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3.09 Pathophysiological Mechanisms of Gastrointestinal Toxicity☆

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3.09.1 Introduction

The alimentary system is an open-ended tubular organ that courses from the oral cavity to the anus. Being open-ended, it is sometimes conceptualized as continuous with the external environment at its proximal and distal termini. The gastrointestinal (GI) system/tract is attended by an interactive complex of glands, tissues, and other organs such as the liver and pancreas. Functions of the gastrointestinal tract include ingestion, propulsion, digestion, absorption, secretion, storage, and elimination of excreta. Proper digestion and assimilation of nutrients is critically important for health and requires carefully coordinated muscular, secretory, absorptive, neurologic, and endocrinologic events. Importantly, a healthy alimentary system requires maintenance of barrier function. These functions are choreographed by one or multiple structural/functional elements acting in concert. Damage to one or more of the components of the alimentary system or its regulatory chemicals may result in pathophysiologic effects.

Alimentary tract lumens are potential spaces lined by polarized epithelium that accommodates ingested substances. This mucus membrane is smooth and shiny (with the exception of the rumen) when intact and healthy. The gastrointestinal system and its ancillary glands are the first line of defense against ingested foreign substances and pathogens of all types. The anatomic, biochemical, physical, secretory, and endocrinologic properties of the alimentary epithelium, resident and blood-borne effector cells, microbiota, genetic polymorphisms, and gut-associated lymphoid tissue (GALT) (comprising one-quarter of the body's total) must be physically or functionally altered for dysfunction such as ptyalism (hypersalivation), regurgitation, emesis (vomiting), abdominal pain, gas production, and/or diarrhea to occur. In most cases, homeostasis is quickly restored without medical intervention. As a result, our understanding of this complex system has been neglected compared to other less well, self-regulated systems. The average human ingests 700 t of antigens in their lifetime. That enteropathy does not occur more often than it does its testimony to the efficacy of gastrointestinal protective systems.

Humans and other animals swallow a great variety and often large amounts of chemicals as nutrients, incidental food additives and contaminants, drugs, and microbes of all sorts exposing the alimentary system to many potentially toxic substances (**Table 1**). It is estimated that there are $>10{,}000$ food additives and residues totaling 1.5 kg year^{-1} capita^{-1} in the typical human diet (Gad, 2007). Concern about these chemicals has led to a surge of the natural/organic/unprocessed food industry.

Table 1 Pathophysiological mechanisms of gastrointestinal toxicity

Mechanisms	*Examples*
Direct effects on cell membrane	Plant lectins, alcohol, NSAIDs, bile acids, sodium chloride
Stimulation of mucosal proliferation	Dioxins, aromatic hydrocarbons, pancreatic enzyme preparations, alcohol
Inhibition of mucosal proliferation	Anticancer drugs
Nerve damage	Surfactants, capsaicin
Reduced blood flow	NSAIDs, alcohol
Activation of emetic pathways	Anticancer agents, 5-HT, dopamine
Disruption of intracellular signal transduction	Cholera toxin
Release of regulatory substances	Antigens, endotoxins, inflammation. NSAIDs
Generation of oxygen free radicals	NSAIDs, laxatives, lipid hydroperoxides, inflammatory responses
Chemotaxis and activation of granulocytes	Endotoxins, antigens
Release of enzymes	Endotoxins, antigens, cytokines
Activation of enzymes	Cholera toxin, nitric oxide
Inhibition of enzymes	NSAIDs, AChEI pesticides
Increase susceptibility to H^+	NSAIDs
Intracellular toxicity	Heavy metals

5-HT, 5-hydroxytryptamine; *AChEI*, acetylcholinesterase inhibitors; *NSAIDs*, nonsteroidal antiinflammatory drugs.

The alimentary mucosa and its ancillary tissues and glands compose a complex and effective barrier to potential damage from xenobiotics. The remarkable integrity of this barrier function enables it to withstand daily assaults by the chemicals to which it is exposed. Because of the intrinsic ability of the gastrointestinal tract to resist toxic chemicals, there is a relative paucity of data regarding gastrointestinal toxicology. It is therefore useful in many cases to extrapolate toxic mechanisms from infectious processes, many of which result in microbial toxin production, inflammation, and ischemia.

Toxic clinical effects in the alimentary system include nausea, emesis, diarrhea, and/or pain. Enzyme insufficiency (lactase, lipase), inflammation, polyps, neoplasms, functional disturbances such as excess mucus production, delayed gastric emptying, or structural damage such as ulcers are more subtle. Therefore, it is critical to understand the anatomy, physiology, and pathophysiology of the gastrointestinal tract to understand direct and indirect toxic injury.

Over three-fourths of documented poisoning cases result from ingestion of xenobiotics (Bronstein et al., 2008). Gastrointestinal disturbances are second only to the neurologic effects of toxins (Olson et al., 2000). The use of animal models in preclinical studies is effective in predicting gastrointestinal system damage over 80% of the time (Betton, 2013).

3.09.1.1 Anatomy

The esophagus, stomach(s), small intestine, cecum, large intestine, colon, and rectum, the tubular portions of the gastrointestinal system, are potential spaces that expand to accommodate ingesta/digesta/excreta. All portions of the digestive system contain mural smooth and/or striated muscle that propel luminal contents. There are three principal motility patterns of the gastrointestinal tract: storage, mixing, and propulsion. Each of these activities is associated with precise changes in muscle contractions.

The length of the system varies among species, shorter tracts in carnivores, and longer more complicated tracts in herbivores. In addition, herbivores require a fermentation chamber to digest cellulose, the forestomachs in ruminants and camelids or an enlarged cecum in equids, rodents, and lagomorphs. A rendering of general gastrointestinal morphology is presented in **Fig. 1**.

3.09.1.2 Oral Cavity

Via "mouth feel" and taste, the oral cavity prevents many harmful substances from entering the body. Caustic substances, some pathogens, heat and electricity may result in thermal and chemical erosions or ulcerations of the oral mucosa, but mucus membranes heal rapidly. At various locations throughout the tract, extraintestinal substances are added to the ingesta. These include saliva that contains electrolytes such as sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate, and iodine, mucus, which serves as a lubricant, antibacterial compounds such as thiocyanate and hydrogen peroxide, secretory immunoglobulin A, epidermal growth factor (EGF) and the digestive enzymes α-amylase, lipase, and kallikrein. Antimicrobial enzymes secreted include lysozyme, lactoperoxidase, proline-rich proteins, acid phosphatases A+B, *N*-acetylmuramoyl-L-alanine amidase, NAD(P)H dehydrogenase (quinone), superoxide dismutase (SOD), glutathione transferase, class 3 aldehyde dehydrogenase, and glucose-6-phosphate isomerase. Saliva also contains a bacteria-rich flora and at least in humans, opiorphin, an analgesic. A partial list of gastrointestinal protective mechanisms is in **Table 2**.

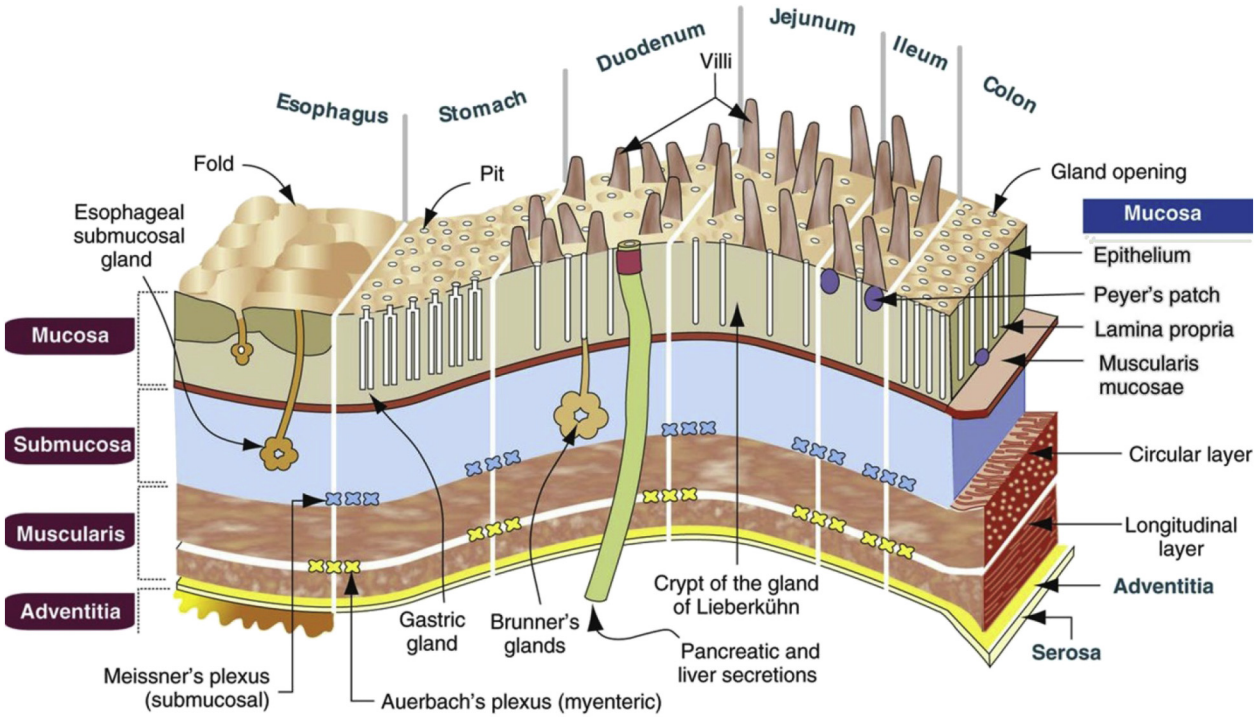

Fig. 1 Schematic diagram of the anatomic and histologic organization of the digestive tube. From Kierzenbaum, A. L. (2002). Histology and cell biology: an introduction to pathology. St. Louis: Mosby. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

Table 2 Defense mechanisms of the gastrointestinal system

Taste buds
Vomiting
Saliva
Flushing action, so potential pathogens are cleared from the oropharynx
Protective coating of the mucosa
Contains antimicrobial lysozyme, lactoferrin, lactoperoxidase, and immunoglobulins
Gastric pH
Microbiota/microbiome—lower GI (damaged by toxicants; carcinogen activation)
100 trillion (anaerobic) bacteria ($10 \times$ host); 3.3 million genes ($150 \times$ host)
Bacteriocins
Compete for nutrients
Compete for attachment sites
Promote immune system maturation
Biotransformation
Enterotype
Secreted immunoglobulins
Extraintestinal secretions from the liver and pancreas
Lactoferrins
Peroxidase
Intestinal proteolytic enzymes
Intestinal biotransforming and metabolic enzymes
Phagocytes and other effector cells within the submucosa
High rate of epithelial turnover
Shedding of receptor laden ALP and catalase containing vesicles from microvilli
Large surface area
Dilution with ingesta
Increased peristalsis resulting in diarrhea
Mucus—contains phages that destroy bacteria $> 1 \times 10^4$
Paneth cells (antimicrobial peptides, lysozymes, phospholipase A2, defensins–cryptdins)
Innate lymphoid cells
Adaptive immune system
Kupffer cells (liver)
Genetic polymorphisms (HLA) and host gene expression

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3.09.1.3 Esophagus

The esophagus is a muscular tube that passes through the neck and thorax and connects the oral cavity with the stomach. It secretes a lubricating mucus to aid in the passage of ingesta. The lining epithelium is keratinized in swine, equids, ruminants, rats, and mice and nonkeratinized in carnivores and humans. The epithelial cell turnover rate is 5–8 days. The muscularis is striated in ruminants and dogs, smooth in equids (distal third) that are unable to vomit, and variably mixed in other species. Mice and rats, that have primarily striated muscle in their esophagus, are also unable to vomit (Treuting et al., 2011). This is principally due to a limiting fold separating the nonglandular from the glandular stomach and diaphragmatic crura that cannot be contracted independently of each other to allow propulsion of contents. Submucosal mucus glands are present throughout the esophagus in swine, dogs, and humans and at the pharyngeal junction in cats, equids, and ruminants. A serosa is absent in all but the abdominal portion of the esophagus. Because the serosa is composed of collagen, it is crucial in holding sutures. Therefore, esophageal resection is seldom attempted and rarely successful. The strong muscular contractions of the esophagus, together with its poor blood supply and lack of serosa, mean that healing from caustic or penetrating wounds renders a poor prognosis for a functional return to normalcy. Insofar as therapy for caustic toxin ingestion what burns going down will also burn coming back up, so generally speaking induction of vomition is contraindicated (Gelberg, 2013).

The contracted musculature of the upper esophageal sphincter relaxes to permit movement of the swallowed bolus into the proximal portion of the esophagus. After the bolus passes, the sphincter regains its previous tone. Relaxation of the sphincter and subsequent restoration of tone require precise control by regulatory systems that may be disrupted by toxins. When the bolus has entered the upper esophagus, an aborally progressing front of contraction of the circular muscle layer begins at the top of the esophageal body, seemingly as a continuation or extension of the forceful contraction of the upper esophageal sphincter that follows its relaxation (Conklin and Christensen, 1994). A ring of contraction above the swallowed bolus moves down the esophagus, propelling the bolus toward the stomach. While the bolus is still progressing through the mid-portion of the esophagus, the contracted ring of circular muscle that forms the lower esophageal sphincter relaxes and is completely relaxed by the time the bolus reaches the gastroesophageal junction. The bolus traverses the lower esophageal sphincter and drops into the stomach. The proximal stomach (fundus and upper corpus) often relaxes, while the swallowed bolus is still in the esophageal body and has not yet passed the lower esophageal sphincter (receptive relaxation). After the bolus has entered the stomach, the fundus and corpus muscles relax so that a large volume can be stored in the upper stomach without significantly increasing intragastric pressure (accommodation). As the volume in the stomach increases, a subjective sensation of satiation develops (Burks and Villar, 1980). In view of the importance of loss of body weight as a sign of chemical toxicity, it is surprising that so little attention has been devoted to disordered sensory perception of gastric fullness as a mechanism of toxicity.

3.09.1.4 Stomach(s)

The forestomachs in ruminants and camelids are esophageal dilations and modifications. They house a digestive flora that produces short chain fatty acids from forage. These fatty acids are directly absorbed into the bloodstream along with sodium and chloride. Most clinical disease of the forestomachs relates to disruptions in coordinated motility and changes in pH. Camelid forestomachs have glandular sacculations. Equids and some rodents have stomachs that are divided into proximal stratified and aboral glandular portions. Swine have only a small stratified portion that directly surrounds the esophageal os. The abomasum/C3 functions similar to the stomachs of monogastric mammals (Gelberg, 2016).

The secretory gastric, small intestinal, and colonic mucosae are lined by a single layer of columnar epithelial cells. The mucosal surface of the stomach is covered by mucus-secreting cells and, in the fundus and corpus, gastric crypts. At the base of the crypts are branching gastric glands (Lloyd and Debas, 1994).

The glandular stomach (abomasum, C3) is responsible for enzymatic and hydrolytic digestion of ingesta. The glandular stomach is composed of a variety of cell types with bidirectional proliferation of cells from the neck of the gastric glands (**Fig. 2**). The epithelial layer is one cell thick, and the turnover rate is 2–4 days. There are three types of exocrine cells in the stomach: parietal, chief, and mucus. The parietal cells produce rennin that coagulates milk protein, intrinsic factor for vitamin B_{12} absorption, and HCL. Chief cells produce zymogen, pepsinogen, and gastric lipase. Mucus cells of the surface and gastric pits produce bicarbonate and an unstirred protective layer on the cell surface. Gastric gland output in humans is 2–3 L day^{-1}. The low luminal pH destroys many ingested pathogens, but there is a resident bacterial flora that cannot be cultured by conventional methods. Enteroendocrine cells (G cells) of the gastric pits produce serotonin, gastrin, ghrelin, somatostatin, endothelin, histamine, enteroglucagon, and other hormones.

Gastric ulcers occur in all species. Although the cause of ulcers, other than caustic agents and those caused by bacteria that can survive the extremely low pH of the stomach (*Helicobacter* spp.), is imprecisely understood, conditions necessary for ulcer development include local disturbances or trauma to the mucosal epithelial barrier, normal or high gastric acidity, and local disturbances to blood flow, including stress-induced and sympathetic-nervous-system-mediated arteriovenous shunts leading to ischemia. These physiologic changes allow pepsin and HCl into the submucosa where they cause chemical erosion of the protective epithelium. In addition, exogenous or endogenous steroids and nonsteroidal antiinflammatory drugs (NSAIDs) depress prostaglandin (PG) E_1 and E_2 decreasing phospholipid secretions that are gastroprotective. Gastric ulcers induced by alcohol consumption are dose related with effects ranging from a decrease in mucus production and bicarbonate secretion to leakage of bicarbonate and electrolytes to vascular damage (Rousseaux and Haschek, 2010). Anecdotal evidence suggests that a propensity towards ulcer formation may be hereditable (Gelberg, 2016). A partial listing of ulcerogenic chemicals is in **Table 3**.

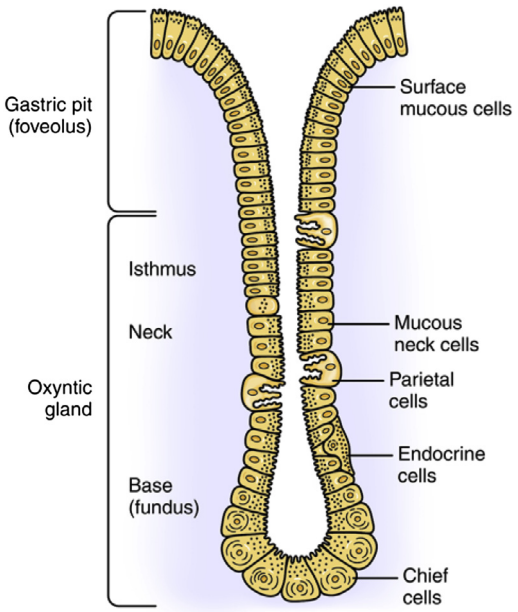

Fig. 2 Schematic illustration, microanatomy of the stomach. Adapted from Ito, S. and Winchester, R. J. (1963). Journal of Cell Biology 16, 541. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

Table 3 Ulcerogenic chemicals

Affecting all parts of the gastrointestinal (G1) tract	*Stomach/duodenum*	*Jejunum/ileum*
Anthracyclines	Acetic acid	Cadmium
β-Cytosine arabinoside	Acetanilide	Clofazimine
Clindamycin	Acrylonitrile	Digoxin
Colchicine	Bisphosphonates	Mercury
Corticosteroids	Bromocriptine	NSAIDs
Cyclophosphamide	Clinitest	Potassium salts
Ferrous salts	Cysteamine	Zirconium
5-Fluorouracin	Coffee	
Sulfur mustard	Diphosphonate	
	Doxapram	
	Ethacrynic acid	
	Ethanol	
	Erythromycin estolate	
	Roxuridine	
	Haloperidol	
	Histamine H_2 receptor antagonists	
	Nitrites	
	NSAIDs	
	Potassium salts	
	Propionitrile	
	Reserpine	
	Spironolactone	
	Taurocholic acid	
	Tobacco	
	Toluenediamine	
	Toluenedithiol	
	Zinc, zinc sulfate	

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Tobacco products increase gastric reflux, pepsinogen-1, and basal acid output while decreasing mucus production and pancreatic bicarbonate secretion resulting in duodenal ulceration (Hojgaard et al., 1996; Maity et al., 2003). Mucus secretion is reduced due to decreases in total mucus neck cell numbers and neck cell mucus volume. Nicotine elevations in vasopressin, platelet-activating factor (PAF), and endothelin and reductions in EGF, PGs, and ornithine decarboxylase-related repair mechanisms contribute to the severity of the ulcerations along with tobacco nitriles and aniline derivatives (Florin et al., 1980).

Gastric mucosa damaged by exposure to high concentrations of sodium chloride can be repaired over a period of 6 h by migration of epithelial cells from the glands (Svanes et al., 1982). Rapid epithelial restitution of the superficial mucosa is one of the primary defense mechanisms of the stomach, small intestine, and colon (Feil et al., 1989). Preservation of the basement membrane in the area of damage is critical for prevention or repair of lesions. Indirect damage may occur when blood flow is compromised.

The liquid component of a mixed meal usually empties faster than the solid component. Low pH or high osmolarity tends to decrease the rate of gastric emptying (Read, 1994). Effective gastric emptying requires coordinated propulsive contractions in the antrum that progress to the pyloric canal along with properly timed relaxation of the upper duodenum (Lin, 1994). Some drugs and toxins reduce the rate of gastric emptying by producing contractions of the duodenum that abolish the antral–duodenal pressure gradient required for effective emptying (Burks, 1994; Malbert et al., 1994).

Because the alimentary lumen is markedly acidic in the stomach and slightly alkaline in the intestine, the degree of ionization of xenobiotics varies by the pK_a(s) of the molecule. Nonionized forms cross cell membranes more readily and can pass into the mucosa depending on the local pH. The gastric mucosa may therefore be a target for acidic compounds such as NSAIDs (Betton, 2013).

3.09.1.5 Intestine

The small intestine functions in digestion, secretion, and absorption. The small intestinal functional surface area is one cell thick but is markedly increased by the presence of numerous mucosal folds that contain finger-like villi (**Fig. 3**). Each villus consists of 2000–8000 epithelial cells and is surrounded by 6–14 crypts of Lieberkuhn. Each absorptive cell on these villi has a microvillus border that further increases surface area and has a glycocalyx housing the digestive enzymes (**Fig. 3**). Damage to any of these structures may result in dysfunction. In humans, it is estimated that the one cell thick surface area of the alimentary system is 300–400 m^2 (Corruzi, 2010).

The "unstirred water layer" adjacent to the GI lumen is a diffusion barrier. When a xenobiotic crosses this unstirred water layer and the acid mucolayer, it reaches the surface epithelium. The two main pathways for substances to enter the membrane are transcellular (through the cells) and paracellular (between the cells) (Gad, 2007).

Transcellular transport mechanisms may be passive such as in diffusion, filtration, osmosis, or facilitated diffusion. Active transcellular transport may be direct or indirect such as by endocytosis, pinocytosis, phagocytosis, receptor-mediated endocytosis, or exocytosis (Kapp, 2007). Paracellular transport pathways allow the passive movement of substances through intercellular epithelial cell spaces regulated by apical tight junctions. These tight junctions permit ion but not large molecule passage. Passage through the tight junction is dependent on xenobiotic molecular size, net charge, intra- and extracellular calcium ion concentration, osmolarity, protein kinase inhibitors, and junctional complex proteins including claudin, occludin, and the junctional adhesion molecule (JAM) (Kapp, 2007).

The epithelial cells rest on a basement membrane subjacent to which is a mesenchymal lamina propria containing blood vessels and a central, blind-ended lymphatic or lacteal (**Fig. 4**). Villus height and crypt depth decrease aborally through the gut, while the number of goblet (mucus) cells increases. The submucosal duodenal (Brunner's) glands secrete an alkaline mucus that helps neutralize gastric acid.

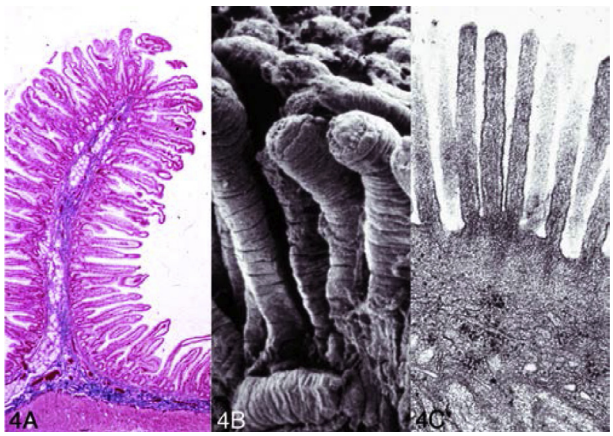

Fig. 3 Organization of the intestine. The digestive and absorptive surfaces of the intestine are markedly increased by the presence of villi and microvilli on the enterocytes. (A) Intestinal villus. Villus epithelial cells are present on basement membranes (not seen) on a core of lamina propria. Hematoxylin and eosin. (B) Small intestine, intestinal villi, scanning electron microscopy. Carbon sputter coat. (C) Enterocyte microvilli. TEM. Uranyl acetate, lead citrate stain. From Damjanov I and Linder J (1996). Anderson's pathology (10 edn.). St. Louis: Mosby. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

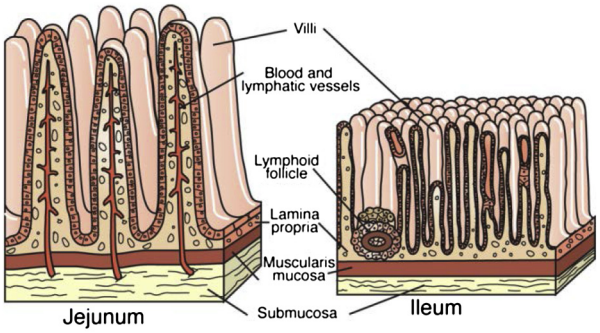

Fig. 4 Schematic diagram of the anatomic and histologic organization of the digestive tube. From Kierzenbaum, A. L. (2002). Histology and cell biology: an introduction to pathology. St. Louis: Mosby. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

Enterocytes are rich in inducible cytochrome P450s (CYPs), microperoxisomes, UDP glucuronosyl transferase, glutathione *S*-transferases (microsomal GSTs), *N*-acetyl transferase, hydrolases of many types, azo-reductases (sulphasalazine is metabolized to 5-aminosalicylic acid in the colon), glucuronidases (metabolizing bile salts, thyroid hormone, drug glucuronides), nitro-reductases, glycosidase (cycasin is metabolized to methylazoxymethanol), and others (Fig. 5). Some of these metabolic activities are present in the gut microflora that can be modified by dietary manipulation (Betton, 2013).

3.09.1.6 Intestinal Blood Flow

The vascular supply to the villi is from the mesenteric arteries. In the lamina propria, the arterioles generate resistance to blood flow, the precapillary sphincters determine perfusion, and molecular exchange occurs across capillaries. Venules are capacitance vessels

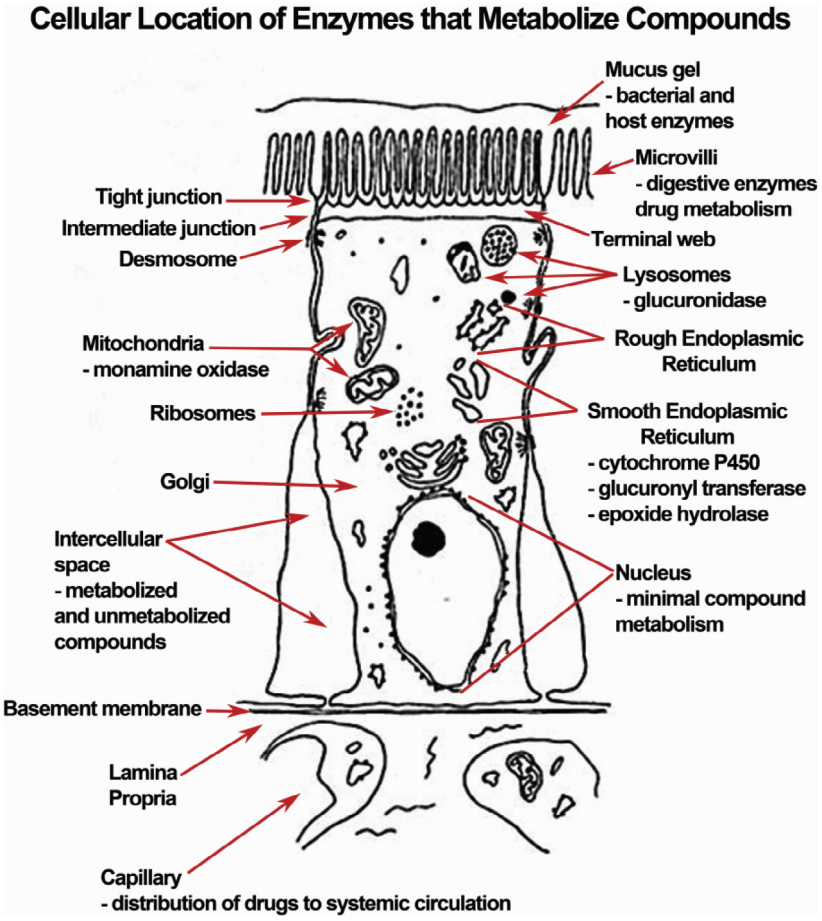

Fig. 5 Cellular location of metabolic enzymes. Haschek, W., Rousseaux, C., and Wallig, M. (2010). Fundamentals of toxicologic pathology (2nd edn.). Copyright © 2010 by Academic Press, an affiliate of Elsevier Inc.

(Tepperman and Jacobson, 1981). Arteriovenous anastomoses are not present; capillary networks cannot be bypassed (Jacobson, 1992). Constant delivery of oxygen and other nutrients to the villus and removal of absorbed materials are critical functions of the mesenteric circulation. Autoregulation of blood flow by villus vessels exceeds that of deeper mucosal or muscular vessels (Tepperman and Jacobson, 1981).

Regulation of mesenteric circulation is via the sympathetic, parasympathetic, and enteric nervous systems; by intrinsic sensory neurons; and by autoregulation (interstitial cells of Cajal). Norepinephrine, adenosine triphosphate, and neuropeptide-Y are vasoconstrictors released directly onto blood vessels by the sympathetic nervous system. Vasoactive intestinal polypeptide (VIP) and substance P may be the primary vasodilator substances released from enteric motor neurons. Calcitonin gene-related peptide (CGRP) and substance P are thought to be released from sensory neurons (Holzer et al., 1994). Capsaicin may cause initial release, then depletion, of the peptide neurotransmitters, substance P, and CGRP. At high concentrations, capsaicin causes neuronal degeneration (Buck and Burks, 1986; Buck et al., 1982b; Holzer, 1991). Topical application of capsaicin to gastric mucosa causes dilation of submucosal arterioles, presumably by initial release of peptides from sensory neurons (Chen et al., 1992). Prior treatment with a neurotoxic (peptide-depleting) dose of capsaicin blocks the vasodilator response. CGRP appears to be the principal neural mediator of gastric mucosal hyperemia (Holzer et al., 1994) and is therefore associated with alimentary mucosal protection (Lippe et al., 1989). The vasodilator and hyperemic effects of CGRP appear to be mediated by nitric oxide (Holzer et al., 1993; Peskar et al., 1991). Endogenous nitric oxide also mediates the vasodilation that accompanies acid secretion (Holzer et al., 1994).

Once chyme passes into the upper small intestine, it is mixed with digestive enzymes. Intimate contact with the absorptive surface of the intestinal mucosa is affected by segmenting or mixing contractions. Contractions of the circular muscle occur more or less randomly but are somewhat fixed by the electrical slow waves or electrical control activity generated in the interstitial cells of Cajal. Propulsive contractions can occur, including random migrating clustered contractions associated with contractile rings that move distally from 5 to 30 cm propelling content aborad (Sarna and Otterson, 1989). Toxic agents that induce excessive migrating clustered contractions may speed propulsion.

The migrating motor complex (MMC) is a band of intense contractile activity that moves aborad over the stomach and small intestine during fasting (Szurszewski, 1969). The MMC sweeps debris from the stomach and intestine in preparation for the next meal. When food is ingested, motility reverts to the digestive pattern. The central nervous system exerts some control of MMC activity, but the MMC cycle appears to be initiated in the enteric nervous system, principally the myenteric plexus (Sarna and Otterson, 1989; Gad, 2007). Premature MMC activity can be induced by opiates, erythromycin, and other agents.

3.09.1.7 Cecum

Patterns of motility in the cecum and colon are complex. In some species, especially in rodents and lapids, the cecum can store large amounts of content that is released intermittently, usually in response to a meal. Stress and corticotropin-releasing factor (CRF) also cause contraction and emptying of the cecum (Peterson and Burks, 1989; Williams et al., 1988). Increased cecal emptying stimulated by CRF contributes to the excess of fecal excretion and diarrhea associated with psychological or chemical stress. Pharmaceuticals that alter intestinal motility include atropine, morphine, clonidine, papaverine, and isoproterenol (Gad, 2015).

3.09.1.8 Colon

The colon absorbs water, electrolytes, short chain fatty acids, and bacterial metabolites. The cell junctions of the large intestinal epithelium are much tighter than those of the small intestine. This prevents back-diffusion of ions through these junctions. As a result, large intestinal enterocytes absorb sodium ions more completely than in the small intestine (Spainhour, 2007). The cecal and colonic walls do not have a continuous layer of longitudinal smooth muscle. The proximal 85% of the colon is therefore incapable of peristaltic contractions. Because the circular muscle is intact, rhythmic segmentation is present. Rhythmic segmentation allows retropulsion but not peristalsis allowing the colon to function as a storage organ (Saur, 2010). Orthograde and retrograde contractions are thought to occur in the proximal and middle colon (Sarna, 1991).

The colon of humans expresses some cytochrome P450 isozymes as well as uridine diphosphate (UDP)-glucuronosyltransferase (McKinnon et al., 1993: Peters et al., 1991). A number of drugs and therapeutic chemicals may alter the colon and or its functions by direct contact or systemic mechanisms (Ernest, 2010).

The microbiota within the colon hydrolyzes some remaining digesta and xenobiotics via bacterial β-glucuronidase. Intestinal microbes facilitate enterohepatic circulation of phenytoin, phenacetin, diethylstilbestrol, digitoxin, and warfarin. These bacteria also convert methylmercury to its inorganic form (Gad, 2015). The colon slowly propels its contents aborally, holds residual material in the distal colon, and expels its contents during defecation (Sarna, 1991). Since the colonic mucosa lacks villi, the absorptive area of the colon relative to the small intestine is small and absorption is slow. Transit of contents through the colon is 2–10 h in small species and 30–50 h in humans.

3.09.1.9 Intestinal Cell Types

There are a variety of epithelial cell types lining the intestine, all of which are produced by progenitor cells in the crypts (**Fig. 6**) via notch signaling (Sander and Powell, 2004). Notch pathways are used by cells (i.e., cell–cell communication) to regulate, via their genes, cell differentiation processes that occur during embryonic and adult life. Enterocyte notch pathways influence

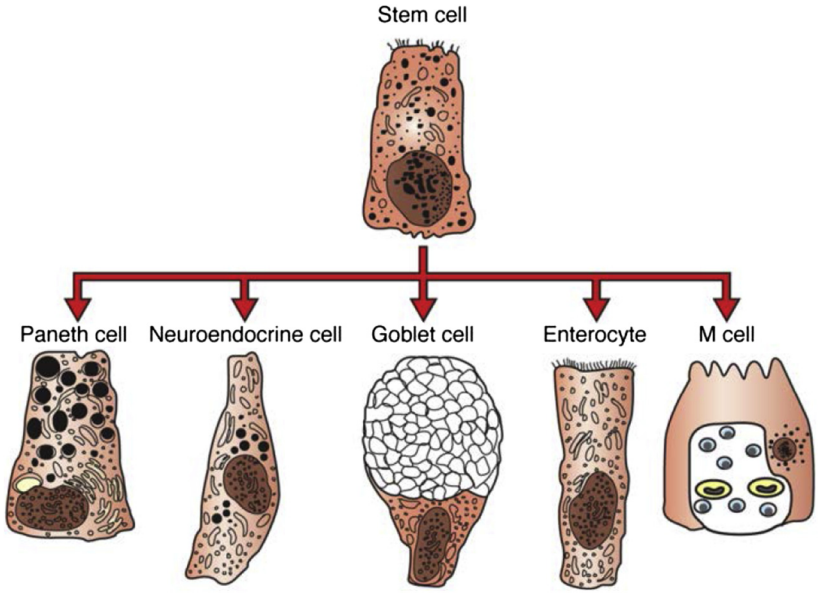

Fig. 6 Schematic illustration of the epithelial cell types of the small intestine. Progenitor cells, located in the intestinal crypts, give rise to all other epithelial cell types lining the crypts and covering the villi. From Damjanov, I. and Linder, J. (1996). Anderson's pathology (10th edn.). St. Louis: Mosby. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

whether intestinal epithelial stem cells differentiate into cells with secretory or absorptive functions. These pathways involve typical ligand–receptor interactions where the ligand is a transmembrane protein expressed in one cell type that binds with a notch receptor (i.e., notch protein) present on/in the cell membrane of another cell type. This binding interaction results in modifications of gene expression in the cell expressing the receptor, such as facilitating its differentiation into an absorptive enterocyte. This ligand–receptor binding appears to result in cells organizing into groups of cell types as needed for their differentiation into specific tissues and organs. (Gelberg, 2016). A combination of Notch and Wnt signals is necessary for proliferation of enterocyte precursors, but differentiation of cell types is independent of Wnt. Wnt and Notch synergy appears to induce intestinal adenomas (Fre et al., 2009).

The intestine has the fastest cell turnover of any of the body's fixed tissues. In a neonatal piglet that has not achieved climax flora, the epithelial turnover rate is approximately 7 days. In the mature gut, the turnover rate is 2–3 days. The immature, recently produced cells, with the exception of the enteroendocrine and Paneth cells, mature as they slide along the basement membrane to the villous tip extrusion zone where senescent cells become part of the fecal mass by a process of apoptosis termed anoikis.

3.09.1.10 Crypt Cells

The crypt or progenitor (stem) cells have short, sparse microvilli and little digestive or absorptive capacity. They proliferate and migrate to replace absorptive cells. Each crypt produces 300–400 cells day^{-1}. The migration rate is dependent on many factors, one of which is adaptation to gut microflora. Thus, in germ-free animals, the replacement rate is similar to neonates. Crypt cells have secretory functions (secretory component, NaCl) and may be involved in IgA and IgM transport.

3.09.1.11 Enterocytes

The absorptive cells (enterocytes) are tall and columnar with approximately 600 luminal microvilli per cell. They harbor a surface glycocalyx that contains the digestive and absorptive enzymes. Unilaminar membrane vesicles are shed into the lumen. These vesicles contain alkaline phosphatase and catalase that are bacteriocidal as well as membrane attachment sites for some pathogens. Enterocytes are connected to each other by an apical junctional complex composed of more than 40 transmembrane proteins and other molecules. Absorptive enterocytes are end stage cells that do not proliferate. They provide negative feedback inhibition to the progenitor cells by secreted chalones. The absorptive cells are pinocytotic early in life for colostrum absorption and passive transfer of antibody in some species. In general, enterocytes are responsible for nutrient and xenobiotic absorption. They contain Class II MHCs and a complement of biotransformation enzymes. In humans with inflammatory bowel disease, there is downregulation of genes encoding enzymes such as cytochrome P450 on colonic enterocytes (Wilke et al., 2012). The colonic mucosa is covered by relatively flat mucus-secreting cells and crypts.

3.09.1.12 Goblet Cells

Mucus producing goblet cells reside in villi and crypts. They increase in number aborally. Mucus exerts a variety of protective effects including trapping of bacteria with resultant passage in the fecal mass, lessening of shear forces of particulate matter on the enterocytes, and housing bacteriophages that reduce the bacterial population of the intestine by 10^4. Aspirin, NSAIDS, bile acids, and cigarette smoke are among the chemicals that decrease gastric and duodenal mucus production and bicarbonate secretion (Watkins and Klaassen, 2010).

3.09.1.13 Paneth Cells

Paneth cells are not present in cats, dogs, raccoons, or pigs. In other species they constitute a cellular mass similar to that of the pancreas. They are believed to have phagocytic and secretory functions. They secrete heavy metals and are injured during this process. They produce bacteriocidal cryptdins, lysins, peptidases, and lysozymes that may serve to protect crypt cells. They migrate toward the crypts rather than the villus tips. Paneth cell function and microbial composition vary among strains of mice suggesting a genetic influence of the host (Sonnenberg et al., 2012). Paneth cells reside in the lower portion of the crypt and have a life span of approximately 21 days.

3.09.1.14 Enteroendocrine Cells

The gastrointestinal system is the largest endocrine organ in the body. Enteroendocrine (argentaffin, enterochromaffin) cells produce a large variety of hormones that are delivered directly into the bloodstream (**Table 4**). These hormones are generated via posttranslational processing of prohormones. Often, more than one molecular form exhibits biological activity. For example, human progastrin consists of 80 amino acids and is cleaved to two biologically active forms, gastrin-34 and gastrin-17. Even smaller fragments of gastrin retain biological activity. Cholecystokinin (CCK) exists in even more biologically active forms. The 115 amino acid precursor peptide is cleaved by endopeptidases to produce CCK-58, CCK-39, CCK-33, CCK-22, CCK-11, and CCK-8, all of which have CCK activity (Reeve et al., 1986). The C-terminal pentapeptide of CCK is identical to that of gastrin and retains gastrin-like activity. Gastrin and CCK are released from gastric or intestinal mucosa, respectively, by ingestion of food. Gastrin acts in the oxyntic area of the stomach to stimulate acid secretion. CCK acts at the gallbladder to produce contractions that stimulate bile flow into the intestinal lumen thus stimulating pancreatic enzyme secretion. CCK has been proposed as a major mediator of the satiety response that leads to cessation of feeding (Della-Fera and Baile, 1979). Endocrine disrupters and other toxicities resulting from increases or decreases in production, processing, gastrin, or CCK release may have effects on gastric acid secretion, digestion, fat absorption, gallbladder contraction, rate of gastric emptying, growth of gastrointestinal mucosa, or appetite.

3.09.1.15 M Cells

M (microfold, membranous) cells occur in most species except rats. They are associated with the dome or follicle-associated epithelium of Peyer's patches or GALT. They play an important role in antigen uptake, including particulate toxins (e.g., asbestos), from the intestinal lumen and in transport to the lymphatic system. M cells have basal recesses that house lymphoid cells. This spatial arrangement allows for more rapid interaction with phagocytosed antigens. M cells allow bidirectional movement of lymphocytes between the lamina propria and intestinal lumen (Nicoletti, 2000). These cells are exploited for the entry of a variety of pathogens such as *Salmonella*, *Yersinia*, *Rhodococcus*, and some viruses (bovine virus diarrhea). **Fig. 7** illustrates the anatomic and mechanistic relationships of M cells to the underlying lymphoid tissue.

3.09.1.16 Submucosa/Lamina Propria

The gastrointestinal mucosa is the largest surface at which mammals interact with the external environment. A single layer of epithelium separates the intestinal lumen from the systemic circulation (Shanahan and Johnson, 1994). Immediately below the epithelial layer of the gastrointestinal mucosa is the loose connective tissue of the lamina propria. Many cellular components of the immune system are present in the lamina propria. Among these is a resident population of lymphocytes that increase with exposure to antigens, especially the microbiota (Gulati et al., 2012).

3.09.1.17 Immune Cells

The immune system and microbiota have profound influences on each other (Mueller et al., 2012). The classification and functions of these innate immune cells are currently being elucidated (Sonnenberg et al., 2012; Walker et al., 2013; **Figs. 8** and **9**). These lymphocytes arise from the same progenitor cell as NK T-cells, do not contain T-cell receptors, and produce a plethora of interleukins and other soluble mediators that parallel those of the antigen-specific immune effector cells. Current theory holds that the innate lymphoid cells hold infections in check until specific immune responses are generated (Leslie, 2012). Dendritic cells may be ILC 3 cells and similar to macrophages have Toll-like receptors (TLRs).

Table 4 Enterochromaffin (enteroendocrine, argentaffin) cells in the gastrointestinal system

Stomach	
Gastrin	Stimulates parietal cells to release HCl, ↑ motility
Ghrelin	Appetite regulator
Neuropeptide Y	↑ Food intake
Somatostatin	↓ Rate of gastric emptying and ↓ smooth muscle contractions and blood flow within the intestine ↓ Release of gastrin, cholecystokinin, motilin, secretin, vasoactive intestinal peptide, gastric inhibitory polypeptide
Enteroglucagon	↓ Release of pancreatic hormones ↓ Exocrine secretory action of the pancreas
Histamine	↑ Gastric acid secretion
Endothelin	Smooth muscle contraction
Glicentin	↑ Glycogenolysis in liver
Glucagon	↑ Blood glucose
Intestine	
Serotonin (90% of body's total from GI tract)	Mood, appetite, sleep
Cholecystokinin	Gallbladder emptying, pancreatic secretion, satiety
Bombesin	Negative feedback for eating
Secretin	Regulates secretions of the stomach, pancreas, and water balance
Enteroglucagon	Delays gastric emptying
Enterogastrone—Brunner's gland	↑ HCl from the stomach
Gastrin	Stimulates parietal cells to release HCl, ↑motility
Fibroblast growth factor 19	Effects on the liver (bile acid production, glucose, glycogen)
Substance P	Stimulates emetic center
Vasoactive intestinal polypeptide	Relaxes smooth muscle of the stomach, internal sphincter, and gallbladder while also inducing contraction of enteric smooth muscle Increases water secretion, inhibits gastrin, and stimulates pancreatic secretion of bicarbonate
Gastric inhibitory peptide = glucose-dependent inhibitory peptide	↓ Gastrin, ↑ insulin
Motolin	Stimulates peristalsis
Peptide YY	↓ Motility
Neurotensin	↑ Pancreatic secretion, ↑blood flow, ↓ motility
Glucagon-like peptide	↑ Insulin, ↓ gastric emptying, ↓ gastric secretion
Glicentin	↑ Glycogenolysis in liver
Glucagon	↑ Increases blood glucose
Urogastrone	↓ HCl
Oxyntomodulin	↓ Gastric secretion, ↓ intestinal mucosal growth
Enkephalins	↑ Smooth muscle contraction, ↓ secretion of water, and electrolytes

GI, gastrointestinal; *HCl*, hydrogen chloride.
Box 2. Pathologic Basis of Veterinary Disease.

The GALT is the largest mammalian lymphoid organ and houses approximately 80% of an individual's immunoglobulin-producing cells (Shanahan and Johnson, 1994). Epithelial cells of the gastrointestinal mucosa are essential components of the immune response in the gut. The close spatial and functional associations between epithelial cells and lymphoid cells suggest that GALT should refer to gut-associated lymphoepithelial tissue (Bockman et al., 1983; Castro and Arntzen, 1993).

Immune system-mediated inflammation is a common result of gastrointestinal toxicity. These cells include lymphocytes, macrophages, neutrophils, eosinophils, basophils, and mast cells. Some lymphocytes also occupy spaces between epithelial cells. Follicular lymphoid aggregates, Peyer's patches, are present in the mucosa. The mucosal immune system is functionally and operationally distinct in several ways from the systemic immune system. In contrast to systemic humoral immunity, IgA is the predominate immunoglobulin generated in the mucosa. Additionally, mucosal lymphocytes have surface markers that are activated relative to the peripheral blood and the systemic immune system.

Many immune system mediators are able to effect changes in cell function or viability associated with responses to toxic substances or conditions. Immune mediators may alter local blood flow and vascular permeability, activate or inhibit intrinsic nerves, alter epithelial cell transport, or are cytotoxic. Some of these immune mediators are cytokines, arachidonic acid derivatives, and amines (e.g., histamine and 5-hydroxytryptamine). Cytokines are peptide mediators released from immune and epithelial cells

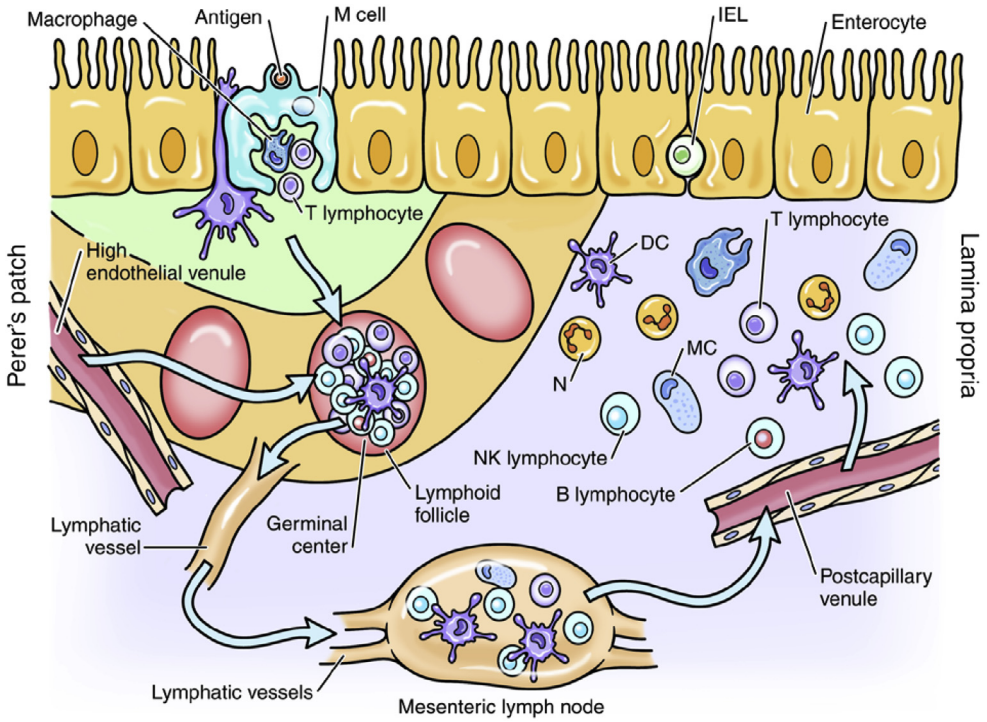

Fig. 7 Schematic illustration of GALT. Dendritic cell (DC), intraepithelial lymphocyte (IEL), microfold cell (M), mast cell (MC), and neutrophil (N). Adapted from Cominelli, F., Arseneau, K.O., Blumberg, RS, et al. (2009). The mucosal immune system and gastrointestinal inflammation. In: Yamata, T. (ed.) Textbook of gastroenterology (5th edn.). West Sussex: Wiley-Blackwell. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

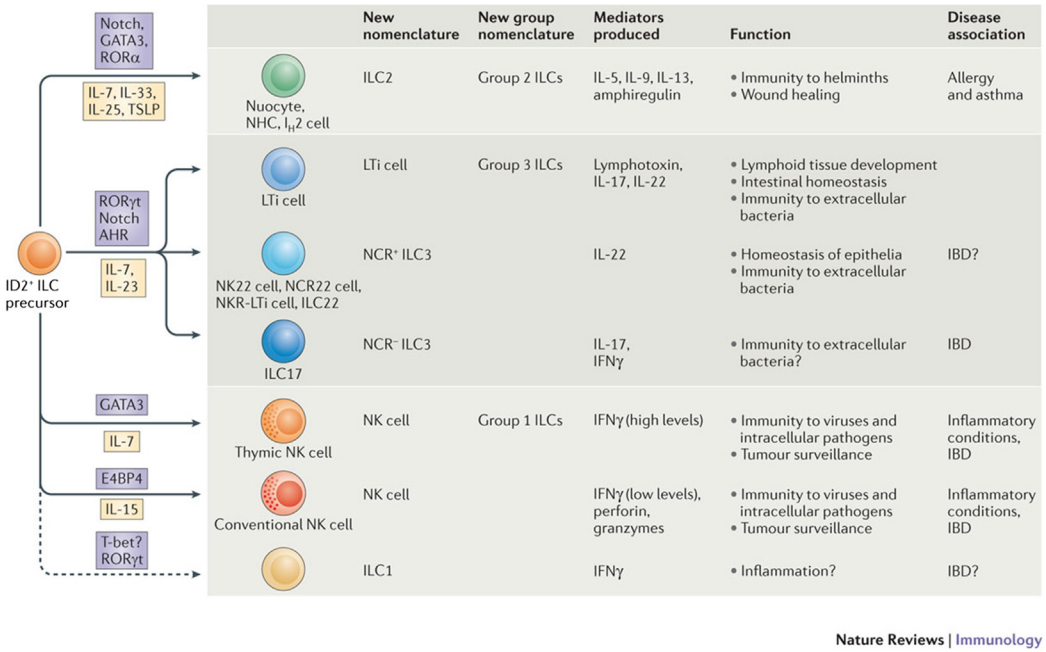

Fig. 8 Innate lymphoid cell subsets, functions, and disease associations. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology. Walker, J. A., Barlow, J. L., and McKenzie, A. N. J. (2013). Innate lymphoid cells—How did we miss them? Nature Reviews Immunology 13, 75–87.

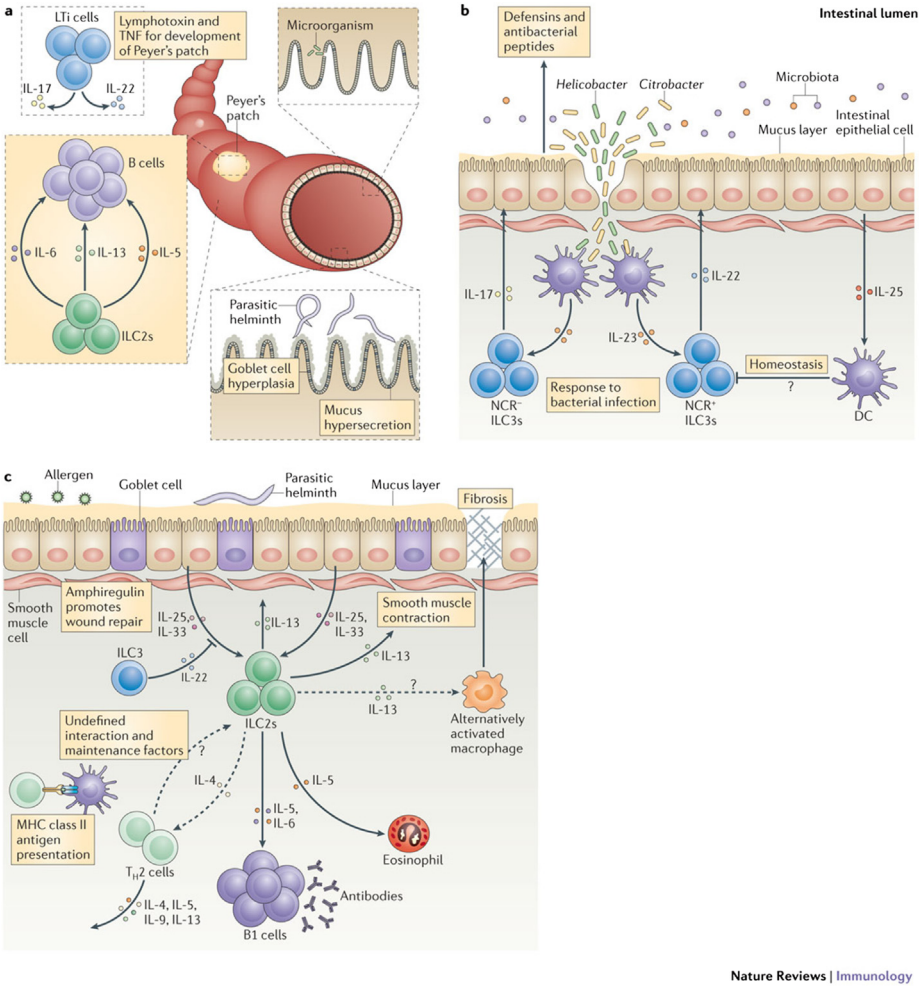

Fig. 9 Schematic of the roles for immune lymphoid cells in intestinal immune function. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Immunology. Walker, J. A., Barlow, J. L., and McKenzie, A. N. J. (2013). Innate lymphoid cells—How did we miss them? Nature Reviews Immunology 13, 75–87.

that activate other immune cells to release mediators, induce chemotaxis, or induce phagocytosis. Macrophages and neutrophils are the cell types most often involved in phagocytosis. The phagocytic activity of these cells is linked to the release of a number of catabolic enzymes (acid hydrolases, neutral proteases, acid phosphatase, lysozyme, and peroxidase), along with superoxide free radicals, arachidonic acid metabolites, and other cytotoxic molecules (Shanahan and Johnson, 1994; Tanner et al., 1984). An increase in intestinal macrophages is often present in inflammatory bowel disease (Shanahan and Johnson, 1994). Macrophages are abundant in granulomas and are a hallmark of Crohn's disease.

3.09.1.18 Mast Cells

Mast cells are very important in maintaining intestinal integrity. They regulate the epithelial barrier; control blood flow, coagulation, smooth muscle contraction; stimulate the enteric nervous system and peristalsis; and play a role in antibody-dependent recognition of parasites and microorganisms. They release proinflammatory paracrine cytokines. Mucosal mast cells differ from mast cells at nonmucosal sites (Enerback, 1987). They lack membrane-bound IgE and contain chondroitin sulfate; peritoneal mast cells contain heparin proteoglycan (Shanahan and Johnson, 1994). Activation of mast cells can lead to effects on mucus secretion, vascular permeability, muscle contraction, and inflammatory cell recruitment (Crowe and Perdue, 1992).

Within the mucosa there is bidirectional communication between intrinsic nerves and mast cells. In the guinea pig colon, histamine causes a recurrent pattern of chloride secretion mediated by activation of histamine receptors and stimulation of cholinergic secretomotor neurons (Cooke et al., 1993). Antigen challenge in sensitized animals may result in release of substance P and CGRP from sensory nerves (Castro and Arntzen, 1993). These neurotransmitters can cause mast cells to release histamine. Histamine causes cholinergic motor neurons to induce muscle contractions or alterations in epithelial ion transport (Castro et al., 1994). Mast cell histamine release affects epithelial transport of water and electrolytes via direct actions on epithelial cells and indirectly

by effects on intrinsic nerves (Castro et al., 1987). Histamine induces a recurrent pattern of chloride secretion from mucosal crypt cells and contractions of smooth muscle (Cooke et al., 1993). In addition to histamine, 5-hydroxy-tryptamine (5-HT) released from enterochromaffin cells and myenteric neurons, as well as mast cells in some rodents, may contribute to mucosal chloride secretion (Cooke et al., 1991). Chloride secretion results in a net transfer of salt and water to the gastrointestinal lumen (Cooke et al., 1993). The actions of 5-HT appear to be indirect and result from release of acetylcholine that acts directly upon mucosal crypt cells. Evidence indicates that 5-HT can induce electrogenic chloride secretion in human jejunal mucosa by direct effects on crypt cells (Kellum et al., 1994). Both the direct effects of 5-HT and histamine on epithelial cells and the indirect effects mediated by cholinergic motor neurons may be augmented and amplified by prostaglandins and other substances released during immune responses or inflammation (Sidhu and Cooke, 1995).

3.09.1.19 Neutrophils

Neutrophils in the lamina propria are transient as they pass through the intestine to become part of the fecal mass and expelled from the body. Human neutrophils spend about 5 days in the bloodstream and about 2 days in tissues. However, there is marked variation in neutrophil lifespan among species. In mice for example, neutrophil lifespan is approximately 0.75 days (Pillay et al., 2010).

3.09.1.20 Globule Leukocytes

Globule leukocytes are present in a variety of submucosal locations including the lamina propria. Their function is largely unknown but may be similar to those of Paneth cells. Theories for their origin include derivation from mast cells, plasma cells, large granular lymphocyte lineages, or a distinct precursor (Spoor et al., 2011). They are most common in parasitic infections and rarely form neoplasms.

3.09.1.21 Microbiota

The microbiota/microbiome of the lower GI system consists of 100 trillion bacteria which is 10 times the number of cells in an animal and 1.3 million times the number of genes in an animal. Due to the enterocytes' low expression of TLRs, such as lipopolysaccharide-responsive TLR4, they do not react to these resident bacteria (Abreu et al., 2003).

Approximately 30–40% of fecal dry matter is of bacterial origin. These bacteria secrete bacteriocins (i.e., proteinaceous toxins that inhibit the growth of other bacteria) and compete for nutrients and for attachment sites thus limiting potential pathogen growth. The microbiome promotes immune system maturation and contains biotransformation enzymes such as β-glucuronidases, β_2-glycosidases, demethylases, hydrolases, and reductases. There appear to be three enterotypes (i.e., type of bacteriologic ecosystem of the gastrointestinal microbiome) in animals, and these biotypes may be, in part, responsible for susceptibility or resistance to certain pathologic conditions.

3.09.1.22 Mucosal Growth and Repair

Several substances including gastrin, TGF-α, and TGF-β are growth factors that stimulate epithelial growth from stem cells. The ingestion and digestion of food are important in maintaining intestinal mucosal growth. Rats maintained on total parenteral nutrition gain body weight, but the weight of the stomach, small bowel, and colon is often only 30–40% of comparable tissues from control animals (Johnson et al., 1975).

3.09.1.23 Mucosal Transport

Xenobiotics enter enterocytes primarily by diffusion. Entry into the systemic circulation is concentration dependent. Molecules >600 MW pass through based on hydrophobicity and other physiochemical properties of the xenobiotic (Gad, 2007). Transport of molecules with molecular weights <200 is hydrostatic and is known as solvent drag. Facilitated diffusion utilizes carriers similar to active transport but does not occur against a concentration gradient. Glucose transport into enterocytes utilizes the Na^+–K^+ pump that generates a Na^+ gradient across cell membranes via a glucose-Na^+ symport protein (Brock and Hobson, 2007).

In order to cross a membrane, a xenobiotic must be lipid soluble; to enter and exit the membrane, it must be water soluble. Many pharmaceuticals are polar or nonpolar. For weak acids or bases, the pK_a of the drug, the pH of the GI tract fluid, and the bloodstream control the solubility and rate of enterocyte absorption (pH partitioning). Weak acids and weak bases are absorbed principally through the gastric mucosa because they are nonionized (Brock and Hobson, 2007).

Absorption is partly passive and dependent on the physiochemical properties of the xenobiotic. Active transport is by way of a family of active "transporter" mechanisms in humans and most common laboratory species (**Fig. 10**). Transport is against concentration gradients using ATP as an energy source (Brock and Hobson, 2007). Active transport of compounds into cells is complicated when structurally related toxicants (e.g., 5-fluorouracil) compete with a nutrient for a transporter protein.

Peptide transporters are present on the brush border membrane of enterocytes and have broad substrate specificity for small peptides and peptidomimetic pharmaceuticals. Many lactam antibiotics and drugs such as cephalexin, ampicillin, amoxicillin, captopril, and other ACE inhibitors exploit this system (the amino acid prodrug of acyclovir has no peptide bond, suggesting

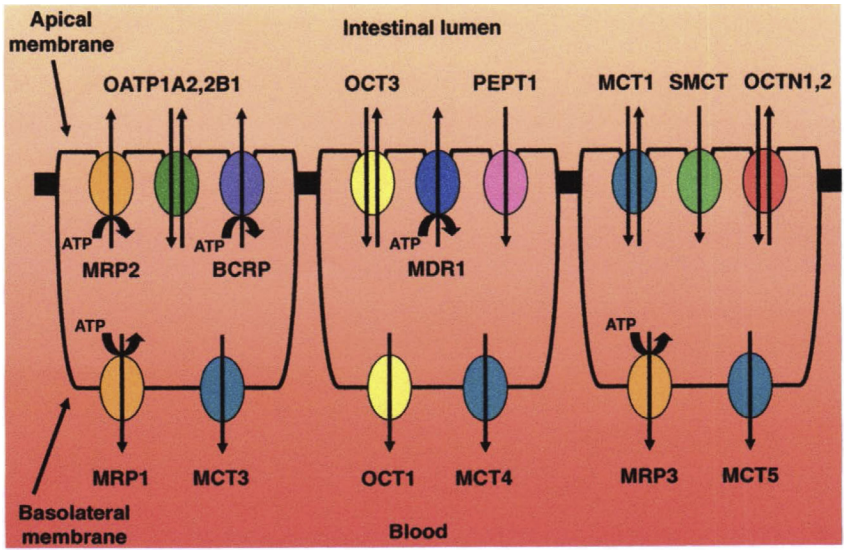

Fig. 10 Transport proteins of the human gastrointestinal tract. Schematic representation of three adjacent enterocytes. Transport proteins are depicted with *arrows* denoting the direction of substrate transport. Dexamethasone (dex), epoxide hydrolase (EH), flavin-containing monooxygenase (FMO), gastrointestinal (GI), glutathione thiolate anion (GS_), glutathione (GSH), glutathione *S*-transferase (GST), monocarboxylate transporter (MCT), multidrug resistance protein 1 (MDR1), 1-methyl-4-phenylpyridinium (MPPfl), messenger RNA (mRNA), multidrug resistance-associated protein (MRP), mitoxantrone resistance protein (MXR), arylamine *N*-acetyltransferase (NAT), nonsteroidal antiinflammatory drug (NSAID), organic anion transporting polypeptide (OATP), organic cation transporter (OCT), organic cation/carnitine transporter (OCTN), 39-phosphoadenosine-59-phosphosulfate (PAPS), peptide transporter (PEPT), P-glycoprotein (P-gp), P-glycoprotein/multidrug resistance protein 1 (P-gp/MDR1), reverse transcriptase-polymerase chain reaction (RT-PCR), solute carrier (SLC), sodium-coupled monocarboxylate transporter (SMCT), sulfotransferase (SULT), uridine diphosphate-glucuronic acid (UDPGA), and UDP-glucuronosyltransferase (UGT). Wolf, K. K., Paine, M. F., Watkins, P. B., et al. (2010). Comprehensive toxicology, V10, pp. 53–75.

a broader spectrum than just without energy input). Active transport requires energy and likely affects absorption of nucleoside analogs used in antiviral and anticancer therapeutics (Gad, 2007).

There are several types of sugar transporters with high affinities for D-glucose and D-fructose and low affinities for D-galactose and mannose. At least one type is facilitated transport. L-sugars affinities are 1000 times lower than D sugars. Bile acid transporters are critical for enterohepatic recirculation (Gad, 2007).

There are many types of intestinal amino acid transporters. Among the drugs utilizing these transporters are a-methyldopa, baclofen, and D-cyclosporin. Organic anion transporters are exploited by carboxylic acids in neutral or basic media. Vitamin transporters are specific such as those for thiamine, vitamin C, folic acid, and B12. The nicotinic acid transporter has affinity for valproic and salicylic acids and penicillins. Foscarnet (antiviral) and fosfomycin (water-soluble antibiotic) utilize phosphate transporters. Bicarbonate transporters exchange bicarbonate, sodium, and chloride and are important regulators of cellular pH through acid–base movement. Choline is the substrate for organic cation transporters (Gad, 2007).

Human efflux transporters of P-glycoprotein are present on enterocyte brush borders. They exorb a large number of drugs and have broad substrate specificity that includes vincristine, taxol, digoxin, some fluoroquinolone antibacterials, quinidine, etoposide, cyclosporine, verapamil, and nifedipine (Gad, 2007).

Long chain fatty acids (palmitate and oleate) can saturate the fatty acid transporter. Bile salts emulsify triglycerides and other fat-soluble molecules forming micelles for absorption into the lymphatics along with other nonpolar compounds. Xenobiotics such as *P*-aminosalicylic acid, tetracycline, 3-methylcholanthrene, polychlorinated biphenyls, and benzpyrene may also be absorbed by this route (Gad, 2007).

Cells can transport macromolecular proteins, polysaccharides, and polynucleotides via phagocytosis or pinocytosis especially in areas of GALT. Phagocytosis is the process of entering cells via cytoskeleton rearrangement forming phagosomes. Phagosomes combine with lysosomes forming phagolysosomes. Pinocytosis is similar to phagocytosis and is selective or nonselective in transporting liquids into cells depending on the involvement of cell surface receptors. This method occurs in association with M cells and is especially effective in neonates and decreases with age. This process is useful for absorbing immunoglobulins but is exploited by some toxins, bacteria, and other xenobiotics. Cadmium, ferritin, and nutrients utilize this pathway. To be absorbed, xenobiotics must survive low gastric pH, be of appropriate molecular structure, molecular size, ionization potential (pK_a, pK_b) and be hydrophilic or lipophilic (Brock and Hobson, 2007).

Xenobiotics with effects on absorption include acetazolamide, a carbonic anhydrase inhibitor involved in HCO_3^- production. Belladonna (atropine), a competitive antagonist of acetylcholine at muscarinic receptors, is an antisecretory agent and decreases GI motility. Clonidine is an adrenergic stimulator with antisecretory and antidiarrheal properties. Domperidone blocks the inhibitory effects of dopamine and increases GI motility, while ezetimibe inhibits cholesterol absorption and blocks the translocation of

dietary and biliary cholesterol into the intracellular spaces of jejunal enterocytes (Toth and Davidson, 2005). Cimetidine, ranitidine, and famotidine (H_2 receptor antagonists) decrease production of gastric acid that may have downstream effects. Loperamide is an opioid receptor agonist and acts as antisecretory agent that also slows colonic motility. Opiates are antisecretory via relaxation of intestinal leiomyocytes. Ouabain Inhibits enterocyte Na^+–K^+–ATPase pumps. Cisapride, metoclopramide, erythromycin, and bethanechol (prokinetic drugs) increase GI motility. Their pharmacologic action and mechanism depend on the specific agent. Lansoprazole, rabeprazole, esomeprazole, omeprazole (proton pump inhibitors) downregulate gastric acid secretion and thus may affect intestinal absorption and motility (Hobson and Hobson, 2007).

3.09.1.24 Mucosal Metabolism of Xenobiotics

The digestion of xenobiotics in preparation for absorption in the GI tract is by hydrolysis of primary organic structures (carbohydrates, lipids, proteins). Differences are in the enzymes required for each type of compound (Spainhour, 2007). Xenobiotics must be absorbed intact or bound to a substance that facilitates absorption by cotransport and metabolized followed by absorption or absorbed intact then metabolized (Brock and Hobson, 2007). Ingested xenobiotics can be metabolized intraluminally or extraluminally. After transport to the portal blood and/or lymphatics, other forms of metabolism may take precedent (Brock and Hobson, 2007).

The majority of low-molecular-weight lipid-insoluble compounds including toxins and drugs enter mucosal epithelial cells by passive diffusion through aqueous membrane pores at epithelial tight junctions. Enterocytes may metabolize pharmaceuticals to presystemic drugs or inactivate toxins. Conversely, they may convert inactive prodrugs or toxins to their active forms (Gad, 2007).

Epithelial cell enzymes conduct oxidative, reductive, hydrolytic, and conjugation reactions. The oxidative reactions are largely catalyzed by cytochrome P450 isozymes. The intestinal mucosa also contains nonspecific esterases and amidases, UDP-glucuronosyltransferases, and reductases. Some enzyme activity, such as nitroreductase and dechlorinase, may be attributable to both mucosal enzymes and luminal microflora (Chadwick et al., 1990). In general, the cytochrome P450 oxidative enzymes are the most important in terms of chemical biotransformation. While it is not known if all cytochrome P450 isozymes are present in enterocytes (Gelboin, 1993), it is clear that most isozymes occur in the intestine (Watkins, 1992). Enterocytic cytochrome P450 activity increases as the cells mature during their migration from crypt to villus. Nearly all cytochrome P450 activity is attributable to villus cells. NADPH cytochrome P450 reductase, a necessary component for the activity of all cytochrome P450 enzymes, is constitutively expressed only in villus cells (Traber et al., 1992). Xenobiotic metabolizing activity is likely retained in enterocytes after they are shed from the villus tip.

Some ingested alcohol is metabolized by gastric alcohol dehydrogenase, prior to systemic absorption. While metabolism in the gastric mucosa probably contributes little to overall alcohol metabolism, gastric alcohol dehydrogenase activity is affected by drugs and by chronic consumption of alcohol (Julkunen et al., 1985).

Xenobiotic metabolism is also a function of luminal microorganisms. The microbiota can affect mucosal enzyme activity. The metabolic activity of the intestinal microflora must be taken into account in biotransformation studies (Rowland, 1988). There are marked animal species differences in microbial composition and metabolism with at least three biotypes. The microbial population can be affected by age and is likely to differ at very young and very old ages. Environmental factors, such as drugs (especially antibiotics), gender, diet, and xenobiotics can modify microbial metabolism and thus the toxicity of foreign compounds.

Xenobiotics are transported from enterocytes to the bloodstream by multidrug resistance-associated monocarbohydrate transporter protein (MCT1), or equilibrative nucleoside transporter (ENT1 and ENT2) (Ciarimboli, 2008). Transport from the enterocytes into the GI lumen is via MRP2, MRP4, breast cancer-resistance protein (BCRP), and P-glycoprotein (Pgp) (Choudhuri and Klaassen, 2006; Toyoda et al., 2008) which are members of the ATP-binding cassette superfamily.

3.09.1.25 Special Environment

The gastrointestinal mucosa is exposed to a hostile environment that can act in concert with toxins to produce mucosal damage. In the stomach and upper duodenum there is a high concentration of hydrochloric acid and pepsin. In the small intestine pancreatic proteases, lipases and bile acids are present. The colonic mucosa is exposed to bacterial toxins and products of fermentation. In view of these environmental challenges, the defensive mechanisms of the gastrointestinal mucosa are remarkably effective and usually protect the mucosa from threats to its integrity.

With that as background, there are a variety of mechanistic targets for intestinal injury and resultant diarrhea. These include diseases of crypt cells, diseases of absorptive cells, abnormalities of the glycocalyx, diseases caused by separation of apical junctional complexes, diseases in which epithelial targets are unknown or nonspecific, diseases of the lamina propria, diseases of the vasculature, and disorders of innervation (Gelberg, 2016). Much is known about the mechanisms of intestinal dysfunction caused by infectious agents that may be extrapolated to xenobiotics in general.

3.09.1.25.1 Diseases of crypt enterocytes

Loss or attrition of crypt enterocytes results in lack of replacement of normal absorptive epithelial cells. In 2–3 days the crypts and villi become depleted. Agents that attack mitotically active cells are termed radiomimetic since they act in a manner similar to radiation. A variety of chemotherapeutic agents, many of which are designed to destroy rapidly dividing neoplastic cells, cause this kind of injury. Similarly, tyrosine kinase inhibitors (growth factor signaling) and cell cycle inhibitors cause apoptosis with resultant crypt

necrosis and secondary enteritis (Betton, 2013). In carnivores and pinnipeds, parvoviruses are radiomimetic. Other tissues, such as those of neonates and hematopoietic precursors, also divide rapidly and are targets of these agents. Conversely, some bacteria such as *Lawsonia* in pigs cause crypt cell proliferation.

3.09.1.25.2 Diseases of absorptive enterocytes

Agents targeting absorptive enterocytes may or may not be fatal depending on the number of cells affected and whether the basement membrane is intact. In the interim, surviving immature enterocytes elongate to cover basement membranes and prevent, among other undesired events, endotoxin absorption from the gut lumen. The lost cells are rapidly replaced by migrating crypt cells, providing they have a basement membrane on which to orient and reconstruct villi. Once basement membranes touch, they fuse resulting in permanently stunted villi and loss of absorptive and digestive surface area. Many agents target absorptive enterocytes including viruses (rotavirus, transmissible gastroenteritis virus of swine, coronaviruses of most mammalian species), intracellular bacteria (*Escherichia coli*), and protozoal parasites such as coccidia and cryptosporidia.

3.09.1.25.3 Diseases of the microvilli and glycocalyx

Agents, including some toxins, selectively destroy the glycocalyx that results in specific or general enzyme deficiencies with a resultant lack of digestive capabilities. An example of this is congenital lactase deficiency (lactose intolerance). Undigested lactose ferments in the intestinal lumen with a resultant osmotic drain and diarrhea. Pathogens such as attaching and effacing *E. coli* damage microvilli and disrupt enzyme systems. The antibiotic neomycin can cause reversible enzyme deficiency via fragmentation of microvilli and destruction of the glycocalyx.

3.09.1.25.4 Separation of apical junctional complexes

This phenomenon is most common in parasitic and bacterial infections where opening of the apical junctional complexes results in transfer of large molecules such as antibody that helps clear the pathogen. This is sometimes called the leaky membrane concept of enteritis.

3.09.1.25.5 Diseases in which epithelial targets are unknown or nonspecific

Intestinal disease is often the result of bacterial or ingested toxins. Some bacteria colonize the small intestine overcoming the washout effect due to pilus antigens. An example is enterotoxic *E. coli* where release of toxins causes the small intestine to secrete electrolytes and water. When secretion exceeds colonic absorption, diarrhea results. There is no histologic evidence of cell damage in these secretory diarrheas. Hypersecretion is a net intestinal efflux of water and electrolytes independent of permeability changes, absorptive capacity, or endogenously generated osmotic gradients. All *Clostridia* spp. produce enterotoxemia but unlike the case with *E. coli*, the toxins are markedly cytolytic causing necrosis of villus absorptive cells and subsequent extension into the lamina propria and blood vessels, much like caustic agents. The result is ulceration and hemorrhage.

3.09.1.25.6 Diseases of the lamina propria

Diseases of the lamina propria include necrotizing processes and space occupying lesions. Often, necrosis of lymphoreticular tissue is how damage commences with extension to the overlying epithelium. This occurs in viral disease such as bovine virus diarrhea of cattle and bacterial diseases such as *Rhodococcus equi* of equids.

By mechanisms that are poorly understood, space occupying lesions of the lamina propria interfere with mucosal diffusion of nutrients into the lacteals (malabsorption) resulting in diarrhea. This occurs whether the lamina propria is filled with immune cells (inflammatory bowel disease), mycobacteria-filled macrophages (Johne's disease of cattle) or neoplastic infiltrates (intestinal lymphoma).

3.09.1.25.7 Intestinal motility

Intestinal motility changes are a part of many mechanisms of diarrhea production but are not considered to be a primary means. Decreased motility allows for bacterial overgrowth; increased motility hinders digestion and absorption.

3.09.1.25.8 Vascular diseases

Endotoxemia may result in injury to the enteric nervous system and the tunica muscularis secondary to vasoconstriction and vasospasm (Oikawa et al., 2007). Lymphangiectasia may be congenital as a result of vascular malformations or acquired secondary to space occupying lesions of the lamina propria. Most often it is idiopathic. It results in malabsorption, steatorrhea, and protein-losing enteropathy.

3.09.1.25.9 Problems with innervation

The alimentary system is second to the CNS in its number of neurons (10^8) and glial cells (4×10^8). Agangliosis and dysautonomia, malfunctions of the cranial nerves, spinal nerves, ganglia, and/or autonomic nervous system may have profound influences on intestinal motility. There are a great variety of agents that cause these changes ranging from botulinum toxin to inflammatory diseases. Many cases are idiopathic or may be hereditary. In addition, there is a bidirectional neurohormonal interchange between intestinal microbiota and the brain. Thus, alteration of the microbiota may result in changes in the gut–brain axis (Collins et al., 2010). Dysbiosis has effects on early brain development in mice, irritable bowel syndrome, Crohn's disease, ulcerative colitis,

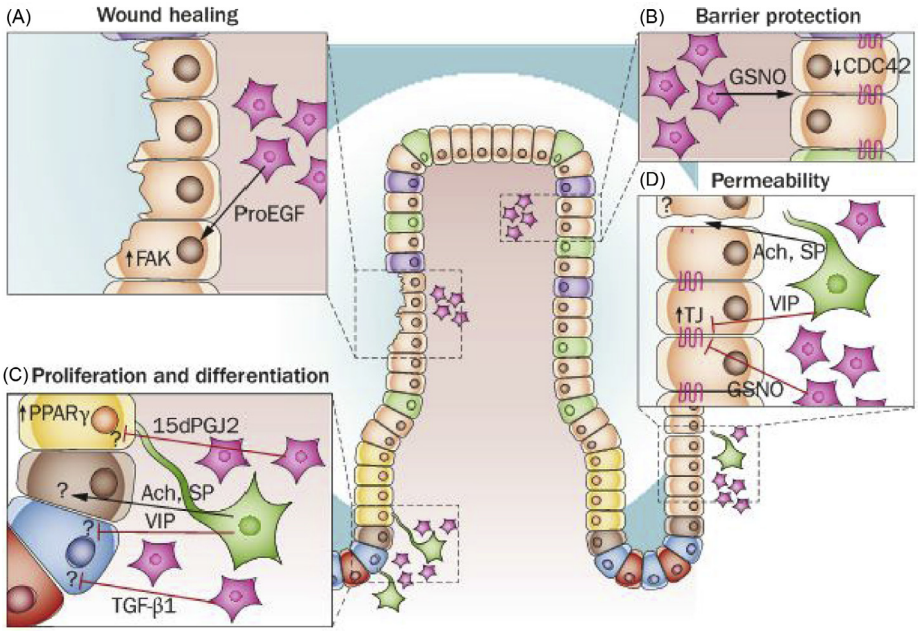

Fig. 11 Soluble factors produced by the ENS regulate IEB functions. Enteric neurons (*green*) and glial cells (*pink*) produce soluble factors that have differential effects on different intestinal epithelial cell types (enterocytes in *light brown*, intestinal stem cells in *blue*, Paneth cells in *red*, enteroendocrine cells in *violet*, and goblet cells in *light green*), thereby regulating IEB proliferation, differentiation, healing, permeability, and protection. (A) Wound healing. Enteric glial cells can enhance wound healing via the release of proEGF, leading to increased activity and expression of FAK. (B) Barrier protection. During infection by pathogens such as *Shigella flexneri*, enteric glial cells release GSNO leading to reduced CDC42 expression, and enhanced intestinal barrier resistance. (C) Proliferation and differentiation. Neurons and glial cells release mediators (such as VIP, or TGF-β1 and 15dPGJ2, respectively) that inhibit intestinal cell proliferation. Conversely, neuromediators (Ach and SP) can increase intestinal cell proliferation. (D) Permeability. Enteric neuromediators can differentially regulate paracellular permeability—VIP reduces paracellular permeability, while Ach increases it. GSNO from enteric glial cells can also reduce paracellular permeability by increasing the expression of key tight junctions associated proteins such as ZO-1. Acetylcholine (Ach), enteric nervous system (ENS), focal adhesion kinase (FAK), S-nitrosoglutathione (GSNO), intestinal epithelial barrier (IEB), peroxisome proliferator-activated receptor γ (PPARγ), substance P (SP), tight junction proteins (TJ), and vasoactive intestinal peptide (VIP). Neunlist, M., Van Landeghem, L., Mahe, M., et al. (2013). Nature Reviews Gastroenterology and Hepatology 10, 90–100.

demyelination in multiple sclerosis, hepatic encephalopathy, and psychiatric disorders such as early-onset autism. The interstitial cells of Cajal are of mesenchymal origin and are the pacemakers of the gut. Inflammation, neoplasia, or loss of these cells affect coordinated movement of the alimentary system.

The digestive neuronal glial epithelial unit (Neunlist et al., 2013) is composed of enteric neurons, glial cells, and intestinal epithelial cells. Enteric neuromediators and gliomediators modulate intestinal epithelial barrier functions such as paracellular permeability, epithelial cell proliferation, and wound healing (Fig. 11). Phenotypical changes in enteric neurons and glial cells are present in certain diseases, but the mechanisms are poorly understood. The neurons and glial cells are organized into two plexi; myenteric (Auerbach's) and submucosal (Meissner's). Myenteric plexi regulate GI motor function, while submucosal plexi regulate mucosal processes. Each villus and colonic unit is innervated by 70–92 submucosal neurons (Song et al., 1995). The enteric nervous system can perform these functions in the absence of the CNS, but the CNS can have an effect on enteric neurons (Fig. 12). The enteric nervous system along with the microflora, the immune system, and fibroblasts help maintain the integrity of the intestinal epithelial barrier.

3.09.2 Abnormal Conditions

3.09.2.1 Emesis

Emesis is associated with rapidly progressive orad propulsion of small intestinal and gastric contents as well as coordinated respiratory, postural, and subjective correlates. Emesis is orchestrated by the diffuse vomiting center in the brainstem that initiates retrograde contractions that typically begin in the mid-portion of the small intestine and migrate orally at a rate of 8–10 cm s^{-1} (Lang et al., 1986; Stewart et al., 1977). Retching movements begin when the wave of retrograde propulsion reaches the stomach. Immediately afterward, the intercostal and diaphragmatic muscles contract to increase intraabdominal pressure, respiration is reflexively inhibited, and the gastric contents are expelled through the oral cavity. While aspiration of vomitus does not often occur in conscious subjects, it can occur any time, especially in those with impaired consciousness such as those under anesthesia. Aspiration

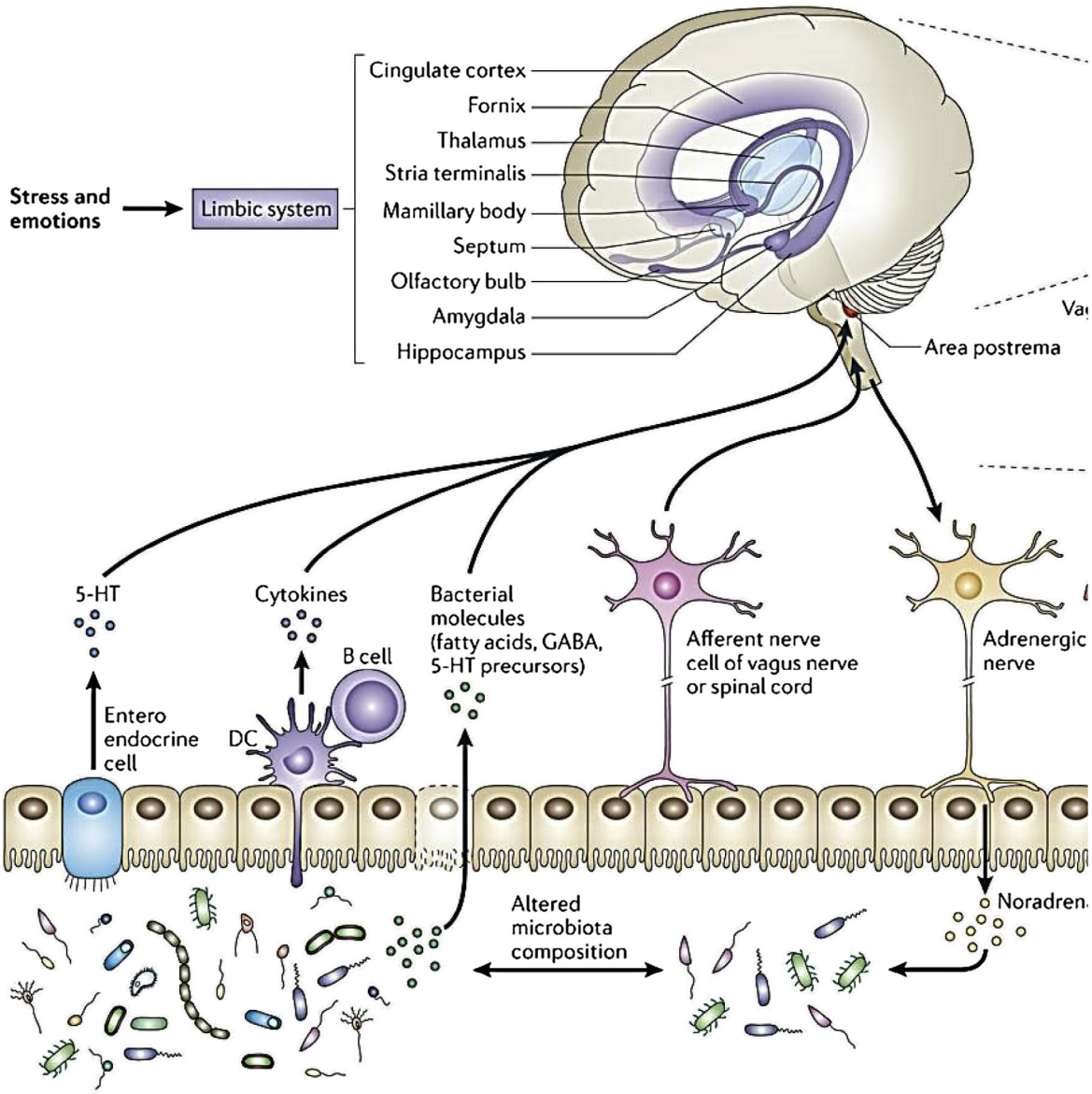

Fig. 12 Bidirectional microbiota–gut–brain axis. Collins, S. M., Surette, M., and Bercik, P. (2012). The interplay between the intestinal microbiota and the brain. Nature Reviews Microbiology 10, 735–742.

is very hazardous particularly if the vomitus contains toxic substances (petroleum products, caustic materials) in addition to the usual potentially damaging content of acid, pepsin, bile, pancreatic enzymes, and microorganisms. Emesis and nausea, which may or may not accompany emesis, can be elicited by both peripheral and central stimuli. In humans, unpleasant sights or smells may be sufficient to trigger emesis. Activation of dopamine D_2 receptors in the chemoreceptor trigger zone or in the vomiting (emetic) center, activation of 5-HT_3 receptors at peripheral vagal sensory pathways, gastritis or gastroenteritis, vection sickness, and other causes can initiate emesis. It is presumed that emesis is largely a protective reflex, designed to empty the upper gastrointestinal tract of toxic substances. Induction of emesis remains a mainstay of treatment of acute ingestion of poisons with the exception of caustic agents that burn mucosa. Examples of emetic agents are in Table 5.

3.09.2.2 Diarrhea

Normal feces are 75% water. Diarrheic feces are more than 85% water. One may conceptualize diarrhea as a result of one of three mechanistic pathways; secretory, inflammatory, and invasive. Thus, there are inflammatory and noninflammatory causes of diarrhea. Noninflammatory causes disrupt absorptive or secretory pathways but do not kill the enterocytes and are generally active in the proximal intestine (Fig. 13). Inflammatory diarrheas are a result of pathogenic processes that are lethal to enterocytes and are generally relegated to the distal small intestine, cecum, and colon (Fig. 14). Diarrhea is usually not associated with increased

Table 5 Substances that induce emesis

Direct mucosal actions	*Central (blood-borne) actions*
$CuSO_4$	Dopamine D_2 agonists
Bacteria	Apomorphine
Viruses	Bromocriptine
Plant and animal toxins	L-Dopa
Cytotoxic agents	Emetine (ipecac)
Irritants	5-HT_3 agonists
5-HT_3 agonists	Cytotoxic agents
Phenylbiguanide	Opiates
2-Methyl-5-HT	

Reproduced from Burk, T. F. (2010). Comprehensive toxicology (10 vols.), pp. 117–144, Elsevier.

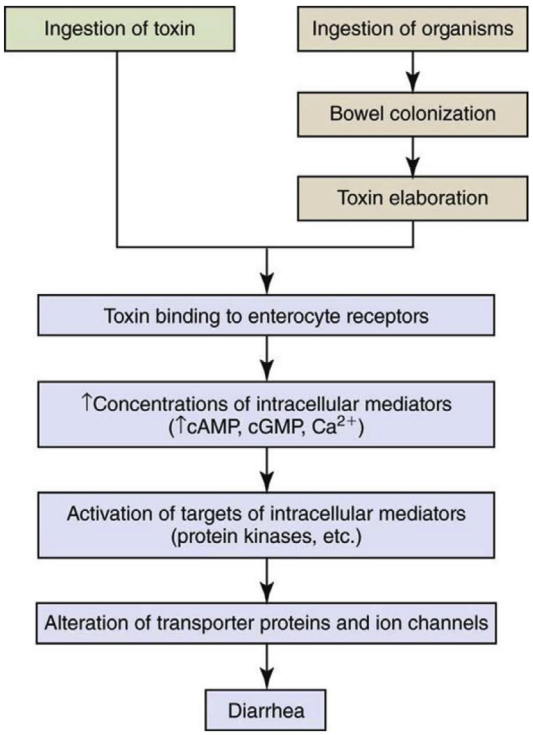

Fig. 13 Schematic diagram of the mechanism of action for enterotoxin-mediated bacterial diarrhea. Adapted from Cominelli, F., Arseneau, K.O., Blumberg, RS, et al. (2009). The mucosal immune system and gastrointestinal inflammation. In: Yamata, T. (ed.) Textbook of gastroenterology (5th edn.). West Sussex: Wiley-Blackwell. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

contractions of the small intestine or colon, despite the prevalence of the erroneous concept that more contractions propel contents more rapidly (Sarna, 1991). In most cases of diarrhea, the incidence and amplitudes of contractions are decreased, not increased. Segmenting contractions retard flow through the bowel; they do not increase flow. However, some diarrheas are associated with powerful propulsive contractions that resemble migrating clustered contractions.

3.09.2.2.1 Mechanisms of diarrhea production

It is unlikely that any one mechanism of diarrhea production is independent of other mechanisms. Additionally, there are confounding variables in diarrhea production including pancreatic disease, liver disease, etc. Combinations of mechanisms are present in specific diseases as follows (Gelberg, 2016).

Malabsorption with or without fermentation leads to osmotic diarrhea whether the cause is loss of digestive enzymes secondary to microvillus disruption, crypt or villus enterocyte death, or space occupying lesions of the lamina propria. Generally, this is a problem of the small intestine, but secondary colonic malfunction can occur because of malabsorption of bile salts and fatty acids that stimulate fluid secretion in the large intestine.

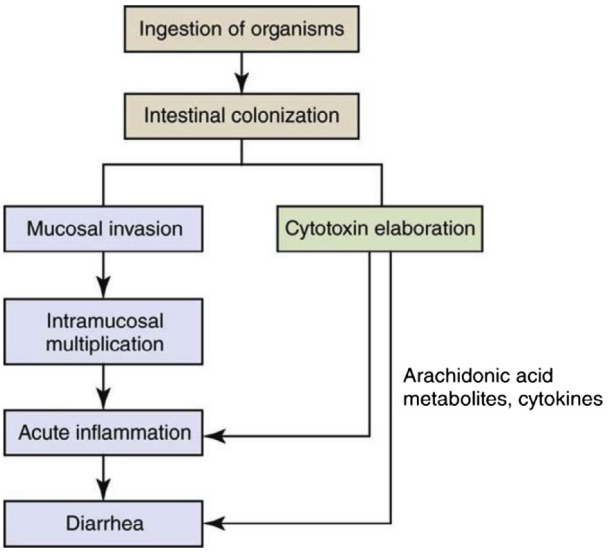

Fig. 14 Schematic diagram of the mechanism of invasive and cytotoxin-mediated bacterial inflammation. Adapted from Cominelli, F., Arseneau, K.O., Blumberg, RS, et al. (2009). The mucosal immune system and gastrointestinal inflammation. In: Yamata, T. (ed.) Textbook of gastroenterology (5th edn.). West Sussex: Wiley-Blackwell. Zachary, J. F. and McGavin, M. D. (2016). Pathologic basis of veterinary disease (6th edn.). Copyright © 2016 by Mosby, Inc, an affiliate of Elsevier Inc.

Chloride (Cl^-) hypersecretion by the cystic fibrosis transmembrane regulator (CFTR) of a structurally intact mucosa. CFTR is regulated by kinases which are dependent on cyclic adenosine monophosphate (cAMP) which acts as a second messenger. Prostanoids, bacterial toxins, and protein kinases all increase cAMP, thus increasing Cl^- secretion. Ca ion (Ca^{2+}) also plays a role in opening Cl^- channels by increasing acetylcholine interaction with epithelial muscarinic receptors via cholinergic nerves in intestinal plexi. Through a different mechanism but also involving the CFTR, bicarbonate secretion is also increased. This osmotic activity results in a net efflux of fluid and electrolytes independent of permeability changes, absorptive capacity, or exogenously generated concentration gradients.

Exudation caused by an increased capillary permeability (protein-losing enteropathy) by leaky tight junctions between enterocytes.

Hypermotility generally is involved in diarrhea but usually not as a primary mechanism in domestic animals. Hypermotility is defined as an increased rate, intensity, or frequency of peristalsis. Theoretically, with decreased mucosal contact time, digestion and absorption of nutrients and water should be less efficient. It is suspected that decreased motility in some diseases allows for increased bacterial proliferation. Conversely, some enterotoxins can stimulate intestinal motility in some motility disorders of humans such as achalasia, Hirschsprung's disease, and inflammatory bowel disease. Diarrhea occurs when there is an alteration in the network of interstitial cells of Cajal within the smooth muscle of the bowel wall. Whether this is a cause or effect of bowel motility disorders is not known.

TLRs and associated molecules produced by enterocytes and leukocytes are very important in the regulation of intestinal inflammation and in the host's response to intestinal pathogens. Intestinal inflammation can lead to neoplasia (Arthur et al., 2012).

M cells regulate the presentation of antigens to GALT.

Other factors (prostaglandins, leukotrienes, and PAF) act on enteric nerves to induce neurotransmitter-induced intestinal secretion by crypt cells.

Cell damage is possibly a consequence of inflammation mediated by T lymphocytes or proteases and oxidants produced by mast cells. T lymphocytes also may affect epithelial cell maturation, causing villous atrophy and crypt hyperplasia.

Cell death can result from pathogen invasion into enterocytes, multiplication of the pathogen, and extrusion of the affected enterocytes. These changes lead to notable distortion of villus architecture with a lack of mature absorptive enterocytes accompanied by nutrient malabsorption and osmotic diarrhea.

Mast cells of the lamina propria are in close association with enteric neurons and the enteric vasculature. They release histamine, prostaglandins, 5HT, and proteolytic enzymes that also play a role in diarrhea production.

The nuts and bolts of the process are complicated. Pathogens enter or attach to enterocytes, and may release enterotoxins. This triggers the enterocytes to release cytokines (IL-8) which activate resident macrophages and recruit new blood-borne macrophages into the lamina propria. The activated macrophages release soluble factors (histamine, serotonin, and adenosine) that increase intestinal secretion of chloride and water and inhibit absorption. Other factors (prostaglandins, leukotrienes, and PAF) act on enteric nerves to induce neurotransmitter-mediated intestinal secretion. The subsequent cell damage is possibly a consequence of inflammation mediated by T-cells or proteases and oxidants secreted by mast cells (**Fig. 15**). T-cells also affect epithelial cell growth producing villus atrophy and crypt hyperplasia. Cell death results from pathogen invasion, multiplication, and extrusion. The end result is marked distortion of villus architecture accompanied by nutrient malabsorption and osmotic diarrhea (Gelberg, 2016).

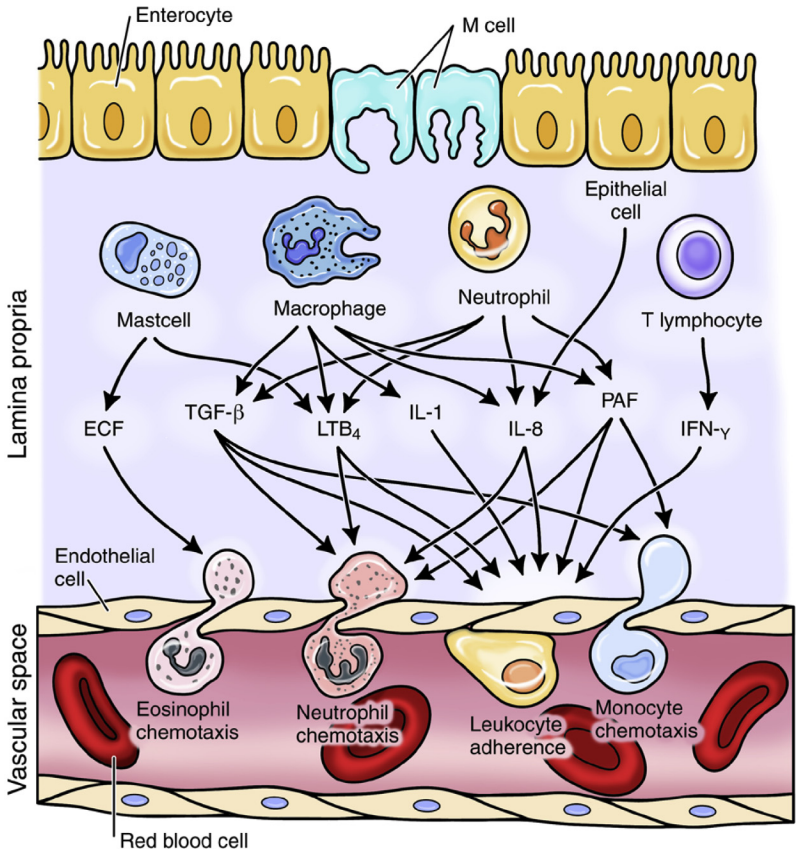

Fig. 15 Schematic diagram of chemotactic factors during intestinal inflammation. Eosinophil chemotactic factor (ECF), interferon-γ (IFN-γ), interleukin (IL), leukotriene B_4 (LTB_4), platelet activating factor (PAF), and transforming growth factor-β (TGF-β). Adapted from Cominelli, F., Arseneau, K. O., Blumberg, R. S., et al. (2009). The mucosal immune system and gastrointestinal inflammation. In: Yamata T. (ed.) Textbook of gastroenterology (5th edn.), West Sussex: Wiley-Blackwell.

3.09.2.2.2 Consequences of diarrhea

Intestinal fluid loss in the absence of replacement therapy gives rise to dehydration and acidosis secondary to electrolyte imbalances. Dehydration also causes hypovolemia and hemoconcentration with inadequate tissue perfusion. Energy generation shifts to anaerobic glycolysis leading to hypoglycemia and ketoacidosis. Acidosis, a lowering of blood and tissue pH, affects pH-dependent enzyme system function which is further exacerbated by HCO_3^- loss in fecal fluid and inadequate renal excretion of H^+ and inadequate absorption of HCO_3^-. The increase in intracellular H^+ and decrease in intracellular K^+ lead to decreased neuromuscular control of myocardial contraction causing a further reduction in tissue perfusion and commencement of a vicious cycle (Gelberg, 2016).

3.09.2.2.3 Intestinal models

A variety of rodent and nonrodent animal models including knockouts have been employed for comparative toxicologic studies. Among these species, pigs are omnivorous like humans. Their digestive physiology is also similar to that of humans. They can be reared in a gnotobiotic state. While monogastric, their stomach has a species specific feature, the torus pyloricus, a prominent muscular outpouching of uncertain function. Porcine Peyer's patches occur in a continuous band along the antimesenteric length of the small and large intestine; a 2-cm-wide lymphoid aggregation is at the ileocecal valve (i.e., cecal tonsil). Xenobiotic-induced injury to the porcine stomach or intestine often results in degeneration, ulceration, or hemorrhage, as in other species. The P450 system of swine has been partially characterized, and its metabolic pathways are similar to humans, with significant overlap in substrate specificity. Total P450 in conventional pigs is similar to that of humans (Swindle et al., 2012).

Intestinal xenografts have been proven to mimic the anatomy, physiology, and disease production of the host species (Thulin et al., 1991). Other models include exfoliated enterocytes (Rolsma et al., 1994), intestinal loops/explants, Ussing chambers, human colonic carcinoma (Caco-2) cell culture (Hidalgo et al., 1989) with high-throughput screening and Parallel Artificial Membrane Permeability Assay (PAMPA) (Avdeef et al., 2004) among others.

3.09.3 Toxic Mechanisms

Some toxic substances produce only one major pathophysiological endpoint, whereas others produce many pathophysiological changes. The most prominent pathophysiological mechanisms of gastrointestinal toxicity are listed in **Table 1** along with examples of causative factors.

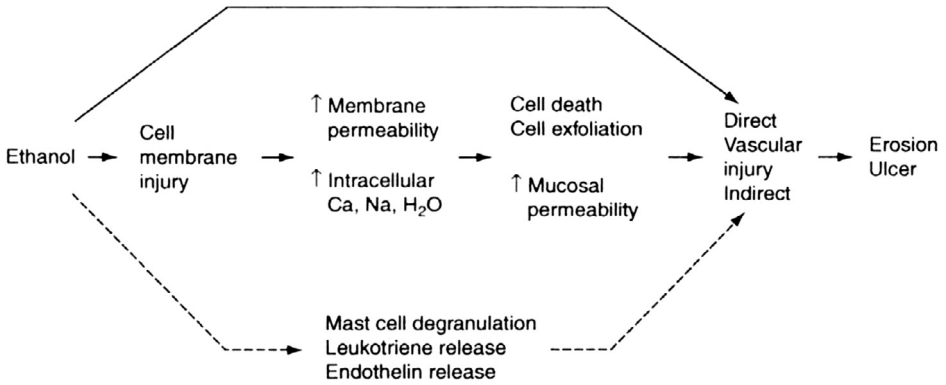

Fig. 16 Pathogenesis of alcohol-related gastric injury. Szabo, S. and Vincze, A. (2010). Comprehensive toxicology (10 vols.), pp. 171–180. Elsevier.

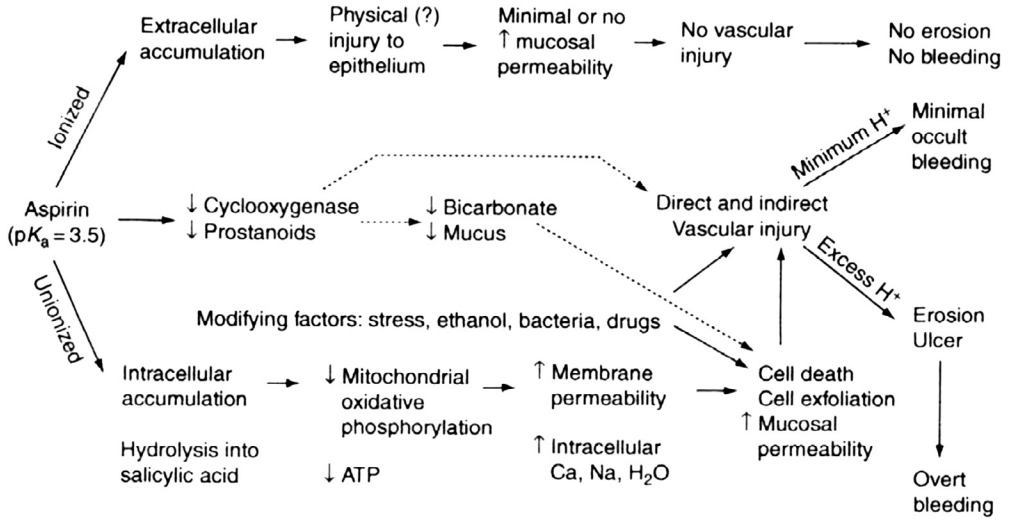

Fig. 17 The pathophysiology of aspirin toxicity. Szabo, S. and Vincze, A. (2010). Comprehensive toxicology (10 vols.), pp. 171–180. Elsevier.

3.09.3.1 Direct Effects on Cell Membranes

3.09.3.1.1 Alcohol

A number of ingested substances can induce toxic effects by direct actions on gastrointestinal epithelial cell membranes. Examples include alcohol (**Fig. 16**) and other organic solvents, aspirin-like drugs (**Fig. 17**), bile acids, sodium chloride, and certain constituents of edible plants. High concentrations of ethyl alcohol (>40%) can cause direct damage to esophageal and gastric epithelial cell membranes. Alcohol disorders the lipids in the outer bilayer of membranes and may increase their fluidity (Geall et al., 1970). Changes in membrane order lead to cell damage that can be measured as a decrease of transmucosal potential difference (Svanes et al., 1982). Bile acids, aspirin, and salicylate exhibit similar effects (Black et al., 1973; Kasbekar, 1973). Aspirin and salicylate can alter production of prostanoids, products of the cyclooxygenase pathway of arachidonic acid metabolism (**Fig. 18**). Exposure of the gastric mucosa to a high concentration of alcohol is associated with increased synthesis of prostaglandins and prostacyclin (Smith et al., 1991). However, the prostanoid substances may help counteract alcohol-induced injury because treatment with indomethacin exacerbates alcohol-induced damage. Other eicosanoids such as leukotriene A_4 may participate in alcohol-induced cell damage since inhibitors of leukotriene synthesis reduce the disruption caused by alcohol (French, 1991). Thus, prostaglandins may protect the gastric mucosa from ethanol damage, while leukotrienes contribute to the damage. Depletion of intracellular glutathione (GSH) has also been implicated in alcohol injury to mucosal cells (Hauser and Szabo, 1991; Victor et al., 1991). The levels of GSH decline in proportion to the degree of alcohol injury and treatment with prostaglandin E_2 can mitigate alcohol injury. *N*-ethylmaleimide prevents prostaglandin-induced protection against alcohol injury.

A single alcohol drinking binge causes a rapid increase in serum endotoxin, bacterial translocation from the intestine, and a prolonged increase in circulation of acute phase proteins. The increased endotoxin levels are associated with increased levels of inflammatory cytokines such TNF-α and IL-6, and the chemokine (monocyte chemotactic protein) MCP-1 (Bala et al., 2014). Chronic administration of alcohol is associated with enhanced expression of a number of growth factors, including EGF and transforming growth factor-alpha (TGF-α). These growth factors are thought to protect the gastric mucosa against acute injury

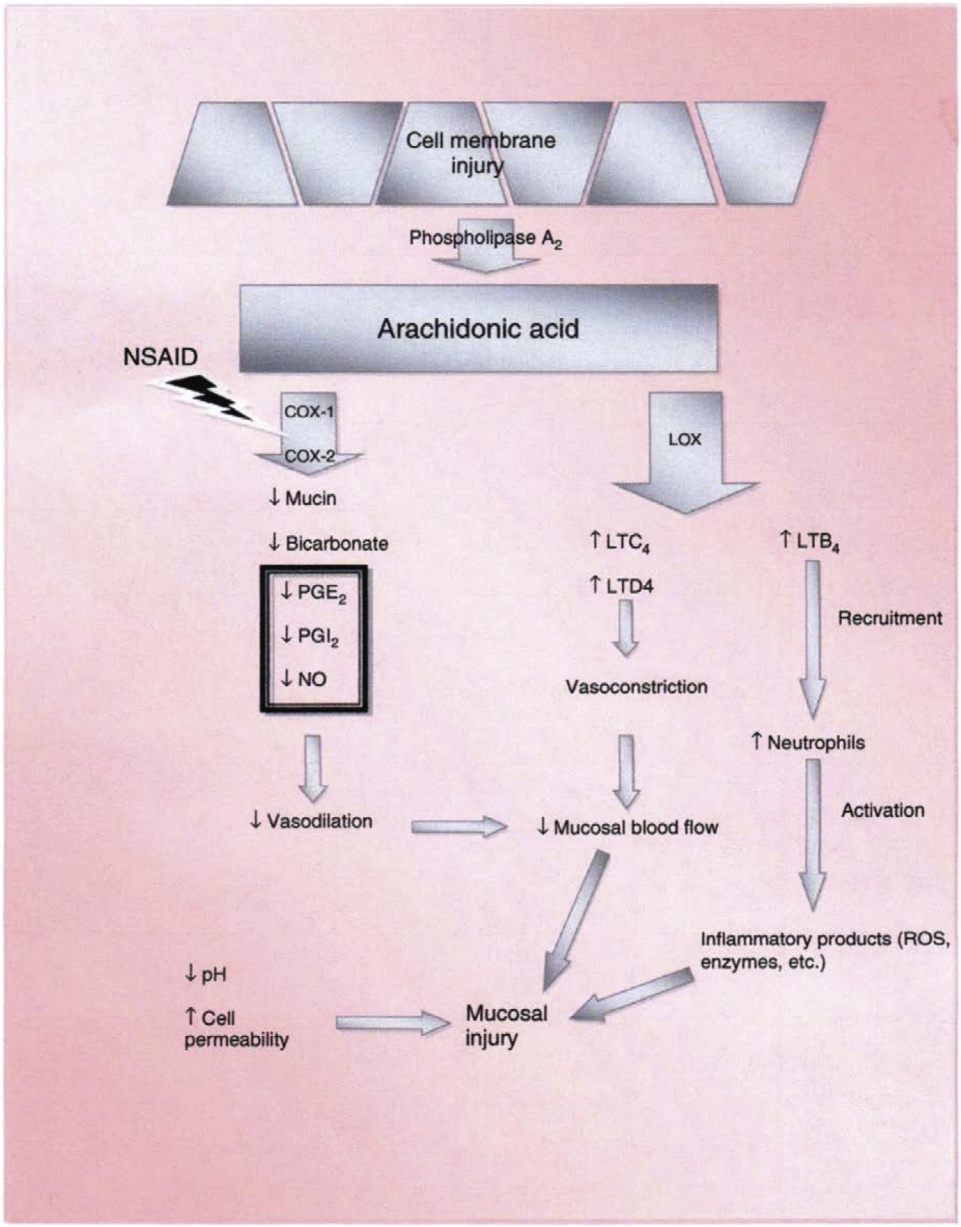

Fig. 18 Mechanism of nonsteroidal antiinflammatory drug (NSAID)-induced gastrointestinal erosion and ulceration. Inhibition of cyclooxygenase (COX) by NSAIDs results in decreased production of mucin, bicarbonate, prostaglandins (PGE_2, PGI_2), and nitric oxide (NO). Decreases of the latter three compounds result in decreased vasodilation within the gastric mucosa, predisposing it to injury. With the COX pathway inhibited, the lipoxygenase (LOX) pathway is favored, increasing production of leukotrienes (LTC_4, LTD_4) that promote vasoconstriction; coupled with decreased vasodilation, there is decreased gastrointestinal mucosal blood flow. Increases in LTB_4 trigger attraction and activation of neutrophils and release of reactive oxygen species (ROS) and enzymes, which promote mucosal injury. Gwaltney-Brant, M. (2010). Comprehensive toxicology (10 vols.), pp. 159–161, Elsevier.

and may explain the observation that adaptation of the gastric mucosa to chronic alcohol administration is associated with increased cell proliferation and increased expression of mucosal EGF and TGF-α (Tarnawski et al., 1992). The ability of chronic alcohol exposure to lead to hyperregeneration of the gastric mucosa could be responsible for the suspected carcinogenic effect of alcohol (Simanowski et al., 1995). Generation of acetaldehyde, in addition to elaboration of growth factors, has also been implicated suggesting a potential role of gastric mucosal alcohol dehydrogenase (ADH) in the deleterious effects of alcohol on gastrointestinal mucosa. The human gastric mucosa contains three isoforms of ADH, and enzyme activity is inversely correlated with age and is higher in males than in females (Moreno and Pares, 1991; Moreno et al., 1994). However the overall contribution of gastric ADH to metabolism of alcohol and generation of acetaldehyde is thought to be slight (Brown et al., 1995; Gugler, 1994; Levitt et al., 1994).

3.09.3.1.2 Lectins

Lectins are plant glycoproteins that can interact specifically with certain carbohydrates on cell membranes, including intestinal epithelial cells (Gelberg et al.,1992). The toxic effects of lectins are dependent on their source, species, and dose. Their potential effects range from depression of growth to lethality (Reddy and Hayes, 1989). The toxicity of ingested lectins often involves their binding to the luminal surface of mucosal epithelial cells where they disturb the function of the brush border membrane (Nakata and Kimura, 1985). Binding of lectins to crypt cells is followed by nonspecific inhibition of both active and passive absorption of all nutrients, including water, across the intestinal mucosa. Inhibition of absorption appears to account for growth depression. Lectins can also cause necrosis of intestinal epithelial cells (King et al., 1980). The mitogenic effects of lectins on lymphocytes can be inhibited by several neuropeptides, including VIP, β-endorphin, and somatostatin. It is not known whether these peptides protect mucosal cells (Krco et al., 1986).

3.09.3.1.3 Cholesterol transport inhibitors

Cholesterol transport inhibitors, such as ezetimibe, reduce circulating cholesterol esters by blocking NPC1L1 receptor essential for lipid micelle uptake and by inhibiting transport across the intestinal epithelium (Hui et al., 2008).

3.09.3.2 Stimulation of Mucosal Proliferation

3.09.3.2.1 Dioxins

Gastrin, EGF, TGF-α, and other endogenous growth factors can stimulate gastrointestinal crypt cell proliferation. Mucosal hyperplasia can also be associated with ingested chemicals, such as 2,3,7,8-tetra-chlorodibenzo-*p*-dioxin (TCDD) and probably other related polychlorinated dioxins (Pohjanvirta and Tuomisto, 1994). TCDD binds to a cytosolic protein called the Ah receptor (Burbach et al., 1992). TCDD binding converts the receptor to its activated, functional form that binds to the DNA of the CYP 1A1 gene for cytochrome P450. Binding of TCDD to the DNA increases the rate of transcription of the cytochrome P450 gene. The intestinal mucosa contains significant levels of Ah receptors and cytochrome P450 CYP 1A1 (Mason and Okey, 1982). TCDD can induce mucosal hyperplasia in some animal species. It has been suggested to exert a disinhibitory effect on gastrin release, which may explain the mucosal hyperplasia (Potter et al., 1983). It also induces a "wasting syndrome" that is characterized by failure of appetite (Pohjanvirta and Tuomisto, 1994). Hypophagia and severe weight loss are the principal signs in most species of TCDD intoxication. Most animals given lethal doses of TCDD die within 2 weeks. Those that live longer often develop tumors (Pohjanvirta and Tuomisto, 1994).

3.09.3.2.2 Alcohol

Chronic ingestion of alcohol can lead to proliferation of mucosal epithelium. The effect of alcohol is likely mediated by peptide growth factors.

3.09.3.2.3 Pancreatic enzyme preparations

Fibrosing colonopathy is a proliferative disorder of the colonic submucosa associated with high doses of pancreatic enzymes (lipase, amylase, and protease) required for management of pancreatic insufficiency in children with cystic fibrosis (Borowitz et al., 1995; Smyth et al., 1995). Inadequate enzyme therapy may be associated with various abdominal symptoms (bloating, abdominal pain), frequent stools or overt diarrhea. Often steatorrhea (excess fat in feces) is a major sign of inadequate enzyme (specifically lipase) treatment. Continued poor digestion leads to retarded weight gain and growth. If signs of poor digestion and malabsorption persist, the practice has been to increase enzyme doses until proper digestion is achieved. Fibrosing colonopathy seems to occur only in children under 12 years receiving >2 mg kg^{-1}, equivalent to 6000 lipase units kg^{-1}, per meal (Borowitz et al., 1995).

Fibrosing colonopathy is characterized by fusiform, long-segment stenosis of the colon that can lead to obstruction. There is submucosal thickening caused by excessive proliferation of fibroblasts resulting in a thick layer of fibrous tissue (Borowitz et al., 1995). The condition may be manifest as colonic obstruction with abdominal pain and may be more common in patients with histories of meconium ileus, distal intestinal obstruction syndrome, and prior colonic surgery. The strictures associated with fibrosing colonopathy require surgical resection.

Many delayed release pancreatic enzyme products are coated with methacrylic acid copolymer, which has been implicated in the toxicity. Theoretically, undissolved plastic polymer migrates across the mucosa, a process that is perhaps facilitated by the presence of sodium lauryl sulfate present in many formulations. Once in the lamina propria, the small particles of plastic stimulate proliferation of fibroblasts that attempt to encapsulate the fragments of methacrylate. The plastic-induced fibrosis eventually results in stricture formation and colonic obstruction. Ironically, the putative fragments cannot easily be visualized by microscopic examination of pathological specimens because the copolymer is crystal-clear.

3.09.3.3 Inhibition of Mucosal Proliferation

The most notable substances that inhibit normal proliferation and turnover of mucosal epithelial cells are the antineoplastic or anticancer drugs. Antineoplastic agents are used in clinical oncology because the drugs are inherently toxic and are designed to kill host cells (O'Keefe and Harris, 1990). The toxic effects of many members of this class of agents occur in tissues with high growth fractions, such as gastrointestinal epithelium. In general, the alkylating agents, such as nitrogen mustards (melphalan,

cyclophosphamide, and chlorambucil) are the most highly toxic to dividing mucosal cells. These agents cause mitotic arrest, cellular hypertrophy, disintegration of epithelial cells, and sloughing of the epithelium. In high-dose chemotherapy protocols they can predispose to bacterial sepsis of the gastrointestinal tract. The nitrogen mustard drugs alkylate DNA and thereby interfere with DNA synthesis and cell division (Chabner et al., 1996). These drugs are most cytotoxic to rapidly proliferating tissues in which a large proportion of the cells are dividing.

Antifolate drugs, such as methotrexate, also produce significant toxicity to the gastrointestinal epithelium. Methotrexate inhibits dihydrofolate reductase and interferes with folate-dependent enzymes required for synthesis of purines and thymidylate. Methotrexate is toxic to all rapidly dividing normal cells including those of the intestinal epithelium. The drug can induce swelling and cytoplasmic vacuolation of epithelial cells within 6 h followed by enterocyte loss and leukocyte infiltration into the submucosa. Mucositis (inflammation) peaks several days after drug administration. Animal studies indicate that methotrexate is extremely toxic to the gastrointestinal tract if elemental liquid diets are the only source of enteral nutrition. It is believed that elemental diets change the pharmacokinetics of methotrexate or further diminish epithelial cell turnover rates (McAnena et al., 1987). Methotrexate-induced gastrointestinal toxicity is also enhanced dramatically in mice by aspirin-like drugs (Badr and Chen, 1985). Interestingly, transgenic mice carrying a mutant dihydrofolate reductase gene display resistance to methotrexate toxicity to the gastrointestinal tract (Isola and Gordon, 1986).

The pyrimidine analog, 5-fluorouracil (5-FU), and related drugs are less likely than alkylating agents or antifolate drugs to induce gastrointestinal toxicity. However, 5-FU is frequently associated with mucosal ulceration and diarrhea. In animal studies, the major toxicity is gastrointestinal disturbance, including decreases in brush border enzyme activity, that requires up to 72 h for recovery after 5-FU administration (Au et al., 1987; Kralovanszky et al., 1993). 5-FU also causes gastrointestinal toxicity, nausea, vomiting, and diarrhea in humans (Palmeri et al., 1990). 5-FU is a biochemical mimic of uracil that blocks thymidylate synthase by preventing thymidylate synthase-mediated methylation. 5-FU also inhibits RNA processing and can be incorporated into DNA as flurodeoxyuridine triphosphate.

cis-Diamminedichloroplatinum (cisplatin) is an inorganic water-soluble, platinum-containing complex that can cause profound gastrointestinal symptoms. In mice, cisplatin causes a significant reduction in crypt cell production leading to villus stunting, loss of digestive enzymes, and diminished function (Allan and Smyth, 1986; Smith et al., 1988). Cisplatin forms adduct with DNA and cross-links adjacent guanine residues. It is known that intracellular levels of glutathione influence the sensitivity of cells to cisplatin. Coadministration of mercaptoethanesulfonate in mice reduces the gastrointestinal toxicity of cisplatin as assessed by cellular architecture, villus recovery rate, and brush border enzyme activity. The antitumor efficacy of cisplatin in mice is not affected (Allan et al., 1986). In higher animals and humans, cisplatin is extremely emetogenic.

Paclitaxel (taxol) is a natural product from the bark of the Western yew tree that is an inhibitor of mitosis. It differs from the vinca alkaloids in that it promotes, rather than inhibits, microtubule formation. In a limited number of patients it induces epithelial necrosis characterized by mitotic arrest that appears to be secondary to accumulation of polymerized microtubules (Hruban et al., 1989).

3.09.3.4 Damage to Intrinsic Nerves/Ganglia/Neurons

3.09.3.4.1 Surfactants

Topical gastrointestinal cationic surfactants, such as benzalkonium chloride (a mixture of compounds) or benzyldimethyltetradecylammonium chloride (BAC), have been reported to destroy intrinsic neurons in the myenteric plexus of the small intestine (Fox et al., 1983). Serosal application of BAC damages longitudinal and circular musculature, myenteric plexi, and extrinsic nerves (Luck and Bass, 1994). Two weeks after treatment with BAC, the number of muscle cells in both the longitudinal and circular muscle layers returns nearly to control values. The damage to nerves is more persistent. It has not been determined whether ingested surface active agents penetrate the mucosal barrier to induce nerve or muscle damage. Experimental treatment with BAC that produced regional loss of myenteric neurons in the rat jejunum was found not to impair gastrointestinal transit through the short region of neural ablation (Luck et al., 1993).

3.09.3.4.2 Capsaicin

Capsaicin, the pungent ingredient in many hot peppers and paprika, produces striking pharmacological effects on sensory neurons in the gastrointestinal tract and elsewhere (Buck and Burks, 1986). Capsaicin occurs naturally in many pepper plants related to *Capsicum annuum*, which grows indigenously in tropical America. Capsicum has been used globally in food for 7000 years (Mózsik et al., 2007) and is especially popular in hot climates where normal dietary intake may reach 1 mg kg^{-1} daily (Szallasi, 1995). The popularity of capsaicin-containing foods in hot climates has been attributed to its ability to cause profuse perspiration, termed gustatory sweating (Lee, 1954). A related compound, resiniferatoxin, was first isolated from *Euphorbia resinifera*, an African plant, but rarely is ingested as food or condiment. Capsaicin activates small diameter sensory nerve fibers in the mucosa and wall of the gastrointestinal tract to induce, especially in the oral mucosa, the sensation of heat, and pungency associated with spicy foods. Activation of sensory neurons by capsaicin promotes release of neurotransmitters from both the central and peripheral terminals of these neurons. The neurotransmitters released are principally substance P and CGRP. The peptides initiate a cascade of proinflammatory events and transmit nociceptive information to the central nervous system (Buck and Burks, 1986). Repeated exposure leads to diminished effects. In high concentrations, capsaicin and related compounds can produce disruption of neural function and eventual destruction of sensory nerves in sensitive species. (Buck et al., 1981, 1982a; Miller et al., 1982a). The neurotoxicity of

capsaicin and related compounds appears to be related to blockage of retrograde transport of nerve growth factor (Holzer, 1991; Miller et al., 1982b). The covalent binding of tritium-labeled capsaicin to hepatic microsomal protein is significantly inhibited by reduced glutathione, implying the formation of a reactive intermediate during metabolism of capsaicin (Miller et al., 1983). Cytochrome P450 2E1 catalyzes the conversion of capsaicin to a reactive species capable of covalent binding to tissue macromolecules (Surh and Lee, 1995). However, no long-lasting deleterious effects on gastrointestinal sensory neurons have been documented in humans, even in those who ingest large amounts of capsaicin daily. Animal studies, on the other hand, indicate that intragastric capsaicin, even in moderate doses, can produce changes in gastrointestinal function. For example, intragastric doses of 0.1 mg kg^{-1} of capsaicin in dogs produces pronounced excitatory effects on colon contractions (Shibata et al., 1995). The contractile effects of intragastric capsaicin in the colon are inhibited by a muscarinic antagonist implying that a cholinergic neural pathway is involved. It is possible that low intragastric concentrations of capsaicin can activate sensory nerves that signal fullness to the brain and thereby initiate the gastrocolic reflex generally associated with ingestion of a meal (Barber et al., 1987).

Capsaicin decreases gastric basal output, enhances the "nonparietal" (buffering) of gastric secretory responses, gastric emptying, and release of glucagon. Capsaicin prevents indomethacin- and ethanol-induced gastric mucosal injury while enhancing gastric transmucosal potential differences (GTPD). The capsaicin reactive receptors, TRVP1, CGRP, and SP, are present in the GI mucosa in patients with a variety of GI disorders. Their presence varies in acute and chronic disorders. Capsaicin sensitive afferent nerves have a key-role in the regulation of glucose absorption from the small intestine (due to a local increase of blood flow), glucose utilization, and release of glucagon (Mózsik et al., 2007).

3.09.3.4.3 Cannabinoids

The endocannabinoid system is widely distributed throughout the gut, with regional variation and organ-specific actions (**Fig. 19**). Among its functions are the regulation of food intake, nausea and emesis, gastric secretion, gastroprotection, gastrointestinal motility, ion transport, visceral sensation, intestinal inflammation, and cell proliferation. Cellular targets include the enteric nervous system, epithelial cells, and immune cells (Izzo et al., 2010).

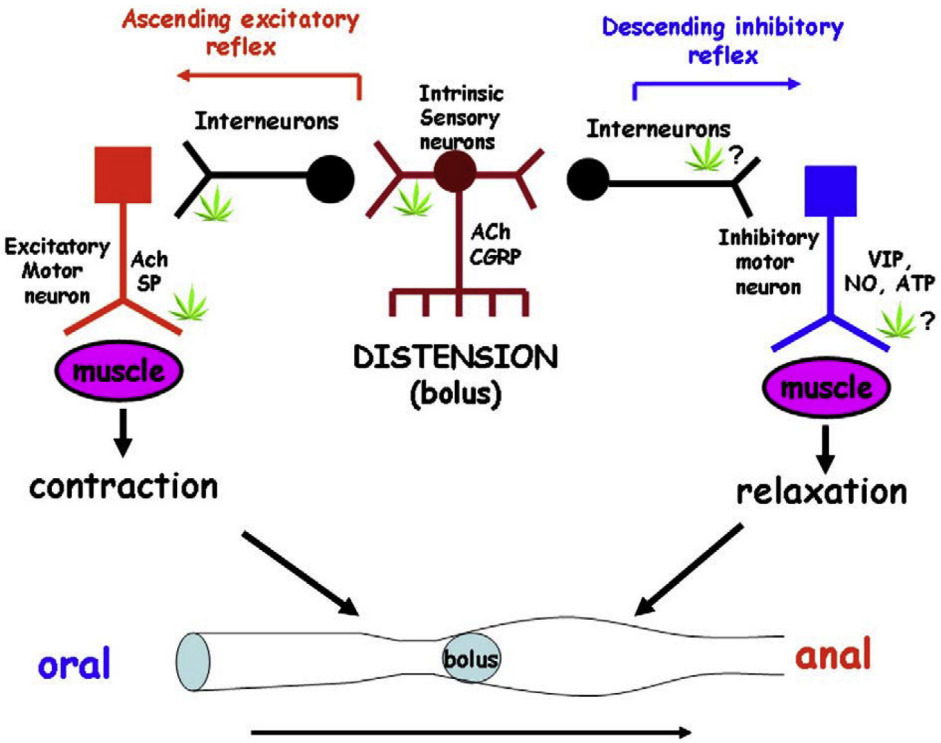

Fig. 19 Sites of action of cannabinoids in the enteric nervous system. Peristalsis, which occurs in response to the radial distension of the intestinal wall, is a coordinated pattern of motor behavior which occurs in the GI tract and allows the contents to be propelled in an anal direction. The pathways mediating peristalsis involve intrinsic sensory neurons and interneurons, as well as excitatory and inhibitory motor neurons. Acetylcholine (ACh) acting through both muscarinic and nicotinic receptors and tachykinins are excitatory neurotransmitters participating in the peristaltic activity, whereas VIP, nitric oxide (NO), and ATP (or a related purine) act as inhibitory mediators. CB1 receptor's (indicated with the marijuana leaf) immunoreactivity has been identified on intrinsic sensory neurons, ascending neurons, and final excitatory motor neurons projecting into longitudinal and circular muscles. Pharmacological evidence suggests that CBs inhibit both the ascending contraction (and concomitant acetylcholine and substance P (SP) release) and descending relaxation (and concomitant VIP release). Additionally, CBs inhibit CGRP release from intrinsic sensory neurons (Izzo and Sharkey, 2010).

CB1 and CB2 are cognate receptors for all types of CB agonist endocannabinoids, phytocannabinoids, and synthetic CBs (Pertwee et al., 1996). CB receptors are largely distributed in the enteric nervous system (Duncan et al., 2005). Both CB1 and CB2 receptors are on enteric neurons, nerve fibers, and terminals. The CB1 receptor is most dense in the myenteric and submucosal plexi (Duncan et al., 2005; Wright et al., 2008). Enteric ganglia consist of motor neurons, interneurons, and intrinsic primary afferent neurons; CB1 and CB2 receptors are on all of the functional classes of enteric neurons (Izzo et al., 2010). CB1 and CB2 receptors are not present on inhibitory motor neurons containing nitric oxide synthase (Kulkarni-Narfa and Brown, 2000; Coutts et al., 2002; Storr et al., 2004; Duncan et al., 2008).

Cannabinoid receptor expression has not been elucidated in any species except for the enteric nervous system. There are regional variations of endocannabinoids in the gut; 2-AG is higher in the ileum than in the colon, and anandamide is higher in the colon than in the ileum (Izzo et al., 2001; Pinto et al., 2002).

The endogenous CB system regulates energy balance and food intake in the brain and other areas including the GI tract (Bellocchio et al., 2008). Food deprivation increases anandamide levels. There is upregulation of CB1 receptor expression in vagal afferent neurons of the GI tract (Gómez et al., 2002; Burdyga et al., 2004). CB1 receptors, located on vagal afferent neurons, may be involved in CB-induced modulation of appetite and anandamide may act as an appetite alarm in the intestine (Storr and Starkey, 2007; Borrelli and Izzo, 2009).

CBs are antiemetic in animal models including those of motion sickness and those given antineoplastic drugs, morphine, and radiation (Darmani, 2001a; Simoneau et al., 2001; Van Sickle et al., 2001, 2003, 2005; Darmani et al., 2007; Ray et al., 2009; Cluny et al., 2008; Parker et al., 2009). CB1 and CB2 receptors are present in the dorsal vagal complex region perhaps explaining their antiemetic effect (Darmani, 2001b; Van Sickle et al., 2001, 2003, 2005; Sharkey et al., 2007).

Activation of CB1 receptors in rodents decreases acid production (Adami et al., 2002, 2004; Coruzzi et al., 2006) via its effects on vagal efferent pathways to the gastric mucosa, not parietal cells. CB1 receptors are present on human parietal cells (Pazos et al., 2008). CB receptor agonists inhibit transient lower esophageal sphincter relaxation in dogs and ferrets via CB1 activation (Lehmann et al., 2002; Partosoedarso et al., 2003; Beaumont et al., 2009). In dogs gastroesophageal reflux is decreased (Lehmann et al., 2002; Beaumont et al., 2009).

CB receptor agonists act on prejunctional CB1 receptors reducing smooth muscle contractility. This occurs in varying areas of the GI tract in animals as compared to humans (Izzo et al., 2008).

In rodents, plant-derived, endogenous and synthetic CB receptor agonists reduce gastric emptying (Calignano et al., 1997; Izzo et al., 1999a; Landi et al., 2002; Di Marzo et al., 2008; Abalo et al., 2009), upper GI transit (Colombo et al., 1998; Izzo et al., 1999b, 2000; Landi et al., 2002; Carai et al., 2006; Izzo and Camilleri, 2009), and colonic propulsion (Pinto et al., 2002).

CB1 and CB2 receptors regulate pathophysiological intestinal motility, apoptosis, and inflammation (Ligresti et al., 2003; Greenbough et al., 2007; Cianchi et al., 2008; Wang et al., 2008; Izzo and Camilleri, 2009; Izzo et al., 2010). Downregulation of CB1 receptors and upregulation of CB2 receptors are present in colon cancer and may have antiproliferative, antimetastatic, and proapoptotic effects either via activation of CB1 or CB2 receptors or through increased endocannabinoids (Izzo et al., 2010). Genetic differences in endocanniboid levels may be related to various gut disorders. In summary, the endocannabinoid system is an important regulatory factor in the GI tract, controlling digestion and host defense (Izzo et al., 2010).

3.09.3.5 Reduction of Mucosal Blood Flow

3.09.3.5.1 Nonsteroidal antiinflammatory drugs (NSAIDS)

The most common and usually most serious toxic effects of NSAIDs are damage to the gastrointestinal tract (Carson and Willett, 1993). The gastrointestinal toxicity of NSAIDs is dose related and increases with age over 60 years (Carson and Willett, 1993; Roderick et al., 1993). Fifteen to twenty percent of patients who regularly take these agents develop esophageal, gastric, or duodenal ulcers. Approximately 3% develop bowel hemorrhage or perforation (Lanza, 1993). In equids, and humans, colonic ulcers may occur. NSAIDs increase the risk of lower GI bleeding and perforation similar to that of the upper GI tract (Sostres et al., 2013). While the gastric and duodenal mucosa are the most frequent targets of NSAID toxicity, lesions in other areas of the small intestine and colon occur (Kirsch, 1994). NSAIDs can induce peptic ulcers, petechiae, blood and protein loss into the gastrointestinal lumen, localized strictures, and bowel wall perforation (Bjarnason et al., 1993). The mechanisms of NSAIDs-induced mucosal damage are complex resulting in reduction in mucosal blood flow and direct and indirect damage to epithelial cells (**Fig. 18**). NSAID-induced mucosal damage follows a 6 h time course: early, neutrophil-independent toxicity and late, neutrophil-dependent toxicity (Nygard et al., 1994). Early changes include alterations in mitochondrial oxidative functions and inhibition of cyclooxygenase (COX). Death is generally from cardiopulmonary disease, terminal cancer, or multiorgan failure (Lanas, 2010).

Because NSAIDs are absorbed across the mucosa they uncouple oxidative phosphorylation (Bjarnason et al., 1993). Mitochondria become energy depleted. Energy depletion leads to disruption of ATP-dependent epithelial cell junctions increasing intestinal epithelial permeability. The increase in permeability and diminution of epithelial barrier functions allow exposure to bile acids, hydrogen ions, and bacteria. Formyl-methionyl-leucyl-phenylalanine (FMLP), a peptide released from bacteria, attracts and activates neutrophils (Granger et al., 1988). Bacteria in the GI lumen increase the ulcerogenicity of NSAIDs (Rainsford, 1989). Activated neutrophils attracted to the mucosal microvessels and lamina propria release reactive oxygen metabolites (ROMs), lysosomal proteases, and leukotriene B_4 (Granger et al., 1994). Myeloperoxidase, a hemoprotein peroxidase release by activated neutrophils into the extracellular medium, interacts with H_2O_2 to form an enzyme substrate complex with great oxidizing potential. Activated neutrophils produce large quantities of OCl^- by means of myeloperoxidase catalyzed oxidation of Cl^- (Granger et al., 1988). HOCl is

a powerful oxidizing agent. ROMs and lysosomal enzymes cause direct damage to epithelial cells. Leukotriene B_4 released by neutrophils is a powerful chemoattractant for additional neutrophils. In addition, leukotriene B_4 causes arteriolar vasoconstriction. As a result of this cascade, gastrointestinal epithelial cells become targets for bile acids, hydrogen ion, ROMs, and lysosomal enzymes.

The other pathway of NSAID toxicity that results in the reduction of mucosal blood flow and damage to epithelial cells is the ability of these drugs to inhibit COX. COX, the first enzyme in the prostaglandin synthetic pathway, converts arachidonic acid to unstable intermediates, cyclic endoperoxides PGG, and PGH. PGG and PGH can be transformed to prostaglandins, such as PGE_2, thromboxane A_2, or prostacyclin. Blockade of COX reduces formation of these prostanoids and shifts metabolism of arachidonic acid toward the lipoxygenase pathway, resulting in the formation of leukotrienes, most notably leukotriene C_4 and B_4. There are two major isoforms of COX, COX-1 and COX-2, which differ in their sensitivity to inhibition by individual NSAIDs (Meade et al., 1993). COX-1 is the constitutive form of the enzyme found in healthy tissues, while COX-2 is an inducible form that can be stimulated by several cytokines and inflammatory mediators (Mitchell et al., 1993). Most NSAIDs inhibit activity of both COX isoforms. Inhibition of COX-2 is associated with most of the beneficial effects of NSAIDs, while inhibition of COX-1 is associated with many of their adverse effects. Drugs that preferentially inhibit COX-2 may have fewer side effects than those that inhibit both isoforms (Meade et al., 1993). Inhibition of COX by NSAIDs results in two significant toxicological effects: reduction of formation of prostaglandins and increased formation of leukotrienes. Prostaglandins protect the gastrointestinal mucosa against luminal acid and other aggressive factors (Leung et al., 1989). The protective effects result from the ability of prostaglandins to stimulate secretion of mucus and bicarbonate (Eberhart and Dubois, 1995; Sababi et al., 1995). Reduction in mucosal synthesis of prostaglandins, including prostacyclin, is associated with mucosal damage (Whittle and Vane, 1984). Prostaglandin E_2 and prostacyclin are also vasodilators. Blockade of prostaglandin and prostacyclin synthesis may favor vasoconstriction and oppose prostaglandin-mediated tonic vasodilation. Increased metabolism of arachidonic acid by the 5-lipoxigenase pathway in the presence of inhibition of COX is thought to contribute to NSAID-induced gastrointestinal toxicity (Peskar et al., 1986). Leukotrienes C_4 and D_4 are vasoconstrictors, and leukotriene B_4 is a powerful chemoattractant of neutrophils (Bjarnason et al., 1993; Eberhart and Dubois, 1995). Leukotriene B_4-stimulated attraction and activation of neutrophils lead to release of lysosomal enzymes and microvascular occlusion. Epithelial cell damage is exacerbated by reduction in mucosal blood flow via a combination of vasoconstriction caused by leukotrienes and occlusion of microvessels by neutrophils (Kitahora and Guth, 1987). Thus, NSAIDs reduce production of protecting substances and increase production of damaging substances. The theoretical advantage of selective inhibitors of COX is their lack of effect on COX-1 and endogenously generated prostaglandins and prostacyclins (Levi and Shaw-Smith, 1994; Seibert and Masferrer, 1994; Seibert et al., 1994). Preservation of prostaglandin and prostacyclin production protects the mucosa via bicarbonate and mucus production, mucosal restitution, and regenerative repair and helps maintain mucosal blood flow. Prostaglandins also decrease secretion of gastric acid by direct effects on oxyntic cell prostaglandin receptors, and lower concentrations of acid at the mucosal surface enhance mucosal restitution (Allen et al., 1993). However, reduction of acid secretion alone is not sufficient to counteract the damaging effects of NSAIDs (Ivey, 1988; Wallace et al., 1993).

3.09.3.6 Emesis

Ingested substances can activate emesis by two mechanisms, locally at the gastrointestinal mucosa and hematogenously at central nervous system sites (**Table 5**). Emesis is usually associated with nausea. Pharyngeal stimulation can induce gagging and emesis without associated nausea, but most chemical substances that induce emesis also produce prodromal nausea.

3.09.3.6.1 Substances acting on mucosa

Toxic substances that act directly on the mucosa to induce emesis activate sensory nerves that travel over vagal and sympathetic afferent pathways to brain medullary centers that control vomiting. The best characterized of the mucosal sensory emetic pathways involves activation of 5-HT_3 receptors at the peripheral ends of sensory nerves (Miller and Nonaka, 1992). 5-HT_3 receptors are ligand-gated ion channels that mediate rapid nerve depolarization including sensory nerves. The vagus nerve is dense with 5-HT_3 receptors and innervates most abdominal viscera, including the gastric and duodenal mucosae (Ireland and Tyres, 1987). Toxic chemicals can act either directly or indirectly at 5-HT_3 receptors to activate sensory nerves or act at other (non-5-HT_3) sites on nerve endings. Certain anticancer drugs, especially the highly emetogenic agents such as cyclophosphamide, carmustine, dactinomycin, and cisplatin, interact with mucosal enterochromaffin cells to promote release of large quantities of 5-HT (Miller and Nonaka, 1992). The 5-HT acts at 5-HT_3 receptors on mucosal sensory nerves to initiate emetic signals. Bilateral abdominal vagotomy and bilateral splanchnic nerve section completely inhibit emesis induced by cyclophosphamide, nitrogen mustard-*N*-oxide, and dactinomycin (Fukui et al., 1993). Emesis induced by antineoplastic chemotherapeutic agents can be reduced or prevented by administration of 5-HT_3 antagonists, such as granisetron or ondansetron. 5-HT_3 antagonists are also effective in blocking emesis induced by irradiation (Smith et al., 1989). However, 5-HT_3 antagonists are not effective against other emetogenic substances, such as copper sulfate, protoveratrine, or apomorphine (Andrews and Hawthorn, 1987; Andrews et al., 1988).

The activation of vagal sensory neurons by 5-HT_3 receptor-mediated events or other mechanisms causes release of proemetic neurotransmitters from the central terminals of sensory fibers in the solitary nucleus and in the area subpostrema. The highest concentration of 5-HT_3 receptors in the mammalian brainstem is the area subpostrema (Pratt and Bowery, 1989; Reynolds et al., 1989). Brainstem 5-HT_3 receptors appear to be associated with presynaptic sites and serve primarily to modulate release of neurotransmitters. Presumably, activation of the central 5-HT_3 receptors enhances release of proemetic substances, thereby activating the chemoreceptor trigger zone of the area postrema and the nearby emetic center. Several cancer chemotherapeutic agents,

most notably cisplatin, appear to act at least in part by effects in the central nervous system that promote release of 5-HT and subsequent activation of 5-HT_3 receptors. Combined vagal and splanchnic nerve transections do not completely prevent vomiting in response to peripherally administered cisplatin, whereas emesis is reduced by administration of 5-HT_3 antagonists (Miller and Nonaka, 1992).

3.09.3.6.2 Substances acting at central sites

Blood-borne emetic agents that act at central sites are direct or indirect agonists at dopamine D_2 receptors. Apomorphine, L-dopa, and bromocriptine are examples of these types of agents. Dopamine D_2 receptors are well represented in the chemoreceptor trigger zone of the brainstem area postrema (Stefanini and Clement-Cormier, 1981). Phenothiazine drugs with significant dopamine D_2 antagonists properties, such as chlorpromazine, prochlorperazine, and promethazine, can block the emetic actions of direct and indirect dopamine D_2 agonists. Emetine, the principal ingredient of ipecac and opiates, such as morphine, appears to act nonspecifically at the chemoreceptor trigger zone to initiate emesis. The area postrema is not protected by a blood–brain barrier, thus allowing blood-borne chemicals to penetrate. The most effective general antiemetic drugs are those that block 5-HT_3 receptors such as ondansetron and granisetron or those that block both dopamine D_2 receptors and 5-HT_3 receptors such as metoclopramide (Stewart, 1990).

3.09.3.7 Disruption of Intracellular Signal Transduction

3.09.3.7.1 Cholera toxin

Intestinal epithelial cells express receptors for certain toxins. Probably the best characterized of such receptors is that for the heat-stable enterotoxin of *E. coli* (Cohen et al., 1988). The *E. coli* enterotoxin (St_a) receptor is a novel transmembrane guanylate cyclase with an extracellular toxin-binding domain. It is expressed primarily in the differentiated cells of the villus. Similarly, cholera toxin specifically binds to a GM_1-ganglioside receptor located on the enterocyte luminal membrane. *Vibrio cholerae* enterotoxin is a high-molecular-weight protein complex that binds to receptors that produce ribosylation of the alpha subunit of the G_s GTP-binding protein regulating adenylyl cyclase activity in epithelial cells (Helper and Gilman, 1992; Lai, 1980). This ribosylation prevents the alpha subunit from hydrolyzing bound GTP causing protracted G_s stimulation of adenylyl cyclase leading to sustained elevations in intracellular cAMP (Kimberg et al., 1971). The elevated intracellular cAMP maintains activation of protein kinase A and protein phosphorylation that inhibit intestinal mucosal sodium absorption while stimulating chloride secretion into the intestinal lumen. The sustained activation of adenylyl cyclase results from enterotoxin-induced failure of the G_s regulatory system.

C cholera toxin also affects pathways in the enteric nervous system altering both mucosal transport of fluid and electrolytes and intestinal motility (Jodal and Lundgren, 1995; Nocerino et al., 1995). Pharmacological agents that interfere with nerve activity (hexamethonium, lidocaine, and tetrodotoxin) produce marked attenuation of cholera toxin-induced fluid secretion (Jodal, 1990). The enteric nervous system is involved in the mucosal secretory response that produces a large fluid load in the intestinal lumen. Moreover, cholera toxin elicits migrating clustered contractions (also known as migrating action potential complexes) that are also inhibited by drugs that block neural activity. Cholera toxin appears to activate a neuronal pathway from the small intestine to the colon that induces secretion of fluid into the colonic lumen (Nocerino et al., 1995). Persistent activation of adenylyl cyclase results in direct alterations in mucosal fluid and electrolyte transport as well as neurally mediated secretory and propulsive motility alterations. The combined effect of these is profuse and life-threatening diarrhea.

The neural pathway activated by cholera toxins has not been precisely identified, but release of VIP has been implicated (Nocerino et al., 1995). Other neurotransmitters and autacoids involved in small intestinal and colonic mucosal transport are candidate mediators of the effects of cholera toxin. For example, histamine acts at H_2 receptors to induce contractions and increase secretion across the mucosa (Andrews et al., 1988). 5-HT and acetylcholine have also been implicated as mediators of mucosal secretion in the small intestine and colon and have pronounced effects on motility (Cooke et al., 1991; Kellum et al., 1994; Sidhu and Cooke, 1995).

3.09.3.8 Release of Regulatory Substances

3.09.3.8.1 Antigens

Hypersensitivity contributes to the adverse GI effects of many environmental toxins (DiPalma et al., 1991). The gastrointestinal mucosa acts as a barrier to antigen absorption but can itself become the site of hypersensitivity responses. Antigens can enter enterocytes by pinocytosis or by interactions with nutrient transport systems. They can also cross the mucosal barrier by paracellular pathways to interact with immune cells in the lamina propria (Atisook and Madara, 1991). Antigens are usually presented to T-cells by macrophages, B-cells, or enterocytes. The antigen-presenting cells release interleukin 1 (IL-1) which activates T-cells to express IL-2. T-cells, in turn, release a number of interleukins, tumor necrosis factor (TNF-α), and interferons, including interferon gamma (macrophage activating factor). Activated macrophages in turn release IL-1 that activates immune cells and nerves, IL-6 that activates lymphocytes, IL-8 that attracts neutrophils, colony stimulating factors that activate immune cells, and prostanoid substances (Shanahan and Johnson, 1994). B-cells are stimulated by antigens and interleukins to proliferate and differentiate into plasma cells that synthesize and secrete immunoglobulins (Hoffman and Waston, 1979). Immunoglobulin-E is one of a number of regulators (cytokines, complement C3a) that can activate gastrointestinal mast cells. Mast cells release neurotransmitters (substance P, CGRP), autacoids (histamine), interleukins, chemotactic factors, PAF, and other substances (**Fig. 15**).

The complex soup of regulatory factors released by cells that participate in the immune response can induce changes in mucosal transport and gastrointestinal motility. Increased fluid secretion stimulated by immune mediators is thought to involve stimulatory effects on enteric nerves with subsequent neurally mediated activation of mucosal secretory mechanisms (Castro et al., 1987). Secretory products of mast cells may act both directly and by means of enteric neurons to increase contractile activity of gastrointestinal smooth muscle (Fargeas et al., 1992; Kirsch, 1994).

Cytokines released from immune and epithelial cells during the immune response may affect mucosal blood flow, induce a chronic inflammatory response, or promote generation of ROMs (Crowe and Perdue, 1992).

3.09.3.9 Generation of Reactive Oxygen Metabolites

3.09.3.9.1 Inflammatory conditions

ROMs, also known as oxygen free radicals, can produce both beneficial and harmful effects in the gastrointestinal tract. ROMs are important in inflammation, hypersensitivities, and mucosal ischemia. Activated neutrophils and macrophages are the major sources of superoxide anion radicals, hydroxyl radicals, peroxyl radicals, and hypochlorous acid (Granger et al., 1994). Nitric oxide (NO) is generated by endothelial cells, platelets, neutrophils, macrophages, and neurons (Schemann and Schaff, 1995). ROMs other than NO are generated in the intestinal mucosa primarily in association with phagocytosis by neutrophils and macrophages. Cytokines and related mediators released in association with the immune response activate neutrophils and macrophages and serve as neutrophil chemoattractants. As a result, ROM generation occurs in acute and chronic inflammation. Adverse gastrointestinal responses after chronic exposure to industrial solvents and aldehydes may result from mucosal inflammation (DiPalma et al., 1991). Infiltration of neutrophils and other granulocytes that release ROMs is stimulated by PAF. PAF is formed by a variety of cell types, including endothelial cells, neutrophils, macrophages, and platelets (Lefer, 1989). SOD, desferrioxamine, and dimethylthiourea all result in attenuated increases in mucosal myeloperoxidase by ROM or PAF (Kubes et al., 1990). A role for xanthine oxidase as a source of superoxide radical is indicated by the ability of xanthine oxidase inhibitors, such as allopurinol, to attenuate mucosal myeloperoxidase activity.

ROMs induce direct cytotoxicity of epithelial cells, net fluid secretion into the intestinal lumen, and alterations in functions of the intestinal microvasculature that lead to increases in permeability (Grisham et al., 1990). ROMs may affect intestinal permeability at concentrations lower than those required for cytotoxicity and affect permeability of microvasculature and enhance platelet aggregation in small vessels leading to thromboses (Salvemini et al.,1989).

Chloramines (RNHCl) can form rapidly in the presence of primary amines or ammonia (both plentiful in the mucosa) from hypochlorous acid (HOCl). Chloramines are relatively long-lived and contain more oxidizing capacity than peroxide or hypochlorous acid (Granger et al., 1994). Interaction between HOCl and NH_3 produces an especially cytotoxic chloramine, NH_2Cl, which readily crosses biological membranes to oxidize intracellular components such as hemoproteins, thiols, and GSH. Other lipophilic chloramines, such as chlorinated histamine, have been shown to injure intestinal epithelial cells when applied exogenously but have not been shown to be produced an inflamed mucosa (Gaginella et al., 1995; Miller et al., 1991,1992).

ROMs are produced in small amounts as metabolic by-products in virtually all tissues. Without adequate protection from ROMs, cells and tissues would suffer oxidative damage (Granger et al., 1994). Chemicals and inflammatory processes can generate toxic amounts of tissue ROMs by overcoming the enzymatic and nonenzymatic antioxidants that prevent or limit oxidative tissue injury. The principal enzymatic antioxidants include SOD, catalase, and GSH peroxidase. Nonenzymatic free radical scavengers include ascorbic acid, which is soluble in water, and the lipid-soluble scavengers, α-tocopherol and β-carotene. In general, the enzymatic antioxidants are intracellular, whereas many nonenzymatic antioxidants are extracellular (Granger et al., 1994).

Chewing tobacco extracts causes lipid peroxidation in addition to DNA damage by producing reactive oxygen species (Bagchi et al., 2002).

3.09.3.9.2 Nitric oxide

NO can be beneficial or toxic. It is synthesized from L-arginine by at least two nitric oxide synthases (Moncada and Higgs, 1993). The two major isoforms of NO synthase are a constitutive enzyme that is calcium and calmodulin dependent and an inducible calcium-independent form. The constitutive form is in the endothelium, nerves, and brain. It seems to continuously generate small amounts of NO. The inducible form is in macrophages and probably other tissues such as gastrointestinal and vascular smooth muscle and vascular endothelium (Moncada and Higgs, 1993; Stark and Szurszewski, 1992). When stimulated by appropriate endotoxins, lipopolysaccharides, or cytokines, the inducible isoform can remain active for many hours and large amounts of NO may accumulate. NO is lipophilic and readily crosses plasma membranes to interact with the heme iron of guanylyl cyclase to stimulate formation of cGMP. Increases in intracellular cGMP decrease cytosolic calcium levels, increase potassium conductance, and evoke membrane hyperpolarization in GI smooth muscle cells and nerves (Stark et al., 1991; Tamura et al., 1993). NO relaxes vascular and GI smooth muscle and perhaps functions as a tonic vasodilator and as the major, nonadrenergic, noncholinergic inhibitory neurotransmitter of the enteric nervous system (Boeckxstrens et al., 1991). NO may serve as an important vasoregulator maintaining adequate mucosal blood flow (Stark and Szurszewski, 1992; Toda and Okamura, 1992). NO released by macrophages and neutrophils is toxic to microorganisms and, in excess, to mammalian cells by interacting with iron-containing enzymes essential for cell function. Once released, NO is oxidized successively to nitrite and nitrate. It is destroyed by interaction with superoxide; SOD is protective. NO avidly binds to oxyhemoglobin by interactions with heme iron.

NO can be inhibited by interfering with synthesis, by binding to hemoglobin, and by blockade of guanylyl cyclase. A number of substituted analogs of L-arginine, L-ornithine, or guanidine are competitive inhibitors of both the constitutive and inducible isoforms of NO synthase (Burks, 1994; Laszlo et al., 1995). L-NAME (N^G-amino-L-arginine methyl ester) is the most commonly used inhibitor of NO synthase. Actions of NO that involve release of the substance from one cell and effects in another can be blocked by exogenously applied hemoglobin, which effectively binds NO in extracellular spaces. Methylene blue prevents expression of NO effects by blocking intracellular guanylyl cyclase.

NO in low concentrations protects the GI mucosa from injury and enhances restitution of damaged mucosa. It vasodilates gastric microvasculature and prevents platelet aggregation. These actions help maintain adequate mucosal blood flow (Whittle et al., 1990). NO also stimulates secretion of mucus helping to maintain protection against luminal acid (Payne and Kubes, 1993; Yanaka et al., 1995). Functional repair of the epithelial barrier after acute injury is enhanced by NO (Miller et al., 1993c; Wang et al., 1994).

NO protects the GI mucosa, maintains mucosal blood flow, and regulates smooth muscle contractions and propulsion. NO can also cause gastrointestinal toxicity. The dual nature of NO is a function of the two major isoforms of NO synthase. NO produced by the constitutive form of the enzyme produces beneficial effects, while NO produced by the inducible form of the enzyme is often implicated in vascular or epithelial cell injury (Boughton-Smith et al., 1993). NO generated by endotoxin lipopolysaccharide on inducible NO synthase increases vascular permeability and contributes to vascular protein leakage (Boughton-Smith et al., 1993). In trinitrobenzene sulfonic acid (TNBS) induced chronic ileitis in guinea pigs enhanced synthesis of NO promotes neutrophil-associated mucosal injury via increases in myeloperoxidase and loss of serum protein into the inflamed tissue (Miller et al., 1993b). The NO synthase inhibitor, L-NAME, ameliorates this inflammatory response (Miller et al., 1993b). Proinflammatory stimuli suggest that low levels of NO protect the vasculature and mucosa, whereas activation of inducible NO synthase produces large amounts of NO over extended periods of time that result in cytotoxicity (Aiko and Grisham, 1995; Laszlo et al., 1994; Miller et al., 1993a; Salzman, 1995). There is evidence that intestinal injury can lead to activation of inducible pulmonary NO synthase (Turnage et al., 1995). Theoretically, toxic and inflammatory injury to the gastrointestinal tract may be reduced by selective inhibition of the inducible form of NO synthase while maintaining the largely protective activity of the constitutive form of the enzyme.

The higher levels of NO produced by the inducible enzyme promote oxidative reactions and the formation of reactive nitrogen intermediates, such as N_2O_3 and N_2O_4; the lower levels of NO produced by the constitutive enzyme simply activate guanylyl cyclase. The nitrogen intermediates form peroxynitrite ($ONOO^-$) that cannot be measured directly in vivo because of its high degree of chemical reactivity. However, nitrotyrosine, formed by the interaction of peroxynitrite with tyrosine, can be measured. In experimental ileitis in guinea pigs by TNBS, inducible nitric oxide synthase and nitrotyrosine are localized in large amounts in superficial epithelium encompassing entire villi but not in crypts (Miller et al., 1995). Enteric neurons contain nitrotyrosine and activation of inducible enzyme may be associated with neuronal injury. Toxic megacolon in humans with ulcerative colitis is associated with the appearance of inducible NO synthase in the muscularis propria that may produce NO that causes smooth muscle relaxation (Mourelle et al., 1995).

Production of NO in the GI mucosa initiates secretory diarrhea (Mascolo et al., 1994a,b). Diarrhea induced in rats by castor oil or sodium choleate is blocked by NO synthase inhibitors. Castor oil and bile salts induce mucosal damage, and the damage is intensified by coadministration of NO synthase inhibitors (Capasso et al., 1994). These data suggest that NO mediates, at least in part, the diarrheal effect of these compounds presumably by increasing secretion of fluid into the intestinal lumen. Simultaneously, NO exerts a protective effect on the intestinal mucosa. Bile acids and castor oil directly damage intestinal mucosa and activate the inducible isoform of NO synthase producing large amounts of NO that are linked to production of diarrhea (Gaginella et al., 1995). Administration of NO synthase inhibitors that nonspecifically block both the constitutive inducible isoforms removes the protective effects of NO provided by the constitutive enzyme and exacerbate mucosal damage. Blockade of the inducible enzyme inhibits excessive NO production responsible for diarrhea. Other laxatives such as phenolphthalein and bisacodyl are similarly associated with electrolyte secretion, changes in mucosal structure, and abnormal motility. These drugs induce NO production through activation of inducible NO synthase (Gaginella et al., 1994).

3.09.3.9.3 Lipid hydroperoxides

Oxidized polyunsaturated fatty acids in vitro are cytotoxic for cultured colonic epithelial cells. Lipid hydroperoxides generated by oxidation produce direct cellular toxicity, especially in immature nondifferentiated cells (Cepinskas et al., 1994). These data suggest that spontaneous oxidation of polyunsaturated fatty acids can produce compounds capable of inducing epithelial cell toxicity. The vulnerability of immature cells to damage may be associated with a lower GSH-dependent detoxification capacity than is present in mature cells. Exogenous GSH promotes lipid hydroperoxide metabolism by rat small intestine and protects against lipid hydroperoxide toxicity (Aw, 1994).

3.09.3.10 Chemotaxis and Activation of Granulocytes

Substances that cause direct or indirect injury to mucosal epithelium, including enterotoxins and antigens, can initiate damage to the gastrointestinal tract by attracting and/or activating granulocytes, especially neutrophils. Neutrophil chemotaxis and activation is a specific mechanism of gastrointestinal toxicity. Enterotoxins and exogenous protein-derived peptides can generate antigens within endosomes of epithelial cells. Constitutively expressed class II major histocompatibility complex (MHC) molecules within these cells can present the antigens to lymphocytes. The class II MHC molecules that permit antigen presentation are expressed on well-differentiated villus enterocytes but not crypt cells (Elson and Beagley, 1994). Antigen presentation by enterocytes is associated

with production of cytokines, particularly IL-1, a chemotaxin for neutrophils that activates neutrophils and macrophages. Activation of macrophages results in generation of IL-8, LTB_4, and PAF. These substances are strongly chemotactic for neutrophils. Leukotriene B_4 increases the number of neutrophils in the area of antigen stimulation and increases local myeloperoxidase activity, a marker for neutrophil infiltration (Mitchell et al., 1993). IL-8 directs neutrophil migration and likely synergizes with IL-1 in mediating neutrophil chemotaxis. Mucosal mast cells, activated by antigen or cytokines, release LTB_4 and PAF to add to neutrophil chemotaxis. Once neutrophils have been attracted to the area of damage or sensitization, IL-1, PAF, and macrophage-generated IL-6 and TNF-α activate the neutrophils to stimulate release of enzymes, ROMs, and other damaging substances. NADPH oxidase, a neutrophil enzyme that reduces molecular oxygen to superoxide, is activated by TNF-α, other cytokines, phorbol esters (such as phorbol 12-myristate 13-acetate), and fluoride salts of aluminum (Maly and Schurer-Maly, 1995).

Several compounds have been evaluated for their ability to inhibit granulocytic chemotoxins. Gamma-hydroxybutyrate, heat-shock protein, inhibitors of 5-lipoxygenase (e.g., ziluten), and experimental antiulcer drugs (e.g., rebamipide) may reduce attraction or activation of granulocytes in inflamed mucosa (Boyd et al., 1994; Kim and Hong, 1995; Mangino et al., 1994; Mascolo et al., 1995; Stojadinovic et al., 1995). The selective 5-lipoxygenase synthesis inhibitor, ziluten, abolishes leukotriene B_4 synthesis and prevents increases in myeloperoxidase activity stimulated by intestinal ischemia and reperfusion injury (Mangino et al., 1994). Heat-shock protein also inhibits leukotriene B_4 (Stojadinovic et al., 1995). Rebamipide inhibits chemotaxis and activation of neutrophils but does not affect superoxide production by either the xanthine oxidase or phorbol ester-stimulated NADPH oxidase pathways (Kim and Hong, 1995). The action of rebamipide may result from attenuation of suppression of SOD in addition to diminished chemotaxis and activation of neutrophils. Gamma-hydroxy butyrate diminishes neutrophil accumulation in an ischemia–reperfusion model (Boyd et al., 1994).

3.09.3.11 Release of Enzymes

Much of the cellular damage that results from granulocyte chemotaxis and activation to areas of tissue injury or inflammation is attributable to ROMs; uncontrolled proteolysis represents another potential pathway by which inflammatory cells may injure the gastrointestinal tract (Caplan et al., 1994; Elson and Beagley, 1994; Granger et al., 1988; Grisham and Granger, 1988). Neutrophil-derived proteases include elastase, collagenase, and gelatinase. These enzymes are potent pathways by which neutrophils may degrade key components of basement membrane and interstitial matrix (Granger et al., 1988). Elastase is intrinsically active. Gelatinase and collagenase are metalloproteinases secreted in inactive forms that require further processing for activation. ROMs have been shown to oxidatively activate these enzymes (Weiss, 1989).

3.09.3.12 Activation of Enzymes

Cholera toxin ("Cholera toxin" section) activates the intracellular adenylyl cyclase resulting in sustained elevations of intracellular cAMP (Helper and Gilman, 1992; Kimberg et al., 1971). Endotoxin lipopolysaccharide and TNBS can activate inducible NO synthase to increase production of NO (Boughton-Smith et al., 1993; Miller et al., 1993b). NO interacts with guanylyl cyclase to increase intracellular concentrations of cGMP (Moncada and Higgs, 1993).

3.09.3.13 Inhibition of Enzymes

3.09.3.13.1 Nonsteroidal antiinflammatory drugs

Aspirin and other NSAIDs produce gastrointestinal toxicity in part by blockade of constitutive and inducible forms of cyclooxygenase (Granger et al., 1994; Rainsford, 1989) (see "Nonsteroidal anti-inflammatory drugs (NSAIDS)" section). The pathophysiology of aspirin toxicity is outlined in **Fig. 17**.

3.09.3.14 Acetylcholinesterase Inhibitors

Chemicals that inhibit acetylcholinesterase produce classical toxic effects in the gastrointestinal tract. These chemicals are rarely ingested as food components. More often they are ingested as a result of direct exposure to pesticides and most often manifest as toxic responses to drugs. Acetylcholinesterase is normally responsible for inactivation of the neurotransmitter acetylcholine at synaptic and neuroeffector endings of cholinergic motor and secretomotor neurons in the enteric nervous system. Inhibition of enzyme activity allows local buildup of acetylcholine at cholinergic sites of neurotransmission and produces classic signs cholinergic crisis. The principal signs of acetylcholine overactivity include excessive segmenting and propulsive contractions in all areas of the tubular GI tract. This increased motor activity is caused primarily by actions of acetylcholine at smooth muscle M_3 muscarinic receptors (Caulfield, 1993). Acetylcholine also acts at M_1 and M_3 muscarinic receptors to increase salivary, gastric, pancreatic, and intestinal secretions. Toxic amounts of acetylcholinesterase inhibitors produce net secretion of large volumes of fluid and electrolytes into the intestinal lumen resulting in profuse, watery diarrhea frequently associated with severe cramping. Natural inhibitors of acetylcholinesterase include physostigmine, also known as eserine, found in the ripe seed of *Physostigma venenosum* (Calabar bean or chop nut). A number of chemicals that inhibit acetylcholinesterase have been synthesized (**Table 6**). These include neostigmine, edrophonium, and pyridostigmine. Acetylcholinesterase inhibitors such as parathion, malathion, and paraoxon are used as insecticides. Some very potent acetylcholinesterase inhibitors have been synthesized for use as extremely toxic military nerve gases. These

Table 6 Inhibitors of acetylcholinesterase

"Reversible" agents	*Organophosphate agents*
Physostigmine	Malathion
Neostigmine	Parathion
Edrophonium	Paraoxon
Pyridostigmine	Tetraethyl pyrophosphate
Demecarium	Fenthion
Ambenonium	Dimpylate
	Diazinon
	Echothiophate
	Isoflurophate
	Tabun
	Sarin
	Soman

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include tabun, sarin, and soman. Isoflurophate (diisopropyl-fluorophosphate, DFP) and echothiophate are potent acetylcholinesterase inhibitors that have been used as therapeutic agents (Taylor, 1996).

Acetylcholinesterase inhibitors are variably reversible. Most of the inhibitors used as drugs, such as neostigmine, are reversible by hydrolysis at the active site of the enzyme. Organophosphate acetylcholinesterase inhibitors, such as most of the insecticides and nerve gases, interact in an essentially permanent and irreversible manner with the active site of the enzyme and produce long-lasting inhibition of enzyme activity. Organophosphate acetylcholinesterase inhibitors account for 80% of pesticide-related hospital admissions in the United States. Pesticide toxicity is a global problem; most poisonings are in developing countries (Bardin et al., 1994). Ingestion is the most common route of exposure.

3.09.3.15 Increased Susceptibility to Acid

The integrity of the surface epithelium of the upper gastrointestinal tract requires an intact barrier to hydrochloric acid, bile acids, and bacteria. The principal defenses against these factors are mucus production, bicarbonate secretion, and the phospholipid hydrophobicity of the surface epithelium (Allen and Garner, 1980; Hills et al., 1983). The adherent mucus gel enhances surface neutralization of acid by bicarbonate (Allen et al., 1993). When the bicarbonate and mucus cap are broken, gastric acid injures cells and destroys capillaries (Ivey, 1988). If the break in the defensive barrier is temporary, epithelial repair is rapid by adjacent cell migration. If injury is more severe or prolonged, reepithelialization may not occur.

The principal regulators of mucus and bicarbonate secretion are locally produced prostanoids, NO, and trefoil peptides (Allen et al., 1993; Babyatsky et al., 1996; Yanaka et al., 1995). Prostanoids, including prostaglandins and prostacyclin, and NO increase mucus and bicarbonate secretion by direct stimulation of gastric and small intestinal epithelial cells.

NSAIDs and alcohol are classic barrier breakers that damage the gastrointestinal epithelium. Their actions are complex and due in part to reduction of mucosal blood flow. NSAIDs also inhibit cyclooxygenase (COX-1 and COX-2) reducing formation of protective prostaglandins and prostacyclin (Bjarnason et al., 1993). These reductions decrease mucus and bicarbonate secretion allowing hydrochloric acid and other luminal compounds to attack epithelial cells.

Trefoil peptides likely play a role in maintaining mucosal integrity by enhancing intestinal cell migration following injury (Babyatsky et al., 1996). Trefoil peptides are present in high concentrations in the mucus gel of the mucosa. Intragastric administration of trefoil peptides provides mucosal protection against injury by alcohol and NSAIDs (Babyatsky et al., 1996). The mechanism is probably topical, suggesting that trefoil peptides interact with mucine glycoprotein to reinforce the mucus gel layer (Rachmilewitz, 1996). Trefoil peptides may protect against gastric damage induced by *Helicobacter pylori*. *H. pylori* penetrate the mucus layer and produce enzymes such as proteases, lipases, and phospholipases, which may degrade and thus deplete mucus (Sarosiek et al., 1988).

3.09.3.16 Intracellular Toxicity

3.09.3.16.1 Arsenic

Trivalent arsenicals, including inorganic arsenite, are sulfhydryl reagents that inhibit many intracellular enzymes (Klaasen et al., 1996). Pentavalent arsenate uncouples mitochondrial oxidative phosphorylation by competing with phosphate in the formation of adenosine triphosphate. Orally ingested arsenicals are taken up in high concentrations by intestinal epithelial cells and act intracellularly to disrupt energy production and enzyme activity. The surface epithelial cells poisoned with arsenic quickly exfoliate, denuding the villus epithelium. Epithelial rupture leads to exudation of plasma into the intestinal lumen where it coagulates. Movement of large volumes of fluid into the intestine produces distention that initiates propulsive contractions. Within 1–12 h of oral

Table 7 Heavy metals

Metal	*Mechanism*	*Main effects*
Arsenic	Intracellular enzyme inhibition	Exfoliation, diarrhea
Cadmium	Epithelial irritation	Emesis, cramps
Mercury	Precipitation of mucosal proteins	Exfoliation, pain
Lead	Competition for calcium ion	Nausea, emesis, pain (lead colic)

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ingestion of arsenicals, gastrointestinal discomfort and characteristic watery diarrhea known as "rice-water stools" occur. Repair of the epithelium is impaired by the arsenic and significant intestinal blood loss may occur (Reddy and Hayes, 1989).

3.09.3.16.2 Other heavy metals (Table 7)

Acute ingestion of cadmium can cause direct irritation of gastrointestinal epithelial cells leading to vomiting, ptyalism, diarrhea, and cramps (Klaasen et al., 1996). Ingestion of ionic inorganic mercury leads to precipitation of mucosal proteins and a corrosive effect on the gastrointestinal mucosa. Acute mercury intoxication is characterized by sloughing of the epithelium and abdominal pain. Acute lead intoxication can produce nausea, abdominal pain, and vomiting. The vomitus may be milky from the presence of lead chloride (Klaasen et al., 1996). Chronic lead intoxication produces gastrointestinal symptoms that are an important sign of exposure, especially in adults. Constipation with severe abdominal pain (lead colic) may be the predominant sign of intoxication. The actions of lead are thought to involve competition with ionic calcium. Intravenous calcium gluconate often produces relief (Klaasen et al., 1996).

3.09.4 Conclusions

Foreign and endogenous chemicals can produce gastrointestinal toxicity by a number of mechanisms that result in alterations in normal function. It is likely that the toxicities are often unrecognized because the changes in function may be minor or may be compensated by the complex regulatory apparatus that protects vital gastrointestinal functions. Compared to other intoxications, there is a dearth of information about GI toxicology. The gastrointestinal system has a number of structures and functions that are in a delicate balance to effect digestion. Because the alimentary route delivers most xenobiotics, it is exposed to high local concentrations of these agents or their biotransformed metabolites directly or after uptake and biliary excretion. The GI system is quite plastic in adapting to dietary or altered metabolic demand. Crypt cells are highly responsive to stimulation and are affected by pharmaceuticals, microbes, and DNA damaging agents. Significant interspecies and target organ differences exist in both the predisposition to toxicants and carcinogens, making the choice of an appropriate animal model critical. A greater mechanistic appreciation and understanding of toxic gastroenteropathy is needed to facilitate effective treatment (Betton, 2013).

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