Distribution, Storage, and Elimination of Toxicants

DISTRIBUTION: Process whereby the absorbed chemical moves away from the site of absorption.

- How do chemicals move through the body?
- Does distribution vary with the route of exposure?
- How fast is a chemical distributed?
- Is a chemical distributed evenly to all organs or tissues?
- Why do some chemicals stay in the blood for a long time whereas others are eliminated quickly?

Distribution

- After a toxicant enters portal circulation, systemic circulation, or lymph, it is available for distribution (translocation) throughout the body, which usually occurs very rapidly.
- Distribution is the process in which a chemical agent, after first gaining entry into the internal body fluid (usually the blood), translocates throughout the fluid compartments of the body.
- The blood carries the toxicant to its
  - sites of biotransformation,
  - site(s) of action,
  - storage, and
  - elimination.

- There are a number of concerns regarding the movement and distribution of toxicants throughout the body.
- These concerns involve:
  - the rate of distribution
  - the role of exposure route on distribution outcome
  - the determinants of equal or unequal distribution to the cells and tissues of the body.

Distribution of toxicants

Distribution can be highly localized, restricted or disperse depending on:

1. Binding and dissolution into various storage sites (fat, liver, bone)
2. Permeability through membranes
3. Protein binding
4. Active transport

If the toxicant accumulates at a site away from a toxic site of action, it is considered as a protective storage site

Factors Affecting Distribution

The rate of distribution to organs or tissues is determined by:

1. Physicochemical properties of the chemical like Lipid solubility, ionisation etc.
2. Ease of crossing cell membranes
3. Rate of diffusion out of the capillary bed into the cells of a organ or tissue.
4. Cardiac output to the specific tissues i.e., Blood flow to the tissue or organ

Factors Affecting Distribution

5. Concentration gradient: Volume of distribution (dose/plasma concentration).
6. Affinity of a xenobiotic for various tissues.
7. Detoxication reactions: Extent of plasma protein binding
8. Tissue sensitivity to the toxicant. Adipose tissue; receptors.
9. Barriers that inhibit migration: Blood-brain and placental.

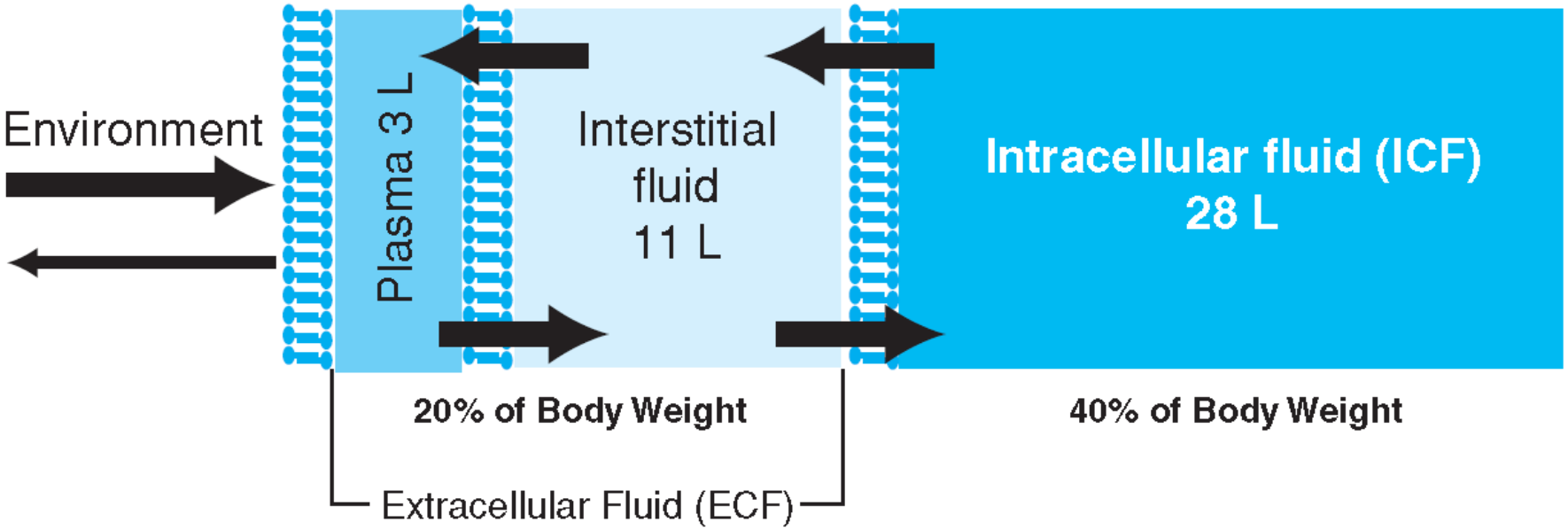
- Penetration of toxicants into cells or tissues occurs by passive diffusion or active transport (as discussed earlier).

How do chemicals move through the body? Or **KINETICS OF DISTRIBUTION**

- Absorption through skin, lung or intestinal tissue is followed by passage into the interstitial fluid. {Interstitial fluid (~15%); intracellular fluid (~40%); Blood plasma (~8%) of b.w.}
- Toxicant leaves interstitial fluid by entering:
  - -local tissue cells
  - -lymph or
  - -blood supply

and is mobilized to other parts of the body.

Distribution of body water and movement of toxicant between compartments.



Body Water and Volume of Distribution

- The process of toxicant distribution results in the movement of the chemical from the exposure site to internal areas of the body.
- Toxicant distribution depends on many factors, including what is referred to as the *apparent volume of distribution* (V_D)

Body Water and Volume of Distribution

- Apparent volume of distribution (V_D) represents the total volume of body fluids in which a toxicant is distributed.
- V_D expressed in litres
- V_D shows how extensively a toxicant is distributed in the body fluids
- $V_D = D/C_p$
- D = dose administered
- C_p plasma concentration

- If a chemical distributes only to the plasma compartment (no tissue distribution), it has a high plasma concentration and a low Vd.
- In contrast, if a chemical distributes throughout the body (into both compartments), it has a low plasma concentration and a high Vd

Apparent Volume of Distribution (V_D)

- This concept is related to the concentration of the toxicant in different fluid compartments within the body.
- It is the theoretical total volume of water required to equally distribute the toxicant throughout the body, expressed in liters/kilogram (L/kg).
- This is important to know because it indicates the extent of the distribution of a toxicant within the body fluids.

Body Water and Volume of Distribution

- Water comprises most of the weight of the body and is primarily distributed into three compartments:
  - *Blood plasma water,* simply referred to here as the plasma, accounts for about 4–5% of total body weight.
  - *Interstitial water* is referred to as interstitial fluid, which is the fluid surrounding the cells of the tissues of the body, and represents approximately 15% of total body weight.
  - *Intracellular water,* or intracellular fluid, is a fluid contained within the cells and represents approximately 40% of total body weight (approximately 28 liters of water).

Plasma Binding, Blood Flow, and Barriers to Distribution

- A toxicant into the blood will move in the plasma either in the unbound or bound form to be distributed to the tissues and organs of the body.
- The distribution of toxicants from the blood to the tissues and organs of the body may not be uniform.
- Based on specializations of the blood vessels and other factors, certain parts of the body such as the placenta, the testes, and brain may serve as “barriers” to the diffusion of certain chemicals in the blood, thereby restricting their entry and reducing potential toxicity.

Plasma Binding, Blood Flow, and Barriers to Distribution

- These barriers should not be viewed as completely restricting the entry of toxicants; instead, they should be viewed as slowing down the rate of entry.
- Once the toxicant has gained entry into the blood, it can be stored, eliminated, and metabolized.
- Unbound and bound toxicants tend to be in equilibrium in the plasma.

Plasma Binding, Blood Flow, and Barriers to Distribution

- Plasma proteins, especially albumin, may act to bind to the toxicant, thereby reducing its potential to enter the cells of the body, because generally only the unbound toxicant is able to cross cell membranes.
- Plasma protein binding therefore affects the distribution of toxicant, the “effective” dose of the toxicant, and its time within the body.
- Lymph generally plays only a minor role in the distribution of toxicants.

Role of plasma protein binding in distribution

- Toxicants bind with plasma proteins like albumin
- Reversible or irreversible
- Thus bound and unbound forms of toxicant
- More binding :
  - less concentration of toxicant in blood
  - Less intensity of toxic effect
  - Elimination rate slowed down
  - Duration of effect prolonged
  - Acts as reservoir for toxicants

Influence of route of exposure

- Important factor as it can affect the concentration of toxicant
- Influences:
  - Degree of biotransformation
  - Storage
  - Elimination
  - Finally toxicity

- If goes to liver first:
  - Biotransformed quickly
  - Blood levels of toxicant downstream decrease or eliminated
  - Potential toxicity is affected

  This is the **first pass effect.**

  Occurs when absorption through
  - GIT
  - ip

  It passes to liver via portal vein.

  Path followed: liver →heart →lung

  Biotransformation or excretion in liver and elimination in lung

- Absorption through liver, skin, iv and im
- Path followed: Blood →heart → systemic circulation → various organs → liver
- Not subjected to first pass effect.

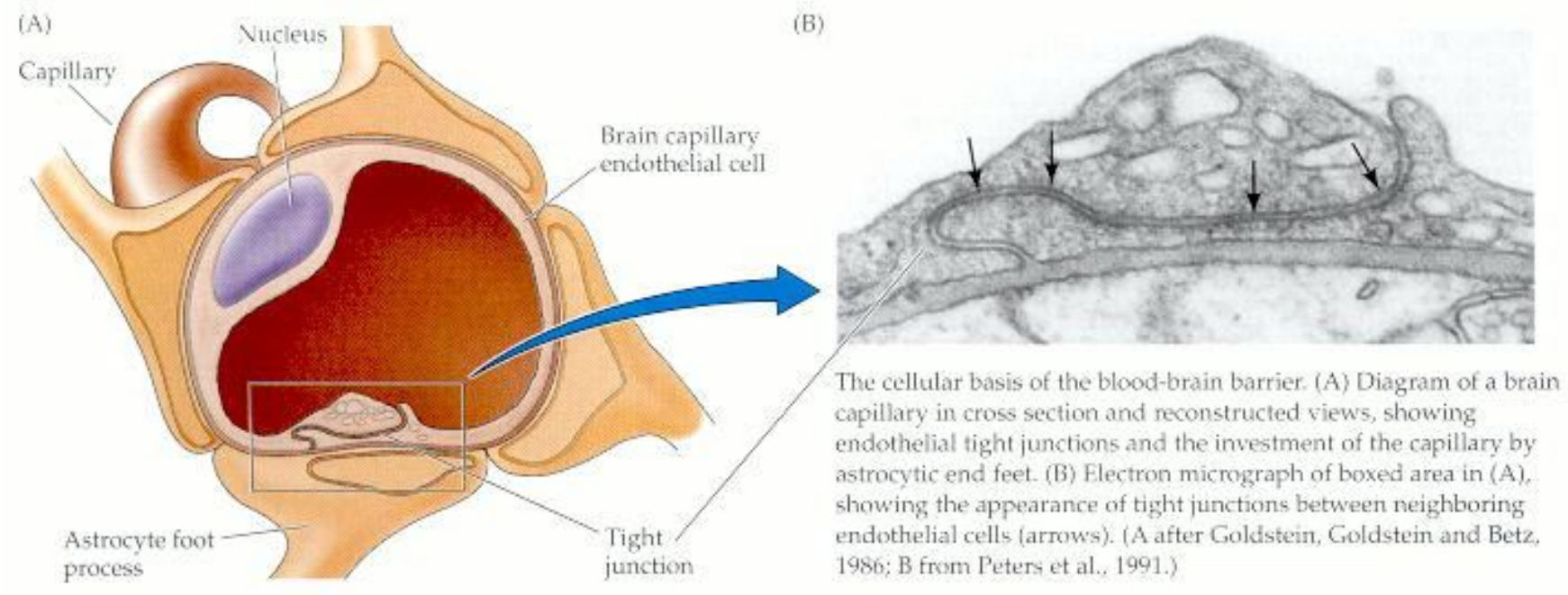
Is a chemical distributed evenly to all organs or tissues?

Depends on

- Volume of Blood
- Tissue affinity
- Structural Barriers to distribution
  - Blood brain barrier
  - Blood testes barrier
  - Placental barrier

- **Volume of Blood:** organs receiving larger blood volume or cardiac output more accumulation e.g. Liver, heart, muscle and brain
- **Tissue affinity:** determines degree of concentration of toxicant eg., adipose tissue (low blood supply) concentrates lipid soluble toxicants
- **Structural Barriers to distribution**
  - **Blood brain barrier**: Astrocytes form barrier b/w capillary endothelium and neurons of brain (slows the rate especially of water soluble)
  - **Blood testes barrier:** prevents the testis
  - **Placental barrier**: slows down and limits the diffusion

Blood-Brain Barrier



The cellular basis of the blood-brain barrier. (A) Diagram of a brain capillary in cross section and reconstructed views, showing endothelial tight junctions and the investment of the capillary by astrocytic end feet. (B) Electron micrograph of boxed area in (A), showing the appearance of tight junctions between neighboring endothelial cells (arrows). (A after Goldstein, Goldstein and Betz, 1986; B from Peters et al., 1991.)

The blood-brain barrier serves to restrict access to many toxicants. It is not an absolute barrier.

It is a site that is less permeable to more hydrophilic substances than are most other areas of the body.

There are four major anatomic and physiologic reasons why some toxicants do not readily enter the CNS.

1. Capillary endothelial cells of the CNS are **tightly joined**, leaving few or no pores between cells.

2. Brain capillary endothelial cells contain an ATP-dependent transporter, the **multi-drug-resistant (mdr)** protein that transports some chemicals back into the blood.

3. Capillaries in the CNS are **surrounded by glial cells** (**astrocytes**) to further restrict access.

4. The **protein concentration in the interstitial fluid** of the CNS is much lower than in other body fluids.

Blood Brain Barrier

- For water-soluble molecules, the tighter junctions of the capillary endothelium and the lipid membranes of the glial cells represent the major barrier.
- Many lipid soluble compounds are restricted due to the many lipid membranes to be crossed (capillary and glial cell membranes) and low protein content.
- The blood-brain barrier is ***more effective against water soluble substances.***

Toxicant Storage

- The storage of toxicants occurs in connective tissues, primarily fat and bone, and in the kidneys and liver.
  - Fat or adipose tissue is located in many parts of the body and is especially accumulated in the subcutaneous tissue.
  - It is here where lipophilic toxicants are stored and are mobilized back into the blood for further distribution, metabolism, elimination, or redeposition.

Toxicant Storage

- The liver and kidneys, with their relatively high blood flow, may store toxicants in amounts greater than other organs.
- The liver has the greatest capacity of all the tissues for metabolism, which may make it especially vulnerable to injury.
- Bone or osseous tissue is also an important site for the deposition of lead, strontium, and fluoride.

Toxicant Storage, cont.

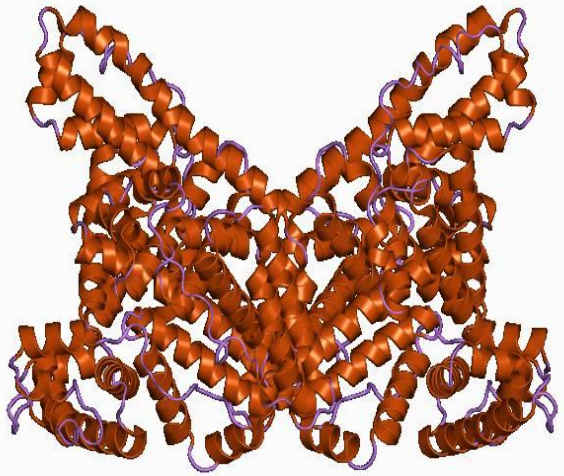
- Although bone has a relatively poor blood supply, mobilization of elements out of the bone matrix does occur, especially during times of extensive bone remodeling (e.g., repair of a broken bone) or during pregnancy when minerals are mobilized from maternal to fetal compartments.
- For example, lead may be substituted for calcium, and fluoride may be substituted for hydroxyl ions.
- Heavy metals stored in the bone may reside there for decades.

Storage of Toxicants in Circulation and Tissues

1. Plasma Proteins as Storage Depot:

   a. **<u>Albumin</u>** -the most abundant protein in plasma- can bind to a very large number of different compounds—(e.g. bilirubin, Ca^{2+}, Cu^{2+}, Zn^{2+}, vitamin C, fatty acids, digitonin, penicillin, sulfonamides, histamine, barbiturates, thyroxine, etc.)

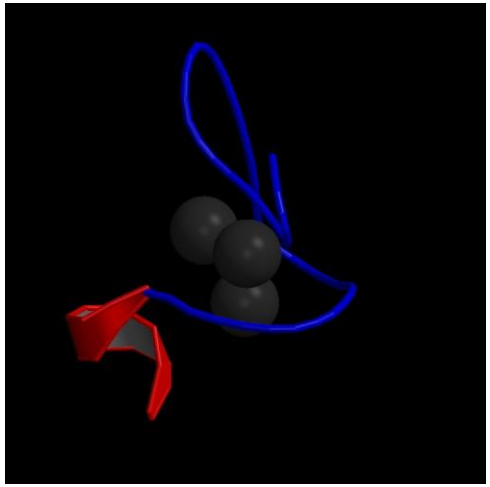
      1. Contains 6 binding regions on the protein
      2. Protein-ligand interactions occur primarily through hydrophobic forces, hydrogen bonding, and van der Waals forces.
      3. Bilirubin, a heme byproduct, is neurotoxic at high levels, but is normally bound to albumin to make it less toxic.



2. Liver and Kidney as Storage Depots—have the highest capacity for binding chemicals.

1. **Ligandin:** this cytoplasmic protein in the liver is a high-affinity binding protein for many organic acids—can bind bilirubin, azodye carcinogens, steroids, etc.

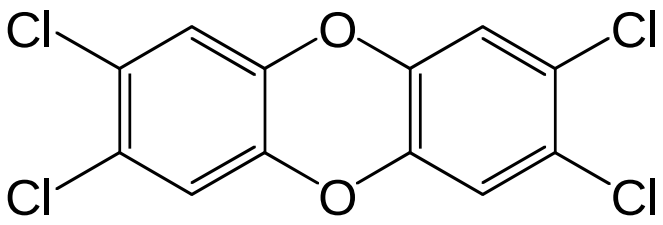
2. **Metallothionein**: found in the kidney and liver and has high affinity for cadmium and zinc--in the liver, metallothionein binds Lead (Pb) and concentrates it to 50-fold more than plasma.



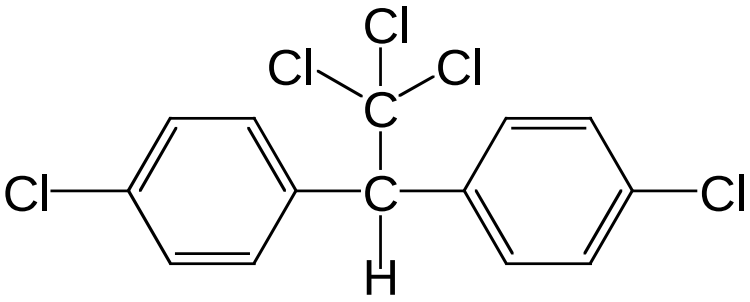
Human metallothionein bound to Cd2+ determined by NMR

3. Fat as a Storage Depot

Many highly lipophilic toxicants are distributed and concentrated in fat (e.g. dioxin, DDT, polychlorinated biphenyls)



dioxin



DDT

-The LD_{50} of a fat-stored compound will be higher in an obese subject.

-However, a quick weight-loss can result in large release of toxicant and toxic effect.

4. Bone as Storage Depot

1. Compounds such as fluoride, lead, and strontium may be incorporated and stored in bone matrix.

2. 90% of lead in the body is eventually found in the skeleton.

3. The mechanism of storage is through exchange of bone components for the toxicant (e.g. F^- may displace ^-OH; Pb^{2+} and Sr^{2+} may substitute for Ca^{2+} in the hydroxyapatite lattice matrix).

Effects of Storage on Toxicity

1. Reduces toxicity of some substances by taking toxic substances out of the sites of action.
2. Increases toxicity if: a)toxicity at storage site, b) displacement of one substance by another (e.g. bilirubin), loss of storage site.
3. Can produce chronic toxicity from prolonged exposure.

Excretion of toxicants

- The processes of toxicant elimination are critical to the reduction of toxicity or potential toxicity in the body.
- The term *elimination* encompasses all of the processes that are used by the body that lead to a decrease in the amount of toxicant, including
  - Renal excretion /elimination
  - Fecal excretion / elimination
  - Biliary excretion
  - Pulmonary excretion / elimination
  - Excretion / Elimination via other routes
    - Sweat and saliva
    - milk,
    - Placenta
    - eggs
    - Nails, hairs

Urinary Excretion

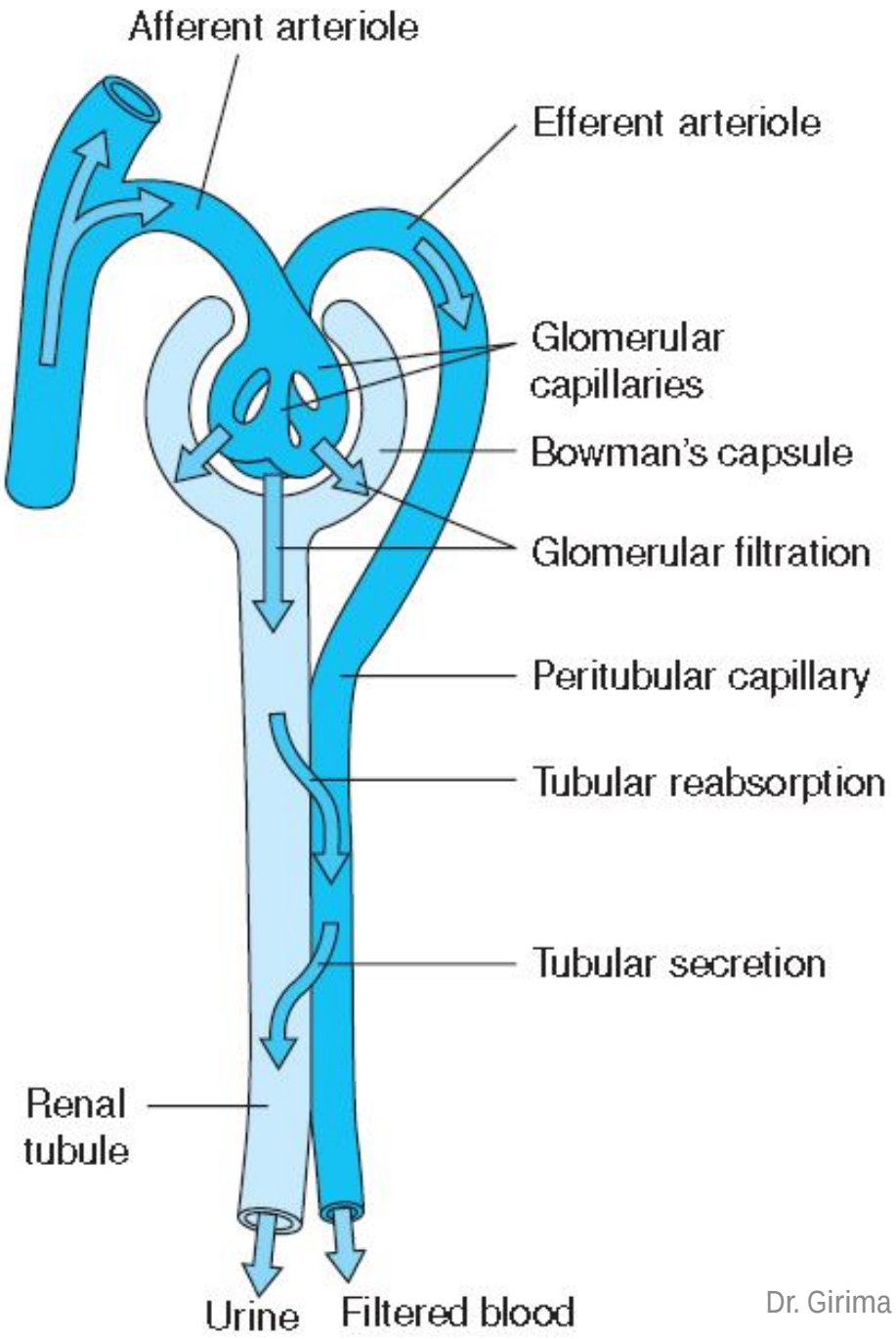
- Elimination of toxicants by renal excretion is one of the most important routes available to the body.
- The kidneys are composed of approximately 1 million functional units referred to as *nephrons.*
- Each nephron is composed of a capillary ball called a glomerulus and a capsule surrounding the glomerulus (Bowman's capsule), leading to the proximal tubule, loop of Henle, distal tubule, and, finally, collecting tubule.

Urinary Excretion

- The urinary excretion of toxicant is influenced by factors that are related to the properties of the toxicant:
  - Molecular size
  - Water solubility
  - Degree of ionization

The Nephron & Toxicant Movement

- For most toxicants size is generally not a problem
  - they are filtered across the glomerulus with relative ease if they are not protein bound in the plasma.
- Ionized toxicants tend to remain within the urine and thus exit when the urine is eliminated from the body.
- Toxicants that are more lipophilic can reenter into the renal circulation through reabsorption, thus increasing their resident time within the body.



The nephron and toxicant movement.

Urinary Excretion: Filtration

- The process of toxicant removal from the blood occurs at the glomerulus of the nephron, where a large amount of blood plasma filters through the large pores of the glomerulus and into the beginning of the nephron tube, Bowman's capsule.

Urinary Excretion: Reabsorption

- Here is where most of the water, electrolytes, amino acids, glucose, and other low-molecular-weight chemicals are returned back to the blood from the glomerular filtrate.
- The process occurs primarily in the proximal convoluted tubule and is driven primarily by simple diffusion.

Urinary Excretion: Secretion

- The process of renal secretion involves the active transport of chemicals from the blood into the proximal tubule of the nephron and is of importance in the conservation of important body ions such as potassium.

Fecal Elimination

- Toxicants can be eliminated in the feces:
  - through their direct discharge into the lumen of the gastrointestinal tract
  - through excretion in the bile
- Toxicants and their metabolites may also be reabsorbed and returned to the liver.
- Biliary excretion is the main route of gastrointestinal elimination of toxicants and their metabolites.
  - Biliary excretion is an active secretory process with specific transporters for organic acids and bases, heavy metals such as lead and mercury, as well as nonionized chemicals.

Fecal Elimination, cont.

- In general, it is the relatively large ionized molecules that are excreted into the bile for elimination.
- Disorders of the liver that may compromise bile secretion could intensify or prolong the effects of some chemicals that would normally be eliminated through this route.
- Toxicants in the bile are transported to the intestinal tract where they are eliminated with the feces or reabsorbed.

Fecal Elimination, cont.

- Excretion of toxicants from the liver generally is accompanied by their biotransformation.
- The enterohepatic circulation is a way in which toxicants can be reabsorbed from the bile that has entered into the gastrointestinal tract at the duodenum and returned to the liver by way of the hepatic portal circulation.
- The recycling of toxicant between intestine and liver has the effect of prolonging its time in the body.
- This is of particular concern because biotransformation in the liver may have produced a metabolite that is more toxic than the parent compound.

Fecal Elimination, cont.

- Toxicants can also be eliminated with the feces through their direct diffusion across the intestinal capillaries of the submucosa to the intestinal lumen where they can be eliminated with the feces.
  - Although this relatively slow elimination pathway is not the primary route of toxicant elimination by way of the gastrointestinal tract, it can be important under conditions where urinary or biliary excretion have become less effective.

Biliary excretion

- Complementary to renal excretion
- Larger molecules are excreted eg., conjugated compounds
- Increases with the molecular weight

Pulmonary Elimination

- The lungs have a large surface area and receive the entire cardiac output
- This makes them an important route for the elimination of volatile liquids and gases.
- Important factors that determine elimination of chemicals from the lungs include:
  - concentration differences between alveolar air and blood plasma
  - vapor pressure
  - plasma solubility

Pulmonary Elimination

- Elimination is by simple diffusion from blood to alveolus, following a concentration gradient if the concentration in capillary blood is greater than the concentration of the chemical in the alveolar air.
- For those gases that have a relatively low solubility in blood, elimination is generally much more rapid than for those that are more soluble.

Pulmonary Elimination, cont.

- As an example, chloroform and ethylene are greatly different in their blood solubilities.
- Ethylene does not dissolve well in the blood and is therefore eliminated much more rapidly than chloroform, which has greater blood solubility.
- Lipophilic gases such as halothane have the potential to accumulate in the body's adipose tissue, and trace amounts in exhaled breath may be present for a long time after the administration of the gas.

Minor Routes of Elimination: Milk

- Toxicants can be transferred from mother's milk to the nursing infant as well as from cow milk to people.
- Chemicals that are lipophilic are of special concern because milk contains a relatively high percentage of fat
  - these chemicals would diffuse from body fat to plasma to mammary gland and be excreted into milk.

Minor Routes of Elimination: Milk

- Chemicals that behave in the body similar to calcium (e.g., lead) can also be excreted along with calcium into the milk.
- Toxicant transport into milk occurs primarily by diffusion of the nonionized chemical.
- The pH difference between blood plasma and milk, about 7.4 and 6.5, respectively, would favor higher concentrations of organic bases in milk compared with organic acids.

Minor Routes of Elimination, cont.

- Saliva: Toxicants that are eliminated to some extent in saliva are usually swallowed, thus prolonging residence time in the body.
- Sweat: Some toxicants that are eliminated via sweat may, if present in sufficient quantities, cause skin irritation.
- Tears
- Semen

Minor Routes of Elimination, cont.

- Hair: Although there is negligible elimination via the hair, some chemicals such as mercury and arsenic may be found there using methods that have been developed primarily for forensic purposes.
- Nails: Same as hair
- Eggs (for birds)
  - For some birds, the elimination of toxicants occurs via the eggs.
  - This poses little hazard to the mother but may greatly endanger the young.

Minor Routes of Elimination: Placenta

- The placenta is not traditionally viewed as an excretory organ for toxicants
- It moves toxicants from maternal compartment to fetal compartment.
  - At the end of a pregnancy, it has a surface area of approximately 10 square meters.
  - It normally functions as an interface, providing oxygen and nutrients to the fetus while eliminating fetal metabolites and carbon dioxide.
  - This occurs by diffusion and active transport.

Minor Routes of Elimination: Placenta

- Maternal elimination of toxicants via the placental route can result in a redistribution of chemicals from maternal tissues to fetal tissues.
- Simple diffusion provides the mechanism to drive lipophilic and low-molecular-weight chemicals across the placenta.
- The placenta is relatively nonprotective to the fetus for lipophilic chemicals, and maternal and fetal tissue levels may be comparable.

Kinetics of excretion

- Rate of elimination is determined by 2 factors:
  - apparent volume of distribution (V)
  - clearance (CL): ratio of rate elimination of chemical to the plasma concentration

CL = rate elimination of chemical (µg/min)/plasma concentration (µg/ml)

Chemical Disposition & Toxicokinetics

- There are specific aspects of disposition that are of primary importance:
  - The duration and concentration of the substance at the site of entry
  - The rate of absorption (determined by the ability of the substance to pass through cell membranes)
  - The total amount of toxicant absorbed
  - The distribution within the body and presence at specific sites
  - The efficiency of biotransformation
  - The toxicity of the metabolites
  - The storage of the toxicant and its metabolites within the body
  - The rate and sites of elimination

Models of Disposition

- The movement of toxicants throughout the body, over time, has been described by the use of models of disposition.
- Models of disposition integrate the processes of:
  - distribution
  - Metabolism
  - elimination

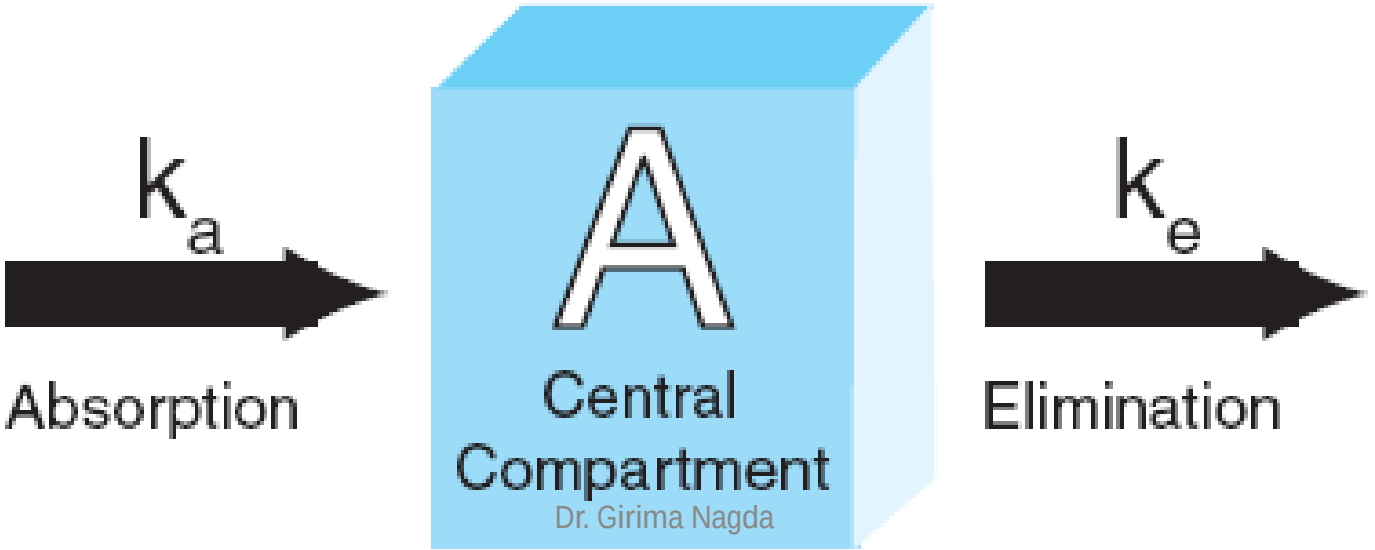
Different theoretical models

- One-compartment model
- Two-compartment model
- Multicompartment model
- Physiologically based model

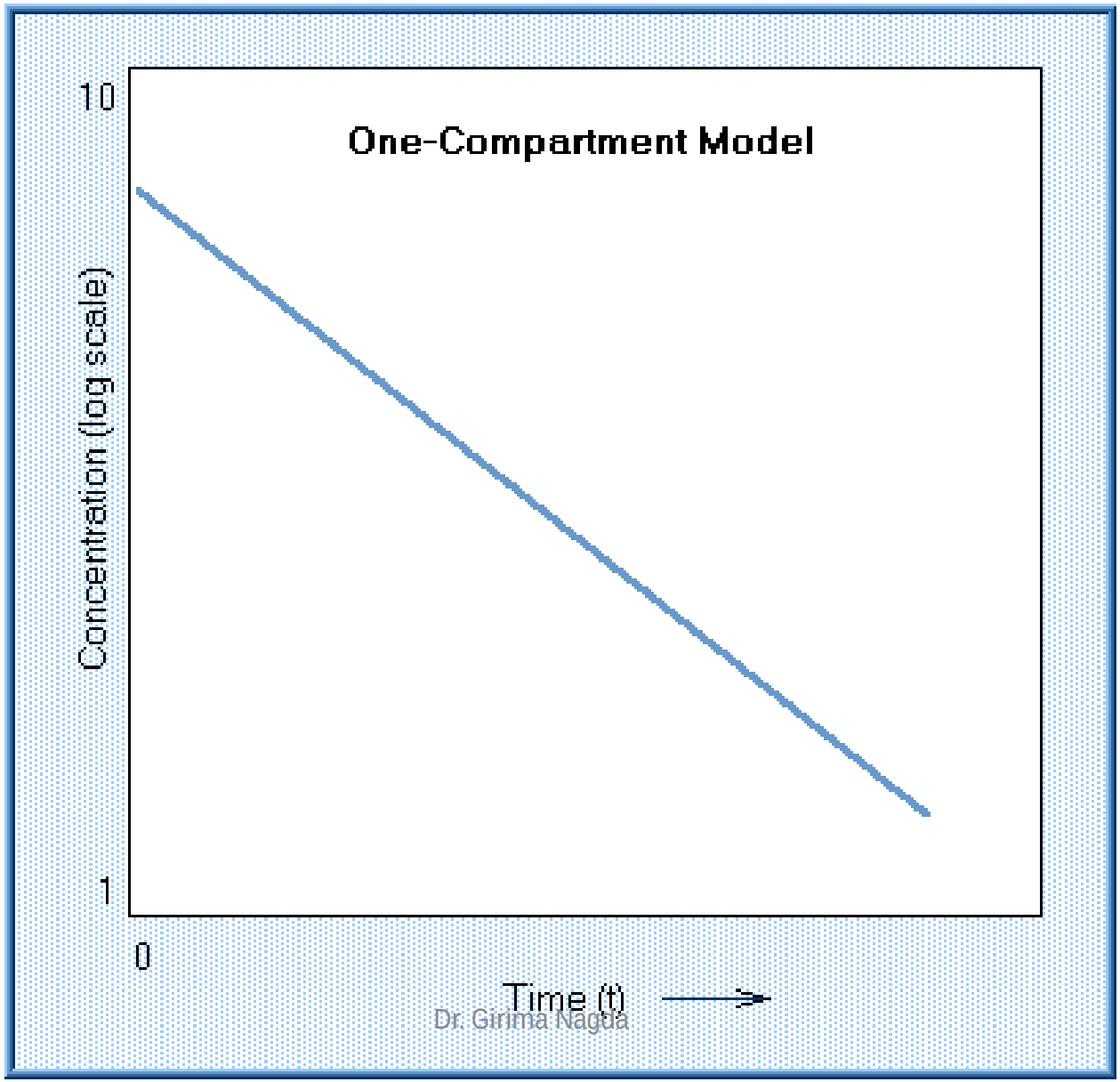
Most toxicants follow a theoretical two- or greater compartment model of disposition.

One-compartment model

- In this simple model, the body is depicted as a single homogeneous compartment.
- The **one-compartment open model** describes the disposition of a substance that is introduced and distributed instantaneously and evenly in the body, and eliminated at a rate and amount that is proportional to the amount left in the body.

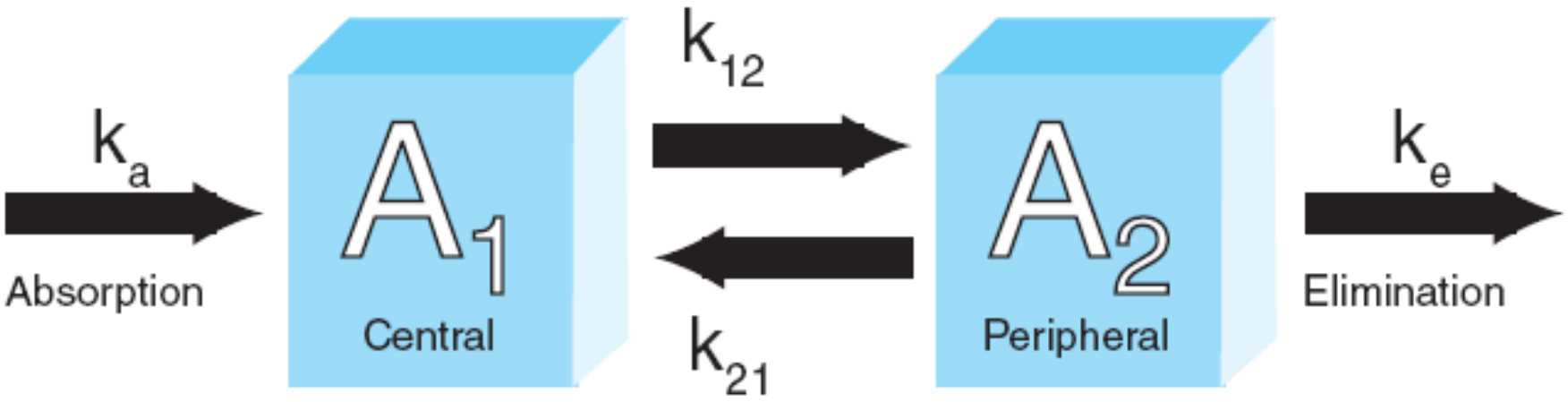


This is known as a "first-order" rate, and represented as the logarithm of concentration in blood as a linear function of time.

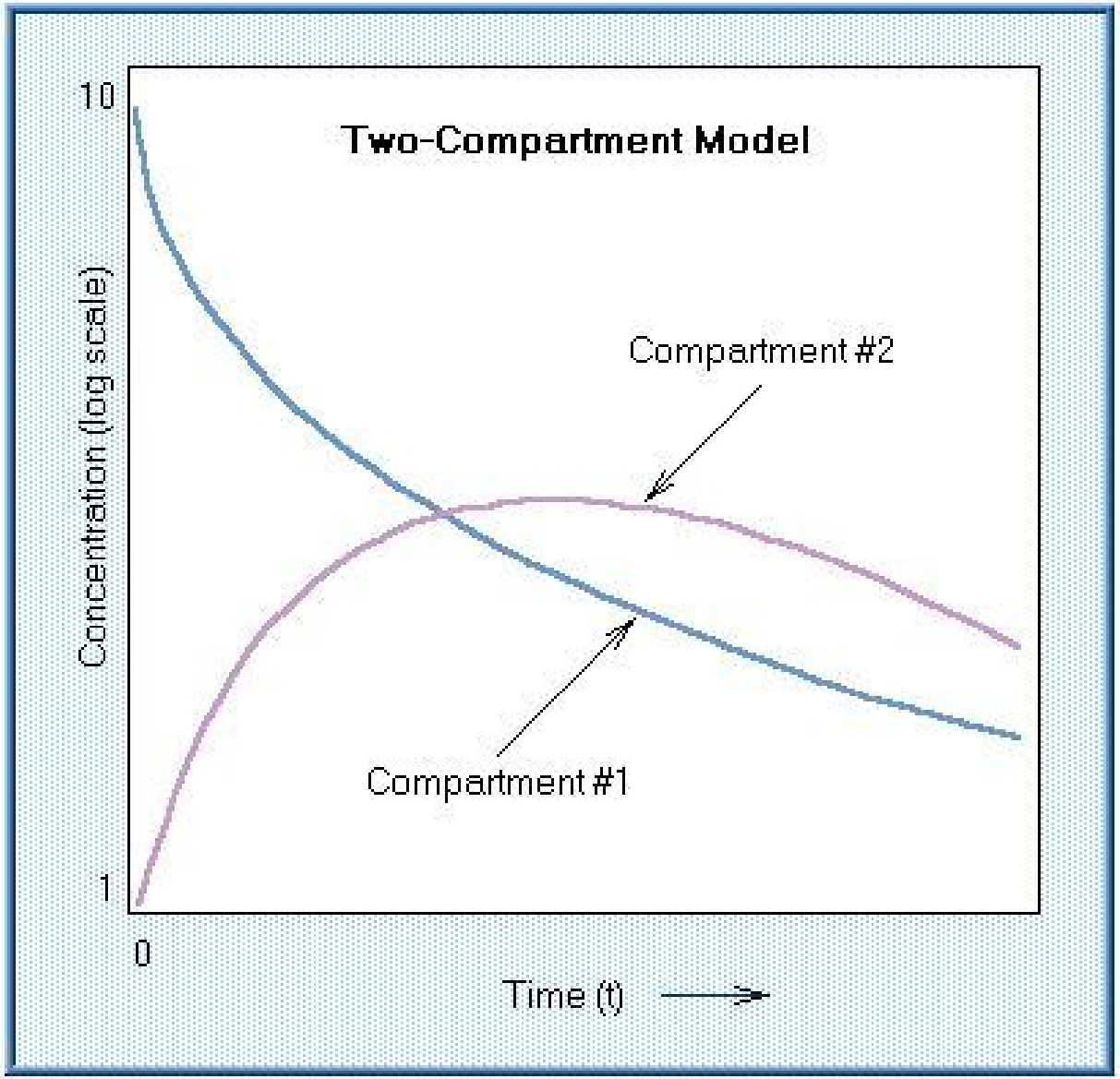


Two-compartment model

- The toxicant is distributed from the blood (central compartment) into a peripheral compartment (e.g., the kidney) where it can be eliminated or returned back to the blood.
- A *half-life* is described as the time required to reduce the blood or plasma concentration by 50%.



- In the **two-compartment open model**, the chemical enters and distributes in the first compartment, which is normally blood. It is then distributed to another compartment from which it can be eliminated or it may return to the first compartment.
- Concentration in the first compartment declines smoothly with time. Concentration in the second compartment rises, peaks, and subsequently declines as the chemical is eliminated from the body.


Two-Compartment Model
10
1
0
Concentration (log scale)
Compartment #2
Compartment #1
Time (t)

One Compartment	Two Compartments
Rapid or prompt equilibrium is attained.	Distribution equilibrium is slow (takes finite time).
There is a single disposition phase	Distribution and post-distribution are two distinct phases.
Linear: drug elimination follows first order kinetics	Linear: distribution and elimination both follow first order

Physiologically based kinetic model

- These models are defined by:
  - physiological volumes
  - blood flows
  - partition coefficients
  - metabolic rate
  - Age
  - Sex
  - body weight
  - percentage of body fat
  - ventilation rate
  - cardiac output
- Thus they can be very complex in nature.