Chapter Six

Drug Absorption from the Small Intestine

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ANATOMY AND PHYSIOLOGY OF THE SMALL INTESTINE

The small intestine is between 5 and 6 metres in length and its main functions are to mix food with enzymes to facilitate digestion, to mix the intestinal contents with the intestinal secretions to enable absorption to occur, and to propel the unabsorbed materials in an aboral direction. The small intestinal epithelium has the highest capacity for nutrient and drug absorption within the gastrointestinal tract, due to the large surface area provided by epithelial folding and the villous structures of the absorptive cells.

Gross morphology

The small intestine is the longest section of the digestive tube and it is arbitrarily divided into three parts. The first 20 to 30 cm is termed the duodenum, the second 2.5 metres the jejunum and the final 3.5 metres the ileum. These regions are not anatomically distinct, although there are differences in absorptive capability and secretion. There is no definite sphincter between the stomach and duodenum although in some studies a zone of elevated pressure between the two regions has been reported to exist. The duodenum has a thick wall with a deeply folded mucous membrane and contains duodenal digestive glands and Brunner's glands. Brunner's glands are found only in the submucosa of the duodenum and produce a protective alkaline secretion which does not contain any enzymes, but serves to neutralize gastric acid. The jejunum is thicker walled and more vascular than the duodenum and has larger and more numerous villi than the ileum. In the ileum, the lymphatic follicles (Peyer's patches) are larger and more numerous than elsewhere in the intestine.

Most of the small intestine is suspended from the body wall by an extension of the peritoneum called the mesentery. The blood vessels which supply the small intestine lie between the two sheets of the mesentery.

Mucosa

The small intestine consists of the serosa, the muscularis, the submucosa and the mucosa (Figure 6.1). The serosa is an extension of the peritoneum, and consists of a single layer of flattened mesothelial cells overlying some loose connective tissue. The muscularis has an outer longitudinal layer and an inner circular layer of muscle. The submucosa consists largely of dense connective tissue sparsely infiltrated by lymphocytes, fibroblasts, macrophages, eosinophils, mast and plasma cells. The submucosa contains an extensive lymphatic network.

The intestinal mucosa itself can be divided into three layers:

a) the muscularis mucosa, which is the deepest layer consisting of a sheet of muscle 3 to 10 cells thick that separates the mucosa from the submucosa.

b) the lamina propria, the middle layer, is mainly connective tissue and forms the core of the numerous villi and surrounds the crypts. The lamina propria usually contains many types of cells, e.g. plasma cells, lymphocytes, mast cells, macrophages, smooth muscle cells and non-cellular elements such as collagen and elastin fibres. The lamina propria provides structural support, and there is increasing evidence that it has an important role in preventing the entry of microorganisms and foreign substances.

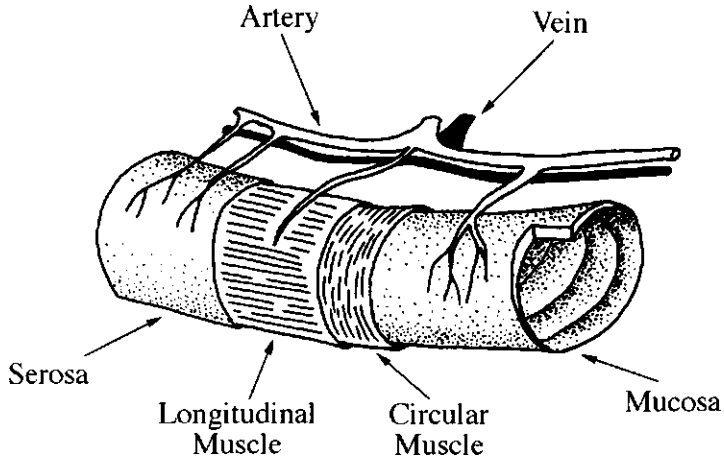

Figure 6.1 Section of the small intestine

c) the epithelium, which is the innermost layer of the mucosa and consists of a single layer of columnar epithelial cells or enterocytes, which lines both the crypts and the villi.

Organisation of the mucosa

The surface area of the small intestinal mucosa is greatly increased by the folds of Kerckring, villi and microvilli (brush border) and is about 200 m^2 (or roughly the size of a tennis court!) in an adult (Figure 6.2).

Folds of Kerckring

A particularly prominent feature in the small intestine is the folding of the epithelium, known as the folds of Kerckring. The folds increase the surface area by a factor of 3. These folds extend circularly most of the way around the intestine and are especially well developed in the duodenum and jejunum, where they protrude by up to 8 mm into the lumen. They also act as baffles which aid mixing of the chyme in the small intestine.

Villi

The surface of the mucous membrane of the small intestine possesses about 5 million villi, each about 0.5 to 1 mm long. Although the villi are often described as "finger-like", their shape changes along the gut and duodenal villi are shorter and broader than those found in the jejunum. Further down the gut the villus height decreases. Diet and environment markedly affect mucosal morphology and intestinal biopsies demonstrate differences between human populations. There is also a species difference, for example, the villi of the chick are pointed and leaf-like.

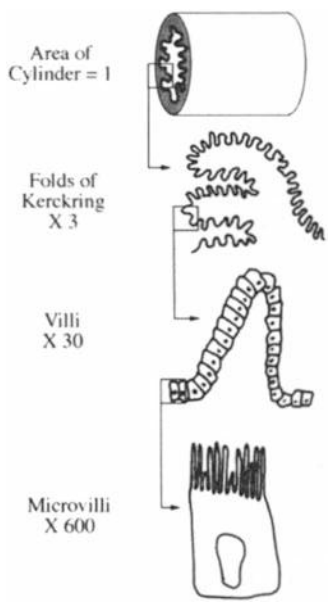

Figure 6.2 Increases in surface area in the small intestine due to folding

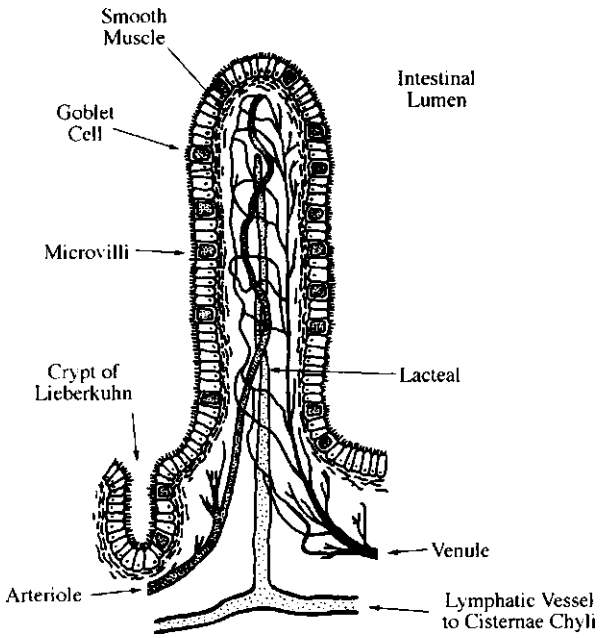

Figure 6.3 Structure of a villus. Note blood and lymph flow

The major features of a villus are illustrated in Figure 6.3. Each villus contains an arteriole and a venule, and also a blind-ending lymphatic vessel called a lacteal. The arteriole and venule do not anastomose in the small intestine as they do in the gastric mucosa. Small molecules absorbed through the villus pass into the descending loop of the villus capillary and diffuse into the ascending vessel. This creates a counter-current exchange system in each villus, which is relatively inefficient since it decreases the concentration gradient for passive diffusion. The efficiency of this process has been estimated to be around 15% and has the net effect of slowing the rate of absorption.

Microvilli

Each enterocyte has about 1000 minute processes or microvilli which project into the intestinal lumen. Multiple actin filaments extending into the interior of each microvillus are believed to be responsible for contraction of the microvilli and thus the movement of the fluid immediately in contact with the surface.

The membrane which forms the microvilli on the outer surface of the absorptive cells is rich in protein, cholesterol and glycolipids and contains enzymes, disaccharidases and peptidases which are localised within the surface membrane. Specific receptor proteins are located on the microvillus membrane-surface coat complex which selectively bind substances prior to their absorption, including the intrinsic factor-vitamin B_{12} complex in the ileum and conjugated bile salts in the distal intestine.

Epithelium

The epithelium which covers the intestinal villi is composed of absorptive cells, goblet cells, a few endocrine cells and tuft or calveolated cells. The goblet and endocrine cells closely resemble those found in the crypts. The tuft or calveolated cells are characterized by long, broad apical microvilli and an intra-cytoplasmic system of tubules and vesicles. These cells

are uncommon and their function is not known. The absorptive cells or enterocytes are tall, columnar cells, with their nuclei located close to their base.

Crypts of Lieberkühn

The cells between the villi form the germinal area known as the crypts of Lieberkühn. The main functions of the crypts are cell renewal and water, ion, exocrine and endocrine secretion. The crypt epithelium consists of at least 4 different cell types:

a) the Paneth cells which secrete large amounts of protein-rich materials, and in the rat are known to phagocytose selected protozoa and bacteria. Human Paneth cells contain lysozyme, IgA and IgG.

b) goblet cells which secrete mucus. These cells are able to tolerate a higher osmotic stress than the enterocytes and are more firmly attached to the basement membrane. Exposure to toxins or hypertonic vehicles leads to accumulation of goblet cells at the apex of the villus, goblet cell capping, and a hypersecretion of mucus presumably as a protective response[1].

c) undifferentiated cells, the most common type, whose major function is in the renewal process of the epithelium. Cells in the enterocyte lineage divide several more times as they migrate up the crypts. As they migrate onto the villi, they will differentiate further into the mature absorptive cells that express all the transport proteins and enzymes characteristic of those cells.

d) endocrine cells which produce hormones and peptides such as gastrin, secretin, cholecystokinin, somatostatin, enteroglucagon, motilin, neurotensin, gastric inhibitory peptide, vasoactive peptide and serotonin.

Stem cells in the crypts divide to form daughter cells. One daughter cell from each stem cell division is retained as a stem cell. The other becomes committed to differentiate along one of the four pathways mentioned above.

The volume of intestinal secretions formed by the cells in the crypts is around 1800 ml per day and is almost pure extracellular fluid, with a pH of between 7.5 and 8.0. The fluid is rapidly absorbed by the villi and provides the watery vehicle required for the absorption of substances from chyme.

The gastrointestinal circulation

The gastrointestinal circulation is the largest systemic regional vasculature and nearly one third of the cardiac output flows through the gastrointestinal viscera, with 10% (500 ml min^{-1}) supplying the small intestine. Each anatomical region (salivary glands, pharynx, oesophagus, stomach, liver and intestine) possesses separate blood vessels. The blood vessels of the jejunum and ileum are derived from the superior mesenteric artery. Arterioles branch off and supply the muscular coat and form plexuses in the submucosal layer. From each plexus, tiny vessels direct blood to the villi and the glands in the mucosa.

The distribution of the blood flow varies according to the metabolic demands of the cells within each region. The highly metabolic villi receive 60% of the blood flow to the mucous layer, while the muscle layer with it's lower demand for oxygen receives only 20% of the blood flow. During periods of enhanced absorption or electrolyte secretion, the blood flow is preferentially distributed to the mucosa, whilst increased intestinal motility causes diversion of the blood flow to the muscle layers. After a meal, blood flow increases by 30 to 130% of basal flow and the hyperaemia is confined to the segment of the intestine exposed to the chyme. Long chain fatty acids and glucose are the major stimuli for hyperaemia, which is probably mediated by hormones such as cholecystokinin released from mucosal endocrine cells[2].

The blood from the small intestine flows into the large hepatic portal vein which takes the blood directly to the liver. The liver has the highest drug metabolising capacity in the body and, in a single pass, can remove a large proportion of the absorbed drug before it reaches the systemic circulation. This process, termed first-pass metabolism, may have a significant effect on drug bioavailability when formulations are given by the oral route. The blood supply from the buccal cavity and the anal sphincter do not drain to the portal vein, and a greater proportion of a drug with a high hepatic extraction may be absorbed from these regions.

The lymphatic system

The lymphatic system is important in the absorption of nutrients, especially fat, from the gastrointestinal tract and provides a route by which electrolytes, protein and fluids can be returned from the interstitial spaces to the blood. It is also responsible for removal of red blood cells lost into tissues as a result of haemorrhage or bacteria which may have invaded tissues.

Structure of the lymphatics

The gastrointestinal tract is richly supplied with lymphatic vessels. Lymphatic vessels are lined by flattened endothelium with an incomplete basement membrane. Smooth muscle and connective tissue also surround the larger lymph vessels. The contractile activity of lymphatic vessels is most likely to be related to the amount of associated smooth muscle. The valves found in the larger vessels assist with the propulsion of lymph.

In the oesophagus, stomach and intestine, there is a plexus of lymph vessels present in the mucosal, submucosal and muscular layers with short vessels linking the networks together, and passing through lymph nodes which act as filters for lymph directed into larger vessels. The major lymphatic trunks are found on the left side of the body with the bulk of the lymph entering the circulation at the left jugulo-sub-clavian tap which is located at the base of the neck.

The lymphatic vessels of the small intestine are called the lacteals. The central lymphatic vessel is a blind-ending tube. The walls consist of a single layer of thin endothelium and resemble blood capillaries, however the small fenestrations seen in the blood vessel walls are not found in the lymphatics. The intestinal villi rhythmically contract and relax which probably serves to pump lymph into the lacteals of the submucosa. The flow of lymph in the thoracic duct is about 1–2 ml min^{-1} between meals but this can increase by 5 to 10 fold during absorption and digestion of a meal.

In addition to its main function of absorption, the gastrointestinal tract is a lymphoid organ. The lymphoid tissue is referred to as the gut-associated lymphoid tissue or GALT. The number of lymphocytes in the GALT is roughly equivalent to those in the spleen.

Lymphatic tissue can be seen in certain areas of the gastrointestinal tract close to epithelial surfaces, or as large aggregates e.g. pharyngeal tonsils and Peyer's patches in the ileum. Peyer's patches are lymphoid follicles located in the mucosa and extending into the submucosa of the small intestine, especially the ileum. Peyer's patches are usually situated on the ante-mesenteric border. Each patch typically comprises of 40 to 50 nodules which are separated from the gut lumen by a layer of epithelial cells, the M-cells or micro fold cells. There is a thin layer of vascularised connective tissue between the nodules and the serosa. The patches have their own blood supply. M-cells lack fully developed microvilli, are pinocytic and contain numerous vesicles. These cells may play an essential role in the intestinal immune response since they transport macromolecules and therefore have a specific role in antigen uptake. At this point in the intestine the mucosal barrier may be breached by pathogens. M cells do not digest proteins, but transport them into the

underlying tissue, where they are taken up by macrophages. The macrophages which receive antigens from M cells present them to T cells in the GALT, leading ultimately to appearance of immunoglobulin A-secreting plasma cells in the mucosa. The secretory immunoglobulin A is transported through the epithelial cells into the lumen, where, for example, it interferes with adhesion and invasion of bacteria.

In adults, B lymphocytes predominate in Peyer's patches. Smaller lymphoid nodules can be found throughout the intestinal tract. Lamina propria lymphocytes are lymphocytes scattered in the lamina propria of the mucosa. A majority of these cells are IgA-secreting B cells. Intraepithelial lymphocytes are lymphocytes positioned in the basolateral spaces between luminal epithelial cells, beneath the tight junctions. These are inside the epithelium, not inside epithelial cells as the name may suggest.

Formation of lymph

Lymph is a component of the extracellular fluid of the body and is largely derived from fluid and solute filtered from the blood circulation across the capillary wall. A mixture of hydrostatic forces and osmotic pressure control the fluid content of the blood. The high pressure within the arteriolar capillaries forces plasma into the intercellular spaces, the majority of which is returned to the bloodstream at the venous end of the capillary. The volume and solute concentration of the filtrate is modified by passage through the tissues and the lymphatic vessel endothelium before becoming lymph. About 10% of the fluid flowing from the arterial capillaries is absorbed by the lymphatic capillaries and returns to the bloodstream through the lymphatic system.

The sparse and incomplete basement membrane of the endothelium of small lymphatics is a weak barrier to the passage of solutes, fluids and large particles. In addition, the intercellular adhesion is poor and hence large particulates and even cells can occasionally pass between them. Specific vesicular transport may also be an important route of entry.

Composition of lymph

All plasma proteins are found in lymph. The protein concentration of lymph from all parts of the alimentary tract tends to be high; e.g. thoracic duct lymph has a protein concentration of 66% of that of serum. Lymph contains relatively less of the larger proteins compared to plasma, suggesting that molecular size is important in lymph filtration. Materials with a molecular weight of less than 10,000 are found in similar concentrations in both lymph and plasma. Additional proteins, mainly immunoglobulins, are added to the lymph on passage through the lymph nodes. Finally, lymph also contains reticulo-endothelial cells (lymphocytes) to destroy bacteria.

Lymph contains all the coagulation factors found in the blood, but it clots less readily. The electrolyte composition is very similar. Cholesterol and phospholipids in lymph are mainly associated with protein as lipoprotein, and together with triglycerides synthesised in the enterocytes form submicrometer droplets known as chylomicrons. This renders the triglycerides water-miscible. The concentration of chylomicrons varies with the amount of protein present in the lymph. The amount of neutral fat in the chylomicrons depends upon the degree of absorption from the gastrointestinal tract. Immediately after meals there are large quantities of lipoproteins and fats in the lymph which have been taken up from the gastrointestinal tract, but this drops to a low level between meals.

Stimulation of lymphatic transport

Many substances given by mouth increase the flow of lymph e.g. olive oil and corn oil[3–5]. In rats, lymph flow is enhanced after intragastric administration of substances such as water, 0.9% sodium chloride, 10% serum albumin or 10% glucose[6]. Of these, sodium chloride is

a particularly potent lymphagogue, and some workers use an intragastric infusion of this solution to maintain a good mesenteric lymph flow in a period immediately after creating a lymph fistula.

Secretions into the small intestine

Glands

Two types of glands are found in the small intestine

1. Brunner's glands which are confined to the duodenum and secrete bicarbonate and mucus.

2. The intestinal cells which are present throughout the small intestine and secrete mucus and a few enzymes.

The intestinal juice or succus entericus produced by the intestinal glands has an electrolyte composition similar to that of extracellular fluid. It has a pH of 7.5 to 8.0. The only enzyme of importance in the succus entericus is enteropeptidase (enterokinase) derived from the microvillous membrane, which converts trypsinogen to trypsin.

Pancreatic secretion

The human pancreas is a large gland, often more than 20 cm long, which secretes approximately 1 litre of pancreatic juice per day. The pancreatic juice has two major components: (i) alkaline fluid and (ii) enzymes. At all rates of secretion pancreatic juice is isotonic with extracellular fluid. The pancreatic acinar cells synthesize and secrete the majority of the enzymes which digest food. All the pancreatic proteases are secreted as inactive enzyme precursors and are converted to the active form in the lumen, whereas pancreatic amylase and lipase are secreted in active forms. The secretion of the aqueous phase and the bicarbonate component is largely regulated by the pH of the chyme delivered into the small intestine from the stomach. The secretion of pancreatic enzymes is primarily regulated by the amount of fat and protein entering the duodenum.

Biliary secretion

The liver secretes bile which is necessary for the digestion and absorption of lipids. All hepatic cells continually form a small amount of bile which is secreted into bile canaliculi. It is stored and concentrated in the gall bladder in man. Approximately 600 ml of hepatic bile is produced per day, but within 4 hours, up to 90% of the water present in the hepatic bile can be removed by the gall bladder. Concentration takes place by removal of sodium ions, and chloride and water then follow passively.

Bile is a variable and complex mixture of water, organic and inorganic solutes. The major organic solutes are bile acids, phospholipids (particularly lecithin), cholesterol and bilirubin. Sodium and potassium ions are found in proportions similar to that found in plasma whilst the concentrations of Cl^- and HCO_3^- are often lower and the bile acids make up the remainder of the ion balance. The bile acids are derivatives of cholesterol in which hydroxyl and carboxylic acid groups are attached to the steroid nucleus, converting it into a powerful natural surfactant. The major pigment of bile is bilirubin. Its formation is of considerable biological significance as it is the most important means by which haem, produced by the breakdown of haemoglobin, is eliminated. Up to 20% of the bilirubin present in bile is produced from other resources such as myoglobin and cytochromes.

Bile salts have two important actions:

(i) emulsification of the fat content of food, producing small droplets of fat in aqueous suspension.

(ii) assisting in the absorption of fatty acids, monoglycerides, cholesterol and other lipids from the intestinal tract by forming submicron clusters of fat and surfactant called mixed micelles.

Brief periodic bursts of bile flow occur under fasting conditions, coincident with the passage of phase 3 of the migrating motor complex (MMC) through the duodenum. When a meal is ingested, the gall bladder contracts and the bile salts are secreted into the duodenum where they can emulsify dietary fat. Bile acids are poorly absorbed in the proximal small intestine, unlike the majority of nutrients, but are absorbed by an active process in the terminal ileum. After absorption, bile acids have a high hepatic clearance and are re-secreted in the bile. This process is known as enterohepatic recirculation.

Secretion and absorption of water

An adult human takes in roughly 1 to 2 litres of dietary fluid every day. In addition, another 6 to 7 litres of fluid is received by the small intestine as secretions from salivary glands, stomach, pancreas, liver and the small intestine itself. By the time the ingesta enters the large intestine, approximately 80% of this fluid has been absorbed. The absorption of water is absolutely dependent on absorption of solutes, particularly sodium.

Within the intestine, there is a proximal to distal gradient in osmotic permeability. Further down the small intestine, the effective pore size through the epithelium decreases, hence the duodenum is much more "leaky" to water than the ileum and the ileum more leaky than the colon. However, the ability to absorb water does not decrease, but water flows across the epithelium more freely in the proximal compared to distal gut because the effective pore size is larger. The distal intestine actually can absorb water better than the proximal gut. The observed differences in permeability to water across the epithelium is due almost entirely to differences in conductivity across the paracellular path as the tight junctions vary considerably in "tightness" along the length of the gut.

Regardless of whether water is being secreted or absorbed, it flows across the mucosa in response to osmotic gradients. In the case of secretion, two distinct processes establish an osmotic gradient that pulls water into the lumen of the intestine. Firstly, the increases in luminal osmotic pressure resulting from influx and digestion of food cause water to be drawn into the lumen. Chyme when passed into the small intestine from the stomach is slightly hyperosmotic, but as its macromolecular components are digested, osmolarity of that solution increases dramatically. For example, starch which is a large molecule, will only contributes a small amount to osmotic pressure when intact. As it is digested, thousands of molecules of maltose are generated, each of which is as osmotically active as the parent molecule. Thus, as digestion proceeds the osmolarity of the chyme increases dramatically and water is pulled into the lumen. Then, as the osmotically active molecules are absorbed, osmolarity of the intestinal contents decreases and water is then reabsorbed.

Secondly, crypt cells actively secrete electrolytes, which leads to water secretion. The apical or luminal membrane of crypt epithelial cells contain a cyclic AMP-dependent chloride channel known also as the cystic fibrosis transmembrane conductance regulator or CFTR because mutations in the gene for this ion channel result in the disease cystic fibrosis. This channel is responsible for secretion of water. Elevated intracellular concentrations of cAMP in crypt cells activate the channel which results in secretion of chloride ions into the lumen. The increase in concentration of negatively-charged chloride anions in the crypt creates an electrical potential which attracts sodium, pulling it into the lumen across the tight junctions. The net result is secretion of sodium chloride into the crypt which creates an osmotic gradient across the tight junction, hence water is drawn into the lumen. Abnormal activation of the cAMP-dependent chloride channel in crypt cells has resulted in the deaths of millions of people. Several types of bacteria produce toxins, the best known of which is the cholera toxin, that strongly and often permanently activate the adenylate cyclase in crypt enterocytes. This leads to elevated levels of cAMP, causing the chloride

channels to essentially become stuck in the "open" position". The result is massive secretion of water which produces the classic symptom of severe watery diarrhoea.

The most important process which occurs in the small intestine which makes absorption possible is maintenance of an electrochemical gradient of sodium across the epithelial cell boundary of the lumen. To remain viable, all cells are required to maintain a low intracellular concentration of sodium. In polarized epithelial cells like enterocytes, a low intracellular sodium concentration is maintained by a large number of sodium pumps or Na^+/K^+ ATPases embedded in the basolateral membrane. These pumps export 3 sodium ions from the cell in exchange for 2 potassium ions, thus establishing a gradient of both charge and sodium concentration across the basolateral membrane. In rats, there are about 150,000 sodium pumps per small intestinal enterocyte, which allows each cell to transport about 4.5 billion sodium ions out of each cell per minute[7]. This flow and accumulation of sodium is ultimately responsible for absorption of water, amino acids and carbohydrates. The transport of water from lumen to blood often occurs against an osmotic gradient, allowing the intestine to absorb water into blood even when the osmolarity in the lumen is higher than osmolarity of blood. The proximal small intestine functions as a highly permeable mixing segment, and absorption of water is basically isotonic. That is, water is not absorbed until the ingesta has been diluted to just above the osmolarity of blood. The ileum and especially the colon are able to absorb water against an osmotic gradient of several hundred milliosmoles.

Digestion and absorption of nutrients

Food assimilation takes place primarily in the small intestine and it is optimized by the increased surface area produced by Kerkring's folds, villi and microvilli. The chyme presented to the duodenum from the stomach consists of a mixture of coarsely emulsified fat, protein and some metabolites produced by the action of pepsin, and carbohydrates including starch, the majority of which would have escaped the action of the salivary amylase.

The chyme is acidic and this is buffered by bile and the bicarbonate present in the pancreatic juice to between pH 6.5 and 7.6. The digestive enzymes are located in the brush border of the glycocalyx and they can be altered by changes in diet, especially by the proportion of ingested disaccharides. The protein content of the diet does not affect the proteases, but a diet deficient in protein leads to a reduction in all enzymes.

It is important to recognize that the epithelium of the gut is not a monotonous sheet of functionally identical cells. As chyme travels through the intestine, it is sequentially exposed to regions having epithelia with very different characteristics. This diversity in function results from differences in the number and type of transporter molecules expressed in the epithelial plasma membrane, and the structure of the tight junctions. Even within a given segment there are major differences in the type of transport that occurs, for example, cells in the crypts have different transporter systems than cells on the tips of villi.

Blood passing through the minute veins of the capillaries is brought into close proximity with the intestinal contents in an area estimated to be about $10m^2$. The capillaries are fenestrated, hence allowing a very rapid exchange of absorbed materials. During digestion and absorption the villi contract fairly quickly at regular intervals and relax slowly. The contraction probably serves to pump lymph into the lacteals of the submucosa and stir the intestinal contents. The veins in the villi ultimately open into the portal vein, which leads directly to the liver and hence all materials carried from the small intestine undergo "first-pass" metabolism.

The site of absorption of the small intestine depends upon the relationship between the rate of transit to that of absorption. This is more apparent for drugs than for food, since excipients may control the rate of drug release. For example the duodenum can be demonstrated to have a high rate of absorption, however the passage through this region

is extremely rapid and so the net absorption in this region is probably quite low. The function of the duodenum is to sample the chyme which is delivered from the stomach and thus regulate the delivery of the food according to its calorific value by a feedback process. Virtually all nutrients from the diet are absorbed into blood across the mucosa of the small intestine. The absorption of water and electrolytes plays a critical role in maintenance of body water and acid-base balance.

Carbohydrates

The principal dietary carbohydrates are starches, sucrose and lactose. Starch is a glucose-containing polysaccharide with a molecular weight which varies from 100,000 to more than 1 million. The two major polysaccharides of starch are amylose and amylopectin. Indigestible carbohydrates e.g. cellulose are the main constituents of dietary fibre.

Salivary and pancreatic amylases initiate the hydrolysis of starch and exhibit their optimal activity near a neutral pH. The salivary amylase is inactivated once it reaches the acid in the stomach. The intraluminal digestion of carbohydrates occurs rapidly in the duodenum due to the large amount of amylase secreted by the pancreas. The final oligosaccharide products of luminal digestion are formed before the chyme reaches the jejunum. The major products of starch digestion are maltose and maltotriose. Carbohydrates are absorbed in the proximal part of the small intestine and they have completely disappeared from the lumen by the time the meal reaches the ileum.

The disaccharides are further digested to monosaccharides by the brush border enzymes lactase, sucrase, maltase and isomaltase during their transfer across the epithelium. It is likely that the enzymes and carriers are so orientated spatially that hydrolysis and and subsequent absorption are sequential events. Both passive diffusion and active transport absorb glucose rapidly and completely. The brush border possesses a sodium-dependent carrier which transports sugars across the membrane in either direction.

Proteins

Most protein digestion occurs principally in the small intestine under the influence of the proteolytic enzymes of the pancreatic secretion. When the proteins leave the stomach they are mainly in the form of large polypeptides. Immediately upon entering the small intestine, the partial breakdown products are attacked by the pancreatic enzymes. Trypsin and chymotrypsin split protein molecules into small polypeptides, carboxypolypeptidase then cleaves individual amino acids from the carboxyl ends of the polypeptides. The brush border of the small intestine contains several different enzymes for hydrolysing the remaining small peptides. The constituent amino acids are then absorbed. Most naturally occurring amino acids are L-isomers which are transported against concentration gradients by sodium-dependent carrier mechanisms. There are four carrier systems for amino acids: for neutral amino acids (histidine), for basic amino acids (lysine), for dicarboxylic acids (glutamic acid) and a fourth transports the amino acids proline, hydroxyproline and glycine.

Enterocytes do not have transporters to carry proteins across the plasma membrane and proteins cannot permeate tight junctions. However, studies suggest that very small amounts of proteins may be absorbed intact. In most instances, the extent of this absorption is small and nutritionally not significant, however it can result in immune reactions, hormonal or toxic effects. This is most clearly seen in neonates. This enhanced ability, which is rapidly lost, is of immense importance because it allows the newborn babies to acquire passive immunity by absorbing immunoglobulins from colostral milk.

Fats

Dietary intake of lipid is mainly in the form of triglycerides which are composed of a glycerol chain and three fatty acids. There are also small quantities of cholesterol, phospholipids and

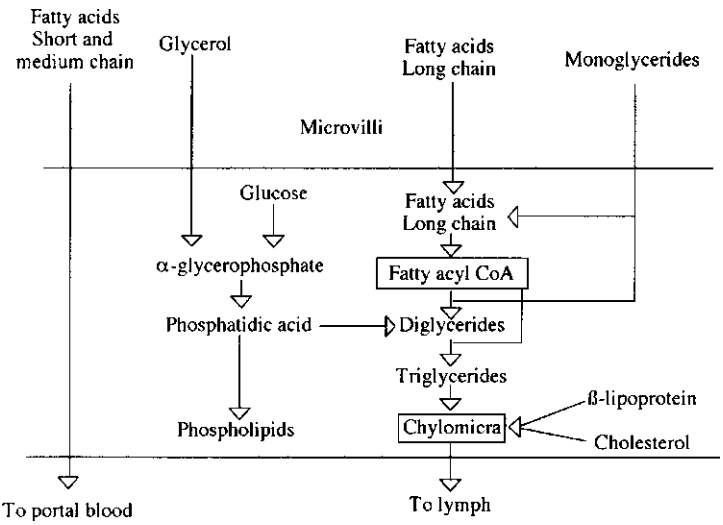

Figure 6.4 Metabolism and transport of fat into the lymphatic and systemic circulation

cholesterol esters in chyme. Fat is emulsified by the bile salts into small droplets which disperse in water allowing access of digestive enzymes.

Lipase in the pancreatic juice and enteric lipase from the epithelial cells of the small intestine both hydrolyse emulsified triglycerides to monoglycerides and fatty acids. The short and medium chain fatty acids are absorbed passively through the epithelium into the blood. The long chain fatty acids and monoglycerides remain as mixed micelles with the bile salts and are internalized by the epithelium. They are reassembled into triglycerides within the cell and excreted into the lymph as small (0.1 μm) droplets called chylomicra (or chylomicrons) (Figure 6.4).

Iron

Heme iron is absorbed from meat more efficiently than dietary inorganic iron and in a different manner[8]. Thus, iron deficiency is less frequent in countries where meat constitutes a significant part of the diet. Proteolytic digestion of myoglobin and hemoglobin results in the release of heme, which is maintained in a soluble form by globin degradation products so that it remains available for absorption. Heme enters the small intestinal absorptive cell as an intact metalloporphyrin. This may be facilitated by a vesicular transport system[9]. In the absorptive cell the porphyrin ring is split by heme oxygenase. The released inorganic iron becomes associated with mobilferrin and paraferritin, which acts as a ferrireductase to make iron available for production of iron-containing end products such as heme proteins. Mucosal transfer of iron into the body occurs competitively with dietary iron that enters the absorptive cell as inorganic iron, because they both share a common pathway within the intestinal cell. Dietary inorganic iron as the ferric iron is solubilized at the acid pH level of the stomach where it chelates mucins and certain dietary constituents to keep them soluble and available for absorption in the more alkaline duodenum.

PATTERNS OF MOTILITY IN THE SMALL INTESTINE

The small intestine, like the stomach, displays two distinct patterns of motility. The fed pattern is characterized by random motor activity, in groups of 1 to 3 sequential contractions, separated by 5 to 40 seconds of inactivity. The physical and chemical nature

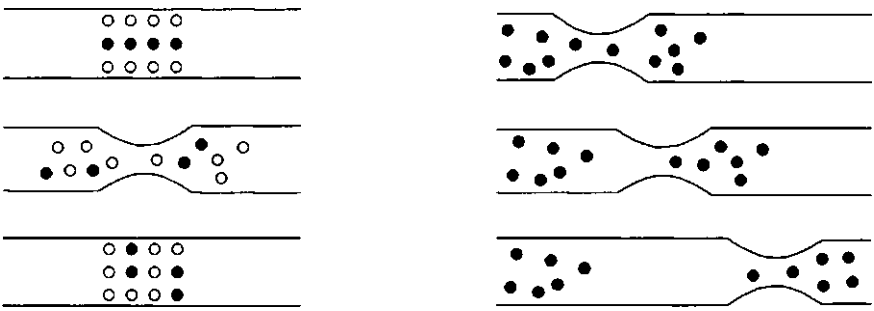

Figure 6.5 Segmental contractions (left) and propulsive contractions (right) of the small intestine

of the food determines the number of contractions; for example, twice as many contractions occur when solid food is ingested than when an equicalorific liquid is consumed[2]. Carbohydrates stimulate the largest number of contractions, followed by proteins and lipids. The fed pattern of motility consists of segmental and peristaltic contractions, the segmental contractions being the most frequent (Figure 6.5). Initially a segment of the bowel, less than 2 cm in length, contracts while the adjacent segments are relaxed; the procedure is then reversed, as the contracted segment relaxes and vice versa. This type of motility mixes chyme by continually moving it in the lumen and increasing contact with the absorbing surface, but since there are less frequent contractions aborally than orally, there is a net movement of chyme towards the large bowel. This movement is enhanced by peristaltic contractions which occur less frequently than segmental contractions, and each move the chyme a few centimetres. The continuous movement shears the chyme resulting in effective mixing (Figure 6.6).

The interdigestive migrating myoelectric complex (Chapter 5) continues from the stomach to the small intestine. Phase I is a period of no activity, Phase II is characterized by random activity and Phase III is a period of intense activity which is associated with the aboral movement of the intestinal contents. The migrating myoelectric complex occurs every 140 to 150 minutes, and as one complex reaches the ileum, another starts at the duodenum. The velocity of the contractile wave decreases as it approaches the ileum and only rarely does it reach the terminal ileum[10].

Motility in the small intestine, as in all parts of the digestive tube, is controlled predominantly by excitatory and inhibitory signals from the enteric nervous system. These local nervous signals are modulated by inputs from the central nervous system, and to some degree by a number of gastrointestinal hormones.

Stagnation at the ileocaecal junction

The ileocaecal junction divides the terminal small intestine from the caecum. The junction or sphincter appears to formed by papillary protrusions into the lumen of the caecum, rather than two flat lips of a valve[11]. Its function seems to be to retain chyme in the small intestine until digestion is largely complete and then to empty its contents into the large bowel. The ileocaecal junction also serves to prevent the spread of the colonic bacteria into the small intestine[12]. Contraction of the ileocolonic sphincter is produced by a-adrenergic agonists including phenylephrine, adrenaline and noradrenaline, and by cholinergic agonists such as bethanechol, whereas pure b-adrenergic agonists, such as isoprenaline, cause relaxation[13].

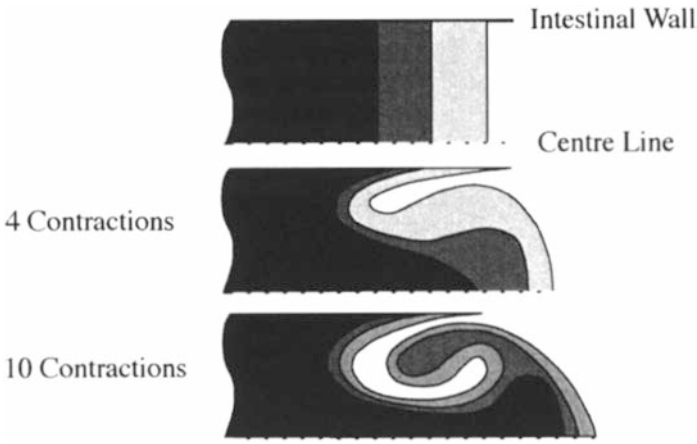

Figure 6.6 Laminar mixing produced by repetitive longitudinal contractions

Scintigraphy often demonstrates accumulation or bunching of material at the ileocaecal junction, followed by spreading of material through the ascending colon[14]. Non-disintegrating matrices may remain at this location for some hours[15].

SMALL INTESTINAL TRANSIT TIMES

Methods for measuring small intestinal transit

There are several methods available for the measurement of small intestinal transit. The hydrogen breath test relies on metabolism of certain carbohydrates, e.g. lactulose, by microbial flora within the large bowel. The carbohydrate must be one which is not absorbed from the small intestine. The gas generated is detected in the expired air. Using this technique, it is possible to estimate the sum of gastric emptying and small intestinal transit times. This test assumes that the unabsorbed carbohydrate encounters fermentative bacteria only in the colon, however bacterial overgrowth into the small intestine will give erroneously short transit times.

Two-dimensional gamma scintigraphy can be used to measure stomach to caecum transit times, but cannot be used to measure the distance travelled by a unit through segments of the small intestine since it is highly coiled. Transit time through segments of the small intestine has been measured using a perspex capsule containing technetium-99m labelled 'Amberlite' resin[16]. External markers were placed on the front, back and sides of volunteers who were then imaged from the front, back and side. This enabled the three-dimensional movement of the capsule through the small intestine to be reconstructed and an estimate to be made of the velocity of the unit. After transit through the duodenum, which was too fast to be accurately measured, the capsule moved through the small intestine at between 4.2 and 5.6 cm per minute. There was no difference in transit times for two capsules with different specific gravities (1.0 and 1.6). The transit rate is in close agreement with the velocity of the migrating myoelectric potential down the small intestine (4.7 cm. min^{-1})[17] and that of 1 to 4 cm per minute for chyme[2].

Many older textbooks quote small intestinal transit times based on barium X-ray contrast measurements, but barium is not a good model of the intestinal contents.

Measurements of transit from patients with some form of organic disease, such as those with ileostomies, should also be treated with caution.

Small intestinal transit times of food

A combination of scintigraphic, x-ray contrast and hydrogen breath techniques to follow the transit of a meal of sausages, baked beans and mashed potato showed that the residues left the small intestine between 2 and 12 hours after ingestion. This suggests that differential transit of meal components occurs[17 18]. There is however some controversy in the literature on this point, since another study failed to find a difference in the small intestinal transit time of the components of a mixed meal of cheese, biscuits, bran and water containing separately radiolabelled liquid and fibre[19]. In this experiment, differences in mouth-to-caecum time of the two labelled components were entirely explained by differences in rates of gastric emptying. A mean small intestinal transit time for a liquid test meal has been reported to be approximately 1.25 h[20]. Further intake of food appears to have little effect on the transit of material already in the small intestine[21].

Physiological and pathophysiological effects on small bowel transit

It has been reported that severe exercise delays the gastric emptying of food whereas moderate exercise accelerates it. In spite of these findings, exercise has little effect on the small intestinal transit of pellets given to fasted individuals[22]. Larger objects such as radioopaque markers showed a marked reduction in whole gut transit time following moderate exercise (jogging and cycling)[23].

In cases of accelerated gastrointestinal transit, administration of a drug in a controlled release formulation instead of a conventional formulation may cause a higher fraction of the dose to escape absorption in the small intestine and enter the colon. This can reduce the availability of the drug either because of slow and erratic absorption or because of inactivation by colonic bacteria (Table 6.1). In contrast, diseases that retard small bowel transit could increase the bioavailability of drugs that are released slowly from controlled-release forms; however, conditions that encourage the overgrowth of bacteria in the small intestine could reduce the bioavailability of drugs susceptible to bacterial degradation (e.g. digoxin).

In patients with partial intestinal obstruction or a narrowed lumen, a single unit may lodge in the gut and expose the intestinal mucosa to high concentrations of drug which may lead to gastric irritation, bleeding, and even perforation[24–26]. For patients with this disease, multiparticulate formulations provide an advantage, because even if pellets lodged within the gut, they would do so over a wide area as they are well dispersed. In addition, each pellet

Table 6.1 Disease causing accelerated and decreased small intestinal transit times (S.I.T.T.)

Faster S.I.T.T.	Slower S.I.T.T.
Secretory diarrhoea	Constipation
Thyrotoxicosis	Myxoedema
Irritable bowel syndrome	Pseudo-obstruction
Chronic pancreatitis	Ileal resection
	Partial gastrectomy
	Jejuno-ileal bypass
	Autonomic neuropathy

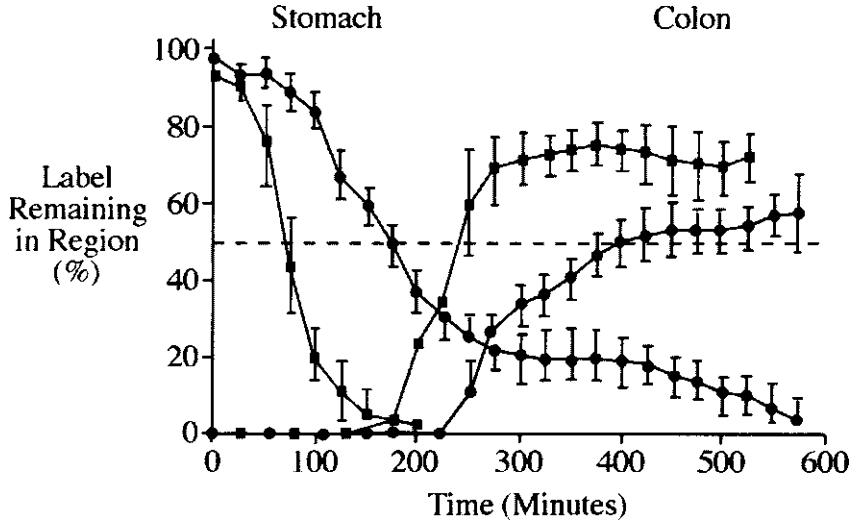

Figure 6.7 Effect of light (■) and heavy breakfast (●) on the gastric emptying and colon arrival of a co-administered multiparticulate formulation

only contains a small fraction of the total dose administered so local concentrations of the drug would be small thus reducing mucosal damage.

Castor oil is believed to decrease the activity of the circular smooth muscle which is thought to produce an increase in intestinal transit. The mechanism by which castor oil produces its effect on the gut could involve inhibition of Na^+, K^+-ATPase, activation of adenylate cyclase, stimulation of prostaglandins and nitric oxide biosynthesis. Castor oil also changes the intestinal permeability and causes histological abnormalities.

Small intestinal transit time of dosage forms

During fasting, both monolithic and multiparticulate dosage forms will be swept rapidly through the small bowel by the migrating myoelectric complex. The action is propulsive and not mixing in nature, thus a capsule containing pellets given on an empty stomach may leave the stomach and pass down the small intestine as a bolus with minimal dispersal[27]. The increased dispersal of pelleted formulations within the small intestine when the formulations are taken with a meal occurs because the pellets become dispersed in the food mass within the stomach[28 29]. As their particle size is small, pellets will continue to be emptied from the stomach as part of the chyme, thus prolonging their delivery to the small intestine (Figure 6.7). Monolithic tablets, on the other hand, depending upon their size, will empty erratically from the stomach after food and as the single unit traverses the small bowel. Hence, the presentation of the drug to the small intestinal mucosa will depend solely upon its dissolution characteristics in each area. The degree of spread of a formulation within the small intestine is particularly important for drugs with poor solubility or for drugs which are slowly transported across the epithelium. Microparticulate dosage forms show longer and more reproducible median transit times compared with single unit tablets[30], giving rise to more predictable and uniform blood levels and reducing the risk of enlodgement and mucosal damage.

A review of data suggests that the small intestinal transit is around 4 hours for solutions, pellets and single unit formulations (Figure 6.8)[31]. Small intestinal transit of a

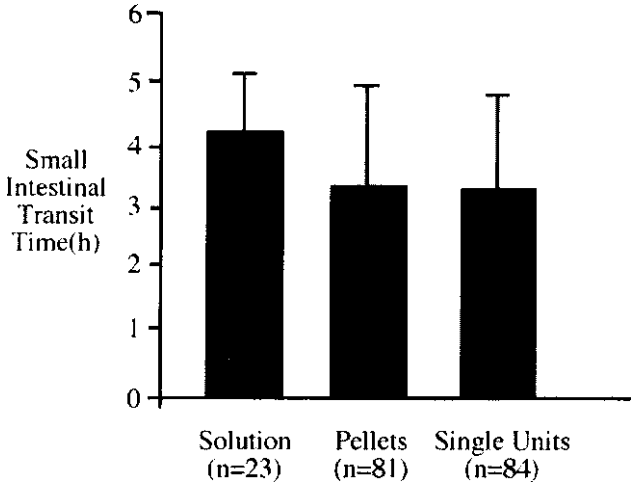

Figure 6.8 Small intestinal transit times of various dosage forms

dosage forms is not affected by their physical state, size or the presence or absence of food, but high calorific loads may slow it slightly although the majority of the effect is on gastric emptying (Figure 6.9)[32]. Small intestinal transit time is remarkably resistant to pharmaceutical intervention and in man physical properties such as shape, density or putative bioadhesive properties are without significant effect on transit. In a study of the spread of controlled-release isosorbide-5-dinitrate within the gastrointestinal tract[33] (Figure 6.10), a deconvolution technique was used to calculate the drug absorption profile, and revealed that isosorbide-5-nitrate was well absorbed from the preparation whilst the pellets resided in the stomach and small intestine. However, absorption was reduced when the preparation entered the colon, hence the absorption window based on an average mouth-caecum transit time (6–8 hours) represents the maximum acceptable time for drug release from this oral controlled-release preparation. These studies suggest that if matrix tablets are

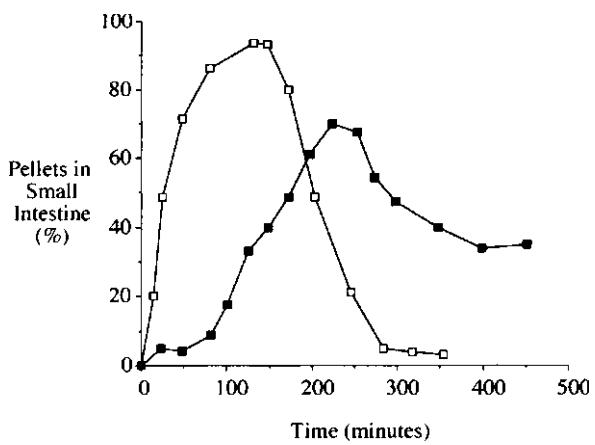

Figure 6.9 Delivery of multiparticulates from the stomach to the small intestine in fasted volunteers (□) after a heavy breakfast (■)

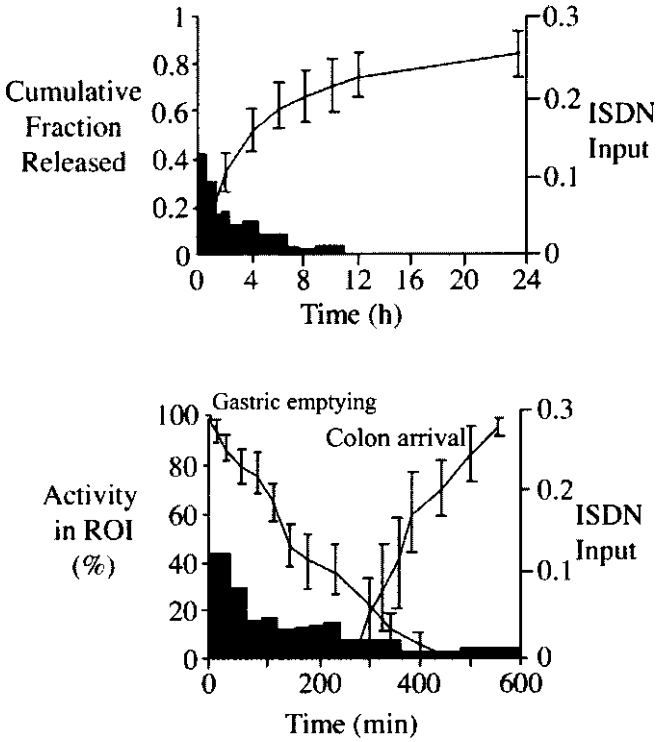

Figure 6.10 Gastrointestinal transit and absorption of isosorbide-5-dinitrate from controlled release pellets using combined gamma scintigraphy and blood sampling (ROI=region of interest)

designed to release their contents over 12 h then colonic absorption of the drug is necessary and the drug must not be degraded by colonic bacteria.

Large amounts of unabsorbable carbohydrate or a large amount of fluid accelerates transit through the small bowel, and could therefore reduce the degree of absorption from a controlled-release formulation taken with the meal[34]. This principle has been used as the basis for a sustained release tablet containing riboflavin and myristyl tripalmitate[35]. Increasing the viscosity of the luminal contents by ingesting viscous polysaccharides such as guar gum or pectin will also prolong small bowel transit time, and may therefore increase the degree of absorption from slowly released or slowly absorbed drugs[36]. For example, the absorption of riboflavin is enhanced after being mixed with a viscous sodium alginate solution[37].

The prolonged mouth-to-caecum transit may explain why the recovery of hydrochlorthiazide in the urine is greater when a controlled-release formulation of the drug is given with food than when given to fasted subjects[38]. Controlled-release lithium preparations are thought to cause diarrhoea by an action on ion transport in the ileum but this does not occur when they are taken with food[39]. This may also be due to enhanced absorption of the drug in the upper part of the small intestine, which occurs with a more prolonged transit in this region produced by the food, thus reducing the amount of lithium reaching the ileum to induce diarrhoea.

Scintigraphy often demonstrates accumulation or bunching of material at the ileocaecal junction. Non-disintegrating matrices may remain at this location for some time[15] [40]. The stagnation at the ileocaecal junction may also cause problems for controlled-release dosage forms which are designed to release drug over a period of 9 to 12 hours, since the concentration of drug could build up within this localized area. This will have no effect on the absorption of most drugs, providing that the rate at which the drug is released from the dosage form is slower than the rate of uptake across the ileal epithelium, and the drug is not degraded by ileal bacteria.

Density and small intestinal transit

Early studies indicated that pellet density affected small intestinal transit in ileostomy patients[41], although subsequent studies were unable to confirm this finding in normal subjects[16] [42]. A further scintigraphic study eventually confirmed that small intestinal transit in patients with ileostomies was not affected by density in the range 0.94 to 1.96 g cm^{-3} (Figure 6.11)[43]. Standard sized units (1.18–1.40 mm) of density 1.5, 2.0 and 2.4 g cm^{-3} administered to healthy volunteers all had similar small intestinal transit times[44].

Small particles with densities close to that of a meal will be emptied continuously with the meal[21] [45]. This means that, for a well designed enteric coated multiparticulate formulation, there is little delay in the onset of plasma levels even when the drug is given with food. As has been discussed previously, buoyant materials and ultra-dense materials do show a slowed gastric emptying. This of course will affect the time course for which they are presented to the small intestine.

ABSORPTION OF DRUGS

The permeability of the epithelium to small ions and water-soluble organic molecules is greater in the duodenum and jejunum than it is in the ileum, which would indicate that there are larger and more numerous water-filled channels high in the small bowel. The variation in absorptive capacity can also, in part, be explained by the higher surface area per unit length in the upper intestine compared with the lower part. A few studies have been carried out to compare the absorption of drugs in the upper and lower small intestine. Human perfusion studies have demonstrated a proximal-to-distal small intestinal absorption

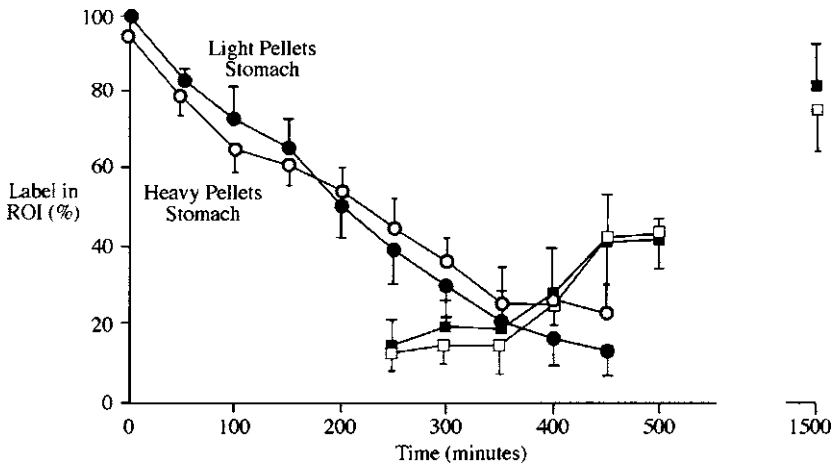

Figure 6.11 The transit of light and heavy pellets in ileostomy patients measured by gamma scintigraphy (ROI=region of interest)

gradient for hydrocortisone but not for triamcinolone[46]. Digoxin[47] and hydrochlorthiazide[38] have also been shown to be absorbed predominantly from the duodenum and upper jejunum. Although there is excellent absorption from the duodenum, transit through this area is extremely rapid, in the order of seconds[16] and hence the actual amount of drug absorbed from this region will be small.

Cell monolayers formed from the human colon cancer cell line Caco-2 have been widely used to study the absorption of drugs[48]. Caco-2 cells form confluent and polarized monolayers when maintained in culture and differentiate towards the mature small bowel enterocyte phenotype. Caco-2 cell monolayers mimic intestinal absorptive epithelium and represent a very useful tool for studying transepithelial drug transport. However, some enzymes and transport systems are expressed to a lesser extent in Caco-2 cells compared to normal enterocytes[49]. Caco-2 cells also express various cytochrome P450 isoforms and phase II enzymes such as UDP-glucuronosyltransferases, sulphotransferases and glutathione-S-transferases, and hence they have been used study presystemic drug metabolism[50].

Specific carriers for the transport of riboflavin and iron are found principally in the duodenum and jejunum, whereas carriers for bile acid and vitamin B_{12} are found mainly in the ileum. The acid microclimate is less obvious in the ileum, favouring the absorption of weak bases while discouraging the absorption of weak acids. Finally, the ileum contains more commensal bacteria than the duodenum and jejunum. In elderly subjects, the bacterial population in the ileum may be high enough to metabolize certain drugs thereby reducing their efficacy.

Absorption and delivery of macromolecules

It is generally accepted that macromolecules, particularly food proteins, do cross the mature small intestinal epithelium in small amounts and reach the systemic circulation. The potential as delivery route for orally administered macromolecular drugs including proteins is being widely explored[51]. There have been several studies on the mechanism and substrate structure-affinity relationship for this transport system. Rapid progress has been made recently in studies on the molecular basis of the intestinal peptide transport system. A protein apparently involved in peptide transport has been isolated from rabbit small intestine, and genes for human intestinal peptide transporters have been cloned, sequenced and functionally expressed[52]. The cellular uptake of small peptides such as di-, tri- and tetrapeptides and peptidomimetic drugs proceeds via specialized proton-coupled transporters[53]. The proton-dependent uptake at the apical cell membrane of the enterocytes results in subsequent exit of intact di- or tri-peptides across the basolateral membrane or, alternatively, intracellular hydrolysis and exit of component amino acids across the basolateral membrane[54]. The peptide carrier has a broad substrate specificity.

Lectins are resistant to digestion and binding to brush-border membranes, hence appreciable amounts of lectins and/or toxins of the general structure of A (toxin)-B (lectin), either free or included in liposomes, may be taken up by and transported through the epithelial cells of the small intestine. As a result tomato lectins have been explored as potential drug delivery agents[55].

Various strategies have been used to target vaccine antigens to the gut-associated lymphoid tissues, such as microspheres prepared from various polymers. Certainly in mice the size of the microspheres has to be less than 5 μm for them to be transported within macrophages through the efferent lymphatics[56]. Transcytosis through Peyer's patches is most suited for highly potent compounds since there are a limited number of Peyer's patches, hence the overall surface area is relatively small. Patch tissue is rich in lymphocytes, thus substances which interact with lymphocytes are best targeted to Peyer's patches when using the oral route[57].

It is known that a number of microorganisms are able to bind selectively to a receptor on the M-cell surface and thereby enter the host. Utilizing the microorganism's ligand could be beneficial for specific targeting to Peyer's patches, bypassing lysosomal degradation in absorptive cells. Moreover, transport of membrane-bound macromolecules by M cells is about 50 times more efficient than that of soluble, non-adherent macromolecules. The colonization of the small intestine by Escherichia coli strains is mediated by cell surface antigens called fimbriae which enable bacteria to adhere to the brush border of epithelial cells. Due to the very close contact between the epithelial cells and the bacteria an enhanced absorption of substances including peptides and proteins can occur. To use bacterial adhesion for the design of drug delivery and drug targeting systems the fimbrial anti-genicity has to be reduced. One approach was to truncate the NH_2-terminal on K99-fimbrial proteins by recombinant DNA-technology[58].

Intestinal pH

A drug administered in a solid form must dissolve in gastrointestinal contents and pass out of the stomach into the small intestine. It then has to gain access to the epithelium by convection of the luminal contents and diffusion across the unstirred microclimate. Finally it must cross the epithelium either by partitioning into the lipid membrane, by passing through water-filled channels or by combining with specific membrane-bound carriers. The residence of the formulation in the small intestine has to be long enough for complete absorption to take place. The principal permeability barrier is represented by the luminal surface of the brush border. Most drugs are absorbed by passive diffusion in their unionised state. The pH of the small intestine determines the degree of ionisation and hence controls the efficiency of absorption; this is the basis of the pH-partition theory of drug absorption which was discussed in Chapter 1. Protein binding at the serosal side of the epithelium helps maintain a concentration gradient by binding the absorbed drug, which is then removed by blood flow from the absorption site.

Gastric pH has been relatively well defined since it is accessible using a Ryle's tube, but fewer studies have investigated intestinal conditions. Data obtained using pH telemetry capsules indicate that the lumen of the proximal jejunum usually lies within the pH range 5.0 to 6.5, rising slowly along the length of the small intestine to reach pH 6 to 7, although high values in the range 7 to 9 have occasionally been found[59].

Measurements using microelectrodes have shown that the pH in the mucosal fluid adjacent to the intestinal epithelium is between 4.5 and 6.0 depending on the luminal glucose concentration. This acid microclimate immediately adjacent to the intestinal epithelium contributes to the absorption of acidic drugs such as acetylsalicylic acid[60]. The majority of the drug is ionised at the pH of the intestinal contents but the molecule is less ionised immediately adjacent to the intestinal epithelium and hence absorption is rapid. Unfortunately, this hypothesis suggests that basic drugs would be poorly absorbed, which is not the case. Bases may be absorbed in an ionised form through the paracellular route, or they may interact with organic cations which have been found to be secreted from the blood into the lumen of the intestine.

Solvent drag and intestinal permeability

The intestine absorbs approximately 10 litres of water a day from the diet and digestive secretions, and only 100–200 ml of water is lost in the stools. The question of whether the water flux influences drug absorption has been raised by many authors. Rat perfusion experiments have shown that the disappearance of the drugs sulphanilamide, sulphisoxazole and metoclopramide from the lumen increases with increasing fluid absorption and decreases when the tonicity of the perfusate increases, which causes intestinal secretion[61]. In

a slight variation of the technique which measured appearance of drug in the plasma, the absorption of both acidic (benzoic, salicylic) and basic drugs (amidopyrine, antipyrine) increased with increasing water absorption[62 63]. This phenomenon is known as solvent drag. It is proposed that it will affect paracellular drug absorption and may affect the absorption of small and hydrophilic drugs. In humans the intestinal steady state perfusion technique using a triple lumen tube passed into the small intestine, combined with simultaneous measurement of drug plasma concentration, has shown that transmucosal water fluxes affect the absorption of paracetamol and ranitidine[64 65].

P-glycoprotein

P-glycoprotein is an ATP-dependent transporter which is capable of transporting an extremely wide variety of drugs out of the cell. The potential role for P-glycoprotein for determining the oral bioavailability of some drugs has only recently been appreciated[66]. P-glycoprotein is expressed in a variety of normal human tissues including the liver, brain, adrenal gland, kidney and intestinal tract epithelia[67]. This suggests a common role as a protective mechanism. In the small intestine it is localised in the apical membranes of the cell, but is not detectable in crypt cells. It is composed of two blocks each containing six trans-membrane regions and a site for binding ATP on each half (Figure 6.12).

The mechanism by which such a wide range of compounds is transported is unknown, but it appears that the drug is effluxed by flipping the drug from the inner to the outer leaflet of the bilayer membrane[68]. This model is consistent with the ability of compounds to penetrate lipid and the common denominator is that the P-glycoprotein substrates are hydrophobic and amphipathic in nature. The number of drugs that can be effluxed from the cell by P-glycoprotein include the immunosuppressive agent cyclosporin A, vinca alkaloids, digoxin, ß-blockers[69], erythromycin, antibiotics and cimetidine. The molecular weight of the compounds transported varies enormously and encompasses a range between 250 to 1850 Daltons (Gramicidin D). P-glycoproteins were originally identified by their ability to transport cytotoxic drugs out of certain types of tumour cells thus conferring resistance. This mechanism appeared to work against a range of drugs and gave rise to the concept of "multi-drug resistance" (MDR).

The therapeutic potential of inactivating this receptor protein has led to the search for non-cytotoxic drugs with the ability to block transport and increase influx to the target cells.

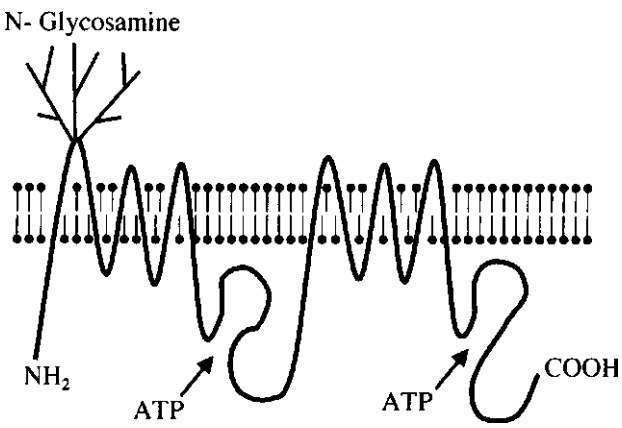

Figure 6.12 Structure of P-glycoprotein in the plasma membrane

It was soon realised that the wide diversity of compounds transported were mutual inhibitors, i.e. these compounds were also substrates for P-glycoprotein. Several of these reversal agents are now under trial in the treatment of acute myeloid leukaemia.

There is some speculation that P-glycoprotein may not prevent the complete absorption of a substrate but may simply control entry at a rate sufficient to ensure intestinal metabolism. The evidence for this is that the P-glycoprotein action appears to work in concert with cytochrome P450 3A4.

Cytochrome P450 3A4 (CYP3A4)

In the past, it was always assumed that the liver, rather than the intestine was the main guardian of the systemic circulation and that metabolism of xenobiotic compounds by the gut was functionally not so important. Although the importance of hepatic metabolism cannot be overstated, there is overwhelming evidence that the intestinal barrier provided by CYP3A4 is a major determinant of systemic bioavailability of orally administered drugs[70], for example, intestinal metabolism may account for as much as 50% of oral cyclosporine metabolism[71]. The cytochrome appears to be identical to that in hepatic cells and produces a similar pattern of Phase 1 metabolites[72].

It appears that CYP3A4 and P-glycoprotein are functionally integrated as there is a great overlap between the substrates for both systems. Secondly, the two complexes are co-localised in tips of the villus and not present in the crypts, and finally the CYP3A4 and P-glycoprotein genes appear to be close to each other on the same chromosome (Figure 6.13)[72].

The inter-relationship of P-glycoprotein and CYP3 A4 operates in a complex manner. Firstly, P-glycoprotein limits the total drug transport across the membrane so that CYP3A4 in the enterocytes is not saturated. Secondly, the slowing of drug absorption by P-glycoprotein increases the duration of exposure of the drug to the CYP3 A4 in the enterocyte, thus providing greater opportunity for metabolism. In addition, the metabolites generated by CYP3A4 are substrates for it. These metabolites are actively transported out of the cell by P-glycoprotein so that they do not compete with the metabolism of the parent drug.

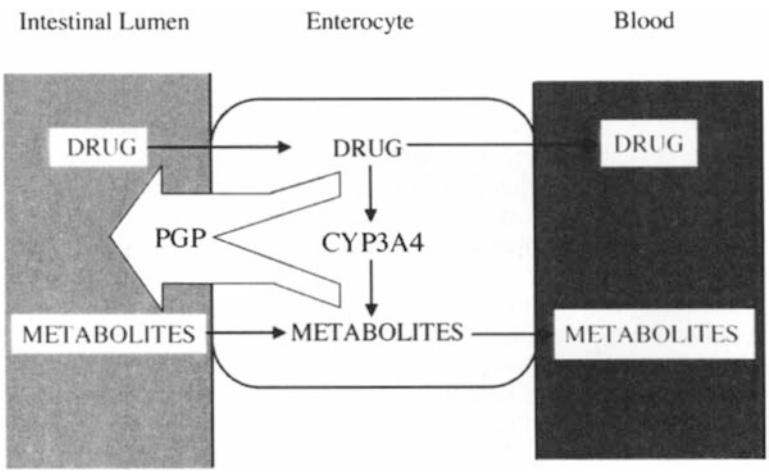

Figure 6.13 Probable mechanism of interaction of P-glycoprotein and cytochrome P4503A4 (CYP3A4)

Intestinal reserve length

If the lumen of the small intestine is occluded by inflating a balloon a short distance below the pyloric sphincter, the plasma levels of glucose from a glucose drink are reduced when compared to levels obtained with free transit of the liquid[36]. Experiments of this type allowed an estimate to be made of the length of intestine required to absorb a particular material. Many nutrients are absorbed almost completely by the time the meal reaches a point 100 cm from the pylorus[73]. This formed the basis of the hypothesis that absorption of the drug from a particular formulation was completed in the upper part of the small intestine, and the remaining length was a "reserve", i.e. unused for drug absorption[74]. If the reserve length was long, this implied that the drug was rapidly absorbed.

In this model the reserve length is defined as the distance from the point at which 95% of the drug has been be absorbed (the absorption length), to the distal end of the small intestine. The drug concentration is assumed to decline continuously with distance from the pyloric sphincter as absorption takes place. If the absorption length is similar to the total intestinal length, then RL approaches zero and the drug will be completely absorbed. The choice of 95% absorption is arbitrary. This hypothesis may apply to some drugs, but it assumes that small bowel contents move down the small intestine at a relatively constant rate and that there are no differences in absorptive capacity along the gastrointestinal tract. This theory is also incorrect from a number of standpoints; there is considerable mixing in the small intestine and individual particles of food can move at independent and widely differing rates. Moreover, values for the median transit times of the same components of a meal in different subjects can show considerable variability. The liquid meals used in the earlier study contained nutrients which could be rapidly assimilated because they were in a simple and well-dispersed form. The intestinal reserve length theory would not necessarily apply to normal meals which are complex and semisolid. Analysis of the effluent from ileostomies, for example, shows that quite high proportions of ingested nutrient enter the terminal ileum. Studies in man and in experimental animals have shown that food may move more rapidly through the jejunum to collect in the lower ileum from where residues are propelled at a more gradual rate to the colon. Thus, it seems likely that most of the small intestinal length is used for the absorption of drugs, particularly when these are given with a meal, and the concept of small intestinal reserve length is not a generally applicable guide to bioavailability. This concept also cannot be applied to certain drugs which are incompletely absorbed; for example the b-blocker atenolol which is absorbed for 3–4 hours following administration. Absorption then stops abruptly, even though 50% of the dose remains unabsorbed, possiblyon entry of the drug into the colon, where it is not absorbed. Similarly hydrochlorothiazide is poorly absorbed in the colon[75] and hence absorption stops abruptly as the bolus enters this region. Although intestinal reserve length is a useful guideline, it makes a number of physiological assumptions, primarily that there is no variation in the absorptive capacity of the small intestine along its length. This assumption may be true for some materials, but in other cases absorption may be carrier-mediated or occur at specific sites.

An alternative approach to the intestinal reserve length theory uses the calculation of the drug dissolution under sink conditions together with physiological variables such as the rate of gastric emptying[76]. The degree of absorption of a drug from the small intestine is directly related to the length of time that the drug remains in contact with the absorptive epithelium. Utilising measurements of drug dissolution under sink or non-sink conditions as appropriate, the time taken for the drug to be released from the formulation, i.e. disintegration and dissolution, can be calculated. This should be less than, or equal to the combined time available for absorption and transit through the potential absorption window in the small intestine. The latter terms will be highly influenced by the degree of

colonic absorption since, in the absence of absorption from the proximal colon, only a maximum of 3–5 hours is available for absorption from the small intestine in the fasting state. Absorption from the proximal colon affords a further six hours of contact time. This model would predict a marked increase in bioavailability for formulations of poorly absorbed drugs such as frusemide taken with a heavy meal since the meal provides a slow input into the upper small intestine, the major site of absorption (Figure 6.14).

Interaction with food

The presence of food may influence the absorption of drugs and can either enhance, delay or reduce absorption[77]. The most serious problem with such studies is that variations in drug absorption may be due to several different effects. Primarily, the effect of food on gastric emptying is considerable, and variations in the rate at which food is presented to the small intestine will change the drug pharmacokinetics. Secondly, the drug can interact with the food in the intestinal lumen, adsorb to food or be or absorbed by it. Metal ions present in food such as milk can chelate drug, or the drug can bind to dietary proteins thus changing its bioavailability. The presence of viscous chyme can act as a physical barrier reducing drug access to the absorbing surface. Finally, food may influence the absorption process by direct interference with the epithelial biochemistry; for example the absorption of a drug that was taken up actively by a carbohydrate transport system would be slowed in the presence of a large carbohydrate meal which would compete for the transporter. In practise it is extremely difficult to disentangle these factors and so most studies simply report an overall effect.

The absorption of drugs such as penicillin V and G, theophylline and erythromycin is reduced by the presence of food, but food delays the absorption of other drugs (cimetidine, metronidazole and digoxin). The effect of food on drug absorption can be dependent on the type of dosage form used, the excipients and the form of the drug, for example erythromycin stearate in film coated tablets demonstrated reduced absorption with food, erythromycin estolate in suspension was unaffected by food, but absorption of erythromycin ethylsuccinate in suspension and erythromycin estolate in capsules was increased by the presence of food[77]. A co-administered meal decreases the oral absorption of bidisomide and does not influence the oral absorption of the chemically-related

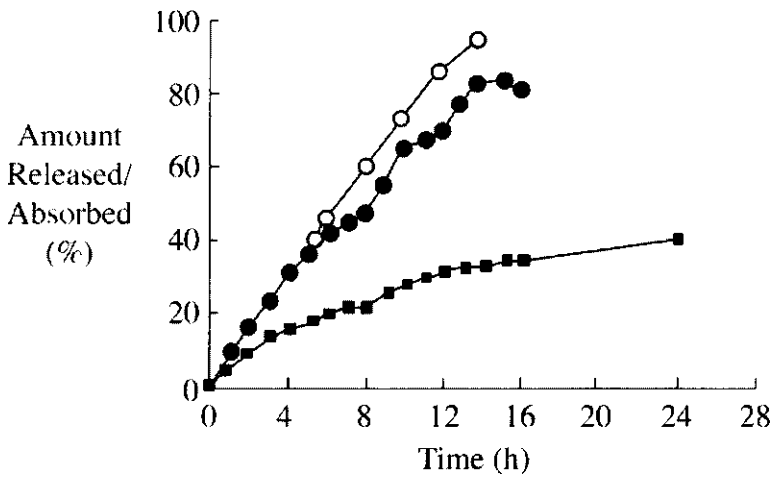

Figure 6.14 Absorption profile for a drug which is poorly absorbed from the colon when administered in a zero-order sustained release dosage form

antiarrhythmic agent, disopyramide[78]. It was postulated that this was due to the bidisomide being well absorbed in the upper but not lower small intestine.

Certain components of food, notably fibre, have a particularly important effect on drug absorption. Fibre is known to inhibit the absorption of digoxin and entrap steroids. It is well accepted that foods such as milk products, which have a high content of polyvalent metals such as calcium, magnesium, iron, aluminium and zinc, inhibit the absorption of tetracycline and reduce availability. Doxycycline has a slightly lesser tendency to form chelates, thus milk reduces its bioavailability somewhat less than other tetracyclines.

Availability of many drugs is determined by their solubility at the local pH. In the stomach this is highly variable, depending on the presence of food, but the small intestine has a relatively constant pH around 7.0. Drug absorption may be modulated by the presence of food which alters the gastric pH, the viscosity and the transit time through various sections of the gut and a single clear effect may not be evident.

Grapefruit juice greatly increases the bioavailability of certain drugs such as lovastatin and simvastatin, but not pravastatin[79]. The increase in bioavailability probably results from downregulation of CYP3A4 in the small intestine[80]. Hence large amounts of grapefruit juice should be avoided, or the dose of affected drugs should be reduced accordingly. The effect appears to be specific to grapefruit juice, since it cannot be reproduced with other citrus juices such as orange juice. It has been suggested that the inhibitory effect of grapefruit juice may be partially counteracted as it may also activate P-glycoprotein efflux of some drugs[81]

Vitamin effects

The consumption of large amounts of certain vitamins (doses of 1 g or more) has become popular with the general public. High doses of vitamin C do not materially affect the clearance of oral antipyrine which might suggest that vitamin C is without action on drug metabolism[82]; however, vitamin C is excreted by conjugation with sulphate. Drugs which are metabolised by sulphation such as ethinylestradiol would be likely to show competitive effects in their metabolism. Concomitant administration of ethinylestradiol with vitamin C resulted in a 50% increase in the steady state concentration of the drug, effectively converting a low dose oestrogen contraceptive pill to a high dose contraceptive pill[83].

Salt effects

It has been postulated that changes in dietary salt might alter metabolism[82]. Volunteers fed a high salt diet (400 mEq/day) compared to a low salt diet (10 mEq/day), during the administration of oral quinidine demonstrated that the bioavialability of quinidine was decreased on the high salt diet[84]. The mechanism of this interaction remains unclear although it could involve an effect on transit or P-glycoprotein status.

First-pass metabolism

Metabolism of drugs administered orally may occur either by gut wall enzymes or by the liver. Hepatic or gut wall enzymes have a limited capacity and the metabolizing enzymes may not necessarily be distributed evenly in the small intestinal epithelium. Estrone sulphokinases. for example, are more prevalent in the duodenal mucosa than the ileum[85]. Thus, oestrogens released lower down in the intestine may reach the blood at more rapid rates and in larger amounts, than the same compounds released in the duodenum and jejunum.

With the exception of the mouth and the terminal rectal area, the venous drainage of the gastrointestinal tract drains enters the liver via the portal vein. Thus, a fraction of the active agent undergoes biotransformation by the liver before reaching the systemic circulation and its site of action. Biotransformation generally inactivates drugs, but it can

also transform drugs to active metabolites or compounds which have a different pharmacological action to the parent drug, or even to toxic metabolites. Extensive first-pass metabolism, that is inactivation of the parent drug in the liver, is often encountered with lipophilic bases, such as propranolol and amitryptiline, but rarely with lipophilic acids such as salicylic acid and penicillin. The exceptions are esters of lipophilic acids such as acetylsalicylic acid and pivampicillin which, are exclusively metabolized before they reach the systemic circulation[86].

Ingestion of food increases the bioavailability of drugs which are metabolized during first pass through the gut wall or liver[87]. Two mechanisms have been put forward to explain this effect. First, the enhanced splanchno-hepatic blood flow will increase the load of drug delivered to saturable enzyme systems so that a greater proportion escapes metabolism. Second, nutrients may compete for hepatic enzymes so that less drug is metabolized. Concurrent food intake particularly enhances the bioavailability of weak bases, including propranolol, metoprolol, labetalol, and hydralazine, which are metabolized by hydroxylation, glucuronidation and acetylation enzyme systems. It has little effect on the hepatic clearance of those weak bases which undergo presystemic dealkylation such as codeine, prazosin, and dextropropoxyphene[88]. Food is not expected to influence first-pass metabolism if drugs are given in sustained release preparations because the delivery of drug to the liver is limited by the release of the drug into the gut lumen and not by changes in blood flow.

RELATIONSHIP BETWEEN DRUG ABSORPTION AND POSITION OF DOSE FORM

Radio controlled capsule

In 1981, a capsule containing a balloon filled with drug was reported, which could be actuated in the gastrointestinal tract when required, by the application of a radio signal[89]. This technique has been used to study absorption at various sites in the gastrointestinal tract. To locate the capsule, it is swallowed with a small dose of barium sulphate to aid its localisation within the gut and is triggered when required. The absorption of frusemide was compared in 5 subjects using the device[90]. The drug was released in the ileo-caecal area in 3 subjects and in the ascending colon in the other two. Maximum plasma concentrations were lower after the release of the drug in the colon, and there was a forty-fold difference between absorption from the stomach and colon with bioavailabilities of 20% and 3% respectively. The capsule was also used to study the absorption of theophylline from the stomach, ileum and colon[91]. The mean relative bioavailability of theophylline was 86% after releasing the drug in the colon. Thus there was no evidence for a so called absorption window for theophylline which has previously been reported in the literature, suggesting that such a "window" might have been related to the pharmaceutical formulations used. The device provides a means of investigating drug absorption under normal physiological conditions but has limitations of single occasion use and the need for repeated x-rays[92]. Although in many studies there have been good correlations between the gamma

scintigraphic data and the plasma concentration profile, there have been examples in the literature where the results have been completely inexplicable. For example, in a study of the transit and disintegration of acetylsalicylic acid from ^{51}Cr-labelled enteric-coated tablets, in four volunteers out of twelve, the absorption of acetylsalicylic acid was delayed more than 10 hours in spite of the fact that complete disintegration and gastric emptying of the tablet seemed to have occurred[93]. In three volunteers, this occurred in the in postprandial state and in one subject it occurred under fasting conditions. In the remaining eight of the twelve subjects, the time of onset of absorption correlated well with the time of disintegration.

Absorption of drugs and foreign substances through the lymphatic system

The lymphatic route has been suggested as a method of by-passing first-pass metabolism for extremely lipid soluble drugs. It has been argued that lipophilic drugs may become incorporated into lipid micelles and transformed into chylomicrons by the epithelium before being released into the lymphatic circulation. All the lymph from the lower part of the body flows up through the thoracic duct and empties into the venous system of the left internal jugular vein, thus avoiding the hepatic portal system. In order to be transported in the chylomicrons, the drug must be extremely lipophilic. The ratio of portal blood flow to intestinal lymph flow in the rat is approximately 500:1. Although lymph is the major transport route for fats, it only contains 1% lipids. The overall effect is to make systemic absorption 50,000 times more efficient than lymphatic absorption. In order for a drug to be transported at equal rates by both lymphatic and systemic circulations, the drug must be 50,000 times more soluble in the chylomicrons than the plasma, i.e have a partition coefficient of 50,000 (log P=4.7)[94].

As expected, in practice the lymphatic route is of little importance, except for those materials which are extremely lipophilic, for example insecticides such as dichlorodiphenyltrichloroethane (DDT), in which the loading of drug in the chylomicrons is as high as 0.6 to 2% by weight[95 96]. These values are approximately 6 to 20% of the saturated solubility of DDT in triglycerides.

Studies of mesenteric lymph and blood plasma levels of p-amino salicylic acid delivered intra-duodenally to rats suggested that the drug was directly transported to the lymphatics[97]. In addition, tetracycline was found in the central lacteal of the villi after administration, but this route was insignificant compared to systemic absorption.

In the rat, the absorption of oestradiol-3-cyclopentyl ether administered in an aqueous solution was mainly via the bloodstream, but when the drug was given in sesame oil (primarily linoleic and oleic acid triglycerides) a greater proportion of the drug was absorbed by the lymphatic route[98]. Addition of glyceryl mono-oleate to the sesame oil augmented this effect. The lymphatic absorption of griseofulvin, a systemic antifungal agent, and probucol, a lipid-lowering agent, is also enhanced by food with high fat content, presumably by dissolution of the drug in the fat prior to absorption[99 100]. The natural extension of this is to dissolve the drug in a lipid administered as an emulsion, an approach which has proven useful with griseofulvin[101].

DRUG INDUCED DAMAGE

All commonly used non-steroidal anti-inflammatory drugs (NSAIDs), apart from aspirin and nalbumetone, are associated with increased intestinal permeability in man. Whilst reversible in the short term, it may take months to improve following prolonged NSAID use[102]. NSAIDs cause quite distinct and severe biochemical damage during drug absorption, with the uncoupling of mitochondrial oxidative phosphorylation proving to be most important. Different NSAIDs and different preparations of the same NSAID may have different effects on small bowel permeability[103]

Small bowel ulceration connected to the use of slow-release potassium chloride tablets has been reported[104]. Most were associated with of 1–2 cm of stenosis, and a significant fraction with perforation of the bowe,l and the mortality rate was 27%. These problems many be reduced if wax-matrix or microencapsulated preparations of potassium chloride are used which slowly release potassium and chloride ions over time[105].

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