

Protein Drug Binding

Presented By
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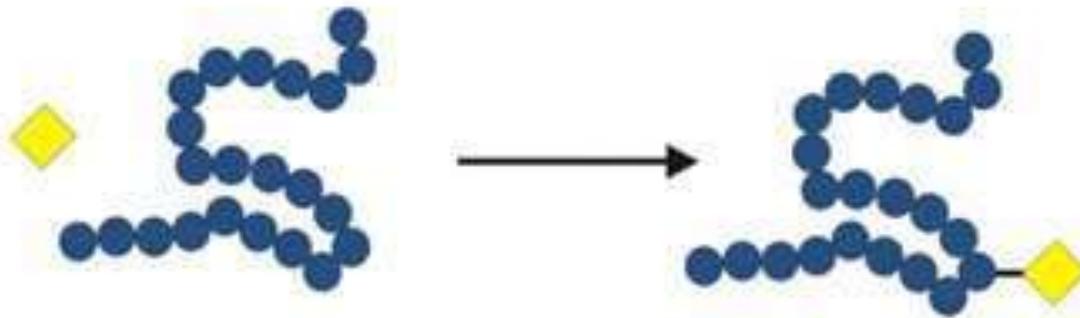
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INTRODUCTION

- The interacting molecules are generally the macromolecules such as protein, DNA or adipose. The protein are particularly responsible for such an interaction.
- The phenomenon of complex formation of drug with protein is called *as protein binding of drug*
- As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to its pharmacokinetic and Pharmacodynamic inertness.
 - **Protein + drug \rightleftharpoons Protein-drug complex**
 - Protein binding may be divided into:
 - 1. Intracellular binding.
 - 2. Extracellular binding.



Drug – Protein binding

MECHANISMS OF PROTEIN DRUG BINDING:

- Binding of drugs to proteins is generally of reversible & irreversible.
- Reversible generally involves weak chemical bond such as:
 1. Hydrogen bonds
 2. Hydrophobic bonds
 3. Ionic bonds
 4. Van der waal's forces.
- Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug.

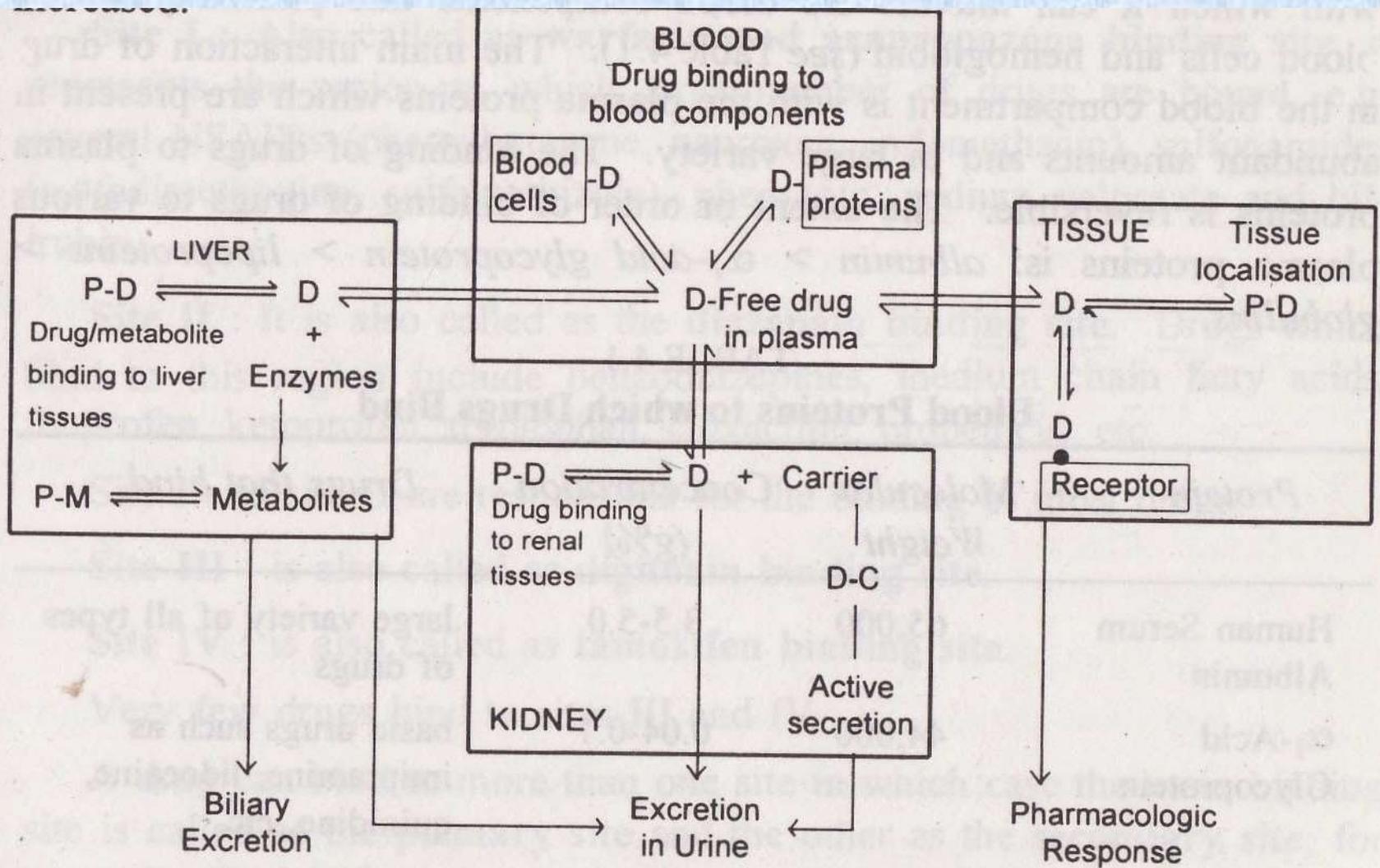


Fig. 4.1 Protein-drug binding: Binding of drugs to various tissue components and its influence on disposition and clinical response. Note that only the unbound drug moves reversibly between the compartments.

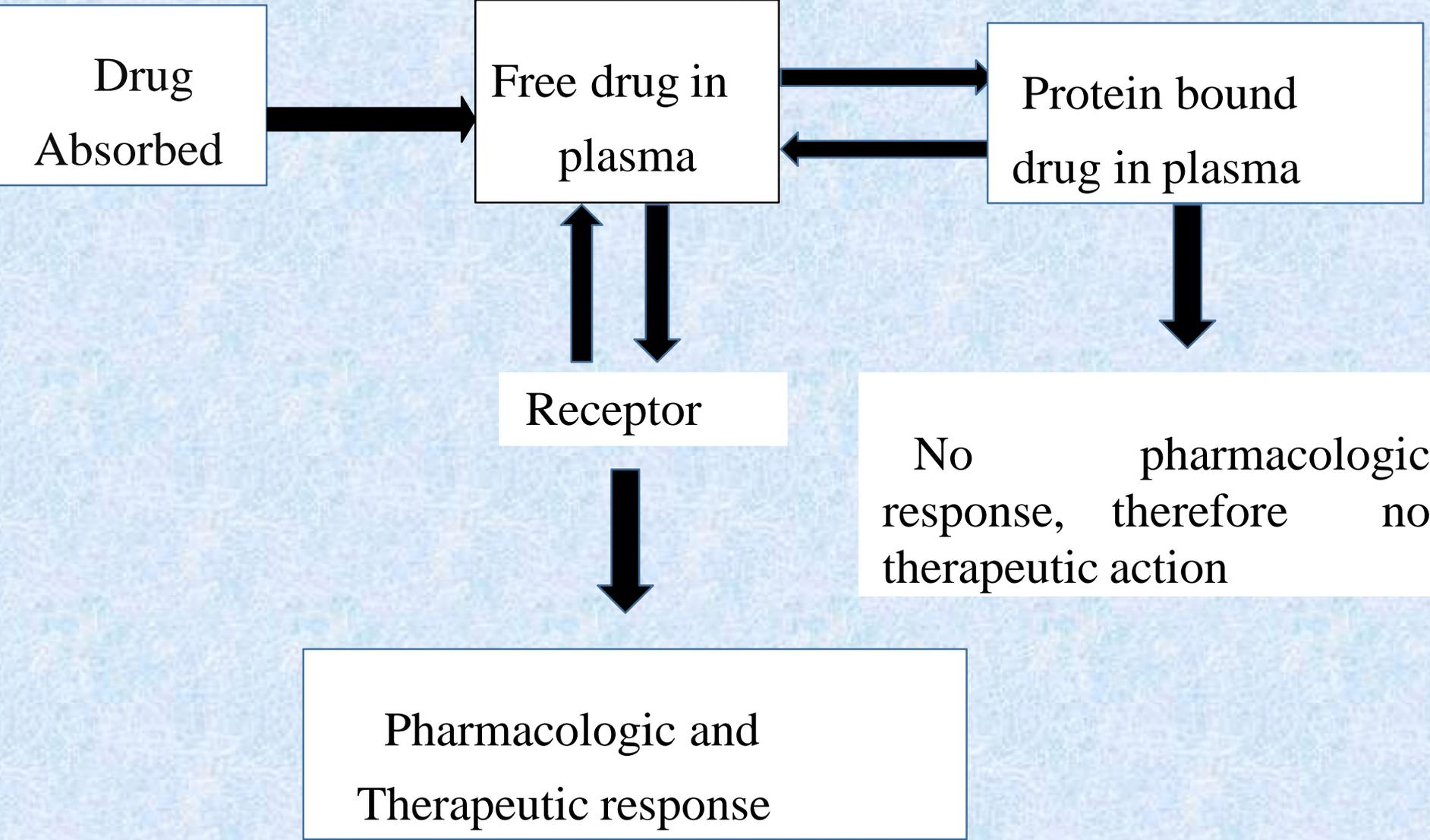


Figure : Protein-Drug Binding

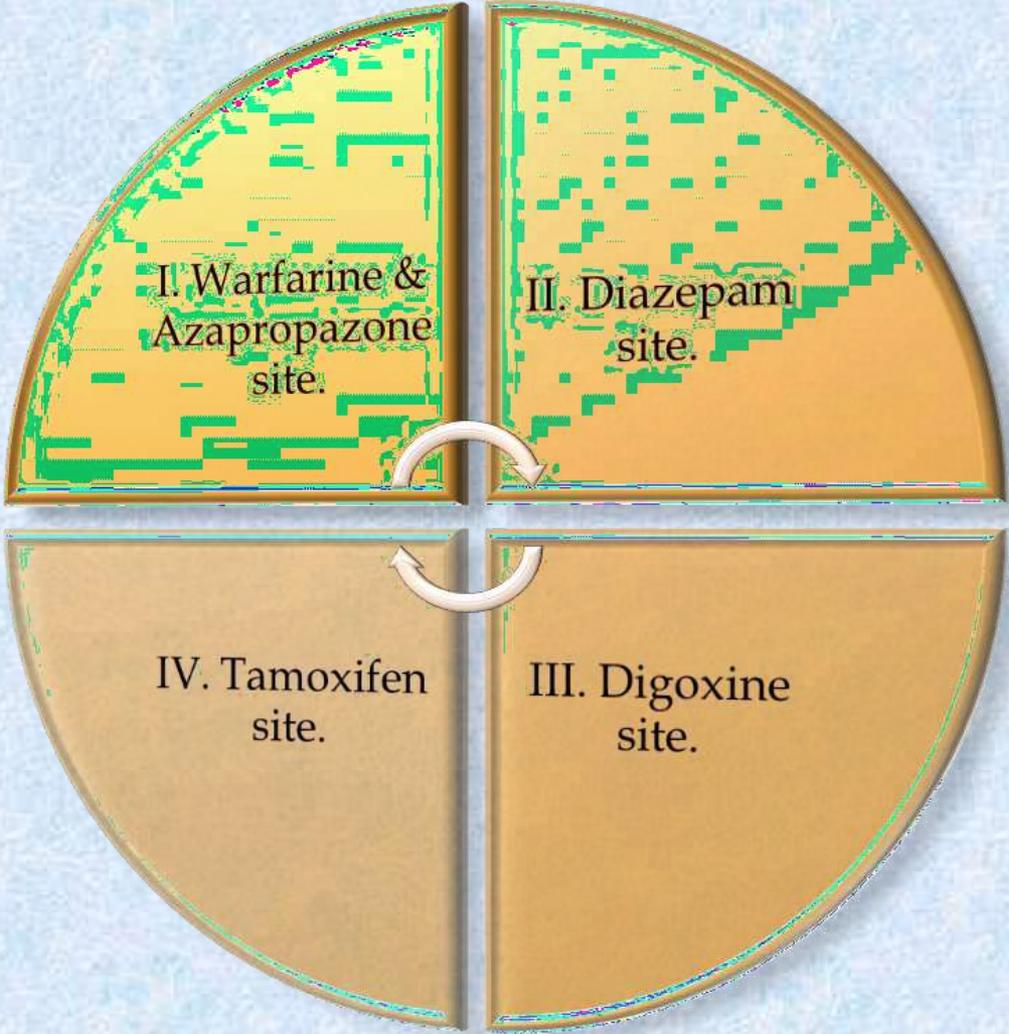
1. BINDING OF DRUG TO BLOOD COMPONENTS

A. Plasma protein-drug binding:-

- The binding of drugs to plasma proteins is reversible.
- The extent or order of binding of drug to plasma proteins is:
Albumin › α 1-Acid glycoprotein ›Lipoproteins ›Globulins.

1. Binding of drug to human serum Albumin.

- It is the most abundant plasma protein (59%), having M.W. of 65,000 with large drug binding capacity.
- Both endogenous compounds such as fatty acid, bilirubin as well as drug binds to HSA.
- Four diff. sites on HSA for drug binding.
 - Site I: warfarin & azapropazone binding site.
 - Site II: diazepam binding site.
 - Site III: digitoxin binding site.
 - Site IV: tamoxifen binding site.



2. **Binding of drug to α 1-Acid glycoprotein:** (orosomuroid)

It has a M.W. 44,000 and plasma conc. range of 0.04 to 0.1 g%. It binds to no. of basic drugs like imipramine, lidocaine, propranolol, quinidine.

3. **Binding of drug to Lipoproteins:**

Binding by: Hydrophobic Bonds, Non-competitive.

Mol wt: 2-34 Lacks dalton.

Lipid core composed of:

Inside: triglyceride & cholesteryl esters.

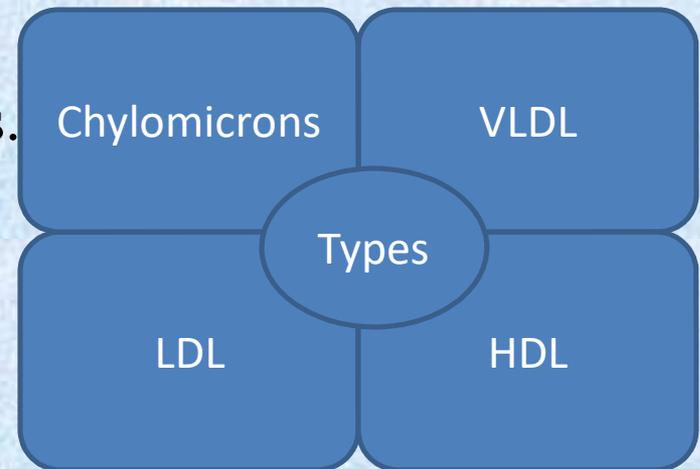
Outside: Apoprotein.

e.g.

Acidic: Diclofenac.

Neutral: Cyclosporin A.

Basic: Chlorpromazine.



4. Binding of drug to Globulins

- It mainly binds to endogenous substances.
- In plasma several globulins have been identified.

Globulin	Synonym		Binds to
1. α 1 Globulin	Transcortine /Corticosteroid globulin	Binding	Steroidal drugs, Thyroxin & Cyanocobalamine.
2. α 2 Globulin	Ceruloplasmine		Vitamin A,D,E,K.
3. β 1 Globulin	Transferin		Ferrous ions
4. β 2 Globulin	---		Carotinoids
5. γ Globulin	---		Antigens

B. BINDING OF DRUG TO BLOOD CELLS

- In blood 40% of blood cells of which major component is RBC (95%). The RBC is 500 times in diameter as the albumin. The rate & extent of entry into RBC is more for lipophilic drugs.
- The RBC comprises of 3 components.
 - a) **Haemoglobin**: It has a M.W. of 64,500 Dal. Drugs like phenytoin, pentobarbital bind to haemoglobin.
 - b) **Carbonic anhydrase**: Carbonic anhydrase inhibitors drugs are bind to it like acetazolamide & chlorthalidone.
 - c) **Cell membrane**: Imipramine & chlorpromazine are reported to bind with the RBC membrane.

Binding of drug to blood cells

Hb

- 7-8 times conc. Of Albumin
- Binds to Phenytoin, Pentobarbital, Phenothiazine

Carbonic
Anhydrase

- Binds to Acetazolamide & Chlorthalidone.

Cell
Membrane

- Binds to Imipramine & chlorpromazine

2. BINDING OF DRUG TO EXTRAVASCULAR TISSUE PROTEIN

- Importance: 1. It increases apparent volume of distribution of drug.
2. localization of a drug at a specific site in body.
- Factor affecting: lipophilicity, structural feature of drug, perfusion rate, pH differences.
- Binding order: *Liver* > *Kidney* > *Lung* > *Muscles*

Tissue	Binding of
1.Liver	Irreversible binding of Epoxides of Halogenated Hydrocarbon & Paracetamol.
2.Lungs	Basic drugs: Imipramine, Chlorpromazine, & AntiHistaminics.

Cont...

Tissue	Binding of
3.Kidney	Metallothionin protein binds to Heavy metals & results in Renal accumulation and toxicity.
4.Skin	Chloroquine & Phenothiazine binds to Melanin.
5.Eye	Chloroquine & Phenothiazine also binds to Eye Melanin & results in Retinopathy.
6.Hairs	Arsenicals, Chloroquine, & Phenothiazine.
7.Bones	Tetracycline(yellow discoloration of teeth), Lead(replaces Ca & cause brittleness)
8.Fats	Lipophilic drugs (thiopental), Pesticides (DDT)
9.Nucleic Acid	Chloroquine & Quinacrine.

FACTORS AFFECTING PROTEIN DRUG BINDING

1. Drug-related factors

a. Physicochemical characteristics of the drug:-

- Protein binding is directly related to the lipophilicity of drug. An increase in lipophilicity increases the extent of binding.

b. Concentration of drug in the body:-

- Alteration in the concentration of drug substance as well as the protein molecules or surfaces subsequently brings alteration in the protein binding process.

c. Affinity of a drug for a particular binding component:-

- This factor entirely depends upon the degree of attraction or affinity the protein molecule or tissues have towards drug moieties.
- For Digoxin has more affinity for cardiac muscles proteins as compared to that of proteins of skeletal muscles or those in the plasma like HSA.

2. Protein/ tissue related factors:

a. Physicochemical characteristics of protein or binding agent:

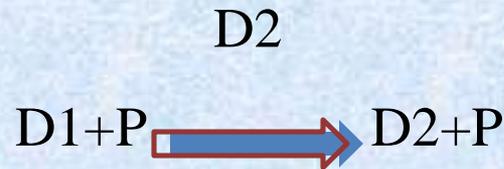
- Lipoproteins & adipose tissue tend to bind lipophilic drug by dissolving them in their lipid core.
- The physiological pH determines the presence of active anionic & cationic groups on the albumin to bind a variety of drug.

b. Concentration of protein or binding component:

- Among the plasma protein, binding predominantly occurs with albumin, as it is present in high concentration in comparison to other plasma protein.
- The amount of several proteins and tissue components available for binding, changes during disease state.

3. Drug interactions

a. Competition between drugs for the binding sites[Displacement interactions]:-



D1: Displaced drug.

D2: Displacer drug.

e.g. Administration of phenylbutazone to a patient on Warfarin therapy results in Hemorrhagic reaction.

b. Competition between drug & normal body constituents:-

The free fatty acids are known to interact with a no. of drugs that binds primarily to HSA. the free fatty acid level increase in physiological, pathological condition.

c. Allosteric changes in protein molecule:-

- The process involves alteration of the protein structure by the drug or its metabolite thereby modifying its binding capacity.
- e.g. aspirin acetylates lysine fraction of albumin thereby modifying its capacity to bind NSAIDs like phenylbutazone.

4. Patient-related factors

a. Age:

1. Neonates: Low albumin content: More free drug.

2. Young infants: High dose of Digoxin due to large renal clearance.

3. Elderly: Low albumin: So more free drug.

b. Intersubject variability: Due to genetics & environmental factors.

c. Disease states:-

Disease	Influence on plasma protein	Influence on protein drug binding
Renal failure	↓ Albumin content	↓ binding of acidic drugs; neutral and basic drugs are un affected
Hepatic failure	↓ Albumin synthesis	↓ binding of acidic drugs; and binding of basic drugs is normal or ↓ depending on AAG levels

SIGNIFICANCE OF PROTEIN/TISSUE BINDING OF DRUG

a. **Absorption-**

- As we know the conventional dosage form follow first order kinetics. So when there is more protein binding then it disturbs the absorption equilibrium.

b. **Distribution-**

- A protein bound drug in particular does not cross the BBB, the placental barrier, the glomerulus.
- Thus protein binding decreases the distribution of drugs.

c. **Metabolism-**

- Protein binding decreases the metabolism of drugs & enhances the biological half life.
- Only unbound fraction get metabolized.
- e.g. Phenylbutazone & Sulfonamide.

d. **Elimination**

- Only the unbound drug is capable of being eliminated.
- Protein binding prevent the entry of drug to the metabolizing organ (liver) & to glomerulus filtration.
- e.g. Tetracycline is eliminated mainly by glomerular filtration.

e. **Systemic solubility of drug**

- Lipoprotein act as vehicle for hydrophobic drugs like steroids, heparin, oil soluble vit.

f. **Drug action-**

- Protein binding inactivates the drugs because sufficient concentration of drug can not be build up in the receptor site for action.
- e.g. Naphthoquinone

g. **Sustain release-**

- The complex of drug protein in the blood act as a reservoir & continuously supply the free drug.

KINETICS OF PROTEIN-BINDING

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



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,

$$\begin{aligned} r &= \frac{[PD]}{P_T} \\ &= \frac{[PD]}{[PD] + [P]} \\ r &= \frac{K_a [P] [D]}{K_a [P] [D] + [P]} = \frac{K_a [D]}{K_a [D] + 1} \end{aligned}$$

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

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