies to inhibit cholera toxin-stimulated intestinal adenylate cyclase, but their potential as prophylactic or therapeutic agents is not known.

Prostaglandin E_1 and CT have a similar effect on electrolyte transport in rabbit ileum. Application of either to the mucosa inhibits sodium absorption and stimulates chloride secretion. One possible explanation for the effects of CT is that it stimulated release of prostaglandin, which then acted on adenylate cyclase, producing cAMP. To test this hypothesis, the effects of inhibitors of prostaglandin release on enterotoxin-stimulated intestinal secretion were investigated. Both indomethacin (Gots *et al.*, 1974) and acetylsalicylic acid (Farris *et al.*, 1976) were shown to be potent inhibitors of enterotoxin-induced intestinal secretion using laboratory animal models. Current evidence does not support the hypothesis that prostaglandins play a primary role in the pathogenesis of cholera or other enterotoxin-induced diarrheal diseases (Schwartz *et al.*, 1975), but the effects of these known prostaglandin inhibitors and other drugs on the intestinal secretory process warrant their evaluation as possible prophylactic and therapeutic agents. In preliminary studies, Jones and his colleagues demonstrated a positive therapeutic response to a new prostaglandin inhibitor (Jones *et al.*, 1977).

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 (Sutherland and Rall, 1960; Schultz *et al.*, 1975), apparently by activating the GTP–GDP system described above (Cassel and Selinger, 1978; Ciment and deVellis, 1978). Adrenergic blocking agents, such as chlorpromazine (Holmgren *et al.*, 1978) and propranolol (Donowitz *et al.*, 1979), have been shown to 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 which may be of therapeutic benefit.

The intestinal "adsorbent" drug Pepto Bismol, a patented medication containing bismuth subsalicylate, and Attapulgite, a heat-treated silicate, have been shown to have antienterotoxic effects (Drucker *et al.*, 1977; Ericsson *et al.*, 1977; Gyles and Zigler, 1978). Controlled therapeutic trials with bismuth subsalicylate have demonstrated significant therapeutic benefit in certain large-volume diarrheal diseases in man suspected of being enterotoxigenic in origin (Portnoy *et al.*, 1976; DuPont *et al.*, 1977; DuPont, 1978). The mechanism of action in inhibiting intestinal secretion has not been determined, but the chemical relation of bismuth subsalicylate to other known prostaglandin inhibitors

is recognized. 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. Recent evidence suggests that salicylates also may stimulate sodium chloride absorption (Powell *et al.*, 1979). These observations taken collectively suggest that new, innovative methods for therapy and control of acute clinical diarrheal disease may be developed in the not too distant future.

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), rota viruses, 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 hydrogen ion and electrolyte disturbances (Dalton *et al.,* 1965; Fisher and McEwan, 1967b; Tennant *et al.*, 1972, 1978).

In acute diarrhea with watery stools of large volume, the fecal fluid originates primarily in 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 which accompanies acute enteritis in the newborn soon produces hemoconcentration and ultimately hypovolemic shock. Such cases are characterized by metabolic acidosis (Dalton *et al.,* 1965; Phillips and Knox, 1969) caused by decreased excretion of H^+ due to renal failure and by increased production of organic acids, the result of decreased tissue oxygenation, which leads to excessive anaerobic glycolysis. Hyperkalemia also is observed characteristically in young, severely dehydrated animals. Hyperkalemia in such cases is the result of increased movement of cellular potassium 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 believed to be a direct cause of death in calves with acute diarrhea (Fisher, 1965; Fisher and McEwan, 1967b). Marked hypoglycemia also has been observed occasionally prior to 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 which occur during acute neonatal diarrhea is summarized in Fig. 12.

In chronic forms of diarrheal disease, excessive fecal losses of electrolyte and fluid may be compensated in part by renal conservation mechanisms and by oral ingestion. If water is consumed without adequate ingestion of electrolytes, hyponatremia and hypokalemia may develop (Tasker, 1967; Patterson *et al.*, 1968). In such cases, the osmolarity 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 situation, that intravenous fluids contain sufficient K^+ to prevent further reduction in plasma concentration. If they do not, additional cardiac irregularities or cardiac arrest may result.

C. Intestinal Malabsorption

Decreased assimilation of nutrients may occur either as a result of defective intraluminal digestion (maldigestion) (Kalser, 1964), or because of defects in mucosal transport (Jeffries, *et al.*, 1969; Floch, 1969; Wilson and Dietchy, 1971). Intestinal malabsorption

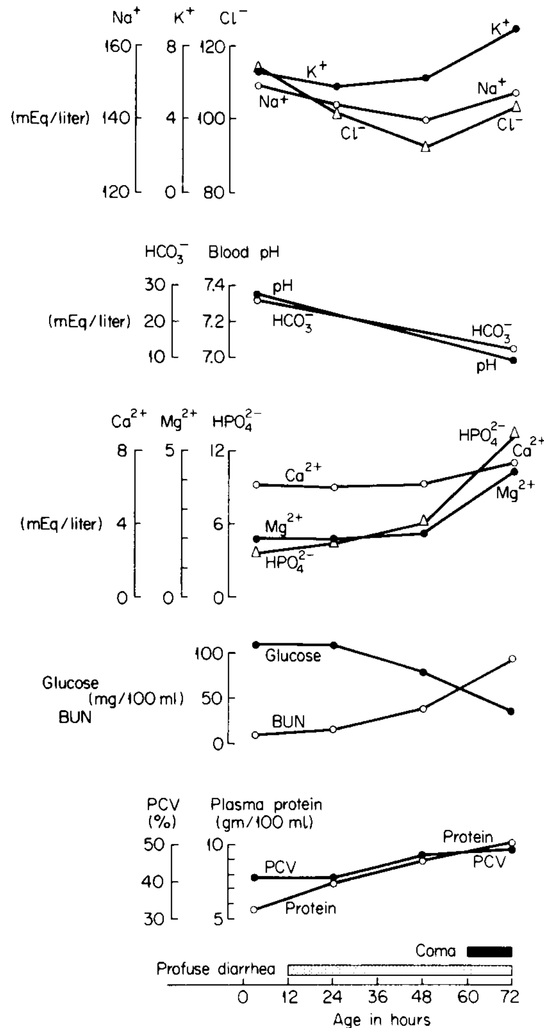


Fig. 12. Metabolic alterations during the course of fatal enteric infection in a neonatal calf (From Tennant *et al.*, 1972.)

or the malabsorption syndrome is observed in several types of intestinal disease, including chronic intestinal granulomatous diseases such as Johne's disease, intestinal parasitic infections, and lymphoma of the intestine. Primary clinical signs include persistent or recurrent diarrhea, nutrient loss in the feces (e.g., steatorrhea), and weight loss. Mucosal cell-enzymatic defects may be accompanied by chronic inflammation, villous atrophy, or cellular infiltrations of the lamina propria of the intestine.

Early reports of primary or idiopathic intestinal malabsorption in dogs (Miller, 1960; Vernon, 1962; Kaneko *et al.*, 1965) were compared to nontropical sprue (adult celiac disease, gluten induced enteropathy) of man, but no convincing association to with gluten sensitivity was demonstrated. Subsequent reports of malabsorption syndromes in the dog have described a variety of causes (Van Kruiningen, 1968; Ewing, 1971; Van Kruiningen

and Hayden, 1972; Hill, 1972; Hill and Kelly, 1974; Schall, 1974; Anderson, 1975, 1977; Burrows *et al.*, 1979), which must be distinguished from the maldigestion caused by pancreatic insufficiency (Anderson and Low, 1965a,b) (juvenile pancreatic atrophy, chronic pancreatitis) and from certain forms of hepatic or gastric disease. Intestinal malabsorption can occur with protozoal enteritis (giardiasis, coccidiosis), lactase deficiency, eosinophilic gastroenteritis, lymphangiectasis, villus atrophy, lymphocytic–plasmacytic enteritis, histoplasmosis, chronic ''bacterial'' enteritis, malignant lymphoma, and intestinal amyloidosis of the bowel. Some authors (Anderson, 1977; Hayden and Van Kruiningen, 1973; Arrick and Kleine, 1978) described malabsorption and pseudoobstruction secondary to hypoplasia of the tunica muscularis of the jejunum in a dog.

Intestinal malabsorption is reported less frequently in the cat than in the dog (Theran and Carpenter, 1968; Wilkinson, 1969).Malabsorption syndromes similar to those recognized in dogs are being recognized with increased frequency in farm animals (Blood *et al.*, 1979). Meuten *et al.* (1978), Cimprich (1974), and Merritt *et al.*, (1976) 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 Berret, 1969).

1. Malabsorption of Fat

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 3–5 gm 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 48 gm/day (Heersma and Annegers, 1948). 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. Small dogs (i.e., less than 10–15 kg body weight) with intestinal malabsorption had fecal fat outputs lower than or equal to published normal values. Fecal fat excretion for normal dogs was 0.24 ± 0.01 gm/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 suggests a lack of pancreatic lipase activity, i.e., exocrine pancreatic insufficiency.

For *free fatty acids,* 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 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, resem-

bling 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 48–72 hours. 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 hours. Merritt *et al.* (1979) have suggested that dogs be fed 50 gm fat per kilogram per day for two to three days prior to 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.

2. *Malabsorption of Other Nutrients*

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 which is a cardinal feature of the disease. There may be malabsorption of vitamin D and/or calcium, resulting in osteomalacia. The anemia sometimes observed may be the result of malabsorption of iron or of the B vitamins, which are required for normal erythropoiesis. Malabsorption of vitamin K can result in hypoprothrombinemia. Glucose malabsorption has been clearly documented by Kaneko *et al.* (1965), and it is likely that amino acids, which are absorbed at a similar level of the small intestine, are also malabsorbed. Carbohydrate and fat malabsorption unquestionably contributes to the calorie deficit which results in weight loss. Amino acid malabsorption may contribute to the development of hypoproteinemia, although this is thought to be due primarily to increased intestinal loss of plasma protein (see Section IV,C).

3. *Differential Diagnostic Considerations*

The diagnosis of idiopathic canine malabsorption can be made only after appropriate diagnostic procedures have ruled out the presence of (1) other primary inflammatory, neoplastic, or parasitic diseases of the intestine and (2) the diseases of the pancreas, liver, or stomach which result in defective intraluminal digestion. The presence of parasitic infection is determined by examining the feces for parasite 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 primary 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, the result of such diseases as chronic pancreatitis or juvenile atrophy. In these diseases, degradation of the major dietary constituents is reduced because of a primary 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. Under certain experimental conditions, diversion of bile flow in the dog actually has a quantitatively small effect on fat absorption (Wells *et al.*, has a quantitatively small effect on fat absorption (Wells *et al.*, 1955; Hill and Kidder, 1972a).

The problems of pancreatic exocrine deficiency are discussed in detail elsewhere in this text (Chapter 7). The most simple and perhaps most widely used test to differentiate intestinal malabsorption from pancreatic exocrine insufficiency is that described by Jasper (1954). The test is employed to detect reduction in trypsin-like activity in the feces of dogs with decreased pancreatic exocrine secretion (Grossman, 1962). There is wide variation in normal activity, making interpretation of the test difficult (Frankland, 1969; Hill and Kidder, 1970; Burrows *et al.*, 1979). The test reveals only the presence or absence of hydrolysis of gelatin and does not differentiate between gelatinase activity produced by intestinal bacteria from that secreted by the pancreas. There is evidence in some species that trypsin is almost completely destroyed by bacteria during its passage through the intestine and that the proteinase activity of the feces is primarily of bacterial origin (Borgström *et al.*, 1959). Despite these theoretical objections, the test has been of clinical diagnostic value in our hands. Fecal gelatinase activity has been detected consistently in cases of intestinal malabsorption and is almost always absent when severe pancreatic exocrine insufficiency is present. Burrows *et al.* (1979) reported that the mean 24-hour trypsin output in dogs with pancreatic insufficiency was significantly lower, and in dogs with malabsorption significantly higher, than clinically normal dogs.

An indirect method to test chymotrypsin activity has been described (Strombeck, 1978). A synthetic peptide, *n*-benzoyltyrosine/*p*-aminobenzoic acid, is administered to test dogs orally. If chymotrypsin is present in the duodenum, hydrolysis of this peptide occurs and *p*-aminobenzoic acid (PABA) is released in a free form, which is absorbed and subsequently excreted in the urine within 6 hours. The urine is analyzed for PABA. Less than 43% PABA excretion identifies dogs with suspected pancreatic exocrine insufficiency.

4. *Tests of Intestinal Absorption*

a. 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 (Turner, 1958; Michaelson *et al.*, 1960), 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 can calculating the percentage of the dose absorbed based on plasma volume.

It is possible to use the results of these two tests, when performed in sequence, to differentiate between steatorrhea caused by a deficiency of pancreatic enzymes and 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 (Fig. 13a,b). The results of this test also may vary depending on the rate of intestinal motility (Tennant *et al.*, 1969b).

b. Vitamin A Absorption. The vitamin A absorption test measures intestinal lipid absorption (Hayden and Van Kruiningen, 1976). Vitamin A absorption requires normal secretion of bile and pancreatic enzymes. Following oral administration of 200,000 units

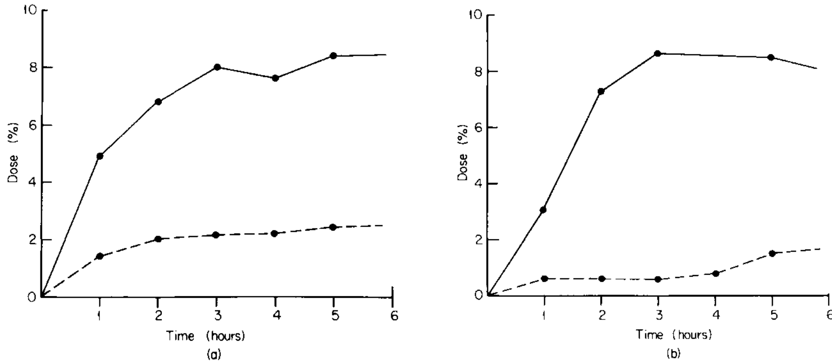


Fig. 13. (a) Absorption of ^{131}I-Labeled oleic acid in normal dogs (3) and dogs with intestinal malabsorption (2). Absorption is expressed as percentage of the dose present in the plasma at intervals following administration. (———) normal; (- - -) intestinal malabsorption. (From Kaneko *et al.*, 1965.) (b) Absorption of ^{131}I-labeled in normal dogs (3) and in dogs with intestinal malabsorption (2). Absorption is expressed as percentage of the dose present in the plasma at intervals following administration. (———) normal; (- - -) intestinal malabsorption. (From Kaneko *et al.*, 1965.)

of vitamin A, mean serum vitamin A concentrations peak at 6–8 hours, with values ranging between three and five times fasting serum levels in normal dogs. Breed differences and delayed gastric emptying will alter results.

c. Glucose Absorption. The absorption of glucose can be measured by means of an oral glucose tolerance test in which a test dose of glucose is given by mouth and the blood glucose level measured at intervals for 3–4 hours following administration. The test has been used in canine malabsorption in which the normal rise in blood glucose level is reduced (Kaneko *et al.*, 1965). The test also has been reported for use in the horse (Roberts and Hill, 1973). Dogs with pancreatic exocrine deficiency may, however, have "diabetic" tolerance curves (Hill and Kidder, 1972b). The major disadvantage of relying on this test alone is that it does not differentiate between decreased intestinal absorption and increased tissue uptake following absorption. This problem can be minimized by comparing results of the oral glucose tolerance test with those obtained with the intravenous tolerance test. The results of this test, however, must be interpreted carefully and in relation to other clinical and laboratory findings. Hill and Kidder (1972) reported that dogs on low-carbohydrate diets can have "diabetic" tolerance curves; test dogs should be on a high-carbohydrate diet 3–5 days before testing.

d. D-Xylose Absorption. The absorption of D-xylose also can be used to evaluate intestinal function. D-Xylose is not metabolized by the body to any significant degree, and the problems of evaluating tissue utilization which occur with glucose are eliminated. Because of the large amounts of D-xylose used in the test, absorption is independent of active transport processes, and the rate of absorption is proportional to luminal concentration.

A D-xylose absorption test for dogs has been described by Van Kruiningen (1968). In this procedure, a standard 25-gm dose of D-xylose is administered by stomach tube. During the 5-hour period following administration, the dog is confined in a metabolism

cage, and urine is collected quantitatively. At the end of the 5-hour test period, the urine remaining in the bladder is removed by catheter, and the total quantity excreted in 5 hours is determined. Normal dogs excreted an average of 12.2 gm during the test period, with a range of 9.1–16.5 gm. The results obtained by this method are dependent not only on the rate of intestinal absorption, but also on the rate of renal excretion, and it is necessary, therefore, to know that kidney function is normal.

The oral xylose tolerance test has received most clinical use (Hill *et al.*, 1970; Hayden and Van Kruiningen, 1973). Dogs are fasted overnight, a blood sample is obtained, and D-xylose is administered by stomach tube at the rate of 0.5 gm/kg. A control test is performed on a normal dog simultaneously with each dog with signs of intestinal malabsorption. The first blood sample is obtained one-half hour after administration. The second sample is obtained 1 hour following administration, and additional samples are taken at hourly intervals for 5 hours. The xylose concentration in the blood is determined by the method of Roe and Rice (1948). Maximal blood levels almost always are reached at 1 hour after administration of the test dose; Hill expects a xylose level of at least 45 mg/dl within 60–90 minutes in a normal dog. In preliminary studies of four dogs with the malabsorption syndrome, maximal blood xylose levels averaged 58% of corresponding control values. In dogs with pancreatic exocrine insufficiency with normal intestinal mucosa, there should be a normal xylose response test. The D-xylose absorption test also has been described for use in differential diagnosis of equine diarrheal diseases (Roberts, 1974). Bolton *et al.* (1976) reported that a dosage of 0.5 gm xylose per kilogram body weight was useful in detecting horses that absorbed the pentose abnormally. Gastrointestinal lesions associated with abnormal results were classified as (1) villous atrophy, (2) edema of the lamina propria, or (3) necrosis of the lamina propria. At this dosage in normal horses, the mean peak plasma concentration is less than one-third that seen in normal dogs given xylose (normal dogs: 60–70 mg % at 60 minutes).

D. Protein-Losing Enteropathy

Albumin, γ-globulin, and other plasma proteins are present 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 that as much as 50% or more of the normal catabolism of albumin (Glenert *et al.*, 1961, 1962; Campbell *et al.*, 1961; Wetterfors, 1964, 1965; Wetterfors *et al.*, 1965) and γ-globulin (Andersen *et al.*, 1963) occurs in the gastrointestinal tract. Others believed that the physiological role of the intestine in plasma protein catabolism is far less significant, accounting for only about 10% of the total catabolism (Waldmann *et al.*, 1967, 1969; Katz *et al.*, 1960; Franks *et al.*, 1963a,b).

Regardless of the questions concerning the relative importance of the gastrointestinal tract in plasma catabolism, it is well established that normal intestinal losses are increased significantly in a variety of gastrointestinal diseases, which are referred to collectively as *protein-losing enteropathies*. The increased loss causes hypoproteinemia (especially hypoalbuminemia), which may be observed in various types of chronic enteric diseases. The excessive losses are produced by ulcerations or other mucosal changes which alter

permeability or by obstruction of 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 now been demonstrated 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 (Nielsen and Nansen, 1967; Halliday *et al.*, 1968; Murray, 1969), and in Johne's disease (Patterson *et al.*, 1967; Nielsen and Andersen, 1967; Patterson and Berret, 1969). In addition to the classic mucosal and submucosal lesions of Johne's disease, Nielsen and Andersen (1967) demonstrated the presence of secondary intestinal lymphangiectasia. Meuten *et al.*, (1978) described protein-losing granulomatous enteritis in two horses and discussed a comparative overview of diseases causing malabsorption in the horse, cow, dog, pig, and man.

Protein-losing enteropathy has been seen with some frequency in the dog (Campbell *et al.*, 1968; Farrow and Penny, 1969; Hill, 1972; Finco *et al.*, 1973; Hayden and Van Kruiningen, 1973; Mattheeus, *et al.;* Hill and Kelly, 1974; Milstein and Sanford, 1977; Barton *et al.*, 1978; Olson and Zimmer, 1978). Intestinal lymphangiectasia was commonly reported. The dog described by Milstein and Sanford (1977) was not hypoproteinemic because the rate of albumin synthesis by the liver was greater than protein loss into the intestine. Protein loss has also been documented in dogs with chronic hypertrophic gastritis (Section IV,A).

Increased intestinal protein loss is the most likely explanation for the hypoalbuminemia associated with certain other enteric diseases, including intestinal malabsorption and lymphoma of the intestine. Munro (1974) demonstrated that protein loss in dogs with experimentally induced protein-losing gastropathy occurs by an intercellular route. Isotope-labeled polyvinylpyrolidine (^{131}I-PVP), ^{67}Cr-labeled ceruloplasmin, and ^{51}Cr-labeled albumin have been used to evaluate enteric protein loss in the dog (Finco *et al.*, 1973; Barton *et al.*, 1978; Hill and Kelly, 1974; van der Gagg *et al.*, 1976; Olson and Zimmer, 1978).

E. Canine Ulcerative Colitis

Canine ulcerative colitis was described originally in the report of Cello (1964). Since that time, ulcerative colitis and its variant form, granulomatous colitis of Boxer dogs, has been reported by several investigators (Van Kruiningen *et al.*, 1965; Kennedy and Cello, 1966; Koch and Skelley, 1967; Sander and Langham, 1968; Ewing and Gomez, 1973; Gomez *et al.*, 1977; Russell *et al.*, 1971). The etiology is generally unknown. Ewing and Aldrete (1973) reported a case of canine giardiasis presenting as chronic ulcerative colitis and cases of ulcerative colitis in dogs have been attributed to trichuriasis, balantidiasis, protothecosis, histoplasmosis, eosinophilic ulcerative colitis, or neoplasia (Lorenz, 1975). Rarely, severe ulcerative colitis is seen in the cat. In some of these cases, feline leukemia virus is demonstrated. Shindel *et al.* (1978) described colonic lesions in cats caused by feline panleukopenia.

Histopathologically, periodic acid–Schiff-positive macrophages are pathognomonic for the granulomatous colitis of boxer dogs. The disease causes chronic, intractable diarrhea, which is often hemorrhagic. In addition, afflicted dogs may vomit and are often emaciated. Fever is usually not present.

Biochemical manifestations of ulcerative colitis depend upon duration and severity of

TABLE VII

Serum Proteins of Dogs with Ulcerative Colitis[a]

Protein	Mean[b] (gm/dl)	Range[b] (gm/dl)	Normal values (gm/dl)
Total serum protein	5.98	4.40–9.10	5.90–6.70
Albumin	2.44	1.14–4.83	3.00–3.70
α-Globulin	0.92	0.39–1.50	0.86
β-Globulin	1.08	0.60–1.86	1.30
γ-Globulin	1.53	0.46–4.60	0.60–0.90
Albumin/globulin ratio	1.00	0.17–2.45	1.00

[a] Ewing and Gomez (1973).
[b] Thirty-six observations on 29 affected dogs.

illness, degree of colorectal involvement, and the presence of systemic complications. In severe cases of long duration with extensive colorectal involvement, hypoalbuminemia and hypergammaglobulinemia (Table VII) are sometimes observed. The pathogenesis of hypoalbuminemia probably involves increased loss of plasma through the denuded and inflammed colorectal mucosa. Hypergammaglobulinemia is probably an associated response to chronic inflammation.

F. Disturbances of Rumen Function

The digestive process of ruminants differs from that of other animals because of microbial digestion and metabolism in the rumen which occurs prior to other normal digestive processes. The short-chain fatty acids (acetic, propionic, and butyric acids) are the primary end products of rumen fermentation and represent the chief dietary source of energy for ruminants (Hungate *et al.*, 1961). The polysaccharide cellulose, which undergoes only very limited digestion in most simple-stomached animals, is readily utilized by ruminants because of the activity of cellulytic bacteria. Significant quantities of nonprotein nitrogen also can be utilized by ruminal bacteria for protein synthesis, and this bacterial protein subsequently can be utilized to meet the protein requirements of the animal.

Bacterial production of vitamins may also meet essentially all the requirements of ruminants. Maintenance of bacterial fermentation within the rumen also presents certain unusual hazards to ruminant animals. When rapid changes in dietary intake occur, the products of fermentation can be released more rapidly than they can be removed. Acute rumen tympany, acute indigestion of D-lacticacidosis, and urea poisoning are diseases which result from such abrupt changes in diet (Hungate, 1966, 1968).

1. Acute Rumen Engorgement (Rumen Overload, Lacticacidosis, Acid Indigestion)

Acute rumen indigestion occurs in sheep or cattle on a high-roughage diet when they inadvertently are allowed access to large amounts of readily fermentable carbohydrate, e.g. grain and 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,b).

As lactic acid accumulates more rapidly than absorption, the rumen pH falls and rumen

atony results. Rumen bacteria produce a racemic mixture of lactic acid. Some L-lactate may be metabolized by the liver and other tissues, but D-lactate cannot be and contributes significantly to the acid load of the body. The excessive lactic acid production results in metabolic acidosis, which is characterized by reduced blood pH and bicarbonate concentration and by a fall in urine pH from a normal value of 8.01 -8.0 to as low as 5.0. Fluid accumulates in the rumen because of increased osmolarity of its contents, causing hemoconcentration, which may lead to hypovolemic shock and death (Hyldgaard-Jensen and Simesen, 1966). If affected animals survive the initial period of explosive fermentation, a chemical rumenitis, caused by lactic acid, may develop. Secondary mycotic rumenitis may also occur and be fatal. Hepatic abscesses also may result from severe rumenitis.

2. *Acute Rumen Tympany (Bloat)*

The rumen of mature cattle can produce 1.2–2.0 liters gas per minute (Hungate *et al.*, 1965). The gas is composed primarily of carbon dioxide and methane, which are products of rumen fermentation. Carbon dioxide is also released when salivary bicarbonate is acted upon by organic acids within the rumen. Under normal conditions, these large amounts of gas are continually removed by eructation.

Any factor which 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 types of bloat is a change in the ruminal contents to a foamy or frothy character. Because of altered surface tension, gas is trapped in small bubbles with the rumen and cannot be eliminated by eructation (Clarke and Reid, 1974).

The chemical changes which cause foam to form within the rumen are not completely clear. Some reports (Nichols, 1966; Nichols and Deese, 1966) suggest that plant pectin and pectin methyl esterase, an enzyme system also from plants, 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. Slime-producing bacteria also have been incriminated in the pathogenesis of frothy bloat. These microorganisms produce an extracellular polysaccharide, which 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 which 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, preventing foam formation by eliminating the products of this enzyme reaction (Nichols, 1963). Much effort is now being directed toward genetic selection of cattle which are less susceptible to rumen tympany and to varieties of legumes which are less likely to produce bloat (Howarth, 1975).

3. *Urea Poisoning*

Unlike monogastric species, ruminants can effectively use nonprotein nitrogen to meet dietary protein requirements. Urea, biuret (Oltjen *et al.*, 1969) and ammonium salts

(Webb *et al.,* 1972) all can serve as dietary supplements. Urea, which is the most frequently used, is hydrolyzed by bacterial urease within the rumen and the free ammonia formed is incorporated into amino acids by microorganisms within the rumen. The bacterial protein so produced is digested and absorbed in the small intestine along with protein from the diet.

Signs of urea poisoning typically develop within minutes after consumption of food containing toxic amounts of urea. Clinical manifestations are the result of excessive ammonia production (Word *et al.,* 1969; Elmer and Barclay, 1971) and are due to the encephalotoxic effects of free ammonia absorbed from the rumen. Tolerance to urea may be significantly increased by gradually elevating the amounts of urea in the diet or by adding readily fermentable carbohydrate. It has actually been possible for ruminants to adapt and thrive on a diet in which urea was the sole source of dietary nitrogen. However, if urea is fed at a level of more than 3% in the diet of unadapted animals, toxic effects are likely. Poisoning may occur when, by accident, animals obtain access to large amounts of urea-containing dietary supplement or in animals receiving bulk feed when there has been an error in formulation 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 ammonia from the rumen. Acetic acid also has been used clinically for the treatment of urea poisoning but under experimental conditions it has more value prophylactically than in animals with frank signs of poisoning (Word *et al.,* 1969).

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