Drug Distribution and Protein Binding

Recommended books

1. Shargel L, Yu A: *Applied Biopharmaceutics & Pharmacokinetics*, 7th ed., McGraw-Hill Education / Medical, 2015
2. S. S. Jambhekar, P. J. Breen: *Basic Pharmacokinetics*, 2nd ed., Pharmaceutical Press, 2012

Chapter Contents

Physiological factors, diffusion and hydrostatic pressure, tissue perfusion and initial drug distribution, drug distribution and pharmacodynamics, effect of protein binding on the apparent volume of distribution, protein binding of drugs, kinetics of protein binding, determination of constant, relationship between protein concentration and drug concentration in protein-drug binding

Learning Outcomes

Students should be able to:

a) Describe the physiology of drug distribution in the body

b) Explain how drug distribution is affected by blood flow, protein, and tissue binding

c) Describe how drug distribution can affect the apparent volume of distribution

d) Explain how volume of distribution, drug clearance, and half-life can be affected by protein binding

e) Determine drug–protein binding constants using *in vitro* methods

f) Evaluate the impact of change in drug–protein binding on free drug concentration.

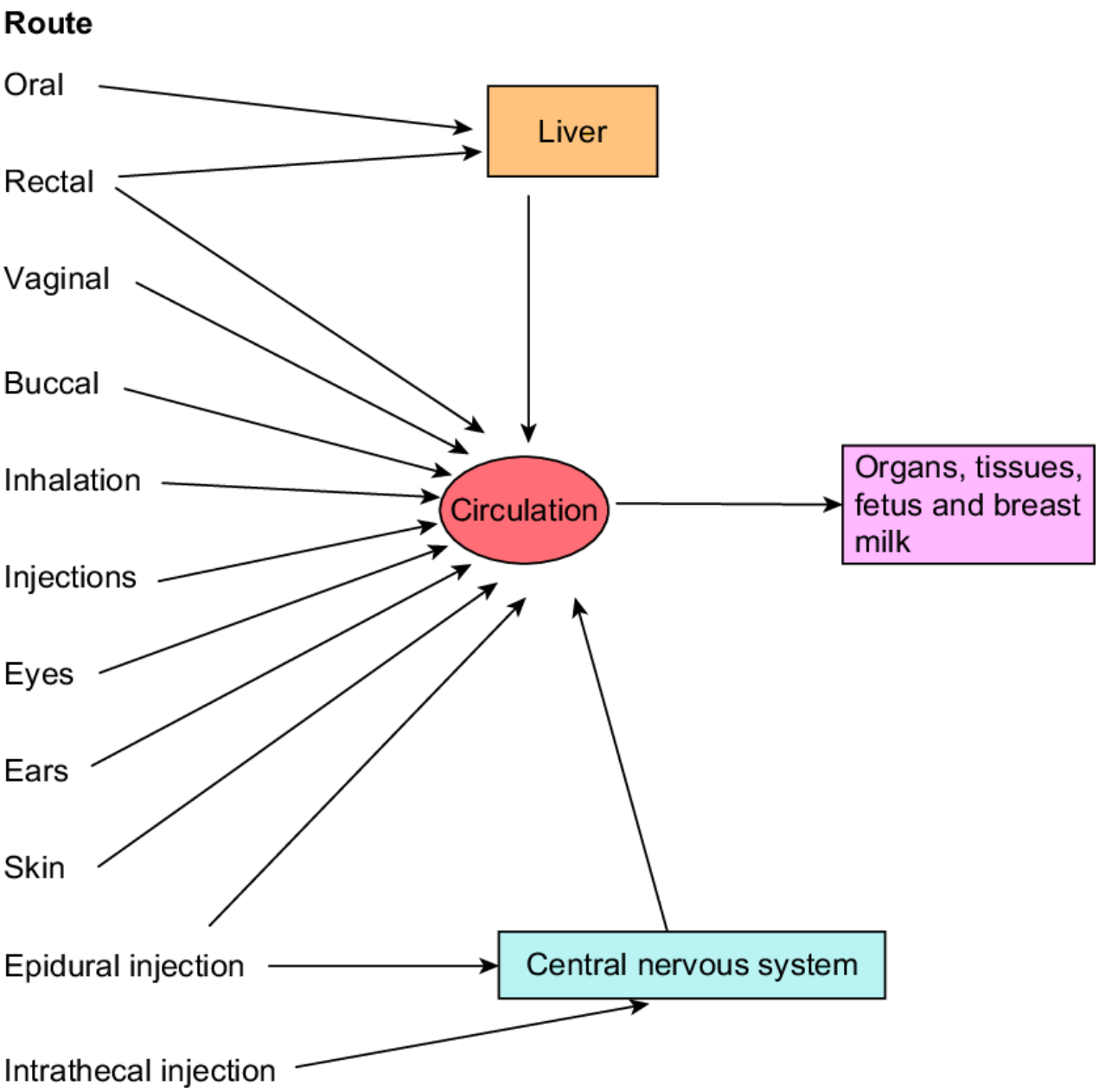
Lecture-1

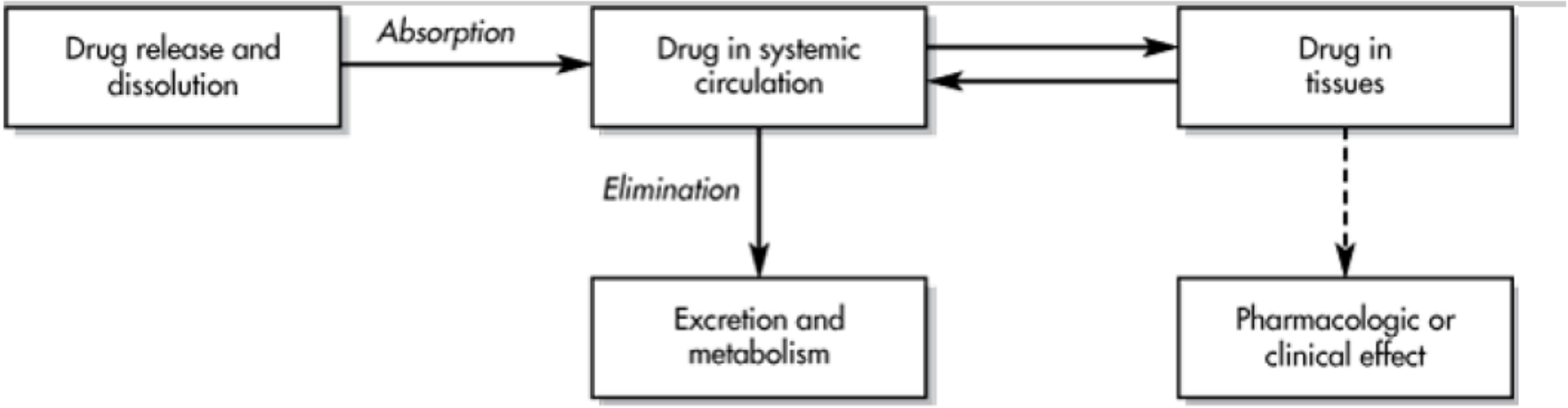
Contents

- Drug Distribution
- Drug Distribution Sites
- Factors Affecting Drug Distribution
- Drug Distribution and Compartment Selection

Drug Distribution

Distribution is the process of reversible transfer of drug to and from the site of measurement (usually blood or plasma).


Route
Oral
Rectal
Vaginal
Buccal
Inhalation
Injections
Eyes
Ears
Skin
Epidural injection
Intrathecal injection
Liver
Circulation
Organs, tissues, fetus and breast milk
Central nervous system


Drug release and dissolution
Absorption
Drug in systemic circulation
Drug in tissues
Elimination
Excretion and metabolism
Pharmacologic or clinical effect

Drug Distribution Sites

- **Target site** (receptor) for drug action
- **Nonreceptor** tissues where side effects or adverse reactions may occur
- **Eliminating organs**, such as the liver and kidney
- **Noneliminating tissues**, such as the brain, skin, and muscle
- **Developing fetus** in pregnancy
- **Mammillary glands** and can be secreted in milk
- **Plasma** and/or tissues by binding with proteins
- **Fat** (in case of lyophilic drug)

Factors Affecting Drug Distribution

The rate and extent of drug distribution is determined by:

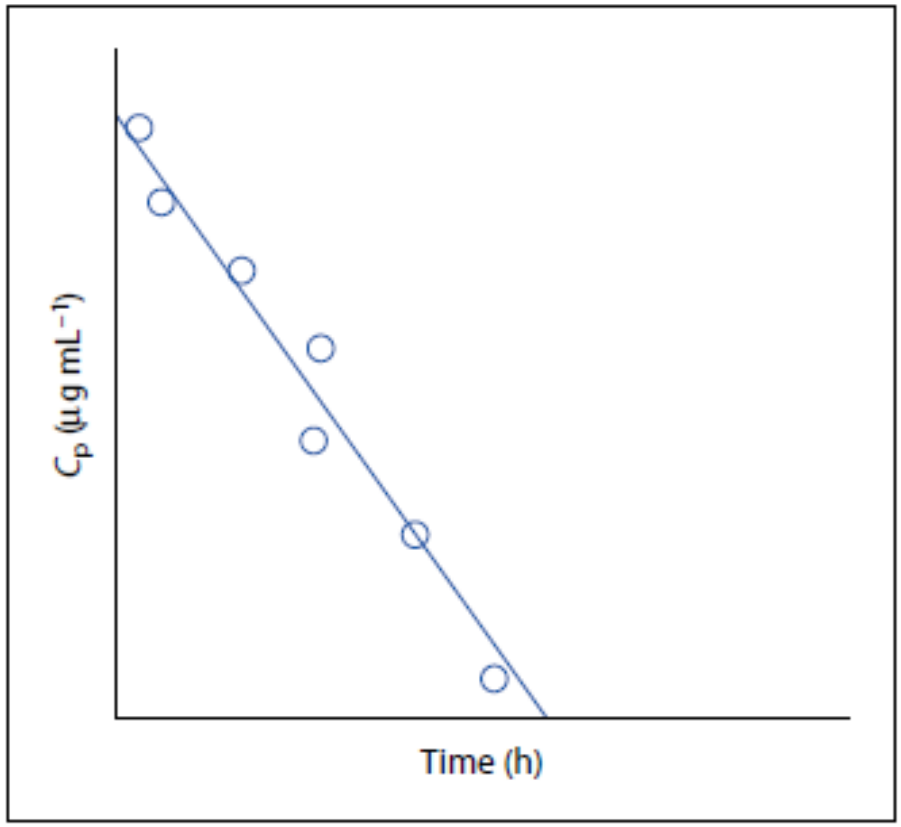
1. How well the tissues and/or organs are perfused with blood
2. The binding of drug to plasma proteins and tissue components
3. The permeability of tissue membranes to the drug molecule.

All these factors, in turn, are determined and controlled by the physicochemical properties and chemical structures (i.e., presence of functional groups) of a drug molecule.

Drug Distribution and Compartment Selection

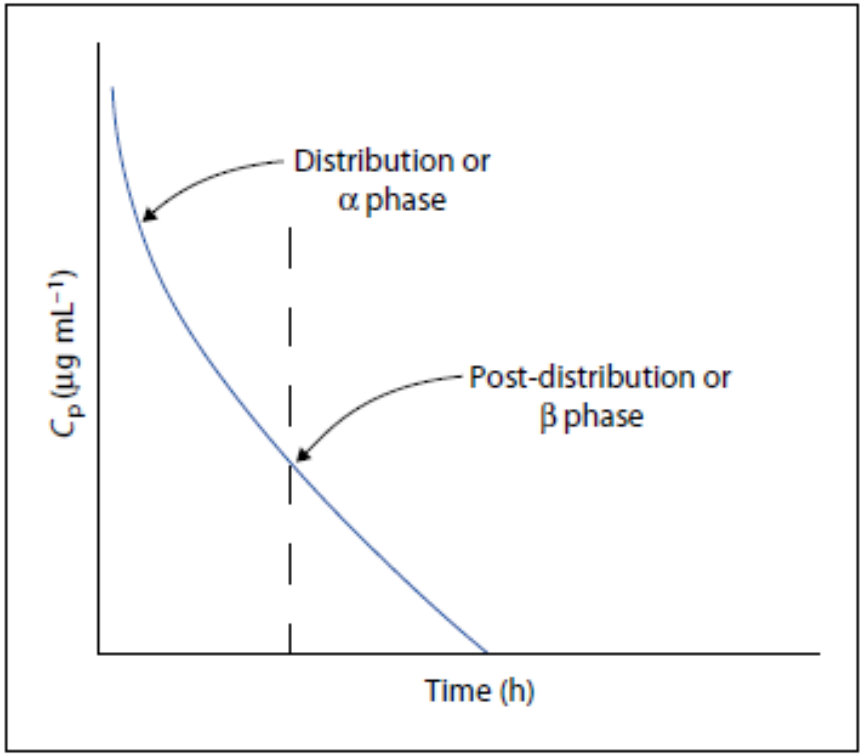
The selection of a compartment model depends solely upon the distribution characteristics of a drug following its administration.

If the drug is rapidly distributed following its administration a one-compartment model will do an adequate job of accurately and adequately characterizing the plasma concentration versus time data.



A typical semilogarithmic plot of plasma concentration (C_p) versus time following the administration of an intravenous bolus dose of a drug that is rapidly distributed in the body.

Generally, the slower the drug distribution in the body, the greater the number of compartments required to characterize the plasma concentration versus time data, the more complex is the nature of the equation employed.



A typical semilogarithmic plot of plasma concentration (C_p) versus time following the administration of an intravenous bolus dose of a drug that is slowly distributed in the body.

Lecture-2

Contents

- Mechanism of Drug Distribution
- Factors affecting passage of drug across the cell membrane
- Drug transverse processes: Passive diffusion and Hydrostatic pressure
- Fluid Balance Between Capillary and Tissue

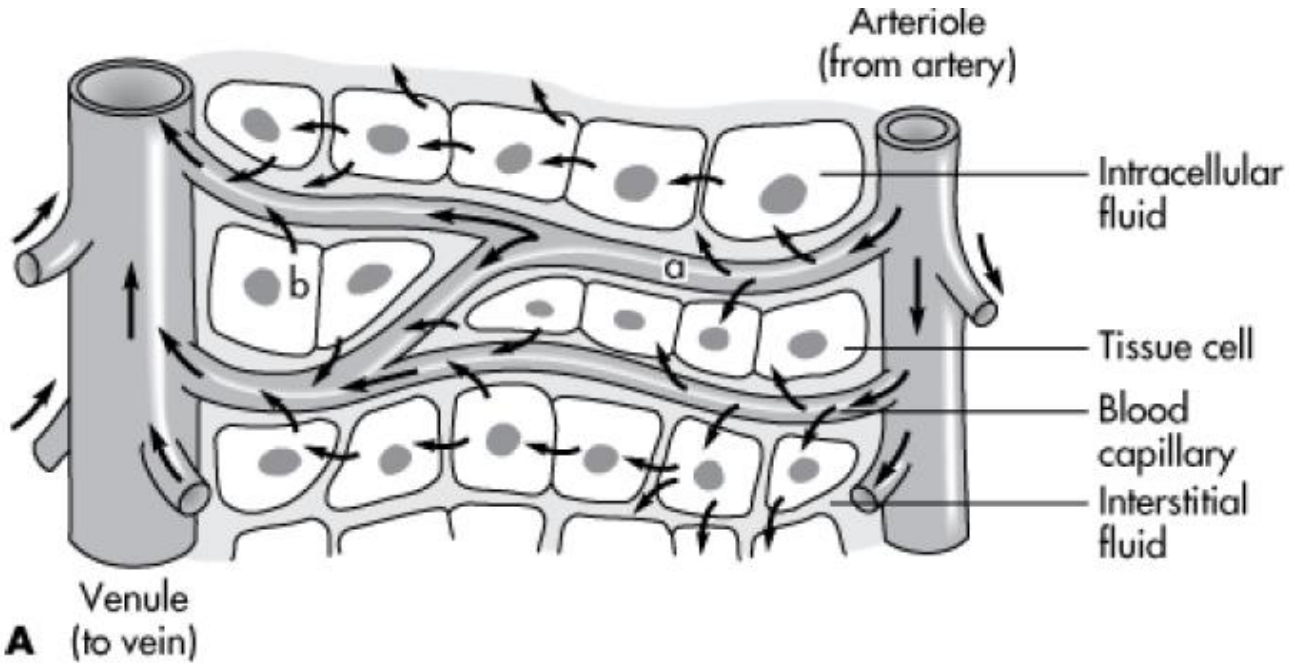
Mechanism of Drug Distribution

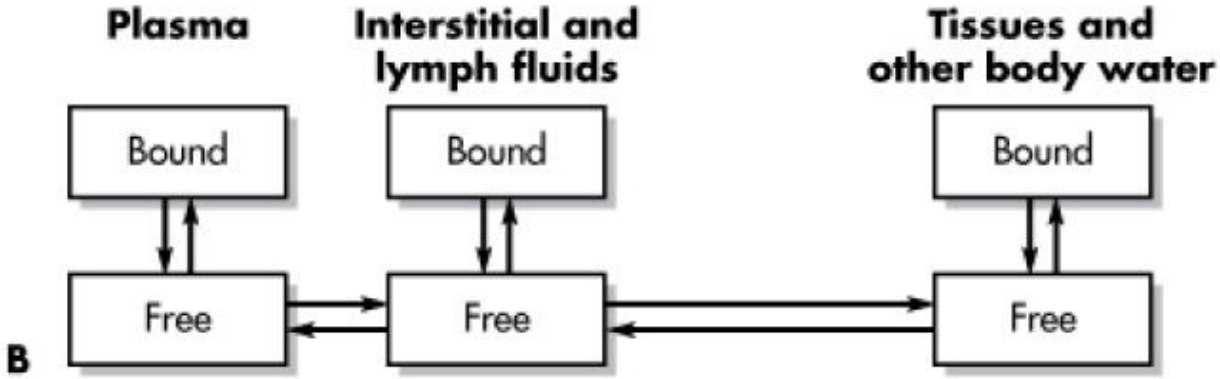
✓ The circulatory system consists of a series of blood vessels; these include the arteries that carry blood to tissues, and the veins that return the blood back to the heart.

✓ Volume of blood pumped by heart is about 5.5 L/min in subjects at rest. The cardiac output may be five to six times higher during exercise. Blood moves at a linear speed of 300 mm/sec through the aorta.

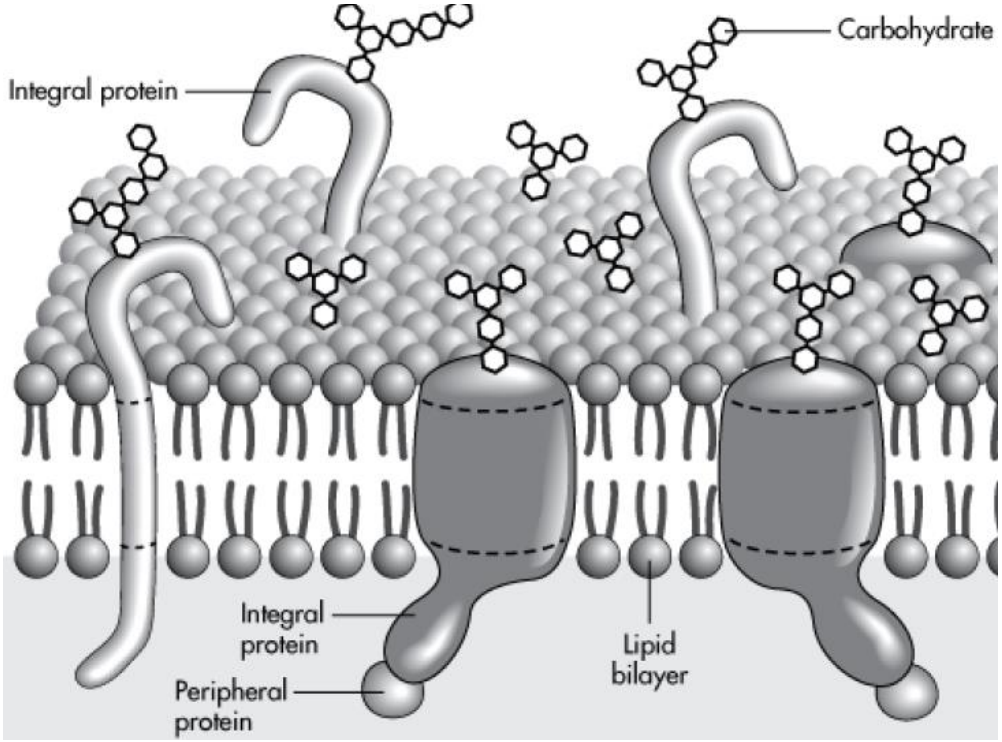
✓ Mixing of a drug solution in the blood occurs rapidly at this flow rate. Drug molecules rapidly diffuse through a network of fine capillaries to the tissue spaces filled with interstitial fluid.

✓ *Drug molecules may further* diffuse from the interstitial fluid across the cell membrane into the cell cytoplasm.

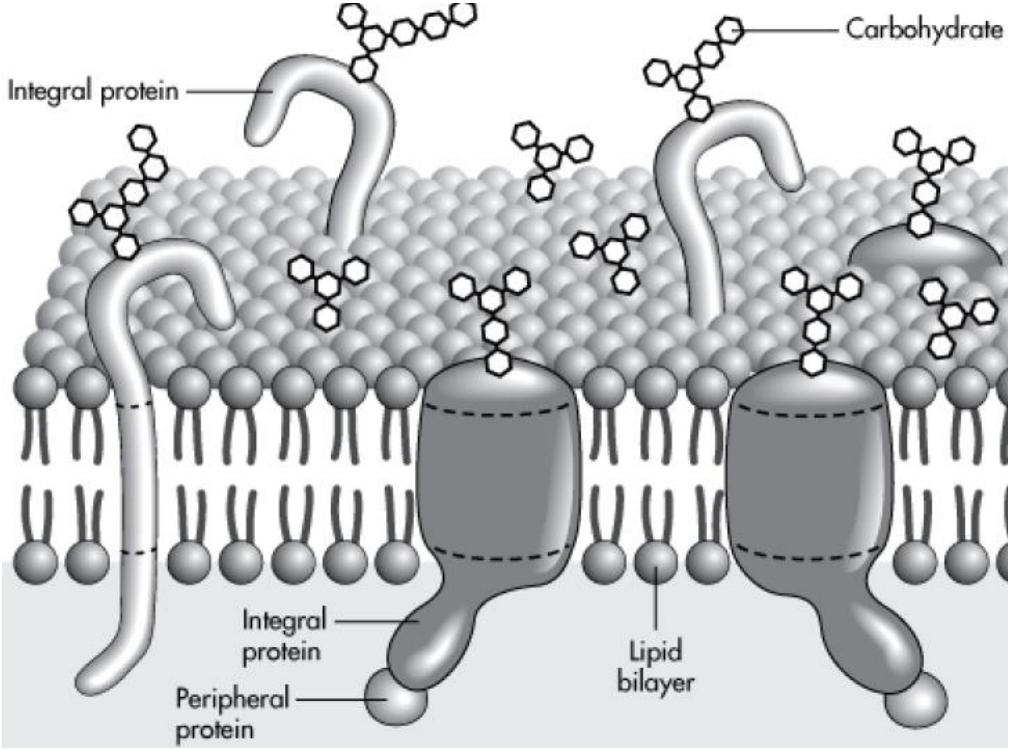

Arteriole
(from artery)
Intracellular
fluid
b
a
Tissue cell
Blood
capillary
Interstitial
fluid
Venule
(to vein)
A


Plasma
Interstitial and
lymph fluids
Tissues and
other body water
Bound
Bound
Bound
Free
Free
Free
B

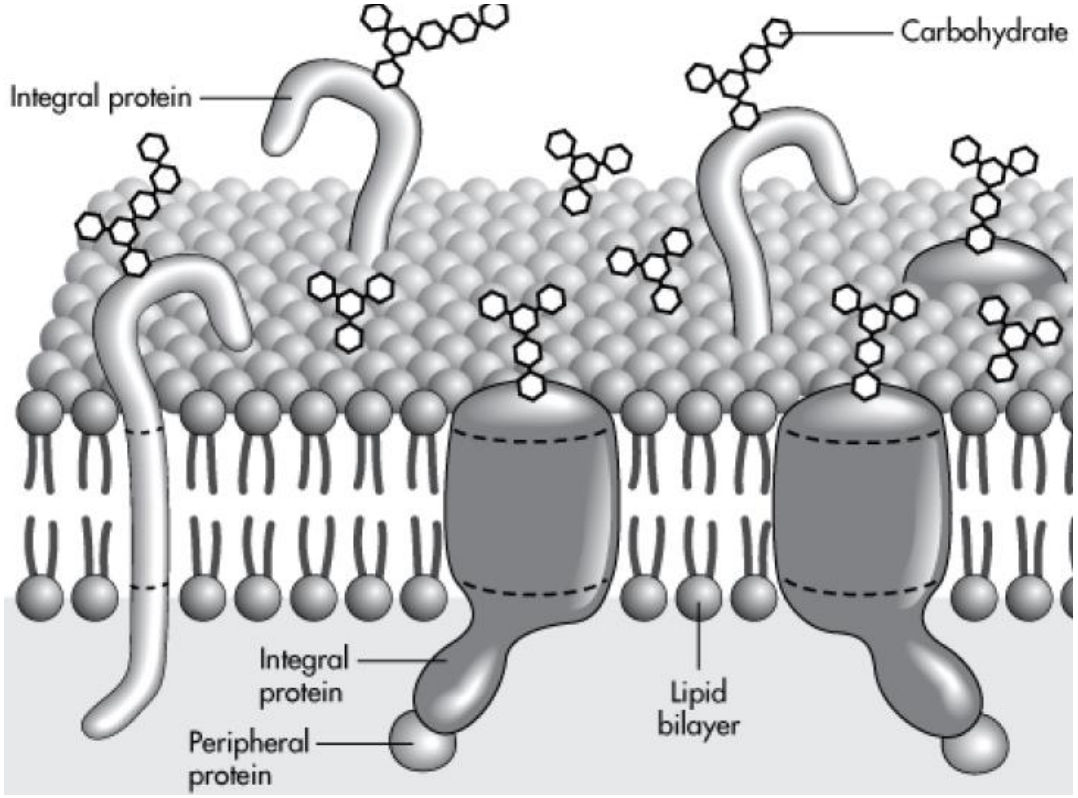
Factors affecting passage of drug across cell membrane



- The passage of drug molecules across a cell membrane depends on the physicochemical nature of both the drug and the cell membrane.



- Cell membranes are composed of protein and a bilayer of phospholipid, which act as a lipid barrier to drug uptake. Thus, lipid-soluble drugs generally diffuse across cell membranes more easily than highly polar or water-soluble drugs.



- Small drug molecules generally diffuse more rapidly across cell membranes than large drug molecules. If the drug is bound to a plasma protein such as albumin, the drug protein complex becomes too large for easy diffusion across the cell or even capillary membranes.

Drug transverse processes through capillary/cell membrane

- Passive diffusion
- Hydrostatic Pressure:

Passive diffusion

Passive diffusion is the main process by which most drugs cross cell membranes. *Passive diffusion* is the process by which drug molecules move from an area of high concentration to an area of low concentration.

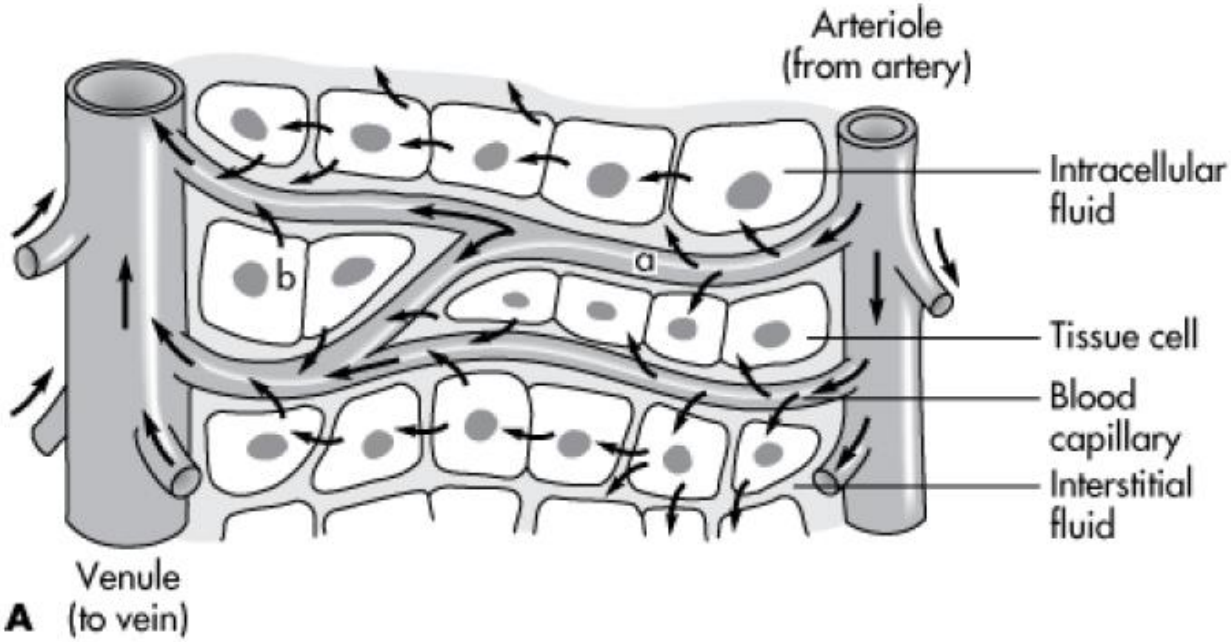
Passive diffusion is described by *Fick's law of diffusion:*

$$\text{Rate of drug diffusion} = \frac{dQ}{dt} = \frac{-DKA(C_p - C_t)}{h}$$

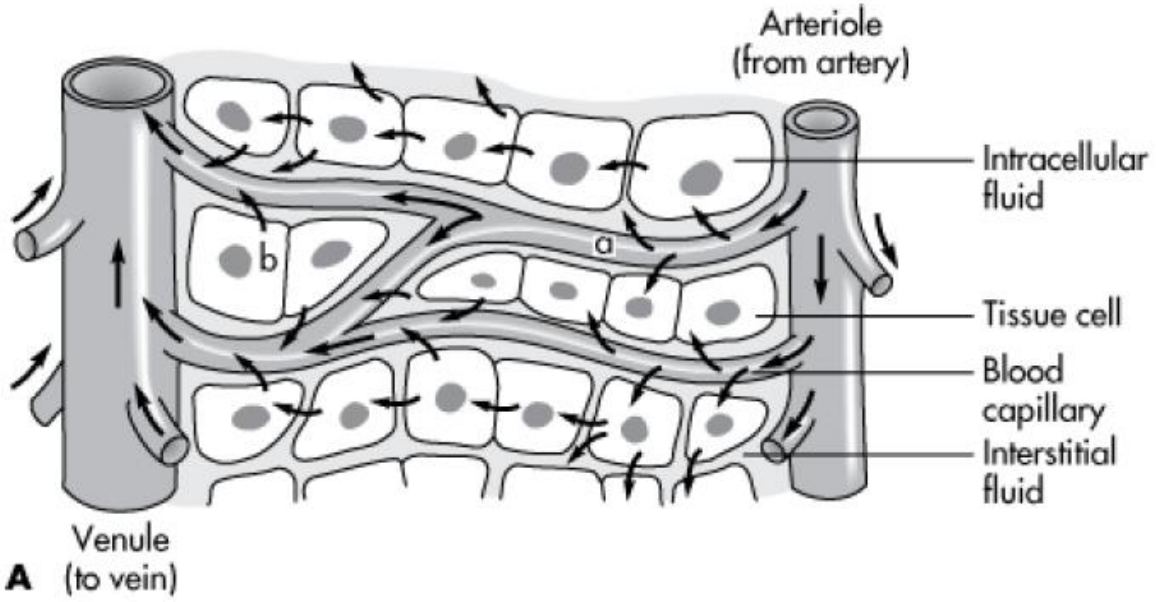
where C_p = *the drug concentration in the plasma*
C_t = *the drug concentration in the tissue*
A = *the surface area of the membrane*
h = *the thickness of the membrane*
K = *the* lipid-water partition coefficient
D = *the diffusion constant.*

Hydrostatic Pressure

Hydrostatic pressure represents the pressure gradient between the arterial end of the capillaries entering the tissue and the venous capillaries leaving the tissue.



- Hydrostatic pressure is responsible for penetration of water-soluble drugs into spaces between endothelial cells and possibly into lymph.
- In the kidneys, high arterial pressure creates a filtration pressure that allows small drug molecules to be filtered in the glomerulus of the renal nephron.

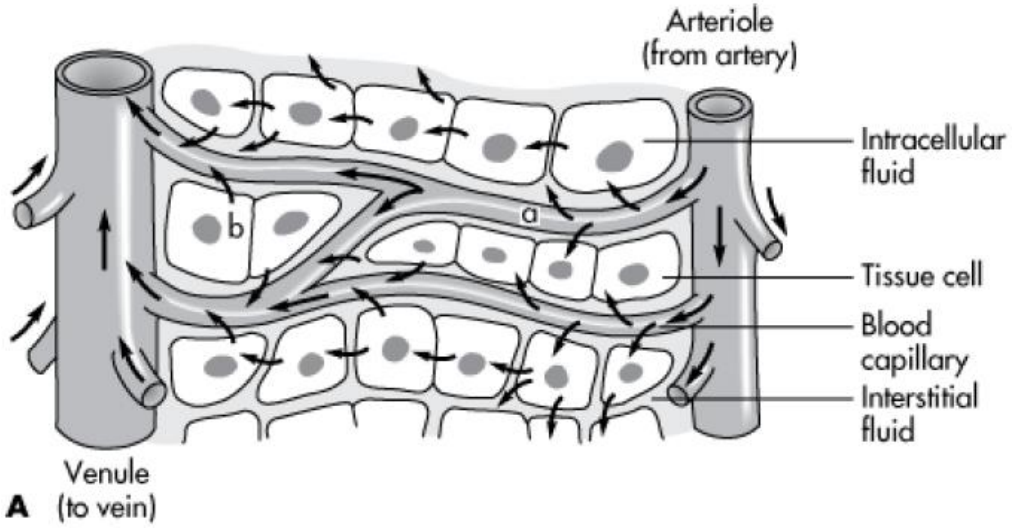


Fluid Balance Between Capillary and Tissue

- The average pressure of the blood capillary is higher (+18 mm Hg) than the mean tissue pressure (-6 mm Hg), resulting in a net total pressure of 24 mm Hg higher in the capillary over the tissue.
- This pressure difference is offset by an average osmotic pressure in the blood of 24 mm Hg, pulling the plasma fluid back into the capillary.
- Thus, on average, the pressures in the tissue and most parts of the capillary are equal, with no net flow of water.

Filtration Pressure

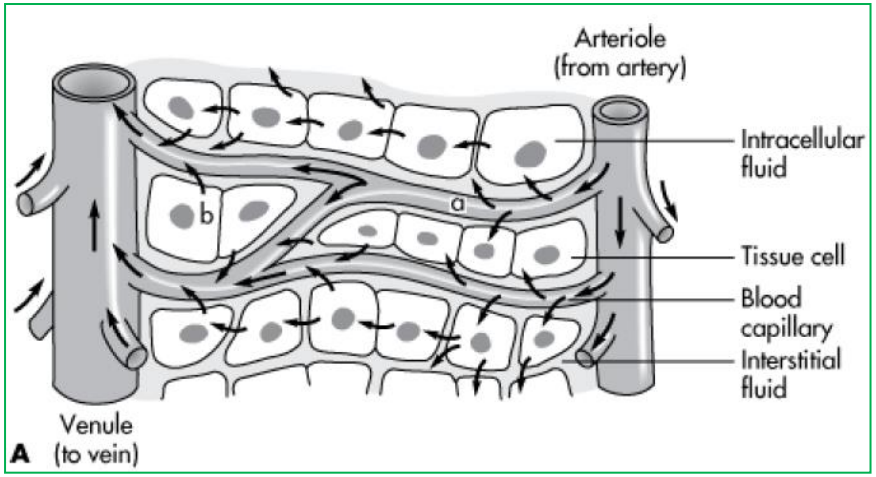
At the arterial end, the pressure of the capillary blood is slightly higher (about 8 mm Hg) than that of the tissue, causing fluid to leave the capillary and enter the tissues. This pressure is called *hydrostatic or filtration pressure*.



Absorptive Pressure

- This filtered fluid (filtrate) in the tissue is later returned to the venous capillary due to a lower venous pressure of about the same magnitude of filtration pressure.

- The lower pressure of the venous blood compared with the tissue fluid is termed *absorptive pressure*. A small amount of fluid returns to the circulation through the lymphatic system.



Contents

- Drug accumulation
- Factors affecting drug accumulation
  - Tissue perfusion
  - Diffusional barrier
  - Affinity of the drug for the tissue
  - Drug-protein/macromolecule binding

Drug Accumulation

- The progressive increase in the concentration of a drug in an organism or tissue, due to higher doses than can be metabolized, or due to poor metabolism of the agent. Drug continues to accumulate until the amount of drug administered in a time period equals that eliminated in the same period.

- In a multiple dose regimen, if the second dose is given early enough so that not all of the first dose is eliminated then the drug will start to accumulate and we will get higher concentrations with the second and third dose.

Illustration

Consider a drug with a half-life of 6 hours. Giving a dose of 100 mg with an apparent volume of distribution of 25 liter the C_p^0 = 4 mg/liter.

- After six hours the plasma concentration will fall to 2 mg/liter.
- After second dose, the plasma concentration will increase by 4 mg/liter from 2 mg/liter to 6 mg/liter.
- Then after another half-life (6 hours) the plasma concentration will fall to 3 mg/liter.
- The third dose will increase the plasma concentration to 7 mg/liter.
- After another half-life the plasma concentration will be 3.5 mg/liter.

Starting concentration/(mgL^{-1})	End concentration/(mgL^{-1})	Concentration lost during dosage interval/(mgL^{-1})
4	2	2
6	3	3
7	3.5	3.5
7.5	3.75	3.75
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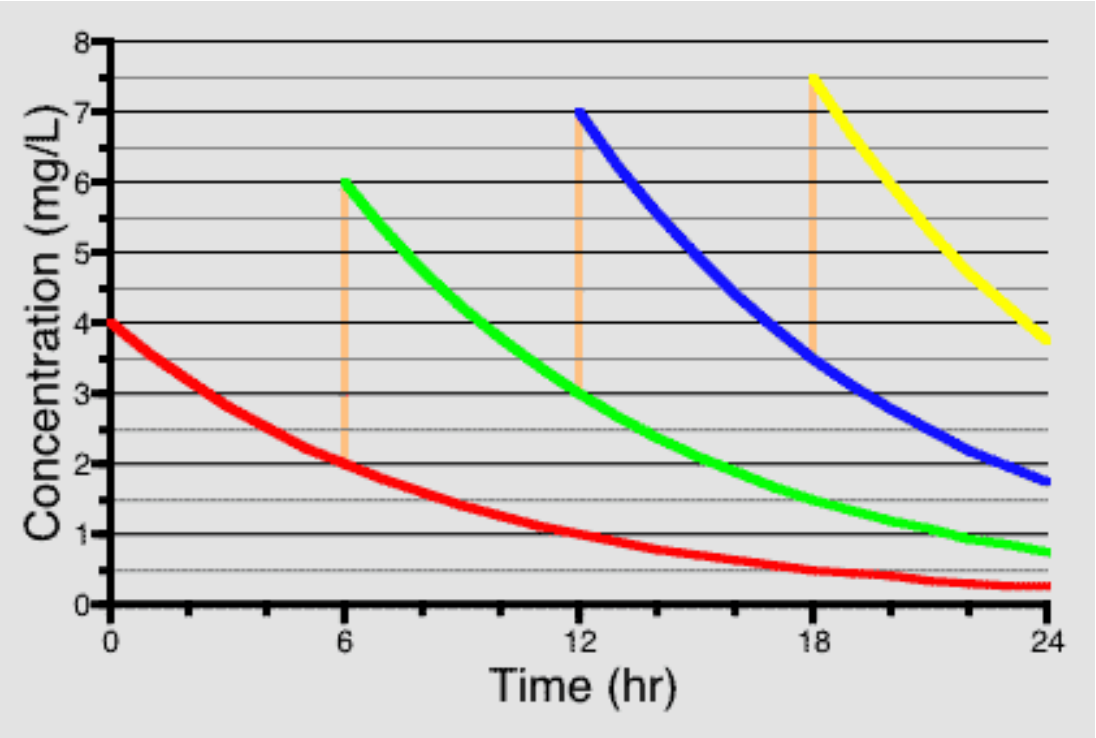
8	4	4, which is the same as the concentration increase caused by each dose



Linear plot of C_p versus time showing doses every six hours

Factors affecting drug accumulation

- **Tissue perfusion.** Tissues receiving high blood flow equilibrate quickly with the drug in the plasma. However, at steady state, the drug may or may not accumulate within the tissue.

❑ **Diffusional barrier.** The deposition or uptake of the drug into the tissue is generally controlled by the diffusional barrier of the capillary membrane and other cell membranes. For example, the brain is well perfused with blood, but many drugs with good aqueous solubility have negligible brain drug concentration. The brain capillaries are surrounded by a layer of tightly joined glial cells that act as a lipid barrier to impede the diffusion of polar or highly ionized drugs.

❑ **Affinity of the drug for the tissue.** Drugs with high tissue affinity tend to accumulate or concentrate in the tissue. Drugs with a high lipid/water partition coefficient are very fat soluble and tend to accumulate in lipid or adipose (fat) tissue.

❑ **Drug-protein/macromolecule binding.** Drugs may accumulate by binding to proteins or other macromolecules in a tissue. In a few cases, the drug is irreversibly bound into a particular tissue. Irreversible binding of drug may occur when the drug or a reactive intermediate metabolite becomes covalently bound to a macromolecule within the cell, such as to a tissue protein.

Lecture-4

Contents

- Apparent Volume Distribution
- Considerations in the Calculation of Volume of Distribution
- Complex Biological Systems and V_D
- Drug–protein binding
- Major Proteins to Which Drugs Bind in Plasma
- Effect of reversible drug–protein binding on drug distribution and elimination

Apparent Volume Distribution

The *apparent volume of distribution*, V_D in a model, is used to estimate the extent of drug distribution in the body. The word "apparent" signifies that the volume determined has the appearance of being true but it is not a true volume.

The V_D represents the result of dynamic drug distribution between the plasma and the tissues and accounts for the mass balance of the drug in the body.

$$V_D = \frac{\text{Amount of drug added to the system } (X)}{\text{Drug concentration in the system after equilibrium } (C_p)}$$

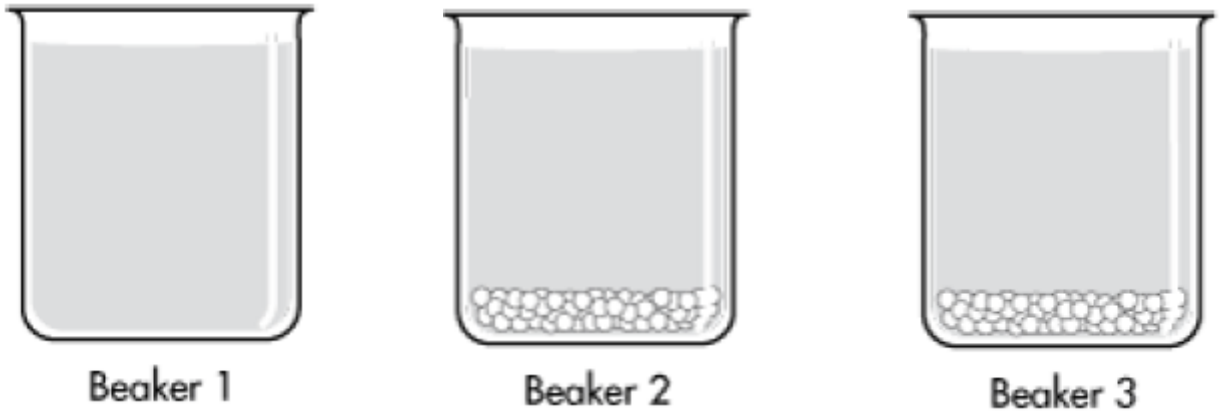
Considerations in the Calculation of Volume of Distribution

Consider three beakers

Beaker 1. Drug distribution in a fluid (water) compartment only, without drug binding and metabolism

Beaker 2. Drug distribution in a fluid compartment containing cell clusters that reversibly bind drugs

Beaker 3. Drug distribution in a fluid compartment containing cell clusters (similar to tissues in vivo) in which the drug may be metabolized and the metabolites bound to cells



Assume that three beakers are each filled with 100 mL of aqueous fluid. Suppose 100 mg of drug is then added to each beaker

CASE 1

The volume of water in beaker 1 is calculated from the amount of drug added (100 mg) and the equilibrated drug concentration. After equilibration, the drug concentration was measured to be 1mg/mL.

$$V_D = \frac{100\,\text{mg}}{1\,\text{mg/mL}} = 100\,\text{mL}$$

The calculated volume in beaker 1 confirms that the system is a simple, homogeneous system and, in this case, represents the "true" fluid volume of the beaker.

CASE 2

Beaker 2 contains cell clusters stuck to the bottom of the beaker. Binding of drug to the proteins of the cells occurs on the surface and within the cytoplasmic interior. This case represents a heterogeneous system consisting of a well-stirred fluid compartment and a tissue (cell). To determine the volume of this system, more information is needed than in Case 1:

Assume that the above measurements were made and that the following information was obtained:

Drug concentration in fluid compartment = 0.5 mg/mL
Drug concentration in cell cluster = 10 mg/mL
Volume of cell cluster = 5 mL
Amount of drug added = 100 mg
Amount of drug taken up by the cell cluster = 10 mg/mL x 5 mL = 50 mg
Amount of drug dissolved in fluid (water) compartment = 100 mg (total) - 50 mg (in cells) = 50 mg (in water)

$$\text{Volume of fluid compartment} = \frac{50\ \text{mg}}{0.5\ \text{mg/mL}} = 100\ \text{mL}$$

$$\text{Apparent volume} = \frac{100\ \text{mg}}{0.5\ \text{mg/mL}} = 200\ \text{mL}$$

CASE 3

Assume that , total drug placed in beaker = 100 mg

Cell compartment:

Drug concentration = 0.2 mg/mL
Metabolite-bound concentration = 9.71 mg/mL
Metabolite-free concentration = 0.29 mg/mL
Cell volume = 5 mL

Fluid (water) compartment:

Drug concentration = 0.2 mg/mL
Metabolite concentration = 0.29 mg/mL

$$V_D = \frac{100\ \text{mg}}{0.2\ \text{mg/mL}} = 500\ \text{mL}$$

Complex Biological Systems and V_D

- The human body is a much more complex system than a beaker of water containing drug metabolizing cells.
- Many components within cells, tissues, or organs can bind to or metabolize drug, thereby influencing the apparent V_D.
- Only free, unbound drug diffuses between the plasma and tissue fluids.
- The tissue fluid, in turn, equilibrates with the intracellular water inside the tissue cells.
- The tissue drug concentration is influenced by the partition coefficient (lipid/water affinity) of the drug and tissue protein drug binding.

Drug–protein binding

Many drugs interact with plasma or tissue proteins or with other macromolecules to form a *drug–macromolecule complex*. The formation of a drug–protein complex is often named *drug–protein binding*.

Types of drug-protein binding

- Irreversible
- Reversible

Irreversible drug-protein binding

- Irreversible drug-protein binding is usually a result of chemical activation of the drug, which then attaches strongly to the protein or macromolecule by covalent chemical bonding.
- Irreversible drug binding accounts for certain types of drug toxicity that may occur over a long period, as in the case of chemical carcinogenesis, or within a relatively short period, as in the case of drugs that form reactive chemical intermediates.

Reversible drug-protein binding

- When drug binds the protein with weaker chemical bonds, such as hydrogen bonds or van der Waals forces.
- The amino acids that compose the protein chain have hydroxyl, carboxyl, or other sites available for reversible drug interactions.

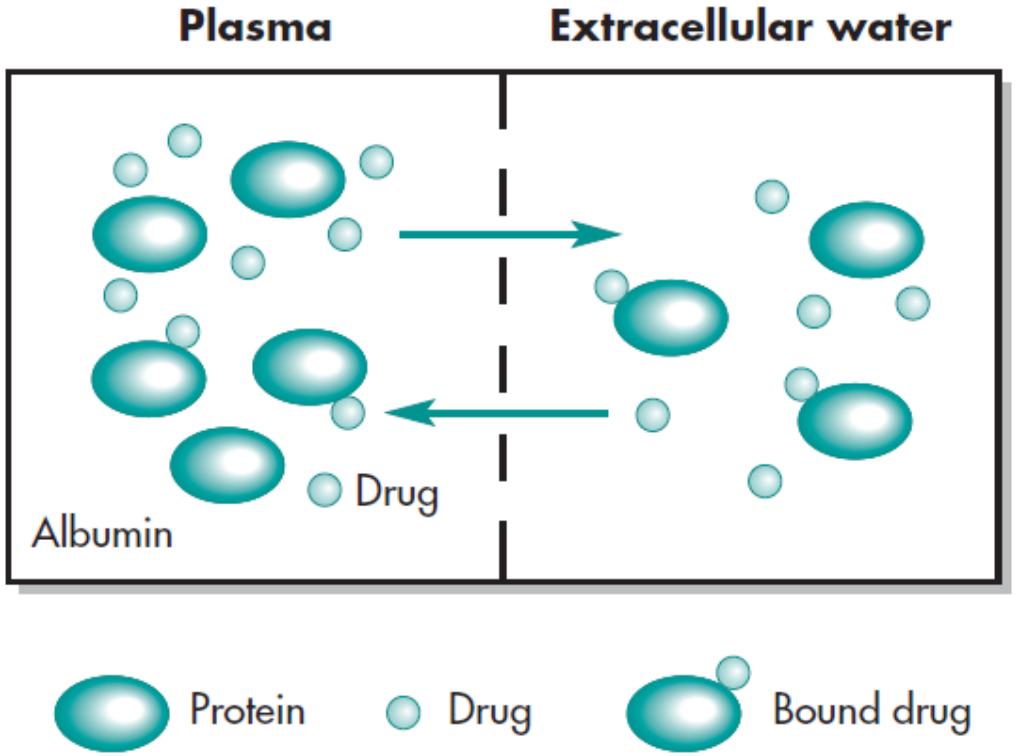


Diagram showing that bound drugs will not diffuse across the membrane but the free drug will diffuse freely between the plasma and extracellular water.

Major Proteins to Which Drugs Bind in Plasma

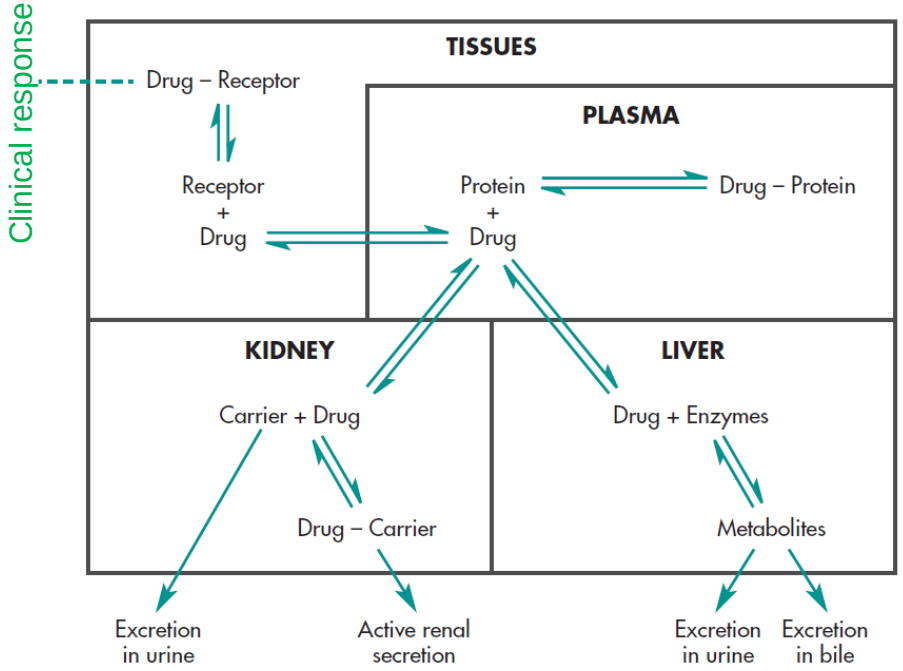
Protein	Molecular Weight (Da)	Normal Range of Concentrations (g/L)	Normal Range of Concentrations (mol/L)
Albumin	65,000	35–50	$5–7.5 \times 10^{-4}$
α_1-Acid glycoprotein	44,000	0.4–1.0	$0.9–2.2 \times 10^{-5}$
Lipoproteins	200,000–3,400,000	Variable	

Albumin

- In the body, albumin is distributed in the plasma and in the extracellular fluids of skin, muscle, and various other tissues.
- The interstitial fluid albumin concentration is about 60% of that in the plasma.
- Albumin is responsible for maintaining the osmotic pressure of the blood and for the transport of endogenous and exogenous substances in the plasma.

Effect of reversible drug–protein binding on drug distribution and elimination

- Drugs may bind reversibly with proteins.
- Free drugs penetrate cell membranes, distributing into various tissues including those tissues involved in drug elimination, such as kidney and liver.
- Active renal secretion, a carrier-mediated system, allows for rapid drug excretion despite drug–protein binding.



- If a drug is displaced from the plasma proteins, more free drug is available for distribution into tissues and interaction with the receptors responsible for the pharmacologic response. Moreover, more free drug is available for drug elimination.

Lecture-5

Contents

- Methods for Studying Drug–Protein Binding
- Considerations in the Study of Drug–Protein Binding
- Effect of protein binding on the apparent volume of distribution
- Effects of displacement of drugs from plasma proteins
- Fraction of unbound drug and apparent volume of distribution, V_{app}

Methods for Studying Drug– Protein Binding

Equilibrium dialysis	Gel chromatography
Dynamic dialysis	Spectrophotometry
Diafiltration	Electrophoresis
Ultrafiltration	Optical rotatory dispersion and circulatory dichroism

- The concentrations of bound drug, free drug, and total protein may be determined by these *in vitro* methods.
- Each method has advantages and disadvantages in terms of cost, ease of measurement, time, instrumentation, and other considerations.

Considerations in the Study of Drug–Protein Binding

- Equilibrium: Maintaining equilibrium between bound and free drug
- Range: Validity of the method over a wide range of drug and protein concentrations
- Extraneous drug binding: Must be avoided or considered
- Denaturation or contamination of the protein : Must be prevented
- pH and ionic concentrations of the media and Donnan effects: Must be considered
- Capability of the method: Detection of both reversible and irreversible drug-protein binding
- Interfering substances: (such as organic solvents) must not be introduced
- Extrapolation of the results: Allow the *in vitro* to the *in vivo* situation

Effect of protein binding on the apparent volume of distribution

- The extent of drug protein binding in the plasma or tissue affects V_D.
- Drugs that are highly bound to plasma proteins have lower V_D
- Drugs with low plasma protein binding have higher V_D

Effect of Changing Plasma Protein Level: An Example

Mouse model

1. Normal
2. Cloned transgenic: Have 8.6 times the normal a_1-acid glycoprotein (AAG) levels

Drug

Imipramine (highly bound to AAG) equal drug doses were administered

	Imipramine Level (ng/mL)	
Mouse Model	**Serum**	**Brian**
Normal	319.9	7307.7
Transgenic	859	3862.6

Volume of distribution, V_D

Effects of displacement of drugs from plasma proteins on pharmacokinetics of the drug

- Directly increase the free (unbound) drug concentration as a result of reduced binding in the blood;
- Increase the free drug concentration that reaches the receptor sites directly, causing a more intense pharmacodynamic (or toxic) response;
- Increase the free drug concentration, causing a transient increase in V_D ;
- Increase the free drug concentration, resulting in more drug diffusion into tissues of eliminating organs resulting in a transient increase in drug elimination.

Fraction of unbound drug and apparent volume of distribution, V_{app}

For a drug that distributes into the plasma and a given tissue in the body, the amount of drug bound may be found by

$$D_B = V_p C_p + V_t C_t \qquad (1)$$

At steady state, unbound drug in plasma and tissue are in equilibration.

$$C_u = C_{ut}$$

Alternatively,

$$C_p f_u = C_t f_{ut} \qquad \text{or,} \qquad C_t = C_p \frac{f_u}{f_{ut}} \qquad (2)$$

where all terms refer to steady-state conditions: f_u is the unbound (free) drug fraction in the plasma, f_{ut} is the unbound drug fraction in the tissue, C_u is the unbound drug concentration in the plasma, and C_{ut} is the unbound drug concentration in the tissues.

Substituting for C_t in Equation (1) using Equation (2) results in

$$D_B = V_p C_p + V_t \left[C_p \left(\frac{f_u}{f_{ut}} \right) \right]$$

or,

$$\frac{D_B}{C_p} = V_p + V_t \left(\frac{f_u}{f_{ut}} \right)$$

As $D_B/C_p = V_{app}$

$$V_{app} = V_p + V_t \left(\frac{f_u}{f_{ut}} \right) \qquad (3)$$

Equation (3) may be expanded to include several tissue organs with V_{ti} each with unbound tissue fraction f_{uti}.

$$V_{app} = V_p + \sum V_{ti} \left(\frac{f_u}{f_{uti}} \right) \qquad (4)$$

Important considerations in the calculation of V_{app}

1. The volume of distribution is a constant only when the drug concentrations are in equilibrium between the plasma and tissue.
2. Values of f_u and f_{ut} are concentration dependent and must also be determined at equilibrium conditions.
3. V_{app} is an indirect measure of drug binding in the tissues rather than a measurement of a true anatomic volume.
4. When f_u and f_{ut} are unity, Equation (3) is simplified to

$$\frac{D_B}{C_p} = V_p + V_t$$

When no drug binding occurs in tissue and plasma, the volume of distribution will not exceed the real anatomic volume.

Lecture-6

Contents

- Kinetics of protein binding
- Determination of binding constants and binding sites (*In Vitro* Methods)

KINETICS OF PROTEIN 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:

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

$$[P] + [D] \Leftrightarrow [PD] \qquad (1)$$

The association constant, Ka (also called the affinity constant), can be expressed as:

$$K_a = \frac{[PD]}{[P][D]} \qquad (2)$$

To study the binding behavior of drugs, a determinable ratio r is defined, as follows:

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

$$r = \frac{[PD]}{[PD]+[P]} \qquad (3)$$

According to Equation (2), $[PD] = K_a\,[P]\,[D]$;

Then,

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

or,

$$r = \frac{K_a[D]}{1+K_a[D]} \qquad (4)$$

If there are *n* identical independent binding sites per protein molecule, then the following equation is used:

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

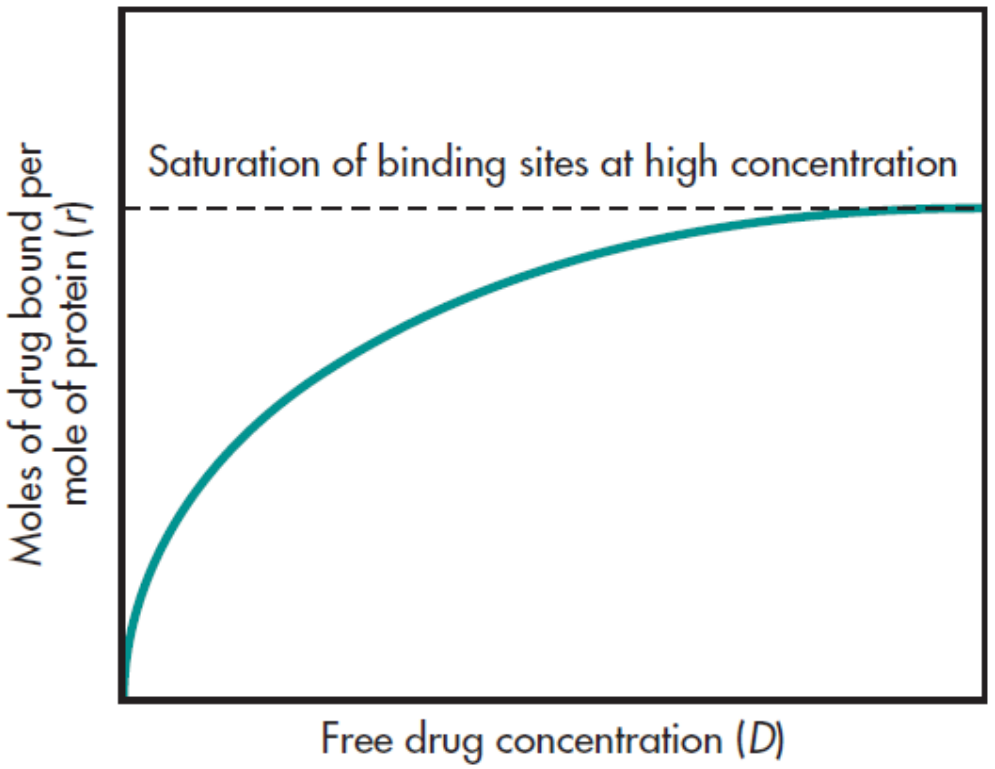
If there is more than one type of binding site and the drug binds independently to each binding site with its own association constant, then

$$r = \frac{n_1 K_1[P]}{1 + K_1[D]} + \frac{n_2 K_2[P]}{1 + K_2[D]} + \cdots \qquad (6)$$

DETERMINATION OF BINDING CONSTANTS AND BINDING SITES BY GRAPHIC METHODS

In Vitro Methods (Known Protein Concentration)

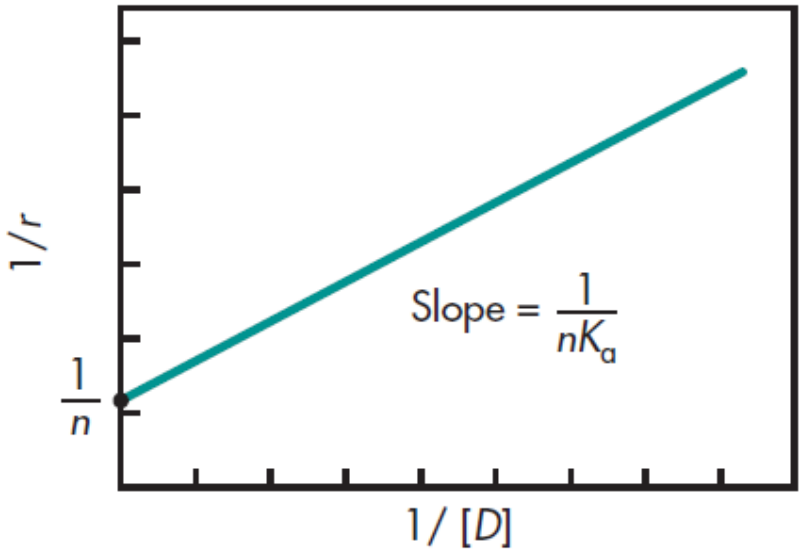
$$r = \frac{nK_a[D]}{1 + K_a[D]}$$



In Vitro Methods (Known Protein Concentration)

$$\frac{1}{r} = \frac{1 + K_a[D]}{nK_a[D]} \qquad \text{or,} \qquad \frac{1}{r} = \frac{1}{nK_a[D]} + \frac{1}{n}$$

Intercept = $\frac{1}{n}$



In Vitro Methods (Known Protein Concentration)

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

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

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

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