DRUG DISTRIBUTION

Introduction

Once a drug has gained excess 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.

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).*

Distribution is a Passive Process, for which the driving force is the concentration gradient between the blood and extra-vascular tissues

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

Distribution of a drug is not uniform throughout the body because different tissues receive the drug from plasma at different rates and to different extents.

Volume of Distribution

The **Volume of distribution** (V_D), also known as **Apparent volume of distribution**, is used to quantify the distribution of a drug between plasma and the rest of the body after oral or parentral dosing.

It is called as Apparent Volume because all parts of the body equilibrated with the drug do not have equal concentration.

It is defined as the volume in which the amount of drug would be uniformly distributed to produce the desired pharmacological action.

Steps in Drug Distribution

- Permeation of free or unbound drug present in blood through capillary wall and entry into ECF.

- Penetration of drug from ECF to tissue cells and intracellular fluid (depends on rate of perfusion to EC tissue and membrane permeability).

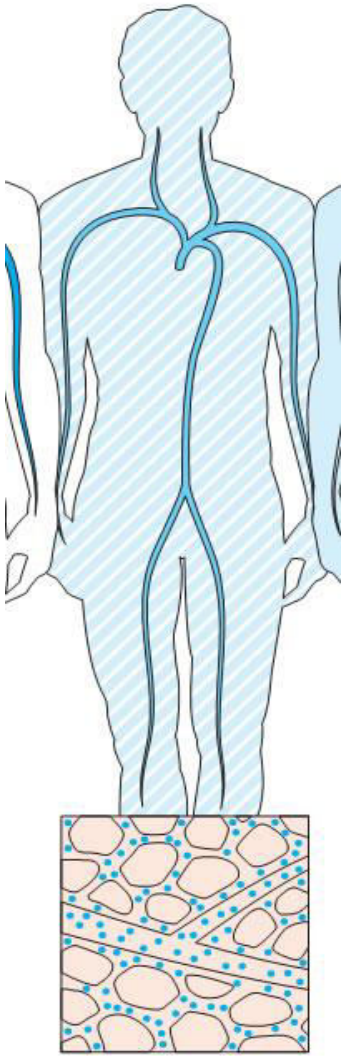
✓ Highly lipid soluble drugs when given by i.v. or by inhalation initially get distributed to organs with high blood flow, e.g. brain, heart, kidney etc.

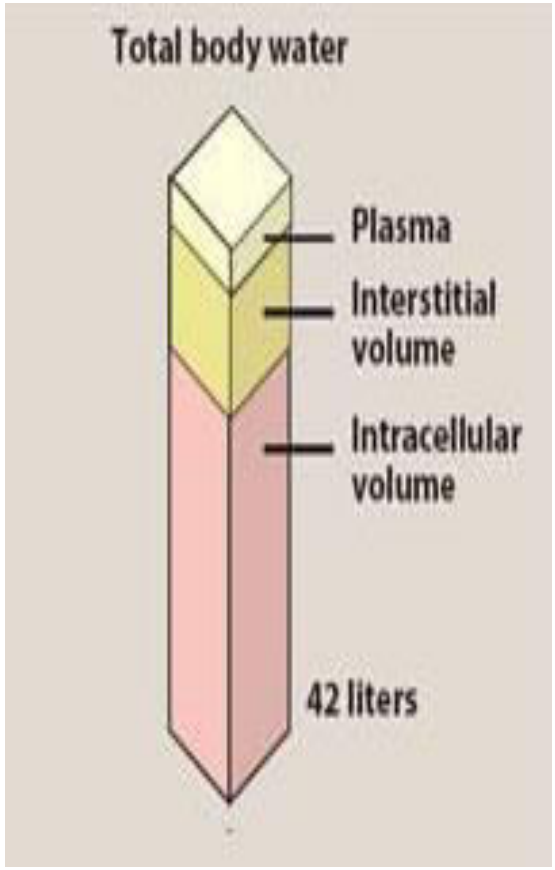
✓ Later, less vascular but more bulky tissues (muscles, fat) take up the drug and plasma concentration falls and drug is withdrawn from these sites.

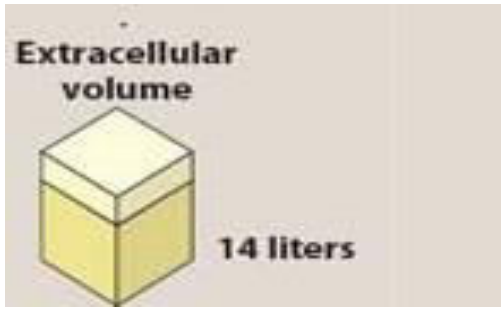
✓ If the site of action of the drug is highly perfused organs, redistribution results in termination of the drug action.

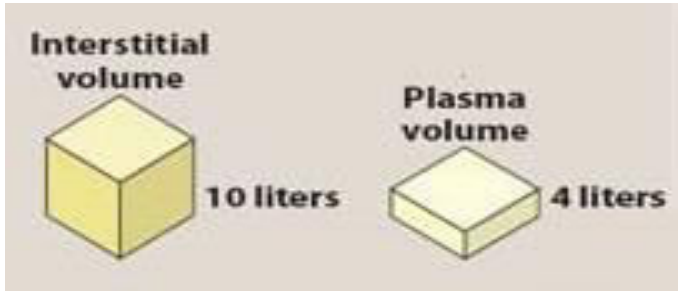
✓ Greater the lipid solubility of the drug, faster is its redistribution.

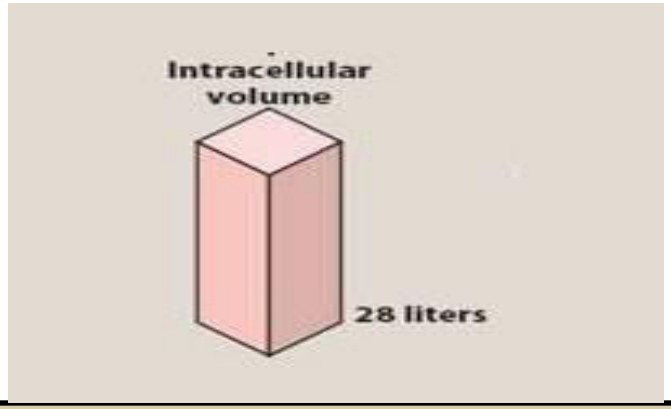
The real volume of distribution has physiological meaning and is related to the Body Water.











The volume of each of these compartments can be determined by use of specific markers or tracers.

Physiological Fluid Compartments the	Markers Used	Approximate volume (liters)
Plasma	Evans Blue, Indocyanine Green	4
Extracellular fluid	Inulin, Raffinose, Mannitol	14
Total Body Water	D_2O, Antipyrine	42

The intracellular fluid volume can be determined as the difference between total body water and extracellular fluid.

Fluid components of a 70 kg adult

BODY FLUID	VOLUME (L)	% OF BODY WEIGHT	% OF TBW
Vascular fluid/ blood (plasma)	4	6	10
Extracelluar-extravascular fluid	10	14	24
Intracelluar fluid	28	40	66
TOTAL BODY WATER	42	60	100

Drugs which bind selectively to Plasma proteins e.g. Warfarin have Apparent volume of distribution smaller than their Real volume of distribution.

The Vd of such drugs lies between blood volume and total body water i.e. b/w 6 to 42 liters.

Drugs which bind selectively to Extra-vascular Tissues e.g. Chloroquine have Apparent volume of distribution larger than their Real volume of distribution.

The Vd of such drugs is always greater than 42 liters.

Differences In Drug Distribution Among Various Tissues Arises Due To a Number of Factors:

- Tissue Permeability of the Drug
  - a. Physiochemical Properties of the drug like Molecular size, pKa and o/w Partition coefficient.
  - b. Physiological Barriers to Diffusion of Drugs.
- Organ / Tissue Size and Perfusion Rate
- Binding of Drugs to Tissue Components

  (Blood components and Extravascular Tissue Proteins)
- Miscellaneous Factors

  Age, Pregnancy, Obesity, Diet, Disease states, and Drug Interactions…

A- Tissue Permeability of the Drugs

Rate limiting steps in distribution of drugs are:

1. Rate of Tissue Permeability, and
2. Rate of Blood Perfusion.

the rate of Tissue Permeability, depends upon Physiochemical Properties of the drug as well as Physiological Barriers that restrict the diffusion of drug into tissues.

Physiochemical Properties that influence drug distribution are:

i. ***Molecular size,***
ii. ***pKa,***
iii. ***o/w Partition coefficient,***
iv. ***Stereochemical nature***

- **Drugs having molecular wt. less than 400 daltons easily cross the Capillary Membrane to diffuse into the Extracellular Interstitial Fluids.**
- **Now, the penetration of drug from the Extracellular fluid (ECF) is a function of :-**
  - ***Molecular Size:***

    Small ions of size < 50 daltons enter the cell through aqeous filled channels where as larger size ions are restricted unless a specialized transport system exists for them.
  - ***Ionization:***

    A drug that remains unionized at pH values of blood and ECF can permeate the cells more rapidly.

    Blood and ECF pH normally remains constant at 7.4, unless altered in conditions like Systemic alkalosis/acidosis.

- ***Lipophilicity:***

  Only unionized drugs that are lipophilic rapidly crosses the cell membrane.

  e.g. Thiopental, a lipophilic drug, largely unionized at Blood and ECF pH readily diffuses the brain where as Penicillins which are polar and ionized at plasma pH do not cross BBB.

  Acidosis results in decreases ionization of acidic drugs and thus increased intracellular drug concentration and pharmacological action. Opposite is the effect of alkalosis.

  sodium bicarbonate induced alkalosis is sometimes useful in treatment of barbiturates.

– *In case of polar drugs, where permeability is the rate limiting step, driving force is the effective partition coefficient of drug.*

Effective Partition Coefficient for a drug is given by:

Effective K o/w = **Fraction unionized at pH 7.4** X **K o/w of unionized drug**

Drug	Relative acidity	Effective partition coff	Relative rate of distribution
Thiopental	Weaker acid	2	80
Salicylic acid	Stronger acid	0.0005	1

- Stereochemical nature of drug influences the drug distribution, when it has tendency to interact with macromolecules, like proteins.

Physiological Barriers to distribution of drugs

- Some 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

Simple Capillary Endothelial Barrier

- All drugs, with molecular size less than 600 daltons, diffuse through the capillary endothelium and into interstitial fluid.
- Only drugs bound to blood components are restricted.

Simple Cell Membrane Barrier

- Cell membrane is similar to the lipoidal barrier in GIT.

PENETRATION OF DRUGS THROUGH BLOOD BRAIN BARRIER

A stealth of endothelial cells lining the capillaries.

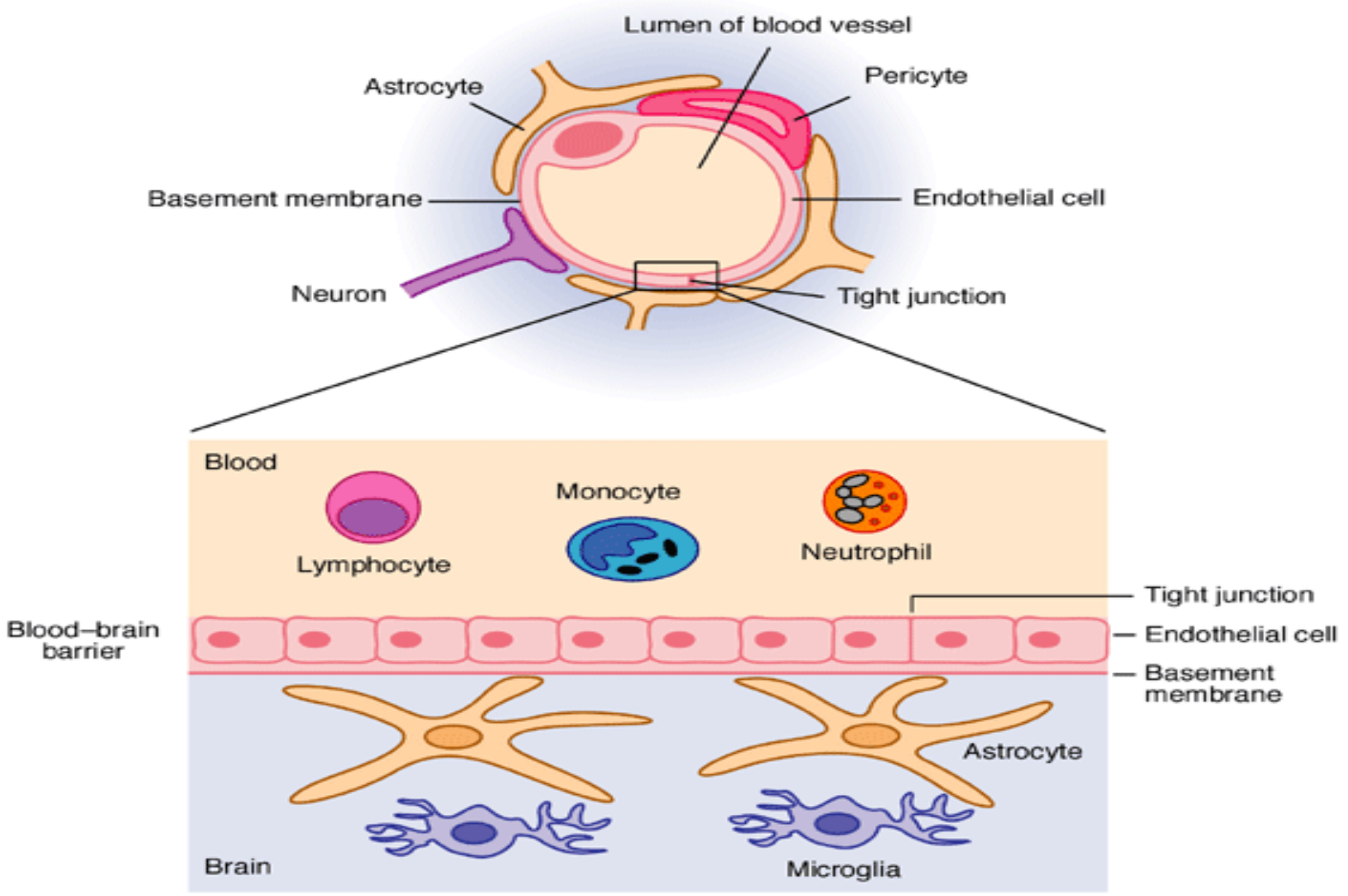
It has tight junctions and lack large intra-cellular pores.

Further, neural tissue covers the capillaries.

- Astrocytes : Special cells / elements of supporting tissue are found at the base of endothelial membrane.

  Together , they constitute the BLOOD BRAIN BARRIER.

- The blood-brain barrier (BBB) is a separation of circulating blood and cerebrospinal fluid (CSF) maintained by the choroid plexus in the central nervous system (CNS).



The blood–brain barrier (BBB)

Since BBB is a lipoidal barrier,

It allows only the drugs having high o/w partition coefficient to diffuse passively where as moderately lipid soluble and partially ionized molecules penetrate at a slow rate.

Cells of the barrier actively transport metabolic products such as glucose across the barrier with specific proteins.

Various approaches to promote crossing BBB:

- Use of Permeation enhancers such as Dimethyl Sulfoxide.
- Osmotic disruption of the BBB by infusing internal carotid artery with Mannitol.
- Use of Dihydropyridine Redox system as drug carriers to the brain (the lipid soluble dihydropyridine is linked as a carrier to the polar drug to form a prodrug that rapidly crosses the BBB)

PENETRATION OF DRUGS THROUGH PLACENTAL BARRIER

Placenta is the membrane separating Fetal blood from the Maternal blood.

- It is made up of Fetal Trophoblast Basement Membrane and the Endothelium.
- Mean thickness in early pregnancy is (25 µ) which reduces to (2 µ) at full term.

- Many drugs having mol. wt. < 1000 Daltons and moderate to high lipid solubility e.g. ethanol, sulfonamides, barbiturates, steroids, anticonvulsants and some antibiotics cross the barrier by simple diffusion quite rapidly .

- Nutrients essential for fetal growth are transported by carrier mediated processes.

STAGES DURING WHICH TERATOGENS SHOW FOETAL ABNORMALITIES

PERIOD	SIGNIFICANCE	HARMFUL EFFECTS
First two weeks	Fertilization and implementation stage	Miscarriage
2-8 weeks	Period of organogenesis	Cleft palate, optic atrophy, mental retardation, neural tube defects, etc.
8 weeks onwards	Groeth and development	Development and functional abnormalities

Blood – Cerebrospinal Fluid Barrier

- The Cerebrospinal Fluid (CSF) is formed mainly by the Choroid Plexus of lateral, third and fourth ventricles.
- The choroidal cells are joined to each other by tight junctions forming the Blood – CSF barrier which has permeability characteristics similar to that of BBB.
- Only high lipid soluble drugs can cross the Blood – CSF barrier.

B. 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.
- Greater the blood flow, faster the distribution.
- Highly perfused tissues such as lungs, kidneys, liver, heart and brain are rapidly equilibrated with lipid soluble drugs.
- The extent to which a drug is distributed in a particular tissue or organ depends upon the size of the tissue i.e. tissue volume.

Miscellaneous Factors

Diet: A Diet high in fats will increase the free fatty acid levels in circulation thereby affecting binding of acidic drugs such as NSAIDS to Albumin.

Obesity: In Obese persons, high adipose tissue content can take up a large fraction of lipophilic drugs.

Pregnancy: During pregnancy the growth of the uterus, placenta and fetus increases the volume available for distribution of drugs.

Disease States: Altered albumin or drug – binding protein conc.
Altered or Reduced perfusion to organs /tissues
Altered Tissue pH

Factor affecting drug-protein binding

Protein binding of drugs

- Intracellular Binding

- Extracellular Binding

Mechanism of protein drug binding

- Usually reversible, suggest involvement of weak chemical bonds like hydrogen bonds, hydrophobic bonds, ionic bonds or vanderwaal's force.
- Irreversible drug binding, usually due to covalent bond formation, associated with carconogenicity or tissue toxicity (eg, hepatotoxicity due to chloroform and paracetamol)

Binding of drugs

- To blood components:
  - Plasma proteins
  - Blood cells
- Extravascular tissue proteins, fats, bones

PLASMA PROTEIN- DRUG BINDING

INTERACTION OF DRUG WITH TO BLOOD PROTEIN

Protein	Molecular Weight (Da)	Conc (g/L)	Drug that bind
Albumin	65,000	3.5–5.0	Large variety of drug
α1- acid glycoprotein	44,000	0.04 – 0.1	Basic drug - propranolol, imipramine , and lidocaine . Globulins (-, -, -globulins corticosteroids.
Lipoproteins	200,000–3,400,000	.003-.007	Basic lipophilic drug Eg- chlorpromazine
α1 globulin α2 globulin	59000 13400	.015-.06	Steroid , thyroxine Cynocobalamine Vit. –A,D,E,K

Binding of drugs to Human Serum Albumin (HSA)

- HSA constitute 59% of total plasma and 3.5-5 gm percent.
- High binding capacity with all drugs and endogenous compounds (fatty acids, bilirubin, tryptophan etc.)
- Four binding sites on HAS:

  - Site I (Warfarin and azapropazone binding site)- several NSAIDs, Sulphonamides, phenytoin, sodium valprorate, bilirubin.

- Site II (Diazepam Binding Site): include benzodiazepines, medium chain fatty acids, ibuprofen, ketoprofen, tryptophan, cloxacillin, probenicid, etc.
- Site III (Digitoxin binding Site)
- Site IV (Tamoxifen binding site)

- Dicuramol: Primary site- site I, Secondary site- Site II

Binding of drugs to $\acute{\alpha}_1$ glycoproteins

- Also known as orosomucoid.
- Binds to number of basic drugs, imipramine, amitrypyline, nortriptyline, propranolol, quinidine and isopyridine.

Binding of drugs to Lipoproteins

- May bind to hydrophobic drugs as HAS and AAG, because of involvement of hydrophobic bonds.
- Concentration of lipoproteins is very less.
- Drug binds by dissolving in lipid core of protein, thus binding capacity depends on lipid content.

- Lipoprotein is classified on the basis of density:

  1. Chylomicrons (least dense and largest in size)

  2. Very low density lipoproteins

  3. Low-density lipoproteins

  4. High density lipoproteins

- The hydrophobic core consists of triglycerides and cholestrol esters. The hydrophillic surface is made up of apoproteins (free cholestrol and proteins).
- Binding of drugs is non-competitive (not dependent on drug concentration).
- All drugs bind to lipoproteins, but lipophillic basic drugs have more affinity
- Lipoprotein binding is significant when
  - Drugs predominantly bind to them
  - Levels of HSA and AAG decreases in blood.

Binding of drug to globulin

α1 globulin bind to a number of steroidal drug cortisone , prednisolone $ thyroxine , cynocobalamine

α2 globulin

(ceruloplasmin) bind to Vit. A D E K

γ- globulin

bind to antigen

β1-globulin

(transferrin) bind to ferrous ion

β2-globulin

bind to carotinoid

Binding of drug to blood cells

- **40% of blood comprises of blood cells.**
- **95% of blood cells comprises of RBCs.**
- **RBC-drug binding plays significant role.**

hemoglobin bind to phenytoin, pentobarbital , phenothiazi--ne

carbonic anhydrase bind to acetazolamid, chlorthalidone

cell membrane – imepramine , chlorpramaz--ine bind to RBCs cell membrane

Tissue binding of drug

Majority of drug bind to extravascular tissue- the order of binding -: liver > kidney > lung > muscle

Liver SS – epoxide of number of halogenated hydrocarbons ,paracetamol

Lung – basic drug imepramine , chlorpramazine , antihistaminies

Kidney – metalothionin bind to heavy metal , lead, Hg , Cd

Skin – chloroquine, phenothizine

Eye – chloroquine, phenothizine

Hairs- arsenicals, chloroquine bind to hair shaft

Bone – tetracycline

Fats – thiopental , pesticide- DDT

Factors affecting drug protein binding

- 1. **Factors relating to the drug**

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

- **2. Factors relating to the protein and other binding components**

a) Physicochemical characteristic of the protein or binding component
b) Concentration of protein or binding component
c) Num. Of binding site on the binding site

- **3. Drug interaction**
- **4. Patient related factors**

Drug related factor

1.a. Physicochemical characteristics of drug

Protein binding is directly related to lipophilicity

↑ lipophilicity = ↑ the extent of binding

- ✓ e.g. The slow absorption of Cloxacilin in comparison to Ampicillin after i.m. Injection is attributed to its higher lipophilicity and the binding of latter 95% as compared to 20%.
- ✓ Highly lipophilic Thiopental tend to localized in adipose tissue.
- ✓ Anionic or acidic drugs like Penicillin, Sulfonamide bind more to HSA
- ✓ Cationic or basic drug like Imepramine and Alprenolol bind to AAG

1. b. CONCENTRATION OF DRUG IN THE BODY

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

Eg. Therapeutic concentration of lidocaine can saturate AAG with which it binding as the conc. of AAG is much less in comprarison to that of HSA in blood.

1.c. DRUG PROTEIN / TISSUE AFFINITY

- Lidocaine have greater affinity for AAG than HSA
- Digoxin have greater affinity for protein of cardiac muscle than skeleton muscles or plasma

2. Protein or tissue related factor

2.a. Physicochemical property of protein / binding component

Lipoprotein or adipose tissue tend to bind lipophillic drug by dissolving them in the lipid core .

The physiological pH determine the presence of anionic or cationic group on the albumin molecule, to bind a variety of drug.

2.b. Concentration of protein / binding component

Mostly all drug bind to albumin as it is present in higher concentration than other proteins.

2.c. Number of binding sites on the protein

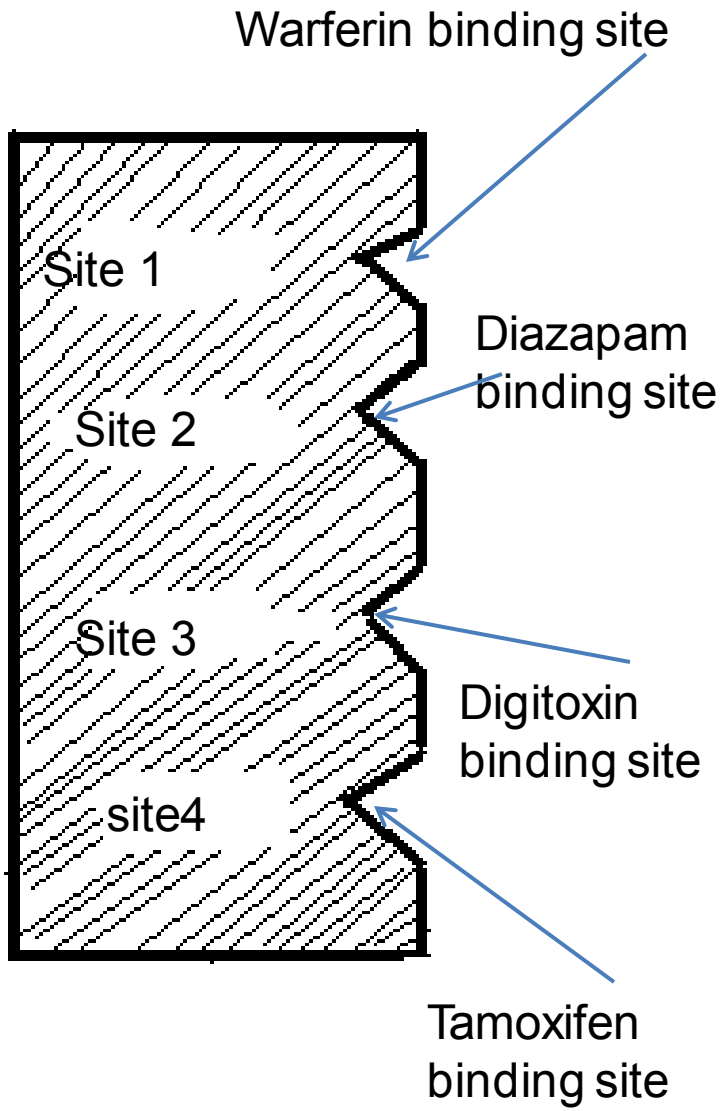
Albumin has a large number of binding site as compare to other protein and it has high capacity binding component.

Several drug capable to binding at more than one binding site

e.g.-flucoxacillin , Flurbiprofen , ketoprofen , tamoxifen and dicoumarol bind to both primary and secondary site of albumin.

Indomethacin binds to three different sites.

AAG is a protein with limited binding capacity because of it low concentration and molecular size.



Drug binding site on HSA

3. Drug interaction

Competition between drugs for binding site (Displacement Interaction)

When two or more drugs are present to the same site , there may be competition between them for interaction with same binding site .

If one of the drug (A) is bound to such a site , then administration of the another drug (B) having high affinity for same binding site result in displacement of drugs (A) from its binding site . This type of interaction is known as displacement interaction .

Where drug (A) here is called as the **displaced drug** and drug (B) as the **displacer .**

Eg. Phenylbutazone displaces Warfarin and Sulfonamide from binding site.

Competition between drug and normal body constituent

The free fatty acids interact with a number of drugs that bind primarily to HSA . When free fatty acid level increases in several conditions – fasting , pathologic – diabeties , myocardial infraction, alcohol abstinence – the fatty acid which also bind to albumin influence binding of several drugs

⇩ binding – diazepam

- propanolol

⇧ binding - warfarin

Acidic drugs like – Sodium salicylate , Sodium benzoate , Sulfonamide displaces bilirubin from its albumin binding sites in neonates and crosses BBB and precipitates toxicity.

Patient related factor

Age

- Neonate – albumin content is low in new born. As a result, there is increase in concentration of unbound drug that primarily bind to albumin eg. Phenytoin , diazepam.
- Elderly -albumin content is lowered leading to increase concentration of unbound drug.

  In old age AAG level is increase thus decrease conc. of free drug that bind to AAG

Disease state

Disease	Influence on plasma protein	Influence on protein drug binding
Renal failure (uremia)	↓ albumin content	Decrease binding of acidic drug , neutral or basic drug are unaffected
Hepatic failure	↓ albumin synthesis	Decrease binding of acidic drug ,binding of basic drug is normal or reduced depending on AAG level.
Inflammatory state (trauma , burn, infection)	↑ AAG levels	Increase binding of basic drug , neutral and acidic drug unaffected

Significance of protein binding of drug

- **Absorption –**the binding of absorbed drug to plasma proteins decrease free drug conc. and disturbs equilibrium . Thus sink condition and conc. gradient are established which now act as the driving force for further absorption
- **Systemic solubility of drug** water insoluble drugs , neutral endogenous macromolecules , like heparin , steroids , and oil soluble vitamin are circulated and distributed to tissue by binding especially to lipoproteins.
- **Distribution -T**he plasma protein-drug binding thus favours uniform distribution of drug throughout the body by its buffer function . A protein bound drug in particular does not cross the BBB, placental barrier and the glomerulus.

Tissue binding, apparent volume of distribution and drug storage

- ✓ A drug that bind to blood component remains confined to blood have small volume of distribution.
- ✓ Drug that show extra-vascular tissue binding have large volume of distribution .
- ❑ the relationship b/w tissue drug binding and apparent volume of distribution-

$$V_d = \frac{\text{amount of drug in the body}}{\text{plasma drug concentration}} = \frac{X}{C}$$

the amount of drug in the body $X = V_d \cdot C$

Similarly , amount of drug in plasma = $V_p \cdot C$

Amount of drug in extravascular tissue = $V_t \cdot C_t$

- The total amount of drug in the body

$V_d \cdot C = Vp.C + Vt.\ Ct$

where , Vp is volume of plasma

Vt is volume of extravascular tissue

Ct is tissue drug concentration

$Vd = Vp + Vt\ Ct/C$ .....(1)

Dividing both side by C in above equation

The fraction of unbound drug in plasma (fu)

$$fu = \frac{\text{conc. of unbound drug in plasma}}{\text{total plasma drug concentration}} = \frac{Cu}{C}$$

The fraction unbound drug in tissue (fut)

$$fut = \frac{Cut}{Ct}$$

Assuming that equilibrium unbound or free drug conc. In plasma and tissue is equal

$$\frac{C_t}{C} = \frac{fu}{fut}$$

mean Cu = Cut then ,

$$Vd = Vp + \frac{Vt \cdot fu}{fut}$$

substituting the above value in equation1

It is clear that greater the unbound or free concentration of drug in plasma larger is its Vd.

Displacement interaction and toxicity

	Drug A	Drug B
% DRUG BEFORE DISPLACEMENT BOUND FREE	 99 1	 90 10
% DRUG AFTER DISPLACEMENT BOUND FREE	 98 2	 89 11
% INCREASE IN FREE DRUG CONCENTRATION	100	10

Eg; kernicterus – DI of bilirubin by NSAID'S drugs

Displacement of digoxine by qunidine

Displacement of warferin by phenylbutazone

Interaction is significant if drug bind more than 95%

Kinetics of protein drug binding

- The kinetics of reversible drug–protein binding for a protein with one simple binding site can be described by the *law of mass action*, as follows:

$$\text{Protein} + \text{drug} \Leftrightarrow \text{drug-protein-complex}$$

or $$[P] + [D] \Leftrightarrow [PD] \quad \text{.....1}$$

The law of mass action, an association constant, K_a, can be expressed as the ratio of the molar concentration of the products and the molar concentration of the reactants. This equation assumes only one-binding site per protein molecule

$$K_a = \frac{[PD]}{[P][D]} \quad \text{.....2}$$

Experimentally, both the free drug [D] and the protein-bound drug [PD], as well as the total protein concentration [P] + [PD], may be determined. To study the binding behavior of drugs, a determinable ratio (r)is defined, as follows

$$r = \frac{\text{moles of drug bound}}{\text{total moles of protein}}$$

moles of drug bound is [*PD*] and the total moles of protein is [*P*] + [*PD*], this equation becomes

$$r = \frac{[PD]}{[PD] + [P]} \qquad \text{.....3}$$

Substituting the value of PD from equa. 2

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

$$r = \frac{K_a[D]}{1 + K_a[D]} \qquad \text{.....4}$$

This equation describes the simplest situation, in which 1 mole of drug binds to 1 mole of protein in a 1:1 complex. This case assumes only one independent binding site for each molecule of drug. If there are *n* identical independent binding sites per protein molecule, then the following is used:

$$r = \frac{nK_a[D]}{1 + K_a[D]} \qquad \text{.....5}$$

- In terms of K_d, which is $1/K_a$, Equation 6 reduces to

$$r = \frac{n[D]}{K_d + [D]} \quad \text{.....6}$$

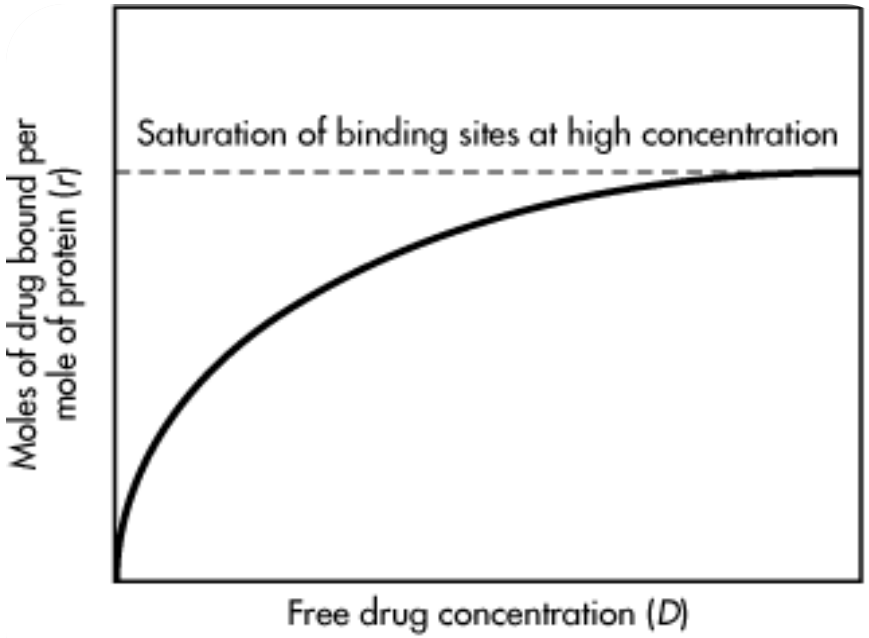
- Protein molecules are quite large compared to drug molecules and may contain more than one type of binding site for the drug. If there is more than one type of binding site and the drug binds independently on each binding site with its own association constant, then Equation 6 expands to

$$r = \frac{n_1 K_1[P]}{1 + K_1[D]} + \frac{n_2 K_2[P]}{1 + K_2[D]} + \cdots \quad \text{.....7}$$

The values for the association constants and the number of binding sites are obtained by various graphic methods.

1. Direct plot

It is made by plotting r vresus (D)



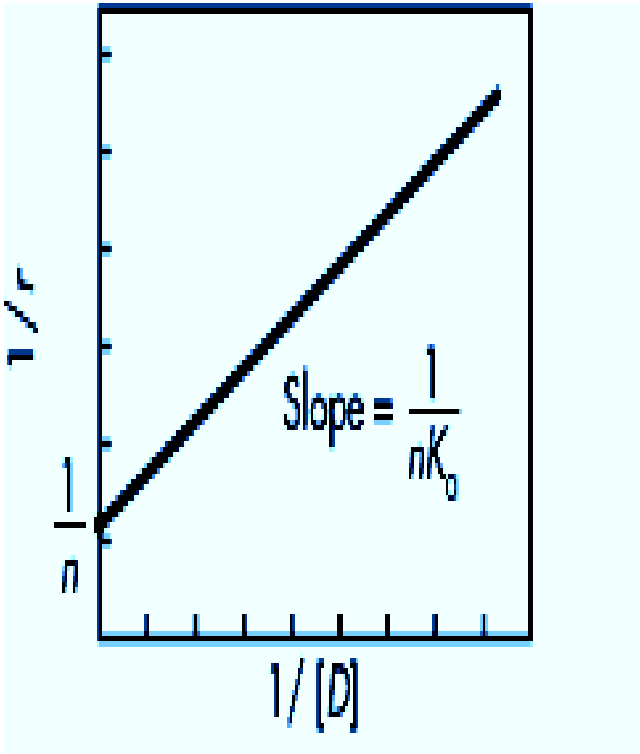
2. Double reciprocal plot

The reciprocal of Equation 6 gives the following equation

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

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

- A graph of 1/*r* versus 1/[*D*] is called a *double reciprocal plot*. The *y* intercept is 1/*n* and the slope is 1/*nKa* . From this graph , the number of binding sites may be determined from the *y* intercept, and the association constant may be determined from the slope, if the value for *n* is known.



3. Scatchardplot

is a rearrangement of Equation 6 The Scatchard plot spreads the data to give a better line for the estimation of the binding constants and binding sites. From Equation 6 , we obtain

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