Name of Unit	Introduction to biopharmaceutics
Course/Subject Name	Biopharmaceutics and Pharmacokinetics
Course/Subject Code	BP604T
Class: B. Pharm. Semester	6th
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Learning Outcome of Unit-1

LO	Learning Outcome(LO)	Course Outcome Code
LO1	Students will learn about the basic concepts in biopharmaceutics and pharmacokinetics.	BP604.1
LO2	Students will learn about the mechanism of drug absorption and various factors affecting the drug absorption in git.	BP604.2
LO3	Students will learn about the distribution of drug in the body fluids and factors effecting this process	BP604.2

CONTENT OF MODULE

Topics
Absorption : • Mechanisms of drug absorption through GIT. • Factors influencing drug absorption though GIT. • Absorption of drug from Non per oral extra-vascular routes. **Distribution :** • Tissue permeability of drugs. • Binding of drugs. • Volume of drug distribution, plasma and tissue protein binding of drugs. • Factors affecting protein-drug binding. • Kinetics of protein binding. • Clinical significance of protein binding of drugs.

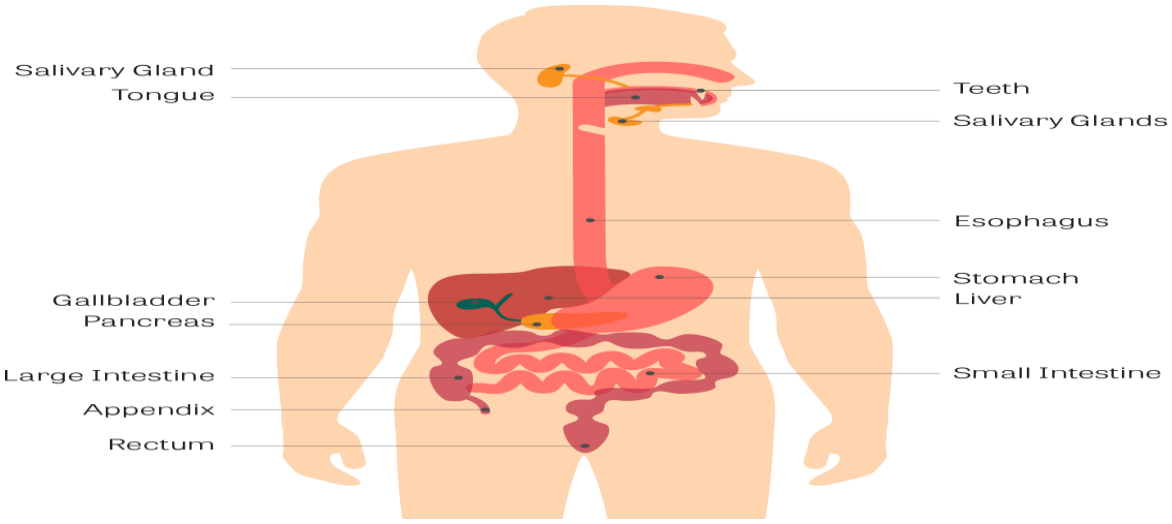
ABSORPTION

Gastrointestinal Tract: The gastrointestinal tract is a muscular tube, approximately 6 m in length with varying diameter. It stretches from the mouth to the anus and consists of four main anatomical areas; the oesophagus, the stomach, the small intestine and the large intestine, or colon. The luminal surface of the tube is not smooth but very rough, thereby increasing the surface area for absorption. The wall of the gastrointestinal tract is essentially similar in structure along its length, consisting of four principal histological layers:

1. The **serosa,** which is an outer layer of epithelium with supporting connective tissues which are continuous with the peritoneum.
2. The **muscularis externa,** which contains three layers of smooth muscle tissue, a thinner outer layer, which is longitudinal in orientation, and two inner layers, whose fibres are oriented in a circular pattern. Contractions of these muscles provide the forces for movement of gastrointestinal tract contents and physical breakdown of food.
3. The **submucosa,** which is a connective tissue layer containing some secretory tissue and which is richly supplied with blood and lymphatic vessels. A network of nerve cells, known as the submucous plexus, is also located in this layer.
4. The **mucosa**, which is essentially composed of three layers: the muscularis mucosae, which can alter the local conformation of the mucosa, a layer of connective tissue known as the lamina propria, and the epithelium.
5. The majority of the gastrointestinal epithelium is covered by a layer or layers of mucus. This is a viscoelastic translucent aqueous gel that is secreted throughout the gastrointestinal tract, acting as a protective layer and a mechanical barrier. Mucus is a constantly changing mix of many secretions and exfoliated epithelial cells. It has a large water component (~95%). Its other primary components, which are responsible for its physical and functional properties, are large glycosylated proteins called mucins. Mucins consist of a protein backbone approximately 800 amino acids long and oligosaccharide side chains that are typically up to 18 residues in length. The mucous layer ranges in thickness from 5 μm to 500 μm along the length of the gastrointestinal tract, with average values of approximately 80 μm. Mucus is constantly being removed from the luminal surface of the gastrointestinal tract through abrasion and acidic and/or enzymatic breakdown, and it is continually replaced from beneath. The turnover time has been estimated at 4 to 5 hours, but this may well be an underestimate and is liable to vary along the length of the tract.

The gastrointestinal tract includes the mouth, pharynx, esophagus, stomach, small intestine, large intestine, and anus.

The extent of drug absorption in a segment of the gastrointestinal tract depends generally on the rate of absorption as well as on the exposed surface area and time available for drug absorption.



Anatomy of gastrointestinal tract

Mechanism of drug absorption:

The three broad categories of drug transport mechanisms involved in absorption are –

A. Transcellular/intracellular transport
B. Paracellular/intercellular transport
C. Vesicular transport

A. Transcellular/Intracellular Transport

It is defined as the passage of drugs across the GI epithelium. It is the most common pathway for drug transport. The 3 steps involved in transcellular transport of drugs are –

- Permeation of GI epithelial cell membrane, a lipoidal barrier – this is the major obstacle to drug absorption
- Movement across the intracellular space (cytosol).
- Permeation of the lateral or basolateral membrane- this is of secondary importance.

The various transcellular transport processes involved in drug absorption are –

2. Passive Transport Processes – These transport processes do not require energy other than that of molecular motion (Brownian motion) to pass through the lipid bilayer. Passive transport processes can be further classified into following types –

A. Passive diffusion.
B. Pore transport.
C. Ion-pair transport.
D. Facilitated- or mediated-diffusion.

3. Active Transport Processes – This transport process requires energy from ATP to move drug molecules from extracellular to intracellular milieu. These are of two types –

a. Primary active transport.
b. Secondary active transport – this process is further subdivided into two –

I. Symport (co-transport).
II. Antiport (counter-transport).

B. Paracellular/Intercellular Transport

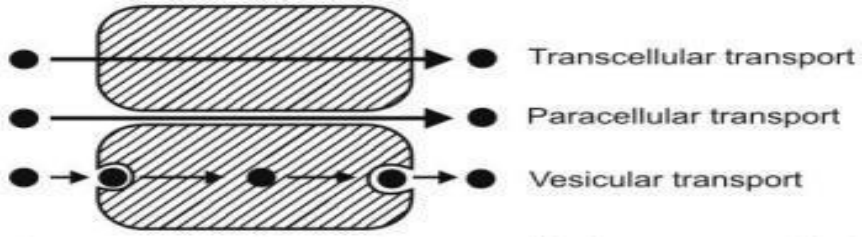
Paracellular/Intercellular Transport – is defined as the transport of drugs through the junctions between the GI epithelial cells. This pathway is of minor importance in drug absorption. The two paracellular transport mechanisms involved in drug absorption are –

1. **Permeation through tight junctions of epithelial cells** – this process basically occurs through openings which are little bigger than the aqueous pores. Compounds such as insulin and cardiac glycosides are taken up this mechanism.
2. **Persorption** – is permeation of drug through temporary openings formed by shedding of two neighbouring epithelial cells into the lumen. Paracellular transport differs from pore transport in that the former involves transfer of drug across epithelium and through the cellular junctions whereas in the case of latter, the molecules are transferred from outside of the epithelial cell into the cell through pores present in the cell membrane.

C. Vesicular or **Corpuscular Transport (Endocytosis)**

Vesicular or **Corpuscular Transport (Endocytosis)** – Like active transport, these are also energy dependent processes but involve transport of substances within vesicles into a cell. Since the mechanism involves transport across the cell membrane, the process can also be classified as transcellular. Vesicular transport of drugs can be classed into two categories –

1. ***Pinocytosis.***
2. ***Phagocytosis***.



compares transcellular, paracellular and vesicular transport mechanisms.

Passive Diffusion

Also called **non-ionic diffusion**, it is the major process for absorption of more than 90% of the drugs. The driving force for this process is the **concentration** or **electrochemical gradient**. It is defined as the difference in the drug concentration on either side of the membrane. Drug movement is a result of the kinetic energy of molecules. Since no energy source is required, the process is called as passive diffusion. During passive diffusion, the drug present in the aqueous solution at the absorption site partitions and dissolves in the lipid material of the membrane and finally leaves it by dissolving again in an aqueous medium, this time at the inside of the membrane.

Passive diffusion is best expressed by **Fick's first law of diffusion**, which states that the drug molecules diffuse from a region of higher concentration to one of lower concentration until equilibrium is attained and that the rate of diffusion is directly proportional to the concentration gradient across the membrane. It can be mathematically expressed by the following equation:

$$\frac{dQ}{dt} = \frac{DAK_{m/w}}{h}(C_{GIT} - C) \qquad (2.1)$$

dQ/dt = rate of drug diffusion (amount/time). It also represents the rate of appearance of drug in blood

D = diffusion coefficient of the drug through the membrane (area/time) A = surface area of the absorbing membrane for drug diffusion (area)

Km/w = partition coefficient of the drug between the lipoidal membrane and the aqueous GI fluids (no units) (CGIT – C) = difference in the concentration of drug in the GI fluids and the plasma, called as the concentration gradient (amount/volume)

h = thickness of the membrane (length)

Based on the above equation, certain characteristics of passive diffusion can be generalized –

1. The drug moves down the concentration gradient indicating *downhill transport.* The process is energy-independent and non-saturable.
2. The rate of drug transfer is directly proportional to the concentration gradient between GI fluids and the blood compartment.
3. Greater the area and lesser the thickness of the membrane, faster the diffusion; thus, more rapid is the rate of drug absorption from the intestine than from the stomach.
4. The process is rapid over short distances and slower over long distance.

Pore Transport

It is also called as **convective transport**, **bulk flow** or **filtration**. This mechanism is responsible for transport of molecules into the cell through the protein channels present in the cell membrane. Following are the characteristics of pore transport –

1. The driving force is constituted by the hydrostatic pressure or the osmotic differences across the membrane due to which bulk flow of water along with small solid molecules occurs through such aqueous channels. Water flux that promotes such a transport is called as **solvent drag**.
2. The process is important in the absorption of low molecular weight (less than 100), low molecular size (smaller than the diameter of the pore) and generally water-soluble drugs through narrow, aqueous-filled channels or pores in the membrane structure—for example, urea, water and sugars.
3. Chain-like or linear compounds of molecular weight up to 400 Daltons can be absorbed by filtration.

Drug permeation through water-filled channels is of particular importance in renal excretion, removal of drug from the cerebrospinal fluid and entry of drugs into the liver.

Ion-Pair Transport

Yet another mechanism that explains the absorption of drugs like quaternary ammonium compounds and sulphonic acids, which ionise under all pH conditions, is ion-pair transport. Despite their low o/w partition coefficient values, such agents penetrate the membrane by forming reversible neutral complexes with endogenous ions of the GIT like mucin. Such neutral complexes have both the required lipophilicity as well as aqueous solubility for passive

diffusion. Such a phenomenon is called as **ion-pair transport** (Fig. 2.5). Propranolol, a basic drug that forms an ion pair with oleic acid, is absorbed by this mechanism.

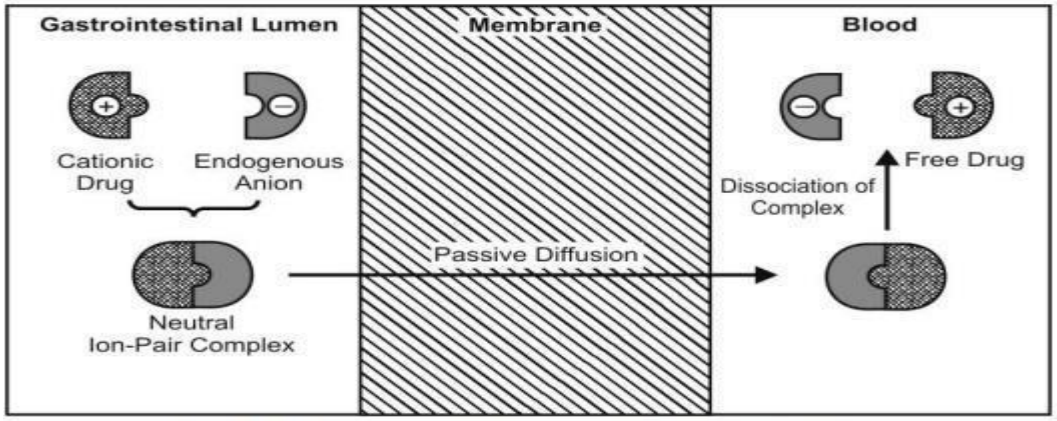


Fig. 2 5. Ion-pair transport of a cationic drug

Carrier-Mediated Transport

Some polar drugs cross the membrane more readily than can be predicted from their concentration gradient and partition coefficient values. This suggests presence of specialized transport mechanisms without which many essential water-soluble nutrients like monosaccharides, amino acids and vitamins will be poorly absorbed. The mechanism is thought to involve a component of the membrane called as the *carrier* that binds reversibly or non- covalently with the solute molecules to be transported. This carrier-solute complex traverses across the membrane to the other side where it dissociates and discharges the solute molecule. The carrier then returns to its original site to complete the cycle by accepting a fresh molecule of solute. Carriers in membranes are proteins (transport proteins) and may be an enzyme or some other component of the membrane. They are numerous in all biological membranes and are found dissolved in the lipid bilayer of the membrane.

Important characteristics of carrier-mediated transport are:

1. A carrier protein always has an uncharged (non-polar) outer surface which allows it to be soluble within the lipid of the membrane.
2. The carriers have no directionality; they work with same efficiency in both directions.
3. The transport process is structure-specific i.e. the carriers have special affinity for and transfer a drug of specific chemical structure only (i.e. lock and key arrangement); generally the carriers have special affinity for essential nutrients.

4. Since the system is structure-specific, drugs having structure similar to essential nutrients, called as false nutrients, are absorbed by the same carrier system. This mechanism is of particular importance in the absorption of several antineoplastic agents like 5-fluorouracil and 5- bromouracil which serve as false nutrients.
5. As the number of carriers is limited, the transport system is subject to competition between agents having similar structure.

Two types of carrier-mediated transport systems have been identified. They are—facilitated diffusion and active transport.

Facilitated Diffusion

It is a carrier-mediated transport system that operates down the concentration gradient (downhill transport) but at a much a faster rate than can be accounted by simple passive diffusion. The driving force is concentration gradient (hence a passive process). Since no energy expenditure is involved, the process is not inhibited by metabolic poisons that interfere with energy production. Facilitated diffusion is of limited importance in the absorption of drugs. Examples of such a transport system include entry of glucose into RBCs and intestinal absorption of vitamins B1 and B2. A classic example of passive facilitated diffusion is the GI absorption of vitamin B12. An intrinsic factor (IF), a glycoprotein produced by the gastric parietal cells, forms a complex with vitamin B12 which is then transported across the intestinal membrane by a carrier system (Fig. 2.7).

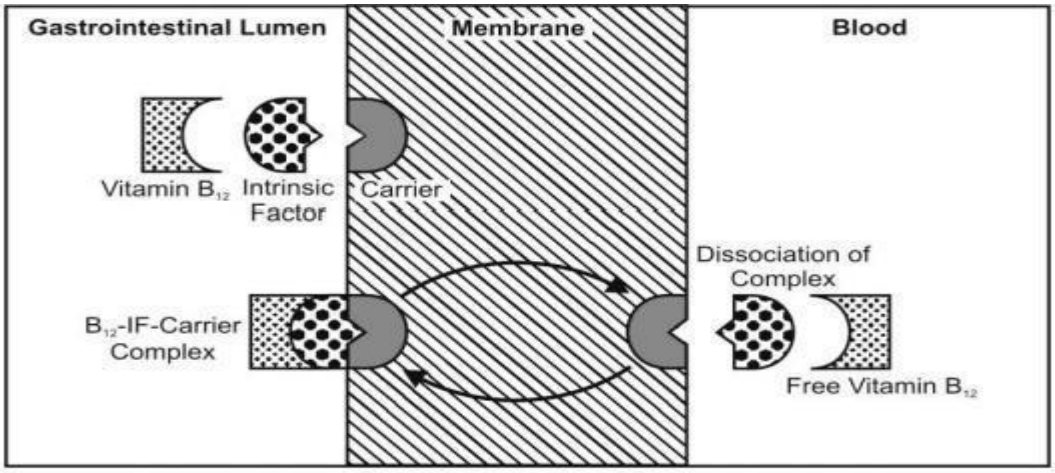


Fig. 2.7. Facilitated diffusion of vitamin B12

Active Transport

This transport mechanism requires energy in the form ATP. Active transport mechanisms are further subdivided into -

Primary active transport – In this process, there is direct ATP requirement. Moreover, the process transfers only one ion or molecule and in only one direction, and hence called as uniporter e.g. absorption of glucose. Carrier proteins involved in primary active transport are of two types –

i. ***Ion transporters –*** are responsible for transporting ions in or out of cells. A classic example of ATP-driven ion pump is proton pump which is implicated in acidification of intracellular compartments. Two types of ion transporters which play important role in the intestinal absorption of drugs have been identified –

a. Organic anion transporter – which aids absorption of drugs such as pravastatin and atorvastatin.

b. Organic cation transporter – which aids absorption of drugs such as diphenhydramine.

ii. ***ABC (ATP-binding cassette) transporters –*** are responsible for transporting small foreign molecules (like drugs and toxins) especially out of cells (and thus called as *efflux pumps*) which make them clinically important. A classic example of ABC transporter is *P-glycoprotein* (P-gp). The latter is responsible for pumping hydrophobic drugs especially anticancer drugs out of cells. Presence of large quantity of this protein thus makmakes the cells resistant to a variety of drugs used in cancer chemotherapy, a phenomenon called as multi-drug resistance. It is for this reason that P-gp is called as multi-drug resistance (MDR) protein. ABC transporters present in brain capillaries pump a wide range of drugs out of brain.

b. Secondary active transport – In these processes, there is no direct requirement of ATP i.e. it takes advantage of previously existing concentration gradient. The energy required in transporting an ion aids transport of another ion or molecule (co-transport or coupled transport) either in the same direction or in the opposite direction. Accordingly this process is further subdivided into –

Symport (co-transport) – involves movement of both molecules in the same direction e.g. *Na*+- *glucose* symporter uses the potential energy of the Na+ concentration gradient to move glucose against its concentration gradient. A classic example of symporter is peptide transporter called as H+-coupled peptide transporter (PEPT1) which is implicated in the intestinal absorption of peptide-like drugs such as β-lactam antibiotics.

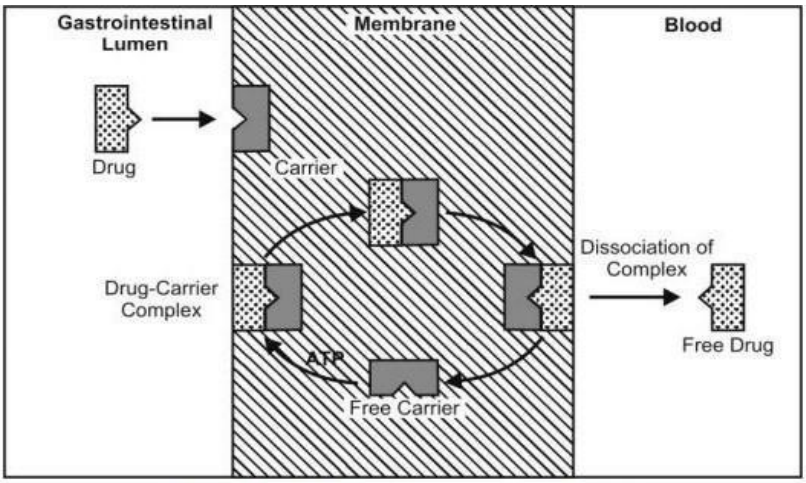


Fig. 2.8. Active absorption of a drug

Antiport (counter-transport) – involves movement of molecules in the opposite direction e.g. expulsion of H+ ions using the Na+ gradient in the kidneys.

Figure 2.8 illustrates active transport of a drug and figure 2.9 represents the types of active transport.

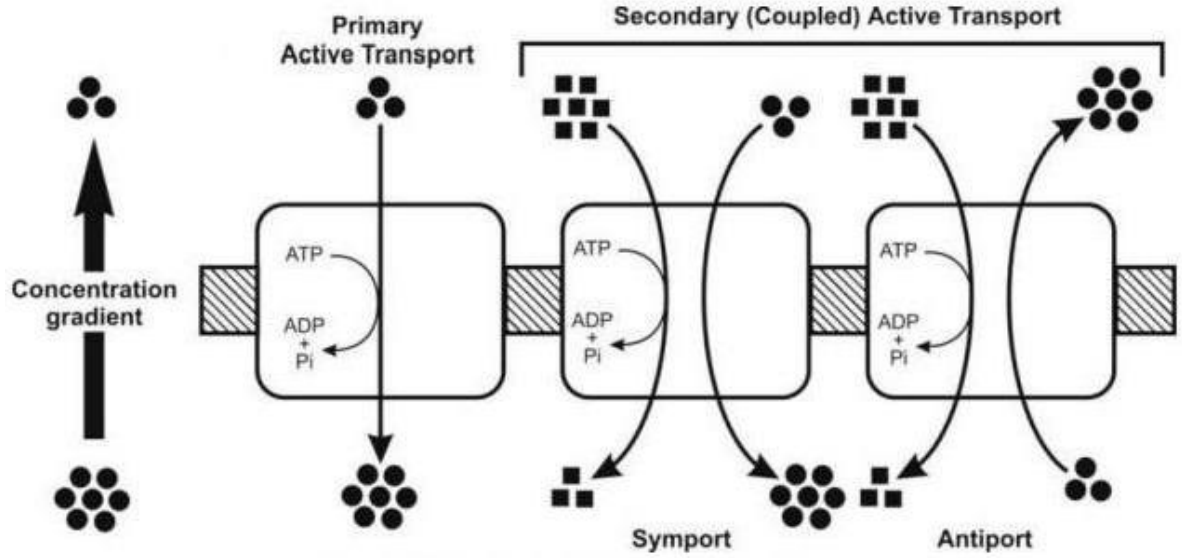


Fig. 2.9. Types of active transport

Active transport is a more important process than facilitated diffusion in the absorption of nutrients and drugs and differs from it in several respects:

1. The drug is transported from a region of lower to one of higher concentration i.e. against the concentration gradient (in the case of ions, against an electrochemical gradient) or *uphill transport*, without any regard for equilibrium.
2. The process is faster than passive diffusion.
3. Since the process is uphill, energy is required in the work done by the carrier.

4. As the process requires expenditure of energy, it can be inhibited by metabolic poisons that interfere with energy production like fluorides, cyanide and dinitrophenol and lack of oxygen, etc. Endogenous substances that are transported actively include sodium, potassium, calcium, iron, glucose, certain amino acids and vitamins like niacin, pyridoxin and ascorbic acid. Drugs having structural similarity to such agents are absorbed actively, particularly the agents useful in cancer chemotherapy. Examples include absorption of 5-fluorouracil and 5- bromouracil via the pyrimidine transport system, absorption of methyldopa and levodopa via an L-amino acid transport system and absorption of ACE inhibitor enalapril via the small peptide carrier system. A good example of competitive inhibition of drug absorption via active transport is the impaired absorption of levodopa when ingested with meals rich in proteins. Active transport is also important in renal and biliary excretion of many drugs and their metabolites and secretion of certain acids out of the CNS.

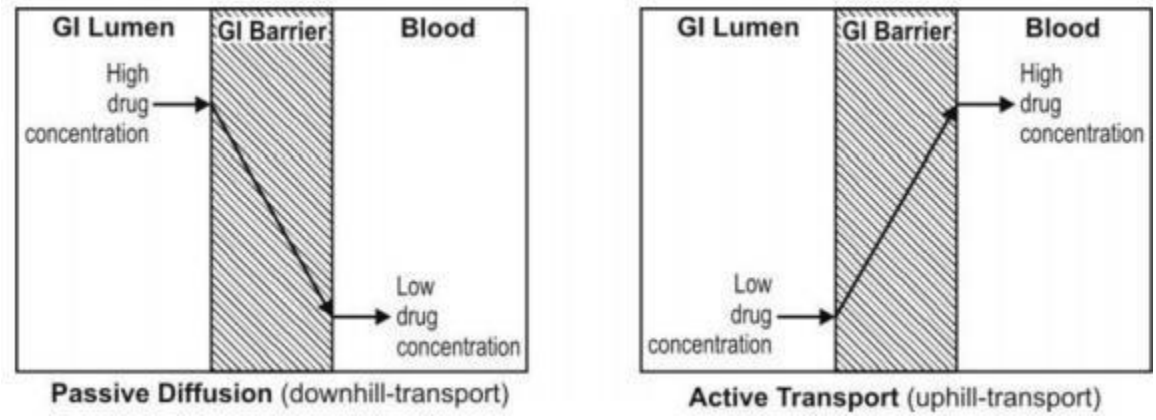


Figure 2.10 compares active and passive transport

Endocytosis

It is a minor transport mechanism which involves engulfing extracellular materials within a segment of the cell membrane to form a saccule or a vesicle (hence also called as **corpuscular** or **vesicular transport**) which is then pinched-off intracellularly (Fig. 2.11). This is the only transport mechanism whereby a drug or compound does not have to be in an aqueous solution in order to be absorbed.

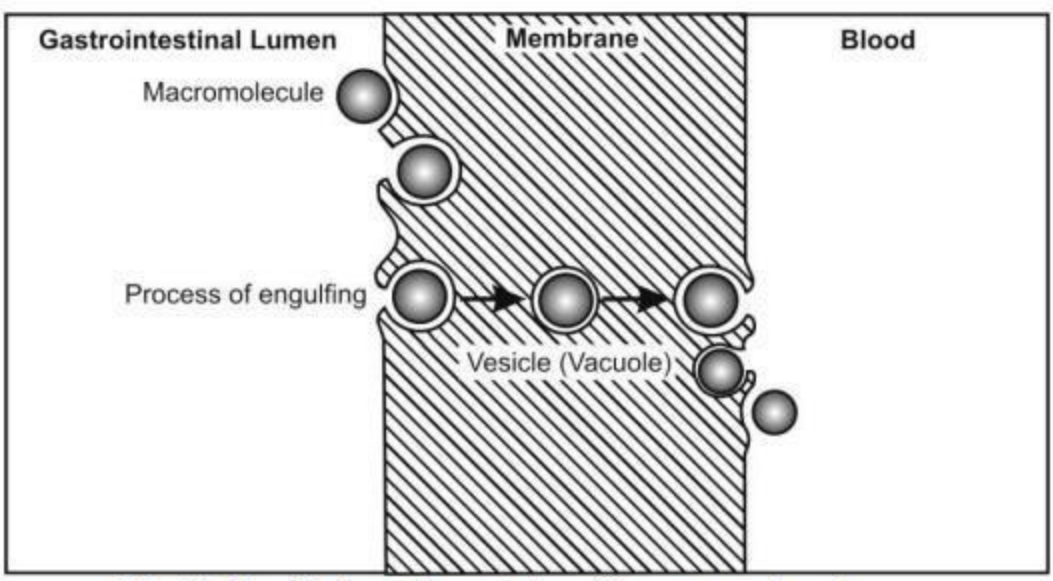


Fig. 2.11. Endocytic uptake of macromolecules.

This phenomenon is responsible for the cellular uptake of macromolecular nutrients like fats and starch, oil soluble vitamins like A, D, E and K, water soluble vitamin like B12 and drugs such as insulin. Another significance of such a process is that the drug is absorbed into the lymphatic circulation thereby bypassing first- pass hepatic metabolism.

Endocytosis includes two types of processes:

1. **Phagocytosis (*cell eating*):** adsorptive uptake of solid particulates, and
2. *Pinocytosis (cell drinking):* uptake of fluid solute.

Orally administered Sabin polio vaccine, large protein molecules and the botulism toxin (that causes food poisoning) are thought to be absorbed by pinocytosis. Sometimes, an endocytic vesicle is transferred from one extracellular compartment to another. Such a phenomenon is called as **transcytosis**.

Factors affecting drug absorption:

A Pharmaceutical factors:

1. **Physicochemical properties of drug:**
   a. Drug solubility and dissolution rate
   b. Particle size and effective surface area
   c. Polymorphism and amorphism
   d. Pseudopolymorphism(hydrates or solvates)
   e. Salt form of the drug

f. Lipophilicity of the drug
g. Drug stability
h. Stereochemical nature of the drug

2. Formulation factors:

a. Disintegration time
b. Manufacturing variables
c. Nature and type of dosage form
d. Pharmaceutical ingredients (excepients)
e. Product age and storage conditions

B. Patient related factors:

a. Age
b. Gastric emptying time
c. Intestinal transit time
d. Gastrointestinal pH
e. Diseased states
f. Blood flow through the GIT
g. Gastrointestinal contents
h. Presystemic metabolism

PHARMACEUTICAL FACTORS

In order to design a formulation that will deliver the drug in the most bioavailable form, the pharmacist must consider –

1. Physicochemical properties of the drug, and
2. Type of formulation (e.g. solution, suspension, tablet, etc.), and
3. Nature of excipients in the formulation.

PHYSICOCHEMICAL FACTORS AFFECTING DRUG ABSORPTION

Drug Solubility and Dissolution Rate:

Except in case of controlled-release formulations, disintegration and deaggregation occur rapidly if it is a well-formulated dosage form. Thus, the two critical slower rate-determining processes in the absorption of orally administered drugs are:

1. Rate of dissolution, and
2. Rate of drug permeation through the biomembrane.

Dissolution is the RDS for hydrophobic, poorly aqueous soluble drugs like griseofulvin and spironolactone; absorption of such drugs is often said to be dissolution rate-limited. If the drug is hydrophilic with high aqueous solubility—for example, cromolyn sodium or neomycin, then dissolution is rapid and RDS in the absorption of such drugs is rate of permeation through the biomembrane. In other words, absorption of such drugs is said to be permeation rate-limited or transmembrane rate-limited.

Factors Affecting Drug Dissolution and Dissolution Rate

Factors of *in vivo* importance that can affect dissolution and hence absorption can be categorized into 2 classes:

1. Physicochemical properties of the drug, and
2. Dosage form factors.

The various physicochemical properties of drug that affect drug dissolution and its rate are—solubility, particle size, polymorphism, salt form, pseudopolymorphism, complexation, wettability, etc. Dosage form factors include several formulation factors and excipients incorporated in the dosage form.

Particle Size and Effective Surface Area of the Drug

Particle size and surface area of a solid drug are inversely related to each other. Smaller the drug particle, greater the surface area. Two types of surface area of interest can be defined:

1. **Absolute surface area** which is the total area of solid surface of any particle, and
2. **Effective surface area** which is the area of solid surface exposed to the dissolution medium.

Polymorphism and Amorphism

Depending upon the internal structure, a solid can exist either in a crystalline or amorphous form. When a substance exists in more than one crystalline form, the different forms are designated as polymorphs and the phenomenon as polymorphism. Polymorphs are of two types:

Enantiotropic polymorph is the one which can be reversibly changed into another form by altering the temperature or pressure e.g. sulphur, and

Monotropic polymorph is the one which is unstable at all temperatures and pressures e.g. glyceryl stearates.

The polymorphs differ from each other with respect to their physical properties such as solubility, melting point, density, hardness and compression characteristics. They can be

prepared by crystallizing the drug from different solvents under diverse conditions. The existence of the polymorphs can be determined by using techniques such as optical crystallography, X-ray diffraction, differential scanning calorimetry, etc.

Depending on their relative stability, one of the several polymorphic forms will be physically more stable than the others. Such a stable polymorph represents the lowest energy state, has highest melting point and least aqueous solubility. The remaining polymorphs are called as metastable forms which represent the higher energy state, have lower melting points and higher aqueous solubilities. Because of their higher energy state, the metastable forms have a thermodynamic tendency to convert to the stable form.

Some drugs can exist in amorphous form (i.e. having no internal crystal structure). Such drugs represent the highest energy state and can be considered as supercooled liquids. They have greater aqueous solubility than the crystalline forms because the energy required to transfer a molecule from crystal lattice is greater than that required for non-crystalline (amorphous) solid— for example, the amorphous form of novobiocin is 10 times more soluble than the crystalline form.

Amorphous > Metastable > Stable.

Hydrates/Solvates (Pseudopolymorphism)

The crystalline form of a drug can either be a polymorph or a molecular adduct or both. The stoichiometric type of adducts where the solvent molecules are incorporated in the crystal lattice of the solid are called as the solvates, and the trapped solvent as solvent of crystallization. The solvates can exist in different crystalline forms called as pseudopolymorphs**.** This phenomenon is called as pseudopolymorphism. When the solvent in association with the drug is water, the solvate is known as a hydrate. Hydrates are most common solvate forms of drugs.

Salt Form of the Drug

Most drugs are either weak acids or weak bases. One of the easiest approaches to enhance the solubility and dissolution rate of such drugs is to convert them into their salt forms.

Drug pKa and Lipophilicity and GI pH—pH Partition Hypothesis

The pH partition theory (Brodie et al) explains in simple terms, the process of drug absorption from the GIT and its distribution across all biological membranes. The theory states that for

drug compounds of molecular weight greater than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by:

1. The dissociation constant (pKa) of the drug.
2. The lipid solubility of the unionised drug (a function of drug Ko/w).
3. The pH at the absorption site.

The lower the pKa of an acidic drug, the stronger the acid i.e., greater the proportion of ionized form at a particular pH. The higher the pKa of a basic drug, the stronger the base .Amount of drug that exist in unionized form and in ionized form is a function of pKa of drug & pH of the fluid at the absorption site and it can be determined by Henderson-hesselbach equation:

For, Acidic drugs

$$pH = pKa + \log [\text{ionized form}]/ [\text{Unionized form}]$$

For, Basic drugs

$$pH = pKa + \log [\text{unionized form}]/ [\text{Ionized form}]$$

Lipophilicity and Drug Absorption

As mentioned earlier, it is the pKa of a drug that determines the degree of ionisation at a particular pH and that only the unionised drug, if sufficiently lipid soluble, is absorbed into the systemic circulation. Thus, even if the drug exists in the unionised form, it will be poorly absorbed if it has poor lipid solubility (or low Ko/w).

In other words, a perfect hydrophilic-lipophilic balance (HLB**)** should be there in the structure of the drug for optimum bioavailability.

DOSAGE FORM (PHARMACO-TECHNICAL) FACTORS

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 deaggregate 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 most widely used conventional solid dosage forms, tablets and capsules. The dosage form related factors that influence dissolution and hence absorption of a drug from such formulations are:

- Excipients (formulation ingredients apart from the active principles), and
- Manufacturing processes.

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 functionality 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.

As a general rule, 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.

PATIENT RELATED FACTORS AFFECTING DRUG ABSORPTION

Age

In infants, the gastric pH is high and intestinal surface and blood flow to the GIT is low resulting in altered absorption pattern in comparison to adults. In elderly persons, causes of impaired drug absorption include altered gastric emptying, decreased intestinal surface area and GI blood flow, higher incidents of achlorhydria and bacterial overgrowth in small intestine.

Gastric Emptying

Apart from dissolution of a drug and its permeation through the biomembrane, the passage from stomach to the small intestine, called as **gastric emptying**, can also be a rate-limiting step in drug absorption because the major site of drug absorption is intestine. Thus, generally speaking, rapid gastric emptying increases bioavailability of a drug.

Rapid gastric emptying is advisable where:

1. A rapid onset of action is desired e.g. sedatives.
2. Dissolution of drug occurs in the intestine e.g. enteric-coated dosage forms.
3. The drugs are not stable in the gastric fluids e.g. penicillin G and erythromycin.
4. The drug is best absorbed from the distal part of the small intestine e.g. vitamin B12.

Intestinal Transit

Since small intestine is the major site for absorption of most drugs, long intestinal transit time is desirable for complete drug absorption.

Gastrointestinal pH

A tremendous 107 fold difference in the hydrogen ion concentration is observed between the gastric and colon fluids. The GI pH generally increases gradually as one move down the stomach to the colon and rectum (*see* Fig. 2.22). GI fluid pH influence drug absorption in several ways:

1. **Disintegration:** The disintegration of some dosage forms is pH sensitive. With enteric-coated formulations, the coat dissolves only in the intestine followed by disintegration of the tablet.

2. **Dissolution:** A large number of drugs are either weak acids or weak bases whose solubility is greatly affected by pH. A pH that favours 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, GI pH is of great significance in the oral bioavailability of drugs. 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.

Absorption of drug from Non per oral extra-vascular routes:

NON PER OS Means other than oral routes which by passes the GIT and reaches to systemic circulation. One of the major advantages of administering drugs by non-invasive transmucosal (& transdermal) routes such as nasal, buccal, rectal, etc. is that greater systemic availability is attainable . Moreover, peptide and protein drugs can also be delivered by such routes.

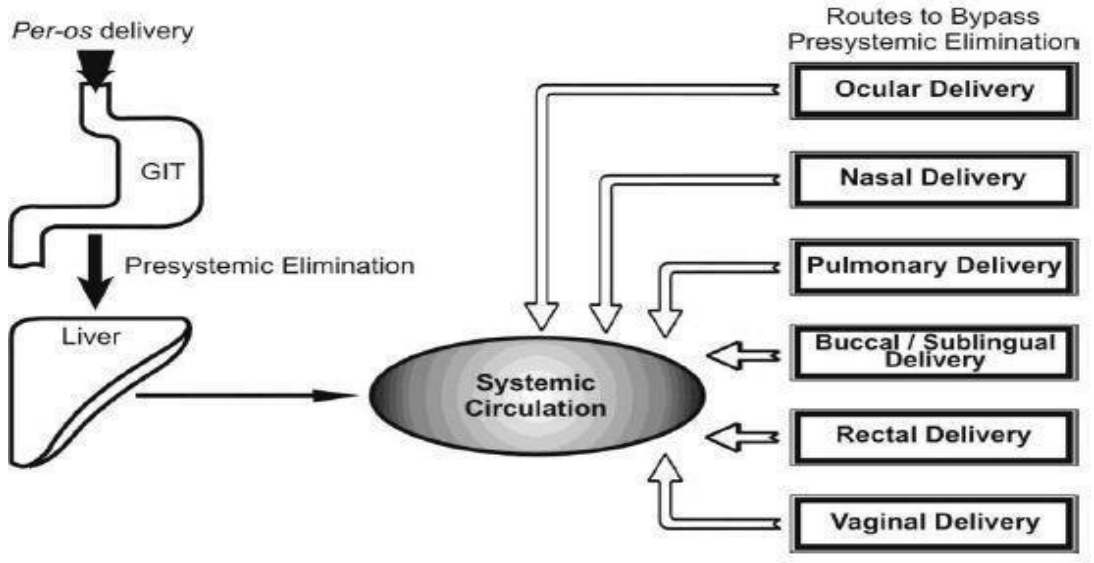


Fig. Various transmucosal non-invasive routes of drug administration to bypass presystemic elimination in GIT/liver

Buccal/Sublingual Administration

The two sites for oral mucosal delivery of drugs are:

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

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.

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 faecal matter retards drug absorption. Though highly vascularised, absorption is slower because of limited surface area.

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 m2 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. Majority of drugs applied topically are meant to exert their effect locally. When topically applied drugs are meant to exert their effects systemically, the mode of administration is called as percutaneous or transdermal delivery. Percutaneous absorption occurs only if the topically applied drug permeates the dermal capillaries and enters the blood stream.

Intramuscular Administration:

Absorption of drugs from i.m. sites is relatively rapid but much slower in comparison to i.v. injections. Factors that determine rate of drug absorption from i.m. 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 (deltoid) > Thigh (vastus lateralis) > Buttocks (gluteus maximus)

2. Since blood flow rate is often the rate-limiting step in absorption of drugs from i.m. sites, most rapid absorption is from deltoid muscles and slowest from gluteal region. The absorption rate decreases in circulatory disorders such as hypotension.
3. Lipid solubility and ionisation of drug: highly lipophilic drugs are absorbed rapidly by passive diffusion whereas hydrophilic and ionised drugs are slowly absorbed through capillary pores.
4. 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. There is some evidence that small peptides and fluids can cross the endothelial tissue of blood capillaries and lymph vessels by transport in small vesicles that cross the membrane, a process called as cytopemphis.

Subcutaneous Administration:

All factors that influence i.m. drug absorption are also applicable to absorption from subcutaneous site. Generally, absorption of drugs from a s.c. site is slower than that from i.m. 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:

1. 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.

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).

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. A faster rate that is dependent upon drug lipophilicity, and

b. A slower rate which is dependent upon drug molecular weight.

In case of lipophilic drugs, rapid absorption by diffusion is observed up to 400 Daltons and satisfactory absorption up to 1000 Daltons.

Intraocular Administration

Topical application of drugs to the eyes is mainly meant for local effects such as mydriasis, miosis, anaesthesia 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 lachrymal fluid influences absorption of weak electrolytes such as pilocarpine. On the other hand, pH of the formulation influences lachrymal output—higher pH decreases tear flow and promotes drug absorption whereas lower pH solutions increase lachrymation and subsequent drug loss due to drainage. Rate of blinking also influences drainage loss.

DISTRIBUTION

Introduction

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. Disposition is defined as the processes that tend to lower the plasma concentration of drug. The two major drug disposition processes are –

1. Distribution which involves reversible transfer of a drug between compartments.
2. Elimination which causes irreversible loss of drug from the body. Elimination is further divided into two processes –

a. Biotransformation (metabolism)

b. Excretion.

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 fluids 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 pharmacological 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.

Steps in Drug Distribution

Distribution of drug present in systemic circulation to extravascular tissues involves following steps -

1. Permeation of free or unbound drug present in the blood through the capillary wall (occurs rapidly) and entry into the interstitial/extracellular fluid (ECF).
2. Permeation of drug present in the ECF through the membrane of tissue cells and into the intracellular fluid. This step is rate-limiting and depends upon two major factors –

a. Rate of perfusion to the extracellular tissue

b. Membrane permeability of the drug.

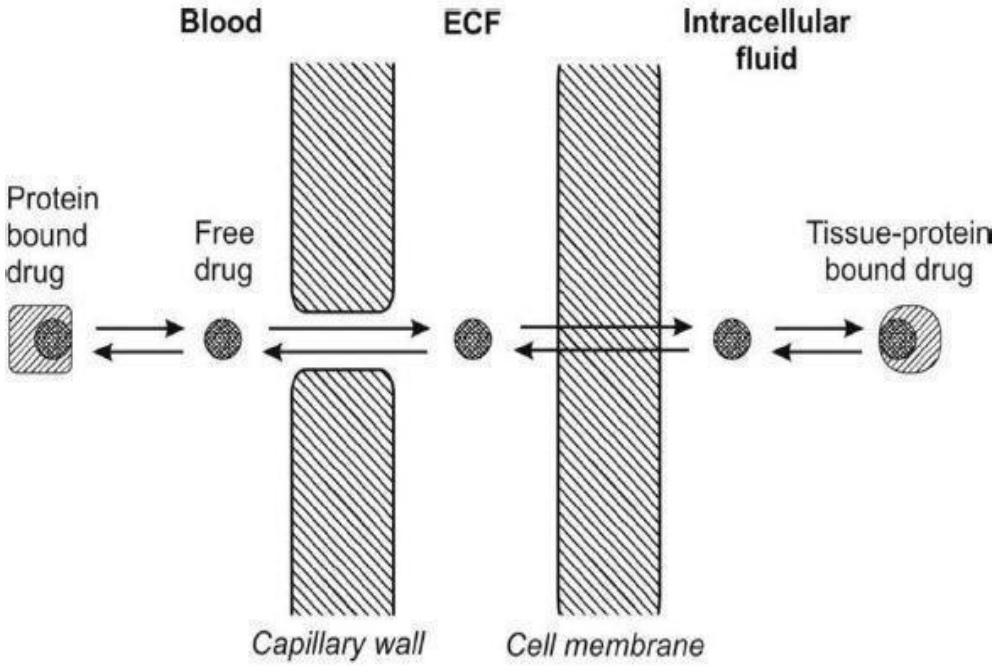


Fig. Schematic of the steps involved in drug distribution

TISSUE PERMEABILITY OF DRUGS:

The two major rate-determining steps in the distribution of drugs are:

1. Rate of tissue permeation, 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 permeation rate-limited. Tissue permeability of a drug depends upon the physicochemical properties of the drug as well as the physiological barriers that restrict diffusion of drug into tissues.

Physicochemical Properties of the Drug:

Important physicochemical properties of drug that influence its distribution are molecular size, degree of ionisation, partition coefficient and stereochemical nature.

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, ionisation constant and lipophilicity of the drug. Only small, water-soluble molecules and ions of size below 50 Daltons enter the cell through aqueous filled channels whereas those of larger size are restricted unless a specialized transport system exists for them.

The degree of ionisation 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 ionisation and diffusion of drugs into cells. A drug that remains unionised 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 ionisation at plasma or ECF pH depends upon their pKa. All drugs that ionise 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 unionised drugs which are generally lipophilic, rapidly cross the cell membrane.

Physiological 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 physiological barriers are:

1. Simple capillary endothelial barrier
2. Simple cell membrane barrier

3. Blood-brain barrier
4. Blood-CSF barrier
5. Blood- placental barrier
6. Blood-testis barrier.

VOLUME OF DISTRIBUTION:

It is defined as the hypothetical volume of body fluid into which a drug is dissolved or distributed. It is called as **apparent volume** because all parts of the body equilibrated with the drug do not have equal concentration.

Apparent volume of distribution = Amount of drug in body/Plasma drug concentration

PROTEIN BINDING OF DRUGS

A drug in the body can interact with several tissue components of which the two major categories are-

1. Blood, and
2. Extravascular tissues.

The interacting molecules are generally the macromolecules such as proteins, DNA or adipose. The proteins are particularly responsible for such an interaction. The phenomenon of complex formation with proteins is called as **protein binding of drugs**.

Protein binding may be divided into –

1. **Intracellular binding** – where the drug is bound to a cell protein which may be the drug receptor; if so, binding elicits a pharmacological response. These receptors with which drug interact to show response are called as **primary receptors**.
2. **Extracellular binding** – where the drug binds to an extracellular protein but the binding does not usually elicit a pharmacological response. These receptors are called **secondary** or **silent receptors**.

Mechanisms of Protein-Drug Binding

Binding of drugs to proteins is generally reversible which suggests that it generally involves weak chemical bonds such as –

1. Hydrogen bonds
2. Hydrophobic bonds

3. Ionic bonds, or
4. Van der Waal's forces.

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.

Binding of drugs falls into 2 classes:

1. Binding of drugs to blood components like—

a. Plasma proteins

b. Blood cells

Binding of drugs to extravascular tissue proteins, fats, bones, etc.

BINDING OF DRUGS TO BLOOD COMPONENTS

Protein	*Molecular Weight*	*Concentration (g%)*	*Drugs that bind*
Human Serum Albumin	65,000	3.5-5.0	Large variety of all types of drugs
α1-Acid Glycoprotein	44,000	0.04-0.1	Basic drugs such as imipramine, lidocaine, quinidine, etc.
Lipoproteins	200,000 to 3,400,000	Variable	Basic, lipophilic drugs like chlorpromazine
α1-Globulin	59,000	0.003-0.007	Steroids like corticosterone, and thyroxine and cyanocobalamin
α2-Globulin	1,34,000	0.015-0.06	Vitamins A, D, E and K and cupric ions
Haemoglobin	64,500	11-16	Phenytoin, pentobarbital, and phenothiazines

Table- Blood Proteins to which Drugs Bind

Plasma Protein-Drug Binding

Following entry of a drug into the systemic circulation, the first things with which it can interact are blood components like plasma proteins, blood cells and haemoglobin. 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:

Abumin > α1-Acid Glycoprotein > Lipoproteins > Globulins.

TISSUE BINDING OF DRUGS
(TISSUE LOCALIZATION OF DRUGS)

The body tissues, apart from HSA, comprise 40% of the body weight which is 100 times that of HSA. Hence, tissue-drug binding is much more significant than thought to be.

A drug can bind to one or more of the several tissue components. Tissue-drug binding is important in distribution from two viewpoints:

1. It increases the apparent volume of distribution of drugs in contrast to plasma protein binding which decreases it. This is because the parameter is related to the ratio of amount of drug in the body to the plasma concentration of free drug and the latter is decreased under conditions of extensive tissue binding of drugs.
2. Tissue-drug binding results in localization of a drug at a specific site in the body (with a subsequent increase in biological half-life). This is more so because a number of drugs bind irreversibly with the tissues (contrast to plasma protein-drug binding); for example, oxidation products of paracetamol, phenacetin, chloroform, carbon tetrachloride and bromobenzene bind covalently 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 site 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 > Muscles

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 or children during odontogenesis results in permanent brown-yellow discoloration of teeth. Lead is known to replace calcium from bones and cause their brittleness.

Table- Comparison Between Plasma Protein-Drug Binding and Tissue-Drug Binding

	Plasma protein-drug binding	*Tissue-drug binding*
1.	Binding involves weak bonds and thus reversible.	Binding generally involves strong covalent bonds and thus irreversible.
2.	Drugs that bind to plasma proteins have small apparent volume of distribution.	Drugs that bind to extravascular tissues have large apparent volume of distribution.
3.	Half-life of plasma protein bound drug is relatively short.	Half-life of extravascular tissue bound drug is relatively long.
4.	Does not result in toxicity.	Tissue toxicity is common.
5.	Displacement from binding sites is possible by other drugs.	Displacement by other drugs generally does not occur.
6.	Competition between drugs for binding to plasma proteins can occur.	Tissue-drug binding is generally non-competitive.

Factors affecting protein-drug binding:

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

1. Drug related factors

a. Physicochemical characteristics of the drug

b. Concentration of drug in the body

c. Affinity of a drug for a particular binding component

Protein/tissue related factors

. Physicochemical characteristics of the protein or binding agent

a. Concentration of protein or binding component

b. Number of binding sites on the binding agent

Drug interactions

. Competition between drugs for the binding site (displacement interactions)

a. Competition between the drug and normal body constituents

b. Allosteric changes in protein molecule

Patient related factors

. Age

a. Intersubject variations

b. Disease states

DRUG RELATED FACTORS

Physicochemical Characteristics of the Drug

As mentioned earlier, 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 sulphonamides bind more to HSA whereas cationic or basic drugs such as imipramine and alprenolol bind to AAG. Neutral, unionised drugs bind more to lipoproteins.

Concentration of Drug in the Body

The extent of protein-drug binding can change with both 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.

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 radio-opaque medium, has so great an affinity for plasma proteins that it has a half-life of 2½ years.

PROTEIN/TISSUE RELATED FACTORS

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.

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.

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.

DRUG INTERACTIONS

Competition Between Drugs for the Binding Sites (Displacement Interactions)

When two or more drugs can bind to the same site, competition between them for interaction 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 sulphonamides from their HSA binding sites.

PATIENT RELATED FACTORS

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, is increased.

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.

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.

IMPORTANT QUESTIONS

Very short answer questions 2 Marks

1. Passive diffusion.
2. Facilitated diffusion.
3. Pinocytosis.
4. Endocytosis.
5. Intestinal Transit.
6. First pass effect.
7. Factors effecting distribution of drugs.
8. Protein binding.
9. Glomerular Filtration.
10. Extra Cellular Fluids.

Short answer questions 5 marks

1. What are different mechanism for drug transport in body.
2. Write note on protein binding of drugs.
3. Highlight Absorption mechanism for drug across biological membrane.
4. Write note on apparent volume of distribution.
5. Significance of plasma protein binding.

Long answer questions 10 Marks

1. Discuss various factors affecting volume of distribution of drug. Also explain its role in Pharmacokinetics of drug.
2. Discuss in detail the passage of drug across biological barrier.
3. Write note on pH partition hypothesis.
4. Discuss various factors affecting absorption of drug from GIT.