Topic –
Absorption

Presented By
Mr. Vishal V. Kalal
Ass. Prof.

JES'S College Of Pharmacy Nandurbar.

BIOPHARMACEUTICS

"Study of factors influencing the rate and amount of drug that reaches to the systemic circulation and the use of this information to optimise the therapeutic efficacy of the drug products"

BIOAVAILABILITY

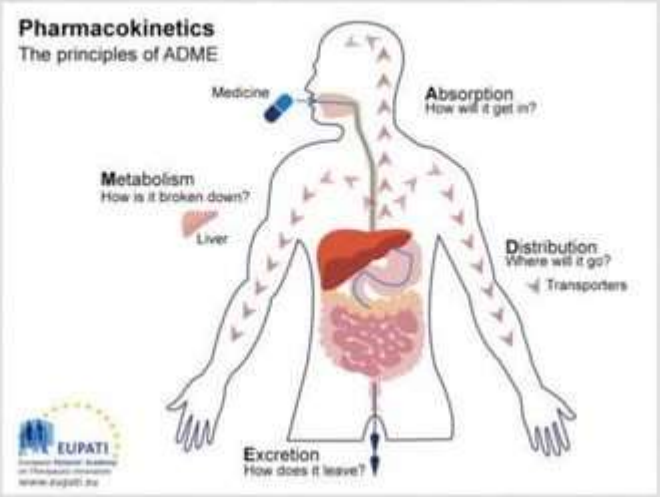
" Rate and extent (amount) of drug absorption. Any alteration in the drug's bioavailability is reflected in its pharmacological effects"

ADME

- The moment of drug from it's site of administration to systemic circulation is called as **ABSORPTION.**
- The movement of drug between one compartment and the other (generally blood and the extravascular tissues) is referred to as drug **DISTRIBUTION.**
- The process that tends to remove the drug from the body and terminate its action is called as **ELIMINATION**. occurs by two processes— biotransformation (metabolism), which usually inactivates the drug, and excretion which is responsible for the exit of drug/metabolites from the body.

PHARMACOKINEICS

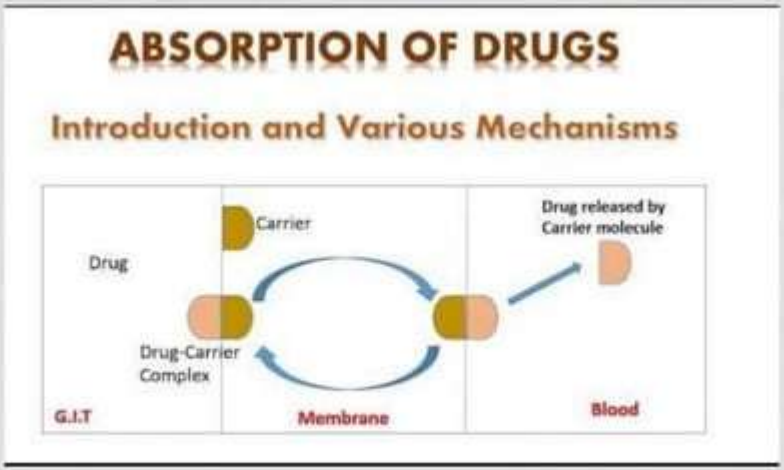
- **Pharmacokinetics** is defined as the study of time course of drug ADME and their relationship with its therapeutic and toxic effects of the drug
- The use of pharmacokinetic principles in optimising the drug dosage to suit individual patient need and achieving maximum therapeutic utility is called as **Clinical Pharmacokinetics.**



1. **Absorption** :Process by which drug enters the body.
2. **Distribution** :Dispersion of drugs throughout the fluids and tissues of the body.
3. **Metabolism** : Irreversible transformation of parent drug compounds into daughter metabolites.
4. **Excretion** :Elimination of drug metabolites from the body.

ABSORPTION OF DRUG

- Drug absorption is defined as the process of movement of unchanged drug from the site of administration to systemic circulation.



MECHANISM OF DRUG ABSORPTION

1. Transcellular / intracellular transport
   - A. Passive Transport Processes
     - I. Passive diffusion
     - II. Pore transport
     - III. Ion- pair transport
     - IV. Facilitated or mediated diffusion
   - B. Active transport processes
     - I. Primary
     - II. Secondary
       - a. Symport (Co-transport)
       - b. Antiport (Counter transport)

2. Para cellular / Intercellular Transport
   A. Permeation through tight junctions of epithelial cells
   B. Persorption

3. Vesicular or Corpuscular Transport (Endocytosis)
   A. Pinocytosis
   B. Phagocytosis

1. TRANSCELLULAR TRANSPORT

- It is define as passage of drugs across the Gastro Intestinal epithelium

A.PASSIVE TRANSPORT

-Not require energy

B.ACTIVE TRANSPORT

- Require energy from ATP

A. PASSIVE TRANSPORT

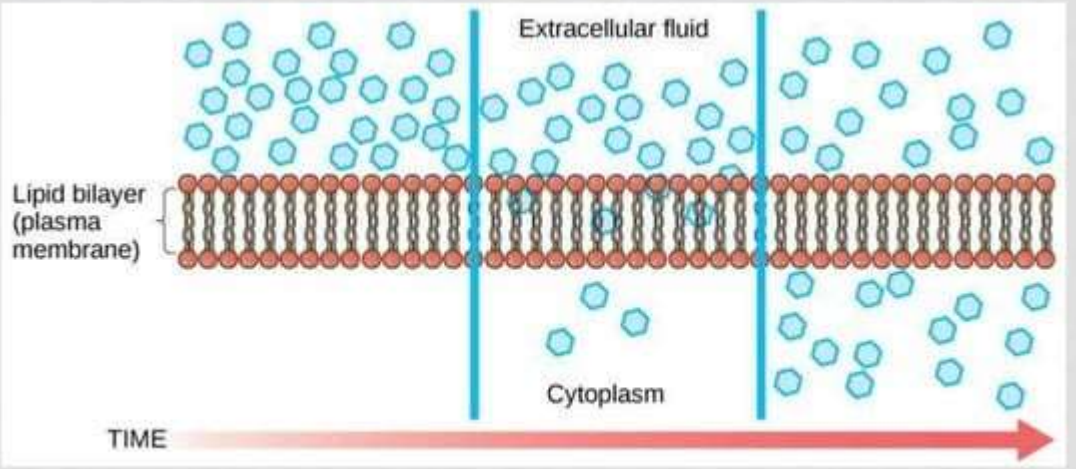
I. Passive diffusion.
II. Pore transport.
III. Ion-pair transport.
IV. Facilitated- or mediated-diffusion

I. PASSIVE DIFFUSION

- Also called non-ionic diffusion, it is the major process for absorption of more than 90% of the drugs. The driving force is concentration or electrochemical gradient. It is defined as the difference in the drug concentration on either side 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.

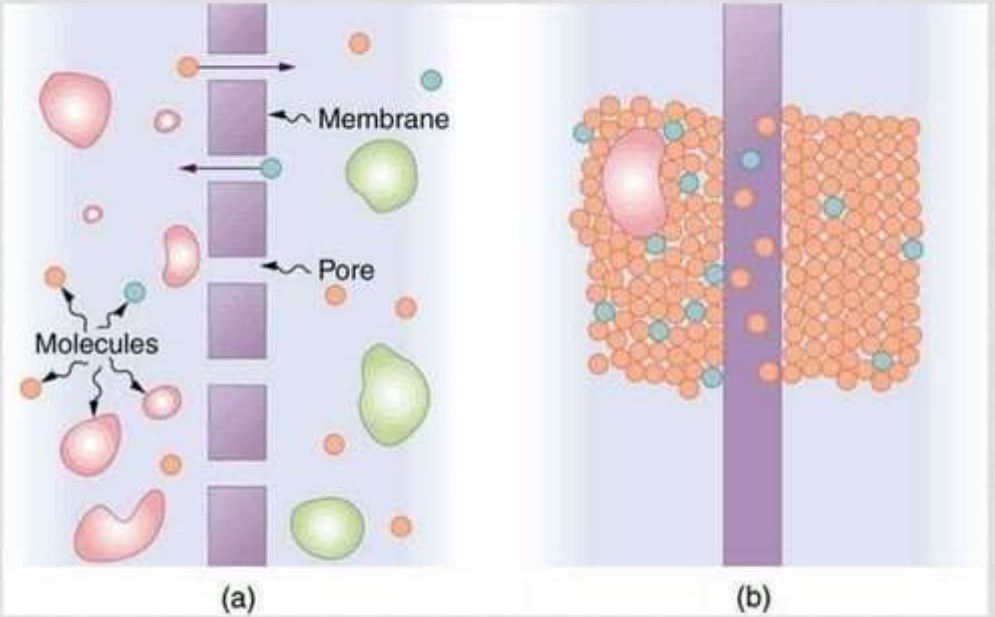
$$\frac{dQ}{dt} = \frac{DAK_{m/w}}{h}(C_{GIT} - C)$$

dQ/dt = rate of drug diffusion
D = diffusion coefficient of the drug
A = surface area of the absorbing membrane
Km/w = partition coefficient of the drug between membrane and the aqueous phase
(CGIT – C) = difference in the concentration of drug in GI fluid & the plasma
h = thickness of the membrane (length)



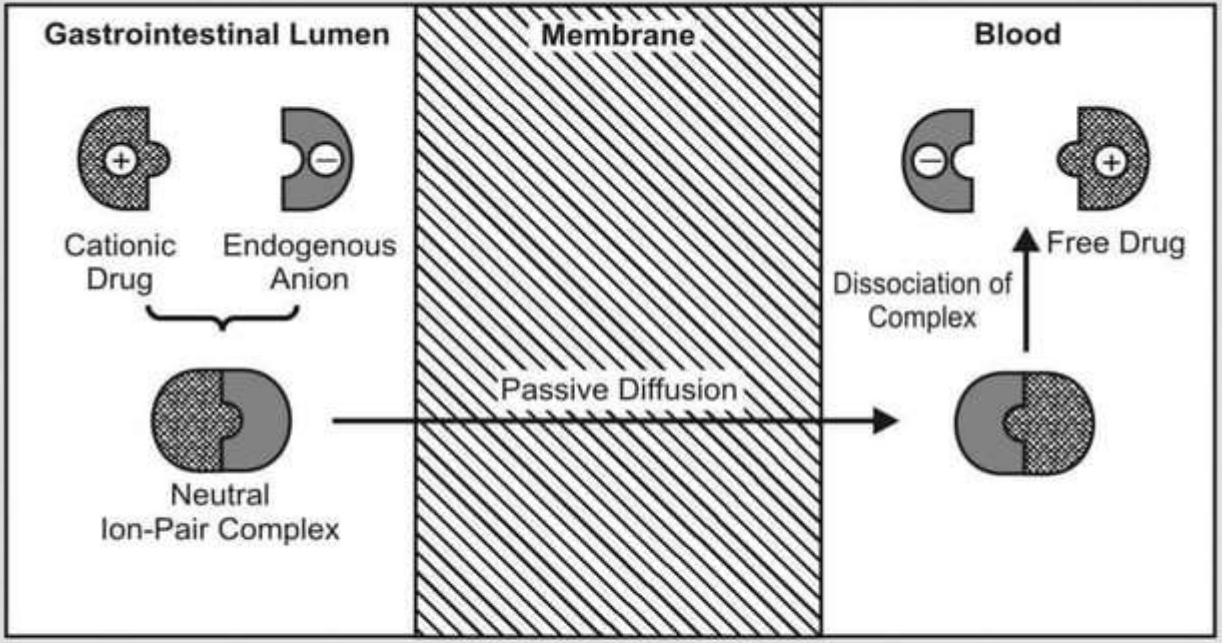
II. PORE TRANSPORT

- It is also called as convective transport, bulk flow or filtration.
- The driving force is hydrostatic pressure or the osmotic differences across the membrane.
- The process is important in the absorption of low molecular weight (less than 100), generally water-soluble drugs through narrow, aqueous-filled channels ex: urea, water and sugars.
- Chain-like or linear compounds of molecular weight up to 400 Daltons can be absorbed by filtration. For example, the straight-chain alkanes.
- Drug permeation through water-filled channels is importance in renal excretion, removal of drug from the cerebrospinal fluid and entry of drugs into the liver.


Membrane
Pore
Molecules
(a)
(b)

III. ION-PAIR TRANSPORT

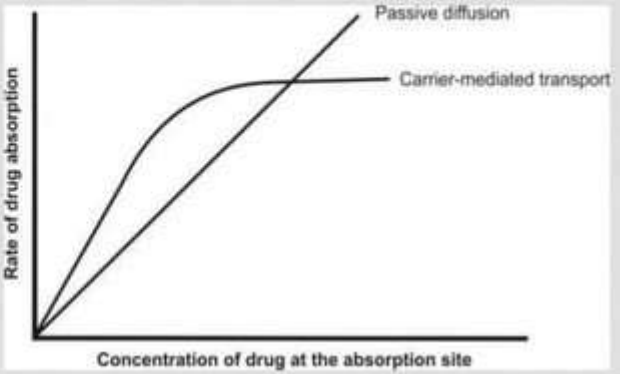
- Absorption of drugs like quaternary ammonium compounds (Examples are benzalkonium chloride, benzethonium chloride) and sulphonic acids (sulfonic acid), which ionise under all pH conditions, is ion-pair transport.
- Despite their low o/w partition coefficient values, such agents penetrate the membrane 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. Propranolol, a basic drug that forms an ion pair with oleic acid, is absorbed by this mechanism.


Gastrointestinal Lumen
Membrane
Blood
Cationic Drug
Endogenous Anion
Free Drug
Dissociation of Complex
Passive Diffusion
Neutral Ion-Pair Complex

IV. CARRIER MEDIATED TRANSPORT

- Some polar drugs cross the membrane more readily than can be predicted from their concentration gradient and partition coefficient values. Like monosaccharides, amino acids and vitamins will be poorly absorbed.
- The mechanism is involved is 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.
- Since the system is structure-specific, drugs having structure similar to essential nutrients, called as false nutrients, are absorbed by the same carrier system.



B. ACTIVE TRANSPORT

This transport system requires energy from ATP to move drug molecules from extracellular to intracellular space.

1. Primary active transport

2. Secondary active transport

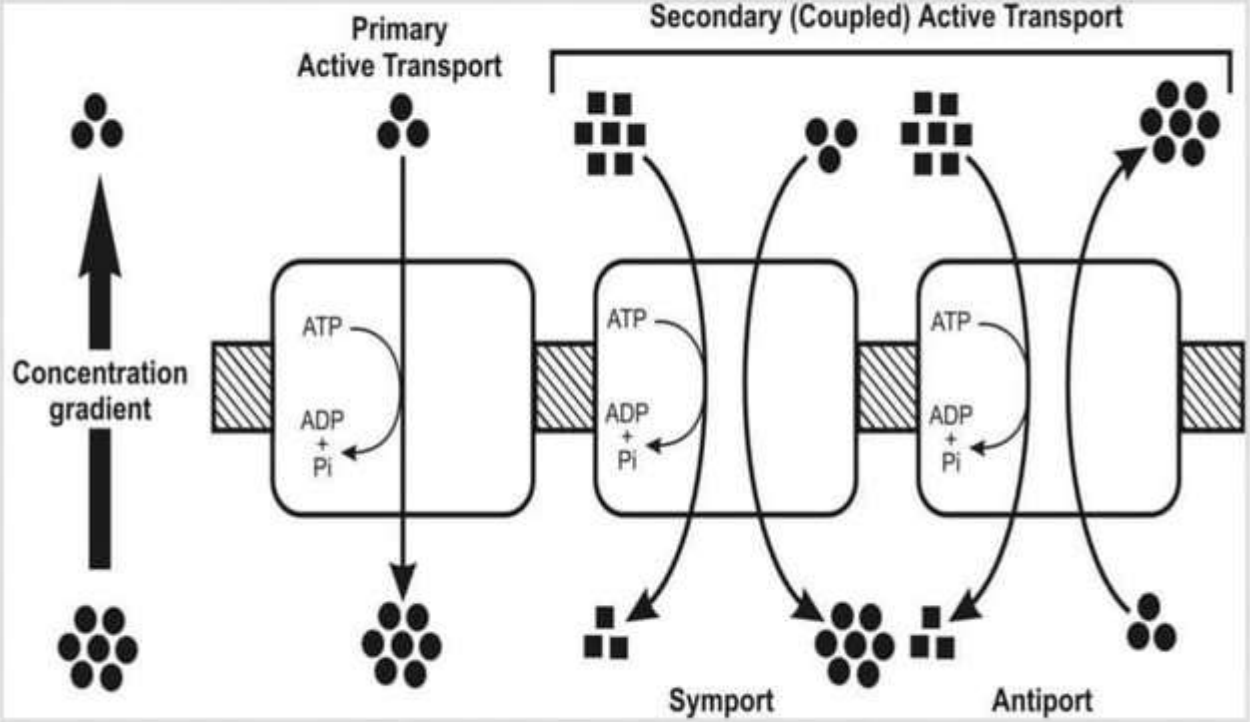
I. Symport (co-transport)

II. Antiport (counter-transport)

1. 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.

- 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.


Primary
Active Transport
Secondary (Coupled) Active Transport
Concentration
gradient
ATP
ADP
+
Pi
ATP
ADP
+
Pi
ATP
ADP
+
Pi
Symport
Antiport

I. **symport (co-transport)** – involves movement of both molecules in the same direction e.g. Na+-glucose symporter.

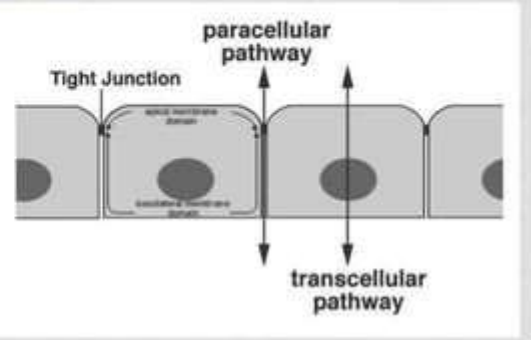
II. **Antiport (counter-transport)** – involves movement of molecules in the opposite direction e.g. H+ ions using the Na+ gradient in the kidneys.

2. PARACELLULAR TRANSPORT

- Transport of drugs through the junctions between the GI epithelial cells. This pathway is of minor importance in drug absorption.

Permeation through tight junctions of epithelial cells

Persorption



- **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.
- **Persorption** – is permeation of drug through temporary openings formed by shedding of two neighbouring epithelial cells into the lumen.

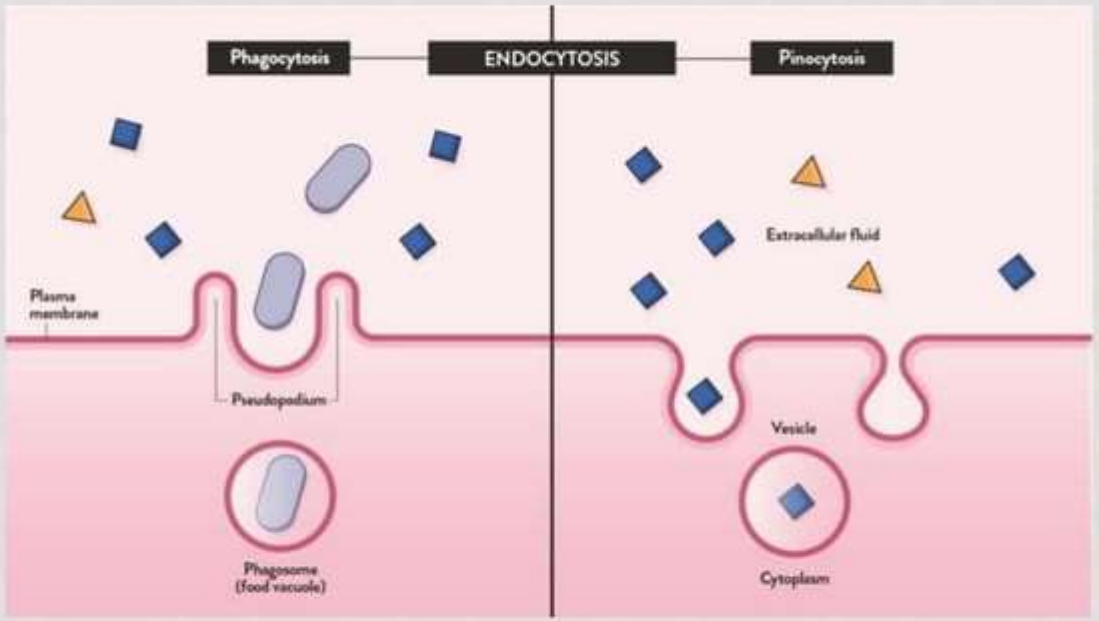
3. VESICULAR TRANSPORT SYSTEM

- 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 –

i. Pinocytosis.

ii. Phagocytosis.

- 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.


Phagocytosis
ENDOCYTOSIS
Pinocytosis
Extracellular fluid
Plasma membrane
Pseudopodium
Vesicle
Phagosome (food vacuole)
Cytoplasm

Topic –
Drug Distribution

Presented By
Mr. Vishal V. Kalal
Ass. Prof.

JES'S College Of Pharmacy Nandurbar.

DRUG DISTRIBUTION

Once a drug enter in to the blood stream,
the drug is subjected to a number of processes
called as Disposition Processes that tend to lower
the plasma concentration.

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

2. **Elimination** which involves **irreversible loss** of drug from the body. It comprises of **biotransformation and excretion.**

CONTENTS

DEFINITION

Drug Distribution is defined as the Reversible transfer of drug between one compartment (blood) to another (extra vascular tissue)

Significance :-

Pharmacological action of drug depends upon its concentration at the site of action

Thus distribution plays important role in

- Onset of Action
- Intensity of Action
- Duration of Action

STEPS IN DRUG DISTRIBUTION

- **Permeation of Free Drug through capillary wall & entry in to ECF.**
- **Permeation of drugs from ECF to ICF through membrane of tissue cell.**

Rate Limiting Steps

- Rate of Perfusion to the ECF
- Membrane Permeability of the Drug

DISTRIBUTION PROCESS

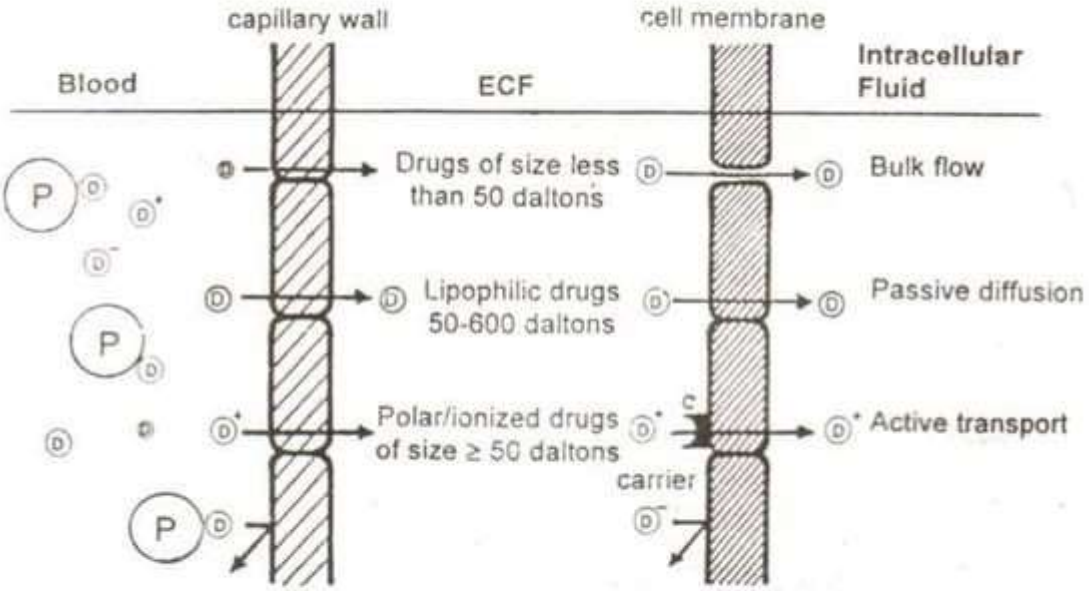


Fig. 3.3 Plasma membrane barrier and drug diffusion across it

Distribution is a Passive Process,

for which the

Driving Force is the Conc. Gradient between the Blood and Extravascular Tissues

- The Process occurs by the Diffusion of Free Drug until equilibrium is established

DISTRIBUTION OF DRUG IS NOT UNIFORM THROUGH OUT THE BODY— WHY ?

Because tissue receive the drug from plasma at different rates & different extents.

Organ perfused	Blood flow (mL/min)	Organ mass (kg)	Normalized blood flow (mL/min/kg)
Liver	1700	2.5	680
Kidney	1000	0.3	3333
CNS	800	1.3	615
Myocardium	250	0.3	833
Fat	250	10	25
Other (muscle...)	1400	55.6	25
total	5400	70	

FACTORS AFFECTING DISTRIBUTION OF DRUGS

1. Tissue Permeability of Drugs
   - ❖ Physicochemical Properties of drug like
     Mol.size, pK_a, o/w Partition Coefficient
   - ❖ Physiological barriers to diffusion of drugs

2. Organ/tissue size and perfusion rate

3. Binding of drugs to tissue components.
   - ❖ binding of drug to blood components
   - ❖ binding of drug to extra cellular components

4. Miscellaneous

TISSUE PERMEABILITY OF DRUGS

Physicochemical Properties of drug

- Molecular size,
- pKa
- o/w Partition Co Efficient.

Physiological barriers to Diffusion of Drugs

- Simple Capillary Endothelial Barrier
- Simple Cell Membrane Barrier
- Blood Brain Barrier
- Blood – CSF Barrier
- Blood Placental Barrier
- Blood Testis Barrier

1). TISSUE PERMEABILITY OF DRUG

a. physicochemical property:

I) Molecular Size;

Mol wt less then 500 to 600 Dalton easily pass capillary membrane to extra cellular fluid.

Penetration of drug from ECF to cells is function of Mol size, ionization constant & lipophilicity of drug

From extra cellular fluid to cross cell membrane through aqueous filled channels need particle size less then 50 Dalton (small) with hydrophilic property .

Large mol size restricted or require specialized transport system

1). TISSUE PERMEABILITY OF DRUG

a. Physicochemical Property

ii) Degree of Ionization (pKa)

♫ The pH at which half of a drug is unionized is called pKa

A weak acid becomes unionized in a strong acidic environment.

A weak acid becomes ionized in a neutral or basic environment.

&

A weak base becomes unionized in a strong basic environment.

A weak base becomes ionized in a neutral or acidic environment.

BUT

The PH of Blood plasma, extra cellular fluid and CSF is 7.4(constant)

Except in acidosis and alkalosis

All the drugs ionize at plasma pH (i.e. Polar , Hydrophilic Drugs)

Can not penetrate the Lipoidal cell membrane

1). TISSUE PERMEABILITY OF DRUG

a. Physicochemical Property

iii) o/w permiability

- Polar and hydrophilic drugs are less likely to cross the cell membrane

Where,,,,,,,,

- **Nonpolar and hydrophobic drugs are more likely to cross the cell membrane**

EFFECTIVE $K_{o/w}$ = Fraction unionized at pH 7.4 x $K_{o/w}$ of unionized drug

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 .....that can be calculated by above formula

- **Lipoidal drug** penetrate the tissue **rapidly.** Among Drugs with same Ko/w but diff in ionization of blood pH.

- One which has **less ionization** show **better distribution.**

  E.g. Phenobarbital > salicylic acid

  Both are having same Ko/w but phenobarbitol have more unionized at blood pH

- highly specialized and less permeable to water soluble drugs.

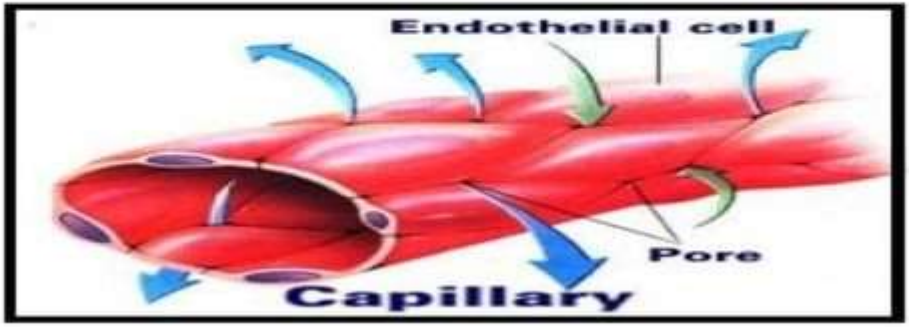
B. PHYSIOLOGICAL BARRIERS

1) The simple capillary endothelial barrier

Capillary supply the blood to the most inner tissue

All drugs ionized or unionized molecular size less than 600dalton diffuse through the capillary endothelium to interstitial fluid

Only drugs that bound to that blood components can't pass through this barrier Because of larger size of complex



B. PHYSIOLOGICAL BARRIERS

2. Simple cell membrane barrier

once the drug diffuse through capillary to extracellular fluid ,its further entry in to cells of most tissue is limited.

Simple cell Membrane is similar to the lipoidal barrier (absorption)

Non polar & hydrophillic drugs will passes through it (passively).

Lipophilic drugs with 50-600 dalton mol size &

Hydrophilic, Polar drugs with <50dalton will pass this membrane

B. PHYSIOLOGICAL BARRIERS

3) Blood brain barrier

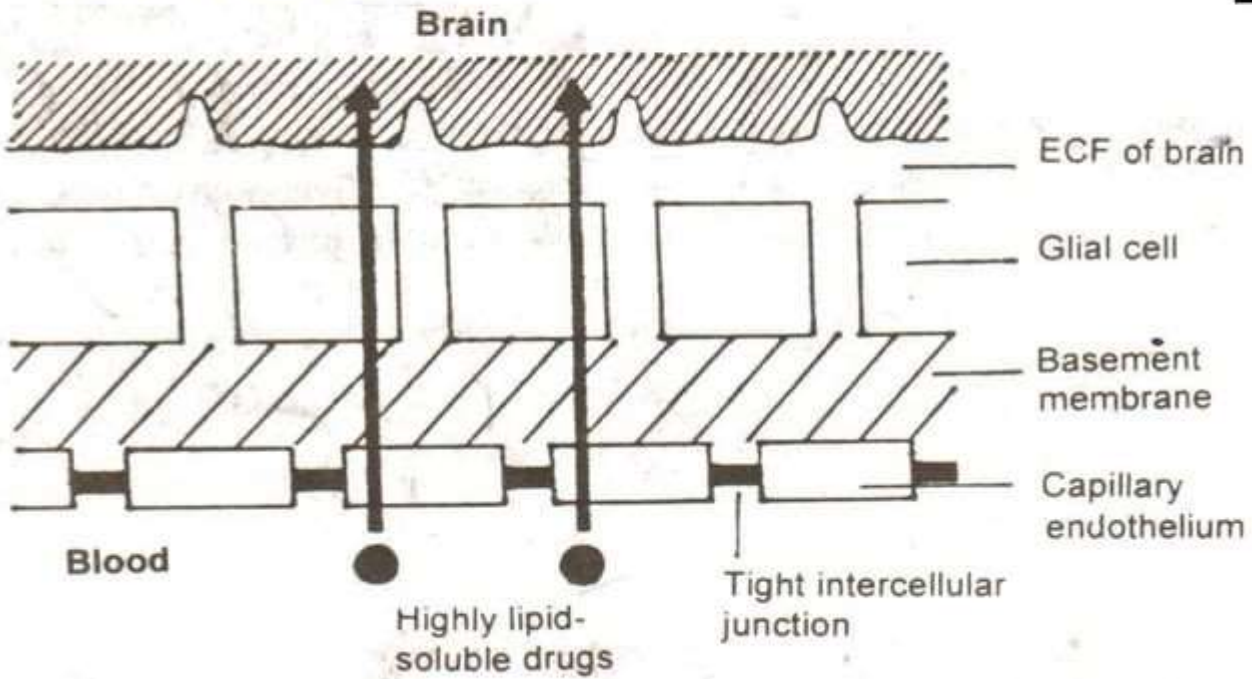


Fig. 3.4 Blood-brain barrier

B. PHYSIOLOGICAL BARRIERS

3) Blood brain barrier

Capillary in brain is highly specialized & much less permeable to water soluble drugs

ENDOTHELIAL CELLS ;- Tightly bonded with each other by intracellular junctions

ASTROCYTES :- present @ the base of endothelial tissue and act as supporting materials

& it Form Envelop around the capillary thus intercellular passage get blocked.

BBB is lipoidal barrier, thus drugs with high o/w partition coefficient diffuse passively others (moderately lipid soluble and partially ionised molecules passes slowly.

Polar natural substance (sugar & amino acid) transported to brain actively thus structurally similar drug can pass easily to BBB.

DIFFERENT APPROACHES TO CROSS BBB

A) Permeation Enhancers ;- Dymethyl Sulfoxide

B) Pro- Drug Approach ;- Dopamine---- Levodopa

(Parkinsonism)

and osmatic disruption of the BBB BY infusing

internal carotid artery with mannitol

C) carrier system ;- Dihydropyridine (Lipid soluble) moiety redox system (highly lipophilic & cross the BBB)

Complex formation (DRUG-DHP). After entering in brain DHP gets metabolize by (CNS) enzyme in brain and drug gets trapped in side the brain.

Polar pyridinium ion can not diffuse back out of the brain.

Ex. Steroidal drug

B. PHYSIOLOGICAL BARRIERS

4) Cerebral spinal fluid barrier ;-

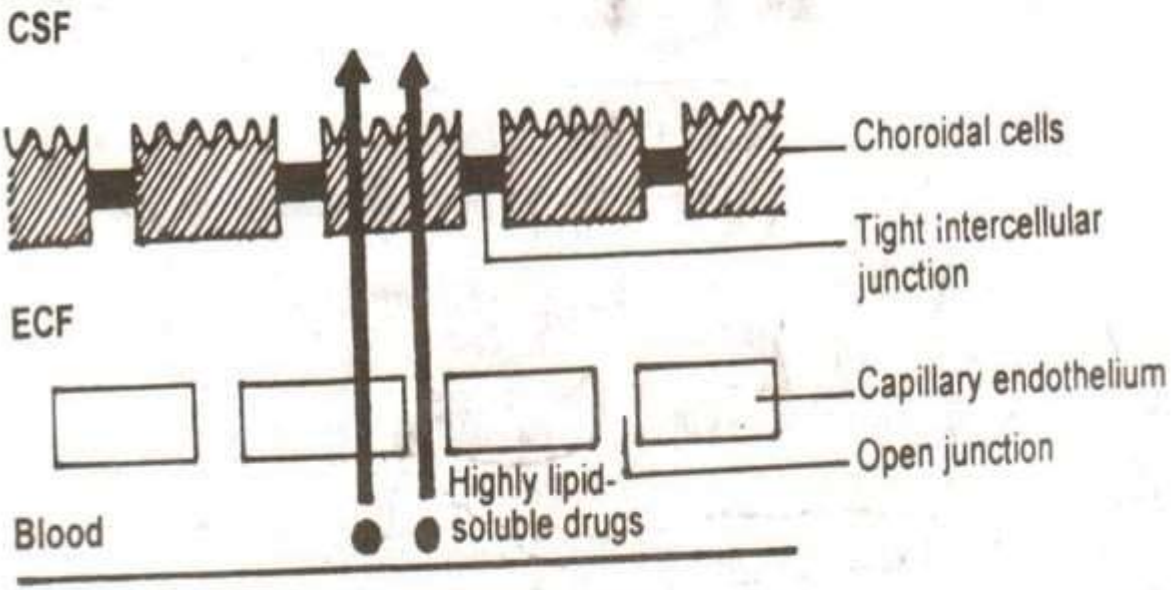


Fig. 3.5 The blood-CSF barrier

B. PHYSIOLOGICAL BARRIERS

4) Cerebral Spinal Fluid Barrier;-

Capillary endothelial cells;- have open junction or gaps so…. Drugs can flow freely b/w capillary wall & choroidal cells.

Choroids plexus;- major components of CSF barriers is choroidal cells which are joined with each other by tight junctions forming the blood-CSF barrier (similar permeability to BBB)

Highly lipid soluble drugs can easily cross the blood-CSF Barrier but moderatly soluble & ionize drugs permeate slowly.

Mechanism of drug transport is similar to CNS &CSF

but the Degree of uptake may vary significantly.

B. PHYSIOLOGICAL BARRIERS

5) Placenta barriers ;-

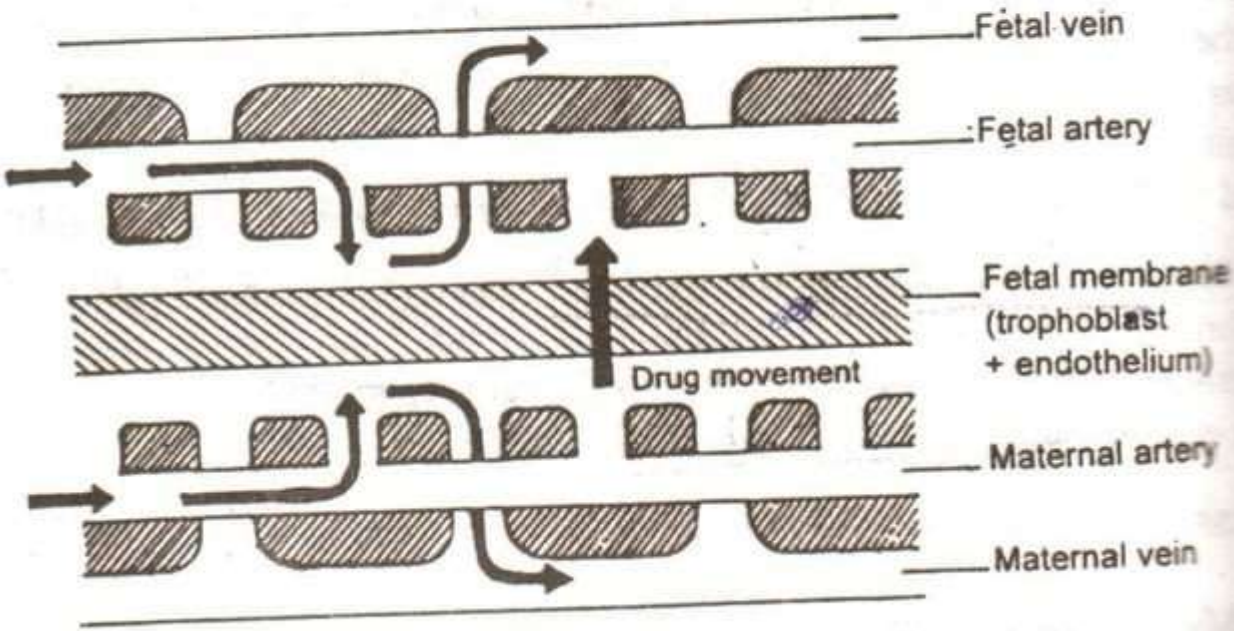


Fig. 3.6 Placental barrier and blood flow across it

B. PHYSIOLOGICAL BARRIERS

- 5) Placenta barriers ;-
- It's the barrier b/w Maternal & Fetal blood vessels
- Both are separated by fetal trofoblast basement membrane & endothelium .
- Thickness *25μ* @ *early pregnancy* later reduce up to *2μ* (even its effectiveness remain unchanged)
- Mol wt <1000 Dalton & moderate to high lipid solubility drugs like….. (Sulfonamides, Barbiturets, Steroids, Narcotic some Antibiotics) cross the barrier by Simple Diffusion rapidly
- Essential Nutrients for fetal growth transported by carrier-mediated processes.
- Immunoglobulines are transported by endocytosis.
- Drugs dangerous to fetus at Two stages
- Its advisable to avoid drugs during 1st trimester (fetal organ development) some drugs produce teratogenic effect
  ex. Phenytoin, methotrexate
- later stage pregnancy affect physiological functions like respiratory depression ex. morphine
- Better to restrict all drugs during pregnancy.

B. PHYSIOLOGICAL BARRIERS

6) Blood - Testis Barrier :-

This barrier not located @ capillary endothelium level.

But at sertoli - sertoli cell junction.

It is the tight junction / barrier b/w neighboring sertoli cells that act as blood-testis barrier .

This barrier restrict the passage of drugs to spermatocytes & spermatids.

2). ORGAN TISSUE SIZE AND PERFUSION RATE

Perfusion Rate :- *is defined as the volume of blood that flows per unit time per unit volume of the tissue (ml/min/ml)*

Perfusion rate – limited *when.....*

1) Drug is highly lipophilic

2) Membrane across which the drug is supposed to diffuse

Above both the cases Greater the blood flow , Faster the distribution

Organ perfused	Blood flow (mL/min)	Organ mass (kg)	Normalized blood flow (mL/min/kg)
Liver	1700	2.5	680
Kidney	1000	0.3	3333
CNS	800	1.3	615
Myocardium	250	0.3	833
Fat	250	10	25
Other (muscle...)	1400	55.6	25
total	5400	70	

- Distribution is permeability rate - limited in following cases
  - When the drug is ionic/polar/water soluble
  - Where the highly selective physiology barrier restrict the diffusion of such drugs to the inside of cell.

- Distribution will be perfusion rate - limited
  - When the drug is highly lipohilic
  - When the membrane is highly permeable.

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

Unit: ml/min/ml

(Distribution Rate Constant) Kt = perfusion rate / $K_{t/b}$

Distribution half life = 0.693/Kt

=0.693$K_{t/b}$/perfusion rate

$K_{t/b}$ tissue/blood partition coefficient

Highly lipophilic drugs can cross most selective barrier like BBB, ex. thiopental,

Highly permeable capillary wall permits passage of almost all drugs (except those bound to plasma protein).

Highly perfused tissues Lungs, Kidneys, Liver, Heart, Brain are rapidly equlibriated with lipid soluble drugs

Drug is distributed in a particular tissue or organ depends upon the **size of tissue** (Volume) & **Tissue/blood** partition coefficient

Ex.Thiopental i.v (liphopillic drug) & high tissue/blood partition coefficient towards brain & adipose tissue

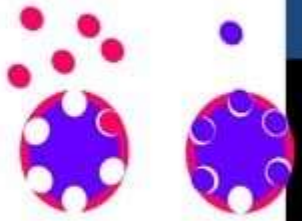
But brain is **highly perfused organ** so drug is distributed **fast** and shows **rapid onset of action than** poorly perfused adipose tissue.

3)Binding of drug to blood and other tissue components

- Binding of drugs to blood components
  - Blood cells
  - Plasma proteins
- Binding of drugs to extra vascular tissues

3).BINDING OF DRUG TO TISSUE COMPONENTS

a) Binding of drug to blood components;-



i) Plasma protein bindings

- Human serum albumin:-all types drug
- $\acute{\alpha}_1$-acid glycoprotein :-basic drugs(impr)
- Lipoproteins :-basic,lipophilic drugs(chlorpromazin)
- $\acute{\alpha}_1$-Globuline :-steroids like corticosterone ,vit-B12
- $\acute{\alpha}_2$-Globuline :-vit-A,D,E,K,cupric ions.
- Hemoglobin :-Phenytoin, phenothiazines.

ii) Blood cells bindings:-

RBC : 40% of blood comprise of blood cells

out of that 95% cells are RBC (RBC comprise of hemoglobin)

drugs like, phenytoin,phenobarbiton binds with Hb

,imipramine,chlorpromazine binds with RBC Cell wall

The major component of blood is RBC

The RBC comprises of 3 components each of which can bind to drugs:

- Hemoglobin
- Carbonic Anhydrase
- Cell Membrane

BINDING OF DRUGS TO PLASMA PROTEINS

- The binding of drug to plasma protein is reversible
- The extent or order of binding of drugs to various plasma proteins is:

Albumin >α_1-Acid Glycoprotein> Lipoproteins > Globulins

Human Serum Albumin

- Most abundant plasma protein with large drug binding capacity
- Both endogenous compounds and drugs bind to HSA
- Four different sites on HSA:
  - **Site I: warfarin and azapropazone binding site**
  - **Site II: diazepam binding site**
  - **Site III: digitoxin binding site**
  - **Site IV: tamxifen binding site**

3).BINDING OF DRUG TO TISSUE COMPONENTS

B. Extra Vascular Tissue proteins

- 40% of total body weight comprise of vascular tissues
- Tissue-drug binding result in localization of drug at specific site in body and serve as reservoir
- As binding increases it also increase bio-logical half life.
- Irreversible binding leads to drug toxicity. (carbamazepin-autoinduction)
- liver>kidney>lungs>muscle>skin>eye>bone>Hair, nail

4). Miscellaneous Factors

- **Age:**
  - a) Total body water
  - b) Fat content
  - c) Skeletal muscles
  - d) Organ composition
  - e) Plasma protein content
- **Pregnancy**
- **Obesity**
- **Diet**
- **Disease states**

4). MISCELLANEOUS FACTORS

a) AGE:-

Difference in distribution pattern is mainly due to

Total body water -(both ICF &ECF) greater in infants

Fat content - higher in infants & elderly

Skeletal muscle - lesser in infants & elderly

organ composition - BBB is poorly developed in infants & myelin content is low & cerebral blood flow is high, hence greater penetration of drug in brain

plasma protein content- low albumin in both infants & elderly

b) PREGNANCY:-

During Pregnancy, due to growth of UTERUS,PLECENTA,FETUS...

Increases the volume available for distribution drug.

fetus have separate compartment for drug distribution, plasma & ECF Volume also increase but albumin content is low.

C) OBECITY :-

In obese persons, high adipose (fatty acid) tissue so high distribution of lipophilic drugs

4). MISCELLANEOUS FACTORS

d) DIET:- A diet high in fats will increases free fatty acid levels in circulation thereby affecting binding of acidic drugs (NSAIDs to albumin)

e) DISEASE STATES:- mechanism involved in alteration of drug distribution in disease states.

i) Altered albumin & other drug-binding protein concentration.

ii) Alteration or reduced perfusion to organ or tissue

iii) Altered tissue pH.

iv) Alteration of permeability of physiological barrier (BBB)

EX- BBB (in meningitis & encephalities) BBB becomes more permeable polar antibiotics ampicilin, penicilin G. &

patient affect CCF, Perfusion rate to entire body decreases it affect distribution.

f) DRUG INTERACTION:-Displacement interaction occurs when two drugs administered which having similar binding site affinity.

Ex.A.Warfarin (Displaced Drug)&B.Phenylbutabutazone (Displacer)HSA

Apparent Volume Of Distribution

The apparent volume of distribution is a proportionality constant relating the plasma concentration to the total amount of drug in the body.

$$X \alpha C$$

$$X = Vd.C$$

$$Vd = X/C$$

Apparent volume of distribution = amount of drug in the body/ plasma drug concentration

Apparent volume of distribution is dependent on concentration of drug in plasma.

Drugs with a large apparent volume are more concentrated in extra vascular tissues and less concentrated intravascular.

In certain pathological cases, the Vd for the drug may be altered if the distribution of the drug is changed.

$Vd = X/C$

$Vd = X_0 / C_o$

=i.v. bolus dose/concentration of drug in plasma

for drugs given as i.v. bolus:

$$Vd_{(area)} = X0/K_E(AUC)$$

For drugs administered extravascularly:

$$Vd_{(area)} = FXo/K_E(AUC)$$

Reference:-

Applied Biopharmaceutics and Pharmacokinetics by *Leon Shargel*

Clinical biopharmaceutics and pharmacokinetics by *Gibaldi*

Biopharmaceutics and Pharmacokinetics by *Brahmankar*


THANK
YOU

Topic –
Factors Affecting Protein Binding of Drug

Presented By
Mr. Vishal V. Kalal
Ass. Prof.

JES'S College Of Pharmacy ,Nandurbar.

FACTORS AFFECTING PROTEIN BINDING OF DRUGS

1. Factors relating to the drug
2. Factors relating to the protein and other binding component
3. Drug interactions
4. Patient related factors

1. DRUG RELATED FACTORS

- **Physicochemical Characteristics of the Drug**

  - Increase in lipophilicity increases the extend of binding.
  - E.g. Cloxacillin 95% bound, Ampicillin 20% bound; hence Cloxacillan is released slowly after i.m. injection.
  - Acidic/anionic drugs bind to HSA; basic/cationic drugs to AAG; neutral/unionized drugs to lipoproteins.

▪ Concentration of Drug in the Body

- At low concentrations, most drugs may be bound to proteins
- At high concentrations, more free drugs may be present owing to saturation of binding sites on protein

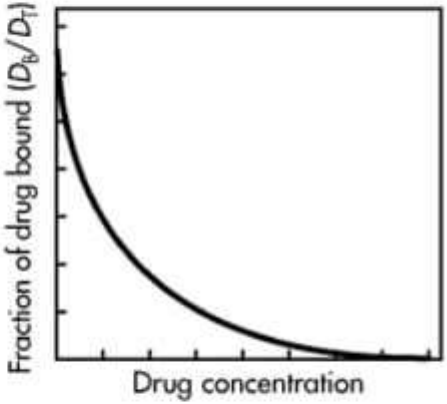


Figure : Fraction of drug bound versus drug concentration at constant protein concentration

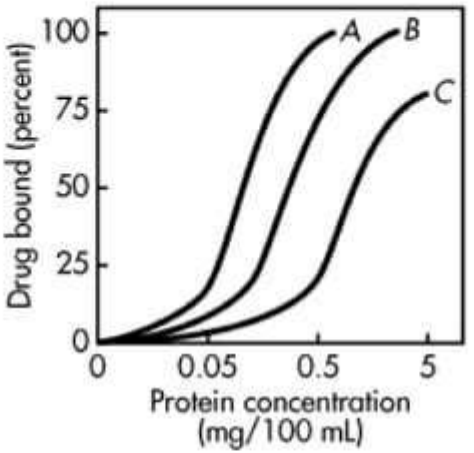
- **Drug-Protein/Tissue Affinity**

- 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 has half life of 2 ½ yrs due to its high affinity to plasma proteins.

2. PROTEIN RELATED FACTORS

- **Physicochemical Properties of Protein/Binding Component**
  - Lipophilicity→lipoproteins bind with lipophilic drugs.
  - Albumin→depend on physiological pH.

- **Concentration of Protein/Binding Components**
  - Disease states affect the concentration of proteins in blood.



Effect of protein concentration on the percentage of drug bound. A, B, and C represent hypothetical drugs with respectively decreasing binding affinity

- **Number of Binding Sites on the Protein**

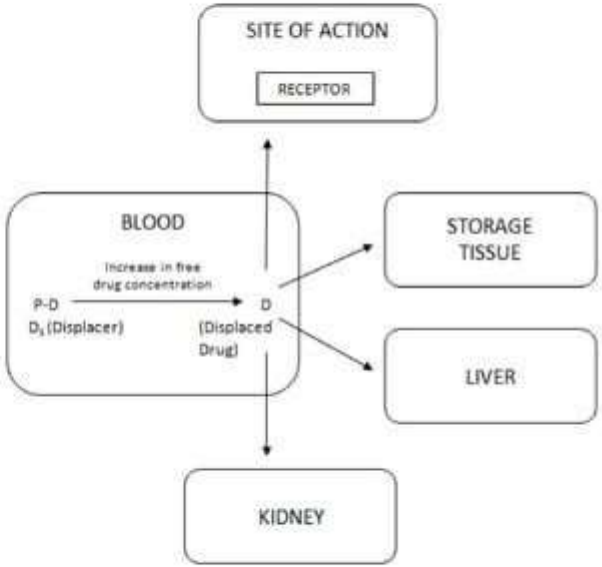
  - Albumin has a large number of binding sites as compared to other proteins.
  - Indomethacin binds to 3 sites on albumin.
  - AAG is a protein with limited binding capacity due to low concentration & molecular size.
  - Lidocaine binds to 2 sites on AAG in presence of HSA.

3. DRUG INTERACTIONS

- **Displacement Interactions**

➢ Competition between Drugs for the Binding Sites.

e.g. warfarin and phenyl butazone



Fate of a drug after displacement interaction

Interactions will result when:

- *The displaced drug* (E.g. warfarin)—
  - is more than 95% bound
  - has a small volume of distribution
  - shows a rapid onset of therapeutic or adverse effects
  - has a narrow therapeutic index

- *The displacer drug* (E.g. phenyl butazone)—
  - has a high degree of affinity as the drug to be displaced
  - competes for the same binding sites
  - the drug/protein concentration ratio is high
  - shows a rapid and large increase in plasma drug concentration

- **Competition between Drugs and Normal Body Constituents:**

  - Interaction with free fatty acids.
  - Free fatty acid levels are increased during fasting, diabetes, MI, etc.
  - Eg.: Interaction of sod. salicylate with bilirubin in neonates.

- **Allosteric Changes in Protein Molecule:**

  - involves alteration of the protein structure by the drug/metabolite ⟶ modify binding capacity.

  - Eg.: Aspirin acetylation of albumin; modify the binding capacity of NSAIDs (increased affinity).

4. PATIENT RELATED FACTORS

- **Age:**
  - Neonates

    Change in albumin content affects the drugs binding to it.
  - Infants
  - Elderly
- **Intersubject Variations:**
- **Disease States:**
  - Hypoalbuminemia severely impair protein-drug binding.

Table 1: Conditions Capable of Altering Plasma Proteins

Decreased Plasma Protein	Increased Plasma Protein
Albumin	**Albumin**
Burns	Hypothyroidism
Chronic liver disease	**α_1-acid glycoprotein**
Cystic fibrosis	Celiac disease
Protein-losing enteropathy	Crohn's disease
Nephritic syndrome	Myocardial infarction
Pregnancy	Renal failure
Chronic renal failure	Rheumatoid arthritis
Trauma	Trauma

Influence of Disease States on Protein-Drug Binding

Disease	Influence on Plasma Protein	Influence on protein-drug binding
1. *Renal failure*	Decreased albumin content; Increased AAG levels	Decreased binding of acidic drugs; neutral and basic drugs unaffected.
2. *Hepatic failure*	Decreased albumin synthesis	Decreased binding of acidic drugs; binding of basic drugs is normal or reduced depending on AAG levels.
3. *Inflammatory states (trauma, surgery, burns, infections, etc.*	Increased AAG levels	Increased binding of basic drugs; neutral and acidic drugs unaffected.

REFERENCE:

- **Leon Shargel**, *Applied Biopharmaceutics and Pharmacokinetics*, 5th edition,Chapter: Physiologic Drug Distribution and Protein Binding.
- **P.L.Madan**, *Biopharmaceutics and Pharmacokinetics*, 1st editoin, p 82-85.
- **D.M.Brahmankar**, *Biopharmaceutics and Pharmacokinetics—A Treatise*, 1st edition, p 97-102.
- **G.R.Chatwal**, *Biopharmaceutics and Pharmacokinetics*, 1st edition, p 55-58.
- **Milo Gibaldi**, *Biopharmaceutics and Clinical Pharmacokinetics*, 4th edition, p 195-200.

REFERENCE:

- **Javed Ali**, *A Textbook of Biopharmaceutics and Pharmacokinetics*, 1st edition, p 51-53.
- **H.P Tripnis, Amritha Bajaj**, *Principles and Applications of Biopharmaceutics and Pharmacokinetics*, 1st edition, p 73-79.
- **V. Venkateswarlu**, *Fundamentals of Biopharmaceutics and Pharmacokinetics*, 1st edition, p 68-71.
- **Shobha Rani**, *Textbook of Biopharmaceutics and Pharmacokinetics*, 1st edition, p 142-143.

THANK YOU

Introduction on Biopharmaceutics

Presented By
Mr. Vishal V. Kalal
Ass. Prof.

JES'S College Of Pharmacy Nandurbar.

INTRODUCTION TO BIOPHARMACEUTICS

CONTENTS

- Introduction
- Definitions
- Diagrammatic presentation
- Processes
- Dosage regimen

INTRODUCTION TO BIOPHARMACEUTICS:

- **Biopharmaceutics:** Is defined as the study of factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimize the therapeutic efficacy of the drug product.
- Thus, biopharmaceutics involves factors that influence the:

1) Protection and stability of the drug within the product.
2) The rate of drug release from the product
3) The rate of dissolution of the drug at the absorption site. and
4) The availability of the drug at its site of action .

DEFINITIONS

- **Pharmacokinetics:** The study of the time course (kinetics) of drug absorption, distribution, metabolism and elimination (ADME) and their relationship with its therapeutic and toxic effect of the drug

  i.e which is study of what the body does to the drug

- Clinical Pharmacokinetic : The use of pharmacokinetic principles in optimizing the drug dosage to suit individual patient need and achieving maximum therapeutic utility is called as clinical pharmacokinetic
- Pharmacodynamic: It deals with of what the drug does to the body

DEFINITIONS

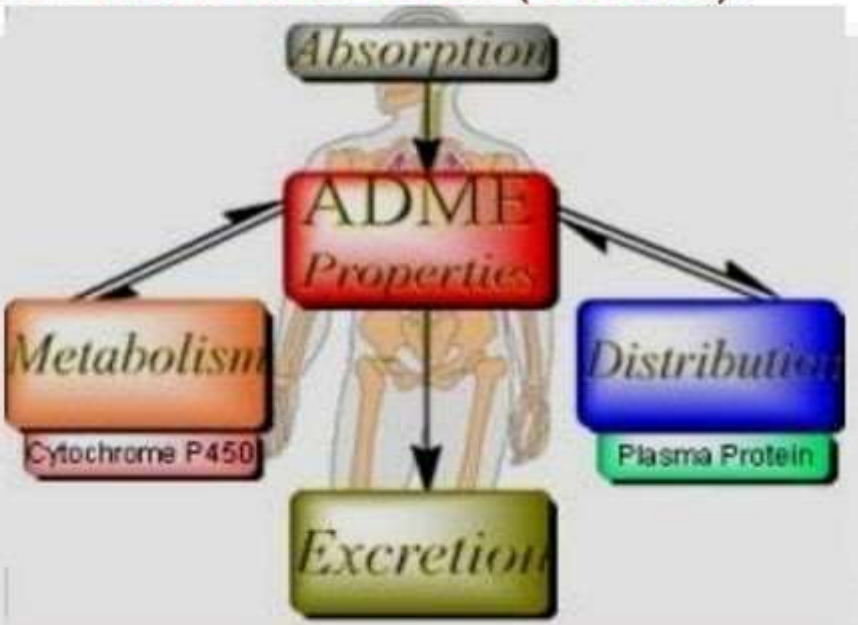
Absorption: The process of movement of drug from its site of administration to the systemic circulation.

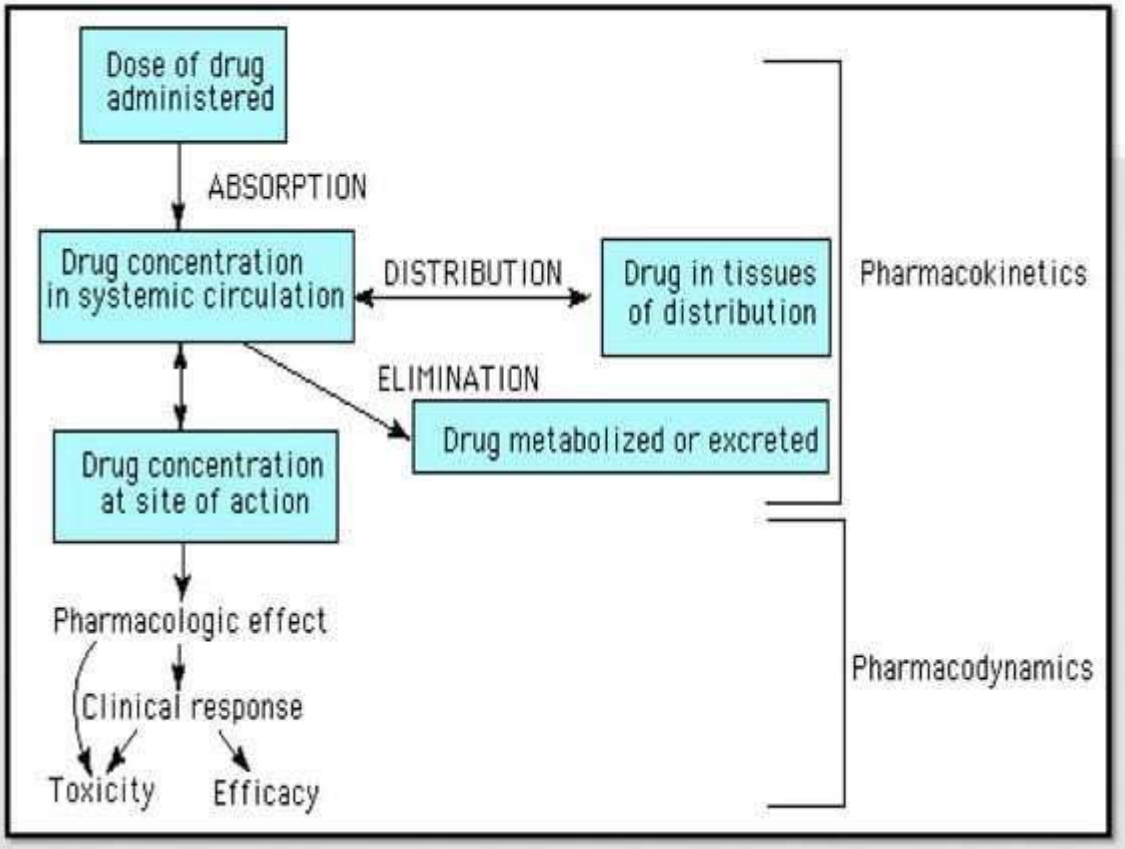
Distribution: The movement of drug between one compartment to and the other(generally blood and the extravascular tissue).

Metabolism (Biotransformation) : is the irreversible transformation of parent compounds into daughter metabolites.

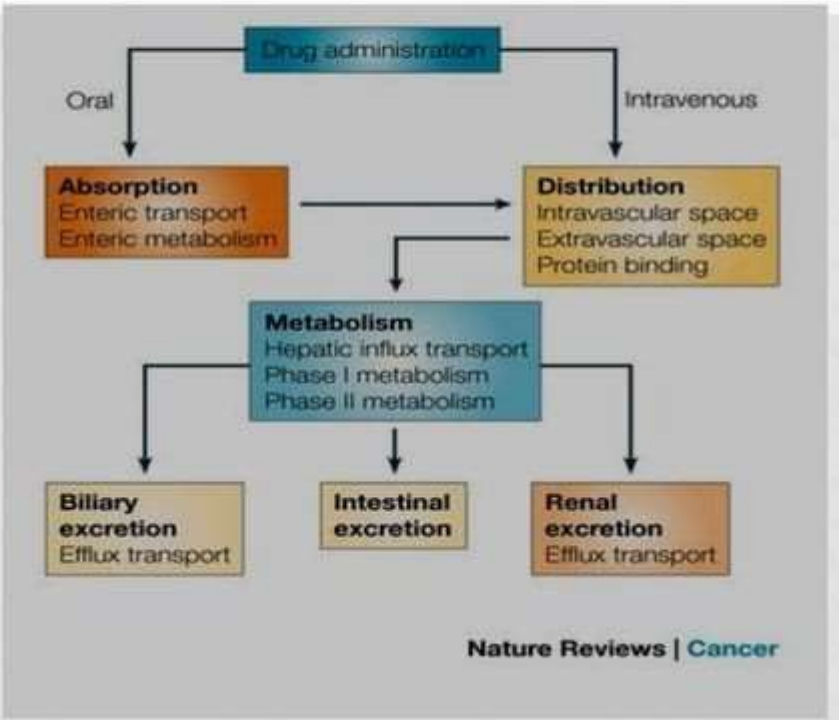
Excretion: is the elimination of the substances from the body.(Which is responsible for the exit of drug/metabolites from the body).

INTRODUCTION TO BIOPHARMACEUTICS (CONT.):



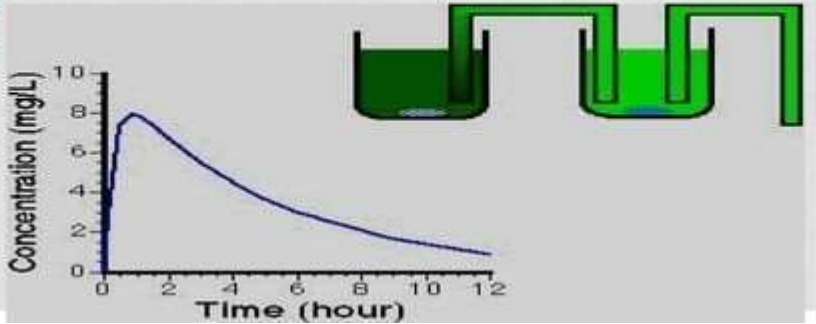

Dose of drug administered
ABSORPTION
Drug concentration in systemic circulation
DISTRIBUTION
Drug in tissues of distribution
Pharmacokinetics
ELIMINATION
Drug metabolized or excreted
Drug concentration at site of action
Pharmacologic effect
Clinical response
Toxicity
Efficacy
Pharmacodynamics

INTRODUCTION TO BIOPHARMACEUTICS (CONT.):



INTRODUCTION TO BIOPHARMACEUTICS (CONT.):

- **Bioavailability:** The rate and extent (amount) of absorption of unchanged drug from its dosage form .
- **Bioavailable dose:** The fraction of an administered dose of a particular drug that reaches the systemic circulation intact.
- **Plasma**



DRUG ADMINISTRATION AND DRUG THERAPY CAN NOW BE CONVENIENTLY DIVIDED IN TO FOUR PHASES OR PROCESSES

1. **The pharmaceutical processes :** formulation of an effective dosage form of the drug for administration by a suitable route.
2. **The pharmacokinetics processes :** ADME of drugs as elicited by the plasma drug concentration-time profile and the dose, dosage form and frequency and route of administration.
3. **The pharmacodynamic processes:** it is concerned with biochemical and physiologic effect of drug and its mechanism of action.
4. **The therapeutic processes:** it is concerned with the translation of pharmacological effect in to clinical effect.

DOSAGE REGIMEN

- Definition
  The manner in which the drug should be taken.
- Time interval
- Dose size

Topic –
Kinetics of Protein Binding

Presented By
Mr. Vishal V. Kalal
Ass. Prof.
JES'S College Of Pharmacy, Nandurbar.

CONTENTS

- Introduction
- Factors affecting protein binding
- Kinetics of protein binding

Introduction:

Drug in the body can interact with several tissue components of which two major categories are blood and extravascular tissue.

Interacting molecules in this process are generally macromolecules such as proteins, DNA & adipose.

The phenomena of complex formation with proteins is called as protein binding of drugs.

The importance of this binding is that the bound drug is pharmacokinetically & pharmacodynamically inert .

Moreover, such a bound drug, because of its molecular size, cannot undergo membrane transport and thus its half life is increased.

Binding of drug involves weak chemical bonds like hydrogen bonds, hydrophobic bonds. Ionic bonds or vander waal's forces which is a reversible forces.

Irreversible binding occurs due to covalent bonding .

Plasma protein-drug binding:

After the drug enters into systemic circulation the first component with which it can interact is blood components i.e; plasma proteins and hemoglobin. This binding is reversible

Order : Albumin > α_1 AG > Lipoproteins > Globulins.

This effect of protein binding is most significant with drugs that are highly protein-bound (>95%) and have a low therapeutic index, such as warfarin.

A low therapeutic index indicates that there is a high risk of toxicity when using the drug. Since warfarin is an anticoagulant with a low therapeutic index, warfarin may cause bleeding if the correct degree of pharmacologic effect is not maintained.

Binding of drugs to human serum albumin:

Human serum albumin (HSA), is the most abundant plasma protein with a molecular weight of 65000.

HSA can bind to various compounds because of its varied structures.

Four different sites are present for drug-binding which are:


I. Warfarine & Azapropazone site.
II. Diazepam site.
IV. Tamoxifen site.
III. Digoxine site.
Very few drugs bind to site 3 & 4

Binding of drugs to α_1 Acid Glycoprotein :

Has a molecular weight of 44,000 and a plasma concentration range of 0.04 – 0.1 g% . Also called as orosomucoid. The drugs which bind are imipramine, amitriptyline, nortriptyline, lidocane, propanolol etc...

Binding of drugs to Globulin:

Globulin	Synonym	Binds to:
1. α_1 Globulin	Transcortine /Corticosteroid Binding globulin	Steroidal drugs, Thyroxin & Cyanocobalamine.
2. α_2 Globulin	Ceruloplasmine	Vitamin A,D,E,K.
3. β_1Globulin	Transferine	Ferrous ions
4. β_2Globulin	---	Carotinoids
5. γ Globulin	---	Antigens

Factors affecting protein-drug binding:

- Factors relating to the drug

a) Physicochemical characteristics of the drug
b) Concentration of the drug in the body
c) Affinity of a drug for a particular binding component

- factors relating to the protein and other binding components

d) physicochemical characteristics of the protein or binding agent
e) Concentration of protein or binding component
f) Number of binding sites on the binding agent

- Drug interaction

g) Competition between drugs for the binding sites
h) Competition between drugs and normal body constituents
i) Allosteric changes in protein molecules

- patient related factors

a) Age
b) Intersubject variations
c) Disease state

Drug related factors

Physicochemical characteristics of the drug:

Its directly related to the lipophilicity of the drug. An increase in lipophilicity increases the extent of binding.

Ex: The slow absorption of cloxacilin in comparison to ampicillin after i.m injection is attributed to its higher lipophilicity.

Anionic or acidic drugs as penicillins and sulfonamides bind to HSA, whereas cationic or basic drugs such as imipramine and alprenolol bind to AAG.

Concentration of drug in the body:

The extent of protein-drug binding can change with both changes in drug as well as protein concentration.

Drug-protein/tissue affinity:

lidocane has greater affinity for AAG than for HAS.
Digoxin has more affinity for proteins of cardiac muscles than those of skeletal muscles or plasma.
Iophenoxic acid, a radioapaque medium, has no great affinity for plasma proteins than it has a half life of two n half years.

Protein/Tissue related factors

Physicochemical properties of protein/binding component:

Lipoproteins and adipose tissue tend to bind

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.
The amount of several proteins and tissue components available for binding, changes during disease states.

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
Ex: fluocloxacillin , flurbiprofen, ketoprofen, tamoxifen and

Indomethacin is known to bind to 3 different sites.

AAG is a protein with limited binding capacity due to its low concentration and low molecular size.
Pure AAG has only 1 binding site for lidocaine but in presence of HSA 2 binding sites have been reported.

KINETICS OF PROTEIN-BINDING

If "P" represents protein and "D" the drug then applying law of mass action to reversible protein-binding binding

$$P + D \rightleftharpoons PD$$

At equilibrium,

$$K_a = \frac{[PD]}{[P][D]}$$

$$[PD] = K_a [P] [D]$$

Where, [P] – concentration of free protein

[D] – concentration of free drug

[PD] – concentration of free - drug complex

K_a – association rate constant

If "P_T" is the total concentration of protein present, unbound and bound, then:

$$P_T = [PD] + [P]$$

If "**r**" is the number of moles of drug bound to total moles of protein, then,

$$r = \frac{[PD]}{P_T}$$

$$= \frac{[PD]}{[PD] + [P]}$$

$$r = \frac{Ka[P][D]}{Ka[P][D] + [P]} = \frac{Ka[D]}{Ka[D] + 1}$$

The above equation holds when there is only one binding site on the protein and the protein – drug complex is a 1:1 complex

If more than one or N number of binding sites are available per molecule of protein then :

$$r = \frac{N Ka [D]}{Ka [D] + 1}$$

The value of association constant, Ka and the number of binding sites N can be obtained by plotting the above equation in four different ways

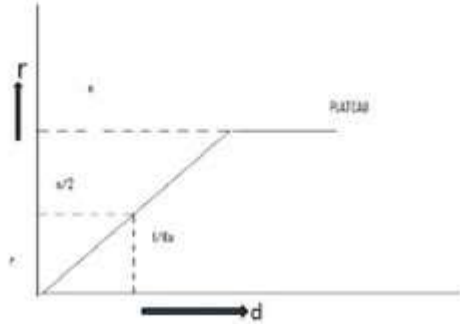
1. Direct plot
2. Scatchard plot
3. Klotz plot
4. Hitchcock plot

PLOTS OF DRUG DISTRIBUTION

1) DIRECT PLOT METHOD:

A direct plot of "r" Vs [D] can be used to find out the no of binding sites on protein 'n' (plateau value).

Ka is obtained by finding drug conc required to saturate the half of the total binding sites available (i.e; n/2).



2) SCATCHARD PLOT:

Obtained by rearranging an equation into linear form.

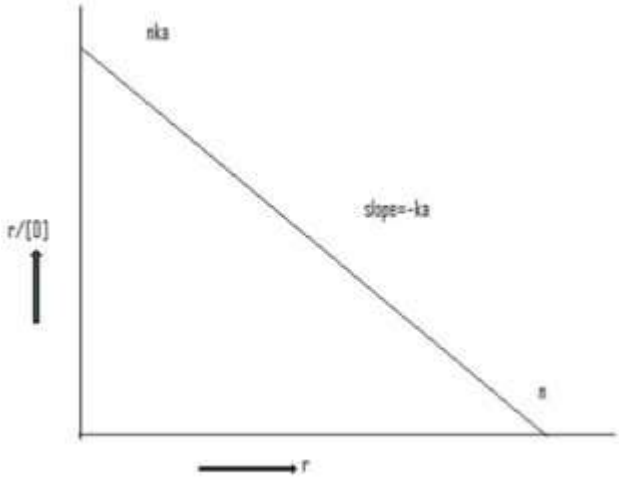
$$r = \frac{nK_a[D]}{K_a[D] + 1}$$

$$r + rK_a[D] = nK_a[D]$$

$$r = nK_a[D] - rK_a[D]$$

$$\frac{r}{[D]} = nK_a - rK_a$$

A plot of r/[D] Vs r yields a st.line with X & Y intercepts equal to 'n' & 'nKa' & the slope is equal to Ka.



Scatchard plot

3) DOUBLE RECIPROCAL PLOT OR KLOTZ PLOT:

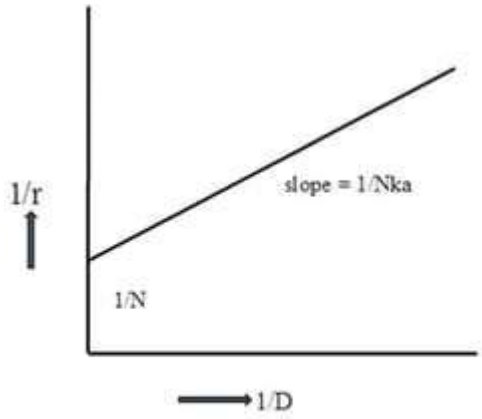
(*LINE WEAVER -BURK PLOT*)

Reciprocal of equation gives-

$$1/r = 1/nka(D) + 1/n$$

A plot of 1/r Vs 1/D yields a double reciprocal plot.

It is straight line with slope 1/Nka and Y-intercept 1/N



Klotz plot

4) HITCHCOCK PLOT

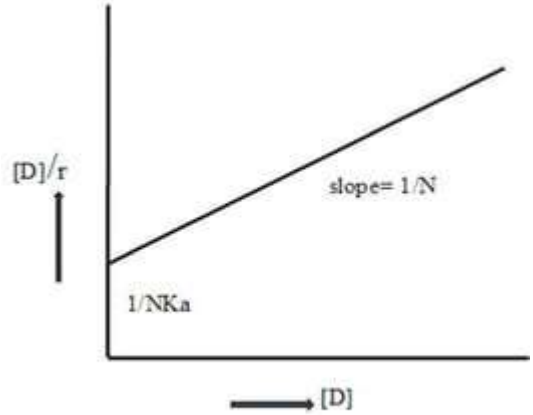
It is made by rearranging the equation as –

$$N\,Ka\,[D]/r = 1 + Ka$$

dividing both sides by Nka gives –

$$[D]/r = 1/NKa + [D]/N$$

A plot of $[D]/r$ Vs $[D]$ yields a straight line with slope $1/N$ and intercept $1/NKa$



Hitchcock plot

References:-

- Biopharmaceutics and pharmacokinetics, D.M.BRAHMANKAR pg no :124,125,134 to 136.


THANK YOU !

Topic –
Volume of Drug Distribution

Presented By
Mr. Vishal V. Kalal
Ass. Prof.

JES'S College Of Pharmacy ,Nandurbar.

Objectives

1. Overview of drug distribution
2. Explain apparent volume of distribution with clinical implications
3Discuss drug binding to plasma proteins and tissues with clinical implications
4. Explain redistribution
5. Discuss blood brain barrier and Placental barrier

Drug Distribution *refers to the Reversible Transfer of a Drug between the Blood and the Extra Vascular Fluids and Tissues of the body (for example, fat, muscle, and brain tissue).*

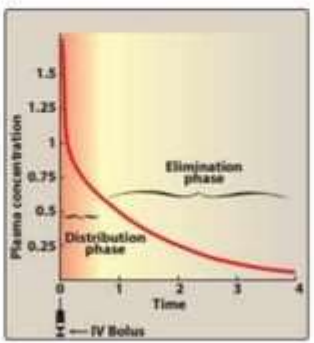
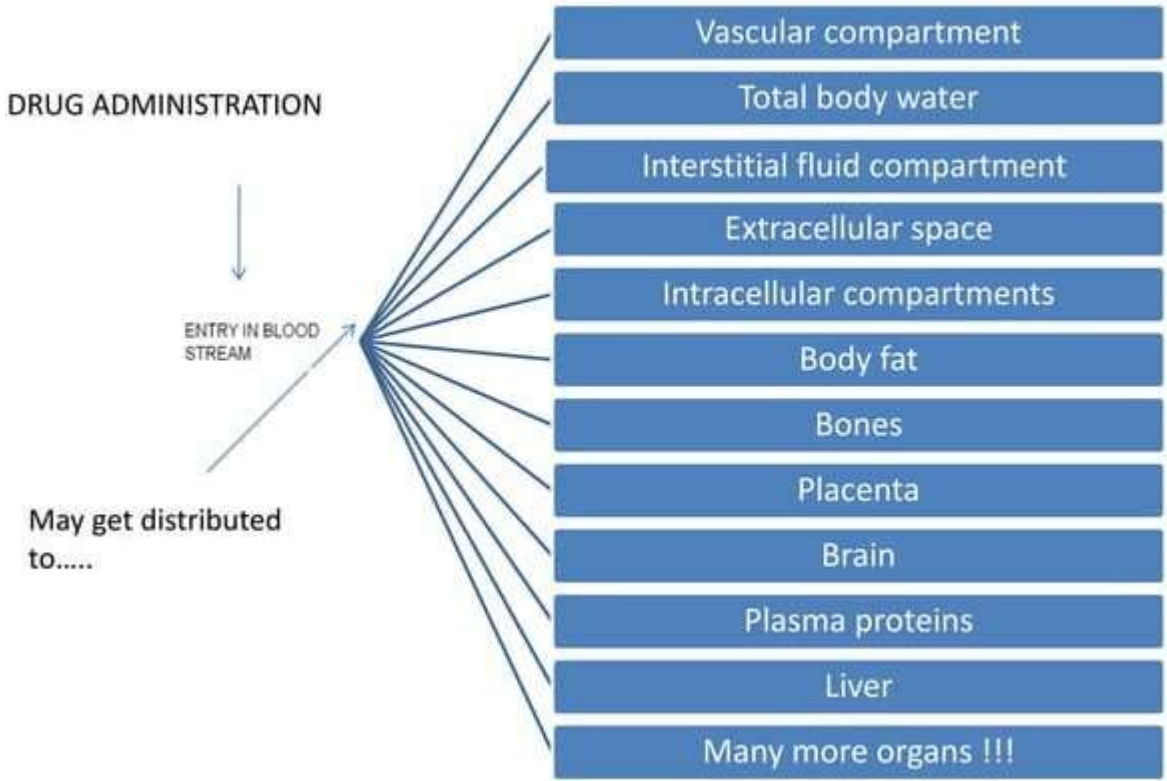
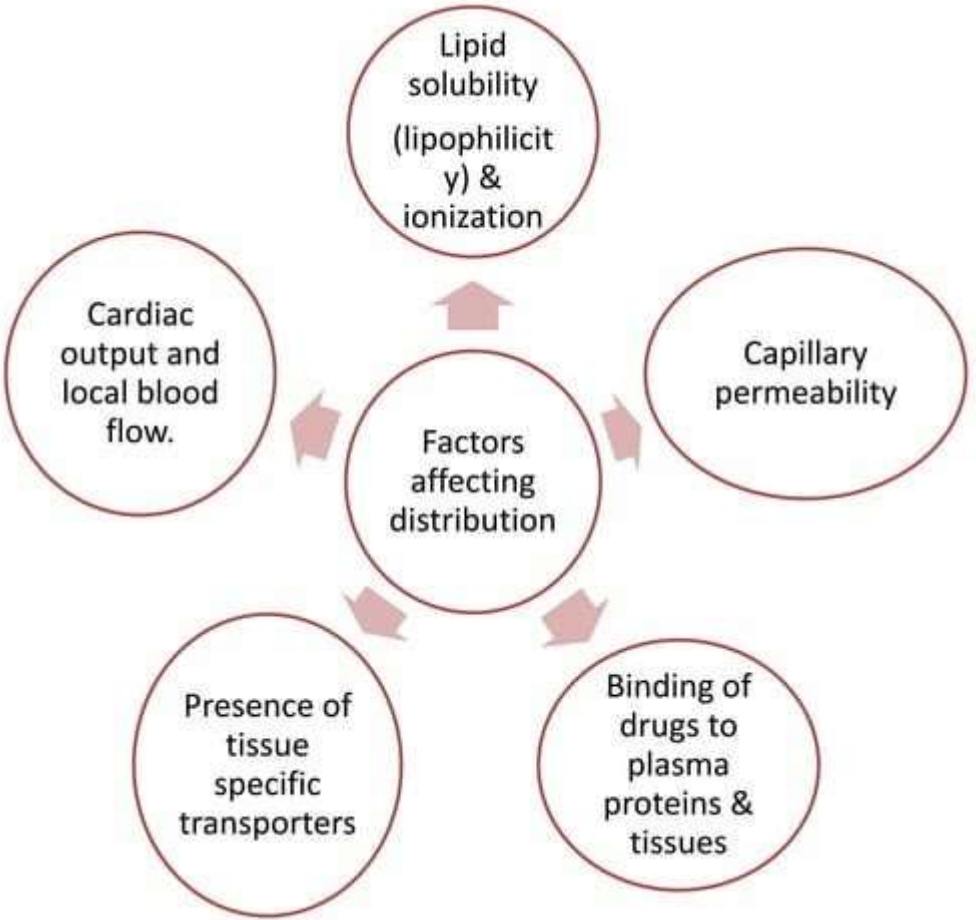


Figure 1.12
Drug concentrations in serum after a single injection of drug. Assume that the drug distributes and is subsequently eliminated.


DRUG ADMINISTRATION
ENTRY IN BLOOD STREAM
May get distributed to.....
Vascular compartment
Total body water
Interstitial fluid compartment
Extracellular space
Intracellular compartments
Body fat
Bones
Placenta
Brain
Plasma proteins
Liver
Many more organs !!!


Lipid solubility (lipophilicity) & ionization
Capillary permeability
Binding of drugs to plasma proteins & tissues
Presence of tissue specific transporters
Cardiac output and local blood flow.
Factors affecting distribution

Volume of distribution

- Fluid volume that is required to contain the entire drug in the body at the same concentration measured in the plasma.
- Calculated by dividing the dose that ultimately gets into the systemic circulation by the plasma concentration at time zero (C_0)

$$V_d = \frac{\text{Amount of drug in the body}}{C_0}$$

Which means

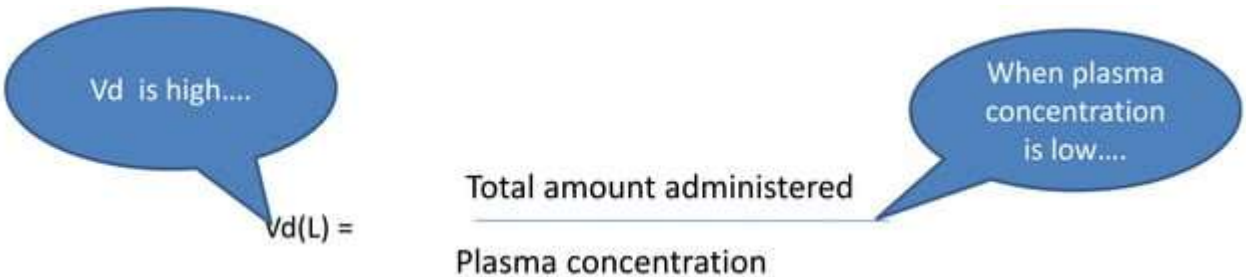
- If 500 mg of drug reaches circulation...(total amount of drug)
- And if plasma concentration is 0.5 mg/ml
- Vd will be 500/0.5 = 1000 ml.
- Which means you require 1000 ml of fluid to accommodate total 500 mg of drug at concentration of 0.5 mg/ml.
- At times it can be larger than total blood volume. (when drug has been **stored in peripheral tissues** so lower blood concentration).
- At times it can be smaller than or equal to total blood volume(when drug **remains in vascular compartment**).

$$Vd(L) = \frac{\text{Total amount administered}}{\text{Plasma concentration}}$$

Vd is low....

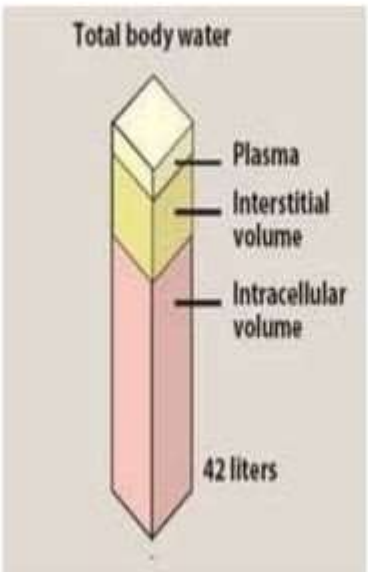
When plasma concentration is high....

$$Vd(L) = \frac{\text{Total amount administered}}{\text{Plasma concentration}}$$

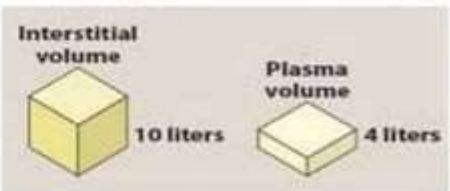

Vd is high....
When plasma concentration is low....
Vd(L) =
Total amount administered
Plasma concentration

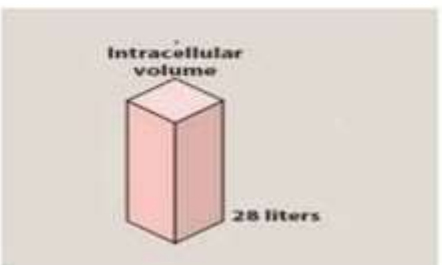
Distribution into the water compartments of body

- Plasma compartment:
  - Drugs having high molecular weight or extensively plasma protein bound like heparin Vd= 4L
- Extracellular fluid:
  - Low molecular weight but hydrophilic drugs
  - Aminoglycosides Vd=14L
- Total body water:
  - low molecular weight and lipophilic,
  - E.g *Ethanol* Vd=42 L


Total body water
Plasma
Interstitial volume
Intracellular volume
42 liters


Extracellular volume
14 liters


Interstitial volume
10 liters
Plasma volume
4 liters


Intracellular volume
28 liters

Apparent Volume of distribution

- A drug rarely associates exclusively with only one of the water compartments of the body.
- Vast majority of drugs distribute into several compartments, often avidly binding cellular components, such as lipids, proteins, and nucleic acids.
- Thus, the volume into which drugs distribute is called the apparent volume of distribution (Vd).

Plasma protein binding

- Most drugs posses physicochemical affinity for plasma proteins
  - Acidic drugs bind to plasma albumin, basic drugs bind to □1 acid glycoprotein
  - Reversible manner
  - Extensive binding serves as a circulating drug reservoir
  - Other proteins to which drugs can bind: globulins, transferrin, ceruloplasmin, tissue proteins & nucleoproteins

Clinical implications of plasma protein

1. Highly plasma protein bound drugs does not cross membranes so largely restricted to vascular compartments (smaller V_d).
2. Temporary storage of the drug which is not available for any action.
3. High degree of protein binding generally makes the drug long acting
4. Plasma concentrations of the drug refer to bound as well as free drug.

Clinical implications of plasma protein

5. One drug can bind to many sites on the albumin molecule. Conversely, more than one drug can bind to the same site.
6. Displacement reactions- (Drug interactions)
   - Salicylates displace sulfonylureas & methotrexate.
   - Indomethacin, phenytoin displace warfarin.
   - Sulfonamides and vit K displace bilirubin(kernicterus in neonates) .
7. In hypoalbuminemia, reduced binding leads to high concentrations of free drug e.g. phenytoin and furosemide.
8. Other diseases: e.g. phenytoin and pethidine binding is reduced in uraemia;

Drugs highly bound to plasma proteins

- To albumin
- Barbiturates
- Benzodiazepines
- NSAIDs
- Valproic acid
- Phenytoin
- Penicillins
- Sulfonamides
- Tetracyclines
- Warfarin

- To α1 acid glycoprotein
- β-blockers
- Bupivacaine
- Lidocaine
- Disopyramide ,
- Imipramine
- Methadone
- Prazosin
- Quinidine
- Verapamil

Clinical implications of volume of distribution

- Dialysis is not very useful for drugs with high Vd e.g digoxin, imipramine
- It helps in estimating the total amount of drug at any time

  amount of drug = Vd X plasma conc of drug at certain time
- Vd is important to determine the loading dose

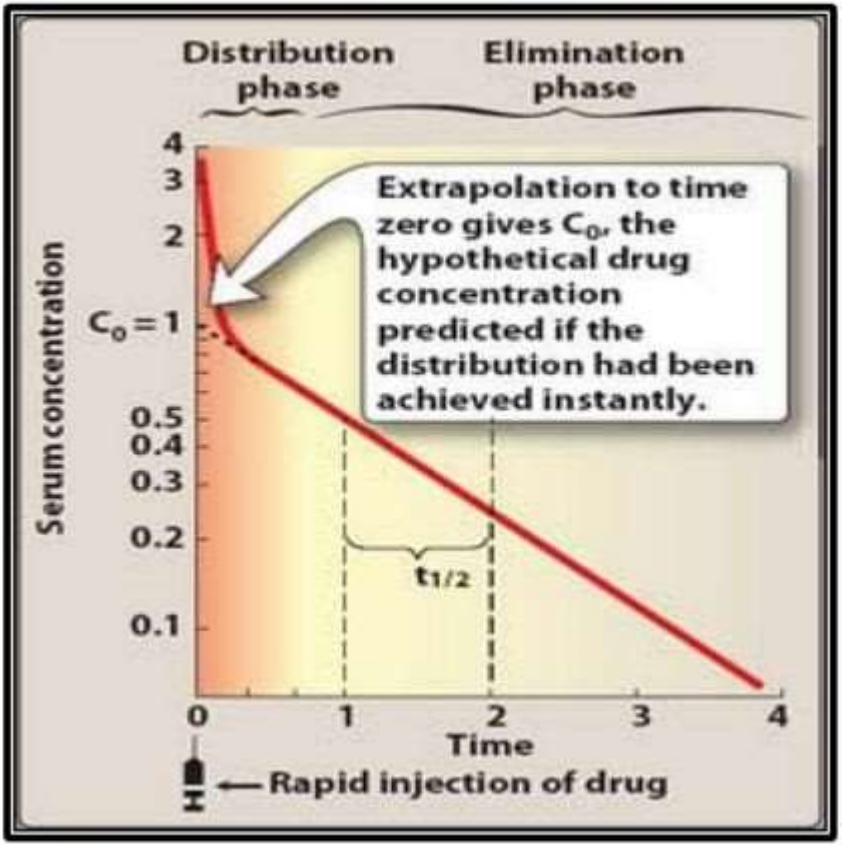
  Loading dose = Vd X desired concentration

Drugs concentrated in body tissues

- Digoxin, emetine: Skeletal muscles, heart, liver, kidney
- Chloroquine: retina and liver
- Iodine: Thyroid
- Chlorpromazine: eye
- Atropine: iris
- Tetracyclines: Bone and teeth
- Thiopentone , DDT: Adipose tissue

Redistribution

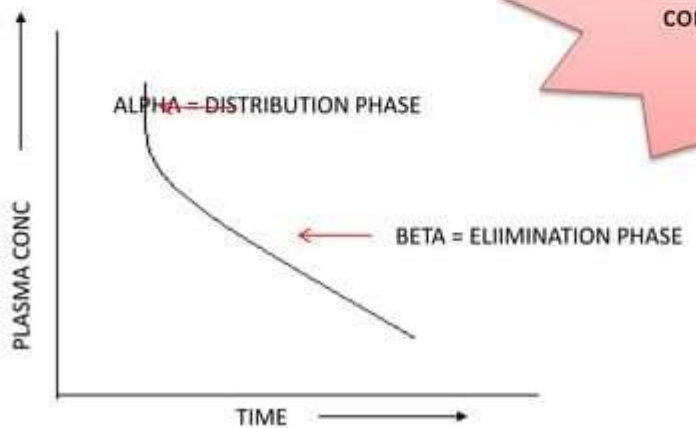
- Highly lipid-soluble drugs get initially distributed to organs with high blood flow (brain, heart, kidney) & later into bulky less vascular tissues (muscle, fat)
- So plasma concentration falls and the drug is withdrawn from these sites
- If the site of action of drug is one of highly perfused organs, redistribution may result in termination of drug action.
- Greater the lipid solubility faster is the redistribution of drug.
- Anaesthetic action of thiopentone sod. injected i.v. is terminated in few minutes due to redistribution.
- To overcome , give continous infusion


Distribution phase
Elimination phase
Extrapolation to time zero gives C_0, the hypothetical drug concentration predicted if the distribution had been achieved instantly.
Serum concentration
$C_0 = 1$
4
3
2
0.5
0.4
0.3
0.2
0.1
$t_{1/2}$
0
1
2
3
4
Time
Rapid injection of drug

PLASMA HALF LIFE

- It is the time taken for the plasma concentration or amount of the drug present in the body to reduce to 50% of previous level

Clinically t ½ that is calculated from BETA ELIMINATION PHASE is considered as t ½ of drug.



At peak → blood concentration will be 100 %

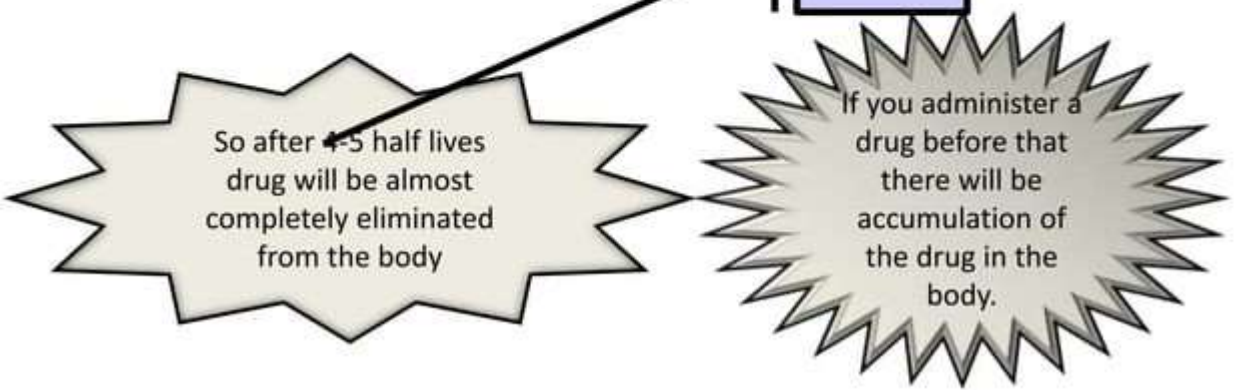
After 1 half life → blood concentration will be 50 %

After 2 half lives → blood concentration will be 25 %

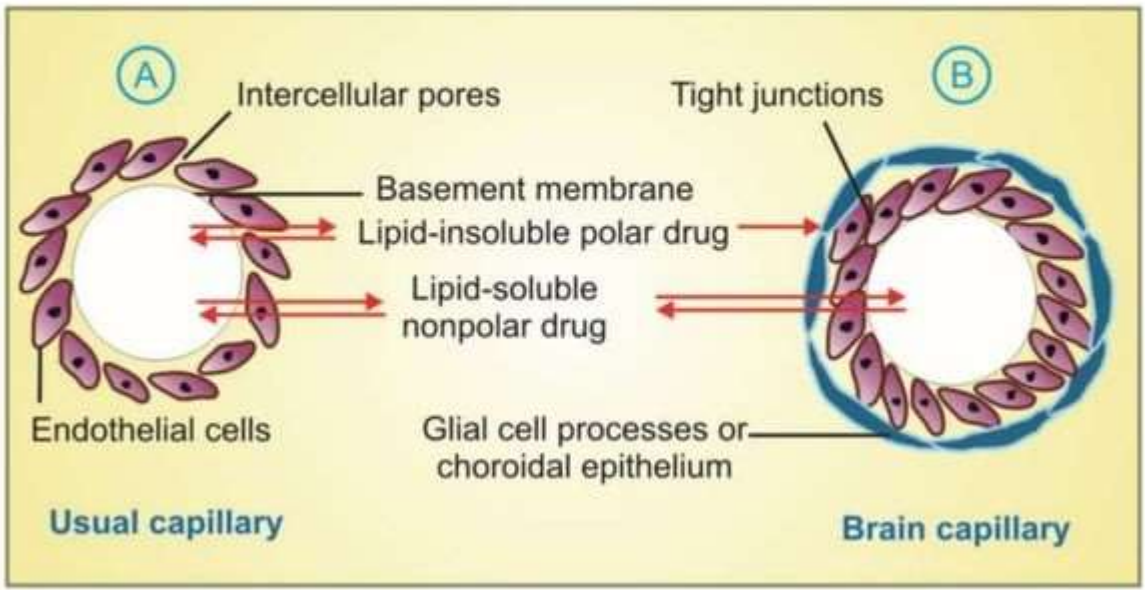
After 3 half lives → blood concentration will be 12.5 %

After 4 half lives → blood concentration will be 6.25 %

After 5 half lives → blood concentration will be 3.125 %



Blood brain barrier



Functions and Properties of the BBB

- Protects the brain from "foreign substances" in the blood that may injure the brain.
- Protects the brain from hormones and neurotransmitters in the rest of the body.
- Maintains a constant environment for the brain.

Properties of drugs that can cross BBB

- low molecular weight
- High degree of lipid solubility
- Non ionized
- Tertiary structure and
- Free drug

Placental Barrier

- Lipoidal and allows free passage of lipophilic drugs
- P Glycoprotein limits exposure to maternally administered drugs
- Also placenta is site of metabolism- lowers exposure to drugs
- Incomplete barrier
- Congenital anomalies

Summary

1. Overview of drug distribution
2. Apparent volume of distribution with clinical implications
3 Drug binding to plasma proteins and tissues with clinical implications
4. Redistribution
5. BBB and Placental barrier