2 Anatomical and Physiological Parameters that Influence Gastrointestinal Absorption

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2.1 SUMMARY

The rate at which ingested compounds are absorbed into the bloodstream from the gastrointestinal (GI) tract and extent to which they are absorbed are governed by the physical–chemical properties of the ingested compounds, the presence and identity of other substances in the GI tract, and the anatomical and physiological characteristics of the GI tract. Humans and laboratory animals share the same basic blueprint for the GI tract, and this chapter describes the organs of the GI tract, the microscopic structure of the GI lumenal epithelium, and the flow of blood and lymph from the GI tract. However, there is remarkable variability across species in the absorptive surface area of the GI tract, the time it takes for materials to move through the GI tract, and the nature of the GI contents. By understanding these differences among species, scientists can better predict the pharmacokinetics of a substance in a species or extrapolate pharmacokinetic data from one species to another.

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2.2 INTRODUCTION

GI absorption entails the passage of materials from the lumen of GI tract (alimentary canal) to the bloodstream. Absorption of nutrients and essential minerals are crucial for a normal, healthy life. Understanding which materials are absorbed, in what specific portions of the alimentary canal, at what rate, and by what mechanisms is important to nutritionists, pharmacologists, and toxicologists. In all cases, scientists want to know how substances pass from the lumen to the bloodstream. Information about the process of absorption and factors that affect it are essential for modeling absorption kinetics and predicting the biodistribution of a substance within a given animal or extrapolating the findings between species (e.g., from rats to humans). Physiologically based pharmacokinetic models and biologically based models are valuable tools for predicting or extrapolating absorption, but they require qualitative—as well as quantitative—knowledge about the biological systems under study.

The rate and extent of absorption of orally ingested compounds are influenced by both properties that are intrinsic to the ingested substances themselves and factors that are associated with the milieu of the alimentary canal and its absorptive surface [1–4]. The most significant intrinsic factors that affect absorption are the physical and chemical properties of a substance, including its lipid solubility, ionization state, and molecular weight or size. These intrinsic factors are independent of the test species. Absorption of a substance is also extensively affected by the presence of other materials within the GI tract, including food eaten before or concomitantly with ingestion of the substance, or in the case of some experimental studies, the vehicle or solvent in which the substance is administered.

It is emphasized that the parameters discussed herein are those that influence the crossing of materials from the GI lumen into the bloodstream of the GI tract. From the major absorptive areas of the GI tract, blood passes by means of the hepatic portal system to the liver, where absorbed materials are subject to possible metabolism or storage. This process is termed the *first-pass effect* because it effectively reduces the concentration or amount of material absorbed from the GI tract that enters the systemic circulation. Thus, while information about absorption of materials from the GI tract is the first step in determining potential biological effects, this does not always provide accurate measures of concentrations found at target tissues that are distant from the GI tract [5].

The factors that contribute most to the interspecies differences in GI absorption are the anatomy and physiology of the GI tract of the species under consideration. Before discussing the differences among species, however, it is important to review their similarities.

2.3 OVERVIEW OF THE ALIMENTARY CANAL

The overall organization of the GI tract of humans is comparable to those of most test species. Detailed information concerning the anatomy and physiology of the GI tract in animals and humans can be found in various sources [6–10] and has been summarized therefrom. Figure 2.1 depicts the regions of the alimentary canals for humans and rats. The alimentary canal is an open-ended tube that extends through the body from the mouth to the anus. One can envision the lumen as a tunnel of the

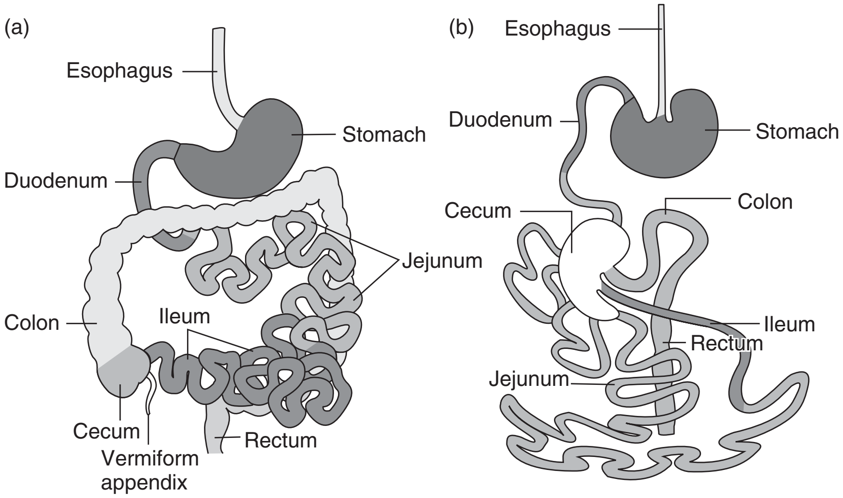

Figure 2.1 Alimentary canals of primates and rodents. In the primate (human depicted in panel a), note the eccentric position (left side) for the entry of the esophagus into the stomach. The portal into the small intestine is nearly horizontal. The small intestine is divided into duodenum (the short initial segment), jejunum, and ileum. The ileum empties into the large intestine. The first part of the large intestine (the cecum) has the largest diameter; the vermiform appendix is attached nears its proximal end. Note that the diameter of the large intestine decreases as material traverses caudally. The rodent GI tract (rat depicted in panel b) shares the same overall organization as the human's, but with a few important differences. Note that the esophagus enters the stomach at a central location and that the entry into the duodenum faces cranially. The relative lengths of the small intestine differ from those of the human in that the jejunum makes up nearly the entire small intestine. The cecum of the rat is extremely large and lacks the presence of a vermiform appendix. Note that figures are not drawn to scale. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

external environment that runs through the body and the wall of the alimentary canal as the interface between the environment and the circulatory system [11,12]. The main functions of the alimentary canal are the digestion of food, the absorption of nutrients and other substances, and the propulsion of material through the digestive tract.

The mouth provides access to the alimentary canal. There, ingested materials are crushed and mixed with saliva by chewing (mastication). While enzymes found in saliva initiate the breakdown of carbohydrates and fats, saliva importantly acts as a lubricant for the ingested material. When a bolus of masticated material attains an appropriate consistency, it is propelled by swallowing (deglutition) into the pharynx.

The pharynx is a conduit that is shared by the respiratory and digestive systems. In humans, the pharynx has an extensive anterior opening that connects to the nasal cavity and mouth in its upper half; inferiorly, the pharynx connects to the esophagus and larynx. For the purposes of this discussion, *pharynx* refers only to the combined oral and laryngeal regions because under normal conditions, the nasopharynx does not participate in ingestion or swallowing. In the rat, mouse, and rabbit, the pharynx is divided into a respiratory region (an elongated nasopharynx) and a digestive region (analogous to the human's laryngopharynx). There is no oropharynx in these species because the epiglottis rests against the palate and separates the nasal cavity from the oral cavity (see Ref. 13 for a comparison of the oral and upper respiratory regions of rats and primates). The inferior end of the pharynx adjoins the esophagus, a hollow muscular tube capable of considerable expansion. Little digestion or absorption occurs in the pharynx and esophagus; rather, these structures serve to rapidly transfer ingested material from the mouth to the stomach.

The stomach is a capacious muscular organ that mixes the ingested material with additional secretions to facilitate digestion. The stomach of rodents and lagomorphs has two grossly discernible regions: the forestomach and glandular stomach. The two are separated by a limiting ridge, which prevents these species from vomiting (Fig. 2.2a). The forestomachs of rodents and lagomorphs serve as an entry from the esophagus and are lined by stratified epithelium. The forestomach of rats and mice (but not rabbits) contains numerous bacteria that are important in their digestive processes. The epithelium that makes up the forestomach surface in these species resembles the

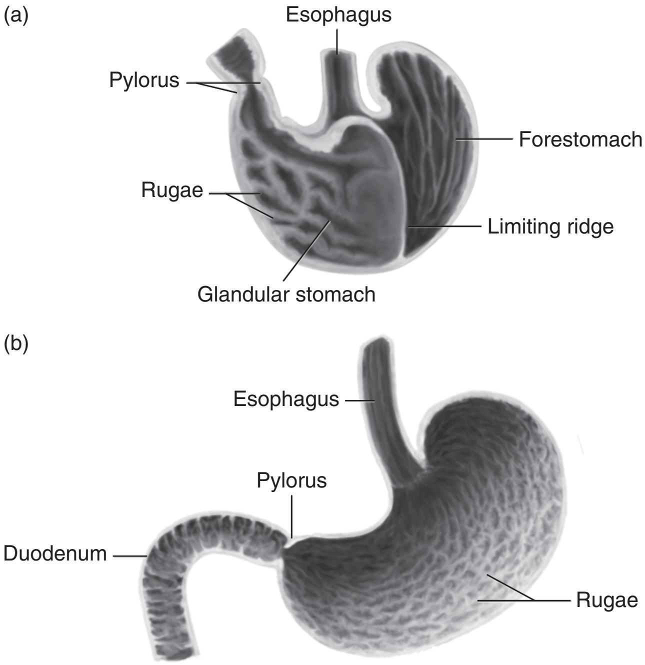

Figure 2.2 Interior structures of rodent and primate stomachs. The interior of the rat stomach (a) exhibits two distinctive regions separated by a prominent limiting ridge, which precludes the rat from vomiting. Food enters the stomach at the forestomach, a nonsecretory region with a hardy epithelium. The portion of the stomach that exits to the duodenum is the glandular stomach, which is lined by a delicate secretory epithelium and exhibits prominent folds (rugae) that are noticeable when the stomach is empty. Entry of food into the duodenum is regulated by a prominent muscular sphincter, the pylorus. The human stomach (b) differs from that of the rat in that the entire lining is secretory and there is no forestomach or limiting ridge. The human stomach has numerous rugae. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

mucous membrane of the oral cavity. The other portion of the stomach, the glandular region, contains acid-secreting glands. In dogs, pigs, and primates (including humans), the stomach is a single chamber that is secretory in nature and contains mucus- and acid-secreting cells. The human condition is depicted in Fig. 2.2b. The predominant gastric secretion in all species considered herein is rich in hydrochloric acid. In humans, the daily volume of gastric secretions can reach 1.5–2 L [4]. Gastric secretions act to continue the disintegration of food particles and to denature proteins. After mixing in the stomach for as many as several hours, small boli of the ingested material (now termed *chyme*) pass through the pyloric sphincter to the small intestine, where digestion is essentially completed and the majority of absorption takes place.

The small intestine is the major site for absorption of nutrients, water, electrolytes, and xenobiotics. The small intestine exhibits three unequally sized portions: the duodenum, the jejunum, and the ileum. These sections differ in length from species to species. The proximal part of the small intestine, the duodenum, receives secretions from numerous sources, including

- the pancreas—high in volume and rich in digestive enzymes and bicarbonate,
- the liver—bile (discussed in Section 2.7.2), and
- enteric (Brunner's) glands, located in the wall of the small intestine—rich in bicarbonate that neutralizes acid from the stomach.

The volume of these secretions in primates, dogs, and pigs more than doubles that from the salivary glands and stomach and serves to make the chyme watery. In contrast, the chyme of rodents remains rather pasty [4]. In humans, the majority of absorption occurs in the duodenum and the proximal half in the jejunum. In rodents and lagomorphs, absorption occurs more uniformly along the duodenum and entire jejunum. At the distal end of the ileum, chyme passes into the large intestine.

In primates, the large intestine is divided into the cecum, ascending colon, transverse colon, descending colon, sigmoid colon, rectum, and anal canal. The other species discussed herein have similar divisions, except that, because of the absence of a true pelvis, they do not have a region designated as the sigmoid colon. The first portion of the large intestine, the cecum, has disparate functions among various species. In primates, fluids and salts that remain after completion of intestinal digestion are absorbed in the cecum. Rabbits, which are herbivores, have a much larger cecum that contains large amounts of bacteria that help digest cellulose. The ceca of carnivores such as dogs use strong muscle contractions to reverse the propulsion of the chyme and bring it back from the more distal regions of the large intestine to mix with newly arrived chyme from the ileum. As the chyme moves through the cecum and the remainder of the colon, it is mixed with intestinal bacterial flora. In fact, most nutrient processing in the large intestine is due to bacteria; little processing occurs as the result of intestinal mucosal/secretory activity. Substances formed as a result of bacterial metabolism that have properties favorable for absorption may be taken up from the large intestine. Water and electrolytes also continue to be absorbed from the large intestine. The progressive absorption of water from the chyme–bacterial matrix results in the formation of solid feces, which are eventually propelled to the rectum and stored until expulsion during defecation.

2.3.1 The Nature of the Protective and Absorptive Interfaces

Histologically, the GI tracts of humans and most test species are quite similar [14]. Detailed descriptions of the lumenal surface of the alimentary canal can be found in textbooks of histology [15–17] and in various reviews [2,4,18]. Briefly, the alimentary canal is lined from the mouth to the anus with a mucosa that functions as the first barrier to entry of materials from the GI tract into the body. The mucosa is composed of an epithelium that is superimposed over a thin layer of loose connective tissue (lamina propria) in which reside blood capillaries and lymphatic vessels. The epithelial cells of the mucosa (discussed in further detail below) are joined together by tight junctions throughout the alimentary canal. The regions of the alimentary canal can be categorized by function: conduits that propel ingested substances through the canal (mouth, pharynx, esophagus, lower rectum, and anus) and regions that operate in digestion and absorption (stomach, small intestine, and all but the distal large intestine).

The mucosae of the conduits are usually a moist, stratified squamous epithelium (five to seven layers of cells) with a poorly vascularized lamina propria. In some conduit regions that are subjected to physical stress (such as the mouth), the epithelium may be keratinized. Conduit regions generally exhibit flat lumenal surfaces with few irregularities that would increase the mucosal surface area. The mucosae of conduit regions are not well adapted for absorption due to their multiple layers of epithelial cells, reduced surface areas, and relatively sparse vascularization.

The mucosae of the absorptive regions differ markedly from those of the alimentary conduits. Absorptive mucosae exhibit simple columnar epithelium (i.e., a single cell layer) with prominent, well-vascularized laminae propriae. The mucosa in the absorptive regions of the alimentary canal is further characterized by modifications of the mucous membrane that increase the absorptive surface area (Fig. 2.3). These modifications include (i) large folds of the mucosal membrane (plicae circulares, also known as *valves of Kerckring*) that are present in some species, (ii) fingerlike projections of mucosal membrane called villi, and (iii) numerous projections from the lumenal surface of the epithelial cell membrane to form a brush border (microvilli). This increased surface area of the mucosa is conducive to the transfer of substances from the lumen to the vascular system.[1]

While the epithelia of the absorptive regions include diverse cell types, the predominant cell type important in the process of absorption is the enterocyte (Fig. 2.4). As previously noted, enterocytes are columnar epithelial cells that are joined to adjacent cells at the lumenal surface by tight junctions. Absorption usually requires passage through the epithelium, the lamina propria, and the walls of blood or lymph capillaries. There are certain normal conditions that allow substances to traverse the junctions between epithelial cells [19–21]. Further, this process can be exacerbated in some disease states, for example, cholera [22]. The apical cell membrane of an enterocyte from the small intestine possesses an estimated 3000–7000 microvilli, which greatly increase the surface area available for absorption [11,23]. There are fewer microvilli on

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[1] The mucosa of the esophagus forms longitudinal folds and the wall of the stomach has ridges (rugae); however, these surface modifications serve to allow expansion during swallowing and after a meal, respectively, rather than increase absorption. Also, some epithelial cells in the stomach have microvilli, although these are secretory in function rather than absorptive. Thus, the surface modifications in the esophagus and stomach do not increase the *absorptive* surface area.

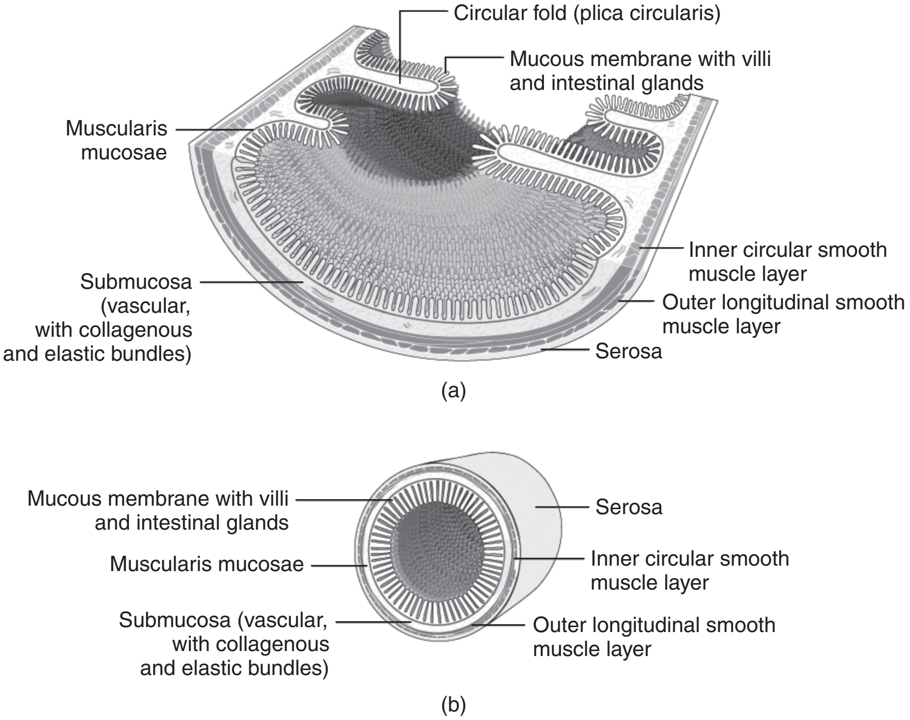

Figure 2.3 Cross sections of proximal small intestines of primates and rodents. Lumenal surface modifications in (a) human and (b) rat. The surface areas for absorption from the lumina of the small intestines of rats and humans are greatly increased by the presence of millions of tiny fingerlike projections (villi). In the rat (b), which has a thick, chalky chyme, the villi project into the lumen from the cylindrical intestinal wall. In humans (a), who have a watery chyme, the absorptive surface area is further amplified by the presence of folds of lumenal epithelium (plicae circulares) that intrude into the lumen, thereby allowing many million more villi to come into contact with chyme. (See also Fig. 2.7 to appreciate the difference in blood supplies to areas with many vs few intestinal folds.) *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

enterocytes of the large intestine. Absorptive epithelial cells rest on a basement membrane and possess a carbohydrate-rich glycocalyx coat on the surface of the microvilli.

The lamina propria in absorptive regions of the alimentary canal possesses a rich supply of blood capillaries and lymphatic vessels. When villi are present (Fig. 2.5), the vascularized lamina propria makes up the core of each villus. The dilated, blind-ending lymphatic capillaries occupy the center of the villus; the blood capillaries form a meshwork of vessels beneath the basement membrane. Absorption may be augmented where fenestrations (small holes) in the capillary endothelial cells or pores in enterocyte membranes are found.

In some absorptive regions of the alimentary canal, such as the small intestine, a thin layer of smooth muscle, muscularis mucosae, lies within the lamina propria of the mucosa. Contractions of the muscularis mucosae appear to assist in the rhythmic movements of the villi that agitate the layer of intestinal secretions and chyme that are in contact with the epithelium (the unstirred layer) and thus promoting absorption.

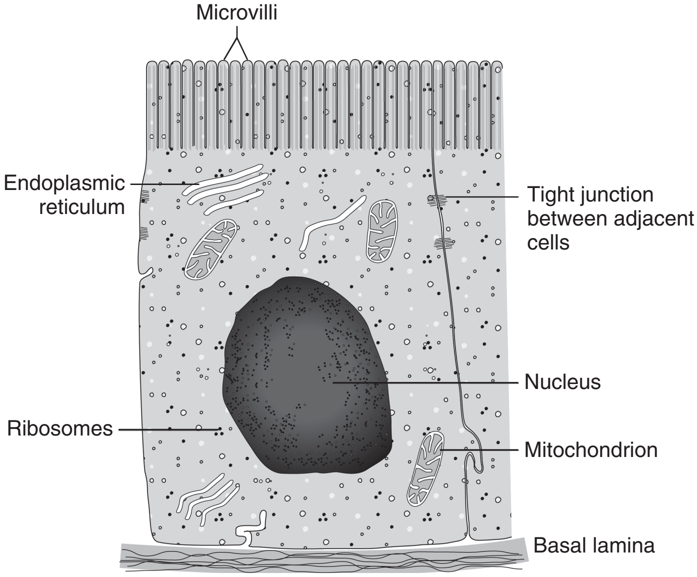

Figure 2.4 A typical intestinal epithelial cell (enterocyte). Enterocytes are high cuboidal to low columnar epithelial cells that rest on a basal lamina. Enterocytes are joined firmly to adjacent cells by tight junctions. The nucleus occupies the basal portion of the cell. The apical surface exhibits numerous microscopic projections (microvilli) that project into the lumen and give the appearance of a "brush border" when seen with a light microscope. Microvilli greatly increase the available absorptive surface area for the enterocyte. Nearly all substances taken up from the intestinal lumen traverse the cytoplasm of an enterocyte. Lipids and fats are absorbed at the base of the microvilli, traverse the cytoplasm, and exit the enterocyte at its lateral aspect, beneath the tight junctions. In contrast, amino acids, triglycerides, and carbohydrates are absorbed throughout the length of the microvillus. These substances traverse the cytoplasm and exit the enterocyte at its base. Under certain conditions, solvent drag (or convection) can be a paracellular route that takes advantage of partial separations of the tight junctions between intestinal cells to allow the passage of large amounts of water and small solutes. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

2.3.2 Sites of and Barriers to Absorption

Throughout the length of the alimentary canal, the lumenal epithelium is both a barrier to the entry of undesirable lumenal contents (such as bacteria and undigested materials such as cellulose) and a facilitator in the absorption of advantageous substances. Detailed discussions of these topics can be found elsewhere [3,4,11,20,24]. While the systemic absorption of orally ingested materials takes place predominantly in the small and large intestines, limited absorption occurs at other sites as well. The most important nonintestinal absorption site is the stomach, which can take up nonionized, lipophilic molecules of moderate size. Compared to that of the intestines, gastric absorption is limited by the comparatively small epithelial surface area, relatively large volume, and the brief amount of time that substances are in contact with the stomach epithelium. Neither the characteristics of the oral/pharyngeal/esophageal epithelium nor the transitory time that substances are in contact with the mucosa of those regions favors absorption. A small amount

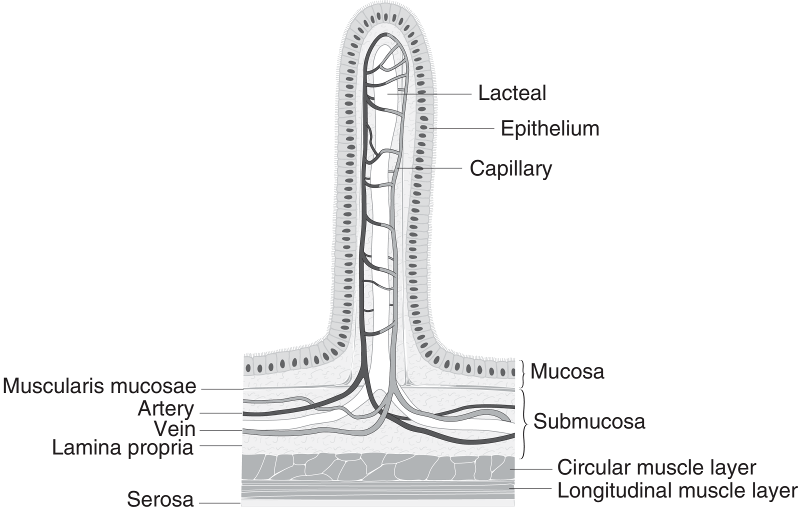

Figure 2.5 Structure of an intestinal villus. The lumenal surface of the entire intestinal tract, including each villus, is lined by enterocytes. Note the fuzzy appearance of the brush border on the surface of the villus. The deep structure of the villus is characterized by a large, blind-ending lymphatic vessel (the lacteal), which absorbs higher molecular weight substances, including fats. The lacteals are tributaries to the intestinal lymphatic channels that coalesce at the cisterna chyli, the origin of the thoracic duct. Materials transported in the chyle (fatty fluid) bypass the liver and avoid the first-pass effect (see Fig. 2.10 to appreciate the route of lymphatic drainage from the GI tract). Note the small plexus of blood capillaries that surround the lacteal. In addition to the vascular structures, the villus contains loose connective tissue and small amounts of smooth muscle (muscularis mucosae). The muscularis mucosae wiggle the villi back and forth in the liquid layer of the lumen adjacent to the intestinal cells, thereby increasing the efficiency of absorption. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

of absorption occurs sublingually (at the underside of the tongue and the floor of the mouth beneath the tongue) and across the buccal membranes; these routes are exploited in the delivery of certain pharmaceuticals (e.g., nitroglycerin and isosorbide dinitrate) [25].

Mechanisms that allow for the passage of materials from the lumen to the bloodstream include (i) passive diffusion across the epithelial membrane, (ii) facilitated (carrier-mediated) diffusion, (iii) active transport, (iv) aqueous pores within the cell membranes or tight junctions, and (v) endocytosis (the transport of material into the cell by folding of the cellular plasma membrane inwards to form a vacuole). These mechanisms of transfer are localized in select portions of the alimentary canal, predominantly in the small intestine.

Most xenobiotic substances are absorbed in the intestines via diffusion across the lumenal surface to gain access to the vasculature of the lamina propria. Passive diffusion follows Fick's law of diffusion [26,27]. Fick's law of diffusion states that bulk diffusion at steady state is proportional to the efficiency of exchange (membrane characteristics) and available surface area. Diffusion requires that a substance comes into intimate contact with the absorptive surface of the alimentary tract. Factors that affect passive diffusion include the expanse of absorptive surface area and the amount of time the substance is in contact with that surface area. Thus, to be able to relate GI absorption in various animal species to humans, it is important to have an understanding of the dimensions of the absorptive regions of the alimentary canal, the available surface areas of those regions, and the transit time of ingesta through each subdivision of the alimentary tract across species. These topics are discussed in Sections 2.5 and 2.6.

2.4 VASCULARITY

The tissues of the alimentary canal are richly vascularized by both the blood and lymphatic systems. Absorbed substances that have passed through the enterocytes gain access to the interstitial fluid of the lamina propria. The interstitial fluid has two sources: fluid in chyme that leaves the lumen of the alimentary canal and an exudate of plasma derived from the capillaries in the lamina propria. The amount of plasma exudate in the lamina propria increases as a result of the physiological hyperemia that occurs when chyme is present in the alimentary tract. The blood and lymphatic vascular systems act as a sink for excess interstitial fluid: absorbed substances move from the interstitium to the vessels by Le Châtelier's principle, depending on the relative flow of fluids in the two systems and the anatomical arrangement and structure of the vascular beds (Figs. 2.6–2.8).

2.4.1 Blood Flow

The GI tract of mammals is richly supplied from three major arteries that emerge from the aorta. The major arteries vascularize the upper (stomach and duodenum), middle (jejunum, ileum, cecum, and the ascending and about half of the transverse colons), and lower (remainder of transverse and descending colons and the rectal regions) regions. Figure 2.6 is a diagram of the arterial supply to the rat intestinal tract. The human pattern is similar; textbooks of anatomy provide great detail for the human condition [10,28].

The perfusion rate for the blood vasculature is about 1000 times that of the lymphatic vasculature for most tissues [11]. Nevertheless, the resting lymph flow rate from the small intestine is measurable (~0.095 mL/min/100 g of intestinal tissue in humans [29]) and increases after meals. Indeed, when the intestine is absorbing at its maximum rate, the lymphatic vasculature transports about 20% of the absorbed fluid [29].

The blood capillaries are organized as a network (plexus) immediately subjacent to the basement membrane of the enterocytes. The degree of vascularity of this plexus is not constant along all regions of the GI tract (Fig. 2.7). A blood capillary comprises a single, thin endothelial cell layer and the surrounding basal lamina. The distance between the basal lamina and the capillary lumen is no more than a few micrometers. Some endothelial cells additionally have 500 Å (0.05 μm) diameter openings (fenestrations) overlain only by the basal lamina; these fenestrations expedite absorption of water and various water-soluble materials [30]. Interestingly, the basal lamina appears to act as sieve; thus, molecules as small as albumin [~75 Å (0.0075 μm) diameter] are excluded from absorption.

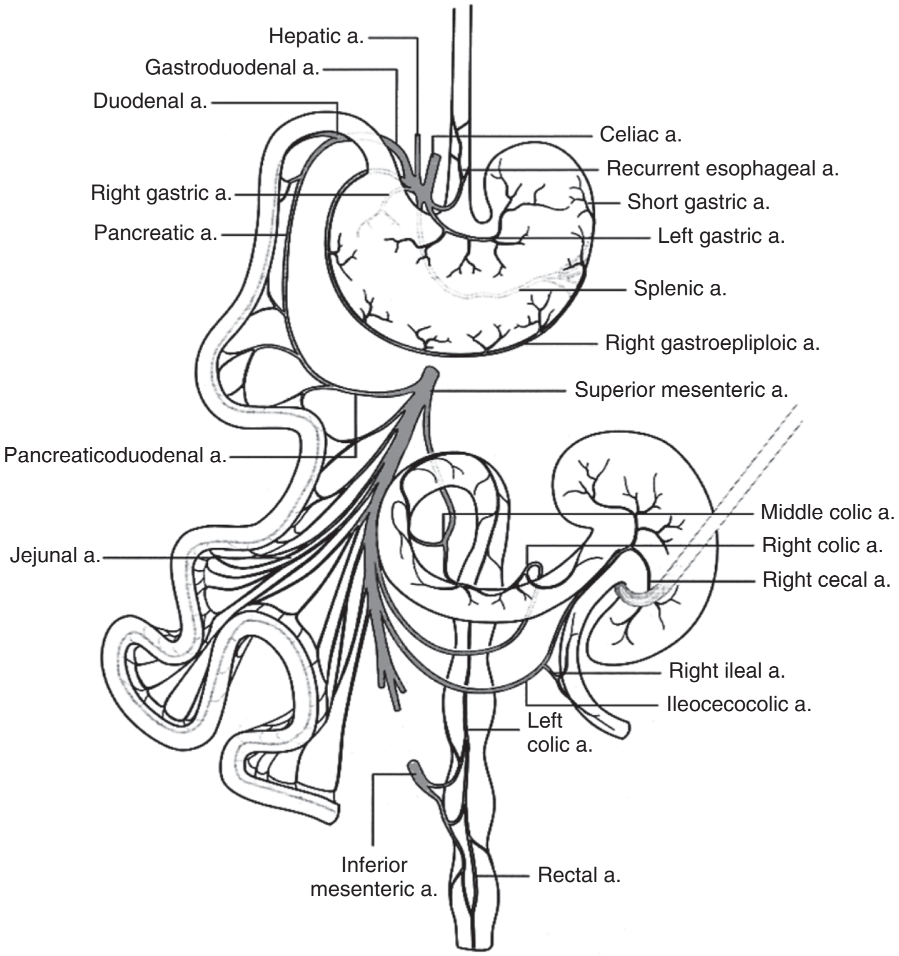

Figure 2.6 Arterial gastrointestinal supply in the rat. The rat arterial pattern is quite similar to that of the human (not shown). In both species, blood is provided by three major branches of the aorta: the celiac artery (which supplies the caudal portion of the esophagus, the stomach, the proximal duodenum, and portions of the pancreas), the superior mesenteric artery (which provides blood to portions of the pancreas, the caudal duodenum, the entire jejunum and ileum, the cecum, and the proximal half of the large intestine), and the inferior mesenteric artery (which furnishes blood to the caudal large intestine and most of the rectum). Note that the arteries enter the intestines along the margins of the organs and travel in tissue folds (mesenteries) that connect the intestines to the body wall. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

2.4.2 Lymphatic Flow

The lymphatic vessels are single saclike vessels (lacteals) located at the core of each villus. Their walls are made of a single endothelial cell layer, but the endothelial cells located here are thinner than blood capillary cells with no overlying basal lamina or fenestrations. Owing to apertures between adjacent endothelial cells, lymphatic vessels are permeable to even the largest chylomicrons [~6000 Å (0.6 μm) diameter

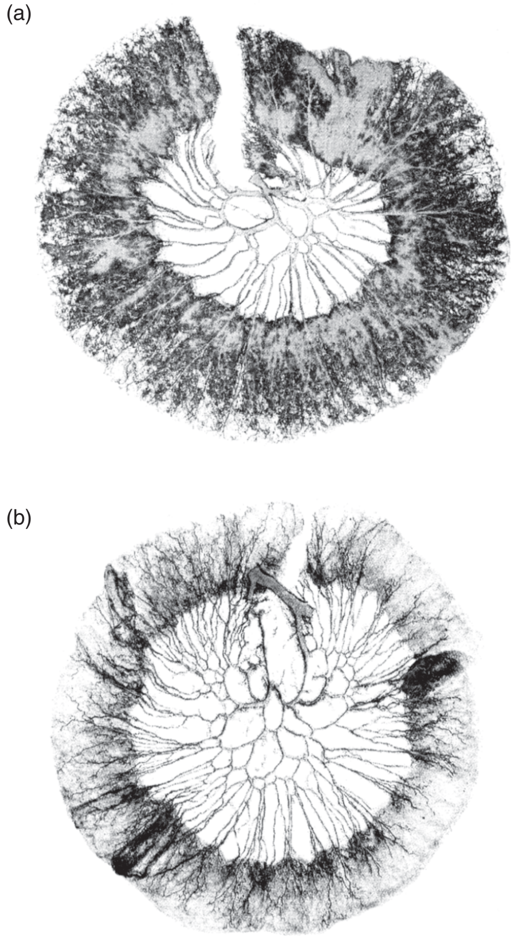

Figure 2.7 Blood supply in proximal versus distal small intestine. (a) Depicts the human proximal jejunum, which can be compared to (b), the distal ileum. The vascularity of these intestinal segments was visualized by injecting the superior mesenteric artery with colored gelatin, followed by clearing of the tissue in benzene. Note the much greater vascularity in the wall of the jejunum (a) versus that of the ileum (b), indicating the greater potential for jejunal absorption. This is a consequence of the presence of numerous plicae circulares in the proximal portion of the human small intestine. *Source*: Drawn after a preparation by M. C. E. Hutchinson [Warwick and Williams, 1973, p. 1285]; Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

emulsion droplets of resynthesized triglycerides, reesterified cholesterol, and phospholipids] [11,31].

2.4.3 Distinctions Between the Vascular Systems

The anatomical differences between the two vascular systems result in segregated routes of absorption for water-soluble materials versus chylomicrons. Water-soluble materials

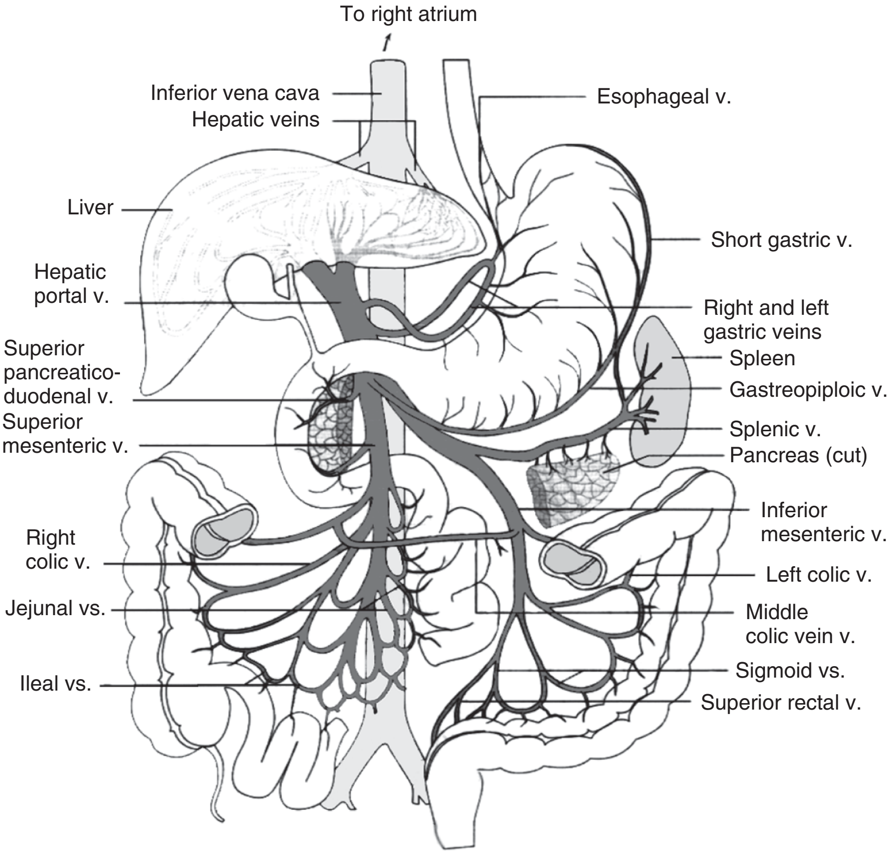

Figure 2.8 Human hepatic portal system. Blood draining from the stomach (via the gastric veins) and the entire small and large intestines (via the superior and inferior mesenteric veins) enters the large hepatic portal vein, which delivers the blood to the liver before the blood returns to the heart by way of the inferior vena cava. This vascular arrangement ensures that all materials absorbed into the blood capillaries of the gastrointestinal tract pass through the capillaries/sinusoids of the liver, where materials can be removed or metabolized before entering the general circulation. This is the anatomical foundation for the phenomenon known as the *first-pass effect*. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

are taken up by the blood vascular route, whereas lipophilic substances associate with chylomicrons and, therefore, follow the lymphatic route [20,32]. Amphipathic substances exhibiting moderate lipophilicity partition between chylomicrons and the aqueous route [12,33].

While both vascular systems serve as pathways that eventually return fluid to the systemic circulation, their routes of return have important differences. Blood vasculature from the gastroenteric organs (stomach, small intestine, and most of the colon) form into veins that ultimately become the hepatic portal vein. The hepatic portal vein drains to the liver, where the blood disperses into the sinusoids of the liver and comes into intimate contact with the liver tissue. Enzymes within the hepatocytes can biotransform xenobiotics to either more or less biologically active substances, concomitantly reducing blood levels of the parent compound. As previously noted, this phenomenon is termed the *first-pass effect*. To leave the liver, the blood follows vessels that recoalesce into the hepatic veins, which drain into the inferior vena cava en route to the heart. Thus, water-soluble substances absorbed from the gastroenteric organs first pass through the liver before entering the systemic circulation. The venous return of the hepatic portal systems for humans and is illustrated in Figs. 2.8 and 2.9, respectively.

High molecular weight and lipophilic substances in the lymphatic fluid draining from the stomach and intestines follow the lymph channels to the saclike cisterna chyli, which is the origin of the thoracic duct (Fig. 2.10). From the thoracic duct, lymph returns to the systemic circulation at the juncture of the left subclavian and left

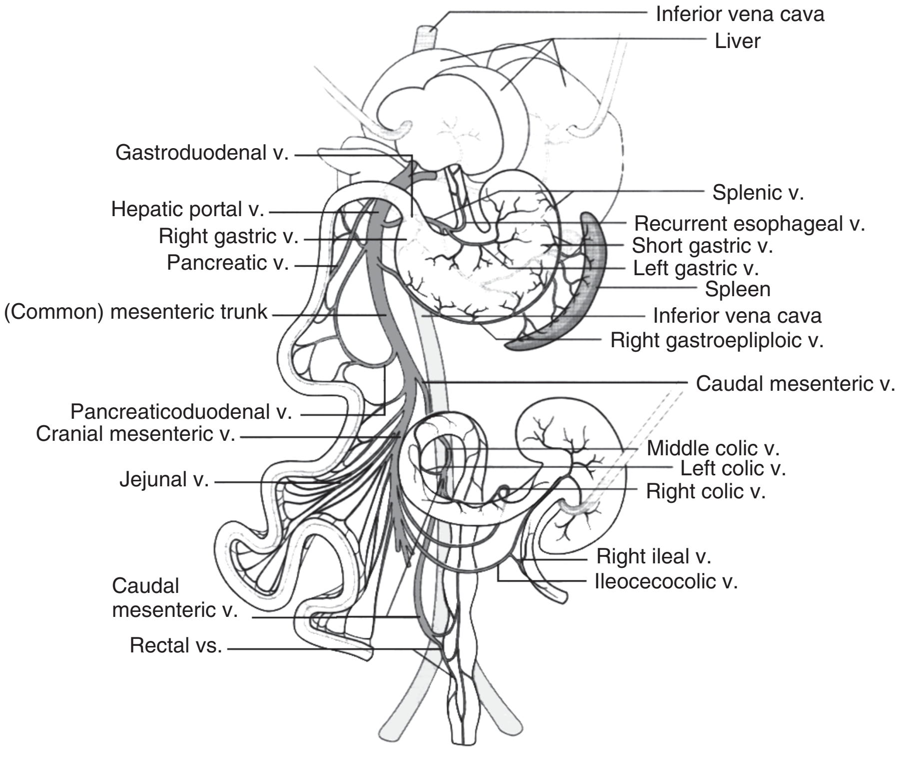

Figure 2.9 Rat hepatic portal system. The pattern of venous return from the gastrointestinal tract in rats exhibits some variability; therefore, for the purposes of this illustration, a common pattern of venous return is shown. In general, the venous return is similar to that of humans in that blood draining from the intestines eventually coalesces into the hepatic portal vein, which obligatorily passes through the liver before entering the inferior vena cava. Note that due to the postural difference between humans (upright) and rats (prone), the names of the analogous mesenteric veins of the rat are the cranial and caudal (rather than superior and inferior) mesenteric veins. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

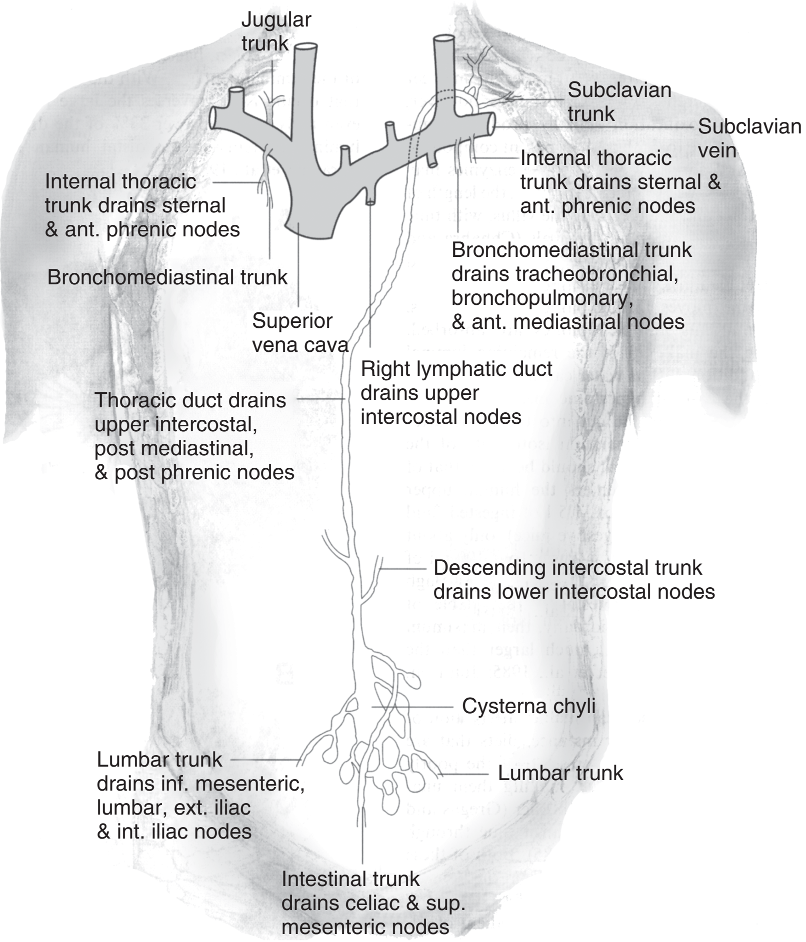

Figure 2.10 Human thoracoabdominal lymphatic system. Some high molecular weight and fatty materials absorbed from the intestinal tract make their way into the lacteals and follow the lymphatic channels through the cisterna chyli to the thoracic duct, to eventually enter the general circulation at the point where the left internal jugular and left subclavian veins join to form the brachiocephalic vein. From this point, the blood and lymph pass directly to the right atrium of the heart. Thus, materials absorbed into the lymphatics bypass the liver and do not undergo first-pass metabolism. Rats have a similar arrangement of lymphatic vessels, and consequently, materials absorbed into the rat lymphatic system also avoid first-pass metabolism. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

internal jugular veins in the root of the neck, thereby circumventing the liver and first-pass metabolism. The first capillary bed traversed by substances that are transported from the alimentary canal into the lymphatic system is at the lungs. Interestingly, any substance (regardless of its molecular weight or lipophilicity) that is absorbed into the bloodstream from the oral cavity (i.e., sublingually or buccally) returns to the heart by way of the superior vena cava, thereby also evading first-pass metabolism.

2.5 DIMENSIONS OF REGIONS OF THE GASTROINTESTINAL TRACT

While the basic organization of the alimentary canal of all mammals is comparable, there are great disparities in the dimensions of some GI structures, including the lengths and surface areas of the various subdivisions. In addition, the "environmental" conditions (e.g., pH and fluidity of the chyme and number and type of bacterial flora) within the lumen of the various subdivisions differ greatly among species. These differences, and their comparison to the human condition, must be taken into account when selecting an animal model for drug disposition studies and when extrapolating the results of studies from animal to human.

The portions of the GI tract considered in the remainder of this document are those involved in absorption of ingested materials, including the stomach, small intestine (duodenum, jejunum, and ileum), and large intestine (cecum, ascending colon, transverse colon, descending colon, and rectum).

2.5.1 Linear Dimensions

This section concentrates on the lengths and percentages of total length of the small and large intestinal regions of the GI tract for seven species (mouse, rat, rabbit, dog, pig, monkey, and human). The data are presented in Table 2.1. The lengths for subdivisions of each region are reported, when available. The data were assembled from multiple sources and, consequently, are presented as ranges when sources disagree.

Inspection of Table 2.1 finds wide ranges in the reported lengths of various portions of the intestinal tract among species. These discrepancies may be due to variations in measurement methods as discussed by Snyder *et al*. [23] and Choi and Chiou [34]. For instance, if the investigators used intubation procedures, their measurements could be too short, either because the intestines tend to gather on the tube or because the propensity of the tube to stray from the center of the lumen by cutting corners. Postmortem measurements may be too long because of the loss of smooth muscle tone in the intestine, which leads to flaccid intestines. If the intestines were fixed, imprecise measurements are likely due to the shrinkage that is attendant to the fixation process.

Potential artifacts notwithstanding, Table 2.1 shows that the rat cecum, which is a primary site for microbial digestion [43], makes up ∼26% of the length of the large intestine, whereas the cecum accounts for only about 5% of the large intestinal length in humans. Interestingly, the rabbit has the longest cecum among the species considered herein. Rabbits are the only herbivores among the subject species. Microbial fermentation of dietary fiber occurs in the rabbit cecum and is the main source of rapidly absorbed volatile fatty acids [44]. Of the species for which data are available, none possesses intestinal length measurements that are proportional to those of the human.

2.5.2 Surface Areas

While comparison of the absolute and relative lengths of the subdivisions of the intestinal tracts reveals some interesting differences among these species, the lengths do not provide adequate information about the surface areas available for absorption. For instance, as noted by DeSesso and Jacobson [4], while the human small intestine is only about 5.5 times the length of the rat small intestine, the human surface area is more than 250 times that of the rat due to modification of the interior surface of the intestine as discussed below.

TABLE 2.1 Comparison of Lengths of the Intestinal Tract and Its Major Subdivisions in Seven Different Species

Region of Intestinal Tract	Mouse			Rat			Rabbit			Dog			Pig			Monkey			Human		
	Length (cm)	% of total	% of Sub-division	Length (cm)	% of Total	% of Sub-division	Length (cm)	% of Total	% of Sub-division	Length (cm)	% of Total	% of Sub-division	Length (cm)	% of Total	% of Sub-division	Length (cm)	% of Total	% of Sub-division	Length (cm)	% of Total	% of Sub-division
Duodenum	5.3^{a}		—	9.5–10^{b}		8	—		—	25^{c,d}		6	—		—	—		—	25^{e}		4
Jejunum	46.2^{a}		—	90–135^{b}		90	—		—	360		90	—		—	—		—	260^{e}		38
Ileum	—		—	2.5–3.5^{b}		2	—		—	15^{c,d}		4	—		—	—		—	395^{e}		58
Total small intestine	45	∼75		125^{b}	83		339–398^{c,f}	61		∼400^{c,g,h}	83		1500–2000^{c,g,h}	70–85		—	—		680^{e}	81	
Cecum	3.4^{a}		—	5–7^{b}		26	41–61^{c,g,i}		28	5–8^{c,d,g}		∼11	20–30^{c,g,h}		—	—		—	7^{e}		5
Colon	10.0^{a}		—	9–11^{b}		42	114–165^{c,g,i,j}		72	25–60^{c,d,g,h}		∼81	400–500^{c,g,h}		—	—		—	93^{e}		60
Rectum	—		—	8^{b}		32	—		—	5^{d}		∼8	—		—	—		—	55^{e}		35
Total large intestine	—	∼25		25^{b}	17		—	39		∼82	17		350–850	15–30		—	—		155^{e}	19	
Total intestinal tract	—			150^{b}			582^{g,h}			482^{c,g}			2350^{c,g}			—	—		835^{e}		

[a] Ogiolda *et al*. [35]; intestinal segments unfixed.

[b] Hebel and Stromberg [8]; anatomical lengths as reported in DeSesso and Jacobson [4].

[c] Kararli [9].

[d] Evans [40].

[e] Snyder *et al*. [23]; anatomical lengths as reported in DeSesso and Jacobson [4].

[f] Pappenheimer [36].

[g] Stevens [37].

[h] Dressman and Yamada [41].

[i] Snipes [38]; formaldehyde fixed segments.

[j] Snipes *et al*. [39]; Bouins solution fixed segments.

Source: Reproduced from DeSesso JM, Williams AL. Annu Rep Med Chem 2008;43:353–371, with permission [23,42].

The small intestinal surface area is important because the vast majority of substances that are absorbed in the GI tract do so in the small intestine, where they gain access to the bloodstream by means of passive diffusion. As noted by DeSesso and Jacobson [4], the absolute surface area of the human small intestine is more than 3800-fold greater than the surface area of the stomach. This enormous difference in surface areas helps explain why gastric absorption of substances is generally small when compared to intestinal absorption. Similarly, because of the modest surface area of the human large intestine ($0.35\,m^2$, which is less than 0.15% that of the small intestine), the large intestine tends to play a minor role in absorption compared with the small intestine.

Because the available surface area is a dominant factor in passive diffusion, Table 2.2 presents the smooth lumenal surface areas of the small intestine for seven species, including humans. Various authors calculated smooth lumenal values by conceiving of the small intestine as a cylinder and using the length and diameter measurements. As noted in Table 2.1, the values listed therein should be considered estimated measures. As previously mentioned, the rate and extent of absorption is increased by expansion of the absorptive surface area. The absorptive surfaces of the small intestine in the subject species exhibit modifications that increase the surface areas by a combined factor of 100–750 depending on the species, as shown in Table 2.2 and illustrated in Fig. 2.11.

TABLE 2.2 Calculated Total Small Intestinal Surface Areas for Seven Species

Species	Body Weights (kg)	Smooth Lumenal Surface Area (m^2)	Fold-Increase Factors			Combined Multiplication Factors	Estimated Total Surface Area (m^2)
			Plicae	Villi	Microvilli		
Mouse	0.032	0.004[a]	(1)[b]	(5)[b]	(20)[b]	100	(0.4)
Rat	0.3	0.016[a]	1[c]	5[c]	20[c]	100	1.6
Rabbit	2.6–3.9	0.087–0.096[a]	1[d]	5.7[e]	24[f]	136.8	11.9–13.1
Dog	10.7–12.6	0.099–0.14[a,g]	1[d]	10[h]	25[i]	250	24.75–35
Pig	47	1.4[g]	1[d]	6[j]	(20–25)[k]	120	(168–210)
Monkey	2.7–3.0	0.091–0.11[b,l]	(3)[m]	(10)[m]	(20)[m]	600	(54.6–66)
Human	70	0.42[a]	3[c]	10[c]	20[c]	600	252

[a] Values as reported in Pappenheimer [36].

[b] Extrapolated from values for rat.

[c] From DeSesso and Jacobson [4].

[d] Value based on the absence of plicae in the small intestine.

[e] Value based on comparison of villi lengths between rabbits and humans as reported in Thomson and Snyder *et al*. [24,49].

[f] As cited in Westergaard and Dietschy [50].

[g] Values as reported in Chivers and Hladik [48].

[h] On the basis of the same size villi as humans as reported in Paulsen *et al*. and Snyder *et al*. [24,51].

[i] As reported in Kararli [9].

[j] On the basis of the comparison of villi lengths between pigs and humans as reported in Shirkey *et al*. and Snyder *et al*.

[k] Extrapolated from values reported for other animal species [24,52].

[l] Values as reported for cynomolgus monkeys.

[m] Extrapolated from values for human.

Source: Reproduced from DeSesso JM, Williams AL. Annu Rep Med Chem 2008;43:353–371, with permission [42].

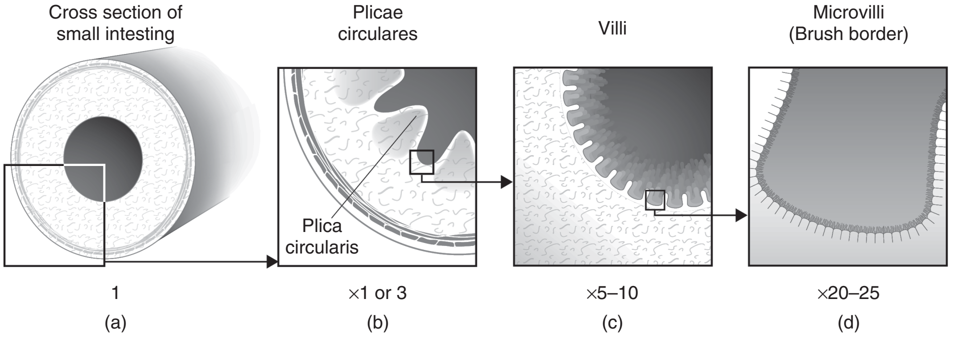

Figure 2.11 Increases in absorptive surface area by anatomic modifications of the intestinal wall. (a) The absorptive surface area of the intestine is depicted as that of a cylinder having a nominal value of 1. Modifications that increase the surface are shown to the right. (b) Folds of mucosa (plicae circulares) are represented. In species that have plicae circulares (monkey and human, among the species considered herein), the surface area is increased approximately threefold. (c) Fingerlike villi are shown projecting from the surface of the intestinal mucosa throughout the length of the intestine. The dimensions (length and width) of the villi vary for each species, as does the number of villi per square millimeter of mucosal surface. The villi increase the absorptive surface area by 5- to 10-fold. (d) The brush border on the surface of individual enterocytes is illustrated. The brush border is composed of microvilli, which further increase the absorptive surface area by 20- to 25-fold. *Source*: Reproduced from DeSesso JM, Williams AL. Annu Rep Med Chem 2008;43:353–371, with permission.

The greatest increase in absorptive surface area due to these modifications occurs in humans. Among the species listed in Table 2.2, only monkeys and humans possess plicae circulares, which increase the lumenal surface area approximately threefold. Intestinal villi increase the lumenal surface area by a factor ranging from 5 to 10. The range in values is due to interspecies variations in the dimensions of the villi (e.g., length, width, and density of the microvilli), as well as changes in dimensions within the different regions of the small intestine [8,23]. That is, the architecture of the villi changes as one progresses distally through the intestine. For example, villi in the duodenum tend to be broader than those of the ileum [45,46], resulting in a gradient of decreased surface area as one moves distally through the small intestine [47]. This gradient is even greater in those species (monkey and human) that possess plicae circulares (which also tend to be larger and more numerous in the proximal intestine). Microvilli, which account for the brush border, further increase the absorptive surface area by a factor of 20–25.

2.5.3 Relative Surface Areas

Animals of dissimilar sizes have different basal metabolic rates and, consequently, have different requirements for caloric intake. Thus, direct comparisons of the absolute surface areas of the GI tracts among various species are not straightforward. One way to normalize the surface areas of the stomach, small intestine, and large intestine across species is to consider these surface areas relative to the total body surface areas of different species. The metabolic rates of most mammals are proportional to body surface area [53,54]; consequently, the relative surface area serves to normalize the effective absorptive surface based on the metabolic requirements of a given species. The surface areas of the GI tract are presented as both absolute surface area and relative surface area in Table 2.3. (The relative surface area is the absolute surface area of a GI tract region divided by the body surface area.)

Humans are the largest species analyzed in Table 2.3; thus, it is not surprising that they have the largest absolute small intestinal surface area. It is noteworthy, however, that when the basal metabolic rates of the various species are taken into consideration, humans also have the largest relative surface area. The impact of the differences in surface areas on absorption of substances can be appreciated by comparing the relative surface areas of the human small intestine to that of the rat. The human relative surface area is approximately four times greater than that of the rat (Table 2.3). Since the amount of a substance that crosses the enteric mucosa is determined by its flux (amount of mass per unit surface area per unit time), the impact of the increased relative intestinal surface area in humans on absorption is twofold. First, substances that are equally well absorbed in both rats and humans are likely to be absorbed more quickly in humans (i.e., exhibit a higher rate of absorption). Second, substances that are poorly or incompletely absorbed by both species are likely to be absorbed to a greater extent by humans.

It should be noted that the values in Table 2.3 can be altered by physiological changes that occur in response to nutritional challenges. For example, during times of starvation or disease (such as diabetes [58]) or during normal physiologic stress brought on by pregnancy, the mucosa of the rat stomach undergoes hyperplasia [59]. In pregnancy, the mucosal surface area of the small intestine increases as gestation progresses due to a temporary, but progressive, increase in villi height [60]. Further, this condition of increased absorptive surface area is maintained throughout lactation [61]. Changes in effective surface areas also occur as animals age. The villi of the small intestine decrease in height during old age [62–64], leading to a decrease in absorptive surface area, and, consequently, reduced efficiency in the absorption of nutrients and other materials from chyme (see further discussion under Section 2.6).

With respect to the often cited concept that metabolic rates of species are proportional to their surface area raised to the two-thirds power [65], it is interesting that the mucosal surface area of the small intestines among nonruminant eutherian mammals increases approximately in proportion to the 0.6 power of body mass [36].

2.6 MOTILITY AND TRANSIT TIMES

As previously noted, absorption of materials from the intestinal tract is determined not only by the total available surface area but also by the amount of time that substances are in contact with the absorptive epithelium. Most of the alimentary canal is invested with at least two layers of smooth muscle. The muscle fibers of the inner layer are arranged circumferentially relative to the lumen, while the outer layer is arranged parallel to the long axis of the canal. The coordinated, rhythmic contractions of these muscle layers produce intestinal motility, which affects the absorption of substances from the intestinal tract. First, intestinal motility thoroughly mixes the chyme to aid in its digestion. Second, it continually agitates the chyme, pushing it against the enterocyte brush border, thus, aiding in absorption. Finally, intestinal motility propels the chyme

TABLE 2.3 Comparison of Absolute Surface Areas and Surface Areas Relative to Resting Metabolic Rates of the Gastrointestinal Tract and Its Major Subdivisions in Seven Different Species

	Mouse		Rat		Rabbit		Dog		Pig		Monkey		Human	
Body weight (kg)	0.032		0.3		2.6–3.9		10.7–12.6		47		2.7–3.0		70	
Body surface area (BSA; m^2)[a]	0.0066		0.03–0.06		0.23		0.39–0.78		—		0.3		1.8	
Region	Absolute SA[b] (m^2)	SA relative to BSA (% total)	Absolute SA (m^2)	SA relative to BSA (% total)	Absolute SA (m^2)	SA relative to BSA (% total)	Absolute SA (m^2)	SA relative to BSA (% total)	Absolute SA (m^2)	SA relative to BSA (% total)	Absolute SA (m^2)	SA relative to BSA (% total)	Absolute SA (m^2)	SA relative to BSA (% total)
Stomach	0.00035[c]	0.05 (0.1%)	0.00062[d]	0.01–0.02 (0.0%)	—	—	0.0344–0.0426[e]	0.04–0.11 (0.1%)	0.016[f]	—	0.0143–0.0306[g]	0.048–0.10 (0.0%)	0.053[h]	0.029 (0.0%)
Total small intestine[i]	0.4	60.61 (99.0%)	1.6	26.67–53.33 (97.9%)	11.9–13.1	51.74–56.96	24.75–35	31.73–89.74 (99.3%)	168–210	—	54.6–66	182–220 (99.8%)	252	140 (99.8%)
Total large intestine	0.0036[j]	0.55 (0.9%)	0.034[k]	0.57–1.13 (2.1%)	0.1606	0.7	0.023–0.245[e,f]	0.029–0.63 (0.5%)	0.5142	—	0.0687–0.0933[g]	0.23–0.31 (0.1%)	0.35[h]	0.19 (0.1%)

[a] As reported in Derelanko, 2000.

[b] Small intestinal surface areas reported as calculated in Table 2.

[c] Ogiolda *et al*. 1998; mean bw = ~43 g; unfixed tissue.

[d] Derived from Snipes, 1997; mean bw = 47.5 g; formaldehyde fixed segments; large intestinal value based on surface areas of cecum and colon combined.

[e] Jarvis and Whitehead, 1980; as reported in DeSesso and Jacobson, 2001.

[f] Young et al., 1991; as reported in DeSesso and Jacobson, 2001.

[g] Derived from Snipes, 1997; mean bw = 3.6 kg; formaldehyde fixed segments; large intestinal value based on surface areas of cecum and colon combined.

[h] Chivers and Hladik, 1980; values from 2 dogs (bw = 10.68 & 12.55 kg).

[i] Derived from Snipes, 1997; mean bw = 13.75 kg; formaldehyde fixed segments; large intestinal value based on surface areas of cecum and colon combined.

[j] Chivers and Hladik, 1997; values based on single pig (bw = 47.98 kg); formaldehyde fix segments; large intestinal value based on surface areas of cecum and colon combined.

[k] Derived from Chivers and Hladik, 1980; values for Cynomolgus monkeys (bw = 2.7–3.1 kg); unfixed tissues.

Source: Reproduced from DeSesso and Williams, Ann Rep Med Chem 2008; 43: 353–371, with permission.

TABLE 2.4 Range of Gastric Emptying Half-Times (in Hours) as Reported for Seven Species of Animals

Species	Liquids	Particulates
Mouse	----1.23[a]----	
Rat	—	—
Dog	1.5[b]	1.5[b]
Rabbit	1.3[b]	12[b]
Pig	2[b]	10[b]
Monkey	0.4–0.5[c]/2.4[d]	—
Human	0.2[e]	0.5–3.4[a,f]

[a] Schwarz *et al*. [70].

[b] Values reported in Kararli and Stevens [10,37].

[c] Kondo *et al*. [71]; measurements from fasted cynomolgus monkeys.

[d] Kondo *et al*. [72]; measurements from fed cynomolgus monkeys.

[e] Granger *et al*. [11].

[f] Degan and Phillips; measurements for males and females, 19–45 years of age.

Source: Reproduced from DeSesso JM, Williams AL. Annu Rep Med Chem 2008;43:353–371, with permission [42,73].

through the GI tract in a net aboral direction (peristalsis), the rate of which influences GI transit times. In addition, the thin layer of smooth muscle in the lamina propria (muscularis mucosae) undulates the intestinal villi, thereby churning the unstirred layer of fluid that is associated with the brush border.

Transit time is the amount of time that elapses as a bolus of food or chyme traverses a particular region of the alimentary canal. In the mouth, transit time is determined by voluntary control of the length of time that is spent chewing food. When the food bolus passes to the pharynx and esophagus, transit time is governed by gravity and peristalsis. In humans, transit time through the pharynx and esophagus is about 6 s. Once in the stomach, transit times depend on the nature of the contents (Table 2.4 [12,66]). In the fasted state, the half-time for saline emptying from the human stomach is 12 min, while the gastric transit time for a full meal is about 4 h [11]. Generally, the various dietary constituents of a meal leave the stomach at varying times, with carbohydrates emptying first, proteins next, and fatty substances last. Liquids drunk with a meal often bypass the solids and enter the duodenum quickly [11]. Table 2.5 presents the ranges of transit times through various portions of the GI tract for each of the subject species. Transit times tend to be shortest in the stomach and longest in the large intestine. Additionally, liquids typically pass through the stomach more quickly than solids. Ingestion of a fatty meal, however, will delay significantly the emptying of stomach contents to the duodenum [10,24]. The fasting/fed state of an individual can also affect transit time, as demonstrated by the great differences in gastric emptying half-times for liquids given to fasted versus fed monkeys. For instance, after prolonged fasting the orocecal transit time is extended, probably because the body needs to absorb additional calories from the ingested material [67]. The amount of fiber in the diet not only influences transit time but also interferes with the access of some substances such as cholesterol to the absorptive surface [68,69].

Physiological status also influences transit time. For example, life stage (e.g., puppy vs adult) influences transit time in dogs. The mean orocecal transit time decreased as large breed puppies grew (Great Dane and Giant Schnauzer). This did not occur, however, in smaller breeds (Miniature Poodle and Standard Schnauzer). It is believed

TABLE 2.5 Range of Gastrointestinal Transit Times (Hours) Through Different Portions of the Gastrointestinal Tract as Reported for Seven Species of Animals

Species		Oro-Cecal				Large Intestine		Total	
		Stomach		Small Intestine					
		Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Mouse		—		—		—		—	
Rat		0.7–2.1^{a}		2.6–3.3^{a}		15.5^{b}		38.9^{c}	
Dog		1.7^{d}		—		18.5^{e}		27.0–37.4^{e,f,g}	
		----14.3^{e}----							
Rabbit		----9.1^{h}----				9.6^{h}		18.1–20.6^{h}	
Pig		0.8–0.9^{i}	1.0–1.3^{i}	3.9–4.4^{i}	3.7–4.3^{i}	24.9–41.3^{i}	35.6–44.4^{i}	29.1–46.6^{i}	40.5–49.7^{i}
Monkey		----1.8^{j}/2.3–2.5^{k}----				—		—	
Human	Child	1.1^{l}		7.5^{l}		17.2–40^{l,m}		—	
	Young adult	1.0–1.6^{d,n}	1.9–2.3^{n}	3.8–3.9^{n}	3.4–3.5^{n}	35.0^{n}		57.3–58.2^{r}	
		1.1^{o,p}		1.8–8.0^{l,o,q}		17.5^{l}			
	Mature adult	0.8–1.1^{n}	1.3–1.8^{n}	2.0–4.2^{n}	3.0–3.8^{n}	33.5–61.5^{n}			

[a] Tuleu *et al*. [74]; measurements from fed rats.
[b] Enck *et al*. [75]; measured by the insertion of dye in the cecum.
[c] Sastry and Rao [76].
[d] Lui *et al*. [77]; liquid transit in fasted Beagle dogs and young adults (21–29 years of age).
[e] Hernot *et al*. [78]; measured in Standard Schnauzers.
[f] Burrows *et al*. [79]; measured in Beagle dogs.
[g] Hernot *et al*. [80]; measured in French Bulldogs, English Cocker Spaniels, and Standard Schnauzers.
[h] Gidenne and Ruckebusch [81].
[i] Wilfart *et al*. [82]; measurements derived from pigs with average weight of 33 kg.
[j] Kondo *et al*. [71]; measurements from fed cynomolgus monkeys.
[k] Kondo *et al*. [72]; measurements from fasted cynomolgus monkeys.
[l] Fallingborg *et al*. [83]; determined in children 8–14 years of age.
[m] Wagener *et al*. [84]; determined in children 4–15 years of age.
[n] Graff *et al*. [85]; determined in adults 20–30 years of age (young) and 38–53 years of age (mature).
[o] Ewe *et al*. [86].
[p] Fallingborg *et al*. [87].
[q] Degan and Phillips [73]; measurements for males and females, 19–45 years of age.
[r] Cummings *et al*., 1978; males only.
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that the decrease is due to the shorter gastric emptying times in large breeds during growth rather than to a faster rate of transport through the intestine [89,90]. In humans, orocecal transit times are significantly longer among older people compared to young adults, but transit time in the large intestine increases significantly only among women [85]. Because the dimensions of intestinal villi change such that the available absorptive surface area decreases with age [91], the increased transit time may be a compensation that improves (or attempts to realize) sufficiency of nutritional absorption. This same group of investigators reported that the overall GI transit time was significantly longer in women than in men at all ages.

2.7 LUMENAL CONTENTS

2.7.1 Physical and Chemical Nature of the Contents

The nature of the lumenal contents in all species is altered as the contents traverse the alimentary canal. This alteration is due to the physical dispersion of ingested solid matter by chewing and the muscular activity of the stomach, the mixing of the ingested matter with imbibed fluid and digestive juices, the action of enzymes on GI contents, the absorption of fluid and materials from the lumen, and the addition of bacterial flora to the contents. There is a dramatic difference in the nature of the chyme that enters the duodenum of rats compared to that of humans [4]. Rat chyme contains many bacteria and has the consistency of a moist paste when entering the duodenum, whereas human chyme is watery and its bacterial content is negligible. The chyme of mice and rabbits is quite similar to that of the rat. The chyme of dogs, pigs, and monkeys is more similar to human chyme than to that of the rat. As chyme moves through the distal portion of the small intestine in all species, it begins to acquire a more viscous consistency, especially at the distal end of the ileum.

The daily volumes and constituents of digestive fluids secreted (under neuronal and hormonal control) by the various portions of the digestive tract and adnexal organs vary greatly based on the species and its physiological status. Estimates of these vary among publications, but the values summarized by Kararli [9] and DeSesso and Jacobson [4] are useful starting points for understanding the differences among species. The quantity and characteristics of these secretions can be altered in response to lumenal contents. In addition to the intestinal secretions, water can move from the blood into the duodenum in response to a hypertonic meal, in order to maintain the isotonicity of lumenal contents with that of plasma [11,12,24].

The pH of the luminal contents also varies widely among species (Table 2.6). As a general rule, however, the pH of luminal contents is the lowest in the stomach and increases as the chyme progresses distally through the GI tract, approaching neutrality. This modification in pH as the chyme travels through the GI tract is accomplished through the secretion of various acidic and alkaline fluids. In most regions of the digestive tract, the secretions are slightly alkaline, and the lumenal contents exhibit a pH of 7–8 as chyme approaches the distal small intestine. The stomach is the lone exception to this general statement. The secretion of acid by the gastric mucosa results in acidification of chyme. In carnivores (including humans, pigs, and dogs) as well as the rabbit (an herbivore), the pH of the stomach is quite low (as low as 1–2 in humans), whereas the gastric pH of rodents (rats and mice) is only slightly more acidic (pH 3–5) than that of the skin. The moderate pH in the stomach of rodents is a consequence of the requirement for microfloral digestion of ingested material that begins before entry to the small intestine [9]. Animals with very acidic stomach contents begin the digestion of proteins therein and have no requirement for a large bacterial colony in their stomach. When chyme enters the duodenum, it is quickly neutralized by the alkaline (bicarbonate) secretion of the Brunner's glands [92]. The pH of chyme affects the ionization state of certain molecules and, therefore, can affect absorption.[2] Despite

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[2] The GI tracts of most elderly humans exhibit the same pH characteristics as do those of younger adults, but there are notable exceptions. Some seniors exhibit elevated gastric pH or even achlorhydria (the inability to secrete gastric acid; Dressman *et al*., 1989). Achlorhydria is often accompanied by delayed gastric emptying times.

TABLE 2.6 Range of pH Values Reported for Different Portions of the Gastrointestinal Tract for Seven Species of Animals[a]

Species	Stomach		Small Intestine[b]			Large Intestine		
	Anterior	Posterior	Duodenum	Jejunum	Ileum	Cecum	Colon	Rectum/Feces
Mouse	4.5	3.1	—	—	—	—	—	—
Rat	4.3–5.1	2.3–4.0	6.5–7.1	6.7–6.8	7.1–8.0	6.4–7.2	6.6–7.6	6.9
Dog	1.5–5.5	1.5–3.4	6.2	6.2–7.3	7.5	6.4	6.5	6.2
Rabbit	1.9–2.2	1.9–2.2	6.0–6.1	6.8–7.5	7.2–8.0	5.7–6.6	6.1–7.2	7.2
Pig	1.6–4.3		6.0	6.2–6.9	7.5	6.3	6.8	7.1
Monkey	4.7–5.0	2.3–2.8	5.6–6.0	5.8–6.0	6.0–6.7	4.9–5.1	5.0–5.9	5.5
Human	1.5–5.0		5.0–7.0	6.0–7.0	7.0–7.4	5.7–5.9	5.5–7.5	6.5–7.0

[a] The range of values reported here are from the following Refs. 10,44,77,87, and 93–95.

[b] Some studies reported pH values for segments 1, 3, 5, and 7 of the small intestine. For the purposes of this table, segment 1 was designated the duodenum, segments 3 and 5 were designated the jejunum, and segment 7 was designated the ileum.

Source: Reproduced from DeSesso JM, Williams AL. Annu Rep Med Chem 2008;43:353–371, with permission [42].

its status as a carnivore, the pH of the GI tract of the dog presents some unique characteristics compared with humans. For instance, the fasting intestinal pH in dogs is higher than that of humans (7.3 in the dog vs 6.0 in humans [77]).

In addition to the pH gradient that exists along the linear axis of the GI tract, a pH gradient also exists from the center of the lumen moving radially toward the epithelial surface. The pH of the lumen is more acidic than the pH of contents at the epithelial surface. This is a function of the unstirred layer of water that is associated with the brush border [4] and the alkaline secretions of the intestinal epithelia [96]. Furthermore, this gradient may affect the rate of uptake of various substances, especially xenobiotics.

The contents of the alimentary canal are also altered by many enzymes found in the secretions of the digestive tract, integrated into or adhered to the cell surface membrane of small intestinal cells, and present within small intestinal cells. Many of these enzymes work to digest carbohydrates, proteins, and fats into readily assimilated molecules necessary to sustain life. Others are involved in the metabolism of xenobiotic compounds. Such compounds may be oxidized, hydrolyzed, and conjugated with large, polar molecules in order to facilitate their excretion. In some instances, however, this attempt at detoxification actually produces compounds that are more toxic than their parent compound. The presence and activity of all these enzymes may vary among species, with age and sex, along the length of the digestive tract and the height of the villus, with diurnal rhythm and diet, and among individuals [33,97–99].

The makeup of the lumenal contents is also changed through the progressive absorption of fluids, electrolytes, nutrients, and xenobiotics as chyme moves through the GI tract. Just as water can flow from the blood to the lumen in the case of hypertonic meals, water can rapidly be absorbed from the gut into the blood after hypotonic meals—again to maintain isotonicity of the lumenal contents with plasma. It should be noted that of the 8–9 L of fluid that enters the human upper digestive tract each day (~1.5 L of ingested fluid plus ~7 L of secreted digestive juice), only ~1 L enters the large intestine and only about 100 mL of water is found in the daily output of feces [4].

2.7.2 Bile and Bile Salts

With regard to interspecies differences in secretions into the intestinal tract, it is important to note that among the subject species in this chapter, the rat is the only one that lacks a gallbladder. While the rat is unique in this respect among typical experimental animals, other common vertebrates, such as pigeons and horses, also lack gallbladders. Species that lack gallbladders do not concentrate bile; rather, bile flows into the lumen of the duodenum on a continuous basis. This means that bile continuously enters the rat duodenum as a dilute solution as it is made. By way of comparison, humans secrete from the gallbladder 2–22 mL of bile per kilogram body weight each day [41]. In contrast, rats secrete 48–92 mL of bile per kilogram body weight each day [20].

The daily flow rate of bile into the duodenum (normalized by body weight in kilograms) for five of the subject species is shown in Table 2.7. The table also lists the concentration of bile salts within the bile for five of the seven subject species. There is no clear relationship between the size of the species and its bile flow rate. Interestingly, the highest rate is found in the herbivorous rabbit.

Bile is a complex mixture of organic and inorganic materials of which the various bile acids are the major component. Bile acids are an extensive group of molecules that share a structural similarity to cholesterol but exhibit differences with regard to substituent side groups. Table 2.8 presents selected physical and chemical properties of bile and bile acids for six of the subject species in this chapter. Inspection of the table reveals that porcine bile has a bile acid composition and other properties that are most similar to human bile. Note that conjugation of bile acids to glycine or taurine is not an excretory pathway, but rather a means to enhance the enterohepatic recirculation of bile acids (see discussion in Section 2.8).

Interestingly, the composition of bile in hamsters, which is not considered in this chapter, is most similar to that of humans [100]. It is also noteworthy that rabbits have the most lipophilic bile of the animals considered, but they are unique in that about 80% of their bile acids are formed by bacterial flora [101].

TABLE 2.7 Comparison of Flow Rates and Composition of Bile from Seven Different Species[a]

Species	Bile Flow Rate (mL/day/kg)	Total Bile Salts (mmol/L)
Mouse	—	—
Rat	48–92	17–18
Rabbit	130	6–24
Dog	19–36	40–90
Pig	—	—
Monkey	19–32	22
Human	2.2–22.2	3–45

[a] Data from Kararli [9].

Source: Reproduced from DeSesso JM, Williams AL. Annu Rep Med Chem 2008;43:353–371, with permission [42].

TABLE 2.8 Bile Acid Characteristics in Various Species

Species	Gallbladder	Lipophilicity of Bile Acid Pool	Bile Acids						Preferred Conjugate Molecule
			CA	DCA	CDCA	HCA	HDCA	MCA	
Rat	Absent	Very low	XX[a]					X[b]	Taurine
Mouse	Present	Very low	XX					X	Taurine
Rabbit	Present	Very high	X	XX					Glycine
Dog	Present	Low	XX	X					Taurine
Pig	Present	Moderate	X		XX	X	X		Glycine/ taurine
Human	Present	Moderate	X	X	XX				Glycine/ taurine

Abbreviations: CA, cholic acid; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; HCA, hyocholic acid; HDCA, hyodeoxycholic acid; MCA, muricholic acid.

[a] Predominant bile acid.

[b] Present.

Source: Data from Hofmann *et al*. (2010), Hofmann (2009), and Hofmann and Roda (1984) [100–102].

2.7.3 Bacterial Flora and Coprophagy

Bacterial flora populates much of the GI tract in all species and contributes to the lumenal contents. The mouth and pharynx have a rich and diversified bacterial population, although oral bacteria are not important in absorption. In rodents, large numbers of microorganisms are found in the stomach and intestines. In humans and rabbits, however, because of the low pH of gastric contents, microorganisms are virtually absent in the stomach and proximal small intestine. In fact, large numbers of bacteria are not encountered until chyme reaches the distal ileum and large intestine [11,94,103,104]. Bacteria are capable of metabolizing substances found in chyme and, thus, can alter constituents found in the luminal contents, which is particularly important in the case of ingested medicines and xenobiotics [105]. Differences between humans and rats with regard to the numbers and types of bacteria, as well as to their geographical location within the digestive tract, affect the site and extent of absorption of some substances [11,106]. Once chyme reaches the colon, bacteria are a major component of the lumenal contents. With the dehydration of chyme that occurs as it traverses the large intestine, bacteria eventually make up over 33% of the dry weight of the lumenal (fecal) content in the distal human large intestine [11].

Many species, especially rodents and rabbits, coprophagize (ingest feces) to aid the digestive process and to ensure absorption of essential components of their diets. This is exemplified by the rabbit, which has a unique digestive metabolism for a monogastric animal [107,108]. Rabbits defecate two types of fecal pellets: hard and soft [107,109]. The soft pellets are known as *cecotrophs* and are the pellets that are consumed by rabbits [110]. Cecotrophs are high in protein content and vitamin B complex [107]. Coprophagy also helps maintain a balanced, healthy population of flora. Impairment of coprophagy can result in deficiency of vitamins, as has been noted for over 80 years [111].

2.8 ENTEROHEPATIC RECIRCULATION

As previously stated, the hepatic portal system involves blood flow from the capillaries of the small intestine that drain to the hepatic portal vein, which flows into the hepatic sinusoids before reaching the heart. The movement of venous blood from the intestine along this specific route is referred to as the *enterohepatic circulation* (Fig. 2.12).

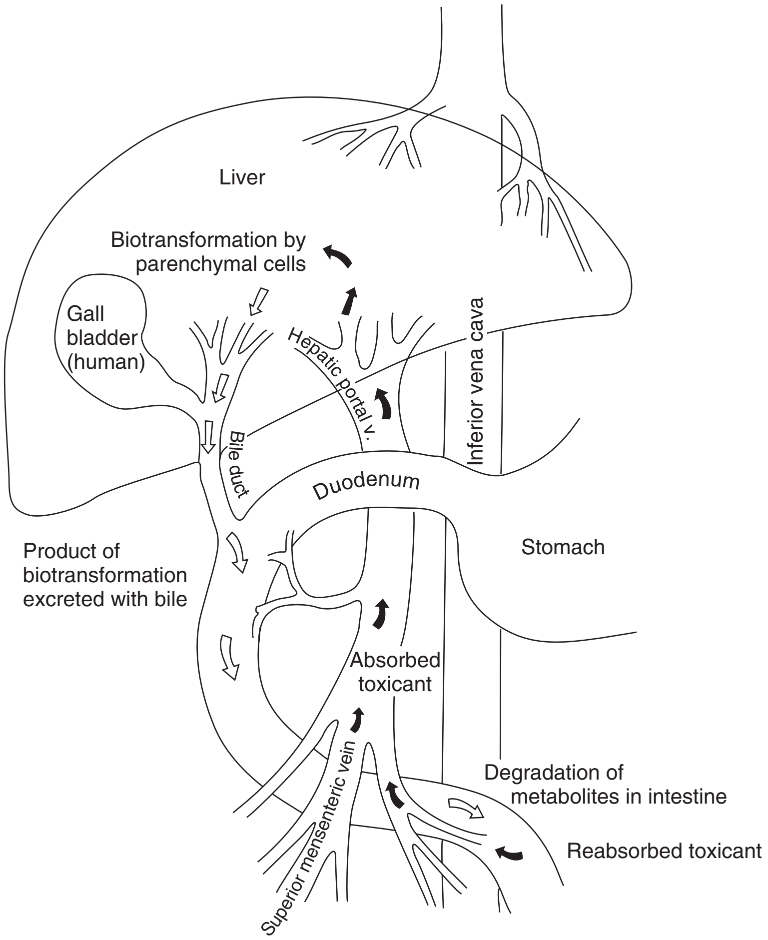

Figure 2.12 Enterohepatic recirculation. This cartoon illustrates the route followed by substances undergoing enterohepatic recirculation. Substances are absorbed from the lumen of the small intestine into the blood capillaries and are carried to the liver by the hepatic portal vein system (black arrows). Within the liver, the substances can be biotransformed by enzymes present in the hepatic cells, often being oxidized and/or conjugated. The biotransformed substances are subsequently excreted into the bile, which collects into the bile duct to empty back into the lumen of the duodenum (open arrows). There, the biotransformed substances may be acted on (usually deconjugated) by digestive enzymes, or, more commonly, by intestinal flora, to re-form the original substances that can then be reabsorbed into the intestinal capillaries, allowing the preceding steps to be repeated. *Source*: Reproduced from DeSesso JM, Jacobson CF. Food Chem Toxicol 2001;39:209–228, with permission.

Many absorbed substances are biotransformed by the hepatic parenchymal cells. Biotransformation products tend to be larger and more polar than the parent compounds, which favors their excretion into the bile. Bile is eventually released into the duodenum. Because large, polar molecules tend not to be reabsorbed from the intestine, bile serves as a primary route of excretion for these molecules into the feces. Under certain conditions, however, some substances may be reabsorbed from the GI lumen, usually due to bacterial degradation of the metabolites in the intestinal lumen. Reentry of these substances into the enterohepatic circulation may be followed by another round of biotransformation, followed by re-excretion into bile and then bacterial degradation and re-uptake. Enterohepatic recirculation can markedly affect the disposition of certain substances and is greatly affected by species-specific composition of bile acids [102].

A substance experiencing enterohepatic recirculation is able to maintain, or even increase, its systemic blood concentration, prolong its half-life in the blood, and even intensify its therapeutic or toxic effects. Alternatively, the compound may remain within the enterohepatic circulation (i.e., does not reach the systemic circulation), being absorbed from the intestine, taken to the liver, and excreted in bile over and over again. In such a case, systemic blood levels remain low, and any toxic effects outside the GI/enterohepatic tract are diminished, while effects within the tract may be increased [112].

2.9 CONCLUSIONS

Several parameters require consideration in any attempt (i) to define and model the events involved in the GI absorption of nutrients and nonnutrients and (ii) to extrapolate the results of these efforts between species. These include anatomical and physiological features of the GI tract (surface area, vascularity, transit time/motility, and enterohepatic recirculation). The physicochemical properties of the substance of interest and of the GI contents must also be considered. Variations in these parameters among different organs of the GI tract and among species affect the sites and rates of absorption, as well as the distribution and metabolism of the absorbed material. By far, the greatest amount of experimental information regarding GI absorption is available for the rat. Owing to similar small intestinal transit times, it is likely that the total amount of material absorbed by the small intestine after oral administration is similar between humans and rats, as has been concluded by others [113]. However, the rate at which ingested materials are absorbed differs between the two species. This is largely a function of the greater amount of surface area found in the proximal portion of the small intestine of humans. This led to the conclusion in our previous paper [4] that humans are likely five times more efficient at absorbing ingested materials than rats. This conclusion compares favorably to the report of Pappenheimer [36], who found that perfused jejunal segments of normal human subjects absorb fluids at a rate that is 5–10 times greater per unit area of mucosa than that of laboratory rats. It is also of interest to note that the oral absorption of a series of 43 drugs in dogs, which share many of the same physiological characteristics as humans, was found to differ significantly from the human [114], whereas the absorption of these substances in rats was much closer to that of humans [113]. The reasons for this remain unclear.

Not all parameters will be important for all chemicals, but a definitive knowledge of the role that each plays in the absorption of a particular substance of interest should allow a quantitative description of the absorption process. Unfortunately, in many cases, the data necessary to evaluate the importance of these parameters is not available, and experiments must be done to measure their effects on absorption. Only by doing so can we effectively understand and accurately model the kinetics of ingested compounds. While the information provided in this chapter is not able to answer the question of which species is the most suitable model to study GI absorption, it is hoped that it will be useful to investigators who must select an animal model in which to test the pharmacological and toxicological effects of compounds for the purpose of extrapolating the findings to humans.

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