Basic Biopharmaceutics

Md. Al- Faruk
B.Pharm (Hons.), M.Pharm, University of Dhaka
Lecturer, Department of Pharmacy
Daffodil International University

CAPITAL BOOK CENTER
DEDICATION

Prof. Dr. Md. Abdur Rashid

(Professor, Dept. of pharmaceutical Chemistry, University of Dhaka

Former Dean, Faculty of Pharmacy, University of Dhaka)
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Understanding the absorption, distribution, metabolism and excretion of drug is important for the drug design and development. Advancement of the diseases has necessitated the need to optimize drug therapy. Along with the accurate dosage form design, it is important to ensure that optimum amount of drug reaches to targeted site. Biopharmaceutics deals with the factors that influence the stability of the drug within the drug product, the release of the drug from the drug product, the rate of dissolution of the drug at the absorption site, and the systemic absorption of the drug.

This book comprehensively describes principles of drug absorption, distribution, metabolism and excretion (ADME) and the factors influence ADME. Concepts of ADME have described with the simple figure and tables. In order to exemplify the text, illustrations have been used liberally throughout the book.

I am thankful to my teachers, colleagues, students and friends for providing their valuable suggestion and inspiration.

Lately, any kind of critical suggestion for improvement and refinement of the text in future will be highly appreciated.

Dhaka
September 14, 2013

Md. Al- Faruk
maruppharm@gmail.com
Biopharmaceutics

All pharmaceuticals undergo extensive research and development prior to approval by the regulators. The physicochemical characteristics of the active pharmaceutical ingredient), the dosage form or the drug, and the route of administration are critical determinants of the \textit{in-vivo} performance, safety and efficacy of the drug product. The properties of the drug and its dosage form are carefully engineered and tested to produce a stable drug product that upon administration provides the desired therapeutic response in the patient. Both the pharmacist and the pharmaceutical scientist must understand these complex relationships to comprehend the proper use and development of pharmaceuticals.

The life cycle of drug inside our body follows the following orders. First, the drug in its dosage form is taken by the patient either by an oral, intravenous, subcutaneous, transdermal, etc., route of administration. Next, the drug is released from the dosage form in a predictable and characterizable manner. Then, some fraction of the drug is absorbed from the site of administration into surrounding tissue and into the body (as with oral dosage forms), or both. Finally, the drug reaches the site of action. If the drug concentration at the site of action exceeds the \textit{minimum effective concentration} (MEC), a pharmacologic response results.

Pharmaceutical scientists have evaluated the relative drug availability to the body \textit{in vivo} after giving a drug product to an animal or human comparing specific pharmacologic, clinical, or possible toxic responses. For example, a drug such as isoproterenol causes an increase in heart rate when given intravenously but has no observable effect on the
heart when given orally at the same dose level. In addition, the bioavailability may differ from one drug product to another containing the same drug, even for the same route of administration. In other words, the nature of the drug molecule, the route of delivery, and the formulation of the dosage form can determine whether an administered drug is therapeutically effective, toxic, or has no apparent effect at all.

**Biopharmaceutics** is the science that examines this interrelationship of the physicochemical properties of the drug, the dosage form in which the drug is given, the route of administration and on the rate and extent of systemic drug absorption.

Thus, biopharmaceutics involves factors that influence (1) the stability of the drug within the drug product, (2) the release of the drug from the drug product, (3) the rate of dissolution/release of the drug at the absorption site, and (4) the systemic absorption of the drug. A general scheme describing this dynamic relationship is described in:

![Diagram](image)

**Figure 1-1.**

The study of biopharmaceutics uses both *in-vitro* and *in-vivo* methods. *In-vitro* methods are procedures employing test apparatus and equipment without involving laboratory animals or humans. *In-vivo* methods are more complex studies involving human subjects or laboratory animals. These methods must be able to assess the impact of the physical and chemical
properties of the drug, drug stability, and large-scale production of the drug and drug product on the biologic performance of the drug. Moreover, biopharmaceutics considers the properties of the drug and dosage form in a physiologic environment, the drug's intended therapeutic use, and the route of administration.

**Pharmacokinetics**

After a drug is released from its dosage form, the drug is absorbed into the surrounding tissue, the body, or both. The distribution through and elimination of the drug in the body varies for each patient which is described by pharmacokinetics.

**Pharmacokinetics is defined as the study of time course of drug ADME and their relationship with its therapeutic and toxic effect of the drug.**

*Pharmacokinetics* is the science of the kinetics of drug absorption, distribution, and elimination (ADME). The description of drug distribution and elimination is often termed *drug disposition*. The study of pharmacokinetics involves both experimental and theoretical approaches. The experimental aspect of pharmacokinetics involves the development of biologic sampling techniques, analytical methods for the measurement of drugs and metabolites, and procedures that facilitate data collection and manipulation. The theoretical aspect of pharmacokinetics involves the development of pharmacokinetic models that predict drug disposition after drug administration. Statistical methods are used for pharmacokinetic parameter estimation and data interpretation ultimately for the purpose of designing and predicting optimal dosing regimens for individuals or groups of patients. Statistical methods are applied to pharmacokinetic models to determine data error and structural model deviations. Mathematics and computer techniques form the theoretical basis of many pharmacokinetic methods.

**Clinical Pharmacokinetics**

During the drug development process, large numbers of patients are tested to determine optimum dosing regimens, which are then recommended by the manufacturer to produce the desired pharmacologic response. However, variations will frequently result in either a sub therapeutic or
toxic response which may then adjust to the dosing regimen. *Clinical pharmacokinetics* is the application of pharmacokinetic methods to drug therapy. Clinical pharmacokinetics involves a multidisciplinary approach to individually optimized dosing strategies based on the patient’s disease state and patient-specific considerations.

*The use of the pharmacokinetic principles in optimizing the drug dosage to suit individual patient needs and achieving maximum therapeutic utility is called as clinical pharmacokinetics.*

The study of clinical pharmacokinetics of drugs in disease states requires input from medical and pharmaceutical research. The influence of many diseases on drug disposition is not adequately studied. Age, gender, genetic, and ethnic differences can also result in pharmacokinetic differences that may affect the outcome of drug therapy. The study of pharmacokinetic differences of drugs in various population groups is termed *population pharmacokinetics.*

Pharmacokinetics is also applied to *therapeutic drug monitoring* (TDM) for very potent drugs such as those with a narrow therapeutic range, in order to optimize efficacy and to prevent any adverse toxicity. For these drugs, it is necessary to monitor the patient, either by monitoring plasma drug concentrations (e.g., theophylline) or by monitoring a specific pharmacodynamic endpoint such as prothrombin clotting time (e.g., warfarin). Pharmacokinetic and drug analysis services necessary for safe drug monitoring are generally provided by the *clinical pharmacokinetic service* (CPKS). Some drugs frequently monitored are the aminoglycosides and anticonvulsants. Other drugs closely monitored are those used in cancer chemotherapy, in order to minimize adverse side effects.

**Pharmacodynamics**

*Pharmacodynamics* refers to the relationship between the drug concentration at the site of action (receptor) and pharmacologic response. The interaction of a drug molecule with a receptor causes the initiation of a sequence of molecular events resulting in a pharmacologic or toxic response. Pharmacokinetic-pharmacodynamic models are constructed to relate plasma drug level to drug concentration in the site of action and establish the intensity and time course of the drug.
Plasma Level–Time Curve

The plasma level–time curve is generated by obtaining the drug concentration in plasma samples taken at various time intervals after a drug product is administered. The concentration of drug in each plasma sample is plotted on rectangular-coordinate graph paper against the corresponding time. As the drug reaches the general (systemic) circulation, plasma drug concentrations will rise up to a maximum ($C_{\text{max}}$). Usually, absorption of a drug is more rapid than elimination. As the drug is being absorbed into the systemic circulation, the drug is distributed to all the tissues in the body and is also simultaneously being eliminated. Elimination of a drug can proceed by excretion, biotransformation, or a combination of both.

![Diagram of Plasma Level–Time Curve](image)

The relationship of the drug level–time curve and various pharmacologic parameters for the drug is shown in the figure. MEC and MTC represent the minimum effective concentration and minimum toxic concentration of
drug, respectively. For some drugs, such as those acting on the autonomic nervous system, it is useful to know the concentration of drug that will produce a pharmacologic effect (i.e., MEC). Assuming the drug concentration in the plasma is in equilibrium with the tissues, the MEC reflects the minimum concentration of drug needed at the receptors to produce the desired pharmacologic effect. Similarly, the MTC represents the drug concentration needed to produce a toxic effect. The onset time corresponds to the time required for the drug to reach the MEC. The intensity of the pharmacologic effect is proportional to the number of drug receptors occupied, which is reflected in the observation that higher plasma drug concentrations produce a maximum pharmacologic response. The duration of drug action is the difference between the onset time and the time for the drug to decline back to the MEC.
Introduction

Drug can enter systemic circulation by three major routes:

1. **Enteral route**: includes gastrointestinal, sublingual and rectal route. GI is the most common route for the majority of drugs.

2. **Parental route**: includes all route of administration under or through the skin. No absorption is required if the drug is administered in IV. But if drug is administered in subcutaneous and intramuscular routes absorption is necessary.

3. **Topical route**: includes skin, eye, etc. Absorption is necessary for topical administered drug.

Intravenous or intra-arterial drugs directly enter the systemic circulation and exert its pharmacologic effect quickly. However, the extra vascular drugs can exert their pharmacologic effect after when they come into the blood circulation from the site of administration. For this, absorption is important step. Drug absorption is defined as the process of movement of unchanged drug from the site of administration to the systemic circulation.

The concentration of drug in the site of action is important parameter of bioavailability as well as therapeutic response. It is difficult to measure exact concentration of drug in the site of action due to presystemic metabolism or fast-pass effect. However, the concentration of drug in plasma can measure more accurately. Therefore,
Drug absorption can define as the process of movement of unchanged drug from the site of administration to the site of measurement i.e. plasma.

Absorption of drug

Absorption of drugs occurs through the barrier between site of administration and systemic circulation. The barrier is made up of cell and drug has to pass the cell membrane to absorb. So, it is important to understand the structure and physiology of cell membrane.

The cell membrane consists of phospholipid bilayer. The phospholipids form a thin, flexible sheet, while the proteins "float" in the phospholipid sheet like icebergs, and the carbohydrates extend out from the proteins.
The phospholipids are arranged in a bilayer, with their polar, hydrophilic phosphate heads facing outwards, and their non-polar, hydrophobic fatty acid tails facing each other in the middle of the bilayer. This hydrophobic layer acts as a barrier to all but the smallest molecules, effectively isolating the two sides of the membrane. Different kinds of membranes can contain phospholipids with different fatty acids, affecting the strength and flexibility of the membrane, and animal cell membranes also contain cholesterol linking the fatty acids together and so stabilizing and strengthening the membrane.

The proteins usually span from one side of the phospholipid bilayer to the other (integral proteins), but can also sit on one of the surfaces (peripheral proteins). They can slide around the membrane very quickly and collide with each other, but can never flip from one side to the other. The proteins have hydrophilic amino acids in contact with the water on the outside of membranes, and hydrophobic amino acids in contact with the fatty chains inside the membrane. Proteins comprise about 50% of the mass of membranes, and are responsible for most of the membrane's properties.

- Proteins that span the membrane are usually involved in transporting substances across the membrane.
- Proteins on the inside surface of cell membranes are often attached to the cytoskeleton and are involved in maintaining the cell's shape, or in cell motility. They may also be enzymes, catalyzing reactions in the cytoplasm.
- Proteins on the outside surface of cell membranes can act as receptors by having a specific binding site where hormones or other chemicals can bind. This binding then triggers other events in the cell. They may also be involved in cell signaling and cell recognition, or they may be enzymes, such as maltase in the small intestine.

The carbohydrates are found on the outer surface of all eukaryotic cell membranes, and are attached to the membrane proteins or sometimes to the phospholipids. Proteins with carbohydrates attached are called glycoprotein, while phospholipids with carbohydrates attached are
called glycolipids. The carbohydrates are short polysaccharides composed of a variety of different monosaccharides, and form a cell coat or glycocalyx outside the cell membrane. The glycocalyx is involved in protection and cell recognition, and antigens such as the ABO antigens on blood cells are usually cell-surface glycoproteins. Aqueous filled pores and perforations of 4 to 10Å in diameter are also present in the membrane surface. Inorganic ions and small organic water soluble molecules like urea can pass.

**Mechanism of drug absorption**

Drug can be transport by both carrier mediated and non carrier mediated system. In carrier mediated system drug pass through by the help of carrier. The principle mechanisms for transport of drug through cell membrane are-

1. Passive diffusion
2. Pore transport
3. Facilitated diffusion
4. Active transport
5. Ion-pair transport
6. Vesicular transport

**Passive diffusion**

It is the major process for absorption. 90% of the drug is transported by this process. Most drugs cross biologic membranes by passive diffusion. Diffusion occurs when the drug concentration on one side of the membrane is higher than that on the other side. The process is passive because no external energy is expended. The driving force for passive diffusion is the difference in drug concentrations on either side of the cell membrane. No carrier and energy required for this process.

**Passive diffusion is the movement of the molecules from high concentration to low concentration due to the concentration gradient.**
The rate of transport of drug across the membrane can be described by Fick's first law of diffusion:-

\[
\text{Rate of diffusion} = \frac{dM}{dt} = \frac{D.A.k.(Ch−Cl)}{x}
\]  \hspace{1cm} (2.1)

Fig: Passive Transport with a Concentration Gradient

Where,

\[
\frac{dM}{dt} = \text{Rate of drug diffusion}
\]

\[D = \text{Diffusion coefficient of the drug through membrane}\]

\[A = \text{Surface area of the membrane}\]

\[K = \text{Partition coefficient of drug between lipophilic membrane and GI fluid.}\]

\[Ch − Cl = \text{Difference in the concentration between GI fluid and plasma; concentration gradient}\]

\[x = \text{Thickness of the membrane}\]

The parameters of this equation are:-

**D: diffusion coefficient.** This parameter is related to the size and lipid solubility of the drug and the viscosity of the diffusion medium. As lipid
solubility increases or molecular size decreases then D increases and thus dM/dt also increases.

**A: surface area.** As the surface area increases the rate of diffusion also increase. The surface of the intestinal lining (with villae and microvillae) is much larger than the stomach. This is one reason absorption is generally faster from the intestine compared with absorption from the stomach.

**X: membrane thickness.** The smaller the membrane thickness the quicker the diffusion process. As one example, the membrane in the lung is quite thin thus inhalation absorption can be quite rapid.

**(Ch -Cl): concentration difference.**

The drug concentration in blood or plasma will be quite low compared with the concentration in the GI tract. It is this concentration gradient which allows the rapid complete absorption of many drug substances.

**k: partition coefficient:** Greater the membrane/water partition coefficient of drug, faster the absorption. Lipophilic drugs diffuses faster rate by solubilizing in the lipid layer of the membrane because the membrane is lipophilic in nature.

Passive diffusion is nonsaturable energy independent process but dependent in molecular size to a lesser extent. Drugs having molecular weight 100-400 daltons can effectively absorb passively.

When drug is taken orally, Ch >>Cl and a large concentration gradient is always exists due to the continuous distribution of drug after absorption to the various part of the body known as **sink condition.**

Under usual condition of absorption $\frac{D.A.k}{x}$ is constant and can be replaced by another constant $k_i$. So from equation (2.1) can be simplified to:

$$\frac{dM}{dt} = k_i \cdot \text{Ch} \quad (2.2)$$

This is a first order equation. So, passive diffusion follows the first order process.
**Pore Transport**

It is called as connective transport. A certain type of protein called transport protein may form an open channel across the lipid membrane of the cell. Very small molecules (less than 100 daltons), such as urea, water and sugars are able to rapidly cross the cell membrane through these pores. The driving force for pore transport is hydrostatic pressure.

**Facilitated diffusion**

It is a carrier mediated system but no energy is required. It is much faster process than passive diffusion. Molecules move from higher concentration to lower concentration due to concentration gradient.

*Facilitated diffusion is the process of spontaneous passive transport (as opposed to active transport) of molecules or ions across a biological membrane via specific transmembrane integral proteins.*

![Facilitated diffusion](image)

Fig: Facilitated diffusion

Facilitated diffusion plays a very minor role in absorption. Glucose enters into RBCs by facilitated diffusion. Absorption of B₁, B₂ and B₁₂ take place by facilitated diffusion.

**Active Transport**

Active transport is more important process compared to facilitated diffusion in absorption of nutrients and drugs. In active transport process molecules are transported against concentration gradient i.e. from a region...
of lower concentration to higher concentration with the help of energy and carrier. Thus,

**Active transport is the movement of all types of molecules across a cell membrane against its concentration gradient with the help of energy and carrier.**

![Active transport of a drug](image)

Different ions (Na\(^+\), K\(^+\), Ca\(^{++}\), Fe\(^{++}\)), glucose certain amino acids and vitamins (niacin, pyridoxine and ascorbic acid) are transported actively. Drugs having structurally similar like to those substance are also active transported. Cancer chemotherapy agents (5-flurouracil, 5-bromouracil), Methyldopa, levodopa, enalpril, etc are also actively transported by active transport. Active transport is also important in renal and biliary excretion of many drugs and their metabolites.

**Ion-pair transport**

Strong electrolyte drugs are highly ionized or charged molecules, such as quaternary ammonium compounds and sulfonic acids.

These drugs penetrate membranes poorly. When linked up with an oppositely charged ion, an ion pair is formed in which the overall charge of the pair is neutral. This neutral complex diffuses more easily cross the membrane due to lipophilicity as well as aqueous solubility. Such phenomenon is called **Ion-pair transport.**
**Vesicular transport**

It is a minor mechanism of absorption which involves engulfing particles or dissolved materials by the cell. It includes two type of process:

1. **Pinocytosis**: refers to the engulfment of small molecules or fluid.
2. **Phagocytosis**: refers to the engulfment of larger particles or macromolecules.

Pinocytosis and phagocytosis are forms of vesicular transport that differ by the type of material ingested. During pinocytosis or phagocytosis, the cell membrane invaginates to surround the material, and then engulfs the material into the cell. Subsequently, the cell membrane containing the material forms a vesicle or vacuole within the cell. Vesicular transport is the proposed process for the absorption of Vitamin A, D, E, and K and drug such as insulin.

![Figure: Vesicular transport](image)

**Factors affecting drug absorption and bioavailability**

Bioavailability depends on the absorption of drug. Lots of factors affect the absorption of drug. Factors include:

1. Physiological factors.
2. Physical-chemical factors.
3. Dosages form factors.
Physiological factors affecting oral absorption

The following physiologic factors affect the absorption of the drugs those will be discussed in the subsequent discussion.

1. Membrane physiology.
2. Passage of drugs across membranes.
3. Age
4. Gastrointestinal physiology.
   a) Characteristics of GIT physiology and drug absorption
   b) Disease state
   c) Presystemic metabolism/First-pass effects
   d) Gastric emptying time, Intestinal Transit and motility
   e) Effect of food on drug absorption

Membrane physiology

The cell membrane is the barrier that separates the inside of the cell from the outside. The cell membrane is made up of phospholipids, proteins, and other macromolecules. The phospholipids make up a bilayer. It contains hydrophilic and hydrophobic molecules. The proteins in the cell membrane are located within the phospholipid bilayer. So, the biologic membrane is mainly lipid in nature but contains small aqueous channels or pores.

The permeability of a drug at the absorption site into the systemic circulation is intimately related to the molecular structure of the drug and to the physical and biochemical properties of the cell membranes. Once in the plasma, the drug may have to cross biological membranes to reach the site of action. Therefore, biological membranes potentially pose a significant barrier to drug delivery. As the cell membrane is lipophilic in nature the lipophilic drugs are easily absorbed.
Passage of drugs across membranes

There are several ways by which drug molecules pass the biologic membrane such as Passive diffusion, pore transport, facilitated diffusion, active transport, ion-pain transport and vesicular transport. Among them drugs are easily absorbed by passive diffusion as the lipophilic nature of the cell membrane. 90% of the drugs molecules are absorbed by passive diffusion. Other absorption process also occurs depending on the nature of the drug molecule but in lesser extent.

Drugs may be absorbed by passive diffusion from all parts of the alimentary canal including sublingual, buccal, GI, and rectal absorption. For most drugs, the optimum site for drug absorption after oral administration is the upper portion of the small intestine or duodenum region. The unique anatomy of the duodenum provides an immense surface area for the drug to diffuse passively (). The large surface area of the duodenum is due to the presence of valve like folds in the mucous membrane on which are small projections known as villi. These villi contain even smaller projections known as microvilli, forming a brush border. In addition, the duodenal region is highly perfused with a network of capillaries, which helps to maintain a concentration gradient from the intestinal lumen and plasma circulation.

Age

Infant’s shows altered absorption pattern compared to adult due to high gastric pH, low surface area of GIT and low blood flow to the GIT. In elderly, altered person gastric emptying, bacterial overgrowth in small intestine causes altered absorption of drug.

Gastrointestinal (GI) Physiology

The normal physiologic processes of the alimentary canal may be affected by diet, contents of the gastrointestinal (GI) tract, hormones, the visceral nervous system, disease, and drugs. Thus, drugs given by the enteral route for systemic absorption may be affected by the anatomy, physiologic functions, and contents of the alimentary tract. Moreover, the physical,
chemical, and pharmacologic properties of the drug itself will also affect its own absorption from the alimentary canal.

The *enteral system* consists of the alimentary canal from the mouth to the anus. The major physiologic processes that occur in the GI system are secretion, digestion, and absorption. Secretion includes the transport of fluid, electrolytes, peptides, and proteins into the lumen of the alimentary canal. Enzymes in saliva and pancreatic secretions are also involved in the digestion of carbohydrates and proteins. Other secretions, such as mucus, protect the linings of the lumen of the GI tract. Digestion is the breakdown of food constituents into smaller structures in preparation for absorption. Food constituents are mostly absorbed in the proximal area (duodenum) of the small intestine. The process of absorption is the entry of constituents from the lumen of the gut into the body. Absorption may be considered as the net result of both lumen-to-blood and blood-to-lumen transport movements.

Drugs administered orally pass through various parts of the enteral canal, including the oral cavity, esophagus, and various parts of the gastrointestinal tract. Residues eventually exit the body through the anus. The total transit time including gastric emptying, small intestinal transit, and colonic transit, ranges from 0.4 to 5 days. The most important site for drug absorption is the small intestine. Small intestine transit time (SITT) ranges from 3 to 4 hours for most healthy subjects. If absorption is not completed by the time a drug leaves the small intestine, absorption may be erratic or incomplete. The small intestine is normally filled with digestive juices and liquids, keeping the lumen contents fluid. In contrast, the fluid in the colon is reabsorbed, and the luminal content in the colon is either semisolid or solid, making further drug dissolution erratic and difficult. The lack of the solubilizing effect of the chyme and digestive fluid contributes to a less favorable environment for drug absorption.

Oral Cavity

Saliva is the main secretion of the oral cavity, and it has a pH of about 7. Saliva contains ptyalin (salivary amylase), which digests starches. Mucin, a
glycoprotein that lubricates food, is also secreted and may interact with drugs. About 1500 ml of saliva is secreted per day.

**Esophagus**

The esophagus connects the pharynx and the cardiac orifice of the stomach. The pH of the fluids in the esophagus is between 5 and 6. The lower part of the esophagus ends with the esophageal sphincter, which prevents acid reflux from the stomach. Tablets or capsules may lodge in this area, causing local irritation. Very little drug dissolution occurs in the esophagus.

**Stomach**

The stomach is innervated by the vagus nerve. However, local nerve plexus, hormones, mechanoreceptors sensitive to the stretch of the GI wall, and
chemoreceptors control the regulation of gastric secretions, including acid and stomach emptying. The fasting pH of the stomach is about 2 to 6. In the presence of food, the stomach pH is about 1.5 to 2, due to hydrochloric acid secreted by parietal cells. Stomach acid secretion is stimulated by gastrin and histamine. Gastrin is released from G cells, mainly in the antral mucosa and also in the duodenum. Gastrin release is regulated by stomach distention (swelling) and the presence of peptides and amino acids. A substance called intrinsic factor for vitamin B-12 absorption and various gastric enzymes, such as pepsin, which initiates protein digestion, are secreted into the gastric lumen to initiate digestion.

Basic drugs are solubilized rapidly in the presence of stomach acid. Mixing is intense and pressurized in the antral part of the stomach, a process of breaking down large food particles described as *antral milling*. Food and liquid are emptied by opening the pyloric sphincter into the duodenum. Stomach emptying is influenced by the food content and osmolality. Fatty acids and mono- and diglycerides delay gastric emptying. High-density foods generally are emptied from the stomach more slowly. The relation of gastric emptying time to drug absorption is discussed more fully in the next section.

**Duodenum**

A common duct from the pancreas and the gallbladder enters into the duodenum. The duodenal pH is about 6 to 6.5, because of the presence of bicarbonate that neutralizes the acidic chyme emptied from the stomach. The pH is optimum for enzymatic digestion of protein and peptide food. Pancreatic juice containing enzymes is secreted into the duodenum from the bile duct. Trypsin, chymotrypsin, and carboxypeptidase are involved in the hydrolysis of proteins into amino acids. Amylase is involved in the digestion of carbohydrates. Pancreatic lipase secretion hydrolyzes fats into fatty acid. The complex fluid medium in the duodenum helps to dissolve many drugs with limited aqueous solubility.

The duodenum is a site where many ester prodrugs are hydrolyzed during absorption. The presence of proteolytic enzymes also makes many protein drugs unstable in the duodenum, preventing adequate absorption.
Jejunum

The jejunum is the middle portion of the small intestine, between the duodenum and the ileum. Digestion of protein and carbohydrates continues after addition of pancreatic juice and bile in the duodenum. This portion of the small intestine generally has fewer contractions than the duodenum and is preferred for in-vivo drug absorption studies.

Ileum

The ileum is the terminal part of the small intestine. This site has fewer contractions than the duodenum and may be blocked off by catheters with an inflatable balloon and perfused for drug absorption studies. The pH is about 7, with the distal part as high as 8. Due to the presence of bicarbonate secretion, acid drugs will dissolve. Bile secretion helps to dissolve fats and hydrophobic drugs. The ileocecal valve separates the small intestine from the colon.

Colon

The colon lacks villi and has limited drug absorption also, because of the more viscous and semisolid nature of the lumen contents. The colon is lined with mucin that functions as lubricant and protectant. The pH in this region is 5.5 to 7. A few drugs, such as theophylline and metoprolol, are absorbed in this region. Drugs that are absorbed well in this region are good candidates for an oral sustained-release dosage form. The colon contains both aerobic and anaerobic microorganisms that may metabolize some drugs. For example, L-dopa and lactulose are metabolized by enteric bacteria. Crohn's disease affects the colon and thickens the bowel wall. The microflora also becomes more anaerobic. Absorption of clindamycin and propranolol are increased, whereas other drugs have reduced absorption with this disease.

Rectum

The rectum is about 15 cm long, ending at the anus. In the absence of fecal material, the rectum has a small amount of fluid (approximately 2 ml) with a pH about 7. The rectum is perfused by the superior, middle, and inferior
hemorrhoidal veins. The inferior hemorrhoidal vein (closest to the anal sphincter) and the middle hemorrhoidal vein feed into the vena cava and back to the heart. The superior hemorrhoidal vein joins the mesenteric circulation, which feeds into the hepatic portal vein and then to the liver.

Drug absorption after rectal administration may be variable, depending on the placement of the suppository or drug solution within the rectum. A portion of the drug dose may be absorbed via the lower hemorrhoidal veins, from which the drug feeds directly into the systemic circulation; some drugs may be absorbed via the superior hemorrhoidal vein, which feeds into the mesenteric veins to the hepatic portal vein to the liver, and be metabolized before systemic absorption.

**Gastrointestinal pH**

Gastrointestinal pH influences drug absorption. The GI pH generally increases gradually from the stomach to the colon and rectum. Disintegration, dissolution and stability of drug affect by acidic and basic environment. Some drugs are acid sensitive and degrade in the in stomach which affects the absorption of drug. A large number of drugs are either weak acids or weak bases whose solubility is greatly affected by pH. A pH that favors formation of salt of the drug enhances the dissolution of that drug. Since drug dissolution is one of the important rate-determining steps in drug absorption.

Table: pH in different region of GIT.

<table>
<thead>
<tr>
<th>Organs</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buccal</td>
<td>6</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>5-6</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.7-3.5</td>
</tr>
<tr>
<td>Duodenum</td>
<td>5-7</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>6-7.5</td>
</tr>
</tbody>
</table>
Weakly acidic drugs dissolve rapidly in the alkaline pH of the intestine whereas basic drugs dissolve in the acidic pH of the stomach. Since the primary site for absorption of most drugs is small intestine, the poorly water-soluble basic drugs must first dissolve in the acidic pH of stomach before moving into the intestine.

Depending upon the, drug pKa and pH of the region influences drug absorption by determining the amount of drug that would exist in the unionized form at the site of absorption. This topic has already been dealt with in sufficient details under pH-partition theory.

**Disease States**

Local diseases can cause alterations in gastric pH that can affect the stability, dissolution and absorption of the drug. Several disease states can influence the rate and extent of drug absorption. The 3 major classes of disease states that can influence the bioavailability of a drug are:

1. Gastrointestinal diseases,
2. Cardiovascular diseases, and
3. Hepatic diseases.

1. **Gastrointestinal diseases:** The influence of achlorhydria (decreased gastric acid secretion and increased stomach pH) on gastric emptying and absorption, of acidic drugs (e.g. aspirin) has been studied. Crohn’s disease and celiac disease (characterized by destruction of villi Crohn’s disease microvilli) are two of the intestinal disorders related with malabsorption syndrome that influence drug absorption. Steatorrhea (impared secretion of bile) and reduced enterohepatic cycling of bile salts can significantly
impair drug absorption. Malabsorption is also induced by alcohol and GI infections like shigellosis, gastroenteritis, cholera and food poisoning. Colonic diseases such as colitis, amebiasis and constipation can also alter drug absorption. Gastrectomy can result in drug dumping in the intestine, osmotic diarrhea and reduced intestinal transit time.

2. Cardiovascular diseases: Several changes associated with congestive cardiac failure influence bioavailability of a drug viz, edema of the intestine, decreased blood flow to the GIT and gastric emptying rate and altered GI pH, secretions mid microbial flora.

3. Hepatic diseases: Disorders such as hepatic cirrhosis influence bioavailability mainly of drugs that undergo considerable first-pass hepatic metabolism e.g. propranolol.

Presystemic metabolism/First-pass effects

For a drug administered orally, the main reasons for its decreased bioavailability first-pass/ presystemic metabolism.

Before a drug reaches blood circulation, it has to pass through eliminating organs namely the GIT and the liver. The loss of drug through biotransformation by such eliminating organs during its passage to systemic circulation is called as first-pass or presystemic metabolism. The enzymes, responsible for presystemic metabolism are

1. Luminal enzymes,
3. Bacterial enzymes
4. Hepatic enzymes
1. Luminal enzymes: These are the enzymes present in the gut fluids and include enzymes from intestinal and pancreatic secretions. The primary enzyme found in gastric juice is pepsin. Lipases, amylases and proteases are secreted from the pancreas into the small intestine. Pepsins and proteases are responsible for the digestion of protein and peptide drugs in the lumen. The intestinal and pancreatic secretions contain hydrolases enzymes which hydrolize ester drugs like chloramphenicol palmitate into active chloramphenicol and peptidases which split amide linkages and inactive protein polypeptide drugs.

2. Gut wall enzymes: Gut wall enzymes are present in stomach, intestine and colon. Alcohol dehydrogenase (ADH) is an enzyme of stomach mucosa that inactivates ethanol. Intestinal mucosa contains both phase I and phase II (predominant) enzymes, e.g. sulfation of ethinyl estradiol and isoprenaline.

3. Bacterial enzymes: The GI microflora is in colon. The colonic microbes generally render a drug more active or toxic on biotransformation—for example sulfosalazine, a drug used in ulcerative colitis, are hydrolyzed to sulfapyridine and 5-amino salicylic acid by the microbial enzymes of the
An important role of intestinal microflora is that in enterohepatic cycle. Their enzymes hydrolyze the conjugates of drugs actively secreted via bile such as glucuronides of digoxin and oral contraceptives. The free drugs are reabsorbed into the systemic circulation.

4. Hepatic enzymes: Several drugs; isoprenaline, propranolol, alprenolol, ptcloxifylline, nitroglycerine, diltiazem, lidocaine, morphine, etc undergo first-pass hepatic metabolism.

**Gastric emptying**

The passage of the ingested material from stomach to the small intestine is called gastric emptying. Gastric emptying rate is the speed at which the stomach components empty into the intestine. The time requires for the gastric component empty into the intestine is called gastric emptying time.

It is the rate limiting step in drug absorption. The major site of absorption is intestine. So, in case of slow gastric emptying, drug stay in the stomach maximum time and drug absorption is less and less bioavailability of the drug. In general, rapid gastric emptying increases the bioavailability of drug. Rapid gastric emptying causes rapid onset of action, more dissolution of drug in intestine and also enhances the stability of drug in gastric fluid (e.g.: penicillin) and increases the absorption of drug in the distal part of the small intestine.

A large number of factors also influence gastric emptying.

1. Volume of meal: An initial rapid rate of emptying observed with a large meal volume and an initial lag phase in emptying of a small volume meal. However, larger the bulk meal, longer the gastric emptying time. So the bioavailability decreases in administration of drug with larger volume of food.

2. Composition of meal: The rate of gastric emptying occurs for various ingested food follow the order: carbohydrates > proteins > fats.
Delayed gastric emptying observed with fatty meal, is beneficial for the absorption of poorly soluble drug griseofulvin. Fat promotes secretion of bile which has inhibitory effect on gastric emptying.

Fig: Dependence of peak acetaminophen plasma concentration as a function of stomach emptying half-life

3. Viscosity and physical state of the meal: Solid meal has high gastric emptying time whereas liquid has less gastric emptying time. High viscous material has low gastric emptying rate compared to less viscous materials.

4. Temperature of the meal: If the temperature of the ingested food is higher or lower than the body temperature then the gastric emptying time increases.

5. Gastrointestinal pH: Gastric emptying become slower in case of low stomach pH and promoted at a higher pH.

6. Electrolytes and osmotic pressure: Water, isotonic solutions and low concentrated solutions empty the stomach rapidly whereas high electrolyte concentration slows the gastric emptying.

7. Body posture: Gastric emptying is rapid while standing and lying on the right side and slow while lying on the left side.

8. Emotional state: Stressful emotional states increase stomach contraction and gastric emptying rate. Depression reduces stomach contraction and gastric emptying.
9. Disease state: Disease like gastroenteritis, gastric ulcer, pyloric stenosis, diabetes and hyperthyroidism retard gastric emptying. Gastrectomy, duodenal ulcer and hyperthyroidism promote gastric emptying rate.

10. Drugs: poorly soluble antacids (Al(OH)$_3$), anticholinergics (atropine, propantheline), narcotic analgesics (morphine) and tricyclic antidepressants (imipramine, amitriptyline) decreases gastric emptying. Dompiridone and some antiemetics stimulate gastric emptying.

**Intestinal Transit**

The most important site for drug absorption is the small intestine. Small intestine transit time (SITT) ranges from 3 to 4 hours for most healthy subjects. If absorption is not completed by the time a drug leaves the small intestine, absorption may be erratic or incomplete. The small intestine is normally filled with digestive juices and liquids, keeping the lumen contents fluid. In contrast, the fluid in the colon is reabsorbed, and the luminal content in the colon is either semisolid or solid, making further drug dissolution erratic and difficult. The lack of the solubilizing effect of the chyme and digestive fluid contributes to a less favorable environment for drug absorption. Transit time of different regions of intestine is given below:

<table>
<thead>
<tr>
<th>Intestinal region</th>
<th>Transit Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Jejunum</td>
<td>2 hours</td>
</tr>
<tr>
<td>Ileum</td>
<td>3 to 6 hours</td>
</tr>
<tr>
<td>Cecum</td>
<td>0.5 to 1 hours</td>
</tr>
<tr>
<td>Colon</td>
<td>6 to 12 hours</td>
</tr>
</tbody>
</table>

Table: Transit time for different regions of intestine.

Like gastric emptying, intestinal transit is influenced by several factors like food, drugs and diseases. Food decreases digestive secretions and pregnancy slow intestinal transit whereas diarrhea promotes it.
Metoclopramide promotes intestinal transit enhance absorption of rapidly soluble drugs. Laxatives also promote the rate of intestinal transit. Anticholinergics slow intestinal transit, promotes absorption of poorly soluble drugs.

**Effect of food on drug absorption**

The presence of food in the GIT can influence the rate and extent of absorption, either directly or indirectly via a range of mechanisms.

1. *Complexation of drugs with components in the diet*: Tetracycline forms non-absorbable complexes with calcium and iron, and thus it is advised that patients do not take products containing calcium or iron, such as milk, iron preparations or indigestion remedies, at the same time of day as the tetracycline.
2. *Alteration of pH*: Food tends to increase stomach pH by acting as a buffer. This liable to decrease the rate of dissolution and absorption of a weakly basic drug and increase that of a weakly acidic one.
3. Alteration of gastric emptying: Fats and some drugs tend to reduce gastric emptying and thus delay the onset of action of certain drugs.

4. Stimulation of gastrointestinal secretions: Gastrointestinal secretions (e.g. pepsin) produced in response to food may result in the degradation of drugs that are susceptible to enzymatic metabolism, and hence a reduction in their bioavailability. Fats stimulate the secretion of bile. Bile salts are surface active agents which increase the dissolution of poorly soluble drugs (griseofulvin). Bile salts can form insoluble and non-absorbable complexes with some drugs, such as neomycin and kanamycin.

5. Competition between food components and drugs for specialized absorption mechanisms: There is a possibility of competitive inhibition of drug absorption in case of drugs that have a chemical structure similar to nutrients required by the body for which specialized absorption mechanisms exist.

<table>
<thead>
<tr>
<th>Delayed</th>
<th>Decreased</th>
<th>Increased</th>
<th>Unaffected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspirin</td>
<td>Penicillins</td>
<td>Griseofulvin</td>
<td>Methyldopa</td>
</tr>
<tr>
<td>Paraetamol</td>
<td>Erythromycin</td>
<td>Diazepam</td>
<td>Propylthiouracil</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Ethanol</td>
<td>Water soluble vitamins</td>
<td>sulfasomidine</td>
</tr>
<tr>
<td>Digoxin</td>
<td>Tetracyclins</td>
<td>levodopa</td>
<td>Iron</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table: influence food on drug absorption.

6. Increased viscosity of gastrointestinal contents: The presence of food in the GIT provides a viscous environment which may result in reduction in the rate of drug dissolution and reduction in the rate of diffusion of drug in solution from the lumen to the absorbing membrane lining the GIT. Hence, there is reduction in drug bioavailability.

7. Food-induced changes in presystemic metabolism: Certain foods may increase the bioavailability of drugs that are susceptible to presystemic intestinal metabolism by interacting with the metabolic process. E.g. Grapefruit juice is capable of inhibiting the intestinal cytochrome P450 (CYP3A) and thus taken with drugs that are susceptible to CYP3A metabolism which result in increase of their bioavailability.
Fig: Effect of fasting *versus* feeding on Propranolol Concentrations

8. *Food-induced changes in blood flow*: Food serves to increase the bioavailability of some drugs (e.g. propranolol) that are susceptible to first-pass metabolism. Blood flow to the GIT and liver increases after a meal. The faster the rate of drug presentation to the liver; the larger the fraction of drug that escapes first-pass metabolism. This is because the enzyme systems become saturated.

**Physico-Chemical Factors Affecting Oral Absorption**

Physical-chemical factors affecting oral absorption include:

a) pH-partition theory
b) Lipid solubility of drugs
c) Dissolution and pH
d) Drug stability and hydrolysis in GIT
e) Complexation
f) Adsorption

**a) pH-partition theory/hypothesis**

Brodie et al. (in 1957) proposed the pH - partition theory to explain the influence of GI pH and drug pKa on the extent of drug transfer or drug absorption. Brodie reasoned that when a drug is ionized it will not be able
to get through the lipid membrane, but only when it is unionized and therefore has higher lipid solubility.

pH-partition hypothesis explains the process of drug absorption from different region of the GIT and the distribution across all over the membrane. According to the pH-partition hypothesis, the gastrointestinal epithelia acts as a lipid barrier towards drugs which are absorbed by passive diffusion, and those that are lipid soluble will pass across the barrier.

According to this theory for *drug compounds of molecular weight greater than 100 and those which are primarily transported across the membrane by passive diffusion, the process of absorption is governed by three important factors like:

1. The dissociation constant i.e. pKa of the drug.
2. The lipid solubility of the unionized drug i.e. partition coefficient (Ko/w).
3. pH at the absorption site.

![Diagram showing transfer across membrane.](image)

As most drugs are weak electrolytes, the unionized form of weakly acidic or basic drugs (the lipid-soluble form) will pass across the gastrointestinal epithelia, whereas the gastrointestinal epithelia is impermeable to the
ionized (poorly-lipid soluble) form of such drugs. Consequently, the absorption of a weak electrolyte will be determined by the extent to which the drug exists in its unionized form at the site of absorption.

The above statement of the hypothesis was based on the assumptions that:

1. The GIT is a simple, lipophilic barrier to the transport of drug.
2. Larger the fraction of unionized drug, faster the absorption.
3. Greater the lipophilicity (Ko/w) of the unionized drug, better the absorption.

The amount of drug that exists in unionized form is a function of dissociation constant (pKa) of the drug and pH of the fluid at the absorption site. The relative amount of ionized and unionized drug in a solution at a particular pH and the percent of drug ionized at this pH is given by Henderson-Hasselbach Equations.

For weak acidic drugs:

\[ HA \rightleftharpoons H^+ + A^- \]

\[ K_a = \frac{a_{H^+} \cdot a_{A^-}}{a_{HA}} \approx \frac{[H^+] \cdot [A^-]}{[HA]} \]

\[ -\log K_a = -\log[H^+] - \log\frac{[A^-]}{[HA]} \]

\[ pK_a - pH = \log\frac{[U]}{[I]} = \log\frac{[HA]}{[A^-]} \]

\[ \frac{[U]}{[I]} = 10^{pKa-pH} \]
For weak basic drugs:

\[ pK_a - pH = \log \left( \frac{[I]}{[U]} \right) = \log \left( \frac{[HB^+]}{[B]} \right) \]

\[ \frac{[U]}{[I]} = 10^{pH-pK_a} \]

From the above equation we see that, when the concentration of ionized and unionized drug becomes equal then pH = pKa (since log I = zero). The pKa is a characteristic of the drug. The pKa value for either the acidic or the basic drug indicates the amount of undissociated drug present available for absorption at the absorption site. The lower the pKa value of the acidic drug, stronger is the acid i.e. greater is the proportion of ionized form at a particular pH. Similarly, higher the pKa value of a basic drug, the stronger is the base.

If we consider the pH range in the GIT from 1 to 8, that of the stomach from 1 to 3 and of the intestine (from duodenum to colon) 5 to 8 then certain generalizations regarding ionization and absorption of drugs can be made by the pH-partition hypothesis:

For acidic drugs:

1. Very weak acids (PKa > 8) such as phenytoin, ethosuximide and several barbiturates are essentially unionized at all pH values and therefore their absorption is rapid and independent of GI pH.

2. Acids in the PKa range 2.5 to 7.5 are greatly affected by changes in pH and therefore their absorption is pH-dependent; e.g. several NSAIDs like aspirin, ibuprofen, phenylbutazone, and a number of penicillin analogs. Such drugs are better absorbed from acidic pH (pH < pKa) where they largely exist in unionized form.
3. Stronger acids with pKa < 2.5 such as cromolyn sodium are ionized in the entire pH range of GIT and therefore remain poorly absorbed.

For basic drugs:

1. Very weak bases (pKa < 5.0) such as caffeine, theophylline and a number of benzodiazepines like diazepam, oxazepam and nitrazepam are essentially unionized at all pH values and therefore their absorption is rapid and pH-independent.

2. Bases in the PKa range 5 to 11.0 arc greatly affected by changes in pH and hence their absorption is pH-dependent; e.g. several morphine analogs, chloroquine, imipramine and amitriptyline. Such drugs are better absorbed from the relatively alkaline pH of the intestine where they largely exist in unionized form.

3. Stronger bases with pKa > 11.0 like mecamylamine and guanethidine are ionized in the entire pH range of GIT and therefore poorly absorbed.

\[
\frac{[U]}{[I]} = 10^{pK_a-pH} = 10^{5.4-3.4} = 10^2 = 100
\]

\[
\frac{[U]}{[I]} = 10^{pK_a-pH} = 10^{5.4-7.4} = 10^{-2} = 0.01
\]

Table: Different drugs and their site of absorption

<table>
<thead>
<tr>
<th>Drugs</th>
<th>pKa</th>
<th>Site of absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very weak acids (pKa &gt; 8.0)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentobarbital</td>
<td>8.1</td>
<td>Unionized at all pH values:</td>
</tr>
<tr>
<td>Hexobarbital</td>
<td>8.2</td>
<td>Absorbed along the entire length of GIT</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>8.3</td>
<td></td>
</tr>
<tr>
<td>Ethosuximide</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td><strong>Moderately weak acids (pKa 2.5 to 7.5)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug</td>
<td>pKa</td>
<td>Description</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>2.7</td>
<td>Unionized in gastric pH and ionized in intestinal pH; Better absorbed from stomach</td>
</tr>
<tr>
<td>Aspirin</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Phenylbutazone</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td><strong>Stronger acids (pKa &lt; 2.5)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disodium cromoglycaye</td>
<td>2.0</td>
<td>Ionized at all pH values: Poorly absorbed from GIT.</td>
</tr>
<tr>
<td><strong>Very weak bases (pKa &lt; 5.0)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thcophyline</td>
<td>0.7</td>
<td>Unionized at all pH values:</td>
</tr>
<tr>
<td>Caffeine</td>
<td>0.8</td>
<td>Absorbed along the entire length of GIT</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Diazepam</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td><strong>Moderately weak bases (pKa 5 to 11.0)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reserpine</td>
<td>6.6</td>
<td>Ionized at gastric pH, relatively unionized at intestinal pH:</td>
</tr>
<tr>
<td>Heroin</td>
<td>7.8</td>
<td>Better absorbed from intestine.</td>
</tr>
<tr>
<td>Codeine</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td><strong>Stronger Bases (pKa &gt; 11)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>11.2</td>
<td>Ionized at all pH values: poorly absorbed from GIT.</td>
</tr>
<tr>
<td>Guanethidined</td>
<td>11.7</td>
<td></td>
</tr>
</tbody>
</table>

Limitations of pH-Partition theory

The pH-partition hypothesis over-simplified though drug absorption is a complicated process. So the theory has its own limitations. Some of the deviations are:

1. Presence of virtual membrane pH

2. Absorption of ionized drug
3. Influence of GI surface area and residence time of drug

4. Presence of aqueous unstimmed diffusion layer

1. **Presence of Virtual Membrane pH:** The pH-partition hypothesis suggested that only the unionized drug at a given GI lumen pH is absorbed. An S-shaped curve, called as the pH-absorption curve denoting the dissociation of drug, is obtained when pH is plotted versus rate of drug absorption.

![pH absorption curve for acidic and basic drugs](image)

Fig: pH absorption curve for acidic and basic drugs

However, differences in the extent of absorption of salicylic acid have been observed at a given GI pH than that predicted by pH-partition theory. The experimental pH partition curves are less steep and shift to the left for a basic drug and to the right for an acidic drug. This led to the suggestion that a virtual pH, also called as the microclimate pH, different from the luminal pH exists at the membrane surface. This virtual membrane pH actually determines the extent of drug ionization and thus, drug absorption.

2. **Absorption of Ionized Drugs:** An important assumption of the theory was that only unionized form of the drug is absorbed and
permeation of the ionized drug is negligible since its rate of absorption is 3 to 4 times less than that of unionized drug. This is true to a large extent as ionized drugs have low lipid solubility and relatively poor permeability. However, the pH-absorption curve shift suggested that ionized forms of ionic drugs also get absorbed to a considerable extent. If such drugs have a large lipophilic group in their structure, despite their ionization, they will be absorbed passively—for example, morphine derivatives. Other mechanisms are also involved in the absorption of ionized drugs such as active transport, ion-pair transport and convective flow.

3. Influence of GI Surface Area and Residence Time of Drug

According to the pH-partition theory, acidic drugs are best absorbed from stomach (acidic pH) and basic drugs from intestine (alkaline pH) in which conditions they are unionized to a large extent. This could be true under conditions where the surface area of stomach and intestine are same. It could also mean that once an acidic drug reaches the intestine, the remaining fraction will be poorly absorbed and that unless a basic drug reaches the intestine and gets absorbed considerably, it may not be able to attain its therapeutic level. But, irrespective of the GI pH and the degree of ionization, both acidic and basic drugs are more rapidly absorbed from the intestine, primarily because of its large surface area and secondly, because of long residence time of the drug in the intestine.

4. Presence of Aqueous Unstirred Diffusion Layer: The pH-shift in the absorption of acidic and basic drugs, as discussed earlier, also accounts for the fact that the bulk of the luminal fluid is not in direct contact with the membrane but a barrier called as aqueous unstirred diffusion layer is interposed between them.
Such a layer has a real thickness and is a barrier to absorption of drugs. In the original pH-partition theory, the rate-limiting step in the absorption of drugs was the partitioning in the lipid barrier. With the incorporation of unstirred aqueous diffusion layer, a drug must diffuse first through this aqueous barrier and then through the lipid barrier.

Thus, drugs having large partition coefficient can rapidly penetrate the lipid membrane but diffusion through unstirred water layer is the rate-limiting step in their absorption. This applies in particular to high molecular weight fatty acids and bile acids.

Despite its limitations, the pH-partition theory is still useful in the basic understanding of drug absorption and movement of drug between various body compartments.

**b) Lipid solubility of drugs**

Some drugs are poorly absorbed after oral administration even though they are non-ionized in small intestine. Low lipid solubility of them may be the reason. The best parameter to correlate between water and lipid solubility is **partition coefficient**.

\[
\text{Partition coefficient (p)} = \frac{[L]}{[W]}
\]

Where, \([L] = \text{concentration of the drug in lipid phase.}\)

\([W] = \text{concentration of the drug in aqueous phase.}\)

The higher p value, the more absorption is observed.
c) Drug Dissolution

Many drugs are given in solid dosage forms and therefore must dissolve before absorption can take place. Except controlled released formulations disintegration and disaggregation occurs rapidly.

Dissolution  Absorption
Solid  Solution  Blood

**Fig:** Dissolution and Absorption

If absorption is slow relative to dissolution then all we are concerned with the absorption of drugs. However, if dissolution is the slow, rate determining step (the step controlling the overall rate) then factors affecting dissolution will control the overall process. This is a more common problem with drugs which have a low solubility (below 1 g/100 ml) or which are given at a high dose, e.g. griseofulvin.

There are a number of factors which affect drug dissolution. One model that is commonly used is to consider this process to be diffusion controlled through a stagnant layer surrounding each solid particle.

**Fig:** Diagram Representing Diffusion through the Stagnant Layer

A physical model is shown in Figure.

First we need to consider that each particle of drug formulation is surrounded by a stagnant layer of solution.
After an initial period we will have a steady state where drug is steadily dissolved at the solid-liquid interface and diffuses through the stagnant layer. If diffusion is the rate determining step we can use Fick's first law of diffusion to describe the overall process.

![Diagram of Concentration Gradient]

**Fig: Plot of Concentration Gradient**

If we could measure drug concentration at various distances from the surface of the solid we would see that a concentration gradient is developed. If we assume steady state we can used Fick's first law to describe drug dissolution.

**Fick's first law**

By Fick's first law of diffusion:

\[
\text{Rate of Solution} = \frac{D \cdot A \cdot (Cs - Cb)}{h}
\]

Equation: Fick's first Law of Diffusion applied to Dissolution

Where, \( D \) is the diffusion coefficient, \( A \) the surface area, \( Cs \) the solubility of the drug, \( Cb \) the concentration of drug in the bulk solution, and \( h \) the thickness of the stagnant layer. If \( Cb \) is much smaller than \( Cs \) then we have so-called "Sink Conditions" and the equation reduces to-
Each term in this equation contributes to the dissolution process.

**Surface area, A**

The surface area per gram (or per dose) of a solid drug can be changed by altering the particle size. For example, a cube 3 cm on each side has a surface area of 54 cm². If this cube is broken into cubes with sides of 1 cm, the total surface area is 162 cm². Actually if we break up the particles by grinding we will have irregular shapes and even larger surface areas. Generally as increases the dissolution rate will also increase. Improved bioavailability has been observed with griseofulvin, digoxin, etc.

\[
\text{Rate of Solution} = \frac{D \cdot A \cdot Cs}{h}
\]

**Equation: Fick's First Law - Sink Conditions**

Fig: Reducing Particle Size Increases Surface Area
Methods of particle size reduction include mortar and pestle, mechanical grinders, fluid energy mills, solid dispersions in readily soluble materials (PEG's).

**Diffusion layer thickness, h**

This thickness is determined by the agitation in the bulk solution. *In vivo* we usually have very little control over this parameter. It is important though when we perform *in vitro* dissolution studies because we have to control the agitation rate so that we get similar results *in vitro* as we would *in vivo*.

![Diagram of concentration versus distance for dissolution into a reactive medium](image)

The apparent thickness of the stagnant layer can be reduced when the drug dissolves into a reactive medium. For example, with a weakly basic drug in an acidic medium, the drug will react (ionize) with the diffusing proton (H\(^+\)) and this will result in an effective decrease in the thickness of the stagnant layer.

The effective thickness is now h′ not h. Also the bulk concentration of the drug is effectively zero. For this reason weak bases will dissolve more quickly in the stomach.

**Diffusion coefficient, D**

The value of D depends on the size of the molecule and the viscosity of the dissolution medium. Increasing the viscosity will decrease the diffusion
coefficient and thus the dissolution rate. This could be used to produce a sustained release effect by including a larger proportion of something like sucrose or acacia in a tablet formulation.

**Drug solubility, Cs**

Solubility is another determinant of dissolution rate. If Cs increases dissolution rate also increases.

**Salt form**

Dissolution profile of different salts of penicillin is presented in the figure.

![Dissolution profiles for three salts and acid penicillin V, pH 4](image)

**Fig:** Dissolution profiles for three salts and acid penicillin V, pH 4

Salts of weak acids and weak bases generally have much higher aqueous solubility than the free acid or base, therefore if the drug can be given as a salt the solubility can be increased and we should have improved dissolution. One example is Penicillin V.

The pH of the diffusion layer would be increased if the chemical nature of the weakly acidic drug was changed from that of the free acid to a basic salt (the sodium or potassium form of the free acid. The pH of the diffusion layer would be higher (5-6) than the low bulk pH (1-3.5) of the gastric fluids because of the neutralizing action of the strong (Na⁺, K⁺) ions present in the diffusion layer.
The drug particles will dissolve at a faster rate and diffuse out of the diffusion layer into the bulk of the gastric fluid, where a lower bulk pH.

Thus the free acid form of the drug in solution, will precipitate out, leaving a saturated solution of free acid in gastric fluid. This precipitated free acid will be in the form of very fine, non-ionized, wetted particles which have a very large surface area in contact with gastric fluids, facilitating rapid redissolution when additional gastric fluid is available.
**Crystal form**

1. **Polymorphism**

Some drugs exist in a number of crystal forms or polymorphs. These different forms may well have different solubility properties and thus different dissolution characteristics. Chloramphenicol palmitate is one example which exists in at least two polymorphs. The B form is apparently more bioavailable.

The recommendation might be that manufacturers should use polymorph B for maximum solubility and absorption. However, a method of controlling and determining crystal form would be necessary in the quality control process. Shelf-life could be a problem as the more soluble (less stable) form may transform into the less soluble form. In time the suspension may be much less soluble with variable absorption.

2. **Amorphous solid**

The amorphous form dissolves more rapidly than the corresponding crystalline form. The more soluble and rapidly dissolving amorphous form of novobiocin antibiotic was readily absorbed following oral administration.
of an aqueous suspension to humans. However, the less soluble and slower-dissolving crystalline form of novobiocin was not absorbed (therapeutically ineffective). The amorphous form of novobiocin slowly converts to the more stable crystalline form, with loss of therapeutic effectiveness. The order for dissolution of different solid forms of the drugs is: amorphous > metastable > stable.

3. Solvates

*Solvates*: If the drug is able to associate with solvent molecules to produce crystalline forms known as solvates. *Hydrates*: drug associates with water molecules. The greater the solvation of the crystal, the lower is the solubility and dissolution rate in a solvent identical to the solvation molecules. Hydrates are most common solvate forms of drugs.

![Fig: Solvates](image)

Generally, the anhydrous form of a drug has greater aqueous solubility than the hydrates. This is because the hydrates are already in interaction with water and therefore have less energy for crystal break-up in comparison to the anhydrates (thermodynamically higher energy state) for further interaction with water. The anhydrous form of theophylline and ampicillin have higher aqueous solubility, dissolve at a faster rate and show better bioavailability in comparison to their monohydrate and trihydrate forms respectively.
On the other hand, the organic (nonaqueous) solvates have greater aqueous solubility than the nonsolvates—for example, the n-pentanol solvate of fludrocortisone and succinylsulfathiazole and the chloroform solvate of griseofulvin are more water-soluble than their nonsolvated forms.

d) Drug stability and hydrolysis in GIT

A drug for oral use may destabilize either during its shelf-life or in the GIT. Two major stability problems resulting in poor bioavailability of an orally administered drug are—degradation of the drug into inactive, form, and interaction with one or more different component(s) either of the dosage form or those present in the GIT to form a complex that is poorly soluble or is unabsorbable. Destabilization of a drug during its shelf-life and in the GIT will be discussed in detail under formulation factors and patient related factors respectively. Drugs that are susceptible to acidic or enzymatic hydrolysis in the GIT, suffer from reduced bioavailability.

e) Complexation

Complexation of a drug may occur within the dosage form and/or in the gastrointestinal fluids, and can be beneficial or detrimental to absorption.

1- Intestinal mucosa (mucin) + Streptomycin = poorly absorbed complex

2- Calcium + Tetracycline = poorly absorbed complex (Food-drug interaction)

3- Carboxyl methylcellulose (CMC) + Amphetamine = poorly absorbed complex (tablet additive – drug interaction)

4- Lipid soluble drug + water soluble complexing agent = well-absorbed water soluble complex (cyclodextrin)
f) **Adsorption**

Certain insoluble substances may adsorb co-administrated drugs leading to poor absorption.

1. Charcoal (antidote in drug intoxication).
2. Kaolin (antidiarrhoeal mixtures)
3. Talc (in tablets as glidant)

**DOSAGES FORM FACTORS AFFECTING DRUG ABSORPTION**

Formulation factors affecting oral absorption include:

a) Disintegration Time  
b) Manufacturing/Processing Variables  
c) Pharmaceutical Ingredients/Excipients (Formulation factors)  
d) Drug stability and hydrolysis in GIT  
e) Complexation  
f) Adsorption

**a) Disintegration Time**

Disintegration time (DT) is of particular importance in case of solid dosage forms like tablets and capsules. In vitro disintegration test is by no means a guarantee of drug’s bioavailability because if the disintegrated drug particles do not dissolve, absorption is not possible. However, if a solid dosage form does not conform to the DT, it portends bioavailability problems because the subsequent process of dissolution will be much slower and absorption may be insufficient. Coated tablets, especially sugar coated ones have long DT. Rapid disintegration is thus important in the therapeutic success of a solid dosage form. DT of a tablet is directly related
to the amount of binder present and the compression force (hardness) of a tablet. A harder tablet with large amount of binder has a long DT. Disintegration can be aided by incorporating disintegrants in suitable amounts during formulation.

After disintegration of a solid dosage form into granules, the granules must disaggregate into fine particles as dissolution from such tiny particles is faster than that from granules.

**Manufacturing/Processing Variables**

Drug dissolution is the single most important factor in the absorption of drugs, especially from the solid dosage forms, tablets and capsules. The dosage form related factors that influence dissolution and hence absorption of a drug from such formulations are:

1. Excipients

The influence of excipients such as binders, lubricants, disintegrants, etc. on drug dissolution will be discussed in the subsequent section of this chapter.

Several manufacturing processes influence drug dissolution from solid dosage forms. Processes of such importance in the manufacture of tablets are:

1. Method of granulation, and
2. Compression force.

The processing factor of importance in the manufacture of capsules that can influence its dissolution is the intensity of packing of capsule contents.
**Method of Granulation:** The wet granulation process is the most conventional technique in the manufacture of tablets and was once thought to yield tablets that dissolve faster than those made by other granulation methods. The limitations of this method include—(i) formation of crystal bridge by the presence of liquid  (ii) the liquid may act as a medium for affecting chemical reactions such as hydrolysis and (iii) the drying step may harm the thermolabile drugs. The method also involves a large number of steps each of which can influence drug dissolution—method and duration of blending, method, time and temperature of drying, etc. The method of direct compression has been utilized to yield tablets that dissolve at a faster rate. One of the more recent methods that has resulted in superior product is agglomerative phase of comminution (APOC). The process involves grinding of drugs in a ball mill for time long enough to affect spontaneous agglomeration. The tablets so produced were stronger and showed rapid rate of dissolution in comparison to tablets made by other methods. The reason attributed to it was an increase in the internal surface area of the granules prepared by APOC method.

**Compression Force:** The compression force employed in tableting process influence density, porosity, hardness, disintegration time and dissolution of tablets. On the one hand, higher compression force increases the density and hardness of tablet, decreases porosity and hence penetrability of the solvent into the tablet. In many cases, promotes higher bonding between the particles which result in slowing of the dissolution rate. On the other hand, higher compression forces cause deformation, crushing or fracture of drug particles into smaller ones or convert a spherical granule into a disc shaped particle with a large increase in the effective surface area. These results in an increase in the dissolution rate of
the tablet. In short, the influence of compression force on dissolution rate is difficult to predict.

**Intensity of Packing of Capsule Contents:** Like the congressional force for tablets, packing density in case of capsule dosage form can either inhibit or promote dissolution. Diffusion of GI fluids into the tightly filled capsules creates a high pressure within the capsule resulting in rapid bursting and dissolution of contents. Opposite is also possible. It has been shown that capsules with finer particles and intense packing have poor drug release and dissolution rate due to a decrease in pore size of the compact and poor penetrability by the GI fluids.

**Pharmaceutical Ingredients/Excipients (Formulation factors)**

A drug is rarely administered in its original form. Almost always, a convenient dosage form to be administered by a suitable route is prepared. Such a formulation contains a number of excipients (non-drug components of a formulation). Excipients are added to ensure acceptability, physicochemical stability during the shelf-life, uniformity of composition and dosage, and optimum bioavailability and function ability of the drug product. Despite their inertness and utility in the dosage form, excipients can influence absorption of drugs. The more the number of excipients in a dosage form, the more complex it is and greater the potential for absorption and bioavailability problems. Commonly used excipients in various dosage forms are vehicles, diluents (fillers), binders and granulating agents, disintegrants lubricants, coatings, suspending agents, emulsifiers surfactants, buffers, complexing agents, colorants, sweeteners, crystal growth inhibitors, etc.

**Vehicle:** Vehicle or solvent system is the major component of liquid orals and parenterals. The 3 categories of vehicles in use are—aqueous vehicles
(water, syrup, etc.), nonaqueous water miscible vehicles (propylene glycol, glycerol, sorbitol) and nonaqueous water immiscible vehicles (vegetable oils). Bioavailability of a drug from vehicles depends to a large extent on its miscibility with biological fluids. Aqueous and water miscible vehicles are miscible with the body fluids and drugs from them are rapidly absorbed. Quite often, a drug is more soluble in water miscible vehicles like propylene glycol (serving as a co-solvent) and show better bioavailability. Sometimes dilution of such vehicles with the body fluids results in precipitation of drug as fine particles which, dissolve rapidly. Solubilizers such as tween 80 are sometimes used to promote solubility of a drug in aqueous vehicles. In case of water immiscible vehicles, the rate of drug absorption depends upon its partitioning from the oil phase to the aqueous body fluids, which could be a rate-limiting step. Viscosity of the vehicles is another factor in the absorption of drugs. Diffusion into the hulk of GI fluids and thus absorption of a drug from a viscous vehicle may be slower.

Diluents (Fillers): Diluents are commonly added to tablet (and capsule) formulations if the required dose is inadequate to produce the necessary bulk. A diluent may be organic or inorganic. Among organic diluents, carbohydrates are very widely used—for example, starch, lactose, microcrystalline cellulose, etc. These hydrophilic powders are very useful in promoting the dissolution of poorly water-soluble, hydrophobic drugs like spironolactone and triarnterene by forming a coat onto the hydrophobic surface of drug particles and rendering them hydrophilic. Among the inorganic diluents, dicalciurn phosphate (DCP) is most common. One classic example of drug-diluent interaction resulting in poor bioavailability is that of tetracycline and DCP. The cause is formation of divalent calcium-tetracycline complex which is poorly soluble and thus, unabsorbable.
**Binders and Granulating Agents:** These materials are used to hold powders together to form granules or promote cohesive compacts for directly compressible materials and to ensure that the tablet remain-intact after compression. Popular binders include polymeric materials (natural, semisynthetic and synthetic) like starch, cellulose derivatives, acacia, PVP, etc. Others include gelatin and sugar solution. In general, like- fillers, the hydrophilic (aqueous) binders show better dissolution profile with poorly wettable drugs like phenacetin by imparting hydrophilic properties to the granule surface. However, the proportion of strong binders in the tablet formulation is very critical. Large amounts of such binders increase hardness and decrease disintegration/dissolution rates of tablets. PEG 6000 was found to be a deleterious binder for phenobarbital as it forms a poorly soluble complex with the drug. Non-aqueous binders like ethyl cellulose also retard drug dissolution.

**Disintegrants:** These agents overcome the cohesive strength of tablet and break them up on contact with water which is an important prerequisite to tablet dissolution. Almost all the disintegrants are hydrophilic in nature. A decrease in the amount of disintegrant can significantly lower bioavailability. Adsorbing disintegrants like bentonite and veegum should be avoided with low dose drugs like digoxin, alkaloids and steroids since a large amount of dose is permanently adsorbed and only a fraction is available for absorption. Microcrystalline cellulose is a very good disintegrant (and a binder too) but at high compression forces, it may retard drug dissolution.

**Lubricants/Antifrictional Agents:** These agents are added to tablet formulations to aid flow of granules, to reduce interparticle friction and sticking or adhesion of particles to dies and punches. The commonly used lubricants are hydrophobic in nature (several metallic stearates and waxes)
and known to inhibit wettability, penetration of water into tablet and their disintegration and dissolution. This is because the disintegrants gets coated with the lubricant if blended simultaneously which can be prevented by adding the lubricant in the final stage. The best alternative is use of soluble lubricants like SLS and carbowaxes which promote drug dissolution.

**Coatings:** In general, the deleterious effect of various coatings on drug dissolution from a tablet dosage form is in the following order: enteric coat > sugar coat > nonenteric film coat. The dissolution profile of certain coating materials changes on aging; e.g. shellac coated tablets, on prolonged storage, dissolve more slowly in the intestine. This can, however, be prevented by incorporating little PVP in the coating formulation.

**Suspending Agents/Viscosity Impartes:** Popular suspending agents are hydrophilic polymers like vegetable gums (acacia, tragacanth, etc.), semisynthetic gums (CMC, MC) and synthetic gums which primarily stabilize the solid drug particles by reducing their rate of settling through an increase in the viscosity of the medium. These agents and some sugars are also used as viscosity impartes to affect palatability and pourability of solution dosage forms. Such agents can influence drug absorption in several ways. The macromolecular gums often form unabsorbable complexes with drugs—for example, sodium CMC forms a poorly soluble complex with amphetamine. An increase in viscosity by these agents acts as a mechanical barrier to the diffusion of drug from the dosage form into the bulk of GI fluids and from GI fluids to the mucosal lining by forming a viscid layer on the GI mucosa. They also retard the GI transit of drugs.

**Surfactants:** Surfactants are widely used in formulations as wetting agents, solubilizers, emulsifiers, etc. Their influence on drug absorption is very complex. They may enhance or retard drug absorption either by
interacting with the drug or the membrane or both. Mechanisms involved in the increased absorption of drug by use of surfactants include:

I. Promotion of wetting (through increase in effective surface area) and dissolution of drugs e.g. tween 80 with phenacetin

2. Better membrane contact of the drug for absorption

3. Enhanced membrane permeability of the drug.

The beneficial effects of surfactants have been observed at pre-critical micelle concentration levels. However, physiologic surfactants like the bile salts (anionic) and lysolecithin (nonionic) promote absorption of hydrophobic drugs like steroids, oil soluble vitamins and griseofulvin by their micellar solubilizing property.

Decreased absorption of drug in the presence of surfactants has been suggested to be due to:

1. Formation of unabsorbable drug-micellic complex at surfactant concentrations above critical micelle concentration

2. Laxative action induced by a large surfactant concentration

**Buffers:** Buffers are sometimes useful in creating the right atmosphere for drug dissolution as was observed for buffered aspirin tablets. However, certain buffer systems containing potassium cations inhibit the drug absorption as seen with vitamin B2 and sulfanilamide. The reason attributed to ii was the uptake of fluids by the intestinal epithelial cells due to which the effective drug concentration in the tissue is reduced and the absorption rate is decreased. Such an inhibitory effect of the various buffer cations on the drug transfer rate is in the following order: K⁺ > NH₄⁺ > Li⁺ >
Na\(^+\) > TRIS\(^+\). Hence, the buffer system for a salt of a drug should contain the same cation as the drug salt and introduce no additional cations.

**Complexing Agents:** Complex formation has been used to alter the physicochemical and biopharmaceutical properties of a drug. A complexed drug may have altered stability, solubility, molecular size, partition coefficient and diffusion coefficient. Basically, such complexes are pharmacologically inert and must dissociate either at the absorption site or following absorption into the systemic circulation. Several examples where complexation has been used to enhance drug bioavailability are:

1. Enhanced dissolution through formation of a soluble complex e.g. ergotamine tartarate-caffeine complex and hydroquinone-digoxin complex,

2. Enhanced lipophilicity for better membrane permeability e.g. caffeine-PABA complex, and

3. Enhanced membrane permeability e.g. enhanced GI absorption of heparin (normally not absorbed from the GIT) in presence of EDTA which chelates calcium and magnesium ions of the membrane.

Complexation can be deleterious to drug absorption due to formation of poorly soluble or poorly absorbable complex e.g. complexation of tetracycline with divalent and trivalent cations like calcium (milk, antacids), iron, magnesium (antacids) and aluminum (antacids). Reasons for poor bioavailability of some complexes are failure to dissociate at the absorption site and large, molecular size of the complex that cannot diffuse through the cell membrane—for example, drug-protein complex.
**Colorants:** Even a very low concentration of water-soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs. The dye molecules get adsorbed onto the crystal faces and inhibit drug dissolution—for example, brilliant blue retards dissolution of sulfathiazole. Dyes have also been found to inhibit micellar solubilization effect of bile acids which may impair the absorption of hydrophobic drugs like steroids. Cationic dyes are more reactive than the anionic ones due to their greater power for adsorption on primary particles.

**Crystal Growth Inhibitors:** In addition to maintaining the initial physical properties of a drug in suspension, crystal growth inhibitors like PVP and PEG inhibit conversion of a high energy metastable polymorph into stable, less soluble polymorph.

**Nature and Type of Dosage Form**

Apart from the proper selection of drug, clinical success often depends to a great extent on the proper selection of dosage form of that drug. For a given drug, a 2 to 5 fold or perhaps more difference could be observed in the oral bioavailability of a drug depending upon the nature and type of dosage form. Such a difference is due to the relative rate at which particular dosage form releases the drug to the biological fluids and the membrane. The relative rate at which a drug from a dosage form is presented to the body depends upon the complexity of dosage form. The more complex a dosage form, greater the number of rate-limiting steps and greater the potential for bioavailability problems.

The bioavailability of a drug from various dosage forms decreases in the following order: Solutions > Emulsions> Suspensions> Capsules > Tablets > Coated tablets > Enteric coated tablets > Sustained Release Products. Thus, absorption of a drug from solution is fastest with least potential for
bioavailability problems whereas absorption from a sustained release product is slowest with greatest bioavailability risk.

Several factors, especially the excipients which influence bioavailability of a drug from its dosage form, have been discussed earlier. Now we will discuss about the how absorption varies in case of different type of dosages forms.

**Solutions:** A drug in a solution (syrups, elixirs, etc.) is most rapidly absorbed since drug dissolution is absent. Factors that influence bioavailability of a drug from solution dosage form include—the nature of solvent (aqueous, water miscible, etc.), viscosity, surfactants, solubilizers, stabilizers, etc.

**Emulsions:** Emulsion dosage forms have been found to be superior to suspensions in administering poorly aqueous soluble lipophilic drugs. It was observed with indoxole (an NSAID) that when it is dissolved in a vegetable oil and emulsified in water, absorption increases 3 fold over its aqueous suspension. Emulsion dosage forms present a large surface area of oil to the GIT for absorption of a drug. Scientists have claimed that a drug administered in oily vehicle, can direct the distribution of drug directly into the lymphatic system thereby avoiding the hepatic portal vein and first-pass metabolism.

**Suspensions:** The major rate-limiting step in the absorption of a drug from suspension dosage form is drug dissolution which is generally rapid due to the large surface area of the particles. Important factors in the bioavailability of a drug from suspensions include particle size, polymorphism, wetting agents, viscosity of the medium, suspending agents, etc.

**Powders:** Though powders are superior to tablets and capsules, they are not in use nowadays due to handling and palatability problems. Major
factors to be considered in the absorption of a drug from powders are particle size, polymorphism, wettability, etc.

**Capsules:** Factors of importance in case of hard gelatin capsules include drug particle size, density, polymorphism, intensity of packing and influence of diluents and excipients. Hydrophilic diluents like lactose improve wettability, disaggregation and dispersion of poorly aqueous soluble drugs whereas inhibitory effect is observed with hydrophobic lubricants like magnesium stearate. A hydrophobic drug with a fine particle size in capsule results in a decrease in porosity of powder bed and thus, decreased penetrability by the solvent with the result that clumping of particle occurs. This can overcome by incorporating a large amount of hydrophilic diluent (up to 50%), a small amount of wetting agent cum lubricant such as SLS (up to 1%) and/or by wet granulation to convert an impermeable powder bed the one having good permeability. Other factors of importance include possible interaction between the drug and the diluent and between drug and gelatin shell.

Soft elastic capsules as such dissolve faster than hard gelatin capsules and tablets. They show better drug availability from oily solutions, emulsions or suspensions of medicaments (especially hydrophobic drugs). One of the best examples of this is the faster dissolution of indoxole (equivalent to that of an emulsion dosage form) when formulated as softgel in comparison to hard gelatin capsule and aqueous suspension. Such poorly soluble drugs can be dissolved in PEG or other suitable solvent with the aid of surfactants and encapsulated without difficulty. Softgels are thus of particular use where the drug dose is low, drug is lipophilic or when oily or lipid based medicaments are to be administered. A problem softgels is the high water content of the shell (above 20%). This moisture migrates into the shell
content and crystallization of drug occurs during the drying stage resulting in altered drug dissolution characteristics.

**Tablets:** Compressed tablets are the most widely used convenience and cost effective dosage form. The bioavailability problems with tablets arise from the reduction in the effective surface area due to granulation and subsequent compression into a dosage form. Since dissolution is most rapid from primary drug particles due to their large surface area, disintegration of a tablet into granules and subsequent disaggregation of granules into fine particles is very important. A number of formulation and processing factors influencing these steps and also the physicochemical properties of drug substance that influence bioavailability.

![Diagram](image)

Fig: sequence in absorption of drug from tablet dosage form.

**Coated Tablets:** The coating acts as yet another barrier which must first dissolve to release the drug molecule. Of the two types of coatings, the film coat, which is thin, dissolves rapidly and does not significantly affect drug
absorption. The sugar coat which though soluble but tough and takes longer to dissolve. The scaling coat which is generally of shellac is most deleterious. Press coated tablets may be superior to sugar coated tablets in such cases.

**Enteric Coated Tablets:** Enteric coated tablets have great potential in creating bioavailability problems because the coat dissolves only in the alkaline pH of the intestine. It may take as long as 2 to 4 hours for such a tablet to empty from the stomach into the intestine depending upon the meals and the GI motility. Hence, the pharmacologic response may eventually be delayed by as much as 6 to 8 hours. The problem of gastric emptying can overcome by enteric coating the granules or pellets and presenting them in a capsule or compressing into a tablet. The thickness of enteric coat is yet another determinant factor in drug dissolution, increasing thickness being more problematic. Aging of the dosage form also affects drug release, especially with shellac. In one of the studies, shellac coated tablets of salicylic acid stored for 2 years showed a 60% decrease in the peak plasma level.

**Sustained Release Products:** Drug release from such products is most unpredictable, the problems ranging from dose dumping to unsatisfactory or no drug release at all. However, with the development of newer innovations and technologies, it is becoming increasingly reliable and the results reproducible with little intersubject variations.

**Product Age and Storage Conditions**

A number of changes, especially in the physicochemical properties of a drug in dosage form, can result due to aging and alterations in storage conditions which can adversely affect bioavailability. With solution dosage form, precipitation of drug due to altered solubility, especially due to conversion
of metastable into poorly soluble, stable polymorph can occur during the shelf-life of the product. Changes in particle size distribution have been observed with a number of suspension dosage forms resulting in decreased rate of drug dissolution and absorption. In case of solid dosage forms, especially tablets, disintegration and dissolution rates are greatly affected due to aging and storage conditions. An increase in these parameters of tablets has been attributed to excipients that harden on storage (e.g. PVP, acacia, etc.) while the decrease is mainly due to softening/crumbling of the binder during storage (e.g. CMC).

Changes that occur during the shelf-life of a dosage form are affected mainly by large variations in temperature arid humidity. In one of the studies conducted on prednisone tablets containing lactose as the tiller, high temperature and high humidity resulted in harder tablets that disintegrated and dissolved slowly.

**ABSORPTION OF DRUGS FROM EXTRA VASCULAR ROUTES**

Drug absorption from all nonoral extravascular sites is governed by the same factors that influence absorption from GIT viz, the physicochemical properties of drug, formulation factors, and anatomic, physiologic and pathologic characteristics of the patient. This is so because the barrier to transport of drugs into the systemic circulation from all such is a lipoidal membrane similar to the GI barrier and the major mechanism is the absorption is passive diffusion. One of the major advantages of administering drugs by noninvasive transmucosal routes h as nasal, buccal, rectal, etc. is that greater systemic availability is attainable for drugs normally subjected to extensive presystemic elimination due to GI
degradation and/or hepatic metabolism. Moreover peptide and protein drugs can also be delivered by such routes.

**Buccal/Sublingual Administration**

The two sites for oral mucosal delivery of drugs are:

1. **Sublingual route**: The drug is placed under the tongue and allowed to dissolve

2. **Buccal route**: The medicament is placed between the cheek and the gum

The barrier to drug absorption from these routes is the epithelium of oral mucosa. Passive diffusion is the major mechanism for absorption of most drugs: nutrients may be absorbed by carrier-mediated processes. Some of the advantages of these routes are:

1. Rapid absorption and higher blood levels due to high vascularization of the region and therefore particularly useful for administration of antianginal drugs

2. No first-pass hepatic metabolism

3. No degradation of drugs such as that encountered in the GIT

4. Presence of saliva facilitates both drug dissolution and its subsequent permeation by keeping the oral mucosa moist

Notable factors to be considered in the oral mucosal delivery of drugs

1. Lipophilicity of drug: Slightly higher lipid solubility than that required for GI absorption is necessary for passive permeation.
2. Salivary secretion: In addition to high lipid solubility, the drug should be soluble in aqueous buccal fluids i.e. biphasic solubility of drug is necessary for absorption: absorption is delayed if the mouth is dry.

3. pH of the saliva: Usually around 6. the buccal pH favors absorption of drugs which remain unionized.

4. Binding to oral mucosa: Systemic availability of drugs that bind to oral mucosa is poor.

5. Storage compartment: For some drugs such as buprenorphine, a storage compartment in the buccal mucosa appears to exist which is responsible for the slow absorption of drugs.

6. Thickness of oral epithelium: Sublingual absorption is faster than buccal since the epithelium of former region is thinner and immersed in a larger volume of saliva.

Factors that limit drug administration by these routes are: limited mucosal surface area, concern for taste of the medicament and discomfort.

Examples of drugs administered by oral mucosal route include antianginals like nitrites and nitrates, antihypertensives like nifedipine, analgesics like morphine and bronchodilators like fenoterol. Certain steroids like estradiol and peptides like oxytocin can also be administered. Apart from tablets, the drugs may be administered as a buccal spray especially to children.

**Rectal Administration**

Despite its diminished popularity, the rectal route of drug administration is still an important route for children and old patients. The drugs may be administered as solutions (microenemas) or suppositories. Absorption is
more rapid from solutions than from suppositories but is more variable in comparison to oral route. Irritating suppository bases such as PEG promotes defecation and drug loss. Presence of fecal matter retards drug absorption. Though highly vascularized, absorption is slower because of limited surface area. The pH of rectal fluids (around 8) also influences drug absorption according to pH partition hypothesis. Absorption of drugs from the lower half of rectum bypasses presystemic hepatic metabolism, Drugs administered by this route include aspirin, paracetamol, theophylline, few barbiturates, etc.

**Topical Administration**

Excluding the respiratory tract’s contact with the inhaled air, the skin is virtually the sole human surface directly interfacing the body with the external environment. It is the largest organ of the body weighing approximately 2 Kg and 2 meter square in area and receives about 1/3rd of total blood circulating through the body. Though tolerant to many chemicals, topically contacted xenobiotics can evoke both local and systemic effects. When topically applied drugs are meant to exert their effects systemically, the mode of administration is called as percutaneous or transdermal delivery.

Anatomically, the skin is made of 3 distinct layers—the epidermis, the dermis and the subcutaneous fat tissue. Epidermis is the nonvascular, multilayered outer region of the skin. The dermis or true skin is a highly vascular region: drugs permeating to this region are taken up into the systemic circulation and sink conditions are maintained.

The principal barrier to the entry of xenobiotics is the most superficial layer of epidermis called as stratum corneum. It is composed of dead, keratinized, metabolically inactive cells that act as the major rate-limiting
barrier to passive diffusion of drugs. In order to act either locally or systemically, a topically applied drug may diffuse through the skin by hair follicles, sweat glands or sebaceous glands but permeation through the multiple lipid bilayers of stratum corneum is the dominant pathway though the rate is very slow. Several factors influence passive percutaneous absorption of drugs:

1. Thickness of stratum corneum: absorption is very slow from regions such as foot and palm where the skin has thickened stratum corneum.

2. Presence of hair follicles: absorption is rapid from regions where numerous hair follicles exist e.g. scalp.

3. Trauma—cuts, rashes, inflammation, mild burns or other conditions in which the stratum corneum is destroyed, promote drug absorption.

4. Hydration of skin: soaking the skin in water or occluding it by using emollients, plastic film or dressing, promote hydration of skin and drug absorption.

5. Environment humidity and temperature: higher humidity and temperature increase both the rate of hydration as well as local blood flow and hence drug absorption.

6. Age: gross histological changes take place as the skin ages. Aged skin is more prone to allergic and irritant effects of topically contacted chemicals as a result of hardening of blood vessels. Infants absorb drug through skin as efficiently as adults. Their ratio of surface area to body weight is 3 times that of adults; hence, systemic toxicity of topically applied drugs is of particular concern in infants.
7. Grooming: the frequency and vigor with which one bathes and the type of soap that is used also contribute to variability in drug absorption.

8. Expose to chemicals: occupational exposure to solvents can accelerate shedding of epidermal cells and enhance drug absorption.

9. Vehicle or base: the vehicle in which the drug is incorporated influences drug absorption: the one in which the drug is dissolved rather than dispersed promotes absorption.

10. Permeation enhancer: incorporation of certain chemicals such as DMSO, propylene glycol, azone, etc. in the topical formulations aid drug penetration.

11. Chronic use of certain drugs: long term use of cortisol or keratolytics like salicylic acid results in enhanced drug penetration.

Drugs that are administered percutaneously include nitroglycerine, lidocaine, betamethasone, estradiol, testosterone, etc. The route is particularly useful for drugs with low oral availability and short duration of action; the effect of the latter category of drugs is prolonged because percutaneous absorption is a slow process.

**Intramuscular Administration**

Absorption of drugs from intramuscular sites is relatively rapid but much slower in comparison to IV injections. Factors that determine the rate of drug absorption from intramuscular sites are:

1. Vascularity of the injection site: the decreasing order of blood flow rate to muscular tissues in which drugs are usually injected is: arm > thigh > buttocks. Since blood flow rate is often the rate-limiting step
in absorption of drugs from IM sites, most rapid absorption is from
deltoid muscles and slowest from gluteal region. The absorption rate
decreases in circulatory disorders such as hypotension.

2. Lipid solubility and ionization of drug: highly lipophilic drugs are
absorbed rapidly by passive diffusion whereas hydrophilic and ionized
drugs are slowly absorbed through capillary pores.

3. Molecular size of the drug: small molecules and ions gain direct access
into capillaries through pores whereas macromolecules are taken up by
the lymphatic system.

4. Volume of injection and drug concentration: a drug in concentrated
injection and large volume is absorbed faster than when given in dilute
form and small volume.

5. pH composition and viscosity of injection vehicle: a solution of drug
in acidic or alkaline pH (e.g. phenytoin, pH 12) or in a nonaqueous
solvent such as propylene glycol or alcohol (e.g. digoxin) when injected
intramuscularly result in precipitation of drug at the injection site
followed by slow and prolonged absorption. Viscous vehicles such as
vegetable oils also slow drug absorption. The principle can however be
utilized to control rate of drug delivery.

**Subcutaneous Administration**

All factors that influence IM drug absorption are also applicable to
absorption from subcutaneous site. Generally, absorption of drugs from a
subcutaneous site is slower than that from intramuscular sites due to poor
perfusion, but this fact is of particular importance for the administration of
drugs for which a rapid response is not desired and for drugs that degrade
when taken orally e.g. insulin and sodium heparin. The rate of absorption of a drug from subcutaneous site can be increased in 2 ways:

I. Enhancing blood flow to the injection site: by massage, application of heat, co-administration of vasodilators locally, or by exercise, and

2 Increasing the drug-tissue contact area: by co-administering the enzyme hyaluronidase that breaks down the connective tissue and permits spreading of drug solution over a wide area.

Absorption can be slowed down by causing vasoconstriction through local cooling or co-injection of a vasoconstrictor like adrenaline or by immobilization of limb. Because of relatively slow drug absorption from subcutaneous tissues, the region is very popular for controlled release medication like implants.

**Pulmonary Administration**

In principle, all drugs intended for systemic effects can be administered by inhalation since the large surface area of the alveoli, high permeability of the alveolar epithelium and rich perfusion permit extremely rapid absorption just like exchange of gases between the blood and the inspired air. However, the route has been limited for administering drugs that affect pulmonary system such as bronchodilators (salbutamol), anti-inflammatory steroids (beclomethasone) and antiallergics (cromolyn). Lipid soluble drugs are rapidly absorbed by passive diffusion and polar drugs by pore transport. Absorption of drugs whose ionization is pH sensitive is dependent upon pH of pulmonary fluids. The drugs are generally administered by inhalation either as gases (volatile/gaseous anesthetics) or aerosol. In the latter case, drug delivery to lungs is largely dependent upon the particle size of the aerosolized droplets—particles larger than 10 microns impact on the mouth, throat or upper respiratory
tract mucosa and do not reach the pulmonary tree whereas very small particles (0.6 microns) from which drug absorption is rapid, penetrate rapidly but are susceptible to easy exhalation. Sometimes, the patient’s inability to inhale a sufficient amount of drug limits drug delivery to lungs.

**Intranasal Administration**

The nasal route is becoming increasingly popular for systemic delivery especially of some peptide and protein drugs. Drug absorption from nasal mucosa is as rapid as observed after parenteral administration because of its rich vasculature and high permeability. The route is otherwise used for drugs to treat local symptoms like nasal congestion, rhinitis, etc.

Two mechanisms for drug transport across the nasal mucosa have been suggested—a faster rate that is dependent upon drug lipophilicity, and a slower rate which is dependent upon drug molecular weight. In case of lipophilic drugs, rapid absorption by diffusion is observed upto 400 daltons and satisfactory absorption upto 1000 daltons. By use of permeability enhancers such as surfactants, even a drug with molecular weight of 6000 daltons shows reasonable bioavailability. For polar compounds absorbed by pore transport, an upper threshold of 200 daltons is the limiting factor. Other factors that may influence nasal permeation of drugs include p11 of nasal secretions (5.5 to 6.5) and its viscosity, and pathological condition such as common cold and rhinitis. Drugs known to influence cleansing function of nasal cilia should not be administered by this route.

**Intraocular Administration**

Topical application of drugs is mainly for local effects such as mydriasis, miosis, anesthesia or treatment of infections, glaucoma, etc. Sterile aqueous solutions of drugs are widely used ophthalmic formulations and administered in the conjunctival cul-de-sac. The barrier to intraocular
penetration of drugs is the cornea which possesses both hydrophilic and lipophilic characteristics. Thus, for optimum intraocular permeation, drugs should possess biphasic solubility. The pH of lacrimal fluid influences absorption of weak electrolytes such as pilocarpine. On the other hand, pH of the formulation influences lacrimal output—higher pH decreases tear flow and promotes drug absorption whereas lower pH solutions increase lacrimation and subsequent drug loss due to drainage. Rate of blinking also influences drainage loss. The volume of fluid instilled into the eyes also affects bioavailability and effectiveness of the drug. Normally, the human eye can hold around 10 mL of fluid; hence, instillation of small volume of drug solution in concentrated form increases its effectiveness than when administered in large volume in dilute form. Viscosity impartsers in the formulation increase bioavailability by prolonging drug’s contact time with the eye. Oily solutions, ointments and gels show sustained drug action for the same reason. Sometimes systemic absorption of a drug with low therapeutic index such as timolol may precipitate undesirable toxic effects. Systemic entry of drugs occur by way of absorption into lacrimal duct which drains lacrimal fluid into the nasal cavity and finally into the GIT. This can be prevented by simple eyelid closure or nasolacrimal occlusion by pressing the finger tip to the inside corner of the eye after drug instillation.

**Vaginal Administration**

Drugs meant for intravaginal application are generally intended to act locally in the treatment of bacterial or fungal infections or prevent conception. The route is now used for systemic delivery of contraceptives and other steroids, without the disadvantage of first-pass metabolism. Controlled delivery and termination of drug action when desired, is possible with this route. Factors that may influence drug absorption from intravaginal site include pH of lumen fluids (4 to 5), vaginal secretions and
the microorganisms present in the vaginal lumen which may metabolize the drug.
INTRODUCTION

After a drug is absorbed into plasma, the drug molecules are distributed throughout the body by the systemic circulation. The drug molecules are carried by the blood to the target site (receptor) for drug action and to other (nonreceptor) tissues as well, where side effects or adverse reactions may occur. Drug molecules are distributed to eliminating organs, such as the liver and kidney, and to noneliminating tissues, such as the brain, skin, and muscle. In pregnancy, drugs cross the placenta and may affect the developing fetus. Drugs can also be secreted in milk via the mammary glands. A substantial portion of the drug may be bound to proteins in the plasma and/or tissues. Lipophilic drugs deposit in fat, from which the drug may be slowly released.

The circulatory system consists of a series of blood vessels; these include the arteries that carry blood to tissues, and the veins that return the blood back to the heart. An average subject (70 kg) has about 5 L of blood, which is equivalent to 3 L of plasma. About 50% of the blood is in the large veins or venous sinuses. The volume of blood pumped by the heart per minute—the cardiac output—is the product of the stroke volume of the heart and the number of heart beats per minute. An average cardiac output is 0.08 L/left ventricle contraction x 69 contractions (heart beats)/min, or about 5.5
L/min in subjects at rest. The cardiac output may be five to six times higher during exercise. Left ventricular contraction may produce a systolic blood pressure of 120 mm Hg, and moves blood at a linear speed of 300 mm/sec through the aorta. Mixing of a drug solution in the blood occurs rapidly at this flow rate. Drug molecules rapidly diffuse through a network of fine capillaries to the tissue spaces filled with interstitial fluid. The interstitial fluid plus the plasma water is termed *extracellular water*, because these fluids reside outside the cells. Drug molecules may further diffuse from the interstitial fluid across the cell membrane into the cell cytoplasm.

![Diagram of body fluid volumes](image)

**Fig:** volume of body fluid in 70kg person.

After entry into the systemic circulation, either by intravascular injection or by absorption from any of the various extravascular sites, the drug is subjected to a number of processes called as disposition processes that tend to loiter its plasma concentration. The two major drug disposition processes are:

1. Distribution which involves reversible transfer of drug between compartments.

2. Elimination which causes irreversible loss of drug from the body. Elimination is further divided into two processes viz, biotransformation (metabolism) and excretion.
As stated above, distribution is defined as the reversible transfer of a drug between one compartment and another.

Since the process is carried out by the circulation of blood, one of the compartments is always the blood or the plasma and the other represents extravascular fluids and other body tissues. In other words, distribution is reversible transfer of a drug between the blood and the extravascular fluid and tissues. Distribution is a passive process, for which, the driving force is Concentration gradient between the blood and the extravascular tissues. The process occurs by diffusion of free drug only until equilibrium is achieved. As the pharmacologic action of a drug depends upon its concentration at the site of action, distribution plays a significant role in the Onset, intensity and sometimes duration of drug action.

Distribution can be thought of as following one of four types of patterns:

1. The drug may remain largely within the vascular system. Plasma substitutes such as dextran are an example of this type, but drugs which are strongly bound to plasma protein may also approach this pattern.

2. Some low molecular weight water soluble compounds such as ethanol and a few sulfonamides become uniformly distributed throughout the body water. Iodine is concentrated by the thyroid gland. The antimalarial drug chloroquine may be present in the liver at concentrations 1000 times those present in plasma. Tetracycline is almost irreversibly bound to bone and developing teeth. Consequently tetracyclines should only be given to young children or infants in extreme conditions as it can cause discoloration and mottling of the developing second set of teeth. Another type of
specific concentration may occur with highly lipid soluble compounds which distribute into fat tissue.

3. A few drugs are concentrated specifically in one or more tissues that may or may not be the site of action.

4. Most drugs exhibit a non-uniform distribution in the body with variations that are largely determined by the ability to pass through membranes and their lipid/water solubility. The highest concentrations are often present in the kidney, liver, and intestine usually reflecting the amount of drug being excreted.

An important parameter to describe the distribution of drug is volume of distribution ($V_D$).

**The volume of distribution ($V_D$), also known as apparent volume of distribution, is a pharmacological, theoretical volume that the total amount of administered drug would have to occupy (if it were uniformly distributed), to provide the same concentration as it currently is in blood plasma.**

Therefore, if $V_D$ is greater, it shows that the drug is more diluted than it should be (in the blood plasma), meaning more of it is distributed in tissue (i.e. not in plasma). It is defined as the distribution of a medication between plasma and the rest of the body after oral or parenteral dosing. It is defined as the theoretical volume in which the total amount of drug would need to be uniformly distributed to produce the desired blood concentration of a drug. The volume of distribution is given by the following equation:
\[ V_D = \frac{\text{total amount of drug in the body}}{\text{drug blood plasma concentration}} \]

\[ V_D = \frac{D_B}{C_p} \]

Apparent volume of distribution (\( V_D \)) is a useful indicator of the type of pattern that characterizes a particular drug. A value of \( V \) in the region of 3-5 liter (in an adult) would be compatible with pattern 1. This is approximately the volume of plasma. Pattern two would be expected to produce a \( V \) value of 30 to 50 liter, corresponding to total body water. Agents or drugs exhibiting pattern 3 would exhibit very large values of \( V \). Chloroquine has a \( V \) value of approximately 115 L/ kg. Drugs following pattern 4 may have a \( V \) value within a wide range of values.

**Factors Influence Drug Distribution**

Distribution of a drug is not uniform throughout the body because different tissues receive the drug from plasma at different rates and to different extents. Differences in drug distribution among the tissues essentially arise as result of a number of factors. Factors influence drug distribution:

A. Rate of distribution:
   1. Membrane permeability of the drug:
      a. Physicochemical properties of the drug like molecular size, pKa and \( K_{o/w} \), partition coefficient
      b. Physiological barriers to diffusion of drugs
   2. Organ/tissue sue and perfusion rate

B. Extent of Distribution
   1. Binding of drugs to tissue components:
      a. Plasma protein binding
b. Tissue drug binding.
c. Lipid solubility
d. pH-pKa

2. Miscellaneous factors:
   a. Age
   b. Pregnancy
   c. Obesity
d. Diet
e. Disease slates
f. Drug interactions

A. Rate of distribution

1. Membrane permeability:

Of the several factors listed above, the two major rate-determining steps in the distribution of drugs are:

1. Rate of membrane permeability, and

2. Rate of blood perfusion.

If the blood flow to the entire body tissues were rapid and uniform, differences in the degree of distribution between tissues will be indicative of differences in the tissue penetrability of the drug and the process will be tissue permeability rate-limited. The tissue permeability, of a drug depends upon the physicochemical properties of the drug as well as the physiologic barriers that restrict diffusion of drug into tissues.

Physicochemical Properties of the Drug

Important physicochemical properties that influence drug distribution are molecular size, degree of ionization and partition coefficient.
Almost all drugs having molecular weight less than 500 to 600 daltons easily cross the capillary membrane to diffuse into the extracellular interstitial fluids. However, penetration of drugs from the extracellular fluid into the cells is a function of molecular size, ionization constant and lipophilicity of the drug. Only small, water-soluble molecules and ions of size below 50 daltons enter the cell through aqueous tilled channels whereas those of larger size are restricted unless a specialized transport system exists for them.

The degree of ionization of a drug is an important determinant in its tissue penetrability. The pH of the blood and the extravascular fluid also play a role in the ionization and diffusion of drugs into cells. A drug that remains unionized at these pH values can permeate the cells relatively more rapidly. Since the blood and the ECF pH normally remain constant at 7.4, they do not have much of an influence on drug diffusion unless altered in conditions such as systemic acidosis or alkalosis.

Most drugs are either weak acids or weak bases and their degree of ionization at plasma or ECF pH depends upon their pKa. All drugs that ionize at plasma pH (i.e. polar, hydrophilic drugs) cannot penetrate the lipoidal cell membrane and tissue permeability is the rate-limiting step in the distribution of such drugs. Only unionized drugs which are generally lipophilic those rapidly cross the cell membrane. Among the drugs that have same o/w partition coefficient but differ in the extent of ionization at blood pH, the one that ionizes to a lesser extent will have greater penetrability than that which ionizes to a larger extent; for example, pentobarbital and salicylic acid have almost the same Ko/w, but the former is more unionized at blood pH and therefore distributes rapidly. The influence of drug pKa and Ko/w on distribution is illustrated by the example that thiopental, a nonpolar, lipophilic drug, largely unionized at plasma pH,
readily diffuses into the brain whereas penicillins which are polar, water-soluble and ionized at plasma pH do not cross the blood-brain barrier.

On the other hand, brain capillaries seem to have impermeable walls restricting the transfer of molecules from blood to brain tissue. Lipid soluble compounds can be readily transferred but the transfer of polar substances is severely restricted. This is the basis of the "blood-brain" barrier.

Since the extent to which a drug exists in unionized form governs the distribution pattern, situations that result in alteration of blood pH affect such a pattern; for example, acidosis (metabolic or respiratory) results in decreased ionization of acidic drugs and thus increased intracellular drug concentration and pharmacologic action. Opposite is the influence of

Fig: The blood-brain barrier
alkalosis. Sodium bicarbonate induced alkalosis is sometimes useful in the treatment of barbiturate (and other acidic drugs) poisoning to drive the drug out and prevent further entry into the CNS and promote their urinary excretion by favoring ionization. converse is-true for basic drugs; acidosis favors extracellular whereas alkalosis, intracellular distribution. In case of polar drugs where permeability is the rate—limiting step in the distribution, the driving force is the effective partition coefficient of drug.

**Physiologic Barriers to Distribution of Drugs**

A membrane (or a barrier) with special structural features can be a permeability restriction to distribution of drugs to some tissues. Some of the important simple and specialized physiologic barriers are:

1. Simple capillary endothelial barrier
2. Simple cell membrane barrier
3. Blood-brain barrier
4. Cerebrospinal fluid barrier
5. Placental barrier
6. Blood-testis barrier

**The Simple Capillary Endothelium Barrier:** The membrane of capillaries that supply blood to most tissues is, not a barrier to moieties which we call drugs. Thus, all drugs ionized or unionized, with a molecular size less than 600 daltons, diffuse through the capillary endothelium and into the interstitial fluid. Only drugs bound to the blood components are restricted because of the large molecular size of the complex.

**The Simple Cell Membrane Barrier:** Once a drug diffuses from the capillary wall into, the extracellular fluid, its further entry into cells of most tissues is limited by its permeability through the membrane that lines such
cells. Such a simple cell membrane is similar to the lipoidal barrier in the GI absorption of drugs.

Blood-Brain Barrier (BBB): Unlike the capillaries found in other parts of the body, the capillaries in the brain are highly specialized and much less permeable to water-soluble drugs. The brain capillaries consist of endothelial cells which are joined to one another by Continuous light intercellular junctions comprising what is called as the blood-brain barrier. Moreover, the presence of special cells called as astrocytes, which are the elements of the supporting tissue found at the base of endothelial membrane, form a solid envelope around the brain capillaries. As a result, the intercellular passage is blocked and for a drug to gain access from the capillary circulation into the brain, it has to pass through the cells rather than between them. (However, there are specific sites in the brain where the BBB does not exist, namely, the trigger area and the median hypothalamic eminence. Moreover, drugs administered intranasally may diffuse directly into the CNS because of the continuity between submucosal areas of the nose and the subarachnoid space of the olfactory lobe.

Since the BBB is a lipoidal barrier, it allows only the drugs having high o/w partition coefficient to diffuse passively whereas moderately lipid soluble and partially ionized molecules penetrate at a slow rate. The effective partition coefficient of thiopental, a highly lipid soluble drug is 50 times that of pentobarbital and crosses the, BBB much more rapidly. Polar natural substances such as sugars and amino acids are transported to brain actively. Thus, structurally similar foreign molecules can also penetrate the BBB by the same mechanism. Most antibiotics such as penicillins which are polar, water-soluble and ionized at plasma pH do not cross the BBB under normal circumstances.
The selective permeability of lipid soluble moieties through the BBB makes appropriate choice of a drug to treat CNS disorders an essential part of therapy for example, Parkinsonism, a disease characterized by depletion of dopamine in the brain, cannot be treated by administration of dopamine as it does not cross the BBS. Hence, levodopa, which can penetrate the CNS where it is metabolized to dopamine, is used in its treatment. Targeting of polar drugs to brain in certain conditions such as tumor had always been a problem. Three different approaches have been utilized successfully to promote crossing the BBB by drugs:

i. Use of permeation enhancers such as dimethyl sulfoxide (DMSO)

ii. Osmotic disruption of the BBB by infusing internal carotid artery with mannitol

iii. Use of dihydropyridine redox system as drug carriers to the brain

In the latter case, the lipid soluble dihydropyridine is linked as a carrier to the polar drug to form a prodrug that readily crosses the BBB. In the brain, the CNS enzymes oxidize the dihydropyridine moiety to the polar pyridinium ion form that cannot diffuse back out of the brain. As a result, the drug gets trapped in the CNS. Such a redox system has been used to deliver steroidal drugs to the brain.
**Blood-Cerebrospinal Fluid Barrier:** The cerebrospinal fluid (CSF) is formed mainly by the choroid plexus of the lateral, third and fourth ventricles and is similar in composition to the ECF of brain. The capillary endothelium that lines the choroid plexus has open junctions or gaps and drugs can flow freely into the extracellular space between the capillary wall and the choroidal cells. However, the choroidal cells are joined to each other by tight junctions forming the blood-CSF barrier which has permeability characteristics similar that of the BBB.

![Image of the blood-CSF barrier](image)

**Fig:** The blood-CSF barrier

As in the case of BBB, only highly lipid soluble drugs can cross the blood-CSF barrier with relative ease; whereas moderately lipid soluble and partially ionized drugs permeate slowly. A drug that enters the CSF slowly cannot achieve a high concentration as the bulk flow of CSF continuously removes the drug. For any given drug, its Concentration in the brain will always be higher than in the CSF.
Although the mechanisms for diffusion of drugs into the CNS and CSF are similar, the degree of uptake may vary significantly. In some cases, CSF drug concentration may be higher than its cerebral concentration e.g. sulfamethoxazole and trimethoprim, and vice versa in other cases, e.g. certain β-blockers.

**Placental Barrier:** The maternal and the fetal blood vessels are separated by a number of tissue layers made of fetal trophoblast basement membrane and the endothelium which together constitute the placental barrier. The flow of blood in the maternal and the fetal blood vessels is shown in Fig.

![Fetal and blood flow across it.](image)

The human placental barrier has a mean thickness of 25 microns in early pregnancy that reduces to 2 microns at full term which however does not reduce its effectiveness. Many drugs having molecular weight less than 1000 daltons and moderate to high lipid solubility e.g: ethanol, sulfonamides, barbiturates, gaseous anesthetics, steroids, narcotic analgesics, anticonvulsants and some antibiotics, cross the barrier by simple diffusion quite rapidly. This shows that the placental barrier is not
as effective a barrier as BBB. Nutrients essential for the fetal growth are transported by carrier-mediated processes. Immunoglobulins are transported by endocytosis.

Drugs are particularly dangerous to the fetus during 2 stages—

I. In the first trimester when the fetal organs develop; it is during this stage that most drugs show their teratogenic effects (congenital defects) e.g. thalidomide, phenytoin, isotretinoin, testosterone, methotrexate, etc.

ii. In the latter stages of pregnancy when drugs are known to affect physiologic functions, e.g. respiratory depression by morphine.

It is; therefore, always better to restrict all drugs during pregnancy because of the uncertainty of their hazardous effects.

**Blood-Testis Barrier:** This barrier is located not at the capillary endothelium level but at sertoli-sertoli cell Junction. It is the tight junctions between the neighboring sertoli cells that act as the blood-testis barrier. This barrier restricts the passage of drugs to spermatocytes and spermatids.

2. **Perfusion rate**

As discussed until now, distribution is permeability rate-limited in the following cases:

   i. When the drug under consideration is ionic, polar or water soluble

   ii. Where the highly selective physiologic barriers restrict the diffusion of such drugs to the inside of the cell

In contrast, distribution will be perfusion rate-limited when

   i. The drug is highly lipophilic
ii. The membrane across which the drug is supposed to diffuse is highly permeable such as those of the capillaries and the muscles.

Whereas only highly lipophilic drugs such as thiopental can cross the most selective of the barriers like the BBB, highly permeable capillary will permits passage of almost all drugs (except those bound to plasma proteins). In both circumstances, the rate-limiting step is the rate of blood flow or perfusion to the tissue. Greater the blood flow, faster the distribution.

Perfusion rate is defined as the volume of blood that flows per unit time per unit volume of the tissue.

In Table, the various tissues are listed in decreasing order of their perfusion rate which indicates the rapidity with which the drug will be distributed to the tissues. Highly perfused tissues such as lungs, kidneys, adrenal, liver, heart and brain are rapidly equilibrated with lipid soluble drugs.

The extent to which a drug is distributed in a particular tissue or organ depends upon the size of the tissue (i.e. tissue volume) and the tissue/blood partition coefficient of the drug. Consider the classic example of thiopental. This lipophilic drug has a high tissue/blood partition coefficient towards the brain and still higher for adipose tissue. Since the brain (site of action) is a highly perfused organ, following IV injection, thiopental readily diffuses into the brain showing a rapid onset of action. Adipose tissues bring poorly perfused, takes longer to get distributed with the same drug. But as the concentration of thiopental in the adipose proceeds towards equilibrium, the drug rapidly diffuses out of the brain and localizes in the adipose tissue whose volume is more than 5 times that of brain and has greater affinity for the drug. The result is rapid termination of action of thiopental due to such tissue redistribution.
Table: Perfusion rate of blood in different organ

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Anatomical Location or Organ</th>
<th>Specific Blood Flow Rate (mm²/sec-gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose tissue</td>
<td>Abdomen, 10-29 mm thick</td>
<td>0.507</td>
</tr>
<tr>
<td></td>
<td>Abdomen, 30-49 mm thick</td>
<td>0.358</td>
</tr>
<tr>
<td></td>
<td>Abdomen, &gt;40 mm thick</td>
<td>0.307</td>
</tr>
<tr>
<td></td>
<td>Thigh, 11 mm thick</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Thigh, 20 mm thick</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Thigh, 43 mm thick</td>
<td>0.15</td>
</tr>
<tr>
<td>Bone</td>
<td>Humerus, marrow flow only</td>
<td>0.055</td>
</tr>
<tr>
<td>Connective tissue</td>
<td>Typical, basal (max)</td>
<td>0.50 (2.5)</td>
</tr>
<tr>
<td>Joint</td>
<td>Knee, avg. @ 303 K skin temp.</td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td>Knee, avg. @ 308 K skin temp.</td>
<td>0.487</td>
</tr>
<tr>
<td>Muscle</td>
<td>Calf, anterior, resting flow (max)</td>
<td>0.46 (9.15)</td>
</tr>
<tr>
<td></td>
<td>Forearm, resting flow (max)</td>
<td>0.53 (8.38)</td>
</tr>
<tr>
<td></td>
<td>Thigh, anterior, resting flow (max)</td>
<td>0.43 (6.00)</td>
</tr>
<tr>
<td></td>
<td>Typical, basal (max)</td>
<td>0.50 (10.0)</td>
</tr>
<tr>
<td>Organ</td>
<td>Brain, basal (max)</td>
<td>9.0 (9.2)</td>
</tr>
<tr>
<td></td>
<td>Gastrointestinal tract, basal (max)</td>
<td>6.7 (26.7)</td>
</tr>
<tr>
<td></td>
<td>Heart, basal (max)</td>
<td>13.3-14.0 (64.0)</td>
</tr>
<tr>
<td></td>
<td>Kidney, basal (max)</td>
<td>67-70 (100)</td>
</tr>
<tr>
<td></td>
<td>Liver, basal (max)</td>
<td>9.6-14.2 (54.5)</td>
</tr>
<tr>
<td></td>
<td>Lung, basal (max)</td>
<td>90 (490)</td>
</tr>
<tr>
<td>Skin</td>
<td>Abdomen, resting flow</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>Arms, resting flow</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>Calf, resting flow</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>Face, resting flow</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>Foot, dorsal surface, resting flow</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>Forearm, sunburned (max)</td>
<td>9.22 (46.7)</td>
</tr>
<tr>
<td></td>
<td>Hands, resting flow</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>Head, resting flow</td>
<td>7.15</td>
</tr>
<tr>
<td></td>
<td>Thigh, resting flow</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>Thorax, resting flow</td>
<td>6.45</td>
</tr>
<tr>
<td></td>
<td>Typical, resting flow (max)</td>
<td>1.7 (25.0)</td>
</tr>
</tbody>
</table>

B. Extent of Distribution

1. Binding of drugs to tissue components:

**Plasma protein binding:** A drug in the body can bind to several components such as the plasma proteins, blood cells and hemoglobin (i.e.
blood components) and extravascular proteins and other tissues. Multiple equilibria occur within plasma where drug can bind to various proteins, examples of which are listed in the table. Acidic drugs commonly bind to albumin, the most abundant plasma protein. Basic drugs often bind to $\alpha_1$-acid glycoprotein, and neutral lipophilic compounds associate with lipoproteins. Proteins, such as $\gamma$-globulin, transcortin, fibrinogen, sex hormone-binding globulin, and thyroid-binding globulin, bind specific compounds. Many large protein drugs also have specific protein carriers. Commonly, more than one plasma protein is involved. Tissue distribution can also involve multiple equilibria. Ionized basic compounds, for example, form ion pairs with the abundant acidic phospholipids in tissues. Their unionized forms may also partition into adipose tissue.

Extensive plasma protein binding will cause more drugs to stay in the central blood compartment. Therefore drugs which bind strongly to plasma protein tend to have lower volumes of distribution. (↑ protein binding = ↓ V). Albumin comprises 50% of the total proteins binds the widest range of drugs. Acidic drugs commonly bind to albumin, while basic drugs often bind to $\alpha_1$-acid glycoproteins and lipoproteins. Groups on the protein molecules that are responsible for electrostatic interactions with drugs include; NH$_3^+$ of lysine, N-terminal amino acids, NH$_2^+$ of histidine, S- of cysteine, COO- of aspartic and glutamic acid residues.

Extensive plasma protein binding will decrease the amount of absorbed drug (decrease peak plasma level). Elimination of a highly bound drug may be delayed. Since the concentration of free drug is low, drug elimination by metabolism and excretion may be delayed. This effect is responsible for prolonging the effect of the drug digoxin.
Table: Representative protein where drugs bind.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight (g/mol)</th>
<th>Normal Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>General</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>67,000</td>
<td>35–50</td>
</tr>
<tr>
<td>α₁-Acid glycoprotein</td>
<td>42,000</td>
<td>0.4–1.0</td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>200,000–2,400,000</td>
<td>Variable</td>
</tr>
<tr>
<td>Specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortisol binding globulin</td>
<td>53,000</td>
<td>0.03–0.07</td>
</tr>
<tr>
<td>(transcortin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex-hormone binding globulin</td>
<td>90,000</td>
<td>0.0036</td>
</tr>
</tbody>
</table>

Changes in the concentration of plasma proteins will influence the effect of a highly bound drug. A low plasma protein level may occur in:

- old age
- malnutrition
- Illness such as liver disease (remember that most plasma proteins are made in the liver), or chronic renal failure where there is excessive excretion of albumin.

In each case the result is a smaller proportion of drug in bound form and freer drug in the plasma. The greater amount of free drug is able to produce a greater therapeutic effect and reduced drug dosages may be indicated in these cases.

**Tissue drug binding:** In addition to plasma protein binding, drugs may bind to intracellular molecules. The affinity of a tissue for a drug may be due to: binding to tissue proteins or to nucleic acids, or in the case of
adipose tissue, dissolution in the lipid material. The concentration of chloroquine in the liver is due to the binding of the drug to DNA. Barbiturates distribute extensively into adipose tissue, primarily because of their high lipid solubility. Tetracyclines bind to bone thus should be avoided in young children or discoloration of permanent teeth may occur.

**Lipid solubility:** Lipid solubility will affect the ability of the drug to bind to plasma proteins and to cross lipid membrane barriers. Very high lipid solubility can result in a drug partitioning into highly vascular lipid-rich areas. Subsequently these drugs slowly redistribute into body fat where they may remain for long periods of time.

**Effects of pH (pH-pKa):** The rate of movement of a drug out of circulation will depend on its degree of ionization and therefore its pKa. Changes in pH occurring in disease may also affect drug distribution. For example, blood becomes more acidic if respiration is inadequate.

2. **Miscellaneous factors affecting drug distribution**

**Age**

Differences in distribution pattern of a drug in different age groups are mainly due to differences in—

a. Total body wafer (both intracellular and extracellular) — is much greater in infants

b. Fat content — is also higher in infants and elderly

c. Skeletal muscles — are lesser in infants and in elderly

d. Organ composition — the BBB is poorly developed in infants, the myelin content is low and cerebral blood flow is high, hence greater penetration of drugs in the brain
e. Plasma protein content — low albumin content in both infants and in elderly

**Pregnancy**

During pregnancy, the growth of uterus, placenta and fetus increases the volume available for distribution of drugs. The fetus represents a separate compartment in which a drug can distribute. The plasma and the ECF volume also increase but there is a fall in albumin content.

**Obesity**

In obese persons, the high adipose tissue content can take up a large fraction of lipophilic drug despite the fact that perfusion through it is low. The high fatty acid levels in obese persons alter the binding characteristics of acidic drugs.

**Diet**

A diet high in fats will increase the free fatty acid levels in circulation thereby affecting binding of acidic drugs such as NSAIDs to albumin.

**Disease\States**

A number of mechanisms may be involved in the alteration of drug distribution characteristics in disease states:

A. Altered albumin and other drug-binding protein concentration
B. Altered or reduced perfusion to organs or tissues
C. Altered tissue pH

An interesting example of altered permeability, of the physiologic barriers is that of BBB. In meningitis and encephalitis, the BBB becomes more permeable and thus polar antibiotics such as penicillin G and ampicillin which do not normally cross it, gain access to the brain. In a patient
suffering from CCF, the perfusion rate to the entire body decreases affecting distribution of all drugs.

**Drug Interactions**

Drug interactions that affect distribution are mainly due to differences in plasma protein or tissue binding of drugs. This topic is discussed under the same heading in subsequent chapter.
PROTEIN BINDING OF DRUG

Introduction

A drug in the body can interact with several tissue components which the two major categories are blood and extravascular tissues. Many drugs interact with plasma or tissue proteins or with other macromolecules, such as melanin and DNA, to form a drug–macromolecule complex. The formation of a drug protein complex is often named drug–protein binding. Drug–protein binding may be a reversible or an irreversible process. Irreversible drug–protein binding is usually a result of chemical activation of the drug, which then attaches strongly to the protein or macromolecule by covalent chemical bonding. Irreversible drug binding accounts for certain types of drug toxicity that may occur over a long time period, as in the case of chemical carcinogenesis, or within a relatively short time period, as in the case of drugs that form reactive chemical intermediates.

For example, the hepatotoxicity of high doses of acetaminophen is due to the formation of reactive metabolite intermediates that interact with liver proteins. Most drugs bind or complex with proteins by a reversible process. Reversible drug–protein binding implies that the drug binds the protein with weaker chemical bonds, such as hydrogen bonds or van der Waals forces.

*The phenomenon of complex formation with proteins is called as protein binding of drugs.*
Drugs may bind to various macromolecular components in the blood, including:

1. albumin,
2. $\alpha_1$-acid glycoprotein,
3. lipoproteins,
4. Immunoglobulins (IgG), and
5. Erythrocytes (RBC).

*Albumin* is a protein with a molecular weight of 65,000–69,000 daltons that is synthesized in the liver and is the major component of plasma proteins responsible for reversible drug binding. In the body, albumin is distributed in the plasma and in the extracellular fluids of skin, muscle, and various other tissues. Interstitial fluid albumin concentration is about 60% of that in the plasma. The elimination half-life of albumin is 17–18 days.
Normally, albumin concentration is maintained at a relatively constant level of 3.5–5.5% (weight per volume) or 4.5 mg/dl. Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances. As a transport protein for endogenous substances, albumin complexes with free fatty acids (FFAs), bilirubin, various hormones (such as cortisone, aldosterone, and thyroxine), tryptophan, and other compounds. Many weak acidic (anionic) drugs bind to albumin by electrostatic and hydrophobic bonds. Weak acidic drugs such as salicylates, phenylbutazone, and penicillins are highly bound to albumin. However, the strength of the drug binding is different for each drug. Table: Major Proteins to Which Drugs Bind in Plasma

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Weight (Da)</th>
<th>Normal Range of Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>65,000</td>
<td>35–50</td>
</tr>
<tr>
<td>α₁-Acid glycoprotein</td>
<td>44,000</td>
<td>0.4–1.0</td>
</tr>
<tr>
<td>α₁- Globulin</td>
<td>59,000</td>
<td>0.003–0.007</td>
</tr>
<tr>
<td>α₂- Globulin</td>
<td>134,000</td>
<td>0.015–0.06</td>
</tr>
<tr>
<td>Lipoproteins</td>
<td>200,000–3,400,000</td>
<td>Variable</td>
</tr>
</tbody>
</table>
\(\alpha_1\)-Acid glycoprotein is a globulin with a molecular weight of about 44,000 Da. The plasma concentration of \(\alpha_1\)-acid glycoprotein is low (0.4–1\%) and binds primarily basic (cationic) drugs such as propranolol, imipramine, and lidocaine. Globulins (\(\alpha\)-, \(\beta\)-, \(\gamma\)-globulins) may be responsible for the plasma transport of certain endogenous substances such as corticosteroids. These globulins have a low capacity but high affinity for the binding of these endogenous substances.

**Lipoproteins** are macromolecular complexes of lipids and proteins and are classified according to their density and separation in the ultracentrifuge. The terms VLDL, LDL, and HDL are abbreviations for very-low-density, low-density, and high-density lipoproteins, respectively. Lipoproteins are responsible for the transport of plasma lipids to the liver and may be responsible for the binding of drugs if the albumin sites become saturated.

**Erythrocytes**, or red blood cells (RBCs), may bind both endogenous and exogenous compounds. RBCs consist of about 45\% of the volume of the blood. Phenytoin, pentobarbital, and amobarbital are known to have a RBC/plasma water ratio of 4 to 2, indicating preferential binding of drug to the erythrocytes over plasma water. Penetration into RBC is dependent on the free concentration of the drug. In the case of phenytoin, RBC drug level increases linearly with an increase in the plasma-free drug concentration (). Increased drug binding to plasma albumin reduces RBC drug concentration. With most drugs, however, binding of drug to RBC generally does not significantly affect the volume of distribution, because the drug is often bound to albumin in the plasma water. Even though phenytoin has a great affinity for RBC, only about 25\% of the blood drug concentration is
present in the blood cells, and 75% is present in the plasma because the drug is also strongly bound to albumin. For drugs with strong erythrocyte binding, the hematocrit will influence the total amount of drug in the blood. For these drugs, the total whole-blood drug concentration should be measured.

The importance of such a binding derive from the fact that the bound drug is both pharmacokinetically as well as pharmacodynamically inert i.e. a protein bound drug is neither metabolized nor excreted nor is it pharmacologically active. A bound drug is also restricted since it remains confined to a particular tissue for which it has greater affinity. Moreover, such a bound drug, because of its enormous size, cannot undergo membrane transport and thus its half-life is increased.

Binding of drugs generally involves weak chemical bonds such as hydrogen bonds, hydrophobic bonds, ionic bonds or van der Wool’s forces and, therefore, is a reversible process. Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug; for example, covalent binding of chloroform and paracetamol metabolites to liver results in hepatotoxicity. Thus, the plasma protein-drug binding is the most significant and most widely studied.

Binding of drugs falls into 2 classes:

1. Binding of drugs to blood components like—
   a. Plasma proteins
   b. Blood cells
2. Binding of drugs to extravascular tissue proteins, fats, bones, etc.
Fig: The influence of binding on drug disposition and clinical response

BINDING OF DRUGS TO BLOOD COMPONENTS

Plasma Protein-Drug Binding

After reaching the systemic circulation drugs can interact with blood components like plasma proteins, blood cells and hemoglobin. The main interaction of drug in the blood compartment is with the plasma proteins which are present in abundant amounts and in large variety. The binding of drugs to plasma proteins is reversible. The extent or order of binding of drugs to various plasma proteins is: albumin > α1- acid glycoprotein > lipoproteins > globulins.
Binding of Drugs to Human Serum Albumin

The human serum albumin (HISA), having a molecular weight of 65,000, is the most abundant plasma protein (59% of total plasma and 3.5 to 5.0 g %) with a large drug binding capacity. The therapeutic doses of most drugs are relatively much smaller and their plasma concentration does not normally reach equimolar concentration with HSA. The HSA can bind several compounds having varied structures. Both endogenous compounds such as fatty acids, bilirubin and, tryptophan as well as drugs bind to HSA. A large variety of drugs, ranging from weak acids, neutral compounds to weak bases bind to HSA. Four different sites on HSA have been identified for drug-binding. They are:

Site I: Also called as warfarin and azapropazone binding site, it represents the region to which large number of drugs are bound, e.g. several NSAIDs (phenylbutazone, naproxen, indomethacin), sulfonamides (sulfadimethoxine, sulfaniethizole), phenytoin, sodium valproate and bilirubin.

Site II: It is also called as the diazepam binding site. Drugs which bind to this region include benzodiazepines, medium chain fatty acids, ibuprofen, ketoprofen, tryptophan, cloxacillin, probenicid, etc. Site I and site II are responsible for the binding of most drugs.

Site III: is also called as digitoxin binding site.

Site IV: is also called as tamoxifen binding site.

Very few drugs bind to Sites III and IV.

A drug can bind to more than one site in which case the main binding site is called as the primary Site and the other as the secondary site; for example, site I is the primary site for dicoumarol and site II the secondary site.
Groups of drug that bind to the same site, compete with each other for binding, but drugs that bind to one site do not competitively inhibit binding of drugs to other sites. However, they may either promote or retard binding of a drug to another site by energetic coupling mechanisms.

**Binding of Drugs to α\textsubscript{1}-acidic Glycoprotein (α\textsubscript{1}-AGP or AAG)**

Also called as the orosomucoid, it has a molecular weight of 44,000 and a plasma concentration range of 0.04 to 0.1 g%. It binds to a number of basic drugs like imipramine, amitriptyline, nortriptyline, lidocaine, propranolol, quinidine and disopyramide.

**Binding of Drugs to Lipoproteins**

Binding of drugs to HSA and AAG involve hydrophobic bonds. Since only lipophilic drugs can undergo hydrophobic bonding, lipoproteins can also bind to such drugs because of their high lipid content. However, the plasma concentration of lipoproteins is much less in comparison to HSA and AAG.

A drug that binds to lipoproteins does so by dissolving in the lipid core of the protein and thus its capacity to bind depends upon its lipid content. The molecular weights of lipoproteins vary from 2 lakhs to 3 lakhs depending on their chemical composition. They are classified on the basis of their density.

The 4 classes of lipoproteins are:

1. Chylomicrons
2. Very low density lipoproteins (VLDL)
3. Low density lipoproteins (LDL) (predominant in humans)
4. High density lipoproteins (HDL)

The lipid core of these macromolecules consists of triglycerides and cholesteryl esters and the outside is made of apoproteins (free cholesterol
and proteins). Predictably, VLDL is rich in triglycerides and HDL is rich in apoproteins. The binding of drugs to lipoproteins is noncompetitive. A number of acidic (diclofenac), neutral (cyclosporin A) and basic drugs (clorpromazine) bind to lipoproteins. Basic, lipophilic drugs have relatively more affinity. Lipoprotein binding becomes significant in cases of drugs that predominantly bind to them and secondarily when levels of HSA and AAG in plasma are decreased.

**Binding of Drugs to Globulins**

Several plasma globulins have been identified and are labeled as α₁-, α₂-, β₁-, β₂- and y-globulins.

1. α₁-globulin: also called as transcortin or CBG (corticosteroid binding globulin), it binds a number of steroidal drugs such as cortisone and prednisone. It also binds to thyroxine and cyanocobalamin.
2. α₂-globulin: also called as ceruloplasmin, it binds vitamins A, D, E and K and cupric ions.
3. β₁-globulin: also called as transferrin, it binds to ferrous ions.
4. β₂-globulin binds to carotinoids
5. y-globulin binds specifically to antigens.

**Binding of Drugs to Blood Cells**

More than 40% of the blood comprises of blood cells of which the major cell component is the RBC. The RBCs constitute 95% of the total blood cells. Thus, significant RBC drug binding is possible. The red cell is 500 times in diameter as the major plasma protein binding component, albumin. The RBC comprises of 3 components each of which can bind to drugs:
1. **Hemoglobin**: It has a molecular weight of 64,500 (almost equal to that of EISA) but is 7 to 8 times the concentration of albumin in blood. Drugs like phenytoin, pentobarbital and phenothiazines bind to hemoglobin.

2. **Carbonic Anhydrase**: Drugs known to bind to it are acetazolamide and chlorhalidone (i.e. carbonic anhydrase inhibitors).

3. **Cell Membrane**: Imipramine and chlorpromazine are reported to bind with the RBC membrane.

It has been shown that the rate and extent of entry into RBC is more for lipophilic drugs, e.g. phenytoin. Hydrophilic drugs like ampicillin do not enter RBC.

**TISSUE BINDING OF DRUGS**

**(TISSUE LOCALIZATION OF DRUGS)**

A drug can bind to one or more of the several tissue components. Tissue-drug binding is important in distribution from two viewpoints: firstly, it increases the apparent volume of distribution of drugs in contrast to plasma protein binding; and secondly, tissue-drug binding results in localization of a drug at a specific site in the body. This is because a number of drugs bind irreversibly with the tissues for example, oxidation products of paracetamol, phenacetin, chloroform, carbon tetrachloride and bromobenzene bind co-valently to hepatic tissues.

Factors influencing localization of drugs in tissues include lipophilicity and structural features of the drug, perfusion rate, pH differences, etc. Extensive tissue-drug binding suggests that a tissue can act as the storage
sire for drugs. Drugs that bind to both tissue and plasma components result in competition between drug binding sites.

For majority of drugs that bind to extravascular tissues, the order of binding is: liver: kidney > lung > muscle. Several examples of extravascular tissue-drug binding are:

1. **Liver**: As stated earlier, epoxides of a number of halogenated hydrocarbons and paracetamol bind irreversibly to liver tissues resulting in hepatotoxicity.

2. **Lungs**: Basic drugs like imipramine, chlorpromazine and antihistamines accumulate in lungs.

3. **Kidneys**: Metallothionin, a protein present in kidneys, binds to heavy metals such as lead, mercury, and cadmium and results in their renal accumulation and toxicity.

4. **Skin**: Chloroquine and phenothiazines accumulate in skin by interacting with melanin.

5. **Eyes**: The retinal pigments of the eye also contain melanin. Binding of chloroquine and phenothiazines to it is responsible for retinopathy.

6. **Hairs**: Arsenicals, chloroquine and phenothiazines are reported to deposit in hair shafts.

7. **Bones**: Tetracycline is a well known example of a drug that binds to bones and teeth. Administration of this antibiotic to infants during odontogenesis results in permanent brown-yellow discoloration of teeth. Lead is known to replace calcium from bones and cause their brittleness.
8. **Fats**: Lipophilic drugs such as thiopental and the pesticide DDT accumulate in adipose tissues by partitioning into it. However, high \( \text{o/w} \) partition coefficient is not the only criteria for adipose distribution drugs since several highly lipophilic (more than thiopental) basic drugs like imipramine and chlorpromazine are not localized in fats. The poor perfusion of adipose could be the reason for such an ambiguity. Reports have stated that adipose localization of drugs is a result of binding competition between adipose and non-adipose tissues (lean tissues like muscles, skin and viscera) and not partitioning.

9. **Nucleic Acids**: Molecular components of cells such as DNA interact strongly with drugs like chloroquine and quinacrine resulting distortion of its double helical structure.

**FACTORS AFFECTING DRUG PROTEIN BINDING**

Factors affecting protein-drug binding can be broadly categorized as—

1. Factors relating to the drug
   a. Physicochemical characteristics of the drug
   b. Concentration of drug in the body
   c. Affinity of a drug for a particular binding component
2. Factors relating to the protein and other binding components
   a. Physicochemical characteristics of the protein or binding agent
   b. Concentration of protein or binding component
   c. Number of binding sites on the binding agent
3. Drug interactions
a. Competition between drugs for the binding site (displacement interactions)
b. Competition between drugs and normal body constituents
c. Allosteric changes in protein molecule

4. Patient related factors
   a. Age
   b. Intersubject variations
   c. Disease states

1. DRUG RELATED FACTORS

   a. Physicochemical Characteristics of the Drug
Protein binding is directly related to the lipophilicity of drug. An increase in lipophilicity increases the extent of binding; for example, the slow absorption of cloxacillin in comparison to ampicillin after i.m. injection is attributed to its higher lipophilicity and larger (95%) binding to proteins while the latter is less lipophilic and just 20% bound to proteins. Highly lipophilic drugs such as thiopental tend to localize in adipose tissues. Anionic or acidic drugs such as penicillins and sulfonamide bind more to HSA whereas cationic or basic drugs such as imipramine and alprenolol bind to AAG. Neutral, unionized drugs bind more to lipoproteins.

   b. Concentration of Drug in the Body
The extent of protein-drug binding can change with the changes in drug as well as protein concentration. The concentration of drugs that bind to HSA does not have much of an influence as the therapeutic concentration of any drug is insufficient to saturate it. However, therapeutic concentration of lidocaine can saturate AAG with which it binds as the concentration of AAG is much less in comparison to that of HSA in blood.
c. Drug-Protein/Tissue Affinity
Lidocaine has greater affinity for AAG than for HSA. Digoxin has more affinity for proteins of cardiac muscles than those of skeletal muscles or plasma. Iophenoxic acid, a radioopaque medium, has so great an affinity for plasma proteins that it has a half-life of 2.5 years.

2. PROTEIN/TISSUE RELATED FACTORS

a. Physicochemical Properties of Protein/Binding Component
Lipoproteins and adipose tissue tend to bind lipophilic drugs by dissolving them in their lipid core. The physiologic pH determines the presence of active anionic and cationic groups on the albumin molecules to bind a variety of drugs.

b. Concentration of Protein/Binding Component
Among the plasma proteins, binding predominantly occurs with albumin as it is present in a higher concentration in comparison to other plasma proteins. The amount of several proteins and tissue components available for binding, changes during disease states. This effect will be discussed in the subsequent sections.

c. Number of Binding Sites on the Protein
Albumin has a large number of binding sites as compared to other proteins and is a high capacity binding component. Several drugs are capable of binding at more than one site on albumin, e.g. fluocloxacillin, flurbiprofen, ketoprofen, tamoxifen and dicoumarol bind to both primary and secondary sites on albumin. Indomethacin is known to bind to 3 different sites. AAG is a protein with limited binding capacity because of its low concentration and low molecular size. Though pure AAG has only one binding site for lidocaine, in presence of HSA, two binding sites have been reported which was suggested to be due to direct interaction between HSA and AAG.
3. DRUG INTERACTIONS

a. Competition Between Drugs for the Binding Sites (Displacement Interactions)

When two or more drugs can bind to the same binding site, competition among them with the binding site results. If one of the drugs (drug A) is bound to such a site, then administration of another drug (drug B) having affinity for the same site results in displacement of drug A from its binding site. Such a drug-drug interaction for the common binding site is called as displacement interaction. The drug A here is called as the displaced drug and drug B as the displacer. Warfarin and phenylbutazone have same degree of affinity for HSA. Administration of phenylbutazone to a patient on warfarin therapy results in displacement of latter from its binding site. The free warfarin may cause adverse hemorrhagic reactions which may be lethal. Phenylbutazone is also known to displace sulfonamides from their HSA binding sites. Displacement interactions can result in unexpected rise in free concentration of the displaced drug which may enhance clinical response or toxicity. Even a drug metabolite can affect displacement interaction.

Clinically significant interactions will result when:

1. The displaced drug (e.g. warfarin) —
   a. is more than 95% bound
   b. has a small volume of distribution (less than 0.13 l/Kg)
   c. shows a rapid onset of therapeutic or adverse effects
   d. has a narrow therapeutic index

2. The displacer drug (e.g. phenylbutazone) —
   a. has a high degree of affinity as the drug to be displaced
   b. competes for the same binding sites
c. the drug/protein concentration ratio is high (above 0.10), and
d. shows a rapid and large increase in plasma drug concentration.

It will be worthwhile to mention here that, both the concentration of the displacer drug and its affinity for the binding site with respect to that of the drug to be displaced, will determine the extent to which displacement will occur.

For a drug that is 95% bound a displacement of just 5% of the bound drug results 100% rise in free drug concentration. If the displaced drug has a small volume of distribution, it remains confined to the blood compartment and shows serious toxic responses. On the contrary, if such a drug has a large Vd, it redistributes into a large volume of body fluids and clinical effects may be negligible or insignificant. The increase in free drug concentration following displacement also makes it more available for elimination by the liver and the kidneys. If the drug is easily metabolizable or excretable, its displacement results in significant reduction in elimination half-life. Displacement also becomes insignificant with the use of more selective, potent, low dose drugs.

b. **Competition Between Drugs and Normal Body Constituents**

Among the various normal body constituents, the free fatty acids are known to interact with a number of drugs that bind primarily to HSA. The free fatty acid level is increased in several physiologic, pathologic (diabetes, myocardial infarction, alcohol abstinence) and pharmacologically induced conditions (after heparin and caffeine administration). The fatty acids, which also bind to albumin, influence binding of several benzodiazepines and propranolol (decreased binding) and warfarin (increased binding). Bilirubin binding to HSA can be impaired by certain drugs and is of great concern in neonates whose BBB and bilirubin metabolizing capacity are not
very efficient. Acidic drugs such as sodium salicylate, sodium benzoate and sulfonamides displace bilirubin from its albumin binding site. The free bilirubin is not conjugated by the liver of the neonates and thus crosses the BBB and precipitates the condition called as kernicterus (characterized by degeneration of brain and mental retardation).

c. Allosteric Changes in Protein Molecule
This is yet another mechanism by which drugs can affect protein binding interactions. The process involves alteration of the protein structure by the drug or its metabolite thereby modifying its binding capacity. The agent that produces such an effect is called as allosteric effector, e.g. aspirin acetylates the lysine fraction of albumin thereby modifying its capacity to bind NSAIDs like phenylbutazone (increased affinity) and flufenamic acid (decreased affinity).

4. PATIENT RELATED FACTORS

a. Age
Modification in protein-drug binding as influenced by age of the patient is mainly due to differences in the protein content in various age groups.

i. Neonates: Albumin content is low in newborn; as a result, the unbound concentration of drug that primarily binds to albumin, for example phenytoin and diazepam.

ii. Young infants: An interesting example of differences in protein-drug binding in infants is that of digoxin. Infants suffering from congestive cardiac failure are given a digitalizing dose 4 to 6 times the adult dose on body weight basis. This is contrary to one’s belief that infants should be given low doses considering their poorly developed drug eliminating system. The reason attributed for use of a large digoxin dose is greater binding of the drug in infants (the
other reason is abnormally large renal clearance of digoxin in infants).

iii. Elderly In old age, the albumin content is lowered and free concentration of drugs that bind primarily to it is increased. Old age is also characterized by an increase in the levels of AAG and thus decreased free concentration is observed for drugs that bind to it. The situation is complex and difficult to generalize for drugs that bind to both HSA and AAG, e.g. lidocaine and propranolol.

b. Intersubject Variations
Intersubject variability in drug binding as studied with few drugs showed that the difference is small and no more than two fold. These differences have been attributed to genetic and environmental factors.

c. Disease States
Several pathologic conditions are associated with alteration in protein content. Since albumin is the major drug binding protein, hypoalbumenemia can severely impair protein-drug binding. Hypoalbumenemia is caused by several conditions like aging, CCF, trauma, burns, inflammatory states, renal and hepatic disorders, pregnancy, surgery, cancer, etc. Every serious chronic illness is characterized by decreased albumin content. Hyperlipoproteinemia, caused by hypothyroidism, obstructive liver disease, alcoholism, etc., affects binding of lipophilic drugs.

Drug interactions and patient related factors that affect protein or tissue binding of drugs influence:

1. Pharmacokinetics of drugs: A decrease in plasma protein-drug binding i.e. an increase in unbound drug concentration, favors tissue redistribution and/or clearance of drugs from the body (enhanced biotransformation and excretion).
2. Pharmacodynamics of drugs: An increase in concentration of free or unbound drug results in increased intensity of action (therapeutic/toxic).

**SIGNIFICANCE OF PROTEIN TISSUE BINDING OF DRUGS**

**Absorption**

The absorption equilibrium is attained by transfer of free drug from the site of administration into the systemic circulation and when the concentration in these two compartments becomes equal. Following equilibrium, the process may stop. However, binding of the absorbed drug to plasma proteins decreases free drug concentration and disturbs such equilibrium. Thus, sink conditions and the concentration gradient are reestablished which now act as the driving force for further absorption. This is particularly useful in case of ionized drugs which are transported with difficulty.

**Systemic Solubility of Drugs**

Water insoluble drugs, neutral endogenous macromolecules such as heparin and several steroids and oil soluble vitamins are circulated and distributed to tissues by binding especially to lipoproteins which act as a vehicle for such hydrophobic compounds.

**Distribution**

Plasma protein binding restricts the entry of drugs that have specific affinity for certain tissues. This prevents accumulation of a large fraction of drug in such tissues and thus, subsequent toxic reactions. Plasma protein-drug binding thus favors uniform distribution of drugs throughout the body.
by its buffer function (maintains equilibrium between the free and the bound drug). A protein bound drug in particular does not cross the BBS, the placental barrier and the glomerulus.

**Tissue Binding, Apparent Volume of Distribution and Drug Storage**

A drug that is extensively bound to blood components remains confined to blood. Such a drug has a small volume of distribution. A drug that shows extravascular tissue binding has a large volume of distribution. A tissue or blood component that has great affinity for a particular drug acts as a depot or storage site for that drug; for example, RBC is a storage site for the lipophilic compound tetrahydrocannabinol.

**Elimination**

Only the unbound or free drug is capable of being eliminated. This is because the drug-protein complex cannot penetrate into the metabolizing organ (liver). The large molecular size of the complex also prevents it from getting filtered through the glomerulus. Thus, drugs which are more than 95% bound are eliminated slowly i.e. they have long elimination half-lives; for example, tetracycline, which is only 65% bound, has an elimination half-life of 8.5 hours in comparison to 15.1 hours of doxycycline which is 93% bound to plasma proteins. However, penicillins have short elimination half-lives despite being extensively bound to plasma proteins. This is because rapid equilibration occurs between the free and the bound drug and the free drug is equally rapidly excreted by active secretion in renal tubules.

**Displacement Interactions and Toxicity**

As stated earlier, displacement interactions are significant in case of drugs which are more than 95% bound. This is explained from the example given
in Table 4.3. A displacement of just 1% of a 99% bound drug results in doubling of the free drug concentration i.e. a 100% rise. For a drug that is bound to a lesser extent e.g. 90%, displacement of 1% results in only a 10% rise in free drug concentration which may be insignificant clinically.

Kernicterus in infants is an example of a disorder caused by displacement of bilirubin from albumin binding sites by the NSAIDs. Another example discussed earlier was that of interaction between warfarin and phenylbutazone. Yet another example of displacement is that of digoxin with quinidine. Digoxin represents a drug with a large volume of distribution (i.e. shows extensive extravascular tissue binding). Since displacement interactions may precipitate toxicity of displaced drug, a reduction in its dose may be called for. This may become necessary for a drug having a small Vd such as warfarin since displacement can result in a large increase in free drug concentration in plasma. With a drug of large Vd such as digoxin, even a substantial increase in the degree of displacement of drug in plasma may not effect a large increase in free drug concentration and dose adjustment may not be required. This is for two reasons- one, only a small fraction of such a drug is present in plasma whereas most of it localized in extravascular tissues, and secondly, following displacement the free drug, because of its large Vd, redistributes in a large pool extravascular tissues. The extent to which the free plasma drug concentration of drugs with different Vd values will change when displaced.

**Diagnosis**

The chlorine atom of chloroquine when replaced with a radioactive I-131 can be used to visualize melanomas of the eye since chloroquine has a tendency to interact with melanin of eyes. The thyroid gland has great affinity for iodine containing compounds; hence any disorder of the same can be detected by tagging compound with a radioisotope of iodine.
Therapy and Drug Targeting

The binding of drugs to lipoproteins can be used for site-specific delivery of hydrophilic moieties. This is particularly useful in cancer therapies since certain tumor cells have greater affinity for LDL than normal tissues. Thus, binding of a suitable antineoplastic to it can be used as a therapeutic tool. EIDL are similarly transported more to adrenal and testes. An example of site-specific drug delivery in cancer treatment is that of estramustine. Estradiol binds selectively and strongly to prostate and thus prostrate cancer can be treated by attaching nitrogen mustard to estradiol for targeting of prostate glands. Drug targeting prevents normal cells from getting destroyed.
Introduction

The ability of humans to metabolize and clear drugs is a natural process that involves the same enzymatic pathways and transport systems that are utilized for normal metabolism of dietary constituents. Humans come into contact with scores of foreign chemicals or xenobiotics (substances foreign to the body) through exposure to environmental contaminants as well as in our diets. Fortunately, humans have developed a means to rapidly eliminate xenobiotics so they do not cause harm. Drugs are considered xenobiotics and most are extensively metabolized in humans. It is worth noting that many drugs are derived from chemicals found in plants, some of which had been used in Chinese herbal medicines for thousands of years. Of the prescription drugs in use today for cancer treatment, many derive from plant species; investigating folklore claims led to the discovery of most of these drugs. It is therefore not surprising that animals utilize a means for disposing of human-made drugs that mimics the disposition of chemicals found in the diet. This capacity to metabolize xenobiotics, while mostly beneficial, has made development of drugs very time consuming and costly due in large part to (1) interindividual variations in the capacity of humans to metabolize drugs, (2) drug-drug interactions, and (3) species differences in expression of enzymes that metabolize drugs. The latter limits the use of animal models in drug development. A large number of diverse enzymes
have evolved in animals that apparently only function to metabolize foreign chemicals. As will be discussed below, there are such large differences among species in the ability to metabolize xenobiotics that animal models cannot be relied upon to predict how humans will metabolize a drug. Enzymes that metabolize xenobiotics have historically been called drug-metabolizing enzymes, although they are involved in the metabolism of many foreign chemicals to which humans are exposed. Dietary differences among species during the course of evolution could account for the marked species variation in the complexity of the drug-metabolizing enzymes. Today, most xenobiotics to which humans are exposed come from sources that include environmental pollution, food additives, cosmetic products, agrochemicals, processed foods, and drugs. In general, these are lipophilic chemicals that in the absence of metabolism would not be efficiently eliminated, and thus would accumulate in the body, resulting in toxicity. With very few exceptions, all xenobiotics are subjected to one or multiple pathways that constitute the phase 1 and phase 2 enzymatic systems. As a general paradigm, metabolism serves to convert these hydrophobic chemicals into derivatives that can easily be eliminated through the urine or the bile.

The onset of pharmacologic response depends upon two pharmacokinetic processes—drug absorption, and drug distribution (since most sites of action are in the extravascular tissues). The duration and intensity of action depend upon the rate of drug removal from the body/site of action or simply speaking, on the rate of elimination and tissue redistribution of drug. Elimination is the major process for removal of a drug from the body and termination of its action. It is defined as the irreversible loss of drug
from the body. Elimination occurs by two processes viz, biotransformation and excretion.

Biotransformation of drugs is defined as the conversion from one chemical form to another inside biological system. The term is used synonymously with metabolism.

**Metabolism is defined as the irreversible biotransformation of drug in the body, typically involves making it more polar to enhance renal excretion.**

The chemical changes are usually affected enzymatically in the body and thus, the definition excludes chemical instability of a drug within the body: for e.g. conversion of penicillin to penicilloic acid by the bacterial penicillinase and mammalian enzymes is metabolism but its degradation by the stomach acid to penicillenic acid is chemical instability. All chemical substances that are not nutrients for the body and enter the body through
ingestion, inhalation or absorption are called as xenobiotics (Greek: xenos = foreign) or exogenous compounds. Drugs are also xenobiotics which enter the body by virtue of their lipophilicity. It is interesting to note that for effective absorption, a drug needs to be sufficiently lipid soluble but it is this same physicochemical property that enables it to bypass excretion. This is because only water-soluble agents undergo renal excretion (major route for exit of drugs from the body) whereas lipid soluble substances are passively reabsorbed from the renal tubules into the blood after glomerular filtration. Thus, if such a phenomenon continues, drugs would accumulate in the body and precipitate toxic reactions. However, to prevent such a consequence, the body is armed with the metabolic system which transforms the water insoluble, lipophilic, nonpolar drugs into polar and water-soluble products that can be easily excreted by the kidneys and are poorly reabsorbed; for instance, hippuric acid, the metabolite of benzoic acid, is 2.5 times more water soluble. Drug biotransformation is thus a detoxification process.

In comparison with xenobiotics, the natural endogenous substances such as neurotransmitters (dopamine, GABA, epinephrine, norepinephrine, etc.), steroids (testosterone, progesterone, cortisol, etc.) and insulin which are also used as therapeutic agents, are inactivated rapidly because of the body’s well developed system for metabolizing such agents. These substances are therefore called as soft drugs. Such soft drugs do not precipitate unexpected toxicity when used in concentrations close to their normal levels.

**Drug Metabolizing Organs**

Liver is the primary site for metabolism of almost all drugs (and other xenobiotics) because of its relative richness in possessing a large variety of enzymes in large amounts. Metabolism by organs other than liver (called as
extrahepatic metabolism) is of minor importance since lower level of drug metabolizing enzymes are present in such tissues. The decreasing order of drug metabolizing ability of various organs is: liver > lungs > kidneys > intestine > placenta > adrenals > skin. Brain, testes, muscles, spleen, etc. also metabolize drugs to a small extent.

Fig: Location of CYP enzymes.
Drug Metabolizing Enzymes

The enzymes that biotransform xenobiotics, are differ from those that metabolize food materials. They are versatile and nonspecific in metabolizing large number of drugs. The enzymes are broadly divided into 2 categories: microsomal and non-microsomal. The microsomal enzymes catalyze a majority of drug biotransformation reactions. The microsomes are basically artifacts which resulted when attempts were first made to isolate endoplasmic reticulum, of the liver homogenate. These vesicular fragments or microsomes are derived from rough endoplasmic reticulum (rough due to the presence of RNA rich ribosomes on the membrane surface whose function is protein synthesis) which shed their ribosomes to become smooth surfaced. The large variety of microsomal enzymes catalyzes a number of oxidative, reductive and hydrolytic and glucuronidation reactions.

Some important characteristics of microsomal enzyme system are:

1. The intact nature of lipoidal membrane bound enzyme of the microsomes is essential for its selectivity towards lipid-soluble substrates.

2. A number of lipid-soluble substrates (xenobiotics in general) can interact nonspecifically with the microsomal enzymes; natural endogenous substances which are generally water-soluble do not interact.

3. The lipid soluble substrate is biontransformed into a water-soluble metabolite by the microsomal enzymes which can be readily excreted.

The nonmicrosomal enzymes include those that are present in soluble form in the cytoplasm and those attached to the mitochondria but not to
endoplasmic reticulum. These are also nonspecific enzymes that catalyze few oxidative reactions, a number of reductive and hydrolytic reactions and conjugation reactions other than glucuronidation. It is interesting to note that, in contrast to microsomal enzymes, the nonmicrosomal enzyme, especially the soluble enzymes, act on relatively water-soluble xenobiotics (as well as endogenous compounds), e.g. oxidases, peroxidases, dehydrogenases, esterases, etc.

**CHEMICAL PATHWAYS OF DRUG BIOTRANSFORMATION**

The pathways of drug metabolism reactions into two general categories:

1. Phase I reactions and
2. Phase II reactions.

**Phase I Reactions**

These reactions generally precede phase II reactions and include oxidative, reductive and hydrolytic reactions. By way of these reactions, a polar functional group is either introduced or unmasked if already present on the otherwise lipid soluble substrate, e.g. -OH, -COOH, -NH₂ and -SF. Thus, phase I reactions are also called as functionalization reactions. These transformations are also called as asynthetic reactions, opposite to the synthetic phase II reactions. The resulting product of phase I reaction is susceptible to phase II reactions.

**Phase II Reactions**

These reactions generally involve covalent attachment’ of small polar endogenous molecules such as glucuronic acid, sulfate, glycine, etc. to either unchanged drugs or phase I products having suitable functional
groups viz. -OH, -COOH, -NH₂ and -SI] and form highly water-soluble conjugates which are readily excretable by the kidneys (or bile). Thus, these reactions are called as conjugation reactions. Since the outcome of such processes are generally products with increased molecular size (and altered physicochemical properties) they are also called as synthetic reactions. Quite often, a phase I reaction may not yield a metabolite that is sufficiently hydrophilic or pharmacologically inert but conjugation reactions generally result in products with total loss of pharmacologic activity and high polarity. Hence, phase II reactions are better known as true detoxification reactions. Since these reactions generally involve transfer of moieties to the substrate to be conjugated, the enzymes responsible are called as transferases.

Fig: Sequence of phase I and phase II reactions.
The biotransformation of drug metabolites, particularly the glutathione conjugates which are excreted via bile in the gut, by the intestinal microflora, is considered by few researchers as phase II reactions. Quite commonly, the biotransformation reactions proceed sequentially and the combination of several phase I and phase II reactions yield a range of metabolites.

The various phase I and phase II reaction are listed below.

**PHASE I REACTIONS**

A. Oxidative Reactions
   1. Oxidation of aromatic carbon atoms
   2. Oxidation of olefins (C=C bonds)
   3. Oxidation of benzylic, allylic carbon atoms and carbon atoms alpha to carbon and imines
   4. Oxidation of aliphatic carbon atoms
   5. Oxidation of alicyclic carbon atoms
   6. Oxidation of carbon-heteroatom systems:
      a. Carbon-Nitrogen systems (aliphatic and aromatic amines)
         i. N-Dealkylation
         ii. Oxidative deamination
         iii. N-Oxide formation
         iv. N-Hydroxylation
      b. Carbon-Sulfur systems:
         I. S-Dealkylation
         ii. Desulfuration
         iii. S-oxidation
      c. Carbon-Oxygen systems (O-dealkylation)
   7. Oxidation of alcohol carbonyl and acid functions
8. Miscellaneous oxidative reactions

B. Reductive Reactions
1. Reduction of carbonyl functions (aldehydes/ketones)
2. Reduction of alcohols and CC bonds
3. Reduction of N-compounds (film, azo and N-oxide)
4. Miscellaneous reductive reactions

C. Hydrolytic Reactions
1. Hydrolysis of esters and ethers
2. Hydrolysis of amides
3. Hydrolytic cleavage of nonaromatic heterocycles
4. Hydrolytic dehalogenation
5. Miscellaneous hydrolytic reactions

PHASE II REACTIONS

1. Conjugation with glucuronic acid
2. Conjugation with sulfate moieties
3. Conjugation with alpha amino acids
4. Conjugation with glutathione and mercapturic acid formation
5. Acetylation reactions
6. Methylation reactions
7. Miscellaneous conjugation reactions

PHASE I REACTIONS

A. OXIDATIVE REACTIONS

Oxidative reactions are the most important and most common metabolic reactions. Almost all drugs that undergo phase I biotransformation undergo oxidation at some stage or the other. Oxidative reactions increase
hydrophilicity of xenobiotics by introducing polar functional groups such as—OH. Such a polar metabolite can thus rapidly undergo phase II reaction or is excretable by the kidneys.

Oxidation of xenobiotics is nonspecifically catalyzed by a number of enzymes located in the microsomes. Such enzymes require both molecular oxygen (O$_2$) and the reducing agent NADPH to effect reaction. They are therefore referred to as the mixed function oxidases. This system requires O$_2$ and NADPH for the overall hydroxylation reaction, which is represented as follows:

$$\text{RH} + \text{O}_2 + \text{NADPH} + \text{H}^+ = \text{ROH} + \text{NADP}^+ + \text{H}_2\text{O}$$

Since only one oxygen atom from the molecular oxygen (dioxygen or O$_2$) is incorporated in the product formed, the mixed function oxidases are also called as monooxygenases. Quite often, the product of such a reaction contains a hydroxyl function; hence, the enzymes are sometimes also called as hydroxylases. The multienzyme mixed function oxidase system, located in the endoplasmic reticulum of hepatic cells, is composed at an electron transfer chain consisting of 3 components:

1. A heme protein known as cytochrome P-450, which is actually a family of enzymes. It plays the important role of transferring an oxygen atom to the substrate RH and converts it to ROH.

2. A second enzyme, the flavoprotein known as cytochrome P-450 reductase (or cytochrome c reductase) which is NADPH dependent. It functions as an electron carrier, catalyzing the reduction of cytochrome P-450 to the ferrous form by transferring an electron from NADPH.
3. A heat stable lipid component known as phosphatidylcholine. Its function is to facilitate electron transfer from, NADPH to cytochrome P-450.

Magnesium ions are also required for maximal activity of mixed function oxidases. The various steps in the oxidation of xenobiotics are:

1. Binding of the substrate (RH) to the oxidized form of the cytochrome P-450 (Fe^{+++}) to form a complex.

2. A one electron transfer from NADPH to the complex by cytochrome P.450 reductase to form reduced (Fe^{++}) P-450 substrate complex; this step is considered as the rate-limiting step in the overall oxidation of xenobiotics.
3. The reduced enzyme-substrate complex combines with a molecule of oxygen to form ternary complex.

Fig: Redox cycle of cytochrome P-450

4. The ternary complex combines with a second electron supplied by NADH in presence of enzyme cytochrome b5 reductase to form a ternary activated oxygen P-450 substrate complex.

5. One atom of oxygen from the activated oxygen complex is transferred to the substrate to yield the oxidized product and the other atom forms water. The free oxidized form of cytochrome P-450 is now ready to attach to yet another molecule of substrate.

1. Oxidation of Aromatic Carbon Atoms (Aromatic Hydroxylation)

This reaction proceeds via formation of a reactive intermediate arene oxide (epoxide) which in most cases undergoes rearrangement to yield arenols and in some cases catechols and glutathionc conjugates.
The arene oxide intermediate is highly reactive and carcinogenic or cytotoxic. Monosubstituted benzene derivatives can be hydroxylated at ortho-, meta- or para-positions but para-hydroxylated product is most common, e.g. conversion of acetanilide to paracetamol and phenylbutazone to oxyphenbutazone.

2. Oxidation of Olefins

Oxidation of nonaromatic carbon-carbon double bonds is analogous to aromatic hydroxylation i.e. it proceeds via formation of epoxides to yield 1,2-dihydrdijols. A better known example of olefinic oxidation is conversion of carbamazepine to carbanIlazepinc \_lo,l 1-epoxide; the latter is Converted to corresponding trans- 10,11 -dihydrodiol.
Oleflic hydroxylation differs from aromatic hydroxylation in that their epoxides are stable and detectable.

3. **Oxidation of Benzylic Carbon Atoms, allylic carbon atom, α-carbon atom to carbonyls and imines.**

Carbon atoms attached directly to the aromatic rings (benzylic carbon atoms) are hydroxylated to corresponding carbinols. If the product is a primary carbinol, it is further oxidized to aldehydes and then to carboxylic acids, e.g. tolbutamide.

Carbon atoms adjacent to olefinic double bonds (are allylic carbon atoms) also undergo hydroxylation in a manner similar to benzylic carbons, e.g. hydroxylation of hexobarbital to 3’-hydroxy hexobarbital.
Several benzodiazepines contain a carbon atom (C-3) alpha to both carbonyl (C=O) and imino (C=N) functions which readily undergoes hydroxylation, e.g. diazepam.
4. Oxidation of Aliphatic Carbon Atoms (Aliphatic Hydroxylation)

Alkyl or aliphatic carbon atoms can be hydroxylated at the terminal methyl group (called as $\omega$—oxidation) and the penultimate carbon atom (called as $\omega$-1 oxidation) e.g. valproic acid.

Terminal hydroxylation of methyl group yields primary alcohol which undergoes further oxidation to aldehyde and then to carboxylic acid quite rapidly. Penultimate carbon atom can be secondary or tertiary e.g. ibuprofen.

5. Oxidation of Alicyclic Carbon Atoms (Alicyclic Hydroxilation)
Cyclohexane and piperidine rings are commonly found in a number of molecules, e.g. acetohexamide and minoxidil respectively. Such rings are generally hydroxylated at C-3 or C-4 positions.

6. Oxidation of Carbon-Heteroatom Systems

a) Oxidation of Carbon-Nitrogen Systems

i. N-Dealkylation: Alkyl groups attached directly to nitrogen atom in nitrogen bearing compounds are capable of undergoing N-dealkylation reactions, e.g. secondary and tertiary aliphatic and aromatic amines.

Tertiary nitrogen attached to different alkyl groups undergoes dealkylation by removal of smaller alkyl group first. A representative example of tertiary aliphatic amine undergoing N-dealkylation e.g. imipramine.
II. Oxidative Deamination: Like N-dealkylation, this reaction also proceeds via the carbinolamine pathway but here the C-N bond cleavage occurs at the bond that links amino group to the larger portion of the drug molecule.

Primary aliphatic amines readily undergo deamination, e.g. amphetamine, while secondary and tertiary amines are deaminated only when bulky groups are attached to nitrogen.

III. N-Oxide Formation: N-oxides are formed only by nitrogen atoms having basic properties. Generally, the tertiary amines yield N-oxides. Example, nicotine.
The N-oxide products are highly water-soluble and excreted in urine.

**IV. N-Hydroxylation:** Converse to basic compounds that form N-oxides, N-hydroxy formation is usually displayed by nonbasic nitrogen atoms such as amide nitrogen. e.g lidocaine.

**b) Oxidation of Carbon-Sulfur Systems**

I. S-Dealkylation: The mechanism of S-dealkylation of thioethers (RSR)
is analogous to N-dealkylation, proceeds via α-carbon hydroxylation. The C-S bond cleavage results in formation of a thiol (RSH’ and a carbonyl product, e.g. 6-methyl mercaptopurine.

II. Desulfuration: This reaction also involves cleavage of carbon-sulfur bond (C=S or thiono). The product is the one with C=O bond. Such a desulfuration reaction is commonly observed in thioamides (RCSNHR’) such as thiopental.

III. S-Oxidation: Apart from S-dealkylation, thioethers can also undergo S-oxidation reactions to yield sulfoxides which may be further oxidized to sulfones.

Several phenothiazines, e.g. chlorpromazine, undergo S-oxidation.
c) Oxidation of Carbon-Oxygen Systems

**O-dealkylation:** The O-conaning functional groups (analogous to amines and amides, the substrates which undergo N-dealkylation) are ethers and esters. However, only the ethers undergo O-dealkylation reaction. Aliphatic ether drugs are rare and aromatic ethers (phenolic) are common. Methyl ethers are rapidly dealkylated in comparison to longer chain ethers such as the one containing i-butyl group. The reaction generally leads to formation of active metabolites, e.g. phenacetin to paracetamol, and codeine to morphine.

![Chemical reaction diagrams]

7. Oxidation of Alcohol, Carbonyl and Carboxylic Acid

These reactions are mainly catalyzed by nonmicrosomal enzymes, dehydrogenases. Primary and secondary alcohols and aldehydes undergo oxidation relatively easily but tertiary alcohols, ketones and carboxylic acids are resistant as such a reaction involves cleavage of C-C bonds.
Primary alcohols are rapidly metabolized to aldehydes (and further to carboxylic acids) but oxidation of secondary alcohols to ketones proceeds slowly. This is because primary alcohols are better substrates for dehydrogenases due to their acidic nature, and secondly, their oxidation products, carboxylic acids, are more water-soluble than ketones. Since primary alcohols are inactivated rapidly, drugs bearing such groups are rare. In case of ethanol, oxidation to acetaldehydes reversible and further oxidation of the latter to acetic acid is very rapid since acetaldehyde is highly toxic and should not accumulate in the body. Compounds with two primary alcohol functions are oxidized stepwise and not simultaneously. With secondary alcohols, the rate of oxidation increases with an increase in alkyl chain length. Compounds with both primary and secondary alcohol groups are oxidized preferentially at the primary group.

**B. REDUCTION REACTIONS**

Bioreductions are also capable of generating polar functional groups such as hydroxy and amino which can undergo further biotransformation or conjugation. A number of reductive reactions are exact opposite of oxidation.

Thus, in this sense, bioreduction comprises one-half of reversible reactions. Such reactions may be catalyzed by the same enzyme (true reversible
reaction) or by different enzymes (apparent reversible reaction). Since reversible reactions usually lead to conversion of inactive ‘metabolites into active drug, they may result in delay of drug removal from the body and hence prolongation of action.

Reduction of Carbonyls (Aldehydes and Ketones)

Depending on their reactivity towards bioreduction, carbonyls can be divided into 3 categories:

1. The aliphatic aldehydes and ketones.
2. The aromatic aldehydes and ketones.
3. The esters, acids and amides.

The order of reactivity of these categories of drugs in undergoing reduction is 1 > 2 > 3 i.e. aliphatic aldehydes and ketones undergo extensive bioreduction whereas esters, acids and amides are least reactive.

Few aldehydes undergo reduction because such a reaction is usually reversible, and secondly, they are susceptible towards oxidation which yields more polar products. Several ketones undergo reduction as it results in more polar metabolites. Reduction of aldehydes and ketones yields primary and secondary alcohols respectively. The reaction is catalyzed by nonmicrosomal enzymes called as aldo-keto-reductases.
A representative example of compounds undergoing reductive reactions is given below. Example: methadone, naltrexone.

1. Reduction of Alcohols and C=C double bonds

Reduction of norethindrone, an α,β-unsaturated compounds results in both reduction of C=C double bond and formation of alcohol e.g. norethindrone.

2. Reduction of N-compounds

The N containing functional groups that commonly undergo bioreduction are nitro, azo and N-oxide. For example, nitrazepam.
C. HYDROLYTIC REACTIONS

These reactions differ from oxidative and reductive reactions in 3 ways:

1. The reaction does not involve change in the state of oxidation of the substrate.

2. The reaction results in a large chemical change in the substrate, brought about by loss of relatively large fragments of the molecule.

3. The hydrolytic enzymes that metabolize xenobiotics are the ones that also act on endogenous substrates; moreover, their activity is not confined to liver as they are found in many other organs like kidney, intestine, etc.

A number of functional groups are hydrolyzed viz, esters, ethers, amides, hydrazides, etc.

1. Hydrolysis of Esters and Ethers.

Esters on hydrolysis yield alcohol and carboxylic acid. The reaction is catalyzed by esterases.

Organic esters with both large acidic and alcoholic groups on hydrolysis loss their activity. Esters where one of the groups is relatively large retain much of their activity when hydrolyzed since such a group is generally a
pharmacophore (having pharmacologic activity). In many cases, such esters are prodrugs which rely on hydrolysis for their transformation into active form, e.g. chloramphenicol palmitate.

Aromatic esters are hydrolyzed by arylesterases and aliphatic esters by carboxyesterases. Examples of various classes of esters undergoing hydrolysis are given below. Examples: aspirin.

2. Hydrolysis of Amides (C-N bond cleavage)

Amides are hydrolyzed slowly in comparison to esters. The reaction, catalyzed by amidases, involves C-N cleavage to yield carboxylic acid and amine.

Primary amides are rare. Secondary amides form the largest group of amide drugs. Examples of amide hydrolysis are given below. Example: procanamide, lidocaine, etc.
3. Hydrolytic cleavage of Nonaromatic Heterocycles

Nonaromatic heterocycles also contain amide e.g. lactams which undergo hydrolysis.

![Chemical structure of penicillin G and its metabolite](image)

Phase II Reaction

Phase II reactions involve transfer of a suitable moiety such as glucuronic acid, sulfate, glycine, etc. in presence of enzyme transferase to drugs or metabolites of phase I reactions having suitable functional groups to form highly polar, readily excretable and pharmacologically inert conjugates. Tissue reactive and carcinogenic metabolites are rendered harmless by conjugation with glutathione. The two phase II reactions viz. acetylation and methylation that do not generate polar products also terminate the pharmacologic activity of xenobiotics. Thus, phase II reactions are the real drug detoxication pathways.

The moieties transferred to the substrates in a phase II reaction possess 3 characteristics:

1. They are simple endogenous molecules.
2. They are of large molecular size.
3. They are strongly polar or ionic in nature in order to render the substrate water-soluble.
An important aspect of conjugation reaction is that they are capacity limited due to the availability of endogenous conjugating molecules. Thus, when doses of drugs are higher than normal levels of conjugating molecules, saturation of metabolism occurs and the unconjugated drug/metabolite precipitates toxicity. The order of capacities of important conjugation reactions is: Glucuronidation > Amino acid conjugation > Sulfation and Glutathione conjugation. The increase in the molecular weight of the drug following conjugation with glucuronic acid, sulfate and glutathione is 176, 80 and 300 daltons respectively. The molecular weight of the conjugate is important in dictating its route of excretion. High molecular weight conjugates are excreted predominantly in bile while the low molecular weight ones are in urine.

**CONJUGATION WITH GLUCURONIC ACID**

Also called as glucuronidation, it is the most common and most important phase II reaction reasons:

1. Readily available source of conjugation moiety.
2. Several functional groups viz alcohols, acids, amines, etc. can combine easily with glucuronic acid.
3. Quantitatively, conjugation with D-glucuronic acid occurs to a high degree.
4. All mammals have the common ability to produce glucuronides.
5. Glucuronic acid has a $p^{Ka}$ in the range 3.5 to 4.0 and hence insoluble at both plasma and urine pH thereby greatly influence water solubility of the conjugated substrate.
6. The glucuronidation enzymes are in close association with the microsomal oxidase, the major phase I drug metabolizing enzyme system, thus a rapid conjugation of phase I metabolism is possible.

7. Lastly, glucuronidation can take place in most body tissues since the glucuronic acid donor, UDPGA is produced in processes related to glycogen, synthesis and thus, will never be deficient unlike those involved in other phase II reactions.

**Oxygen or O-Glucuronides**

Xenobiotics with hydroxyl and/or carboxyl functions form O-glucuronides.

1. **Hydroxyl Compounds:** These form ether glucuronides. Several examples of such compounds are given below. Examples: chloramphenicol, trichloroethanol, hydroxylated hexobarbital, morphine, paracetamol etc.

2. **Carboxyl Compounds:** These form ester glucuronides. Example: salicylic acid, fenoprofen, etc.

**Nitrogen or N-Glucuronides**

Xenobiotics with amine, amide and sulfonamide functions form N-glucuronides. Example: desipramine, tripelennamine, etc.

**Carbon or C-Glucuronides**

Xenobiotics with nucleophilic carbon atoms such as phenylbutazone form C-glucuronides.

Certain endogenous compounds such as steroids, bilirubin, catechols and thyroxine also form glucuronides.
CONJUGATION WITH SULFATE MOIETIES

Sulfation is similar to glucuronidation but it is catalyzed by nonmicrosomal enzymes and occurs less commonly as the moiety that transfers sulfate to the substrate is easily depleted. Thus, this process is saturable and predominates at low substrate concentration whereas glucuronidation is dominant at high concentration.

Functional groups capable of forming sulfate conjugates include phenols, alcohols, arylamines, N-hydroxylamines and N-hydroxyamides. The reaction product is a sulfate ester, also called as ethereal sulfate. Examples of compounds undergoing sulfation are paracetamol, sulbutamol, etc.

Sulfoconjugates can be tissue reactive, e.g. the o-sulfate conjugate of N-hydroxy phenacetin covalently binds to hepatic and renal tissues. Endogenous substances can also undergo sulfation, e.g. steroids, biologic amines, etc.

CONJUGATION WITH ALPHA AMINO ACIDS

This reaction also occurs to a limited extent because of limited availability of amino acids.

Conjugation occurs commonly with glycine. Glutamine conjugation occurs to a lesser extent. Conjugation with other amino acids like aspartic acid, serine and taurine is still uncommon. The substrate is generally an acid (aromatic in particular) and the reaction product is an amide.

Examples of drugs forming glycine or glutamine conjugates are isopropoxyacetic acid, cholic acid, salicylic acid, nicotinic acid, etc.
Amino acid conjugation occurs extensively in the liver mitochondria and thus can be used to estimate hepatic function. The diagnostic marker used is benzoic acid which on conjugation with glycine yields hippuric acid. Flippuric acid is rapidly excreted in urine. Thus, the rate and extent of urinary excretion of hippuric acid following oral or i.v. administration of benzoic acid indicates functioning of liver. A decreased output indicates hepatic disorder.

**CONJUGATION WITH GLUTATHIONE AND MERCAPTURIC ACID**

Glutathione (γ-glutamyl cysteinyl glycine or GSH) is a tripeptide with a strongly nucleophilic character due to the presence of a -SH (thiol) group in its structure.

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glutathione (GSH)
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Thus, it has great affinity for electrophilic substrates, a number of which are potentially toxic compounds. It is important to note that a highly electrophilic metabolite has a tendency to react with tissue nucleophilic groups such as -OH, -NH₂ and -SH and precipitate toxicities such as tissue necrosis, carcinogenesis, mutagenesis, teratogenesis, etc. Conjugation with glutathione protects the tissue from such reactive moieties and thus the reaction is an important detoxication route.
ACETYLATION

This reaction is basically an acylation reaction and thus similar to conjugation with α-amino acids. The analogy also lies in the fact that both reactions yield amide products. Acetylation however differs from a-amino acid conjugation in that the substrates are exogenous amities (and not carboxylic acids) and the acylating agent is endogenous acetyl CoA (CH₃COSCoA). The general sequence of reaction is similar to that for a-amino acid conjugation. The enzyme involved is the nonmicrosomal N-acetyl transferase.

Acetylation is an important metabolic pathway for drugs containing primary amino groups. Alcohols (e.g. choline) and thiols (e.g. CoASH) also undergo acetylation.

Examples of drugs undergoing acetylation are histamine, procainamide, PABA, sulfanilamide, etc.

Acetylation may sometimes lead to toxic products, e.g. acetyl derivatives of some sulfonamides (cause renal toxicity due to decreased water solubility of the metabolites formed) and reactive arylacetamides.

METHYLATION

This reaction differs from general characteristics of phase II reactions in several ways:

1. The metabolites formed are not polar or water-soluble.

2. The metabolites, in a number of instances, have equal or greater pharmacologic activity than the parent drug, e.g. morphine formed from normorphine.
3. The reaction is of lesser importance in metabolism of xenobiotics. It is more important in the biosynthesis (e.g. adrenaline, melatonin) and inactivation of endogenous amines (e.g. noradrenaline, serotonin, histamine).

Methylation can be considered as intermediate of phase I and phase II reactions. It can be called as a phase I reaction as it is reverse of demethylation reaction and can be classed as a phase II reaction because of its mechanism.

Examples of the substances undergo methylation are morphine, norephedrine, nicotine, histamine, etc.

**MISCELLANEOUS CONJUGATION REACTIONS**

Some of the rare conjugation reactions are mentioned below.

**Conjugation of Cyanide**

The toxicity of cyanide ion is due to ability to arrest enzymes involved in cellular respiration and convert hemoglobin to cyanometemoglobin which lacks the ability to transport oxygen to tissues. Conjugation of cyanide ion involves transfer of sulfur atom from thiosulfate to the cyanide ion in presence of enzyme rhodanese to form inactive thiocyanate.

**Conjugation with Ribose**

- Endogenous purine and pyrimidine bases conjugate with ribose to form nucleotides.
FACTORS AFFECTING BIOTRANSFORMATION OF DRUGS

The therapeutic efficacy, toxicity and biological half-life of a drug greatly depend upon its metabolic rate. A number of factors may influence the rate of drug metabolism; they are:

1. Physicochemical properties of the drug
2. Chemical factors:
   a. Induction of drug metabolizing enzymes
   b. Inhibition of drug metabolizing enzymes
   c. Environmental chemicals
3. Biological factors:
   a. Species differences
   b. Strain differences
   c. Sex differences
   d. Age
   c. Diet
   f. Altered physiologic factors:
      i. Pregnancy
      ii. Hormonal imbalance
      iii. Disease states
4. Temporal factors:
   i. Circadian rhythm
   ii. Circannual rhythm

PHYSICOCHEMICAL PROPERTIES OF THE DRUG

Just as the absorption and distribution of a drug are influenced by its physicochemical properties, so is its interaction with the drug metabolizing enzymes. Molecular size and shape, pKa, acidity/basicity, lipophilicity and
steric and electronic characteristics of a drug influence its interaction with the active sites of enzymes and the biotransformation processes to which it is subjected. However, such an interrelationship is not clearly understood.

CHEMICAL FACTORS

Induction of Drug Metabolizing Enzymes

The phenomenon of increased drug metabolizing ability of the enzymes (especially of microsomal monooxygenase system) by several drugs and chemicals is called as enzyme induction and the agents which bring about such an effect are known as inducers. Most inducers are in general lipophilic compounds with long elimination half-lives.

Two categories of inducers have been defined:

1. Phenobarbital type inducers: includes several drugs and pesticides which increase the rate of the metabolism of a large number of drugs.

2. Polycyclic hydrocarbon type inducers such as 3-methyl cholanthrene and cigarette smoke which stimulate the metabolic rate of few drugs.

Some drugs (such as carbamaazepine) stimulates their own metabolism, the phenomenon is called as auto-induction or self-induction.

Metabolism involved in enzyme induction may be increased enzyme synthesis, decreased rate of enzyme degradation, enzyme stabilization or enzyme activation.

Table: Inducers of Drug Metabolizing Enzyme System and Drugs Commonly Affected by them

<table>
<thead>
<tr>
<th>Inducers</th>
<th>Drugs with Enhanced Metabolism</th>
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Enzyme induction results in decreased pharmacologic activity of most drugs, increased activity where the metabolites are active and altered physiologic status due to enhanced metabolism of endogenous compounds such as sex hormones. Some examples of inducers and drugs affected by them are given in table.

### Inhibition of Drug Metabolizing Enzymes

A decrease in the drug metabolizing ability of an enzyme is called as enzyme inhibition. The process of inhibition may be direct or indirect.

1. Direct Inhibition: may result from interaction at the enzymic site, the net outcome being a change in enzyme activity. Direct enzyme inhibition may be competitive or noncompetitive.

   A. Competitive Inhibition: results when structurally similar compounds compete for the same site on an enzyme. Such an inhibition due to substrate competition is reversible and can be overcome by high Concentration of one of the substrates, e.g., methacholine inhibits metabolism of aectyl choline by competing with it for cholinesterase.

   B. Noncompetitive Inhibition: arises when a structurally unrelated agent interacts with the enzyme and prevents the metabolism of drugs. Since the interaction is not structure-specific, metals like lead,
mercury and arsenic and organophosphorus insecticides inhibit the enzymes noncompetitively.

2. Indirect Inhibition is brought about by one of the two mechanisms:
   
a. Repression: is defined as the decrease in enzyme content. It may be due to a fall in the rate of enzyme synthesis as affected by ethionine, puromycin and actinomycin D or because of rise in the rate of enzyme degradation such as by carbon terrachloride, carbon disulfide, disulfiram, etc.

   b. Altered Physiology: due to nutritional deficiency or hormonal imbalance.

Direct enzyme inhibition is usually rapid (a single dose of inhibitor be sufficient) and therefore more important than enzyme induction. Enzyme inhibition generally results in prolonged pharmacologic action of a drug.

**Environmental Chemicals**

Several environmental agents influence the drug metabolizing ability of enzymes. Halogenated pesticides such as DDT and polycyclic aromatic hydrocarbons contained in cigarette smoke have enzyme induction effect. Organophosphate insecticides and heavy metals such as mercury, tin, nickel, cobalt and arsenic inhibit drug metabolizing ability of enzymes.

Other environmental factors that may influence drug metabolism are temperature, altitude, pressure, atmosphere, etc.

**BIOLOGICAL FACTORS**

**Species Differences**

Screening of new therapeutic molecules to ascertain their activity and toxicity requires study in several laboratory animal species. Differences in
drug response due to species differences are taken into account while extrapolating the data to man.

Species differences have been observed in both phase I and phase II reactions. In phase I reactions, both qualitative and quantitative variations in the enzyme and their activity have been observed. An example of this is the metabolism of amphetamine and ephedrine. In men and rabbit, these drugs are predominantly metabolized by oxidative deamination whereas in rats the aromatic oxidation is the major route. In phase II reactions, the variations are mainly qualitative and characterized either by the presence of, or complete lack of certain conjugating enzymes; for example, in pigs, the phenol is excreted mainly as glucuronide whereas its sulfate conjugate dominates in cats. Certain birds utilize ornithine for conjugating aromatic acids of glycine.

**Strain Differences/Pharmacogenetics**

Enzymes influencing metabolic reactions are under the genetic control. A study of intersubject variability in drug response (due to differences in, for example, rate of biotransformation) is called as pharmacogenetics. The intersubject variations in drug biotransformation may either be monogenically or polygenically controlled. A polygenic control has been observed in studies in twins. In identical twins (monozygotic), very little or no difference in the metabolism of phenylbutazone, dicoumarol and antipyrine was detected but large variations were apparent in fraternal twins (dizygotic; twins developed from two different eggs) for the same drugs.

Differences observed in the metabolism of a drug among different races are called as ethnic variations. Such a variation may be monomorphic or
polymorphic. When a unimodal frequency distribution is observed in the entire population the variations are called as continuous or monomorphic; for example, the entire human race acetylate PABA and PAS to only a small extent. A polymodal distribution is indicative of discontinuous variation (polymorphism). An example of polymorphism is the acetylation of isoniazid (INH) in humans. A bimodal population distribution was observed comprising of slow acetylator or inactivator phenotypes (metabolize INH slowly) and rapid acetylator or inactivator phenotypes (metabolize INH rapidly).

**Sex Differences**

Sex related differences in the rate of metabolism can be attributed to regulation of such processes by sex hormones since variations among male and female are generally observed following puberty. Such sex differences are widely studied in rats; the male rats have greater drug metabolizing capacity. In humans, women metabolize benzodiazepines slowly than men and several studies show that women on contraceptive pills metabolize a number of drugs at a slow rate.

**Age**

Differences in the drug metabolic rate in different age groups is main due to variations in the enzyme’ content, enzyme activity and hemodynamics. In neonates (up to 2 months), the microsomal enzyme system is not fully developed and many drugs are biotransformed slowly; for example, caffeine has a half-life of 4 days in neonates in comparison to 4 hours in adults. A major portion of this drug is excreted unchanged in urine by the neonates. Conjugation with sulfate is well developed (paracetamol is excreted mainly as sulfate) but glucuronidation occurs to a very small extent. As a result, hyperbilirubinemia precipitates kernicterus and chloramphenicol leads to
cyanosis or Gray baby syndrome in new born. Similarly, sulfonamides cause renal toxicity and paracetamol causes hepatotoxicity. Infants (between 2 months and one year) show almost a similar profile as neonates in metabolizing drugs with improvement in the capacity as age advances and enzyme activity increases. Children (between one year and 12 years) and older infants metabolize several drugs much more rapidly than adults as the rate of metabolism reaches a maximum somewhere between 6 months and 12 years of age. As a result, they require large mg/Kg doses in comparison to adults; for example, the theophylline half-life in children is two-third of that in adults. In very elderly persons, the liver size is reduced, the microsomal enzyme activity is decreased and hepatic blood flow also declines as a result of reduced cardiac output all of which contribute to decreased metabolism of drugs. Drug conjugation however remains unaffected.

**Diet**

The enzyme content and activity is altered by a number of dietary components. In general, low protein diet decreases and high protein diet increases the drug metabolizing ability. This is because the enzyme synthesis is promoted by protein diet which also raises the level of amino acids for conjugation with drugs. The protein carbohydrate ratio in the diet is also important; a high ratio increases the microsomal mixed function oxidase activity. Fat free diet depresses cytochrome P-450 levels since phospholipids, which are important components of microsomes, become deficient. Dietary deficiency of vitamins (e.g. vitamin A, B2, L3t, C and E) and minerals such as Fe, Ca, Mg, Cu and Zn retard the metabolic activity of enzymes. Starvation results in decreased amount of glucuronides formed than under normal conditions. Malnutrition in women results in enhanced
metabolism of sex hormones. Alcohol ingestion results in short term decrease followed by an increase in the enzyme activity.

**Altered Physiologic Factors**

Pregnancy: Studies in animals have shown that the maternal drug metabolizing ability (of both phase I and phase II reactions) is reduced during the later stages of pregnancy. This was suggested as due to high levels of steroid hormones in circulation during pregnancy. In women the metabolism of promazine and pethidine is reduced during pregnancy or when receiving oral contraceptives. Higher rate of hepatic metabolism of anticonvulsants during pregnancy is thought to be due to induction of drug metabolizing enzymes by the circulating progesterone.

Hormonal Imbalance: The influence of sex hormones on drug metabolism has already been discussed. The effect of other hormones is equally complex. Higher levels of one hormone may inhibit the activity of few enzymes while inducing that of others. Adrenolectomy, thyroidectomy and alloxan induced diabetes in animals showed an impairment in the enzyme activity with a subsequent fall in the rate of metabolism. A similar effect was observed with pituitary growth hormone. Stress related changes in ACTH levels also influence drug biotransformation.

Disease States: As liver is the primary site for metabolism of most drugs, all pathologic conditions associated with it result in enhanced half-lives of almost all drugs. Thus, a reduction in hepatic drug metabolizing ability is apparent in conditions such as hepatic carcinoma, hepatitis: irrhosis, obstructive jaundice, etc. Biotransformations such as glycine conjugation of salicylates, oxidation of vitamin D and hydrolysis of procaine which occur in kidney, are impaired in renal diseases. Congestive cardiac failure and myocardial infarction which result in a decrease in the blood flow to the
liver, impair metabolism of drugs having high hepatic extraction ratio e.g. propranolol and lidocaine. In diabetes, glucuronidation is reduced due to decreased availability of UDPGA.

**Temporal Factors**

Circadian Rhythm: Diurnal variation in the enzyme activity with light cycle is called circadian rhythm in drug metabolism. It has been observed that the enzyme activity is maximum during early morning (6 to 9 am) and minimum in late afternoon (2 to 5 p.m.) which was suggested to correspond with the high and low serum levels of corticosterone (the serum corticosterone level is dependent upon the light-dark sequence of the day). Clinical variation in therapeutic effect of a drug at different times of the day is therefore apparent. The study of variations in drug response as influenced by time is called as chronopharmacology. Time dependent change in drug kinetics is known as chronokinetics. Drugs such as aminopyrine, hexobarbital and imipramine showed diurnal variations in rats. The half-life of metyrapone was shown to be 2.5 times longer during the night than in the day, in rats.
EXCRETION OF DRUGS

Introduction

Drugs and their metabolites are removed from the body by excretion. Excretion is defined as the process whereby drugs and their metabolites are irreversibly transferred from internal to external environment. Excretion of unchanged or intact drug is important in the termination of its pharmacologic action. The principle organs of excretion are kidneys. Excretion by organs other than kidneys such as lungs, biliary system, intestine, salivary glands and sweat glands is known as non renal excretion.

RENAL EXCRETION OF DRUGS

Almost all drugs and their metabolites are excreted by the kidneys to some extent or the other. Some drugs such as gentamicin are exclusively eliminated by renal route only. Agents those are water-soluble, nonvolatile, and small in molecular size (less than 500 daltons) and which are metabolized slowly are excreted in the urine.

The basic functional unit of the kidney involved in excretion is the nephron. Each kidney comprises of one million nephrons. Each nephron is made up of the glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the collecting tubule.
The principal processes that determine the urinary excretion of a drug

1. Glomerular filtration,
2. Active tubular secretion, and
3. Active or passive tubular reabsorption.

Glomerular filtration and active tubular secretion tend to increase the concentration of drugs in lumen and hence facilitate excretion whereas tubular reabsorption decreases it and prevents the movement of drug out of the body. Thus, the rate of excretion can be given by equation:

\[
\text{Rate of excretion} = \text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}
\]

**Glomerular Filtration**

Glomerular filtration is a nonselective, unidirectional process whereby most compounds, ionized or unionized, are filtered except those that are bound to plasma proteins or blood cells and thus behave as macromolecules. The glomerulus also acts as a negatively charged selective barrier promoting retention of anionic compounds. The driving force for filtration through the glomerulus is the hydrostatic pressure of the blood flowing in the capillaries. Out of, the 25% of cardiac output ‘or 1.2 liters of blood/mm that goes to the kidneys via renal artery, only 10% or 120 to 130 ml/min is filtered through the glomeruli, the rate being’ called as the glomerular filtration rate (GFR).

Though some 180 liters of protein and cell free ultrafiltrate pass through the glomeruli each day, only about 1.5 liters is excreted as urine, the remainder being reabsorbed from the tubules.
Fig: A simplified diagram of urinary excretion of drugs.

The GFR can be determined by an agent that is excreted exclusively by filtration and is neither secreted nor reabsorbed in the tubules. That excretion rate value of such an agent is 120 to 130 ml/min. Creatinine, nulin, mannitol and sodium thiosulfate are used to estimate GFR ‘of which the former two are widely used to estimate renal function.

**Active Tubular Secretion**

It is a carrier-mediated process which requires energy for transportation of compounds against the concentration gradient. The system is capacity-limited and saturable. Two active tubular secretion mechanisms have been identified:
1. System for secretion of organic acids/anions like penicillins, salicylates, glucuronides, sulfates, etc. It is the same system by which endogenous acids such as uric acid are secreted.

2. System for secretion of organic bases/cations like morphine, mecamylamine, hexamethonium and endogenous amines such as catecholamines, choline, histamine, etc. Both the systems are relatively nonselective and independent of each other but both can be bidirectional i.e. agents may both be secreted as well as reabsorbed actively—for example, uric acid.

Active secretion is unaffected by changes in PH and protein binding since the bound drug rapidly dissociates the moment the unbound drug gets excreted. But in contrast to glomerular filtration, it is dependent upon renal blood flow. Drugs undergoing active secretion have excretion rate values greater than the normal GFR value of 130 ml/min; for example, penicillin has renal clearance value of 500 ml/min. Such a high value is indicative of both glomerular filtration as well as tubular secretion.

Agents that are used to measure active tubular secretion are the ones that are filtered as well as secreted to such an extent that they are removed from the blood in a single pass through the kidneys i.e. their clearance reflects the renal plasma flow rate which is 600 to 700 ml/min. Para amino hippuric acid (PAH), a highly polar agent and iodopyracet are used to determine active secretion. Active secretion occurs predominantly in the proximal tubule region of the nephron.

Any two structurally similar drugs having similar ionic charge and employing the same carrier-mediated process for excretion enter into competition. A drug with greater rate of clearance will retard the excretion of the other drug with which it competes. The half-life of both the drugs is
increased since the total sites for active secretion are limited. This may result in accumulation of drugs and thus, precipitation of toxicity. However, the principle of competition can be exploited for therapeutic benefits. An interesting example of this is the anionic agent probenecid. Probenecid inhibit the active tubular secretion of organic acids such as penicillins, PAS, PAH, l7-keto steroids, etc. thus increasing their concentration in plasma by at least two fold. A 50% reduction in penicillin G dose is suggested, especially when the drug is meant to be consumed in large doses as in gonococcal infections. The actively secreted and filtered probenecid, if unionized in tubular fluid, is highly lipid soluble and therefore will get reabsorbed passively. Inhibition of drug secretion by pobenecid is undesirable in case of nitrofurantoin since the latter is used as a urinary tract antiseptic (organic bases can also interfere with tubular secretion of cationic drugs but are not in therapeutic use). While inhibiting the active secretion of anionic drugs on one hand, probenecid is known to suppress the carrier-mediated reabsorption of the endogenous metabolite, uric acid and is thus of therapeutic value as a uricosuric agent in the treatment of gout.

**Tubular Reabsorption**

Tubular reabsorption occurs after the glomerular filtration of drugs. It takes place all along the renal tubule. Reabsorption of a drug is indicated when the excretion rate values are less than the GFR of 130 ml/9in. An agent such as glucose that is completely reabsorbed after filtration has a clearance value of zero. Contrary to tubular secretion, reabsorption results in an increase in the half life of a drug.
Tubular reabsorption can either be an:

1. Active process, or
2. Passive process.

Active tubular reabsorption is commonly seen with high threshold endogenous substances or nutrients that the body needs to conserve such as electrolytes, glucose, vitamins, amino acids, etc. Uric acid is also actively reabsorbed (inhibited by the uricosuric agents). Very few drugs are known to undergo reabsorption actively e.g. oxopurinol.

Passive tubular reabsorption is common for a large number of exogenous substances including drugs. The driving force for such a process i.e. the concentration gradient is established by the back diffusion or reabsorption of water along with sodium and other inorganic ions. Understandably, if a drug is neither secreted nor reabsorbed, its concentration in the urine will be 100 times that of free drug in plasma due to water reabsorption since less than 1% of glomerular filtrate is excreted as urine.

The primary determinant in the passive reabsorption of drugs is their lipophilicity. Lipophilic substances are extensively reabsorbed while polar molecules are not. Since a majority of drugs are weak electrolytes (weak acids or weak bases), diffusion of such agents through the lipoidal tubular membrane depend upon the degree of ionization which in turn depends on two important factors: —

1. pH of the urine
2. pKa of the drug
**Urine pH:** It is an important factor in the sense that it is not constant like the plasma pH but varies from 4.5 to 7.5. Thus, a large pH gradient may exist between urine and plasma.

The pH of the urine is dependent, upon diet, drug intake and pathophysiology of the patient. Food rich in carbohydrates result in higher urinary pH whereas proteins lower it. Drugs such as acetazolamide and antacids such as sodium bicarbonate produce alkaline urine while ascorbic acid makes it acidic. More significant alteration in urine pH is brought about by IV infusion of solutions of sodium bicarbonate and ammonium chloride which are used in the treatment of acid-base imbalance. Respiratory and metabolic acidosis and alkalosis result in 'acidification and alkalinization of the urine repectively.

**Drug pKa:** The significance of pH dependent excretion for any particular compound ir greatly dependent upon its pKa and lipid solubility. A characteristic of drugs, pKa values govern the degree of ionization at a particular pH. A polar and ionized drug will be poorly reabsorbed passively and excreted rapidly Reabsorption is also affected by the lipid solubility of drug; an ionized but lipophilic drug will be reabsorbed while a unionized but polar one will be excreted.

The combined effect of urine pH and drug pKa and lipid solubility on reabsorption of drugs is summarized as follows:

1. An acidic drug such as penicillin or a basic drug such as gentamicin which is polar in its unionized form is not reabsorbed passively, irrespective of the extent of ionization in urine. Excretion of such drugs is independent of pH of urine and its flow rate. Their rate of excretion is the sum of rate of filtration and rate of active secretion.
2. Very weakly acidic, nonpolar drugs (pKa> 8.0) such as phenytoin or very weakly basic, nonpolar drugs (pKa< 6.0) such as propoxyphene are mostly unionized throughout the entire range of urine pH and are therefore extensively reabsorbed passively at all values of urine pH. The rate of excretion of such drugs is always low and insensitive urine pH.

3. A strongly acidic drug (pH < 2.0) such as crornoglycic acid or a strongly basic drug (pKa > 12.0) such as guanethidine, is completely ionized at all values of urine pH and are, therefore, not reabsorbed. Their rate of excretion is always high and insensitive to pH of urine.

4. Only for an acidic drug in the pKa range 3.0 to 8.0 (e.g. several NSAIDs) and for a basic drug in the pKa range 6.0 to 12.0 (e.g. morphine analogs, tricyclic antidepressants, etc.) the extent of reabsorption is greatly depend upon urine pH and varies from negligible to almost complete; for example, the amount of dexamphetamine excreted in the urine varies from 3 to 55% of the administered dose as the urine pH varies from 8.0 to 5.0.

The toxicity due to overdosage of drugs, whose excretion is sensitive to pH change, can be treated by acidification or alkanlinization of the urine with ammonium chloride and sodium bicarbonate respectively. Thus crystallization caused by precipitation of sulfonamides in the renal-tubules and subsequent kidney damage can be overcome by alkanlinizing the urine. Excretion of basic drugs can be promoted by acidification of urine. The therapeutic activity of the urinary antiseptic hexamine also depends on the urine pH. It is not converted to active form i.e. formaldehyde unless the urine is acidic.
**Urine Flow Rate:** In addition to urine pH and pKa, the rate of urine flow also influences the extent of reabsorption. Polar drugs whose excretion is independent of urine pH and are not reabsorbed, are unaffected by urine flow rate. An increase in urine flow in case of such drugs will only produce more dilute urine. Only those drugs whose reabsorption is pH-sensitive, for example, weak acids and weak bases; show dependence on urine flow rate. For such agents, reabsorption is inversely proportional to the urinary flow. These compounds can be divided into two types based on their extent of reabsorption in relation to that of water:

1. Drugs which are reabsorbed to an extent equal to or greater than the reabsorption of water e.g. phenobarbital. In such cases, the relationship between renal clearance and urinary excretion is linear.

2. Drugs which are reabsorbed to an extent lower than the reabsorption of water e.g. theophylline and many more drugs. In these cases, the relationship between renal clearance and urinary excretion is convex curvilinear.

In addition to modification of pH, urinary elimination of an agent can also be enhanced by forced diuresis. Forced diuresis is the increase in urine flow induced by large fluid intake or administration of mannitol or other diuretics. The principle can be used in an intoxicated person to remove excessive drug by promoting its excretion and decreasing the time for reabsorption.

Both urine pH control and forced diuresis can be used to treat toxicity with drug overdose when:

1. Urinary excretion is the major route for elimination of drug
2. The drug is extensively reabsorbed passively from the renal tubules
3. The reabsorption is sensitive to urine pH (and urine flow rate)
Apart from the foregoing discussion on the passive reabsorption of drugs, the process is also important in the reabsorption of low threshold substances such as urea, certain phosphates and sulfates, etc.

**CONCEPT OF CLEARANCE**

The clearance concept was first introduced to describe renal excretion of endogenous compounds in order to measure the kidney function. The term is now applied to all organs involved in drug elimination such as liver, lungs, the biliary system, etc. and referred to as hepatic clearance, pulmonary clearance, biliary clearance and so on. The sum of individual clearances by all eliminating organs is called as total body clearance or total systemic clearance. It is sometimes expressed as a sum of renal clearance and nonrenal clearance.

Clearance is defined as the hypothetical volume of body fluids containing drug from which the drug is removed or cleared completely in a specific period of time. It is expressed in ml/min and is a constant for any given plasma drug concentration. In comparison to apparent volume of distribution which relates plasma drug concentration to the amount of drug in the body, clearance relates plasma concentration to the rate of drug elimination.

\[
\text{Clearance (Cl)} = \frac{\text{Elimination rate}}{\text{Plasma drug concentration}}
\]

Renal Clearance (\(\text{Cl}_R\)) : It can be defined as the volume of blood or plasma which is completely cleared of the unchanged drug by the kidney per unit time. It is expressed mathematically as:
Physiologically speaking, renal clearance is the ratio of “sum of rate of glomerular filtration and active secretion minus rate of reabsorption” to “plasma drug concentration C”.

\[ \text{Cl}_R = \frac{\text{Rate of urinary excretion}}{\text{Plasma drug concentration}} \]

The contribution of each of the above physiologic processes in clearing a drug cannot be determined by direct measurement. It can however be determined by comparing the clearance values obtained for a drug with, that of an agent such as creatinine or inulin which is cleared by glomerular filtration only. The ratio of these two values is called as renal clearance ratio or excretion ratio.

\[ \text{Renal Clearance Ratio} = \frac{\text{Renal clearance of drug}}{\text{Renal clearance of creatinine}} \]

Thus, depending upon whether the drug is only filtered, filtered and secreted or filtered and reabsorbed, the clearance ratio will vary. The renal clearance values range from zero to 650 ml/mm and the clearance ratio from zero to five.

**FACTORS AFFECTING RENAL EXCRETION OR RENAL CLEARANCE**

Apart from the three physiologic processes that govern the urinary excretion, other factors influencing renal clearance of drugs and metabolites are:

1. Physicochemical properties of the drug
2. Plasma concentration of the drug
3. Distribution and binding characteristics of the drug
4. Urine pH
5. Blood flow to the kidneys
6. Biological factors
7. Drug interactions
8. Disease states

**Physicochemical Properties of the Drug**

Important physicochemical factors affecting renal excretion of drugs are: molecular size, pKa and lipid solubility. The molecular weight of a drug is very critical in its urinary elimination. An agent of small molecular size can be easily filtered through the glomerulus. Compounds of below 300 daltons, if water soluble, are readily excreted by the kidneys. Drugs in the molecular weight range 300 to 500 daltons can be excreted both in urine and bile. Molecules of size greater than 500 daltons are excreted in urine to a lesser extent.

The influence of drug pKa on excretion has already been discussed. Urinary excretion of an unchanged drug is inversely related to its lipophilicity. This is because; a lipophilic drug is passively reabsorbed to a large extent.

**Plasma Concentration of the Drug**

Glomerular filtration and reabsorption are directly affected by plasma drug concentration since both are passive processes. A drug that is not bound to plasma proteins and excreted by filtration only, shows a linear relationship between rate of excretion and plasma drug concentration. In case of drugs which are secreted or reabsorbed actively, the rate process increases with an increase in plasma concentration to a point when saturation of carrier
occurs. In case of actively reabsorbed drugs, excretion is negligible at low plasma concentrations. Such agents are excreted in urine only when their concentration in the glomerular filtrate exceeds the active reabsorption capacity, e.g., that are actively secreted, the rate of excretion increase with increase in plasma concentration up to saturation level.

**Distribution and Binding Characteristics of the Drug**

Clearance is inversely related to apparent volume of distribution of drugs. A drug with large Vd is poorly excreted in urine. Drugs restricted to blood compartment have high excretion rates.

Drugs that are bound to plasma proteins behave as macromolecules and tfiis V fl be filtered through the glomerulus. Only unbound or free drug appear in the glomerular filtrate. An earlier equation given for renal clearance is:

\[
Cl_R = \frac{\text{Urine concentration}}{\text{Plasma concentration}} \times \text{Urine flow rate}
\]

Since only free drug can be excreted in the urine, the fraction of drug bound to plasma proteins is important.

Drugs extensively bound to proteins have long half-lives because the renal clearance is small and urine flow rate is just 1 to 2 ml/min. The renal clearance of oxytetracycline which is 66% unbound is 99 ml/min while that of doxycycline (7% unbound) is just 16 ml/min.

Actively secreted drugs are much less affected by protein binding, e.g. penicillin. The free fraction of such drugs are filtered as well as secreted actively and dissoCiation of drug-protein complex occurs rapidly.

The influence of urine pH on renal clearance has already been discussed.
Blood Flow to the Kidneys

The renal blood flow is important in case of drugs excreted by glomerular filtration only and those that are actively secreted. In the latter case, increased perfusion increases the contact of drug with the secretory sites and enhances their elimination. Renal clearance in such instances is said to be perfusion rate-limited.

Biological Factors

At Age, sex, species and strain differences, differences in the genetic make-up, circadian rhythm, etc. alter drug excretion. Renal excretion is approximately 10% lower in females than in males. The renal function of newborns is 30 to 40% less in comparison to adults and attains maturity between 2.5 to 5 months of age. In old age, the GFR is reduced and tubular function is altered, the, excretion of drugs is thus slowed down and half-life is prolonged.

Drug Interactions

Any drug interaction that results in alteration of binding characteristics, renal flood flow, active secretion, urine pH and intrinsic clearance and forced diuresis would alter renal clearance of a drug. The renal clearance of a drug extensively bound to plasma proteins is increased after displacement with another drug. An interesting example of this is gentamicin induced nephrotoxicity by furosemide. Furosemide does not precipitate this effect by its diuretic effect but by displacing gentamicin from binding sites. The increased free antibiotic concentration accelerates its renal clearance. Acidification of urine with ammonium chloride, methionine or ascorbic acid enhances excretion of basic drugs. Alkalization of urine with citrates, tartarates, bicarbonates and carbonic anhydrase inhibitors promote excretion of acidic drugs. Phenylbutazone competes with hydroxyhexamide,
the active metabolite of antidiabetic agent acetohexamide, for active secretion and thus prolongs its action. Urinary excretion of digoxin is reduced by diazepam. All diuretics increase elimination of drugs whose renal clearance gets affected by urine flow rate.

**Disease States—Renal Impairment**

Renal dysfunction greatly impairs the elimination of drugs especially those that are primarily excreted by the kidneys. Some of the causes of renal failure are hypertension, diabetes mellitus, hypovolemia (decreased blood supply to the kidneys), pyelonephritis (inflammation of kidney due to infections, etc.), nephroallergens (e.g. nephrotoxic serum) and nephrotoxic agents such as aminoglycosides, phenacetin and heavy metals such as lead and mercury.

Uremia, characterized by impaired glomerular filtration and accumulation of fluids and protein metabolites, also impairs renal clearance of drugs. In both these conditions, the half-lives of drugs are increased. As a consequence, drug accumulation and toxicity may result. Determination of renal function is therefore important in such conditions in order to monitor the dosage regimen.

Renal function can be determined by measuring the GFR. Both endogenous and exogenous substances have been used as markers to measure GFR. In order to be useful as a marker, the agent should entirely get excreted in unchanged form by glomerular filtration only and should be physiologically and pharmacologically inert. The rate at which these markers are excreted in urine reflects the GFR and changes in GFR reflects renal dysfunction. Inulin (the exogenous fructose polysaccharide) and serum creatinine level have been used successfully for such purposes.
Inulin clearance provides an accurate measure of GFR but has the disadvantage of being a tedious method. Clinically, creatinine clearance is widely used to assess renal function.

Creatinine is an endogenous amine produced as a result of muscle catabolism. It is excreted unchanged in the urine by renal filtration only. An advantage of this test is that it can be correlated to the steady state concentration of creatinine in plasma and needs no collection of urine. The method involves determination of serum creatinine levels. Since creatinine production varies with age, weight and gender. Different formulae are used to calculate creatinine clearance from the serum creatinine values.

A direct method for determining creatinine clearance is determination of the amount of creatinine excreted in urine in 24 hours (to calculate the rate of creatinine excretion) and the mean of serum creatinine from blood samples taken just before and immediately after the urine collection period. Following formula is used:

$$C_{\text{cr}} = \frac{\text{Rate of creatinine excretion}}{\text{Serum creatinine in mg\%}}$$

The normal creatinine clearance value is 120 to 130 ml/min. A value of 20 to 50 ml/mm denotes moderate renal failure and values below 10 ml/mm indicate severe renal impairment.

The renal function, RF is calculated by the equation:

$$RF = \frac{\text{Creatinine clearance of patient}}{\text{Creatinine clearance of a normal person}}$$

**Dose Adjustment in Renal Failure**

Generally speaking, drugs in patients with renal impairment have altered pharmacokinetic profile. Their renal clearance and elimination rate are
reduced, the elimination half-life is increased and the apparent volume of distribution is altered. Thus, dose must be altered depending upon the renal function in such patients. However, except for drugs having low therapeutic indices, the therapeutic range of others is sufficiently large and dosage adjustment is not essential. Dosage regimen need not be changed when the fraction of drug excreted unchanged, is 0.3 and the renal function RF is 0.7 of normal. This generalization is based on the assumption that the metabolites are inactive and binding characteristics and drug availability is unaltered and so is the renal function in kidney failure conditions. When the f value approaches unity and RF approaches zero, elimination is extremely slowed down and dosing should be reduced drastically. The significance of nonrenal clearance increases in such conditions.

The required dose in patients with renal impairment can be calculated by the simple formula:

\[
\text{Normal dose} \times \text{RF}
\]

The dosing interval in hours can be computed from the following equation:

\[
\frac{\text{Normal interval (in hours)}}{\text{RF}}
\]

When the drug is eliminated both by renal and nonrenal mechanisms, the dose to be administered in patients with renal failure is obtained from the equation:

\[
\text{Normal dose (RF} \times \text{Fraction excreted in urine + fraction eliminated nonrenally)}
\]
Dialysis and Hemoperfusion

In severe renal failure, the patients are put on dialysis to remove toxic waste products and drugs and their metabolites which accumulate in the body.

Dialysis is a process in which easily diffusible substances are separated from poorly diffusible ones by the use of semi permeable membrane.

There are two procedures for dialysis: -

1. Peritoneal dialysis, and
2. Hemodialysis.

In the former, the semi permeable membrane is the natural membrane of the peritoneal cavity. The method involves introduction of the dialysate fluid into the abdomen by inserting the catheter and draining and discarding the same after a certain period of time. In hemodialysis, the semi permeable membrane is an artificial membrane. Since the system is outside the body, it is also called as extracorporeal dialysis. The equipment is referred to as artificial kidney or hernodialyzer. Apart from the removal of toxic waste from the body, hemodialysis is also useful in the treatment of overdose or poisoning situations where rapid removal of drug becomes necessary to save the life of the patient. Patients of kidney failure require dialysis of blood every 2 days. Each treatment period lasts for 3 to 4 hours.

Factors that govern the removal of substances by hemodialysis are:

**Water Solubility**: Only water soluble substances are dialyzed; lipid soluble drugs such as glutethimide cannot be removed by dialysis.

**Molecular Weight**: Molecules with size less than 500 daltons are dialyzed easily. e.g. many unbound drugs having large molecular weight such as vancomycin cannot be dialyzed.
**Protein Binding:** Drugs bound to plasma proteins or blood cells cannot be dialyzed since dialysis is a passive diffusion process.

**Volume of Distribution:** Drugs with large volume of distribution are extensively distributed throughout the body and therefore less easily removed by dialysis, e.g. digoxin.

Fig.: Shows schematic representation of hemodialysis.

The dialyzing fluid contains sodium, potassium, calcium, chloride and acetate ions, and dextrose and other constituents in the same concentration as that in plasma. The unwanted metabolites in the patient’s blood such as urea, uric acid, creatinine, etc. diffuse into the dialysate until equilibrium. Since the volume of dialysate is much greater than that of blood and since it is replenished with fresh fluid from time to time, almost complete removal of unwanted substances from the blood is possible. Drugs which can be removed by hemodialysis are barbiturates, aminoglycosides, chloral hydrate, lithium, etc.
The rate at which a drug is removed by the dialyzer depends upon the flow rate of blood to the machine and its performance. The term dialysance, also called as dialysis clearance, is used to express the ability of machine to clear the drug from blood. It is defined in a manner similar to clearance by equation:

\[ C_{ld} = \frac{Q(C_{in} - C_{out})}{C_{in}} \]

Where,

- \( C_{ld} \) = Dialysance or dialysis clearance
- \( Q \) = Blood flow rate to dialyzer
- \( C_{in} \) = Concentration of drug in blood entering the dialyzer
- \( C_{out} \) = Concentration of drug in blood leaving the dialyzer

In hemoperfusion, the blood is passed through a bed of adsorbent such as charcoal or resin; as a result, drugs and other unwanted molecules are adsorbed while plasma proteins are not. It is also useful in treating severe drug intoxication. The limitation of hemoperfusion is that it also removes the blood platelets, white cells and endogenous steroids.

**NON-RENAL ROUTES OF DRUG EXCRETION**

Drugs and their metabolites may also be excreted by routes other than the renal route, called as the extrarenal or nonrenal routes of drug excretion. The various such excretion processes are:

1. Biliary excretion
2. Pulmonary excretion  
3. Salivary excretion  
4. Mammary excretion  
5. Skin/dermal excretion  
6. Gastrointestinal excretion  
7. Genital excretion

**Biliary Excretion of Drugs—Enterohepatic Cycling**

The hepatic cells lining the bile canaliculi produce bile. The production and secretion of bile are active processes. The bile secreted from liver, after storage in the gall bladder, is secreted in the duodenum. In humans, the bile flow rate is a steady 0.5 to 1 ml/mm. Bile is important in the digestion and absorption of fats. Almost 90% of the secreted bile acids are reabsorbed from the intestine and transported back to the liver for resecretion. The rest is excreted in feces.

Being an active process, bile secretion is capacity-limited and subject to saturation. The process is exactly analogous to active renal secretion. Different transport mechanisms exist for the secretion of organic anions, cations and neutral polar compounds. A drug whose biliary concentration is less than that in plasma, has a small biliary clearance and vice versa. In some instances, the bile to plasma concentration ratio of drug can approach 1000 in which cases, the biliary clearance can be as high as 500 ml/mm or more.

Compounds that are excreted in bile have been classified into 3 categories on the basis of their bile/plasma concentration ratios:

- **Group A compounds** whose ratio is approximately 1, e.g. sodium, potassium and chloride ions and glucose.
Group B compounds whose ratio is >1, usually from 10 to 1000, e.g. bile salts, bilirubin glucuronide, creatinine, sulfobromophthalein conjugates, etc.

Group C compounds with ratio < 1, e.g. sucrose, inulin, phosphates, phospholipids and mucoproteins.

Drugs can fall in any of the above three categories. Several factors influence secretion of drugs in bile:

1. **Physicochemical Properties of the Drug**
   The most important factor governing the excretion of drugs in bile is their molecular weight. Its influence on biliary excretion is summarized in the Table.

   Polarity is the other physicochemical property of drug influencing biliary excretion. Greater the polarity, better the excretion. Thus, metabolites are more excreted in bile than the parent drugs because of their increased polarity. The molecular weight threshold for biliary excretion of drugs is also dependent upon its polarity. A threshold of 300 daltons and greater than 300 daltons is necessary for organic cations (e.g. quaternaries) and organic anions respectively. Nonionic compounds should also be highly polar for biliary excretion, e.g. cardiac glycosides.

   **Table: Influence of Molecular Weight on Excretion Behavior of Drugs**

<table>
<thead>
<tr>
<th>Below 300 daltons</th>
<th>Excreted mainly in urine; less than 5% is excreted in bile</th>
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<tbody>
<tr>
<td>Above 500 daltons</td>
<td>Excreted mainly in bile; less than 50.0 is excreted in urine</td>
</tr>
</tbody>
</table>
2. Nature of Biotransformation Process

A metabolic reaction that greatly increases the polarity as well as the molecular weight of drug favors biliary excretion of the metabolite. Thus, phase II reactions, mainly glucuronidation and conjugation with glutathione, result in metabolites with increased tendency for biliary excretion (increase the molecular weight by 176 and 300 daltons respectively). Examples of drugs excreted in the bile as glucuronides are morphine chloramphenicol and indomethacin. Stilbestrol glucuronide is almost entirely excreted in bile. Glutathione conjugates are exclusively excreted via bile and are not observable in the urine because of their large molecular size. Conjugation with amino acids and acetylation and methylation reactions do not result in metabolites with greatly increased molecular weight and therefore have little influence on biliary excretion of xenobiotics. For a drug to be excreted unchanged in the bile, it must have a highly polar functional group such as -COOH (cromoglycic acid), -SO$_3$H (amaranth). –NH$_4^+$ (oxyphenonium), etc. Clomiphene citrate, an ovulation inducer, is almost completely removed from the body via biliary excretion.

3. Other Factors

Miscellaneous factors influencing biliary excretion of drugs include sex and species differences, protein-drug binding, disease states, drug interactions, etc.
Substances having high molecular weight show good excretion in bile in case of rats, dogs, and hen and poor excretion in rabbits, guinea pigs and monkeys. The route is more important for the excretion of drugs in laboratory animals than in man. Protein bound drugs can also be excreted in the bile since the secretion is an active process. In cholestasis, the bile flow rate is reduced thereby decreasing biliary excretion of drugs. Agents such as phenobarbital stimulate biliary excretion of drugs, firstly, by enhancing the rate of glucuronidation, and secondly, by promoting bile flow. The route of drug administration also influences biliary drug excretion. Orally administered drugs which during absorption process go to the liver, are excreted more in bile in comparison to parenterally administered drugs. Food also has a direct influence on biliary excretion of drugs. Protein and fat rich food increase bile flow.

The efficacy of drug excretion by the biliary system and hepatic function can be tested by an agent that is exclusively and completely eliminated unchanged in the bile, e.g. sulfobromophthalein. This marker is excreted within half an hour in the intestine when the hepatic function is normal. A delay in its excretion is indicative of hepatic V and biliary malfunction. The marker is also useful in determining hepatic blood flow rate.

The ability of liver to excrete the drug in the bile is expressed by biliary clearance.

\[
\text{Biliary clearance} = \frac{\text{Biliary excretion rate}}{\text{Plasma drug concentration}}
\]

Just as the major portion of bile salts excreted in intestine is reabsorbed, several drugs which are excreted unchanged in bile are also absorbed back into the circulation. Some drugs which are excreted as glucuronides or as glutathione conjugates are hydrolyzed by the intestinal or bacterial enzymes to the parent drugs which are then reabsorbed. The reabsorbed
drugs are again carried to the liver for resecretion via bile into the intestine. This phenomenon of drug cycling between the intestine and the liver is called as enterohepatic cycling or enterohepatic circulation of drugs.

![Diagram of Enterohepatic Circulation]

**Fig: Enterohepatic Circulation**

Such a recycling process continues until the drug is biotransformed in the liver or is excreted in the urine or both. The drugs which are secreted via bile in the intestine but not reabsorbed are finally excreted in the feces.

Enterohepatic circulation is important in the conservation of important endogenous substances such as vitamin B12, vitamin D3, folic acid, several
steroid hormones and bile salts. The process results in prolongation of half-lives of several drugs (e.g. carbenoxolone) which are extensively excreted in bile. The half-life of agents such as DDT, which are resistant to biotransformation and are highly lipophilic, may increase to several days due to such a recycling phenomenon. The prolonged therapeutic activity of oral contraceptives (upto 12 hours) is also due to such a recirculation. Other examples of drugs undergoing enterohepatic circulation are cardiac glycosides, rifampicin, chiorpromazine and indomethacin. Drug interactions affecting enterohepatic cycling occur when agents such as antibiotics kill the intestinal microflora and thus retard hydrolysis of drug conjugates and their subsequent reabsorption, or the unabsorbable ion exchange resins such as cholestyramine which bind strongly to the acidic and neutral drugs (e.g. digitoxin) and thus prevent their reabsorption. The principle of adsorption onto the resins in the GIT can however be used to treat pesticide poisoning by promoting their fecal excretion.

Biliary excretion of drugs can be assessed by giving the drugs parenterally and detecting their presence in feces. This also rules out the doubt about the incomplete absorption of such drugs when given orally and observed in feces.

**Pulmonary Excretion**

Gaseous and volatile substances such as the general anesthetics (e.g. halothane) are absorbed through the lungs by simple diffusion. Similarly, their excretion by diffusion into the expired air is possible. Factors influencing pulmonary excretion of a drug include pulmonary blood flow, rate of respiration, solubility of the volatile substance, etc. Gaseous anesthetics such as nitrous oxide which are not very soluble in blood are excreted rapidly. Generally intact gaseous drugs are excreted but metabolites are not. Compounds like alcohol which has high solubility in
blood and tissues are excreted slowly by the lungs. The principle involved in the pulmonary excretion of benzene and halobenzenes is analogous to that of steam distillation.

**Salivary Excretion**

Excretion of drugs in saliva is also a passive diffusion process and therefore predictable on the basis of pH-partition hypothesis. The pH of saliva varies from 5.8 to 8.4. The mean salivary pH is 6.4. Unionized, lipid soluble drugs at this pH are excreted passively in the saliva. Amount of drug will be excreted to saliva depends on percent ionization and saliva/plasma drug concentration ratio (S/P).

The S/P ratios have been found to be less than 1 for weak acids and greater than 1 for weak bases i.e. basic drugs are excreted more in saliva as compared to acidic drugs. The salivary concentration of some drugs reaches as high as 0.1%. Since the S/P ratio is fairly constant for several drugs, their blood concentration can be determined by detecting the amount of drug excreted in saliva. e.g. caffeine, theophylline, phenytoin, carbamazepine, etc. Some drugs are actively secreted in saliva, e.g. lithium, the concentration of which is sometimes 2 to 3 times that in plasma. Penicillin and phenytoin are also actively secreted in saliva.

The bitter after taste in the mouth of a patient on medication is an indication of drug excretion in saliva. In few instances, the process is responsible for side effects such as black hairy tongue in patients receiving antibiotics, gingival hyperplasia due to phenytoin, etc. Some basic drugs inhibit saliva secretion and are responsible for dryness of mouth. Drugs excreted in saliva can undergo cycling in a fashion similar enterohepatic cycling, e.g. sulfonamides, antibiotics, clonidine, etc.
Mammary Excretion

Excretion of a drug in milk is important since it can gain entry into the breast feeding infant. Milk consists of lactic secretions originating from the extracellular fluid and is rich in fats and proteins. About 0.5 to 1 liter/day of milk secreted in lactating mothers.

Excretion of drugs in milk is a passive process and is dependent upon pH-partition behavior, molecular weight, lipid solubility and degree of ionization. The pH of milk varies from 6.4 to 7.6 with a mean pH of 7.0. Free, unionized, lipid soluble drugs diffuse into the mammary alveolar cells passively. The extent of drug excretion in milk can be determined from milk/plasma drug concentration ratio (M/P). Since milk is acidic comparison to plasma, as in the case of saliva, weakly basic drugs concentrate more in milk and have M/P ratio greater than 1. The opposite is true for weakly acidic drugs. It has been shown that for acidic drugs, excretion in milk is inversely related to the molecular weight and partition coefficient and that for basic drugs, is inversely related to the degree of ionization and partition coefficient. Drugs extensively bound to plasma proteins, e.g. diazepam, are less secreted in milk. Since milk contains proteins, drugs excreted in milk can bind to it. The amount of drug excreted in milk is generally less than 1% and the fraction consumed by the infant is too less to reach therapeutic or toxic levels. But some potent drugs such as barbiturates, morphine and ergotamine may induce toxicity in infants. Discoloration of teeth with tetracycline and jaundice due to interaction of bilirubin with sulfonamides are examples of adverse effects precipitated due to drug excretion in the milk. Nicotine is also secreted in the milk of mothers who smoke. Thus, wherever possible, nursing mothers should avoid drugs and smoking. If medication is unavoidable, the infant should be bottle fed.
**Skin Excretion**

Drugs excreted through the skin via sweat also follow pH-partition hypothesis. Passive excretion of drugs and their metabolites through skin is responsible to some extent for the urticaria and dermatitis and other hypersensitivity reactions. Compounds such as benzoic acid, salicylic acid, alcohol and antipyrine and heavy metals like lead, mercury and arsenic are excreted in sweat.

**Gastrointestinal Excretion**

Excretion of drugs into the GIT usually occurs after parenteral administration when the concentration gradient for passive diffusion is favorable. The process is reverse of GI absorption of drugs. Water soluble and ionized forms of weakly acidic and basic drugs are excreted in the GIT, e.g. nicotine and quinine arc excreted in stomach. Orally administered drugs can also be absorbed and excreted in the GIT. Drugs excreted in the GIT arc reabsorbed into the systemic circulation and undergo recycling.

**Genital Excretion**

Reproductive tract and genital secretions may contain the excreted drugs. Some drugs have been detected in semen. Drugs can also get excreted via the lacrymal fluid. A summary of drugs excreted by various routes is given in Table.
References:
