Course: *Toxicology, Path-438*

Pharmacology Basics:

Cytochrome P-450, Bioconjugation Reactions, and Renal Elimination

Learning Objectives:

- Define the term ”Biotransformation”
- Describe the phrase ”First Pass Metabolism”
- Describe “Phase-I Reactions” & “Phase-II reactions” of drug metabolism in the liver.
- Describe Cytochrome P450 enzyme system and mention the four isozymes responsible for the vast majority of P450-catalyzed reactions.
- Explain why some individuals obtain no benefit from certain drugs while the same drugs are effective in other individuals.
- Describe inducer and inhibitor drugs interaction. Give examples.
- Explain why neonates are vulnerable to certain drugs e.g. chloramphenicol as compared to adults.
- List the factors that affect drug metabolism.
- List the types of drug excretion.
- List the three processes of renal elimination of drugs. Describe each process.
- Explain how manipulation of urine pH can affect drug reabsorption in kidney distal tubules and increase renal excretion of the drug.
- List the factors that affect renal excretion of drugs.

Overview:

- Once the drug/toxin is administered to the body, four pharmacokinetic properties determine ***the speed of onset of drug action***, ***the intensity of the drug's effect***, and ***the duration of drug action***:

  - **Absorption**: First, the drug is absorbed through the site of administration and entered into plasma.

  - **Distribution**: Second, the drug may then reversibly leave the bloodstream and distribute into the interstitial and intracellular fluids.

  - **Metabolism**: Third, the drug may be biotransformed by metabolism by the liver, or other tissues.

  - **Elimination**: Finally, the drug and its metabolites are eliminated from the body in urine, bile, or feces.

- The pharmacokinetic parameters allow the clinician to design and optimize treatment regimens, including decisions as to the route of administration for a specific drug, the amount and frequency of each dose, and the duration of treatment.

Where are we today?

Last week:

- **Absorption**- drug gets into bloodstream.
- **Distribution** - drug gets to site of action.

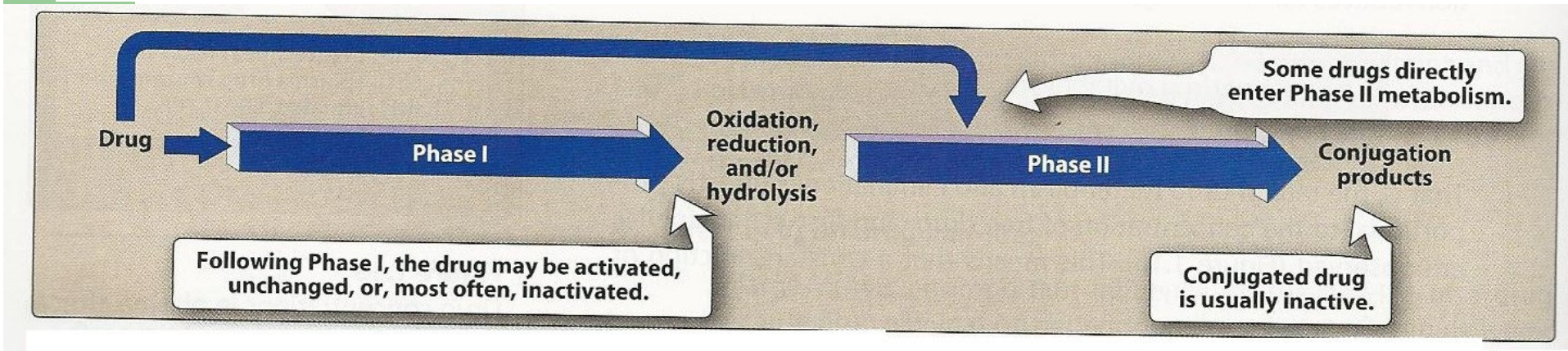
Today:

- **Metabolism** – drug is "changed" so that it can be excreted.
- **Elimination** - drug leaves the body.

Drug Clearance Through Metabolism:

- The ***kidney cannot efficiently eliminate lipophilic drugs*** that readily cross cell membranes and are reabsorbed in the distal convoluted tubules.

- ***Lipid soluble agents must first be metabolized into more polar (hydrophilic) substances in the liver*** (**Biotransformation**) using two general sets of reactions, called **Phase I** ***(functionalization)*** and **Phase II** ***(conjugation).***



Drug Clearance Through 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).
- Biotransformation is generally applicable to metabolism of all ***xenobiotics*** ***(chemicals not normally produced or expected to be present in the body)*** as well as ***endogenous compounds*** such as steroids, vitamins and fatty acids.
- Biotransformation is ***enzymatic in nature***; enzyme systems involved are ***localized in liver***.
- Every tissue has some metabolic activity; other organs with significant metabolic capacity are ***GIT, kidneys and lung***.

Drug Clearance Through Metabolism - *Biotransformation:*

- **First Pass Metabolism**: Following nonparenteral administration of a drug, a significant portion of the dose may be metabolically inactivated in either the ***intestinal endothelium*** or ***the liver*** before it reaches the systemic circulation.

- **First-Pass Metabolism** limits oral availability of highly metabolized drugs.

- With respect to drug metabolizing reactions, two sub cellular organelles are quantitatively the most important: the **endoplasmic reticulum** and the **cytosol*** *(*intracellular fluid (ICF) or cytoplasmic matrix is the liquid found inside cells).*

  - **Phase I oxidative enzymes are almost exclusively localized in the endoplasmic reticulum.**

  - **Phase II enzymes are located predominantly in the cytosol.**

Reactions of Drug Metabolism- *Phase –I Reactions:*

- **Phase-I reactions** convert lipophilic molecules into more polar molecules by introducing or unmasking a ***polar functional group*** such as –OH or –NH2.

- **Phase-I metabolism** includes ***oxidation, reduction, hydrolysis, and hydration.*** It may ***increase, decrease, or leave unaltered the drug's pharmacologic activity (mostly inactivate):***

  - Phase-I reactions utilizing **the P450 system**: The phase-I reaction most frequently involved in drug metabolism are catalyzed by cytochrome P450 system (also called ***microsomal mixed-function oxidases***):

$$\text{Drug} + O_2 + \text{NADPH} + H^+ \longrightarrow \text{Drug}_{\text{modified}} + H_2O + \text{NADP}^+$$

$NADP^+$=Nicotinamide adenine dinucleotide phosphate

  - The oxidation proceeds by the drug binding to the oxidized form of cytochrome P450, and then oxygen is introduced through a reductive step, coupled to NADPH:cytochrome P450 oxidoreductase.

  - **The P450 system is important for the metabolism of many endogenous compounds** (e.g. steroids, lipids, etc) and **for biotransformation of exogenous substances (xenobiotics).**

Phase –I Reactions – *Cytochrome P450 System*:

- **Cytochrome P450**, designated as **CYP**, is a superfamily of ***heme-containing isozyme*** that are located in most cells but ***primarily in the liver & GIT***.

- Involved in metabolism of diverse endogenous and exogenous compounds: drugs, environmental chemicals, and other xenobiotics.

- The family name is indicated by **CYP** added to an Arabic number, followed by a **capital letter** for the **subfamily,** e.g. CYP3A. **Another number** is added to indicate the **specific isozyme** as in CYP3A4.

- ~1000 currently known cytochrome P450s, **about 50 active in humans**.

- Because there are many genes that encode multiple enzymes, there are likewise many different P450 isoforms. These enzymes can modify a large number of drugs and one drug can a be a substrate for more than one isoenzyme.

- ***Four isoenzymes are responsible for the vast majority of P450-catalyzed reactions***: **CYP3A4/5, CYP2D6, CYP2C8/9 & CYP3A2. CYP3A4** is found in intestinal mucosa, accounting for the ***first pass metabolism*** of drugs such as ***clonazepam***.

Phase –I Reactions – *Cytochrome P450 System* – ***Genetic Variability:***

- **Genetic variability:** P450 enzymes exhibit considerable variability among individuals and racial groups.

- Variations in P450 activity may alter a drug's efficacy and the risk of adverse events.

- **CYP2D6**, in particular, has been shown to exhibit genetic polymorphism . e.g. some individuals obtain no benefit from the ***opiod analgesic codeine***, because they lack CYP2D6 enzyme that activates the drug. *Racial difference: 5-10% prevalencee in European Caucasian as compared to 2% in Southeast Asians.*

Phase –I Reactions – *Cytochrome P450 System – Drugs Interactions:*

❖ **Inducers:** Drug interactions can induce selected CYP isozymes. Xenobiotics may induce the activity of these enzymes by ***inducing the expression of the genes encoding the enzyme or by stabilizing the enzymes***:

- e.g. certain drugs (***Phenobarbital, rifampin, and carbamazepine***) are capable of increasing the synthesis of one or more CYP isozymes increasing biotransformation of drugs metabolized these isozymes and decreasing their effect:

  - ✓ ***Rifampin (anti T.B.) significantly decreases plasma concentration of HIV proteases inhibitors (antiviral), thereby diminishing their ability to suppress HIV.***

Phase –I Reactions – *Cytochrome P450 System – Drugs Interactions:*

- **Inhibitors:** Drug interactions inhibiting CYP isozyme (the opposite of inducers):
  - e.g. ***Omeprazol is a potent inhibitor of three CYP isozymes responsible for warfarin metabolism increasing the plasma concentration of warfarin causing increased inhibition of coagulation and hemorrhage.***

- **Phase-I Reactions not involving the P450 system** include ***amine oxidation, alcohol dehydrogenation, esterases, and hydrolyses.***

Phase –I Reactions – *Cytochrome P450 System:*

❖ These are the types of reactions performed by the Cytochrome P450 system:

• **Aromatic hydroxylation**	**Phenobarbital, amphetamine**
• **Aliphatic hydroxylation**	**Ibuprofen, cyclosponine**
• **Epoxidation**	**Benzo [a] pyrene**
• **N-Dealkylation**	**Diazepam**
• **O- Dealkylation**	**Codeine**
• **S- Dealkylation**	**6-Methylthiopurine**
• **Oxidative Deamination**	**Diazepam, amphetamine**
• **N-Oxidation**	**Chlorpheniramine**
• **S-Oxidation**	**Chlorpromazine, cimetidine**
• **Phosphothionate oxidation**	**Parathion**
• **Dehalogenation**	**Halothane**
• **Alcohol oxidation**	**Ethanol**

Reactions of Drug Metabolism- *Phase –II Reactions:*

- This phase consists of **conjugation reactions.** If the metabolite from Phase-I metabolism is sufficiently polar, it can be excreted by the kidney otherwise many Phase-I metabolites are still too lipophilic and need Phase-II reactions to be excreted in the Kidney.

- Conjugation reaction with an endogenous substrate, such as ***glucoronic acid, sulfuric acid, acetic acid, or amino acid*** *results* in ***polar usually more water soluble*** *compounds that are most often* ***therapeutically inactive*** ***(a notable exception is mporphine-6-glucuronide, which is more potent than morphine)***. ***Glucuronidation is the most common and most important conjugation reaction.***

Reactions of Drug Metabolism- *Phase –II Reactions:*

- ***Neonates are deficient in this conjugation system***, making them particularly vulnerable to drugs such as ***chloramphenicol,*** which is inactivated by the addition of glucuronic acid, resulting in ***gray baby syndrome***.

- Drugs already possessing an **–OH, -NH_2, or –COOH group may enter Phase-II directly** and become conjugated without prior Phase-I metabolism.

- Not all drugs undergo Phase-I and Phase-II reactions in that order. e.g., ***isoniazid is first acetylated (a Phase-II reaction) and then hydrolyzed to isoicotinic acid (a Phase-I reaction).***

Factors Affecting Drug Metabolism:

- **Environmental Determinants**: exposure to certain exogenous compounds; drugs dietary micronutrient (food additives, nutritional or preservative), environmental factors (pesticides, industrial chemicals). This can be in the form of induction or inhibition.

- **Disease Factors:**
  - **Liver diseases** (cancer, alcohol liver disease…etc):
    - ✓ *Dysfunction can lead to impaired drug metabolism-decreased enzyme activity; First pass metabolism affected – may increase 2-4X bioavailiability. Results in exaggerated pharmacological responses and adverse effects.*
  - **Cardiac failure** causes decreased blood flow to the liver.
  - ***Hormonal diseases, infections and inflammation*** can change drug metabolizing capacity.

Factors Affecting Drug Metabolism:

❖ **Age and Sex:**

- *Full maturity appears in second decade of life* and *slow decline in function associated with aging*.
- Responsiveness to certain drugs is *different for men and women*.
- **Pregnancy** – ***induction*** of certain drug metabolizing enzymes occurs in ***second and third trimester*** also hormonal changes during development have a profound effect on drug metabolism.

❖ **Genetic Variation:** genetic diversity is the rule rather than the exception with all proteins, including drug metabolizing enzymes.

Excretion of Drugs:

- Excretion is defined as the process where by drugs or metabolites are irreversibly transferred from internal to external environment through renal or non renal route.

- Excretion of unchanged or intact drug is needed in termination of its pharmacological action.

- The principal organ of excretion are kidneys.

Excretion of Drugs – *Types of Excretion:*

- **RENAL EXCRETION.**

- **NON RENAL EXCRETION:**
  - **Biliary excretion.**
  - **Pulmonary excretion.**
  - **Salivary excretion.**
  - **Mammary excretion.**
  - **Skin / Dermal excretion.**
  - **Gastrointestinal excretion.**
  - **Genital excretion.**

Renal Excretion of Drugs:

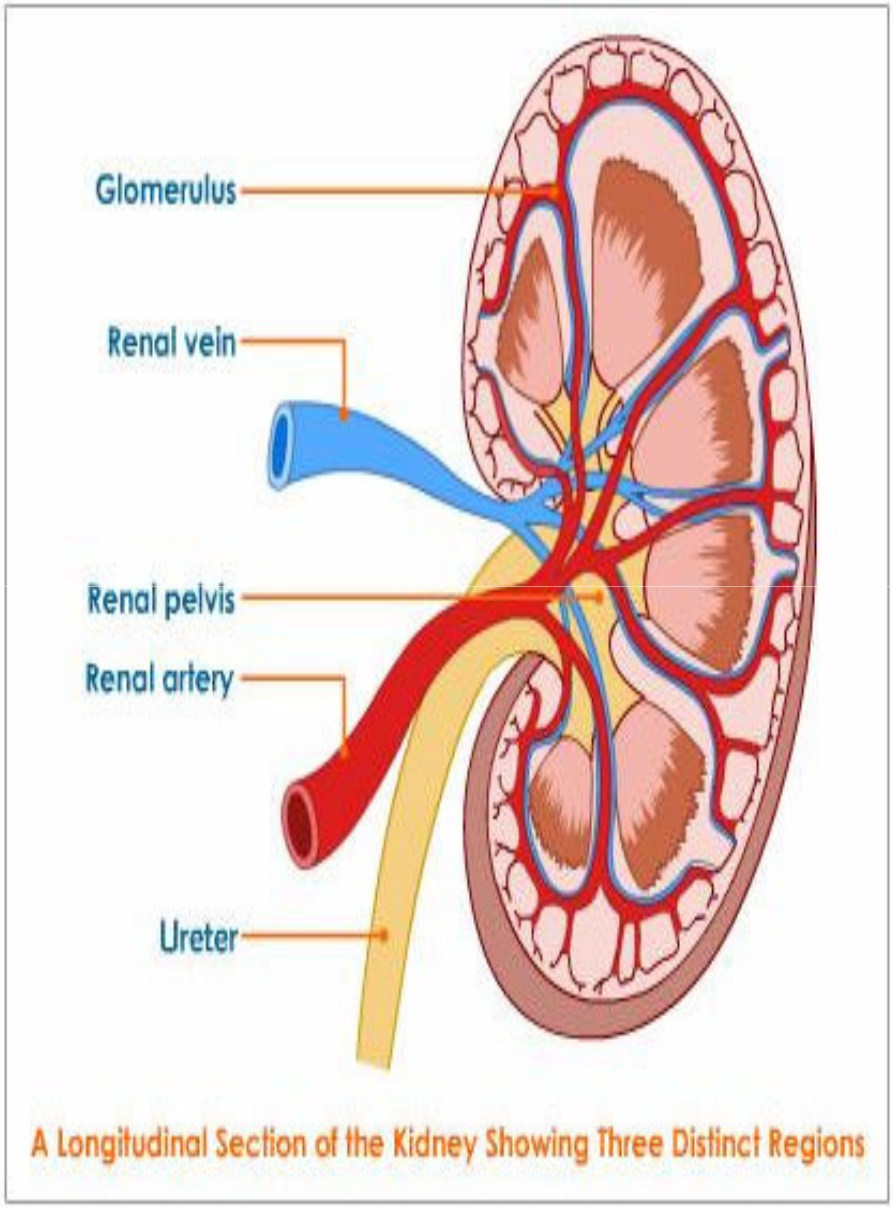
- Elimination of drugs from the body requires the agents to be ***sufficiently polar*** for efficient excretion.

- Elimination of drugs via the kidneys into urine involves the three processes of:

  - **Glomerular Filtration.**
  - **Active tubular secretion.**
  - **Passive tubular reabsorption.**

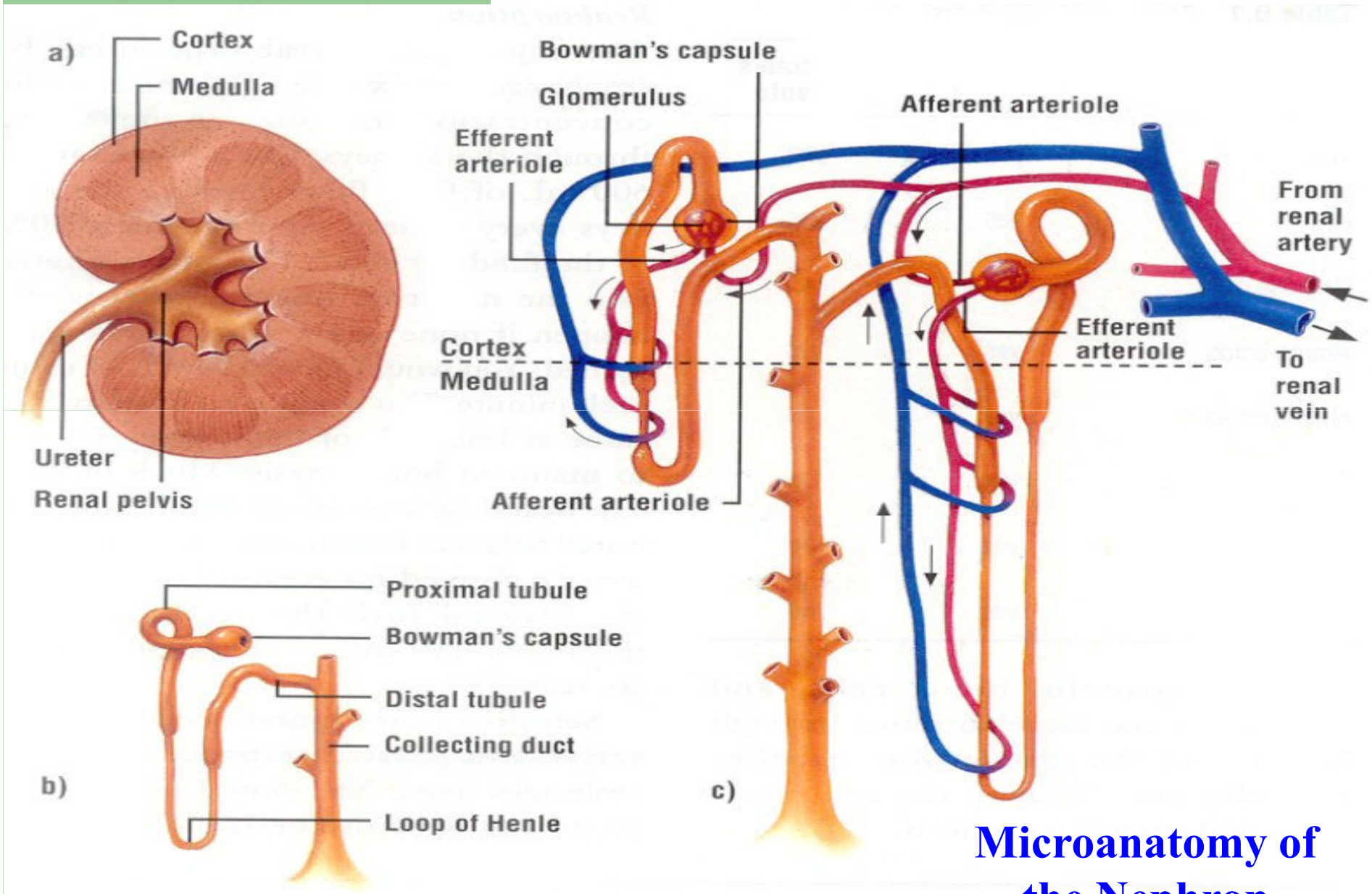
Renal Excretion of Drugs:

Microanatomy of the Kidney



A Longitudinal Section of the Kidney Showing Three Distinct Regions

Renal Excretion of Drugs:



Microanatomy of the Nephron

Renal Excretion of Drugs – *Glomerular Filtration:*

- It is non selective, unidirectional process.
- Ionized or unionized drugs are filtered, ***except those that are bound to plasma proteins.***
- Driving force for GF is ***hydrostatic pressure of blood*** flowing in capillaries.
- **Glomerular Filtration Rate (GFR)**: GFR (125ml/min) is about 20% of renal plasma flow (600mL/min) is filtered through glomeruli.
- ***Lipid solubility & pH do not influence the passage of drugs*** into the glomerular filtrate.

Renal Excretion of Drugs – *Proximal (Active) Tubular Secretion:*

- Drugs that were not transferred into the glomerular filtrate leave the glomeruli through efferent arterioles, which divide to form a capillary plexus surrounding the nephric lumen in the proximal tubule.

- Active tubular secretion ***mainly occurs in proximal tubule***. It is ***carrier mediated process which requires energy*** for transportation of compounds against concentration gradient.

- **Two secretion mechanisms** are identified:
  - ***System for secretion of organic acids/anions***, e.g. Penicillin, Salicylates.
  - ***System for organic base / cations***, e.g. Morphine, Mecamylamine hexamethonium.

- Active secretion is ***unaffected by change in pH and protein binding.***

- Drug undergoes active secretion have ***excretion rate values greater than normal GFR,*** e.g. Penicillin.

Renal Excretion of Drugs – *Distal Tubular Reabsorption:*

- As a drug moves toward the distal convoluted tubule, its concentration increases and exceeds that of the perivascular space.

- The drug, if unchanged, may diffuse out of the nephric lumen, back into the systemic circulation.

- Tubular reabsorption can be ***active or passive processes***, ***it results in increase in the half life of the drug:***

  - **Active Tubular Reabsorption**: Its commonly seen with endogenous substances or nutrients that the body needs to conserve e.g. electrolytes, glucose, vitamins.

  - **Passive Tubular Reabsorption**: It is common for many exogenous substances including drugs. The driving force is concentration gradient which is due to reabsorption of water, sodium and inorganic ions.

Renal Excretion of Drugs – *Distal Tubular Reabsorption:*

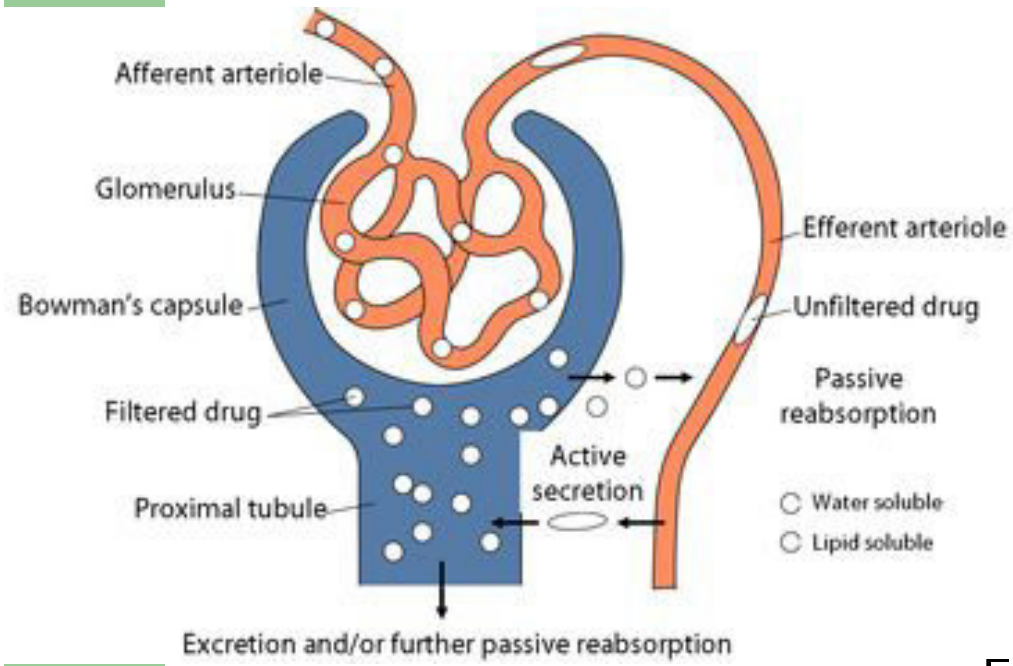
- Manipulating the pH of the urine to increase the ionized form of the drug in the lumen may be done to minimize the reabsorption and increase the clearance of undesired drug:

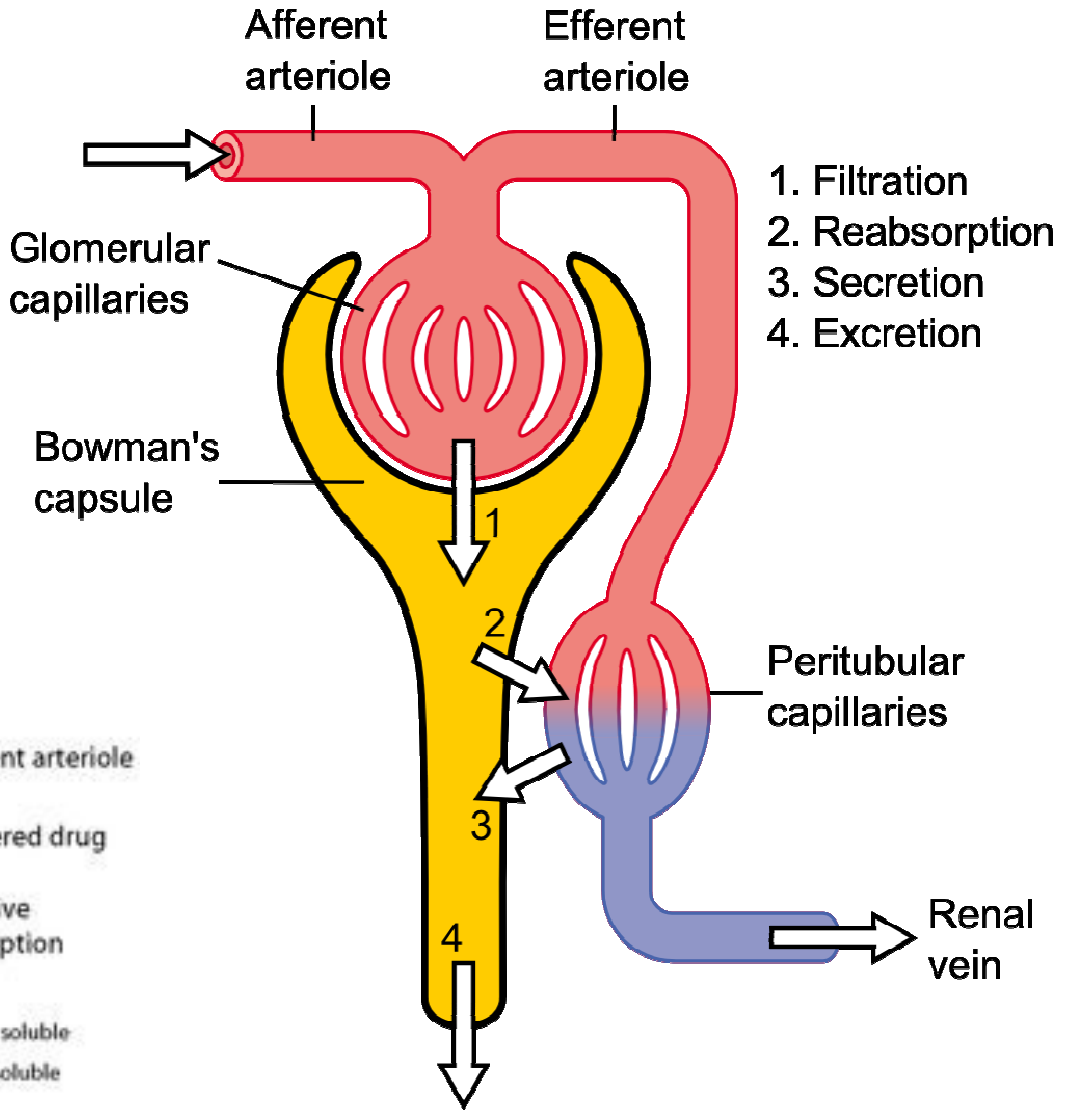
  - As a general rule, ***weak acids can be eliminated by alkalinization of the urine, whereas elimination of weak bases may be increased by acidification of the urine***. This process is called "***ion trapping***" :

    - ✓ e.g. Patients with *Phenobarbital* (*weak acid*) overdose can be given *bicarbonate* (*alkalinizes urine*) and keeping the drug ionized decreasing its reabsorption.

    - ✓ e.g. An overdose of a *weak base* (e.g. *amphetamine*), acidification of the urine with *NH4Cl* leads to protonation of the drug (become charged) and enhances it excretion.

Renal Excretion of Drugs:





Excretion = Filtration – Reabsorption + Secretion

Renal Excretion of Drugs – *Factors Affecting Renal Excretion:*

- **Physical properties of the drug**: Drugs with Mol.wt <300, water soluble are excreted in kidney. Mol.wt 300 to 500 Dalton are excreted both through urine and bile. Drugs that are ***bound to plasma proteins*** behave as macromolecules and ***cannot be filtered through glomerulus***.

- **Biological factors:**

  - **Sex:** Renal excretion is 10% lower in female than in males.

  - **Age:** The renal excretion in newborn is 30-40 % less in comparison to adults. In old age the GFR is reduced and tubular function is altered which results in slow excretion of drugs and prolonged half lives.

- **Drug interaction**: Any drug interaction that results in alteration of binding characteristics, renal blood flow, active secretion, urine pH, intrinsic clearance and forced diuresis would alter renal clearance of drug.

- **Disease state**: Renal dysfunction greatly impairs the elimination of drugs especially those that are primarily excreted by kidney. Some of the causes of renal failure are ***Hypertension, Diabetes, Pyelonephritis.***