CHAPTER 1

Anatomy and Physiology of Gastro Intestinal Tract (GIT) and Drug Absorption

Human digestive tract is developed for digestion of food and absorption of nutrients. Drugs that are administered through oral route are absorbed via similar pathways available for nutrients. The major parts of digestive system are: Mouth, stomach, small intestine, colon and rectum. Orally administered drugs first enter the stomach and then move along the GIT. A brief study of anatomy and physiology of GIT is essential for understanding drug absorption and hence, bioavailability from dosage form.

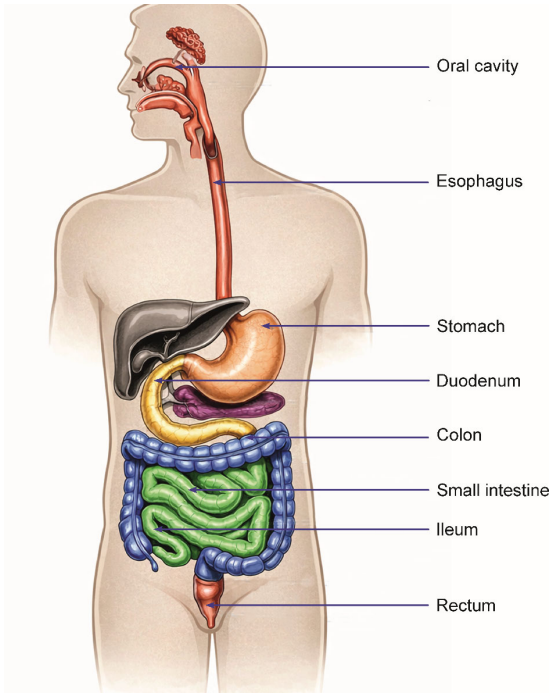


Figure 1.1: Human Gastro-Intestinal Tract.

Stomach

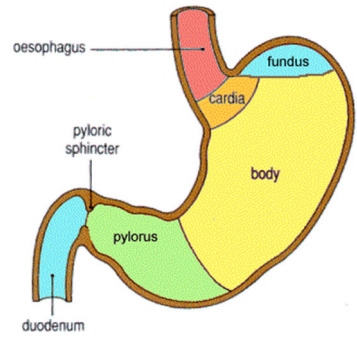


Figure 1.2: Various regions of stomach.

Stomach is the first organ that receives any swallowed material via oesophagus. The received material is stored in stomach and subjected for digestion process. Stomach is divided into cardia, fundus, body and pylorus and different functions are performed by each region. The body of the stomach secretes hydrochloric acid and pepsinogen enzyme while pylorus secretes gastrin, pepsinogen and gastrin. Hydrochloride acid kills microorganism and protects from infections and it activates pepsinogen into pepsin, an enzyme, for the digestion of proteins. The lower end of stomach contains pylorus sphincter that connects to the duodenum, the first part of small intestine.

Histology of Stomach

Details of cross-sectional areas of stomach wall are:

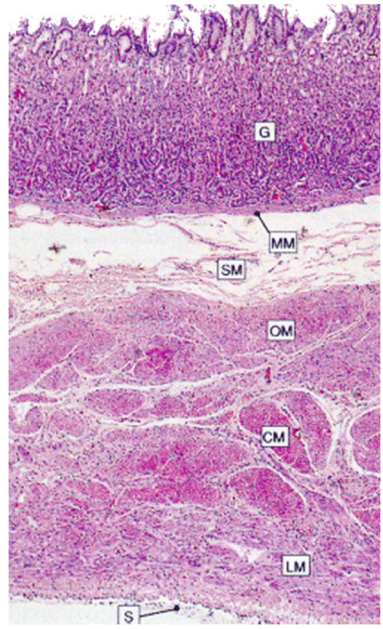


Figure 1.3: Cross sectional view of stomach wall.

Gastric mucosa (G) contains glandular tissue that secretes various compounds that aid digestion process. Hydrochloric acid is secreted by parietal cells, pepsin is produced by chief cells and regulatory hormones are secreted by enteroendocrine glands. Muscularis mucosa (MM) is located between lamina propria and submucosa (SM). It consists of two layers of muscle fibers: an inner circular layer and an outer longitudinal layer. The movements in the stomach are due to this muscle layer for proper mixing and digestion of food in stomach. The three important muscles of stomach are inner oblique (OM), outer longitudinal (LM) and circular muscles (CM).

The pH of luminal fluids is acidic in the stomach (pH 1.5–3.5) and this influences the stability of some drugs.

The gastric emptying time, measured by magnetic resonance imaging as the time to get back to fasted gastric volumes, is approximately 45 min after a glass of water (240 mL) and more than 6 h after a standardized food as per Food and Drug Administration (FDA) high-caloric breakfast. Gastric emptying time influences the bioavailability of drugs from drug products.

Gastric Emptying

Stomach acts as a chamber to store the food, partial digestion of food called chyme, and controls the movement of chyme into the duodenum. In order to perform above functions, stomach fundus and proximal corpus of the stomach act as reservoir and a pressure pump. The mixing of stomach contents is done by distal corpus and proximal antrum and terminal antrum and the pyloric sphincter work together as grinder and filter. The gastric emptying is regulated differently based on the state of the digestive process.

Gastric Emptying during the Digestive Period or Fed State

The food ingested is filled in the stomach during filling phase during which stomach accommodates the food without increase in pressure and peristaltic contractions are almost nil. Once the filling phase is completed, digestive phase starts by rhythmic contractions of the stomach that creates mixing of food with stomach digestive enzymes and break down of food into chyme. During the digestive process the pyloric sphincter is in closed condition and the digested food reaches antrum of stomach. Once the quantity of partly digested food level reaches certain threshold, propulsion of food into duodenum begins due to peristaltic movement initiated by stomach muscles and pyloric sphincter relaxes to allow the food to enter duodenum. The time between entry of food into the stomach and the start of stomach emptying is called lag time. Stomach forms a functional path along with the lesser curvature of the stomach, through which liquids are shunted into duodenum directly. The pylorus and pylorus sphincter control the movement of food from stomach to duodenum. The antrum generates forceful contractions to propel the food to pyloric grinder where the food is digested partially and is called “chyme”. The chyme is delivered into the duodenum due to powerful contractions of pylorus and opening of the pylorus sphincter in synchronous. The duodenum relaxes to receive the chyme. The duodenum bulb contracts to propel the received chyme to next portion of the duodenum and is ready to receive another chyme. The pyloric complex acts as both a grinder and a variable filter, it can facilitate or inhibit gastric emptying in the digestive period. Various factors influence the gastric emptying process and gastric emptying time.

Gastric Emptying in the Inter-Digestive Period or Fasting State

After digestion of food, the stomach attains fasting state. In order to clear indigestible food left after digestion, a cyclical motor activity called Migrating Motor Complex (MMC) is initiated that occurs in four phases.

Phase I: The peristaltic pump shows slow electrical waves and hence, muscle contractions are not involved. Motor quietness is due to tonic inhibition of the motor activity. This phase can last for about 45 to 60 minutes.

Phase II: The stomach shows frequent phasic contractions due to slow waves created in the muscles.

Phase III: This phase is neurally mediated and has nothing to do with the slow waves. The phase III is the cleaning stage where a front of large amplitude contractions that propagate towards pyloric sphincter are created for 5-15 minutes. The pylorus and duodenum are relaxed and pyloric sphincter is open to allow the sweep of food remains in stomach into duodenum.

Phase IV: The stomach comes to resting stage due to Vagus nerve stimulation that immediately abolishes the gastric motor and neuro-hormonal activity. The MMC cycle repeats from phase I.

Effect of Stomach Emptying on Bioavailability of Dosage Forms

The size of the undissolved solid particles generated form dosage form or size of undigestible solid dosage forms are affected by gastric emptying process. Solids can empty from the stomach only if they are about 2 mm in diameter, whilst larger digestible particles are reduced to this size by the digestive contractions of the stomach.

Larger indigestible solids are retained in the stomach, and emptied by the contractions of the migrating myoelectric complex (MMC) of the fasted stomach. Tablets larger than the critical value will be retained in the stomach and will be emptied by the more powerful contractions of the MMC. Studies concluded that non-disintegrating tablets of up to 11 mm in diameter can empty from the fed stomach, whereas tablets with a diameter greater than 11 mm are more likely to empty only during the contractions of the MMC. The diameter of the resting pylorus sphincter is 12.8 mm and hence, larger tablets bioavailability is highly variable due to variation in stomach emptying. The reason for bringing size and shape guidance by FDA is to address this issue.

Multi-particulate System

Multi-particulate systems are smaller multi-particulate systems of pellets or micro-tablets filled into capsules or compressed into tablets which

disintegrate into the original particles when taken. The residence time in different parts of the gastrointestinal (GI) tract is often an important feature of such formulations. The gastric emptying and small intestinal transit of single- and multiple-units has been compared in several studies. Gastric emptying of pellets in fasting state is fairly predictable while in fed state lot of variability is observed. After an initial period of approximately 2-3 h with no emptying of the pellets (0.4-0.6 mm) in most subjects, the pellets moved rapidly into the small intestine in an approximately zero order rate process. A similar delay in gastric emptying has also been shown for larger pellets (0.8-1.1 mm) given together with a radiolabelled meal.

Hence, in generic product development attention has to be paid for the number of pellets, size and density of pellets in comparison with reference product in order to achieve similar gastric emptying and subsequent drug availability in GIT. The fasting and fed state of stomach exhibit different emptying patterns based on the physical form of material. For example, liquids and small particles of less than 2mm are emptied quite rapidly from stomach irrespective of digestive state of the stomach. However, large size tablets have shown significantly different emptying rates based on condition of stomach. In fasted state, the gastric emptying rate was higher than fed state. The type of food also influences the gastric emptying rate. Light breakfast has shown faster gastric emptying rate than heavy breakfast.

Small Intestine

The small intestine is the longest part of GIT that further digests the chyme by chemical and mechanical means. Digestive juices including bile juice is mixed with the chyme and intestine causes mechanical digestion by rhythmic contractions called “peristalsis”. All the useful materials from the food are absorbed from the intestine.

Small intestine contains three specific sections that have specific functions.

The Duodenum: This part of small intestine receives the highly acidic chyme from stomach and it neutralizes the acid with an alkaline mucus secreted from its submucosal glands called *Brunner glands*. This neutralisation helps in protecting the surface of duodenum and also the further surfaces of small intestine. The common bile duct connects to the duodenum at a common opening known as the **Ampulla of Vater**, which also receives the pancreatic duct, allowing bile and digestive enzymes to enter together for digestion. Anatomically it surrounds the head of the pancreas and looks like “C” alphabet.

The Jejunum: It starts from the end of the duodenum and ends with the beginning of the ileum. Jejunum is middle small intestine that is approximately 2.5 meters long and is coiled extensively. The junction between jejunum and ileum is not clearly demarked. The mucosa of jejunum is folded extensively called "*plicae circulares*" upon which finger like projections called "*villi*" are present. This arrangement of mucosa increases the surface available for absorption of useful materials effectively.

The Ileum: The ileum is the most distal small intestine segment, measuring around 3 meters and terminating at the cecum. The ileum has more mesenteric fat than the jejunum. Histologically, the ileum has more lymphoid nodules (Peyer patches) than the jejunum. The mucosa has high surface area similar to jejunum due to the presence of *Plecae circulares* and *villi*.

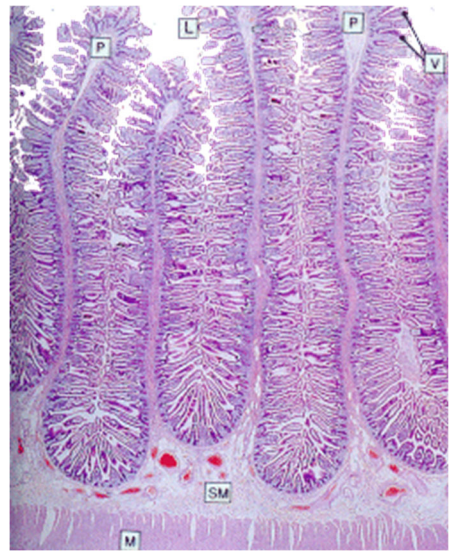


Figure 1.4: Cross sectional view of Jejunum wall.

The wall structure of Jejunum shows the presence of *Plicae circulares* (P) and *villi* (V) due to which the absorption surface available in jejunum is increased several folds compared with tube of similar length. Each villus contains several microvilli on its surface to increase the surface area further. Each villus has its own blood supply and are connected to blood vessels present in sub-mucosa (SM). All the absorbed materials are collected into mesenteric vein and finally enter into hepatic portal system. The movement of food in the jejunum and ileum happens due to the peristaltic movements caused by the muscular layers (M) present in the walls of them.

Small Intestinal Transit Time

There were no differences that could be attributed to dosage form, or stomach contents. The physical form of dosage form has no impact on transit time. It was observed that solutions, particles and single unit such as tablets have similar intestinal transit time. Even fasting and fed state have no impact on intestinal transit time of dosage forms or food. It indicates that intestinal transit time is fairly constant when compared with stomach emptying that showed significant variation based on physical form and digestive state of stomach.

The mean transit time of about three to four hours agrees with the recent studies on the transit of food (mean transit solid food = 3.6±0.3 h, n=15) and water (4.0 $\pm$ 0.8 h). It has been suggested that drugs, whether present as a particular dispersion, or as a micellar or molecular solution are considered to be propelled along the small intestine at the same net propulsive rate as food particles. However, in case of multi-particulate systems, unlike single unit drug product, particles or pellets are present at different locations in GIT at a given point of time. Single unit dosage form is present at one location at a given point of time. Therefore, drug release from pellets or particles and subsequent absorption happens from several locations in GIT while absorption happens only from one site in GIT for single unit dosage form.

Many sustained or controlled release drug delivery systems are tested for drug release for 12 to 24 hours in in-vitro tests under standard conditions. The relevance of this in-vitro test is questionable since the intestinal transit time is fairly constant. It is evident that the dosage form would reach colon in 4-5 hours from the intestine. Hence, the drug release is expected to be more in colon due to higher residence time for a 24-hour controlled release product. Therefore, formulation scientist has to consider these physiological aspects while developing a controlled or sustained release drug product.

Intestinal Fluid Composition

Consideration of intestinal fluid composition is critical for drug bioavailability. In order to have bioavailability from dosage form, the dosage form has to disintegrate into granules or particles, drug particles have to go into solution and the soluble drug only eligible for absorption. Hence, drug solubility in intestinal fluids is the primary criterion for drug absorption. In addition, stability of drug in intestinal fluids and its permeability influences drug absorption. Intestinal fluid contains mainly water and other important components such as electrolytes, bile and pancreatic secretions.

In addition, the GI tract is dynamic and the composition is continuously changing through a cycle of fasted and fed states superimposed upon the

inherent biological variability of human subjects. In order to assess the impact of the complex and variable composition of gastrointestinal fluids on drug solubility and therefore absorption, two general approaches have been adopted: the aspiration of human intestinal fluid (HIF) for direct solubility measurements or the development of simulated intestinal fluids (SIF) based on the aspirated samples. Regional fluid volumes over duodenum to ileum ranges from 5.6 to 20.38 mL in the proximal small intestine, 36.4 to 44.08 mL in distal small intestine, and from 42 to 64.46 mL in total small intestine.

In order to achieve bioequivalence of generic drug product, the bioavailability of reference product has to understood with the GIT transit time, fluid composition, dosage form behaviour in different regions of GIT and drug solubility in different locations in GIT. Development of generic drug product should use this understanding in designing the product so that its in-vivo behaviour is expected to be similar to that of reference product. The concept of biorelevant media is derived from these biological features of GIT.

The Colon or Large Intestine

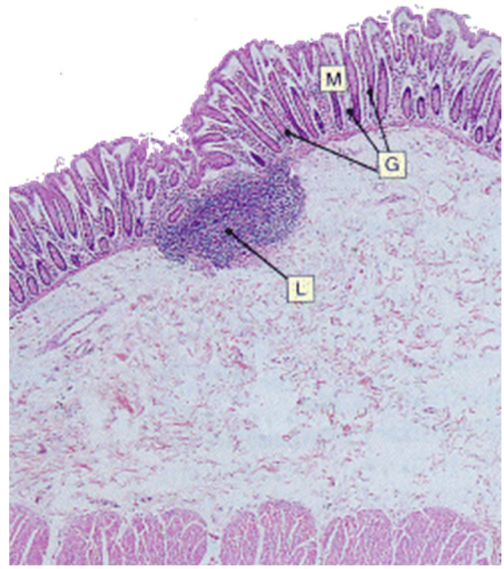


Figure 1.5: Cross section of Colon wall.

All the useful nutrients and other materials are almost completely absorbed in small intestine and the unabsorbed material reaches colon via ileo-caecal valve. The conservation of water is done by Colon by absorbing from the material reached from small intestine and the semi-solid faeces moves into

the rectum where it is stored until expelled from the body through the anus. The mucosa (M) of colon contains straight tubular glands (G) that are packed tightly in mucosa. The tubular glands contain cells that are specialised in the absorption of water and also mucus-secreting goblet cells to lubricate the surface of the colon to aid smooth transit of faeces. In addition, large intestine contains lymphoid tissue (L) at various areas.

The human large intestine is divided into ascending, transverse and descending colon and is about 1.5 meters long with about 5-7.5 cm in diameter. The distal part of large intestine forms a small portion called sigmoid colon that connects with rectum that stores faeces. The lumen of the colon is coated with mucus secreted from goblet cells present in the mucosa.

The physiology and physical properties of the colonic contents differ significantly compared with small intestine. In addition, microbial flora of colon also plays a role in maintaining the conditions in lumen of colon. Colonic delivery of drugs is influenced by the pH and composition of contents in ascending, transverse and descending colon that exhibit inter and intra subject variability. In addition, the microbial constitution of the subject significantly affects the colonic absorption of drugs from Colon Drug Delivery Systems (CDDS). The residence time of the dosage form in colon is also varies from person to person.

Colon Transit

After the gastric emptying the contents transit in small intestine with less variability but the contents of intestine are emptied into colon at ileocecal valve. The dosage forms emptying is controlled by this valve. Once the contents of intestine are entered in to ascending colon, the transit in colon starts.

The environment of the large bowel differs along its length and it is only in the ascending colon where conditions are sufficiently favourable to allow drug absorption. As the contents of ascending colon travel in transverse colon, water is absorbed further leading to very low water and also the gases generated from microbial fermentation are present making this region of colon unsuitable for drug dissolution and absorption. In descending colon, the consolidation of matter happens leading to formation of faeces and almost negligible drug absorption happens.

The colon transit time measurements have shown a bimodal distribution with high variability. In one study, the mean colonic transit time is 39.6 ± 21.4 hours. Hence, colon targeted drug delivery systems exhibit high variability in pharmacokinetics of drugs.

Pathological effects on colon transit are well documented, as the generation of normal clinical ranges for physiological factors has to be established as a function of age and gender. Colon transit is influenced by central, local and hormonal influences as the integration of gut motility occurs through intrinsic and extrinsic pathways.

Colonic Fluid Volume

The presence of specific cells that absorb water from the contents reached from small intestine enables colon to absorb almost 90% of water leaving only few millilitres of water ranging from 1 to 44 ml and the average is about 13 ml. The drug delivery systems have to undergo disintegration and dissolution in this small colonic fluid for drug absorption making it very challenging to design CDDS.

Viscosity of Colonic Luminal Contents

As the contents in the colon transit through ascending, transverse and descending colon, water is absorbed continuously and hence, the viscosity of contents increases gradually and becomes almost a semi-solid at the end of descending colon. This poses further challenge to develop CDDS. As the viscosity increases the dissolution of drug from CDDS diminishes leading to slow and incomplete absorption of drug. Hence, the CDDS has to be designed such that most of the drug is released and exists in dissolved state in the early parts of colon. If the CDDS is meant for local antimicrobial action, then it has to be designed effectively to deliver sufficient concentration of drug in low fluid environment so that microbes receive minimum inhibitory concentration of drug.

Colonic pH

The pH of the colonic contents influences the pharmacokinetics and pharmacodynamics of a CDDS since the solubility of drugs and/or excipients in the dosage form are affected by pH. If the CDDS has a pH-sensitive polymer, then the drug release is more significantly affected by colonic pH. The pH of contents in GIT varies at different anatomical location. In stomach the pH can be as low as 1 to 2, in duodenum the neutralisation of gastric contents brings the pH to 4.5 to 5.5. The pH in Jejunum and ileum is about 6.5 but reaches to about 7.5 at the terminal ileum. The contents from jejunum reach the ascending colon having pH about 7.5 and gradually decreases due to the fermentation of polysaccharides present in the undigested contents by colonic bacteria and also due to the formation of short chain fatty acids. These variations in pH across the colon exhibit inter and intra subject variability leading to significant variations in bioavailability from colon. This phenomenon is clearly evident with delayed release Mesalamine drug delivery systems.

Colonic Enzymes and Metabolism

The colon hosts a microbial flora based on the food habits of the human being. The symbiosis of microbes in colon is an important requirement for human survival. Both aerobic and anaerobic microorganisms are present in colon and it is estimated that colon can inhabit more 400 different microorganism species. These wide variety of microorganisms can have enzymes that can metabolise drugs, undigested food containing carbohydrates, polysaccharides, fats and proteins. Drugs are also metabolised by the enzymes of bacteria leading to the formation of active metabolites, inactive metabolites or harmful metabolites. This metabolism by bacteria in colon is used to develop "prodrugs" that are converted into active form of drug for local or systemic action. Certain colonic drug delivery systems are developed with materials that can only metabolised by bacterial enzymes in colon. Polymers such as chitosan, pectin and guar gum are used for CDDS whose performance is based on their metabolism by colonic microorganisms.

One common requirement for CDDS is that they have to be protected in stomach and small intestine so that the delivery system reaches colon. Subsequently, the drug release from the delivery system utilizes the specific conditions prevailing in colon such as pH, microbial enzymes, bacterial degradation of dosage form, conversion of prodrug into active moiety etc.

Tablets versus Pellets Colon Transit

Studies were conducted by co-administering radio labelled tablets and pellets and transit of dosage forms is observed. Firstly, formulations administered after overnight fasting tend to arrive at the colon approximately 5 h after dosing which was also confirmed in later studies. Second, once in the colon the tablet moved ahead of the labelled pellet mass with an average residence time of 4.7 h in the ascending colon (range 0.7–7.5 h). The tablet reached the transverse colon before 86% of the pellets. Transit was longer in the fed subjects versus the subjects who remained fasted during the study. At 24-h, the tablet had been excreted or was at the rectosigmoid junction with pellets distributed between the ascending and the descending colon with little material in the transverse colon.

For whole colon transit the differences are 26.2 ± 8.3 h for the tablet and 35.7 ± 6.0 h for the [111In]-labelled pellets. This difference reflects the differential transit of tablets and pellets: effectively a sieving mechanism. This suggests that the propulsive movements are less effective for particulates, which perhaps stick to the colon wall rather than remain in the luminal flow.

Morning versus Evening Dosing

Total transit time was compared for OROS devices dosed at 0800 or 2200 h using faecal excretion of the unit. Whereas a unimodal distribution of excretion with maxima at 24–32 h after dosing was seen with morning administration, evening dosing resulted in a bimodal distribution with peaks around 12 and 34 h. In another study reported median transit times using scintigraphy of 29 h in one study and 35 h in another following night time dosing versus median transit times following morning dosing of 24 and 26 h.

The Pancreas

The function of Pancreas with respect to GIT is to provide enzymes to aid digestion of food in the small intestine. Any food basically contains carbohydrates, proteins and fats. Pancreas secretes amylases, peptidases and lipases and releases into duodenum via common bile duct that digest the carbohydrates, proteins and fats respectively.

The Liver

The liver is present below the lungs in the right side of abdomen and is divided into four lobes which cover spleen, stomach and small intestine. The average weight of human Liver is 3.0-3.5 lbs. The major function of Liver in digestion process is to provide bile juice from gall bladder. Bile juice is secreted and collected in gall bladder which is emptied into duodenum in response to food via common bile duct. Bile juice contains bile acids and bile salts that are critical for fat digestion.

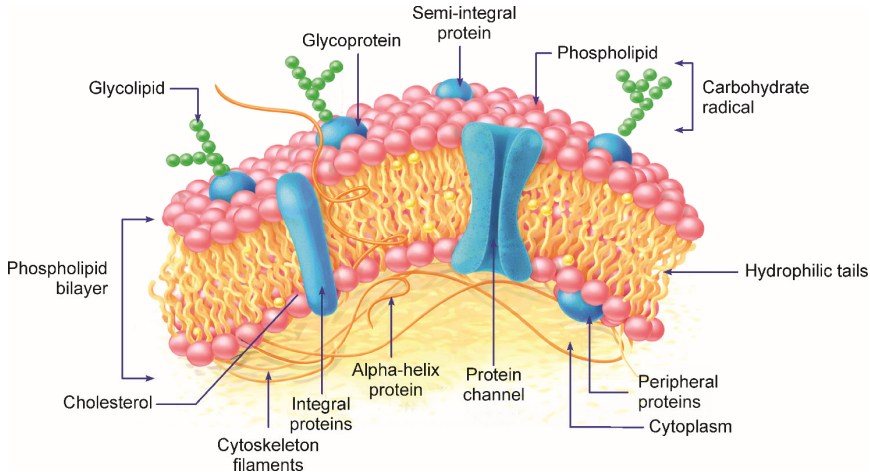
Mechanisms of Drug Absorption

When a tablet is administered by oral route it has to undergo disintegration and dissolution in GI fluids so that the drug exists in molecular or ionic state. Drug in molecular level or ionic state in the gastrointestinal (GI) fluids can only get absorbed into systemic circulation. The drug molecules in the GI fluids must cross the unstirred aqueous layer present on mucus, mucus layer and glycocalyx to reach the apical membrane of cells. The passage of drug molecules through apical membrane is achieved by several mechanisms.

Structure of Plasma Membrane

The figure below shows the structure of the membrane through which the drug molecules have to be transported from luminal contents of GIT to the systemic circulation. Fluid mosaic model proposed by Garth L. Nicolson and S.J. Singer in 1972 explains the absorption processes in GIT. The apical membrane is made up of lipid bilayer mainly consisting of phospholipids and

cholesterol where the hydrophobic portion of two lipids are inside the structure while their hydrophilic heads are outside. As an integral part of membrane, glycoproteins and glycolipids are anchored on the apical surface (towards lumen of GIT) of bilayer. There are some semi-integral proteins also present that act as transporters and pore forming proteins create very small pores that connect the luminal side of GIT and cell cytoplasm.



The absorption mechanisms are listed below:

- Passive Diffusion
- Active Transport or Carrier mediated Transport
- Facilitated Diffusion
- Ion-pair Formation
- Convective Transport or Pore Transport
- Vesicular Transport or Pinocytosis

1. *Passive Diffusion*

Most of the drugs are absorbed by passive diffusion mechanism followed by active transport. The passive diffusion mechanism is solely depending on the concentration gradient of drug from luminal side to the cytoplasm. In order to cross the lipid bilayer, the drug molecule has to partition between luminal contents and lipid layer. Again, the drug in lipid layer partitions into cytoplasm and then partitioning between cytoplasm and lipid layer on the other side of the cell. The driving force in this process is concentration gradient of drug across all positions in the path. There is no involvement of energy in this process and apical membrane is not playing any role and hence, called passive transport. This process of partitioning between aqueous fluid

to lipophilic membrane continues till the drug reaches a blood capillary present in the lamina propria of wall of GIT. In passive diffusion process drug molecules spontaneously diffuse from a region of high drug concentration to a region of low drug concentration. This process is called passive because no external energy is expended. The passive diffusion process continues till the drug concentration in the GI fluids and in the cell membrane are equal. Since the absorbed drug enters systemic circulation, the drug levels in absorbing cells are always kept low and the concentration gradient is maintained till all the drug in GI fluids is absorbed.

Drugs in unionized form only can cross the lipophilic cell membrane while ions cannot cross the membrane by passive diffusion. Drug molecules that have proper hydrophilic and lipophilic groups are absorbed quickly and completely. Drugs that are weakly acidic or basic undergo dissociation into unionized and ionized forms in GI fluids. The extent of ionization of a weak electrolyte drug depends on both the dissociation constant of the drug (i.e., pKa) and the pH of GI fluids in which the drug is present. Since only unionized form of the drug is absorbed by passive diffusion, the extent of unionized form in the solution determines the absorption rate. The fraction of unionized form of drug can be estimated by Henderson-Hasselbach equation that relates the pKa of the drug and pH of the GI fluids to the fraction of unionized and ionized forms of the drug.

In passive diffusion process drug at the site of absorption ultimately enters the blood after crossing several cells and membranes called biological barrier. **Figure 1.6** shows the process of passive diffusion across the biological barrier. Passive diffusion of drug across the gastrointestinal/blood barrier can often be described mathematically by Fick's Law of Diffusion. Accordingly, the rate of absorption of drug into the blood is given by,

$$dx/dt=DA/h * (K1\ Cg – K2\ Cb) \quad (1.1)$$

Where:

Dx/dt = the rate of drug transport across the gastrointestinal barrier

D = the diffusion coefficient of the drug in the gastrointestinal barrier

A = the surface area available for the drug absorption

h = the thickness of gastrointestinal barrier

K1 = the apparent partition coefficient of the drug between the gastrointestinal barrier and the GI fluids

Cg = the concentration of the drug in solution form in the GI fluids

K2 = the apparent partition coefficient of the drug between the gastrointestinal barrier and blood

Cb = concentration of the drug in the blood at the site of absorption

According to equation 1.1, the rate of drug absorption is directly proportional to the diffusion coefficient (D), the surface area available for absorption (A), the concentration gradient (K1Cg-K2cb) and inversely proportional to the thickness of the gastrointestinal barrier (h). A drug molecule should have solubility in GI fluids and should have sufficient lipid solubility to cross the gastrointestinal barrier and finally should partition into blood from barrier membrane. Once the drug enters blood through blood capillaries walls it will be carried into systemic circulation and will be diluted by:

1. Distribution in a large volume of blood
2. Distribution into body tissue and fluids
3. Metabolism and excretion

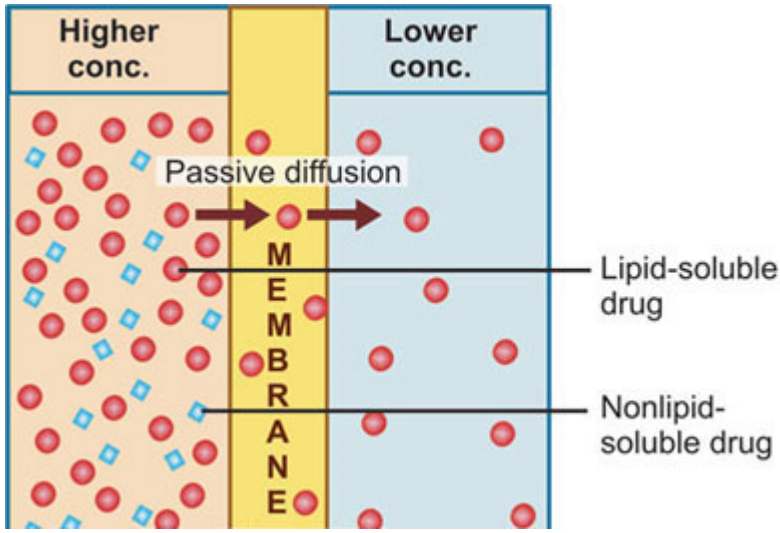


Figure 1.6: Passive diffusion of drug molecules across gastrointestinal barrier. Solid arrows indicate direction of net movement of drug molecules.

Further, the drug in blood can bind with plasma proteins and also components of tissues in which it is present. This binding of drug leads to low concentration of free drug in blood. This is called "sink" condition in which the drug concentration gradient is maintained due to drug concentration at absorption site (Cg) is very high than in the blood (Cb), i.e., Cg>>Cb.

Under such conditions, where K1Cg>>K2Cb and thus (K1Cg-K2Cb) approximates to K1Cg, and the equation 1.1 may be reduced to:

$$dx/dt=DA/h*(K1Cg) \quad (1.2)$$

For a drug and biological membrane under consideration, the parameters D, A, K1 and h can be considered as constants and all can be combined into a new constant called permeability constant, P. Hence, equation 1.2 can be written as,

$$dx/dt = P*Cg \quad (1.3)$$

Where $P = DA/h*K1$ (1.4)

Equation 1.3 is an expression for a first order kinetic process and indicates that the rate of passive drug absorption will be proportional to the concentration of the unionized (absorbable form) form of the drug in the GI fluids at the site of absorption.

2. ***Active Transport or Carrier Mediated Transport***

Most of the drugs are absorbed by passive diffusion process but drugs that are highly hydrophilic such as 5-fluorouracil and many important nutrients are absorbed by an active transport mechanism. In contrast to passive diffusion, active transport involves active participation by the apical cell membrane of the columnar absorption cell. A specific component of cell membrane such as protein acts as a 'carrier' for the transport of nutrient or chemical or drug. Carrier mediated transport is thought to be developed in living systems for the absorption of essential nutrients from the food irrespective of their concentration. For example, essential amino acids are present in the human blood but more are required for the survival, hence, even if small amount of essential acid is present in the food, it has to be absorbed which is possible only by active transport. Drugs are not essential components for survival of human and hence, this mechanism is not for the absorption of drugs. The carrier molecule couples with certain groups of nutrients only and if the drug contains similar groups in its structure, then the carrier molecule does not discriminate between nutrient and drug. Hence, those drugs that share similar functional groups to that of nutrients are transported by active transport mechanism. Active transport seems to be limited to drugs structurally similar to endogenous substances (eg, ions, vitamins, sugars, amino acids). These drugs are usually absorbed from specific sites in the small intestine. **Figure 1.7** depicts the active transport process in GIT. The drug molecule or an ion form of drug forms a complex with the 'carrier' on the surface of the apical cell membrane of a cell. The drug-carrier complex then moves across the membrane and liberates the drug or an ion form of drug on the other side of the membrane. The carrier that is freed from drug or an ion form of drug returns to its initial position in the membrane and is now ready to transport another molecule or an ion.

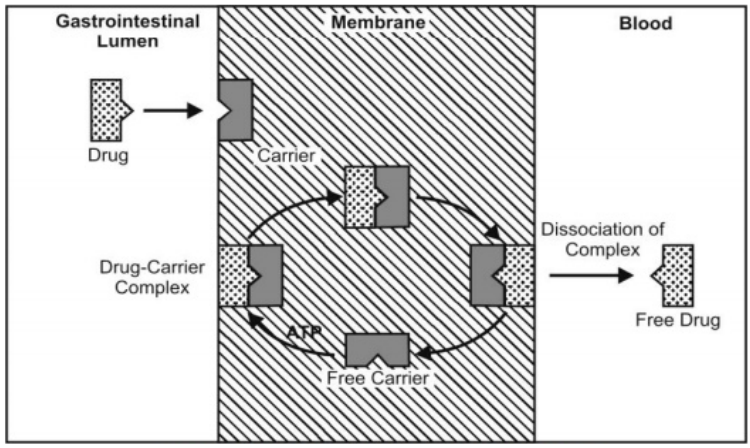


Figure 1.7: Active transport of a drug across a cell membrane.

Many nutrients such as sugars and amino acids are transported by active transport mechanism and vitamins such as thiamine, nicotinic acid, riboflavin and pyridoxine are also absorbed by carrier mediated transport mechanism. Drugs methyldopa and nicotinamide are absorbed by active transport. Active transport also plays an important role in the renal and biliary secretion of many drugs and metabolites. The characteristic features of carrier mediated transport mechanism are:

1. ***Specific location in GIT*:** Specific carrier system does not present in entire GIT but only at particular part of GIT. Because of this, specific substances are absorbed only from certain location in the GIT where the density of carrier system is high. For example, riboflavin is preferentially absorbed from proximal portion of the small intestine.
2. ***Selectivity*:** The specific carrier molecule interacts with specific groups present in the nutrients and transports by active transport mechanism. It is like a lock and key type of interaction. In order to undergo active transport by this specific carrier molecule, the drug should have specific functional groups that resembles the nutrients with which it can interact with carrier molecule. The drug levodopa shows structural similarity with the amino acid tyrosine and phenylalanine. Hence, levodopa is transported by that carrier system which transports the amino acids, tyrosine and phenylalanine.
3. ***Transportation against concentration gradient*:** The driving force in the passive diffusion is the concentration gradient of the drug across GIT to blood but an active transport is characterized by the transport of the drug against a concentration gradient- that is, from the regions of low concentration to the regions of high concentrations. Hence, energy is spent for the active transport process.

4. ***Saturation of Transport*:** The active transport is based on the number of carriers present per unit surface of the cell membrane in which they are present. Therefore, the rate of drug absorption by active transport mechanism is increased as the concentration of drug available for absorption increases until all the carrier molecules are engaged in the transport and beyond this critical concentration, the rate of drug absorption is constant or saturated (Figure 1.8). The rate of absorption of a drug by passive diffusion is directly proportional to the concentration of the drug at the site of absorption in GI fluids. Hence, the rate of drug absorption increases linearly with drug concentration in the GI fluids as shown in Figure 1.8.
5. ***Competitive Inhibition*:** Each carrier-mediated transport system is specific to a particular chemical environment on a molecule with which it binds during transportation. Therefore, chemicals having such specific chemical environment in their structure are transported by same carriers. Two chemicals having similar chemical environment compete for the same carrier involved in the active transport and hence, the transport of one chemical is inhibited by the other chemical. This phenomenon is called competitive inhibition. The extent of absorption of one chemical relative to the other depends on the relative affinity of these chemicals to the binding site of carrier.

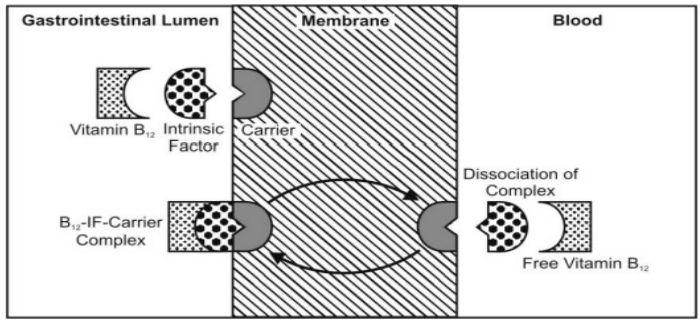


Figure 1.8: Relationship between the drug concentration at the absorption site and rate of drug absorption by passive diffusion (Line A) and active transport (Line B).

The kinetics of drug absorption by active transport mechanism is best described by Michaelis - Menten equation since the number of carrier molecules in the membrane are limited.

$$\text{Absorption Rate} = (V_{max} * C_g)/(K_m + C_g) \quad (1.5)$$

Where Cg is the drug concentration at the absorption site, Vmax is the maximum velocity of the absorption process and is a constant for a given drug.

Km is called Michaelis-Menten constant. At low drug concentration at the absorption site, such that Km>>Cg, then equation 1.5 can be reduced to:

$$\text{Absorption rate} = (\text{Vmax}*\text{Cg})/\text{Km} \quad (1.6)$$

As long as Km>>Cg, the absorption process follows the first-order kinetics since the rate of drug absorption is directly proportional to the concentration of the drug at the absorption site. As the concentration of the drug increases significantly, the linearity between the drug concentration and rate of absorption diminishes and drug concentration at the site of absorption (Cg) becomes much more than Km. When Cg>>Km, the equation 1.5 reduces to

$$\text{Absorption rate} = \text{Vmax}*\text{Cg}/\text{Cg} = \text{Vmax} \quad (1.7)$$

At this stage the active transport system is saturated and a constant rate of drug absorption is observed beyond a certain drug concentration.

3. *Facilitated Diffusion*

Facilitated diffusion is the transport of substances across a biological membrane from an area of *higher* concentration to an area of *lower* concentration with the help of a transport molecule. The difference between an active transport and facilitated diffusion is that the active transport requires energy and can transport drug molecules against a concentration gradient, while the facilitated diffusion does not need energy and cannot transport drug molecules against a concentration gradient. The molecules move along with the concentration gradient which is the driving force in facilitated diffusion. The difference between passive diffusion and facilitated diffusion is that no carrier molecule is required for passive diffusion while a carrier molecule is required for facilitated diffusion. However, both the processes work with concentration gradient as driving force. Glucose is transported by facilitated diffusion using glucose transporters into the blood from GIT. It shows all other characters of an active transport such as specific location in GIT, selectivity, saturation and competitive inhibition.

4. *Ion-Pair Formation*

Some compounds such as quaternary ammonium compounds are ionized over the entire gastrointestinal pH and hence exists as ions in GI fluids. Therefore, ionized form cannot partition directly into the lipid membrane and the bioavailability is negligible. There is a limit of charged species size for pore transport. In general, ionic form of drug is bigger than available pore size in the cell membrane, hence, can't be transported by aqueous channels. However, the interaction of such drug ions with the oppositely charged organic ions either available in the food, endogenously or added externally leads to the formation of an ion-pair whose overall charge is neutral. This neutral complex (ion-pair) diffuses more easily across the lipid membrane of

the cell due to its lipid solubility. Therefore, attempts have been made to improve the bioavailability of drugs that form ions in the GIT by combining them with organic counter ion to form absorbable ion-pairs. The drugs propranolol and quinine show a good absorption when paired with oleic acid and hexylsalicylate, respectively. Several other drugs were also converted into ion-pairs to improve their absorption from GIT. These ion-pairs again dissociate to liberate active drug ion in the blood.

5. ***Convective Transport or Pore Transport***

The cell membranes contain aqueous filled pores or channels that are connected to intra-cellular fluids. The effective radius of these channels has been estimated to be of the order of 0.4nm. Very small molecules such as water, urea and low molecular weight sugars and organic electrolytes are able to cross the cell membranes rapidly as if there is no barrier for their passage though these aqueous filled channels. As a result of molecular size limitation due to effective radius of channels, this mechanism of absorption appears to be of minor importance with respect to the gastrointestinal absorption of large water-soluble drug molecules or ions. However, convective absorption is involved in the renal excretion of drugs and the uptake of drugs into the liver.

6. ***Vesicular Transport or Pinocytosis***

Pinocytosis (cell drinking) or vesicular transport is the process of engulfing particles or dissolved materials by the cell. The mechanism is comparable to phagocytosis and involves invagination of the material by the apical cell membrane of the columnar absorption cells lining the gastrointestinal tract to form vacuoles containing the material. These vacuoles then cross the columnar absorption cells. This mechanism of the absorption appears to be of little importance for drugs but is important for the absorption of macromolecules such as protein. Vesicular transport is the proposed mechanism for the absorption of orally administered Sabin Polio Vaccine and various large proteins.

Sublingual Route

Sublingual, meaning literally “under the tongue” refers to a method of administering drugs via the mouth in such a way that the drugs are rapidly absorbed via blood vessels under the tongue. There is a considerable evidence that most drugs are absorbed by passive diffusion from sublingual route.

The salivary glands consist of lobules of cells which secrete saliva through the salivary ducts into the mouth. The three pairs of salivary glands are the parotid, the submandibular and the sublingual which lie on the floor of the mouth. In an average 1 to 1.5 litres of saliva is secreted per day in normal

human. The volume of saliva which is available constantly is around 1.1ml, thus providing a relatively low fluid volume available for drug release from dosage form. Increased salivary secretion in response to acidic stimulation is documented and the salivary secretion is increased in response to food. The average pH of saliva is maintained at 6.0 and hence, drugs selected for sublingual delivery should have good solubility at this pH. Saliva is composed of a variety of electrolytes, including sodium, potassium, calcium, magnesium, bicarbonate, and phosphates. Also found in saliva are immunoglobulins, proteins, enzymes, mucins, and nitrogenous products, such as urea and ammonia. These components interact in related function in the following general areas: (1) bicarbonates, phosphates, and urea act to modulate pH and the buffering capacity of saliva; (2) macromolecule proteins and mucins serve to cleanse and contribute to plaque metabolism. (3) Antibacterial action of saliva is due to the presence of immunoglobulins and enzymes.

Design of sublingual dosage forms should consider the very low volume available in sublingual route and pH of 6.0. Further, the time available for drug release and subsequent absorption is limited to 5-7 minutes since human can't hold the dosage form for long without swallowing the contents of mouth. The excipients selected may stimulate release of saliva and use of permeation enhancers may improve the bioavailability of drugs. Keeping the reference product composition into consideration, generic product has to be manufactured to achieve bioequivalence. Innovative biorelevant tests using 2-7 ml of media that mimics saliva composition has to be developed for comparative evaluation of test product with reference product.