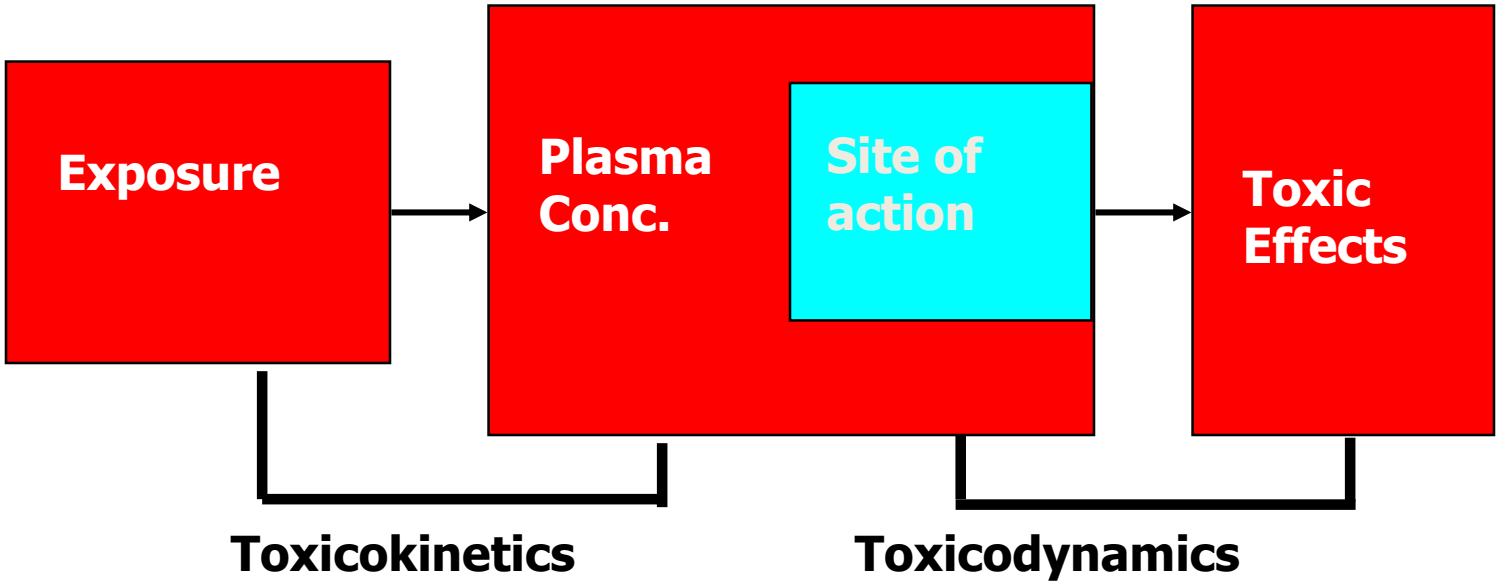
- **INTRODUCTION**
- **TRANSMEMBRANE TRANSPORT OF XENOBIOTICS**
- **ABSORPTION-FACTORS EFFECTING**

  ABSORPTION-MAIN ROUTES OF ABSORPTION
- **DISTRIBUTION**
- **EXCRETION**


Exposure
Plasma Conc.
Site of action
Toxic Effects
Toxicokinetics
Toxicodynamics

TOXICOKINETICS

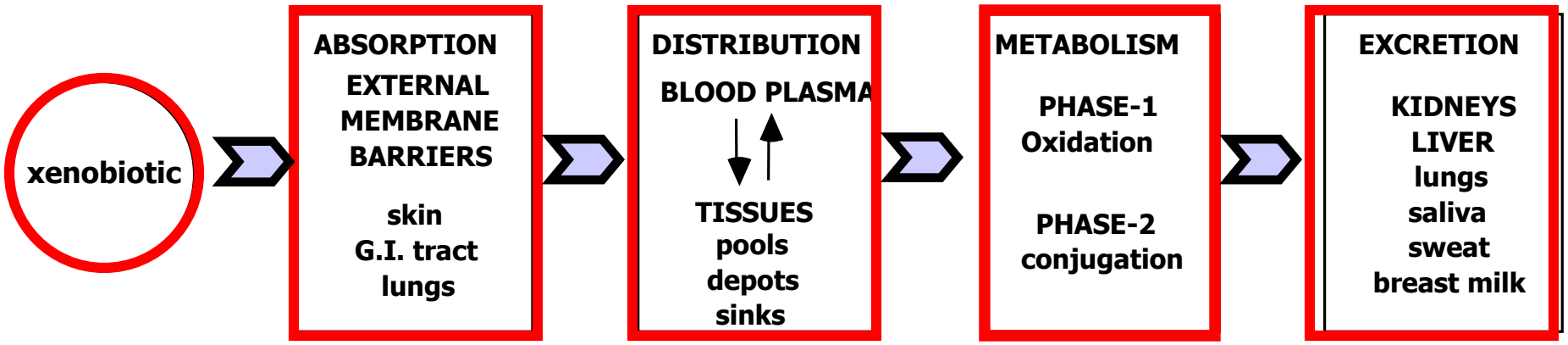
Characterization (Quantitation) of the time course of disposition (ADME) of xenobiotics in the whole organism

"a substance gets into the body and what happens to it in the body".

The end result of these toxicokinetic processes is a **biologically effective dose of the toxicant.**

TOXICODYNAMICS

- Toxicodynamics is the study of toxic actions of xenobiotic substances on living systems.
- Toxicodynamics is concerned with processes and changes that occur to the drug at the target tissue, including metabolism and binding that results in an adverse effect.
- Simply, TD is concerned with what the toxicant do to the body


xenobiotic
ABSORPTION
EXTERNAL MEMBRANE BARRIERS
skin
G.I. tract
lungs
DISTRIBUTION
BLOOD PLASMA
TISSUES
pools
depots
sinks
METABOLISM
PHASE-1
Oxidation
PHASE-2
conjugation
EXCRETION
KIDNEYS
LIVER
lungs
saliva
sweat
breast milk

ABSORPTION
DISTRIBUTION
METABOLISM-BIOTRANSFORMATION
ELIMINATION

- Absorption- xenobiotics gets into bloodstream
- Distribution - gets to site of action
- **Metabolism - is "changed" so that it can be excreted**
- Elimination - leaves the body

How toxicokinetics can influence the toxicity?

❖ ***Absorption***.

- A toxic xenobiotic which is poorly absorbed may not cause toxicity

❖ ***Distribution***

- The distribution of a toxicant to a tissue other than the target organ decreases its toxicity.

- ***Metabolism (Biotransformation)***
  - Two substances with equal absorption rate may differ in toxicity depending on their biotransformation.

- ***Elimination***
  - The toxicity of xenobiotic depends on its elimination rate from an organism.

I. ABSORPTION

Absorption is the transfer of a xenobiotic from the site of exposure into the systemic circulation. During this process, xenobiotics cross body membranes and enter the bloodstream.

It is ***the first rate limiting step*** in the toxicokinetics of a xenobiotic.

No absorption, no toxicity

MAIN ABSORPTION ROUTES/BARRIERS ARE;

1. **ORAL-GASTRO INTESTINAL TRACT (GI)**
2. **INHALATION-LUNG**
2. **DERMAL-SKIN**

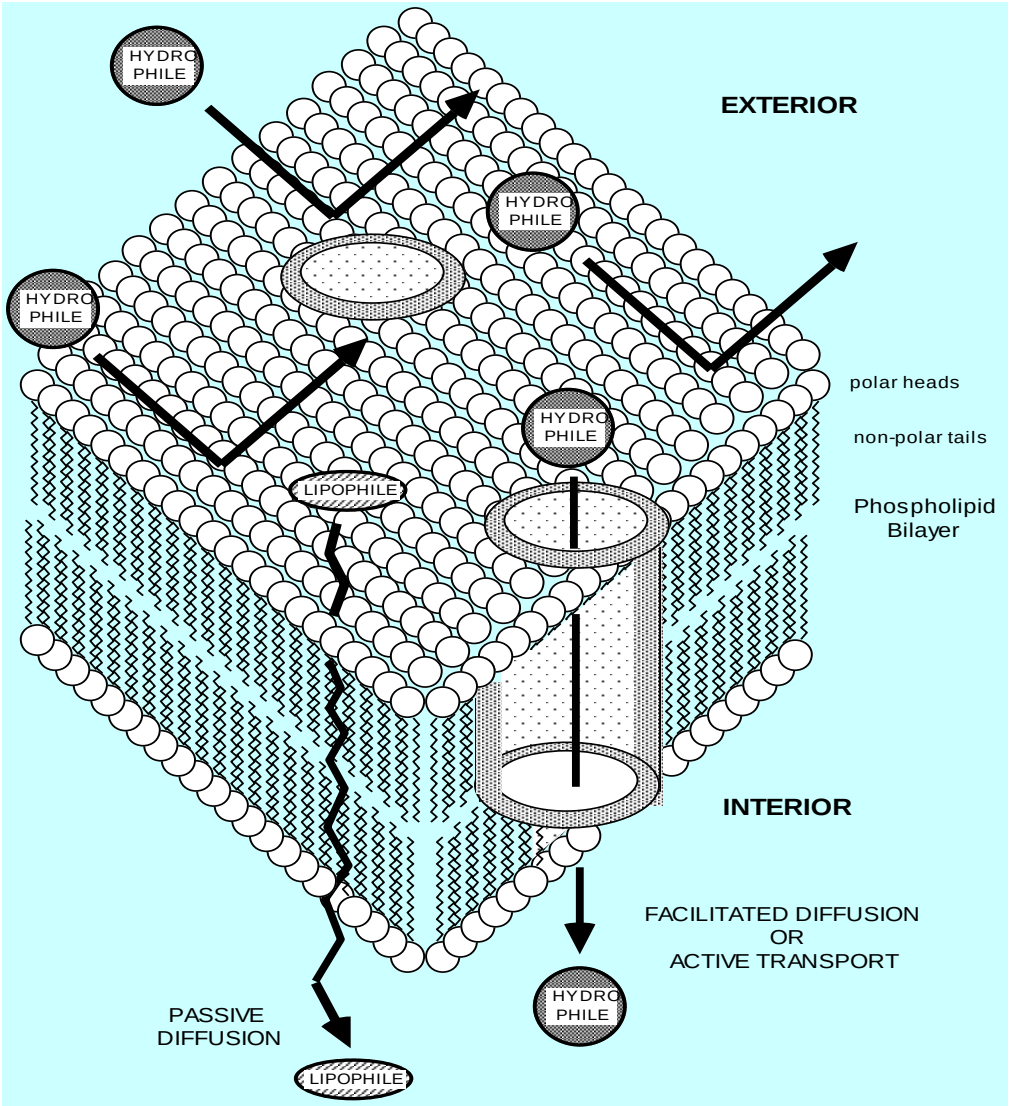
Xenobiotics must cross one of these barriers to exert their toxicities in the body. However during this process they usually pass through various cell membranes.

TRANSMEMBRANE TRANSPORT OF XENOBIOTICS

Xenobiotics must pass a number of cell membrans to enter systemic circulation, to move within and leave the body.

Structure of a cell membrane

- ✓ The basic unit of the cell membrane is a ***phospholipid bilayer***
- ✓ Different proteins are inserted or embedded in membrane.
- ✓ Some of these proteins serve as **important biological receptors** or **aqueous pores** and **ion channels**.


HYDRO PHILE
HYDRO PHILE
HYDRO PHILE
HYDRO PHILE
EXTERIOR
polar heads
non-polar tails
Phospholipid Bilayer
LIPOPHILE
INTERIOR
FACILITATED DIFFUSION OR ACTIVE TRANSPORT
HYDRO PHILE
PASSIVE DIFFUSION
LIPOPHILE

BASIC MECHANISMS OF XENOBIOTIC TRANSMEMBRANE TRANSPORT

A xenobiotic may pass through a membrane by;

1. Passive transfer (simple diffusion)
2. Facilitated diffusion
3. Active transport
4. Endocytosis (phagocytosis and pinocytosis)

Passive transfer (simple diffusion)

Most xenobiotics cross membranes by passive transfer.

*It does not need cellular energy or assistance.

*The driving force force for the transport across the membrane is the concentration gradient (from higher to lower concentration) between the two compartments.

*Lipid soluble chemicals diffuse across the lipid domain of membranes.

*Small hydrophilic molecules (up to m.w. 600) cross membranes through aqueous pores.

Protein carriers can be divided into two categories:

(1) active, energy-dependent transporters of the large superfamily known as ATP-binding cassette (ABC) transporters

(2) solute carriers (SLCs) that predominantly function through facilitative diffusion

Facilitated diffusion

* Similar to active transport, but

*Xenobiotic does not diffuse against a concentration gradient,

* No energy is needed and

* Protein carrier is used.

Solute carriers (SLCs):

- Solute carriers transfer endogenous compounds, including glucose, neurotransmitters, nucleotides, essential metals, and peptides.

- Facilitated diffusion rules are valid.

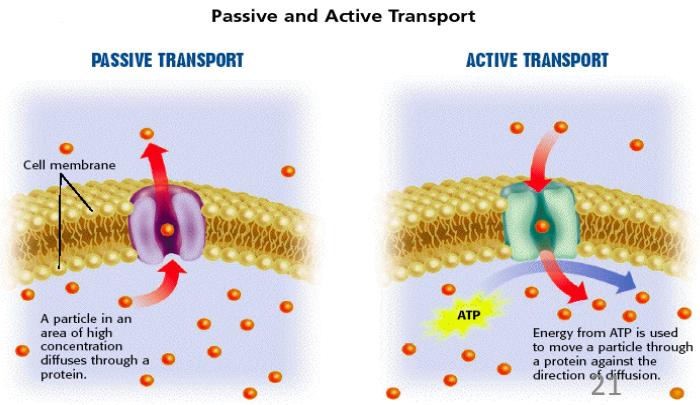
- **Organic-anion transporter (OAT*)*** and ***organic-cation transporter (OCT)*** are important in the renal and hepatic uptake of xenobiotics.

Active transport

* These type of chemicals are transported against a concentration gradient.

* Specific membrane *carrier proteins* are used.

* This process is energy dependent(ATP)



Active energy-dependent transporters of the large superfamily known as ATP-binding casette (ABC) transporters.

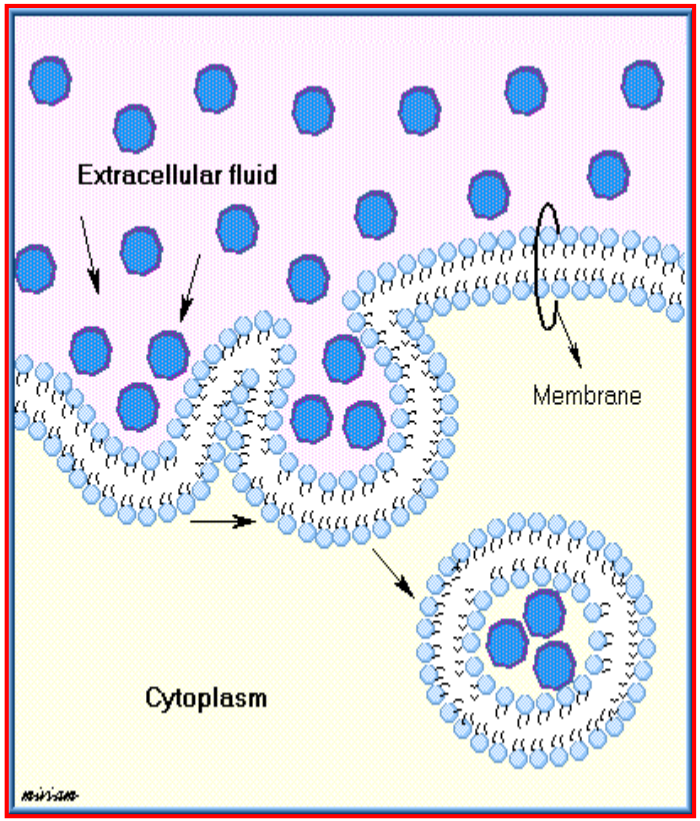
Phosphoglycoprotein (P- glycoprotein, P-gp):

* It is a specific xenobiotic transporter.
* This transporter exude chemicals out of the cells (efflux transporters). In chemotherapy, they contributes to their resistance to anticancer drugs.

Multiresistant drug protein (MRP):

* These transporters are also important in the efflux of xenobiotic metabolites, particularly conjugates of glucuronic acid and glutathione.

Endocytosis (phagocytosis and pinocytosis)



- Endocytosis is a process for moving the large molecules and particles into the cells.
- ***Phagocytosis*** *(cell eating)*: Substance is engulfed by the cell membrane and carried into the cell by pinching of the newly formed vesicle.
- ***Pinocytosis*** *(cell drinking)*: This is similar to phagocytosis, but substance is liquid or very small particles.

Factors affecting absorption

1. Factors related with chemical.

a. Physiochemical properties

b. Concentration at absorption site

c. Physical form

2. Factors related with site of exposure

a. Blood flow

b. Surface area and permeability

1. Factors related with chemical

a. Physiochemical properties

i. Mol. weight: Small molecules are readily absorbed.

ii. Lipid solubility: Lipid solubility increases the absorption.

Lipid solubility is determined by lipid/water partition coefficient (log P). Octanol and water mixture is used to determine this value.

Positive log P value = high lipid solubility
Negative log P value = water solubility

.

iii. Ionization degree of a chemical

The ionized form usually has low lipid solubility and thus does not cross *readily through the lipid domain of a membrane*.

The degree of ionization of a chemical depends on its pK_a and the pH of the solution.

The pH at which a weak organic acid or base is 50% ionized is called its pK_a or pK_b.

pK_a is defined as the negative logarithm of the ionization constant of a weak organic acid or base.

For strong acids, it is near to zero, for strong bases it is near to 14.

Weak acids are polar in alkaline urine and usually excreted faster whereas weak bases are polar in acidic urine and thus excreted faster.

This is called as "***ion trap***" and this is utilized in the elimination of xenobiotics in acute intoxication cases.

The relationship between pKa and pH is described by the Henderson-Hasselbalch equations

Henderson-Hasselbalch

Weak Acid / Weak Base

Acid

$$pKa - pH = \text{Log} \frac{[\text{nonionized drug}]}{[\text{ionized drug}]}$$

Base

$$pKa - pH = \text{Log} \frac{[\text{ionized drug}]}{[\text{nonionized drug}]}$$

For acids: p*Ka* − *pH = log [nonionized]/*[ionized]

For bases: p*Ka* − *pH = log [ionized]/*[nonionized]

pH of the medium:

Effects ionization of xenobiotics.

Weak acids → best absorbed in stomach.

Weak bases → best absorbed in intestine.

b. Concentration at exposure site

Generally high concentration results with higher absorption.

c. Physical form

Solid chemicals are absorbed slowly, as they have to be dissolved first.

2. Factors related with site of exposure

a. Blood flow rate at site of exposure

High perfusion results high rate absorption rate

b. Surface area and permeability

There is a linear relationship between exposed surface area and absorption.

Main exposure routes

1. Oral (GI tract)

* One of the most important absorption site.

* Important for food/water contaminants and drugs.

* Absorption of xenobiotics can take place along entire GI tract.

* To enter the body via the GIT, chemicals must pass through the GIT lining and capillary membranes before entering the blood.

Mouth and oesophagus: Little absorption occurs in here.

Stomach (pH: 1-3):Weak organic acids are absorbed here due to asidic media. (Surface area:<5m^2)

Small intestine(pH:7-8): The greatest absorption of xenobiotics takes place in here (Surface area: 200m^2) particularly alkaline ones and food. The rate of absorption increases with the residency time. The residency time of a xenobiotic in here depends on intestinal motility.

Colon and rectum: Absorption is negligible in here.
(Surface area : <m^2).

Other Routes of Exposure

- Intraperitoneal
- Intramuscular, subcutaneous, intradermal
- Intravenous

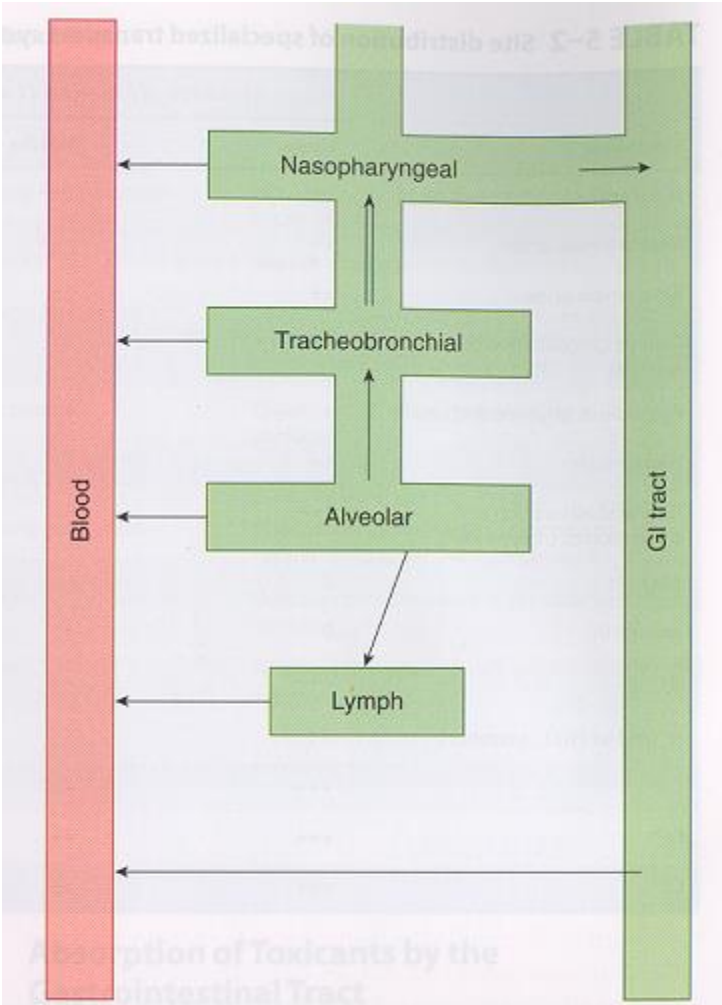
2. Inhalation (lung:respiratory tract)

√ Xenobiotics such as gases, vapors of volatile liquids and aerosols are absorbed by this route.

√ The absorption of gas depends on its solubility in blood.

√ The physical form and particle size of the xenobiotic determines penetration.

√ *Particular very small particles (<1 µm in diameter)* are able to reach alveoles and can enter bloodstream.



Schematic diagram of the absorption and translocation of chemicals by lungs.

3. Dermal (Skin)

√ Human skin comes into contact with many toxic agents such as pesticides and other *environmental and occupational chemicals*.

√ A multilayered barrier not very permeable.

√ The rate-determining barrier is the stratum corneum, the upper layer of epidermis.

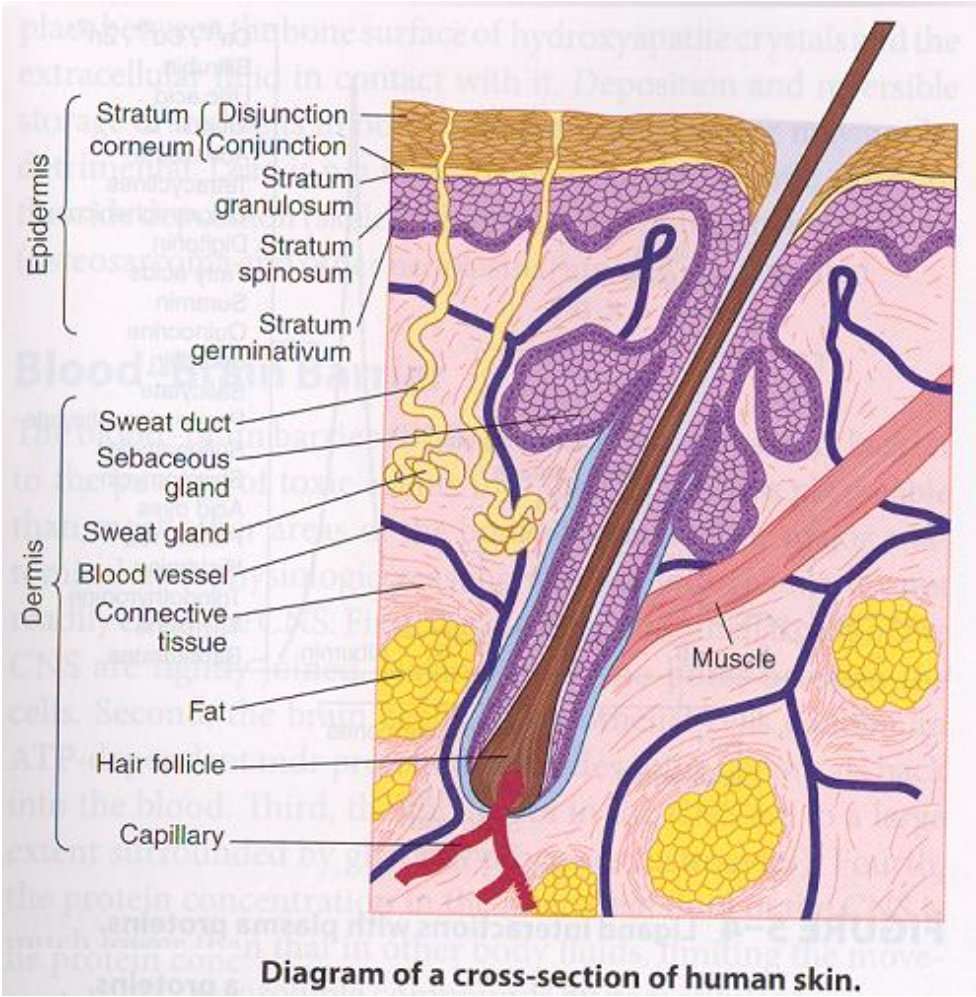


Diagram of a cross-section of human skin.

- Factors important here are:

  lipid solubility

  hydration of skin

  site (e.g. sole of feet vs. scrotum)

II. DISTRIBUTION

After entering the blood, a xenobiotic may distribute throughout the body to organs or tissues.

Only the free fraction of the xenobiotic is active.The xenobiotics are often concentrated in a specific tissue which may or may not be their site of toxic action.

As a xenobiotic is biotransformed or excreted from the body, more is released from the storage site.

Distribution

- Rapid process relative to absorption and elimination

- Extent depends on
  - blood flow
  - size, M.W. of molecule
  - lipid solubility and ionization
  - plasma protein binding
  - tissue binding

Storage of xenobiotics in body constituents:

Binding to the plasma proteins

- Xenobiotics bind to several plasma proteins such as albumin and globulins.
- In general, binding is reversible.
- Protein-binding in the plasma greatly affects distribution, prolongs the half-life within the body, and affects the dose threshold for toxicity.
- Important drug interaction leading toxicity is seen at plasma protein binding level.

Liver and Kidney as storage depot

- These organs have a high capacity for binding multitute of xenobiotics.
- Proteins such as metallothionein have high affinity for metals namely Cd. Such bindings serve as a storage depot and cause the accumulation of the toxicants.

Fat as a storage depot

- Many lipid-soluble xenobiotics are stored in body fat.
- DDT and polychlorinated biphenyls (PCBs) are stored in adipose tissue.
- Due to the relatively low blood flow, fat is a rather stable depot.

Bone as storage depot

- Deposition and reversible storage of xenobiotics in bone is dynamic and may not be detrimental (toxic).

- Lead (Pb) is stored in bone but not toxic to bone.

- However, chronic effects of fluoride deposition (skeletal fluorosis) and radioactive strontium (osteosarcoma) are well documented.

Redistribution

- Redistribution is the movement of a chemicals from its initial distribution site to high affinity sites with time.

- For example, the initial distribution of lead (Pb) is to liver and in a month it is redistributed to bone, its storage site.

Volume of distribution (Vd)

- The apparent V_d (liters) is the total volume of body fluids in which a xenobiotic is distributed.

- The V_d is determined in order to know how extensively a xenobiotic is distributed in the body fluids.

- The V_d can be calculated by the formula:

V_d = dose (mg) / plasma con. (mg/L)

Volume of distribution (Vd)

- The larger the Vd, the greater is the extent of the distribution.

- At larger V_d, it is concluded that this xenobiotic is distributed to human fat, muscle, etc.

- A highly plasma protein binding will have a small V_d while a high tissue protein binding (digoxin) will have a large Vd.

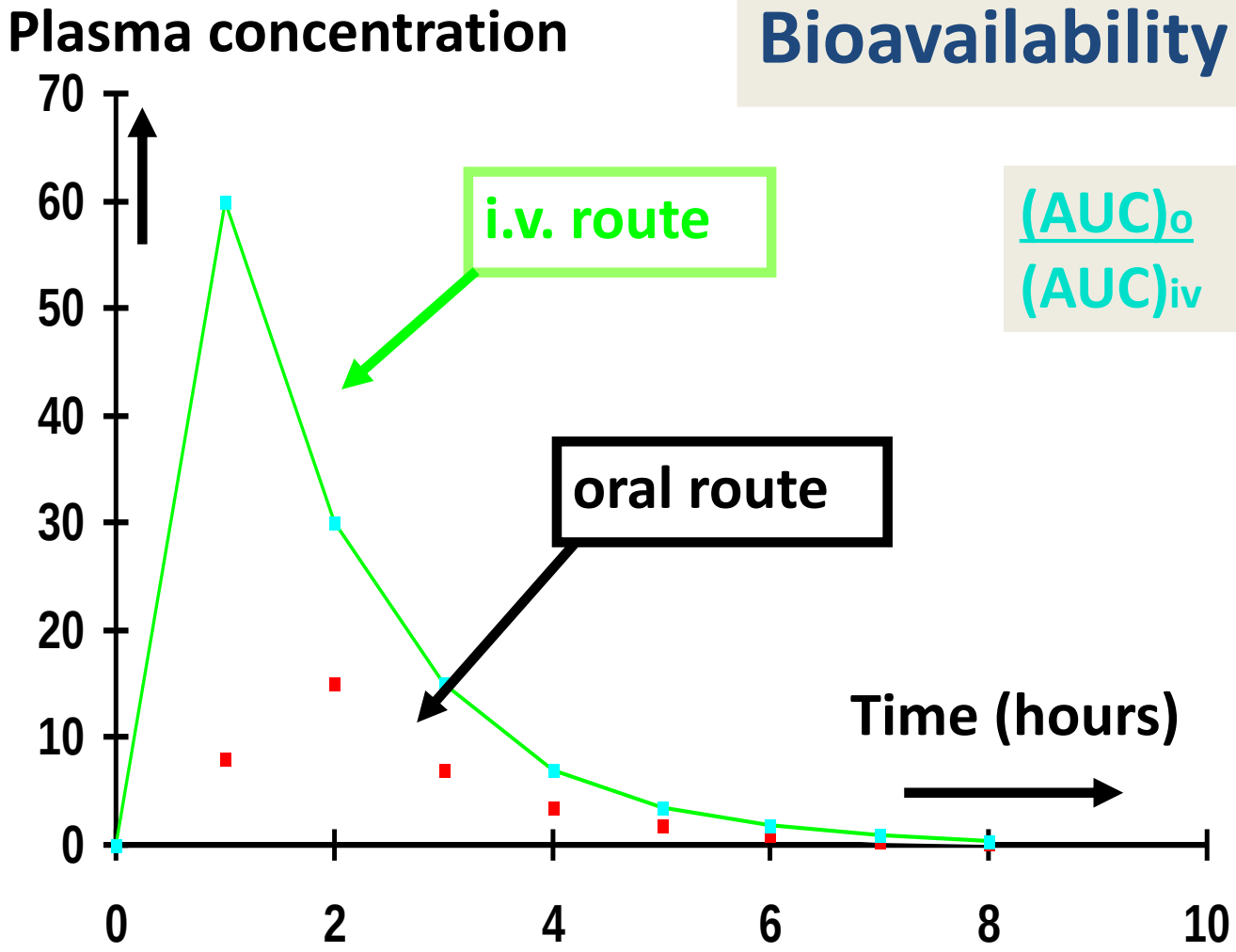
Bioavaibility is the fraction of the administered dose reaching the systemic circulation

for i.v.: 100%
for non i.v.: ranges from 0 to 100%

✓ Bioavailability is 1 (100% absorption) for intravascular drug administration and usually less than 1 for oral drug administration.

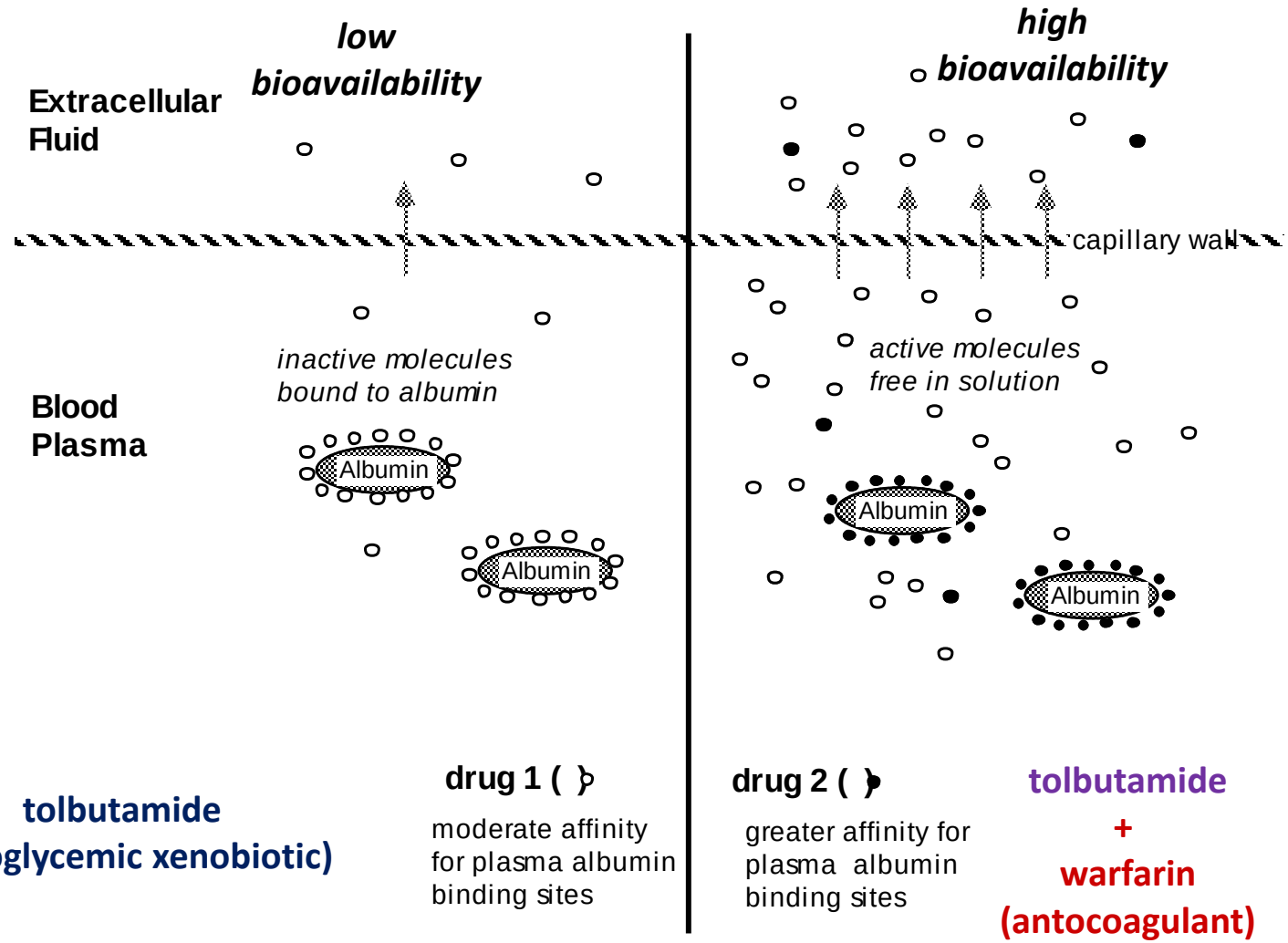
✓ Bioavailability is a key factor in the onset of xenobiotics action.

Bioavailability (F)



$$\frac{(AUC)_o}{(AUC)_{iv}}$$

Competition-displacement between xenobiotics



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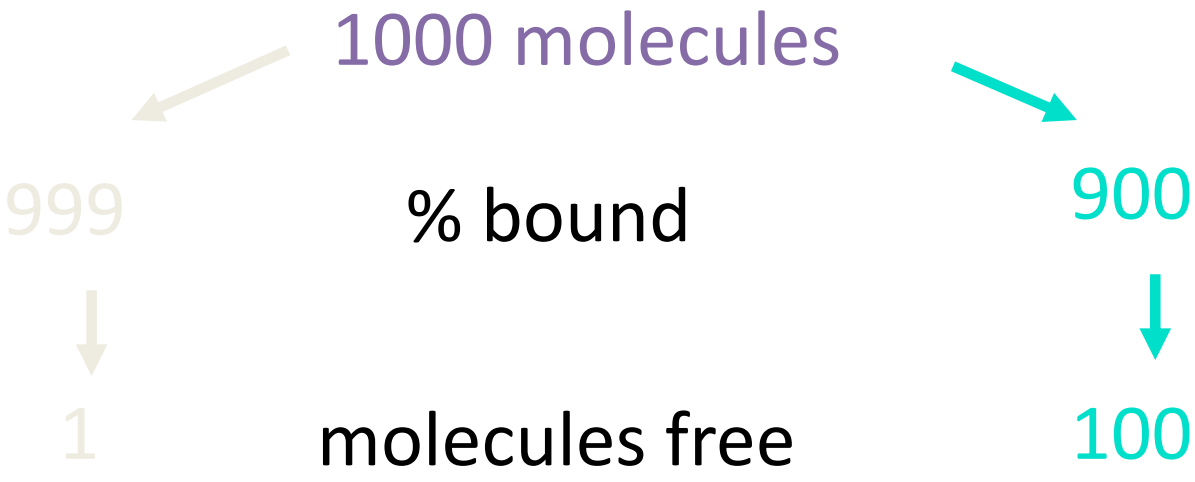
Alter plasma binding of drugs



100-fold increase in free pharmacologically active concentration at site of action.

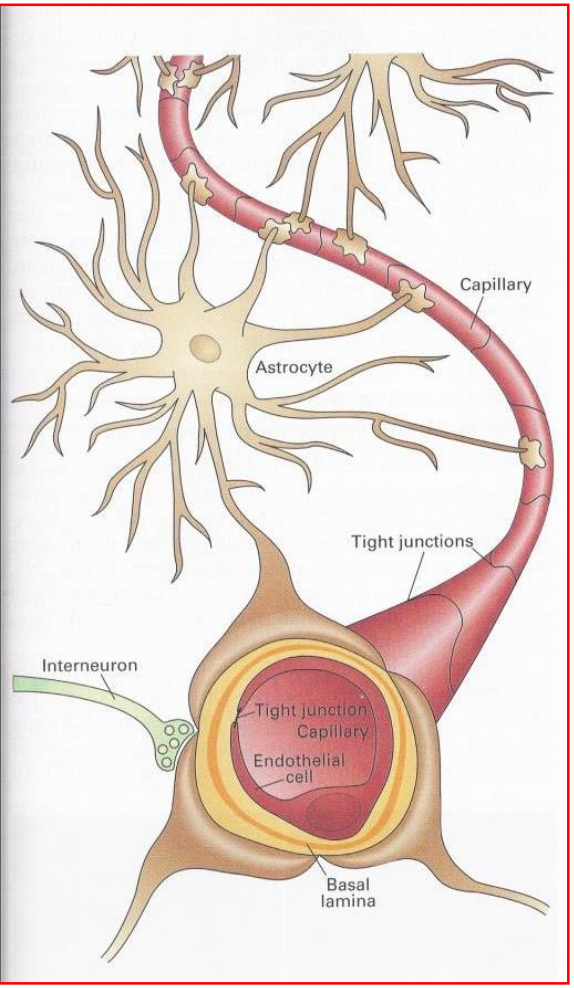
Effective → TOXIC

Physiological barriers

1. **The blood-brain barrier**

2. **The placental barrier**

1. The blood-brain barrier (BBB)



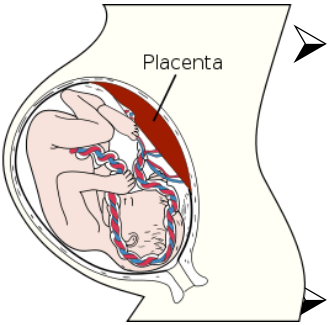
BBB is less permeable than most other areas of the body.

Composed of capilleries formed by *endothelial cells with tight junctions with astrocytes* surroundind these capilleries.

Only lipid soluble xenobiotics cross this barrier.

Not fully developed at birth, and thus some xenobiotics could be more toxic to newborns than to adults.

2. The placental barrier



- It consists of several cell layers (almost 6) between the maternal and fetal circulatory vessels in the placenta.
- Placenta is thought to be an absolute barrier to drugs and other chemicals until thalidomide disaster in 1960.
- Xenobiotics can cross this barrier. (e.g. Ethanol,Pb-lead) but should be having molecular weight less than 1000.
- Major factors in xenobiotic transfer to the fetus:
  - Lipid solubility
  - Plasma protein binding

Biotransformation

“Biotransformation of xenobiotic is defined as

the **conversion from one chemical form to another”.**

« the term is used synonymously with ***metabolism***»

Biotransformation

- ✓ Generates more polar (water soluble), inactive metabolites
- ✓ Readily excreted from body
- ✓ Metabolites may still have potent biological activity (or may have toxic properties)
- ✓ Generally applicable to metabolism of all xenobiotics as well as endogenous compounds such as steroids, vitamins and fatty acids

Phase I and Phase II

- Phase I
  - functionalization reactions
- Phase II
  - conjugation reactions

III. EXCRETION

Xenobiotics are excreted/eliminated from the body in the form of the parent compounds, their metabolites and/or their conjugates.

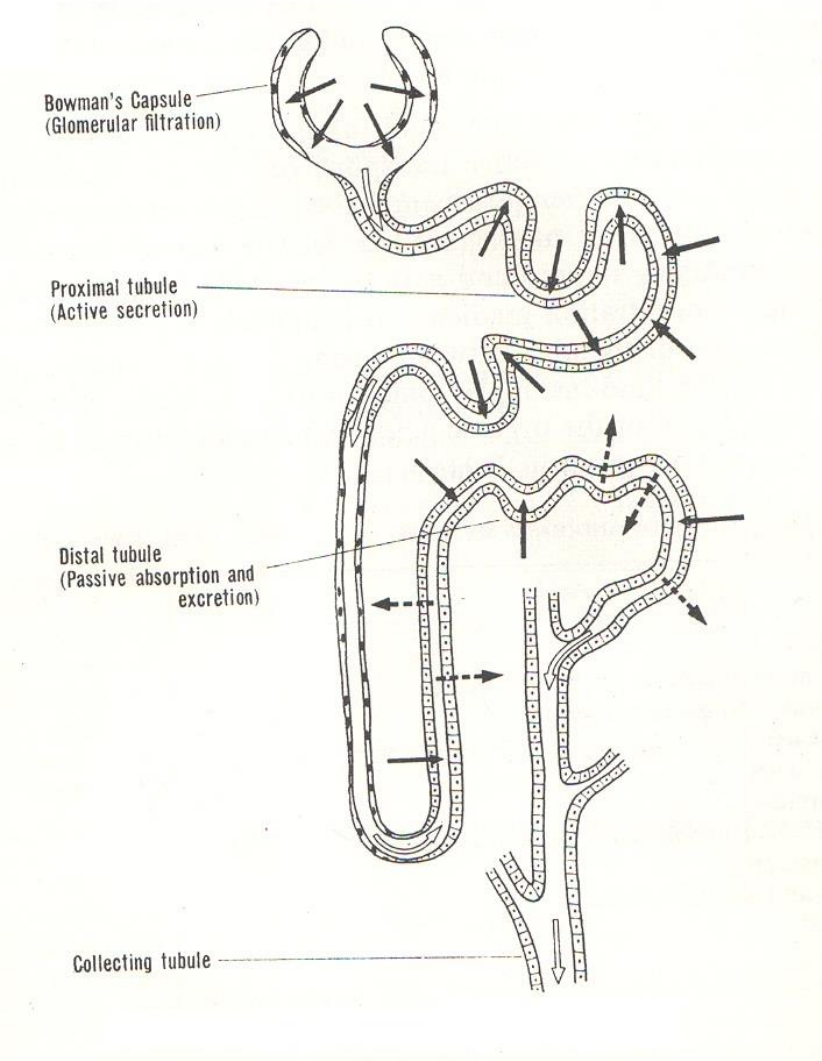
The primary excretion/elimination routes:

- Urinary-Renal
- Fecal
- Respiratory- Exhalation
- Other

1. Urinary-renal excretion

Renal excretion consists of 3 distinct processes namely

- Glomerular filtration
- Active tubular secretion (takes place at proximal tubule of the nephron)
- Tubular excretion (takes place at distal tubule of the nephron)

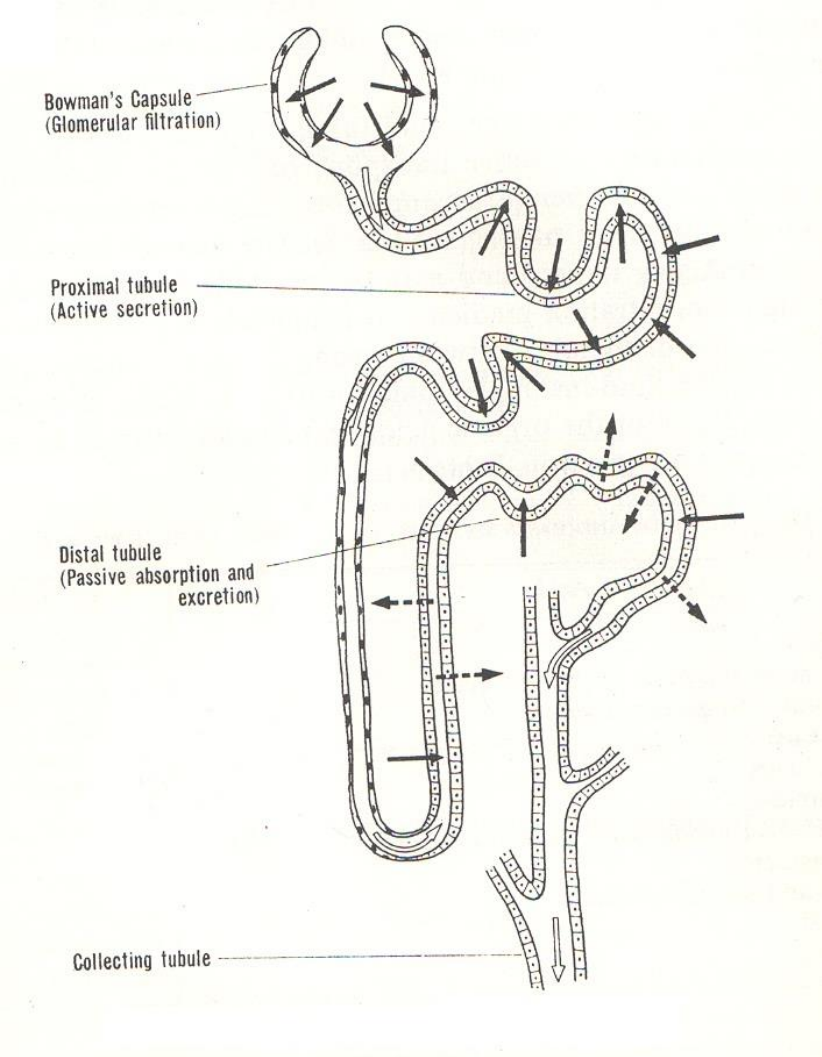


Glomerular filtration

*Molecular weights larger than 60 kDa and plasma protein bound xenobiotics can not be filtered at the glomeruli.

*About 99% of the filtrate is reabsorbed into blood, the remaining 1% is excreted as urine.

*Presence of albumin or blood cells in urine is the indication of glomerular damage.

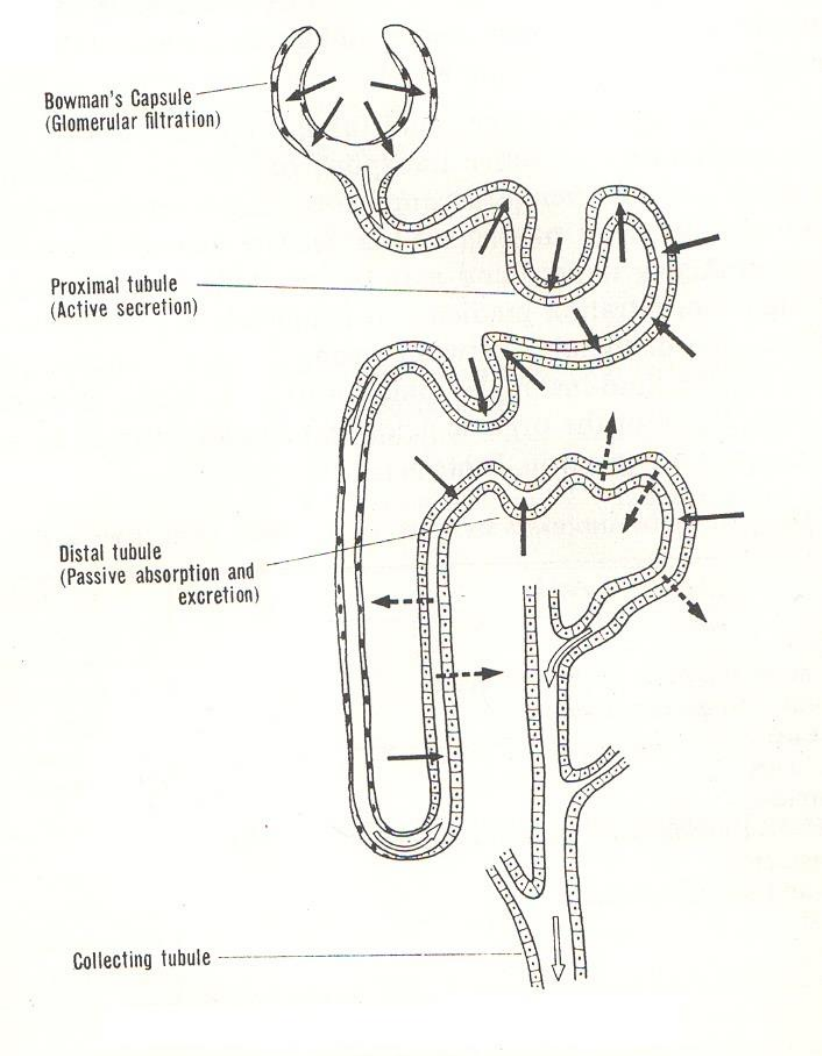


Active tubular secretion

*Xenobiotics can be excreted into urine by active secretion at proximal tubule of the nephron.

* Protein bound xenobiotics, polar metabolites and conjugates are readily secreted by this mechanism

* Secretion occurs by active transport mechanisms



Tubular excretion-reabsorption

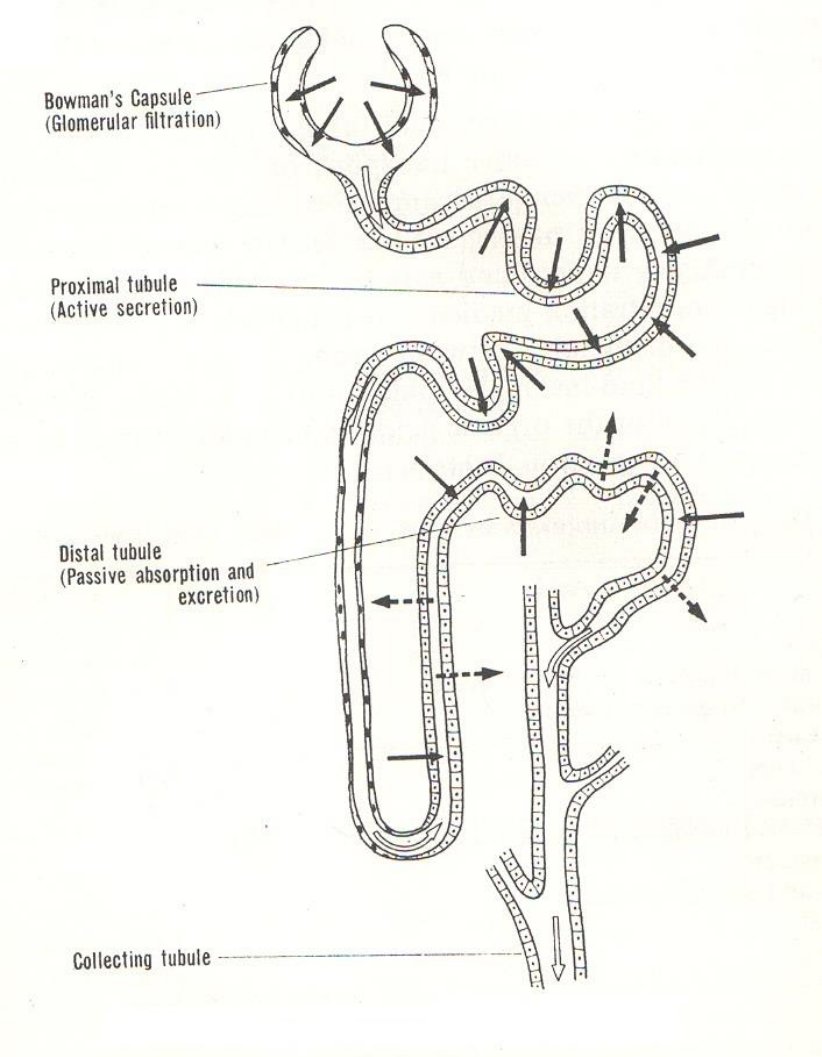
Xenobiotics can be reabsorbed from urine into blood capillaries via passive diffusion at distal tubule of the nephron.

Urine pH greatly affects reabsorption or urinary excretion.Thus, by altering the pH of the urine xenobiotic reabsorption back to bloodstream or remain in the tubular lumen and excreted with urine can be accomplish.

If the urine is alkaline, weak acids are less lipophilic/more ionized and thus excreted largely.

If the urine is acidic, weak acids are more lipophilic/less ionized and reabsorbed largely. As a result renal excretion is reduced.

- This is utilized in acute poisonings to enhance elimination of toxicants.



2. Fecal *excretion*

Excretion in feces occurs by two ways

a.Biliary excretion
b.Direct intestinal excretion

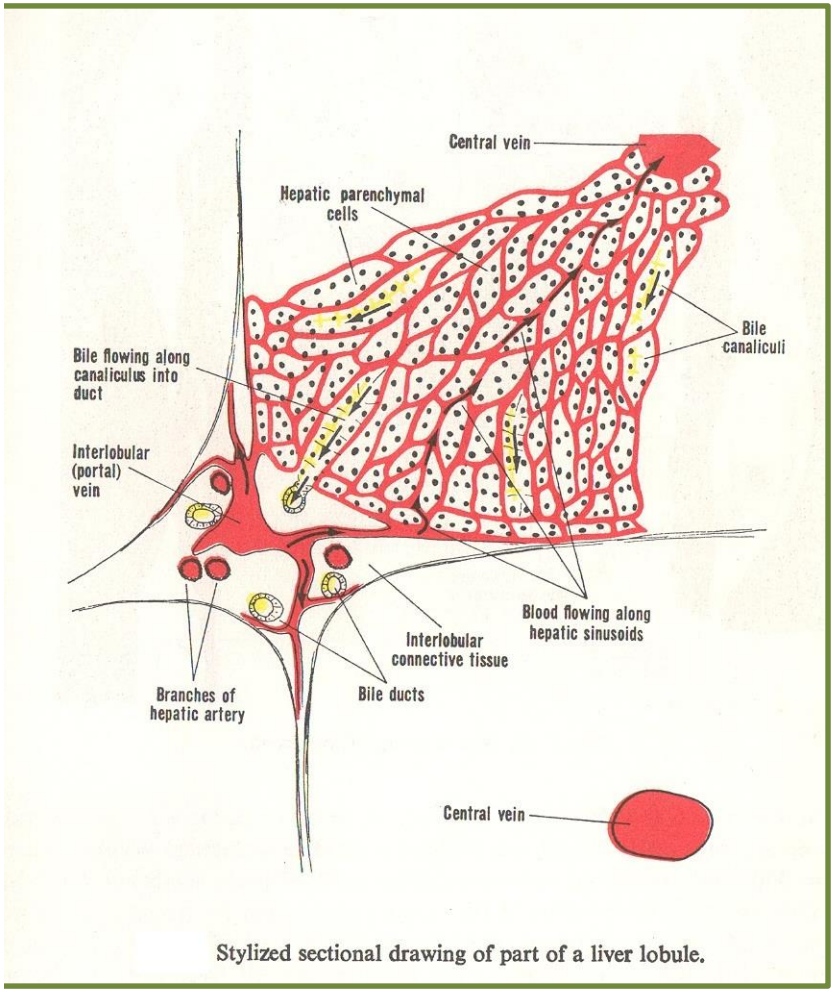
a.Biliary excretion

In liver xenobiotics as metabolites or conjugates are transferred into the bile and thereafter the intestine.

Xenobiotics can either be excreted with feces or undergo an enterohepatic circulation

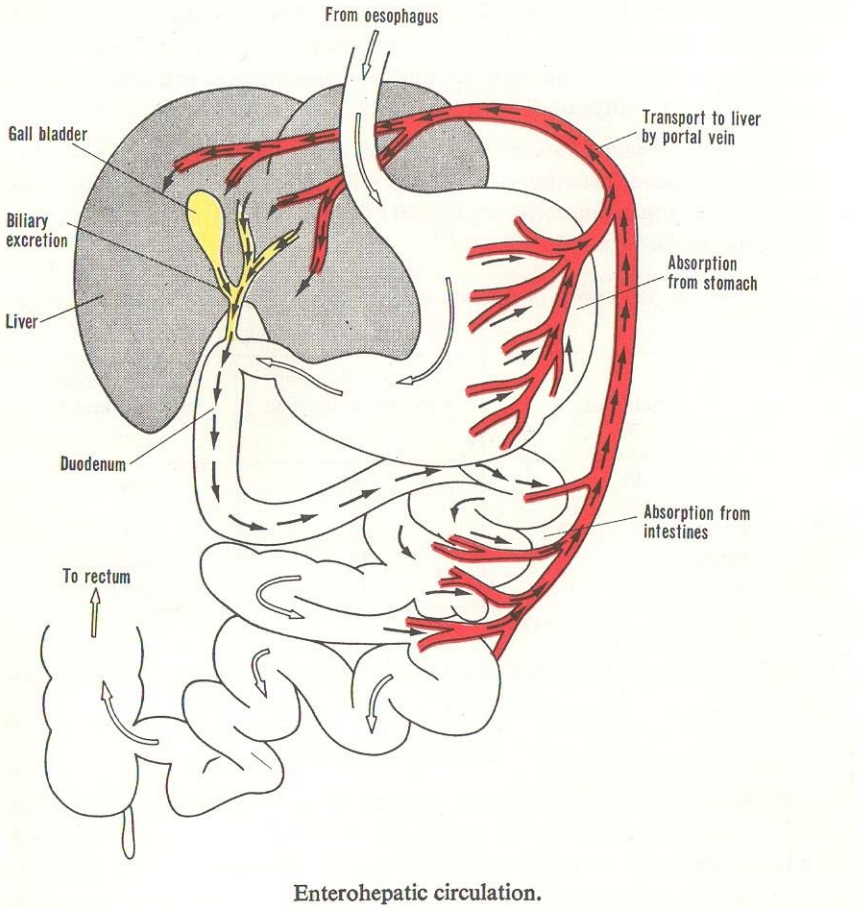
Regulated by active transport. e.g.

- Organic bases and acids, neutral substances, and metals (As, Pb, Hg) are excreted by biliary route.



Stylized sectional drawing of part of a liver lobule.

Enterohepatic circulation



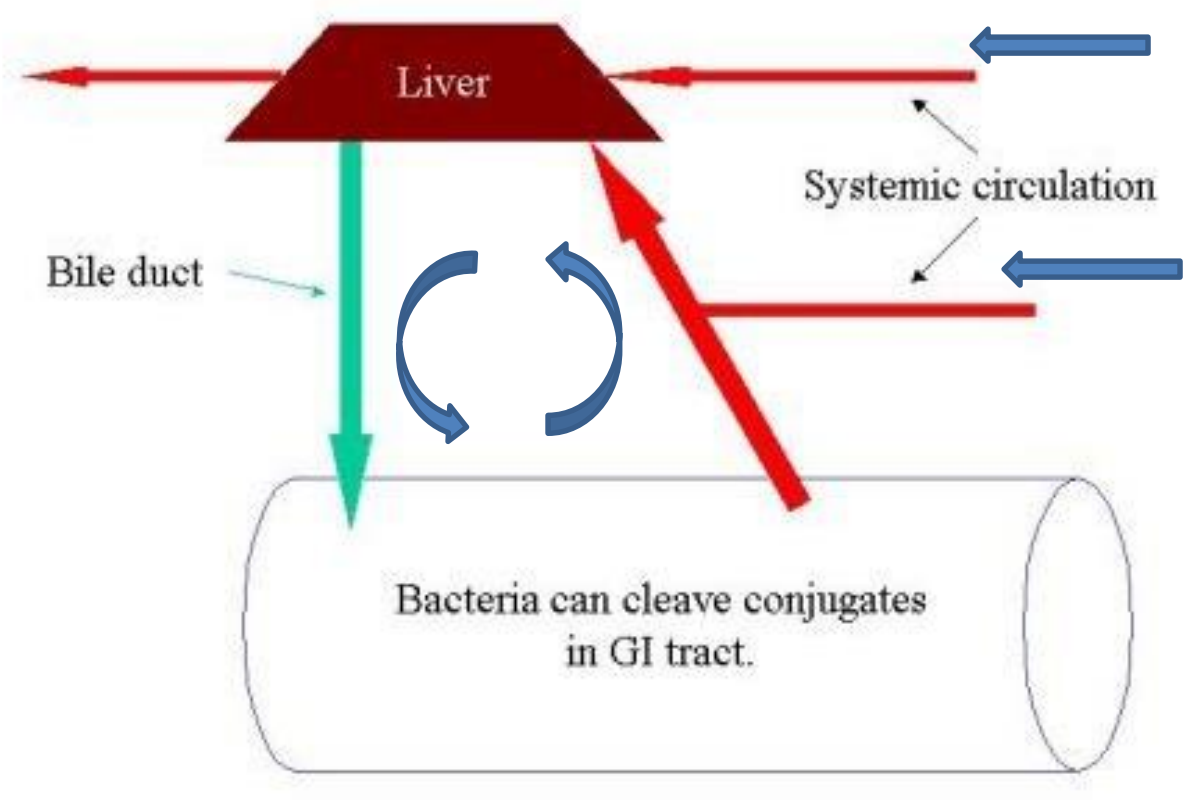
Enterohepatic circulation.

Hydrolytic enzymes in the intestinal flora can hydrolize polar glucuronide and sulfate conjugates making them lipophilic for reabsorption

Then, reabsorption is occured and xenobiotic returns to the liver again by the portal vein.

This process is repeated and called ***enterohepatic circulation*** and half-life of a chemical is prolonged and toxicity can be enhanced.

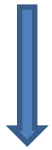
Enterohepatic circulation



= drug molecule paths

Enterohepatic circulation

- Drug taken up by liver cells
- Drug or phase II conjugate excreted in bile
- Drug reabsorbed from intestine



- Volume of distribution is increased
- It takes longer to eliminate the drug than you might expect

b. Direct intestinal excretion.

In some instances some xenobiotics can be eliminated via the feces by *direct intestinal excretion.*

Relatively slow process.

A major elimination route for compounds that have low rates of biotransformation and/or low renal or biliary clearance.

b. Direct intestinal excretion.

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Relatively slow process.

A major elimination route for compounds that have low rates of biotransformation and/or low renal or biliary clearance.

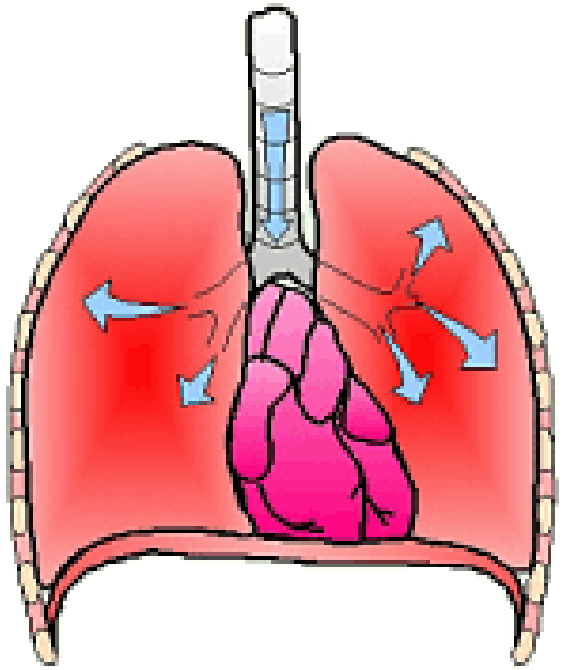
3. Respiratory- Exhalation- *excretion*

The lungs are also an important excretion route for xenobiotics (and/or their metabolites) in the gaseous phase of blood.

Elimination of gases is roughly inversely proportional to the rate of their absorption.

Xenobiotics are eliminated by passive diffusion.

e.g. Ethanol, anesthetic gases



4.Other excretion routes

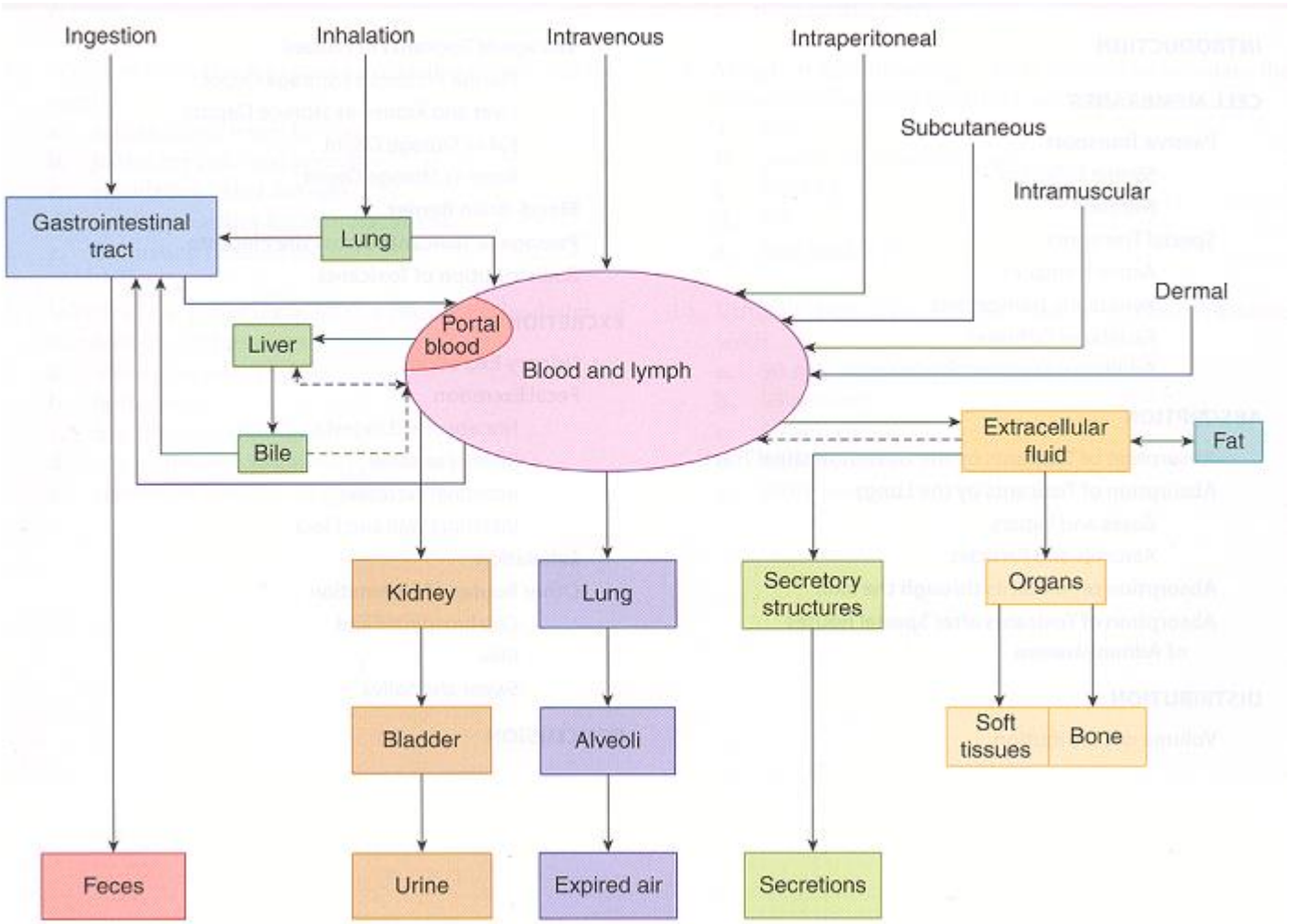
Mother's milk, sweat and saliva are minor excretion routes.

Excretion of xenobiotics into milk is extremely important to the nursing offspring.

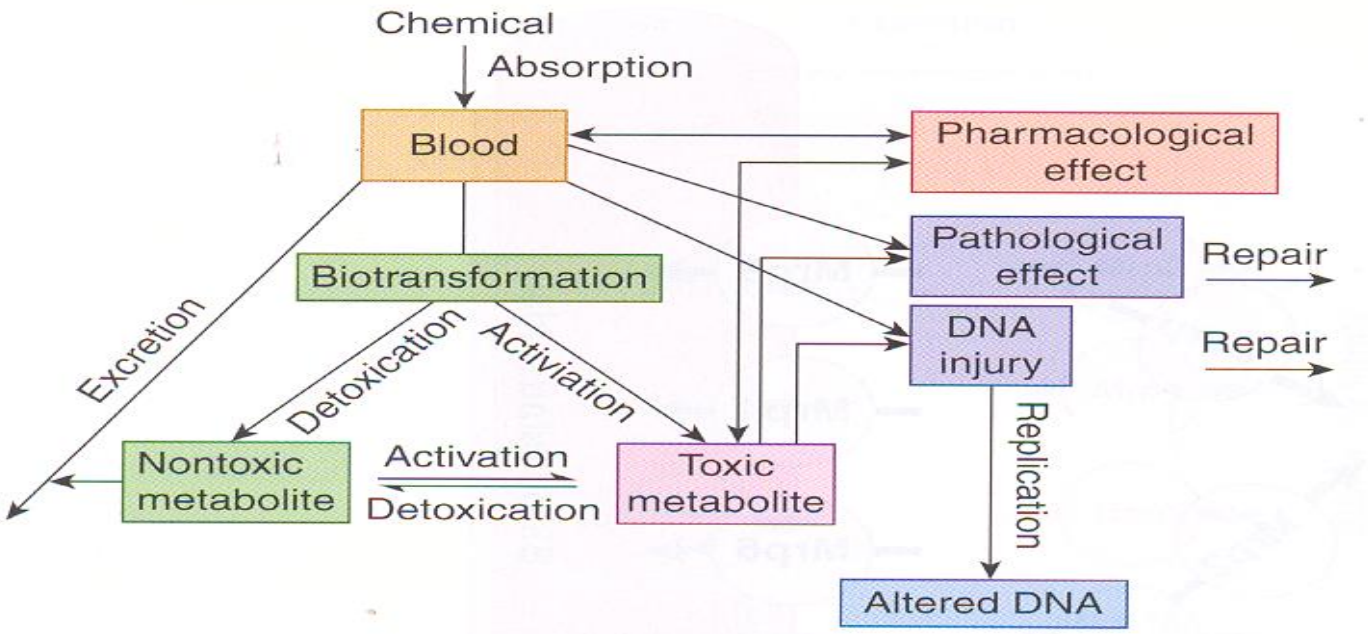
- It is more acidic than plasma (~pH=6.5).
- Lipid soluble xenobiotics diffuse along with fats from plasma into the mammary gland and are excreted with milk during lactation.
- Toxic substances may be passed from cow's milk to people.

Under great perspiration, sweat may be significant excretion route. Toxic subtances excreted into sweat may produce dermatitis.

VARIOUS FACTORS OVERALL AFFECTING DISPOSITION ARE SHOWN BELOW



Routes of absorption, distribution, and excretion of toxicants in the body.



Schematic representation of the disposition and toxic effects of chemicals.

*Depending on their physical and chemical properties xenobiotics may be absorbed by GI tract, lung and/or skin.

*Body has the ability to biotransform and excrete these compounds

*When rate of absorption exceeds the rate of elimination, the xenobiotic may accumulate, reach critical concentration at a target site and toxicity may ensue.

*Hence, whether xenobiotic elicit toxicity depends not only on its inherent potency and site specificity but also on how an organism can dispose of that toxicant.

*Therefore, the toxic response exerted by xenobiotics is critically influenced by the rates of **Absorption, Distribution, Biotransformation** and **Excretion**.