PASSAGE OF DRUG MOLECULES ACROSS THE CELL MEMBRANE AND DISTRIBUTION

Dr. UMER SUFYAN.M
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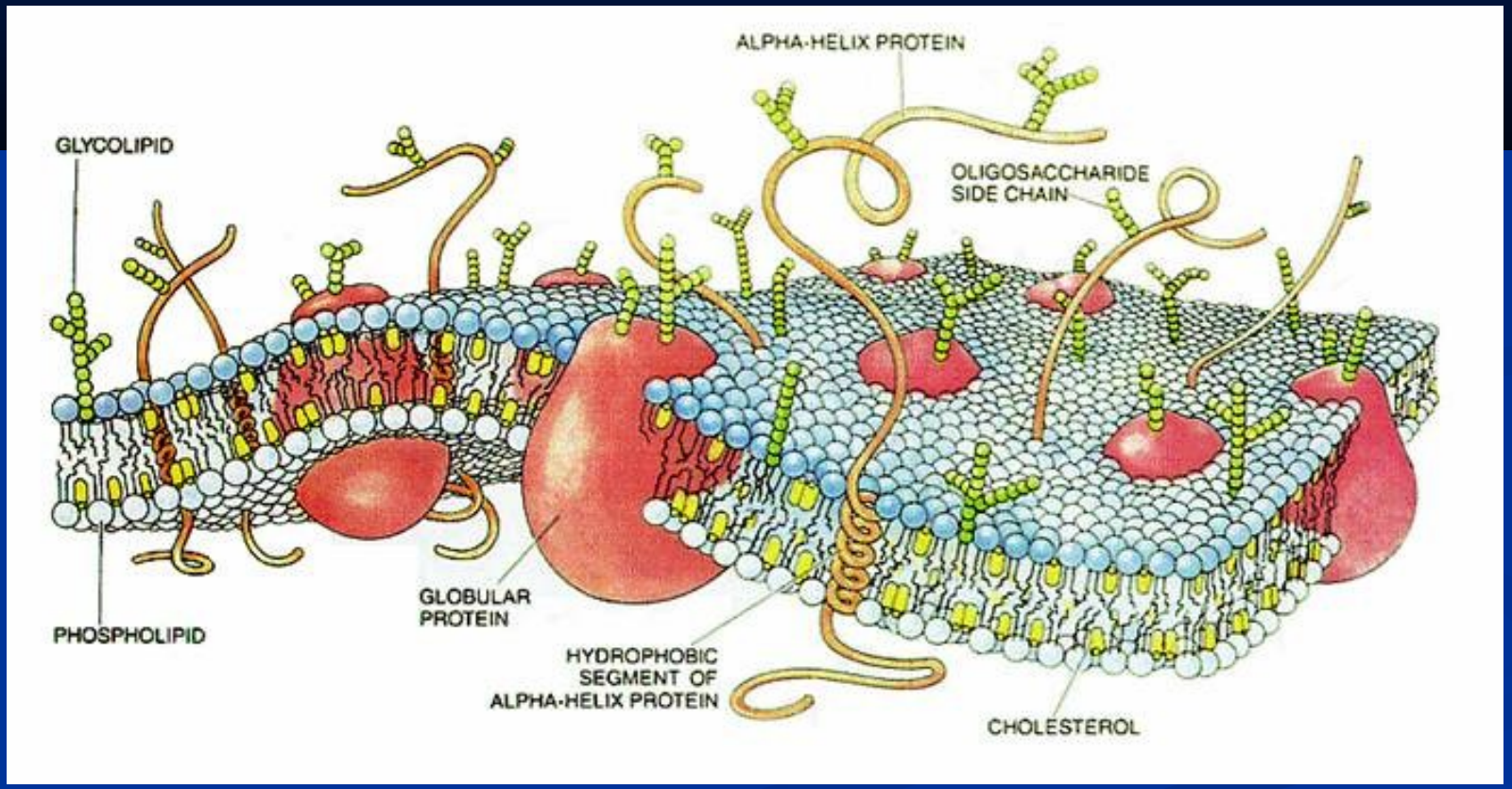

ALPHA-HELIX PROTEIN
GLYCOLIPID
OLIGOSACCHARIDE
SIDE CHAIN
GLOBULAR
PROTEIN
PHOSPHOLIPID
HYDROPHOBIC
SEGMENT OF
ALPHA-HELIX PROTEIN
CHOLESTEROL

INTRODUCTION

- The movement or translocation of drug from one side of biological barrier to other is called Biotransport and the mechanism underlying the transfer of drug across biological barriers are called the Transport mechanism.

The major transport mechanism are :

1. Passive Diffusion
2. Carrier Mediated transport
    - A. Facilitated Diffusion
    - B. Active Transport
3. Pinocytosis or Phagocytosis
4. Filtration

1. PASSIVE DIFFUSION

- Passive diffusion is the process by which the drug molecules pass through a biological barrier from a phase of higher concentration to the phase of lower concentration without requiring any expenditure of energy.

- Nonionised drugs can diffuse passively across the biological barrier at a rate proportional to their lipid : water partition coefficient.

- For a weak electrolytes Diffusion depends upon the degree of ionisation of the drug, the PH of the surrounding environment and the lipid :water partition coefficient

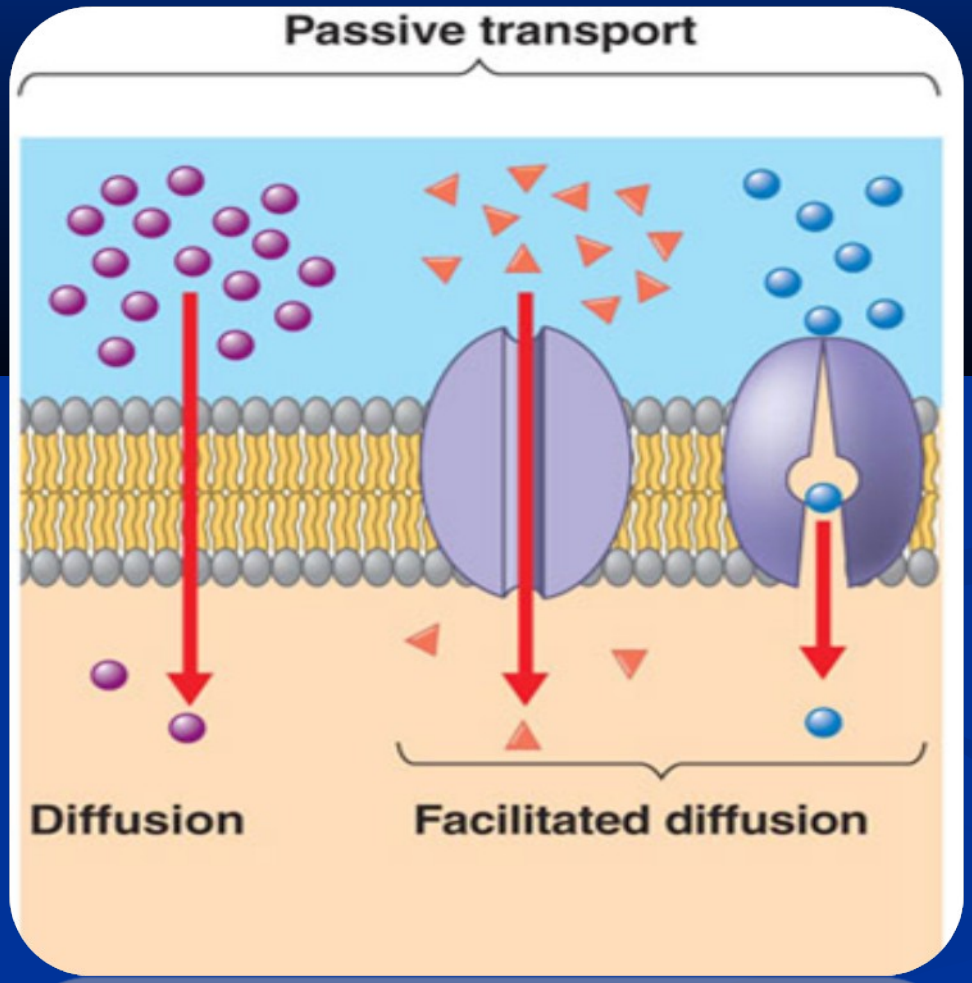
- Many drugs are acidic or basic compounds, which are ionized to a certain degree in aqueous medium. Their degree of ionization depends on their dissociation constant (pKa) and the pH of the environment and extension of ionisation.
- Henderson-Hasselbach equation:

For Acidic drug:

$$pKa = pH + \log \frac{\text{Conc. Of nonionised Acid}}{\text{Conc.Of ionised Acid}}$$

For Basic drug:

$$pKa = pH + \log \frac{\text{Conc. Of ionised base}}{\text{Coc. Of non ionised base}}$$


Passive transport
Diffusion
Facilitated diffusion

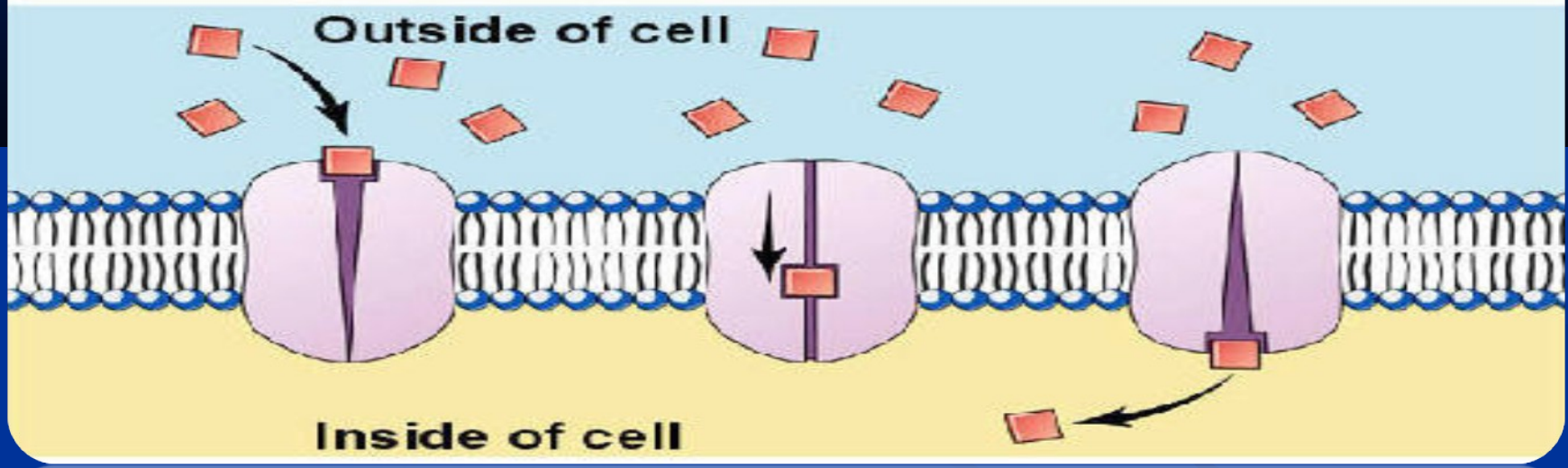
Carrier-mediated transport

- Polar compounds like sugar and amino acids and certain drugs of therapeutic interest cannot penetrate through membrane by passive diffusion but are moved by a carrier system present on the membrane surface
- Carrier molecules are usually proteins which combine with a drug substrate and form a complex
- After the complex crosses the membrane ; carrier dissociates from the drug and carrier returns to the original side of membrane for reuse.

Facilitated diffusion :

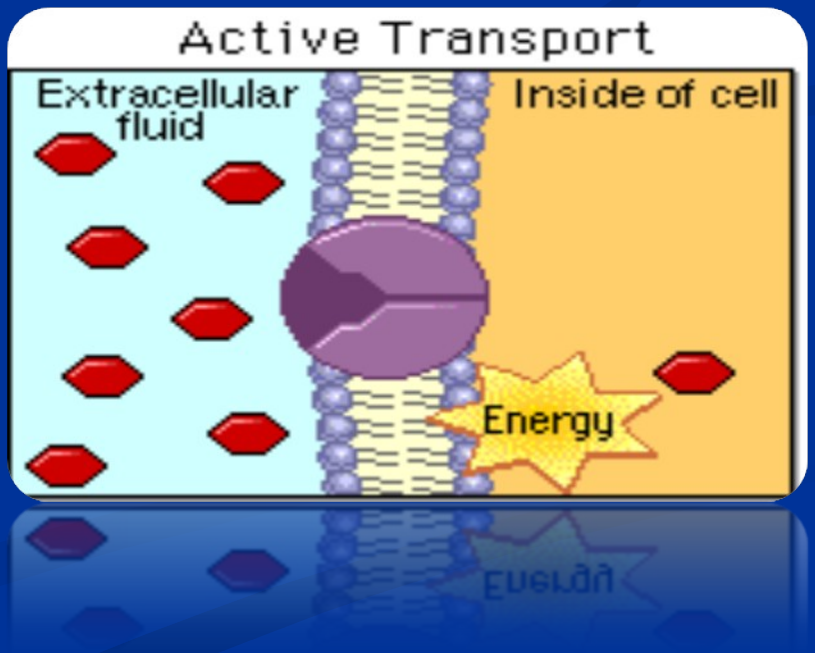
- Carrier-mediated transport from higher to lower concentration without needing energy and translocates the substrate in the direction of electrochemical gradient.
- e.g. GLUT 4 enhances the permeation of glucose across a muscle cell membrane

Facilitated Diffusion



Active transport

- Transport of drug is energy dependent
- Carrier mediated transport against a concentration gradient
- From lower to higher concentration



- Depending upon the driving force. the active transport can be subdivided into primary or secondary active transport

Primary active transport :

- The bio-transportation of drugs is directly coupled with ATP hydrolysis for deriving the energy and is usually carried by ABC group of biotransportrs

Secondary active transport :

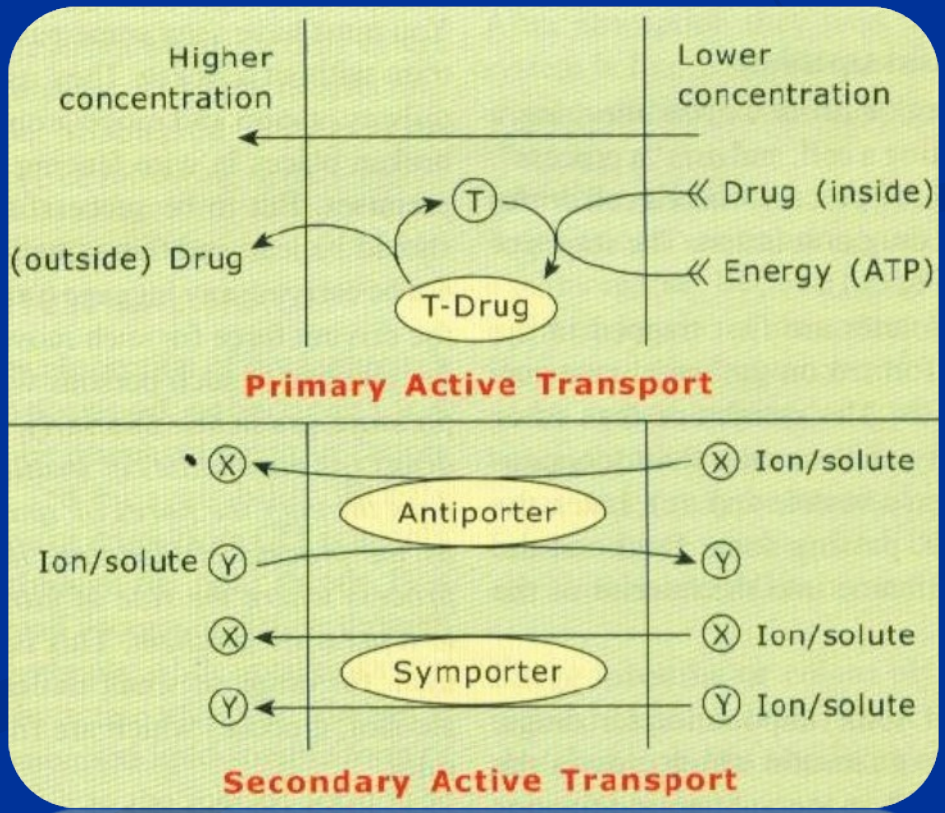
- One ion or the solute (X) supplies the driving force for the transport of other ion/solute (Y).
- Depending on the direction of flux of X and Y, the transporter can be called either a symporter or an antiporter

- Symporter transports X and Y in the same direction .

  Eg: $Na^+/K^+ /2Cl^-$

- Antiporter transports X and Y in the opposite directions

  Eg: Na^+ and H^+ exchanger



Pinocytosis :

- Process where a cell drink or engulfs a fluid or a drug in solution.

  E.g: -Insulin crosses the BBB by this process

Phagocytosis:

- Process were particulate matter transferred by local invagination of cell membrane

  Eg: -Poisoning by botulinum toxin

ATP-binding cassette transporter

- Serve as channels to transport molecules across cell membranes.
- Facilitate the import of nutrients into cells or export toxic products into the surrounding medium, which are essential for cellular homeostasis, cell growth, cell divisions, and bacterial immunity.
- Hydrolyze ATP and use it to move molecules against the concentration gradient or transport substrates across lipid membranes.

- ABC transporters transporter molecules such as

  -ions, sugars, amino acids, vitamins, peptides, polysaccharides, hormones, lipids.

- ABC transporters are involved in diverse cellular processes such as

  - maintenance of osmotic homeostasis,
  - nutrient uptake,
  - antigen processing,
  - cell division,
  - bacterial immunity,
  - pathogenesis

P-glycoprotein

- P-glycoprotein (permeability glycoprotein, abbreviated as P-gp or Pgp) is an ABC-transporter of the MDR/TAP subfamily;
- It was first discovered in Chinese hamster ovary cell mutants by Juliano R. L. and Ling. V. in 1976;
- Function: pump out substrates through plasma membrane with the combination and hydrolysis of ATP;
- Clinical related: drug efflux.

Biological Aspect

- Activity:
  - ATPase activity
  - Substrate transport: wide specificity
- Activators & **Inhibitors**
- Multidrug resistance===>chemotherapy

Expression and Functions of Pgp

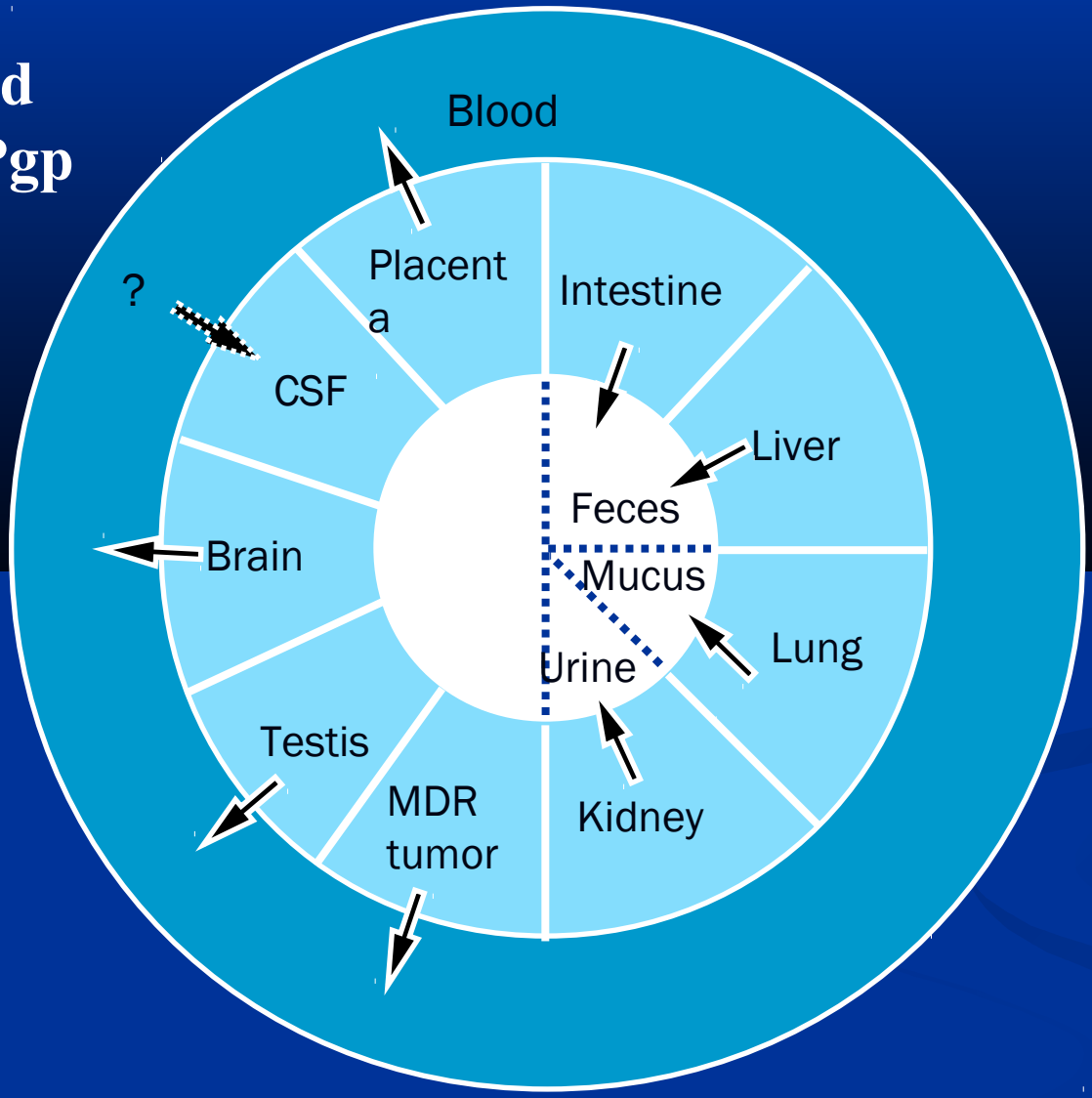


Figure 1. Expression and functions of P-glycoprotein in various organs of the human body. The bold solid arrows indicate the known direction of transport, while the one marked "?" means unsure function. Red indicates vasculature, blue represents tissue, and white indicates excreta. CSF, cerebrospinal fluid; MDR, multidrug resistance.

Drawn by Keynote. Cited from P Kannan et al. Imaging the Function of P-Glycoprotein With Radiotracers: Pharmacokinetics and In Vivo Applications Clin Pharmacol Ther. 2009;86:368–377.

- P-gp transports various substrates across the cell membrane including:
- Drugs such as tacrolimus and quinidine
- Chemotherapeutic agents such as etoposide and vinblastine
- Cardiac glycosides like digoxin
- Immunosuppressive agents
- Glucocorticoids like dexamethasone
- HIV-type 1 antiretroviral therapy agents like protease inhibitors and nonnucleoside reverse transcriptase inhibitors.

It is inhibited by many drugs, such as:

- Azithromycin , Captopril , Clarithromycin , Cyclosporine
- Quinidine, Quinine , Reserpine , Ritonavir , Verapamil

Absorption

Absorption is movement of a drug from site of administration to the central compartment and the extent to which this occurs.

- Only lipid soluble drugs can cross the biological Membranes
- Lipid soluble drugs is unionised ,ionised form is water soluble

Absorption via GI tract

Mouth :

Saliva pH is slightly acidic.

lipid soluble or non ionised basic drugs can be absorbed from this site.

After sublingual absorption directly reaches systemic circulation bypassing first –pass metabolism.

Eg :Isosorbide dinitrate

Stomach :

pH is Acidic

lipid soluble unionised acidic drug can be absorbed

Absorption pass through hepatic portal system –First pass metabolism

Intestine :

pH Alkaline

drug have to go hepatic portal before reaching systemic circulation

Colon :

pH is alkaline

From external haemorrhoidal vein major amount of drug enter directly to systemic circulation

Parentral route :

Drug injected I V completely and rapidly distributed

Reaches blood stream directly without crossing any membrane

Lungs :

Vapourised form and spray of suspended microfined particles are absorbed by simple diffussion from pulmunary epithelium and mucous membrane of trachea and lungs

Eg : Salbutamol , General anaesthetics

Topical site :

- Absorption throgh intact skin.
- Keratinised epidermis behaves like a barrier to permeability
- Dermis is quit permeable to lipid soluble drugs.
- Significant absorption can occur if skin is abraded.

Eg : Transdermal : Nitroglycerin , scopolamine

Mucous membrane : Oxytocin , vasopressin

Ophthalmic drugs : eye drops

Factors that Affect the rate and Extent of Drug Absorption

1. Dosage form / Drug formulation
2. Physicochemical Properties of the Drug
    - molecular weight
    - pH
    - lipophilic vs hydrophilic
        * Partition coefficient

- most drugs are weak acids or bases that are present in solution as both the nonionized and ionized forms
- nonionized substances are usually more lipid-soluble and can diffuse readily across the cell membrane
- ionized molecules have low lipid solubility and are unable to penetrate the lipid membrane

3. Physiologic variables

- gastric motility
- pH at the absorptive site
- area of absorbing surface
- mesentric blood flow
- presystemic elimination / first pass

BIOAVAILABILITY

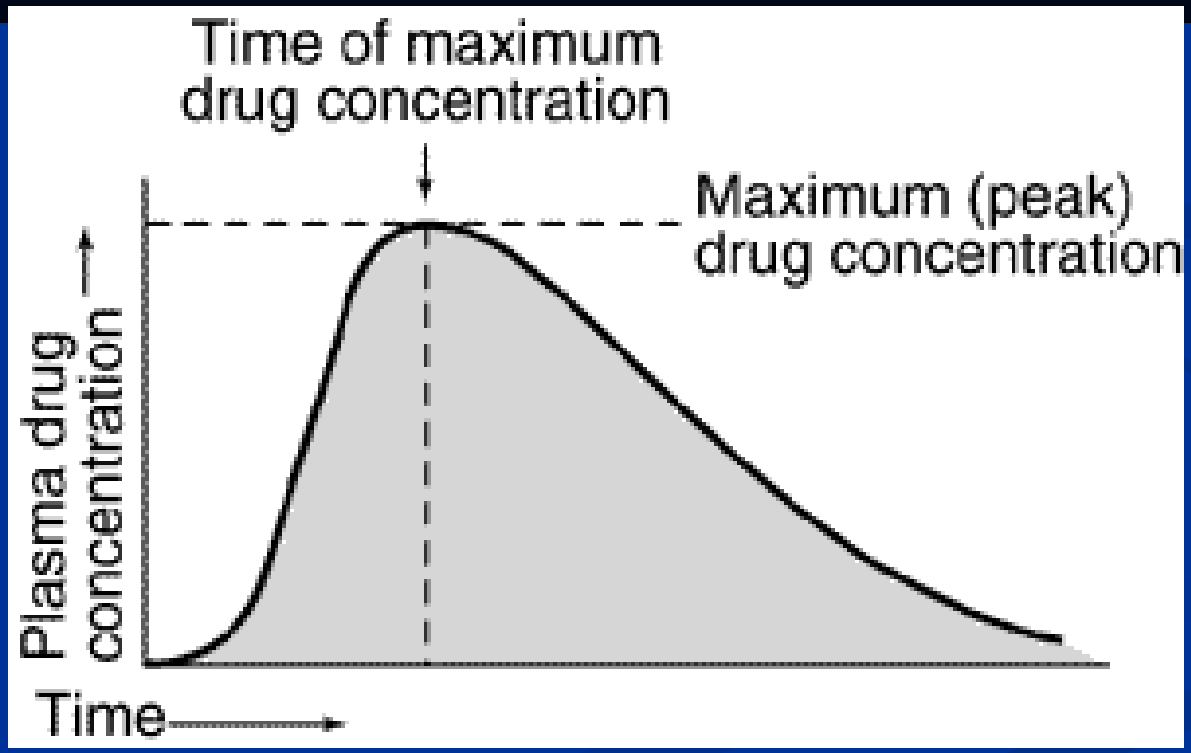
- measures the rate and extent by which a drug reaches systemic circulation
- fraction of unchanged drug that reaches systemic circulation

Factors that Affect Bioavailability

1. Biopharmaceutical factors
   - Dosage form
   - Physicochemical properties
2. Physiologic factors
   - Gastric motility
   - Presystemic metabolism
3. GIT contents – Food, drugs, fluid
4. Disease states

Determinants of Bioavailability

1. Plasma data



- AUC is the most reliable measure of bioavailability.
- It is directly proportional to the total amount of unchanged drug that reaches the systemic circulation
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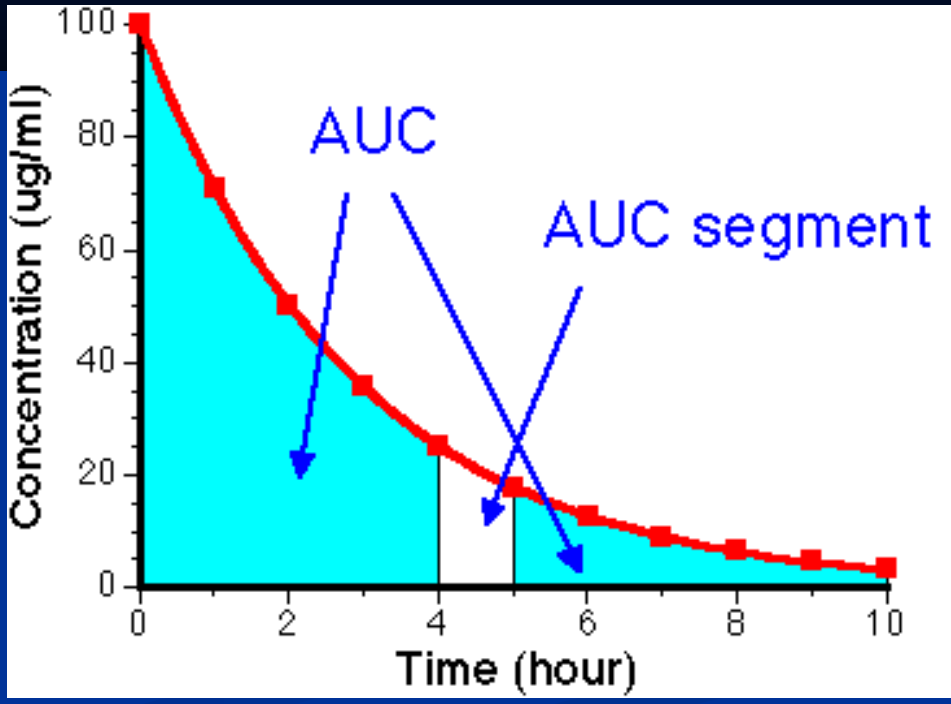
$$F = \frac{\text{AUC oral}}{\text{AUC injection}} \times 100$$



2. Urine data

- maximum urinary excretion rate
- time for maximum excretion rate
- cumulative amount of drug excreted in the urine

3. Pharmacologic effect

Bioequivalence:

- when two related drugs show comparable bioavailability

Therapeutic Equivalence:

- when two similar drugs have comparable efficacy and safety

Routes of Administration, Bioavailability, and General Characteristics

Route	Bioavailability (%)	Characteristics
Intravenous (IV)	100 (by definition)	most rapid onset
Intramuscular (IM)	75 to ≤ 100	large volumes often feasible; may be painful
Subcutaneous (SC)	75 to ≤ 100	smaller volumes than IM; may be painful; slower onset than IV or IM
Oral (PO)	5 to < 100	most convenient; first-pass effect may be significant
Rectal (PR)	30 to < 100	less first-pass effect than oral
Transdermal	80 to ≤ 100	usually very slow absorption; used for lack of first-pass effect; prolonged duration of action

DISTRIBUTION

- Process by which a drug reversibly leaves the systemic circulation and enters the interstitial space or the cells of the tissues
- Once in the blood stream, the drug is distributed to the different tissues

The extend and pattern of distribution of a drug depends on :

- Lipid solubility
- Ionisation at physiological pH
- Extend of binding to plasma and tissue proteins
- Presence of tissue-specific transporters
- Difference in regional blood flow

Permeability of barriers

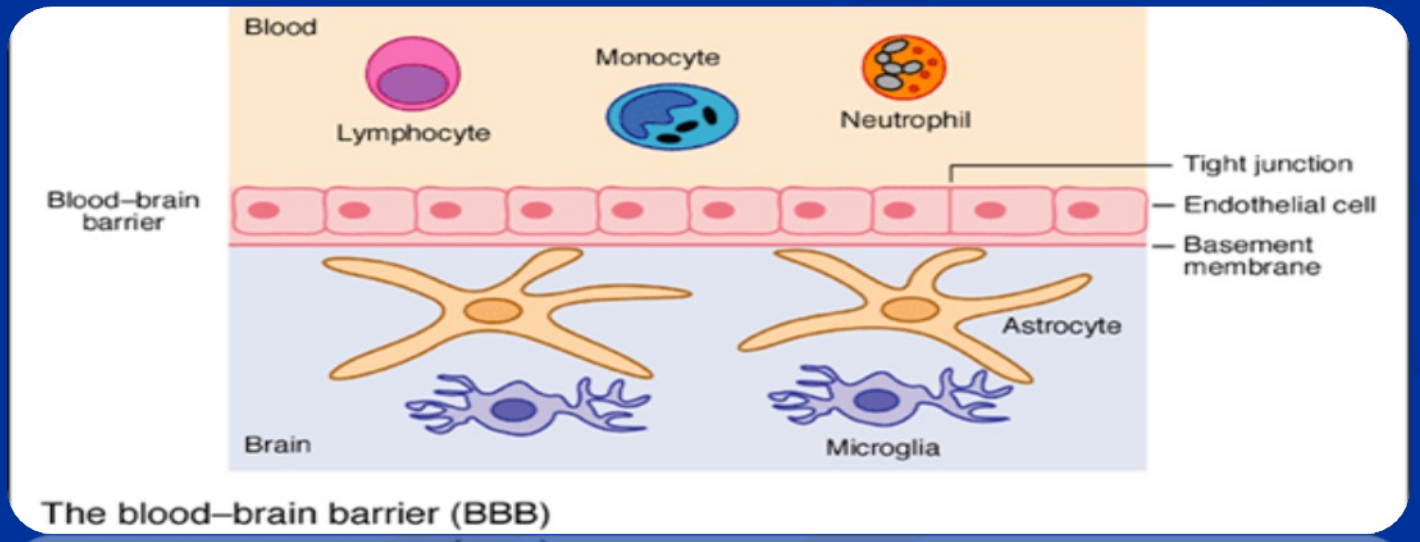
Blood brain barrier :

- Endothelial cells of capillaries are continues tight junction.
- lipid soluble non ionised form of drugs penetrate more easily to brain.
- More lipophilic a drug, more likely to cross the blood brain barrier.
- BBB involves membrane transporters limits access of drugs to tissues

BBB involves membrane transporters

- limits access of drugs to tissues.
- Inflammatory condition alter permeability characteristics of BBB allowing entry of drugs that are restricted.

  E.g- Penicillin, Chloramphenicol, Ampicillin



The blood–brain barrier (BBB)

DRUGS - BBB

- Volatile anesthetic - Ether, chloroform.
- Ultra short acting barbiturates - Thiopental.
- Narcotic analgesic - Morphine, heroin.
- Dopamine precursor - L-dopa
- Propranolol & Diazepam

DOES NOT CROSS

- Dopamine, serotonin& Streptomycin
- Quaternery substances - d-tubocurarine,
- Hexamethonium, Neostigmine, Acetylcholine

Blood CSF Barrier :

- CSF is secreted by the epithelial cells of choroid plexus
- Lined by a occluding zonulae
- Epithelial cells joined by tight junctions allows non ionised lipid soluble drugs

CSF Brain Barrier:

Extremely permeable to drug molecules from CSF to brain cell

Eg : pencillin is less lipid soluble ,poor penetration through BBB but when given Intrathecal route it can cross CSF Brain barrier

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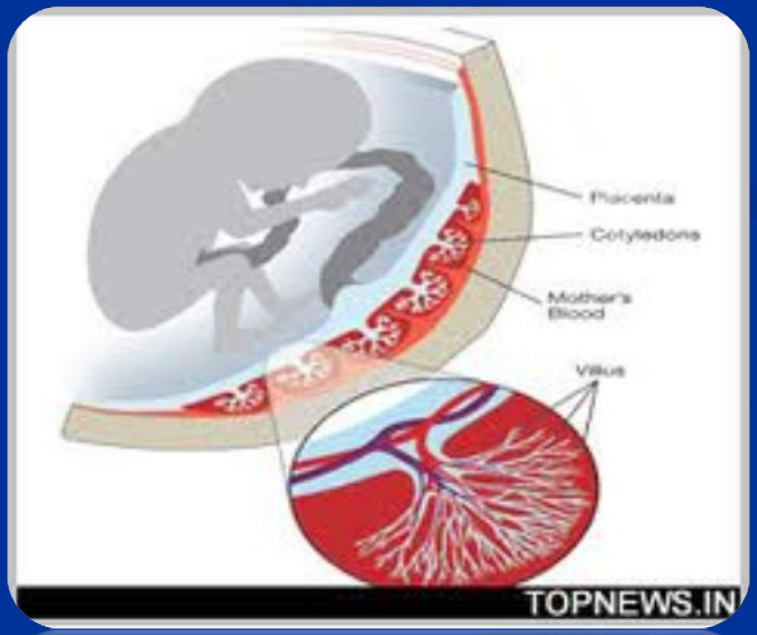
Placental barrier

It is lipid in nature Lipid solubility, extent of plasma binding & degree of ionization of week acids & bases are determinants in drug transfer



- Fetal plasma is slight acidic than the mother
- As in brain ,P-gp & other export transporters present in - placenta limits fetal exposure to toxic agents

Eg: pentobarbital, alcohol , certain antibiotics.

Binding of drugs-

Plasma protein binding :

- It's a non linear & saturable process.
- prolongs the drug availability & Duration of action.
- Delays metabolic degradation & excretion of drugs
- Fraction of total bound drug in plasma determined by drug concentration, affinity of binding sites, number of binding sites

- Diminishes its penetration into the CNS.
- Hypoalbuminemia , secondary severe liver disease or nephrotic syndrome ↓ binding.
- Cancer, Arthritis, MI, Crohn's disease -↑ level of α1 acid glycoprotein & enhanced binding of basic drugs.
- As free drug is active & gets eliminated to replace the lost drug ,bound drug dissociates.

- Therapeutic range of plasma concentration is limited, extent of binding & unbound fraction is constant
- acidic drugs binds to plasma albumin
  Eg - Penicillins, Sulfonamides, Tolbutamide & Salicylic acid.
- basic drugs binds to α_1 acid glycoprotein
  Eg - Propranolol, Lignocain, Quinidine.
- Displacement interactions where drug bound with higher affinity will displace the one having lower affinity.

Eg - Phenylbutazone, Salicylates & Sulfonamides displaces Tolbutamide → hypoglycemia

- Salicylates,Indomethacin,Phenytoin & Tolbutamide displaces Warfarin → haemorrhage.

- Sulfonamides & vitamin K displace endogenous ligands like bilirubin→ kernicterus in neonates.

- Drug extensively protien binding has smaller apparent volume of distribution.

- warfarin- 99% bound, Tolbutamide- 98% bound,
- Phenytoin- 90% bound causes toxicity after getting displaced from plasma protein binding sites.

Compartments of drug distribution:

Cellular reservoir :

- A drug may have a great affinity for plasma proteins, yet be primarily distributed in tissues, This situation would occur if the tissue have higher affinity for drug.
- Eg: - Digoxin and Emetin in skeletal muscles, heart, liver and kidney

  Iodine in thyroid

Fat Reservoir :

- Highly lipid- soluble drugs like thiopentone selectively accumulated in fat and adipose tissue.

Transcellular Reservoir :

- Aqueous humour – eg Chloramphenicol and Prednisolone
- CSF – Eg: Aminosugars and Sucrose
- Endolymph, joint fluids – Eg: Ampicillin

Bones and Connective Tissue Reservoirs :

- Many drugs like Tetracycline ,Lead, Arsenic and Fluoride , form a complex with bone salt and get deposited in nails, bones and in teeth.

Plasma Protein Binding Drug Reservoir:

- Drugs bind to plasma and cellular proteins in a reversible manner and in dynamic equilibrium.
- Free drug + Protein <=>Drug-Protein complex

Apparent volume of distribution

- The volume of distribution does not represent a real volume but regarded as the size of the pool of the body or fluids

- Digoxin is widely distributed in the body including muscles and adipose tissue, leaving a small fraction to be distributed in plasma

$$aVd = \frac{\text{The total amount of drug in the body (mg/kg}}{\text{Conc. Of the drug in the plasma(mg/L)}}$$

- Relates the amount of a given drug in the body to the concentration of the drug in the blood

Drugs highly bound to plasma proteins have a low aVd value

- Eg : Tolbutamide, Furosemide, Warfarin

Drugs lesser the plasma protein binding greater is the aVd

- Eg : Chloroquine and Metoprolol.

- aVd for many drug may be much more than the actual body volume

  Eg : Digoxin, Imipramine and analogues

- The drugs that are widely distributed in body including muscle and adipose tissue. Such drugs are difficult to be removed by haemodialysis if toxicity appear.

  Eg., Warfarin, Aspirin, Tolbutamide

- Value of aVd < *5* L implies that the drug is retained within the vascular compartment.

  Eg :Warfarin and furosemide

- Value of aVd approximating *15* L suggests that the drug is restricted to the extracellular fluid

  Eg : Aspirin, Tolbutamide. Gentamicin and Amoxycilline

Redistribution-

- Termination of drug effect, after the withdrawal of drug, because of its redistribution in to vicera, muscle mass, lean tissue and fat
- when highly lipid soluble drugs given by I.V enters the brain rapidly .

  Eg : Thiopentone

References :

- Pharmacological basis of Therapeutics – Goodman & Gilman 12th Edition.
- Basic & Clinical Pharmacology 11th Edition.
- Essential Medical Pharmacology– K. D. Tripathi 7th Edition.
- Principles of pharmacology – HL Sharma & KK Sharma.