Principles of Pharmacology & Toxicology

Week 1

Toxicology Defined

- Study of adverse effects of chemical or physical agents on living organisms

Casarett & Doull's *Toxicology*

- The study of the adverse effects of chemical, physical, or biological agents on people, animals, and the environment

Society of Toxicology (web page)

- The study of poisonous chemicals, drugs, etc., and how a person or other living thing reacts to them

Merriam-Webster online

Terminology

- Toxic Agents

  any substance producing toxicity
- Toxins

  toxic agent produced by biological systems

  plant, animal, bacteria, fungi
- Toxicants

  toxic agents produced by or by-product of human activity
- Exposure

  physical contact or dosing with a toxic agent

Toxicologic

- Chemical Allergy
  - immunologically mediated reaction to a chemical
  - may involve sensitization (pre-exposure)
  - hypersensitivity reaction observed thereafter
  - chemicals not sufficiently immunogenic, so combines with protein as hapten to be immunogenic
- Chemical Idiosyncracy
  - abnormal reaction to chemical
  - genetically based
    - long duration of succinylcholine metabolism because of slow-metabolizing pseudocholinesterase phenotype
    - NADH-cytochrome b5 reductase deficiency: susceptibility to nitrites, leading to methemoglobin formation, low oxygen carrying capability

Toxicologic

- Immediate vs Delayed Toxicity
- Some toxic effects may not be seen for years, such as carcinogens causing disease decades later
  - Vaginal cancers in young adult females whose mothers used diethylstibestrol (DES) with in utero exposure (20-30 y later)
  - Organophosphorus insecticides (e.g. triorthocresylphoshate [TOCP]) causing degeneration of long axons in PNS & CNS over several days

Overlap with Pharmacology

- Principles of toxicology overlaps a great deal with the fundamentals of pharmacology
- Drugs both natural and synthetic can be given at one dose to achieve a desired or intended therapeutic effect, but at a higher dose can produce a toxic effect
- Thus, concepts in pharmacology are presented in concert with instruction in toxicology

Good Things & Bad Things

A Philosophical Consideration

- Bad Things Always Bad Things (Or Are They?)
- Snake venom always thought bad
- But many venoms or their refined molecular products used for medical therapy
- Thus: Good Things Sometimes Bad Things

A lesson?

- Be prepared to evaluate the "incontrovertible facts" from time to time

Toxicant Chemistry

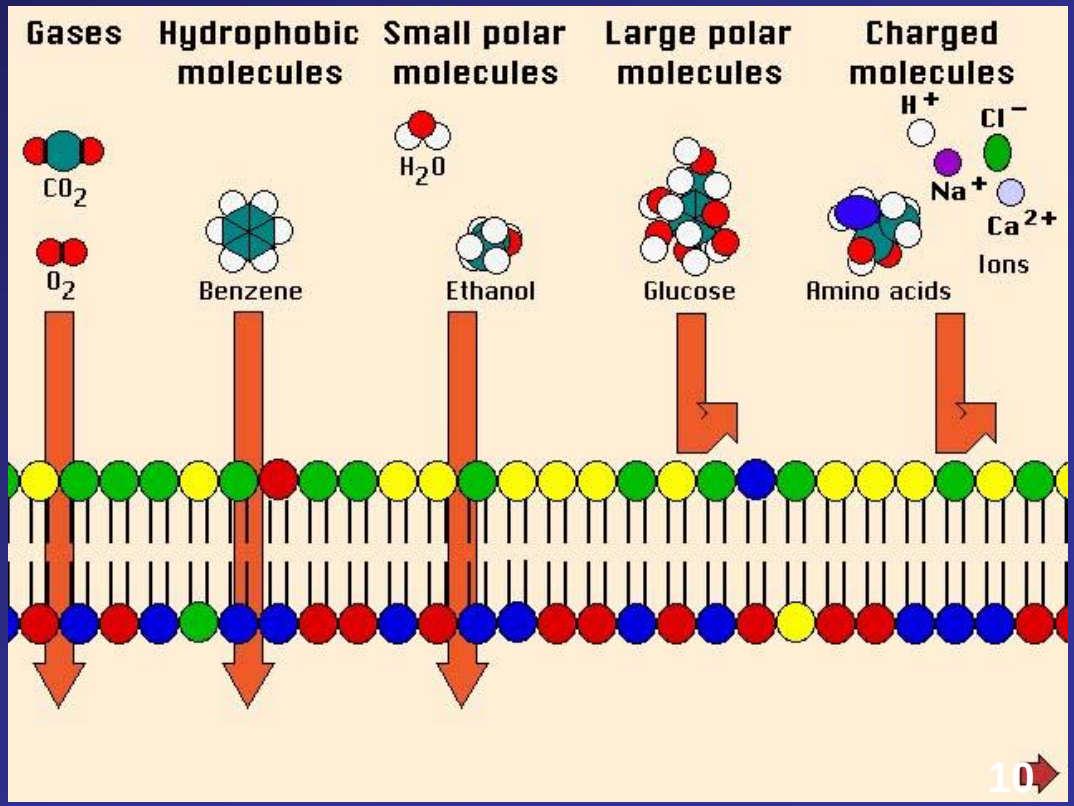
- Lipophilic / Hydrophobic / Fat-Soluble
  - alkyl (methylene: $-CH_2-$)
- Hydrophilic / Water-Soluble
  - Polar Nonionic: hydroxyl –OH groups
  - Ionic (pH-dependent and –independent)
    - Cationic (+ charged): amine $-NH_2$ groups
    - Anionic (– charged): carboxyl –COOH groups

Toxicant Permeability

- Fully lipophilic toxicants can easily cross cell membrane phospholipid bilayers
- Toxicants with any ionized (positively or negatively electrically charged) functional groups cannot freely cross the lipid bilayer
- This includes zwitterionic toxicants that may have a net charge of zero on the molecule
- An absence of charged groups does not guarantee membrane permeability, since functional groups may have significant hydrophilic polar groups

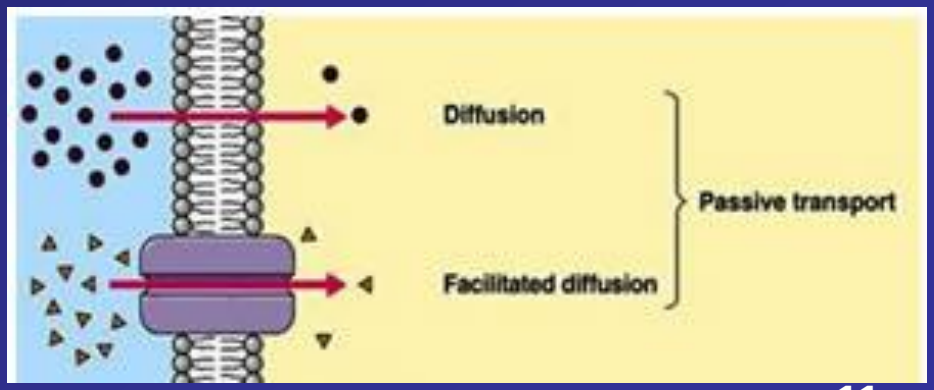
Chemistry of Membrane Permeability

- Molecules having no net polarity ("dipole"), especially hydrophobic or lipophilic, cross the lipophilic lipid bilayer of cell membranes without difficulty
- Molecules having a net polarity or having many polar groups or having electric charges (ions) do not cross the membrane
- Impermeable molecules must have special channel or transport proteins in membrane to open gate for them to cross



Getting Across A Membrane

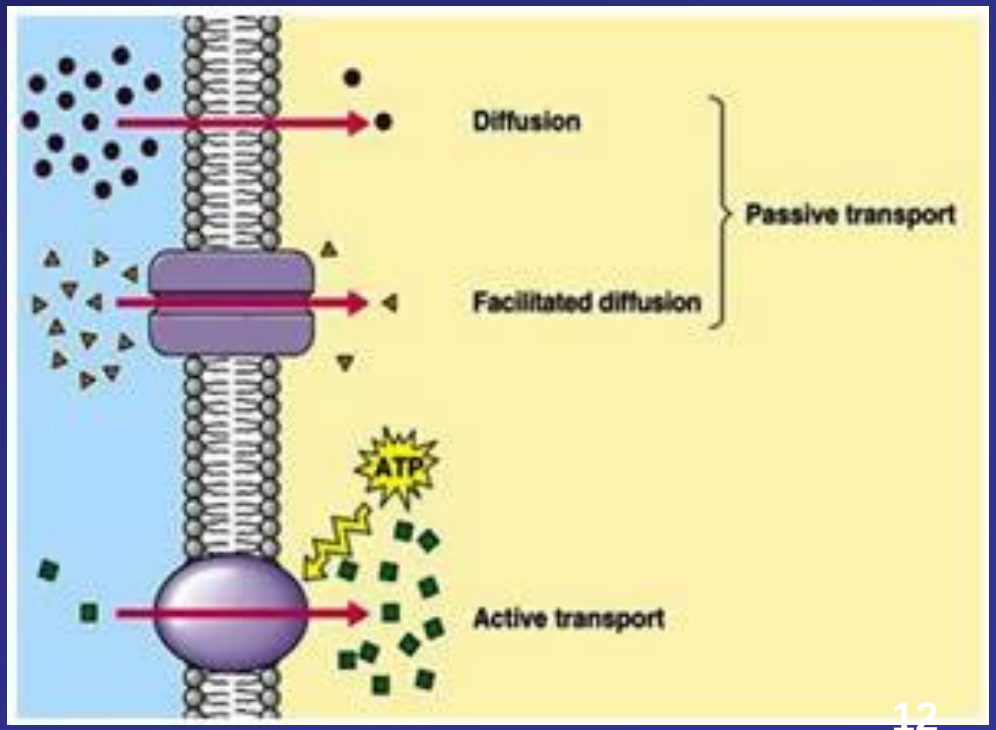
- Thermodynamic laws explain that molecules will diffuse within a space (volume) to distribute themselves evenly within that space: it's same for the volume of the body
- Cell membranes represent a barrier to this diffusion for most, not all molecules
- For those that can cross membranes, the simple diffusion process works
- For others, proteins move with their concentration gradient through channels in membrane proteins
-



http://pharmafactz.com/wp/wp-content/uploads/2014/12/active-transport.jpg

Getting Across A Membrane

- Active transport uses the cell's energy (ATP) to move molecules against their concentration gradient
- The figure shows an ATP-dependent transport membrane protein moving molecules *into* a cell, but there are many ATP-dependent transporters that use energy to move molecules *out* of cells (e.g., the drug efflux transporters mentioned in this course)

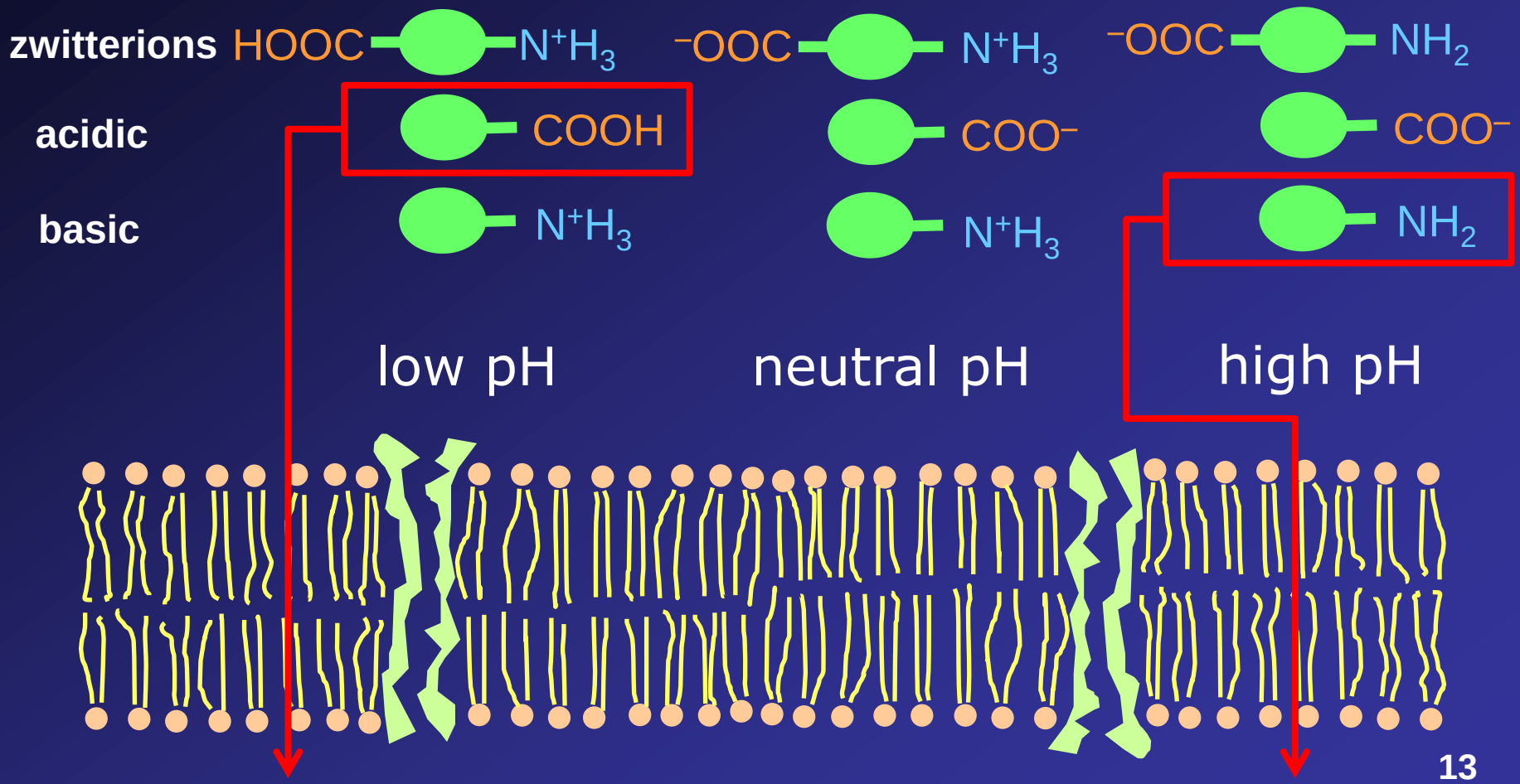


http://pharmafactz.com/wp/wp-content/uploads/2014/12/active-transport.jpg

Permeability & pH-Dependence

Toxicant Class

red boxed toxicants not guaranteed permeability, but have stronger potential

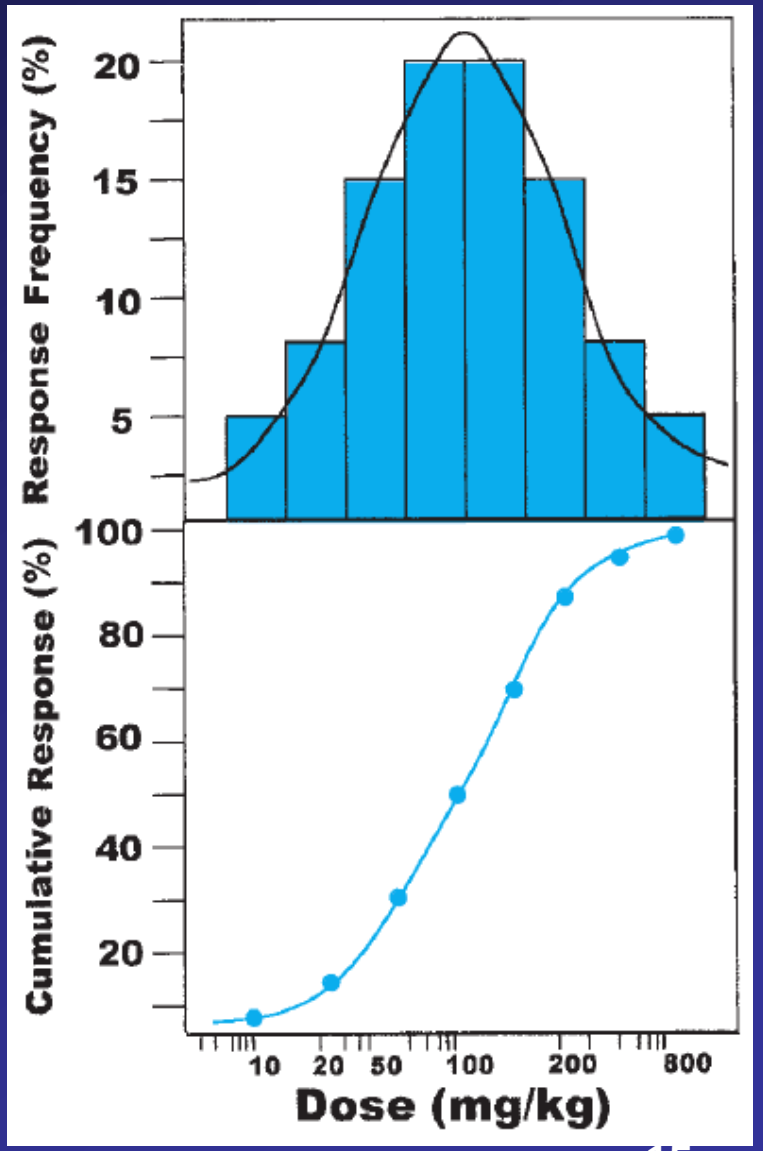


Dose-Response

- Dose: administer substance (drug, poison) in specific quantity
- Response: observe a variety of effects, whether intended/expected or unintended/unexpected
- The "therapeutic" effect: a desired effect from a drug (not an effect for toxicology)
- The "side" effect: the willingness to tolerate unpleasant, uncomfortable, and sometimes painful effects in order to obtain the therapeutic effect
- The "toxic" effect: the intolerable circumstance where health or life is endangered; generally an acute effect; chronic effects less perceptible

Responses in Populations

- The dose at which individuals in a population show a response (effect) to a drug or toxicant is *normally distributed* generally
  top panel in figure
- The plot of the cumulative percentage showing the response to the dose has value in determining the dosage for drug
- This is the Dose-Response curve/plot
  bottom panel in figure



The Dose-Response Curve

- Dose-Response relationships are plotted as the cumulative percentage of a population showing an effect as a particular dose
- They are used for setting the appropriate dose of a drug
- The ED_{50} parameter refers to the "effective dose" for 50% of the population
- That is, the ED_{50} is the dose at which the desired effect is produced in 50% of the population

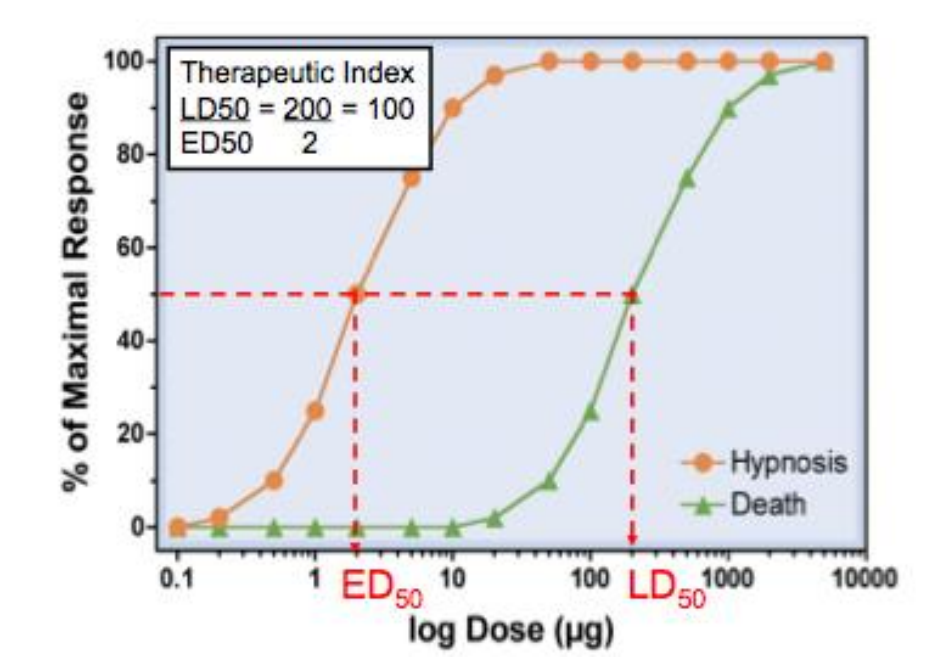
Toxic Doses / Lethal Doses

- The Dose-Response curves apply for all manner of responses:
- toxic response
  - toxic response 1 (TR1): suppose loss of vision
  - toxic response 2 (TR2): suppose hemiplegia
  - the dose producing toxicity in 50% of the population: **TD_{50}**
- lethal response
  - the ultimate toxic response
  - the dose producing death in 50% of the individual in the population is the **LD_{50}**

Therapeutic Index

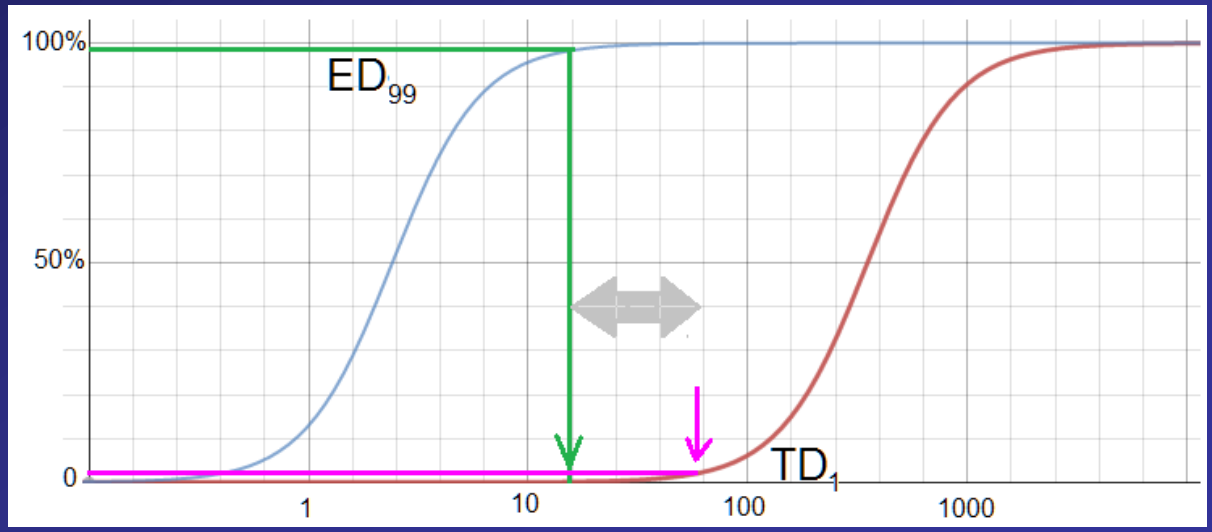
- In pharmacology, this value is a measure of how safe a (therapeutic) drug is with respect to obtaining the intended effect and risking seeing a toxic effect, especially the lethal effect
- This is the ratio TD_{50} / ED_{50}

Agonist	TI
THC (cannabis)	1000
diazepam	100
morphine	70
digoxin	2



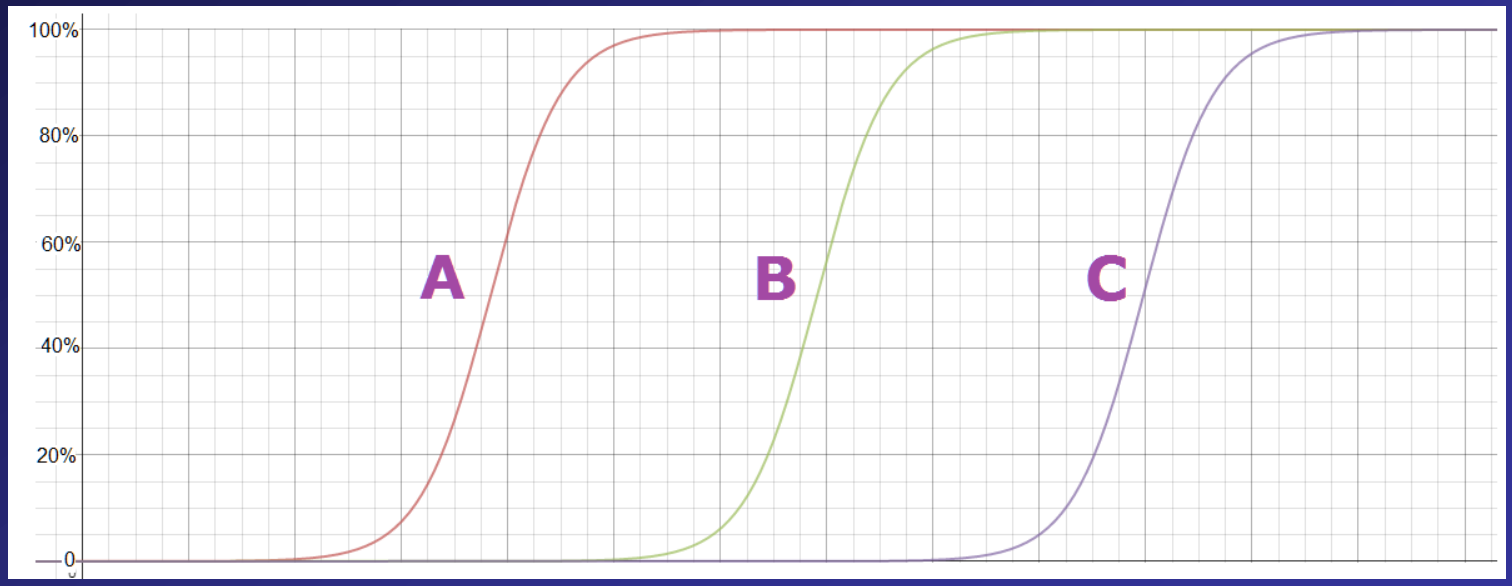
Margin of Safety: $TD_1 \div ED_{99}$

- Another parameter of the dose-response curve is the margin of safety, the ratio of $TD_1 \div ED_{99}$
- If the TD_1 is really the LD_1, then this would be of more value than a therapeutic index, in the even that the ED curve near 100% could even overlap the LD curve at close to 0%



Potency

- Potency reflects the amount of drug/toxicant necessary to produce the effect
- It is a comparative tool for drugs/toxicants producing the same effect
- Suppose A, B, C are toxicants causing tumors in test animals: as the curve shows, A produces the effect at lower doses than B or C, and so it is more potent than either B or C



Toxicokinetics / Pharmacokinetics

- The study of how a drug enters the body, is changed by the body, and is eliminated by the body
- Four processes describe toxico/pharmacokinetics:

1. Absorption

   The process where a toxicant passes barriers (GI tract, skin, lung aveoli) to get into the systemic circulation

2. Distribution

   The process where the toxicant increases its concentration in vascular, interstitial and intracellular volume

Toxicokinetics / Pharmacokinetics (cont)

3. Metabolism (Biotransformation)

   The process where the cell reduces the level/concentration of toxicant (de-toxifies) by chemically modifying it

4. Elimination

   The process in which the body attempts to excrete the toxicant or a modified form of it (its detoxified products)

Absorption

- This is about getting a toxicant/drug past the barriers to the systemic circulation
- Ingestion / Oral
  - Most any toxicant in a pill/tablet/liquid
- Inhalation
  - Gaseous/vapor toxicants
- Dermal
  - lipophilic toxicants particularly
- Rectal
  - suppositories

does not include intravenous (i.v.) administration, since that is a direct route past the barriers

Chemistry of Absorption

- The unionized (electrically uncharged) toxicant more readily passes a cell membrane barrier than an ionized toxicant
- Absorption of pH-dependent toxicants affected by pH

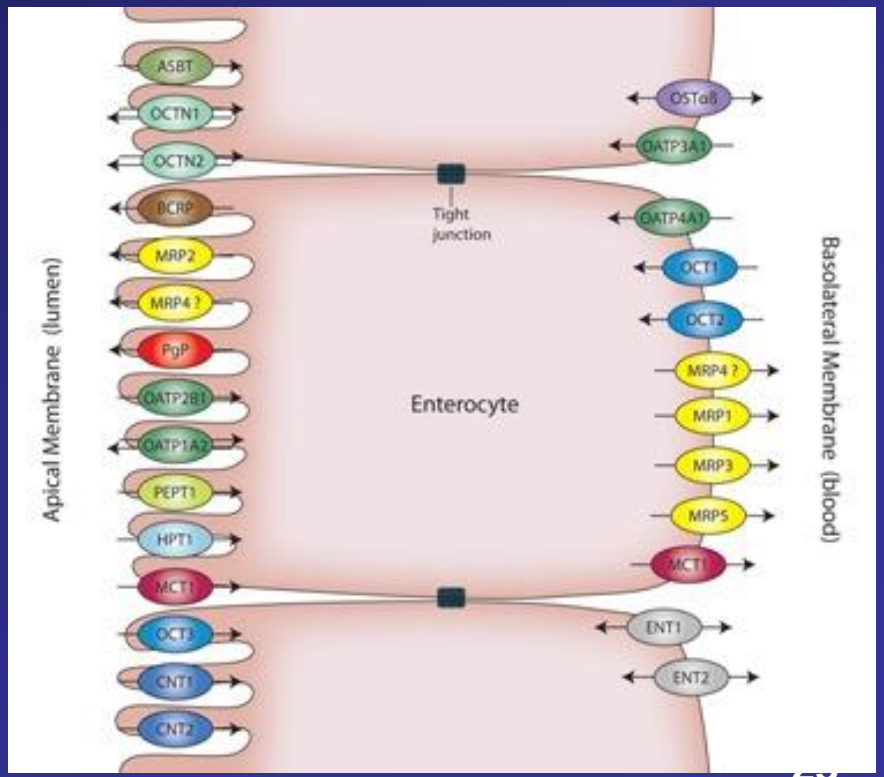
- [note: this information is a repeat of previous slides]

GI Tract

- The intestines represent an initial barrier to absorption
- Intestinal epithelial cells (enterocytes) form "tight junctions" as a barrier to simple diffusion of toxicants into blood
- Additionally they have membrane proteins actively transporting xenobiotics back into intestinal lumen

left side of cell in figure

while you don't need to memorize names of the efflux or exchange transporter proteins in the figure, you will likely encounter their mention in the research you do for your presentations



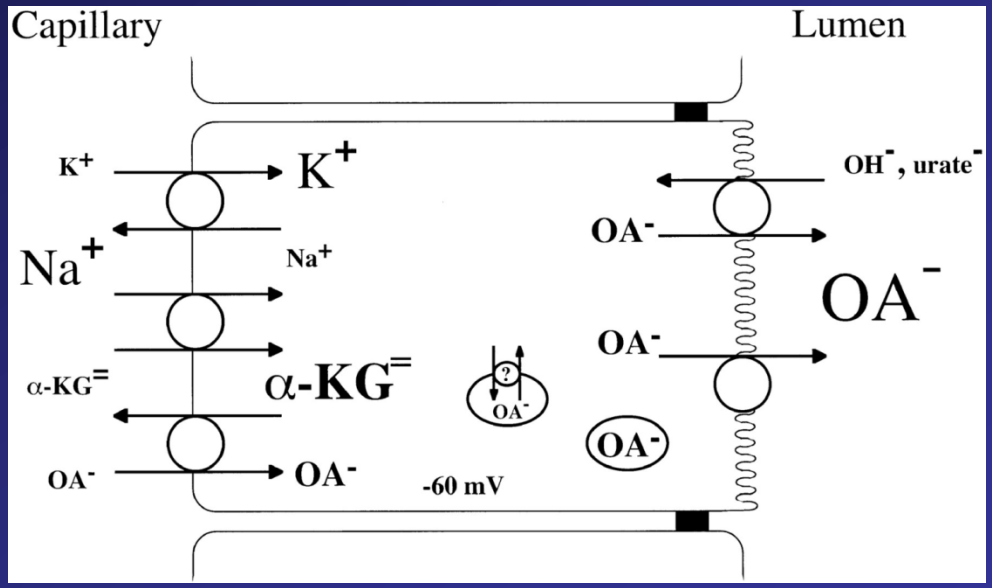
http://www.solvobiotech.com/images/uploads/Enterocytes1.jpg

MDR Efflux Transporters

- Certain membrane proteins (P-glycoprotein) use ATP to drive out cytotoxic xenobiotics, some of which are cancer drugs
- These transporters pump out multiple drugs (multi-drug resistance [MDR]) with broad specificity and are present in a large variety of cell types
- High expression of these proteins is likely in cells whose organs function as the first guard in exposure to toxicants
  - intestinal epithelium
  - liver hepatocytes

Coupled Ionic Transporters

- This type of membrane protein transporters are also involved in the efflux of toxicants to prevent absorption into systemic circulation
- Negatively charged toxicants (organic anions) are transported to the lumenal (excretory) side of epithelial cells in kidney and intestine



http://ajprenal.physiology.org/content/281/2/F197

Distribution

- Distribution is the process of a drug/toxicant circulating the vascular volume and reaching the tissues, intracellular and interstitium
- Factors affecting distribution include
  - blood flow: flow to organs (liver, kidney, brain) is greater than to other parts of body
  - capillary permeability
  - binding affinity of drugs/toxicants to plasma or tissue proteins

Plasma Protein Binding

Proteins in plasma will bind to many drugs, affecting and limiting their distribution outside vascular volume

Ligand-binding preferences

- α & β lipoproteins
  many lipophilic substances
- α_1-acid glycoprotein
  basic substances
- metal-binding proteins
  drugs/toxicants with metal cations

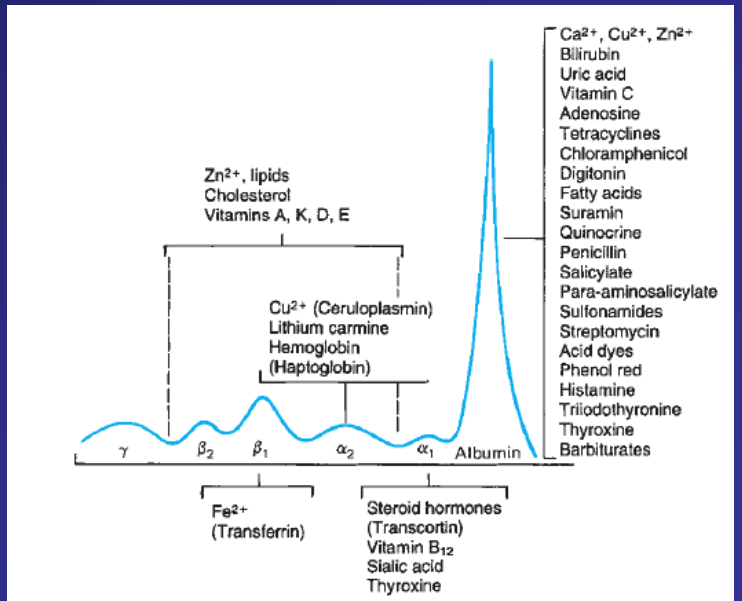


Figure 5-8. Ligand interactions with plasma proteins.

Blood-Brain Barrier

- Distribution to CNS tissue & fluid space limited by blood-brain barrier
- Endothelial cells in brain capillaries forming tight junctions to inhibit polar substances entering CNS
- Xenobiotic efflux transporters in cells actively keep lipophilic substance out

  Pgp + BCRP (cations+neutral)
  Mrp2 + Mrp4 (anions + neutral)
- Glial cells (astrocytes + microglia) extend processes on endothelium to act as (reinforce) barrier

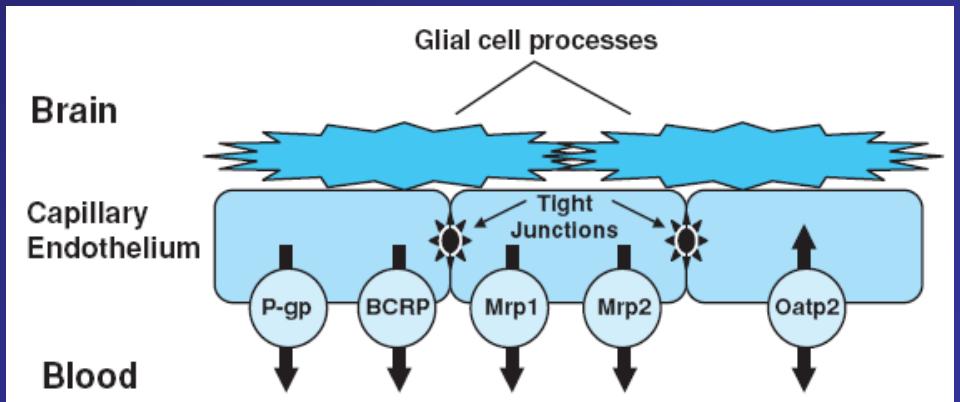


Figure 5-10. Schematic model showing the xenobiotic transporting systems that contribute to the blood–brain barrier.

Blood-CSF Barrier

- Epithelial cells of choroid plexus on other side of capillary epithelium form part of barrier. Cells have xenobiotic efflux transporters (as do endothelial cells)

  Luminal P-gp and basolateral Mrp1

  Oatp3, Oat3, Oct, and Pept2 also involved

  ("Oat"=organic anion transporter, "Oct"=org cation transporter)
- The low protein content of CSF also prevents toxicity of xenobiotics that are protein binders

Facts about CNS Barriers

- BBB not as well developed in newborns compared to adults so xenobiotics/drugs might enter brain
- Some areas of the brain more accessible: cortex, hypothalamus lateral nuclei, neurohypophysis
  - bilirubin and morphine more toxic
  - due to increased blood supply to the these parts or is barrier more permeable?

Crossing The Placenta

- Humans have a hemochorial placenta: the fetal placental tissues are cellular but the maternal cellularity is gone
- Most nutrients move from maternal blood to fetal circulation by active transport

Volume of Distribution (V_d)

- Distribution is the process of a drug/toxicant leaving the circulation and reaching the tissues (intracellular and interstices)
- Factors affecting distribution include
  - blood flow: flow to organs (liver, kidney, brain) is greater than to other parts of body
  - capillary permeability
  - binding affinity of drugs/toxicants to plasma or tissue proteins

$$V_d = \frac{Dose}{C_0}$$

where C_0 is the plasma concentration at "time zero"

If possible, administer dose intravenously, then sample blood several times quickly after administration to approximate C_0

Bioavailability

- Bioavailability is the fraction of drug/toxicant reaching systemic circulation compared to dose given
- *F* = computed amount in circulation / dose
- If 80 mg of a 100 mg dose reach circulation, then F = 0.80
- This is important for determining doses of drugs/toxicants that enter circulation by non-intravenous routes
- It is assumed that a drug/toxicant given intravenously has *F* = 1.0
- Bioequivalence: If two drugs/toxicants show similar bioavailability and pharmacokinetics, they are bioequivalent

Bioavailability Factors

- If toxicant/drug injected intravenously, F = 1.0 (bioavailability = 100%)

Oral administration of drug imposes factors:

- First-pass hepatic metabolism

  All drugs & toxicants absorbed in gut enter the portal vein to the liver, so are exposed to liver enzymes expressly designed to filter out and detoxify xenobiotics

- Drug solubility
  - molecules should not be very lipophilic (insoluble in aqueous compartments) nor very hydrophilic (cannot easily cross membrane barriers
  - Best are those that are weak acids and bases

Bioavailability Factors (continued)

- Chemical stability

  For pharmacologically significant drugs, the molecular nature cannot be altered if it is to remain effective

- Drug formulation characteristics
  - size of particles/grain: smaller size generally improves absorpition
  - salt vs free acid/free base form: site of absorption (stomach vs intestine)
  - enteric coating: site of absorption
  - binders, dispersing agents: affect dissolution

Detoxification

- All cells of the body have the ability to chemically modify the molecules, including toxicants, that enter them
- Most modifications are enzymatic, but non-enzymatic reactions can occur

- Cells that form a lining that is a barrier between where toxicants can pass to a compartment will have mechanisms to detoxify or thwart passage

  capillary endothelium, GI tract epithelium
- The liver is the gate to the systemic circulation of substances that manage to get past the epithelial cells of the GI tract. It is a major organ of detoxification

Biotransformation

- The response of the body (cells) to alter the toxicity of xenobiotics
- Used for useful drugs and toxicants alike
- Occurs in two phases (Phase I and Phase II)
- Biotransformation involves biochemical reactions to chemically alter toxicants so that they become excretable
- They also provide the possible additional benefits of detoxifying the toxicant and preventing its ability to easily cross membranes of cells and thus distribute itself throughout the body
- All biotransformations are mediated by enzymes with but few exceptions where reactions are spontaneous

Phase I Biotransformation

- Phase I involves chemical reactions that can radically alter the molecular structure of the toxicant
- The intention is to add functional groups that "put a handle" on the molecule so it can excreted
- The transformation also make the molecule polar or ionized, preventing the molecule from easily crossing cell membranes
- Additionally the toxic nature can be neutralized
  - However in some cases, the resulting metabolite might be even more toxic

Phase I Reactions

Fall into three basic classes

1. Oxidation

   Many of these reactions are catalyzed by the cytochrome P450 enzymes

2. Reduction

   Many oxidoreductases and mono-oxygenases that depend on the co-enzymes NAD, NADP, FAD, and FMN are involved here

3. Hydrolysis

   Enzymes that perform these reactions include carboxylesterases, peptidases, phosphatases, and other hydrolases

Phase II Biotransformation

- In this phase, the toxicant undergoes conjugation
- Conjugation is the "tagging" or "putting a handle" on the toxicant
- The toxicant does not have to have undergone Phase I biotransformation: it may be in a state that allows it anyway

Hydrolysis

- The H_2O molecule is introduced into a bond-breaking reaction

  This includes trans-esterifying enzymes, where an alcohol (ROH) and not an HOH molecule used
- (Carboxyl)esterases
  - 60 kDa glycoproteins found in serum, lysosomes, cytosol and particularly high concentrations in liver ER
  - 5 types in liver, 30 types in brain
  - These hydrolyze esters
    $RC(=O)\text{-}OR' + H_2O \rightarrow RCOOH + HOR'$
  - Includes acetylcholinesterase & butyrylcholinesterase
- Paraoxonases (Lactonases)

  hydrolyze organophosphates, cyclic carbonates, lactones, aromatic carboxylic acid esters

Hydrolysis

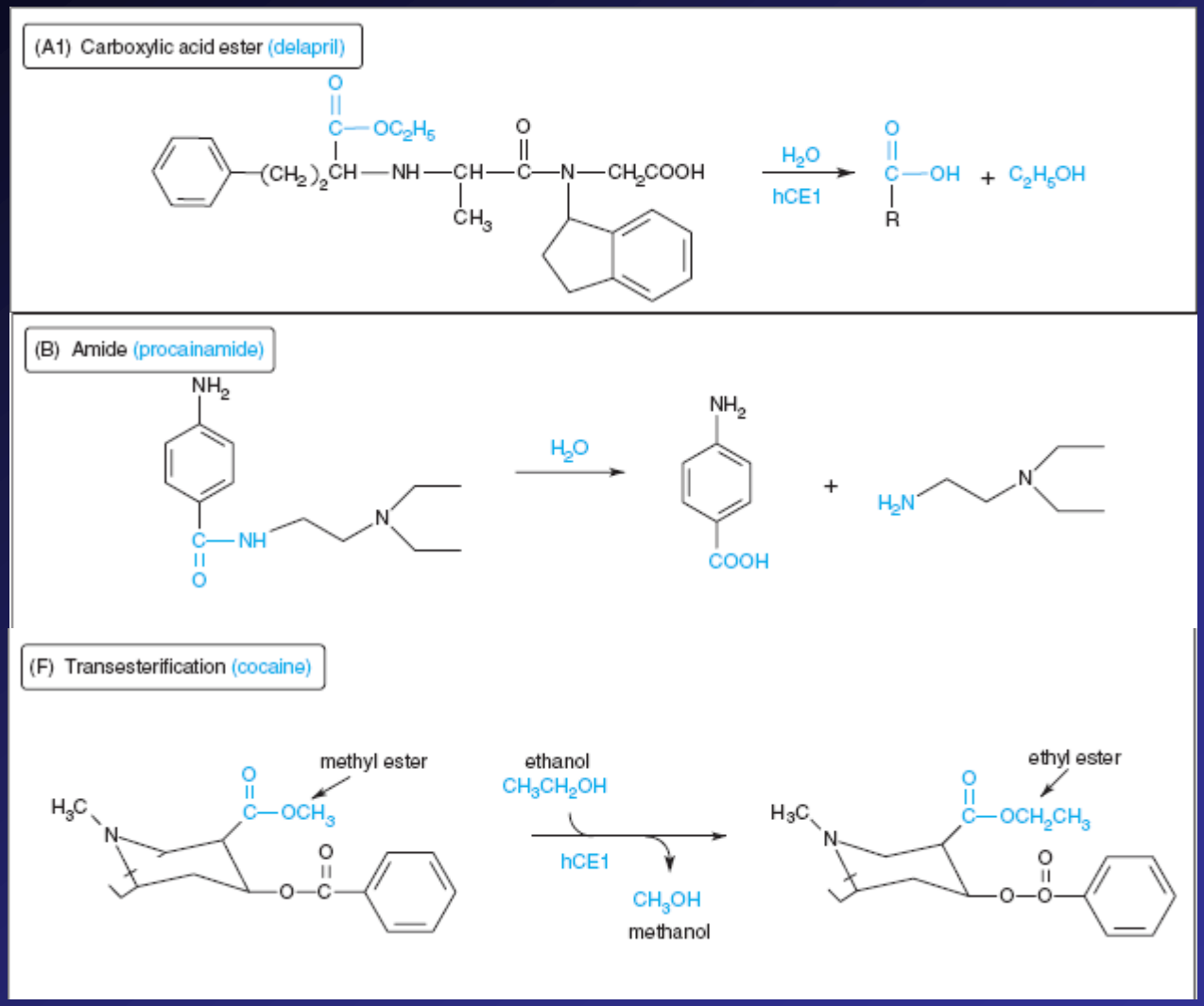
- Epoxide hydrolases

  Cytochrome P450 enzymes (discussed later) can form epoxides, which should be hydrolyzed so conjugation (also discussed later) can occur

- Peptidases

  These enzymes can hydrolyze amino acids from either end of the polypeptide (aminopeptidases and carboxypeptidases) or within the strand (serine and cysteine proteases)

Specific Hydrolytic Reactions

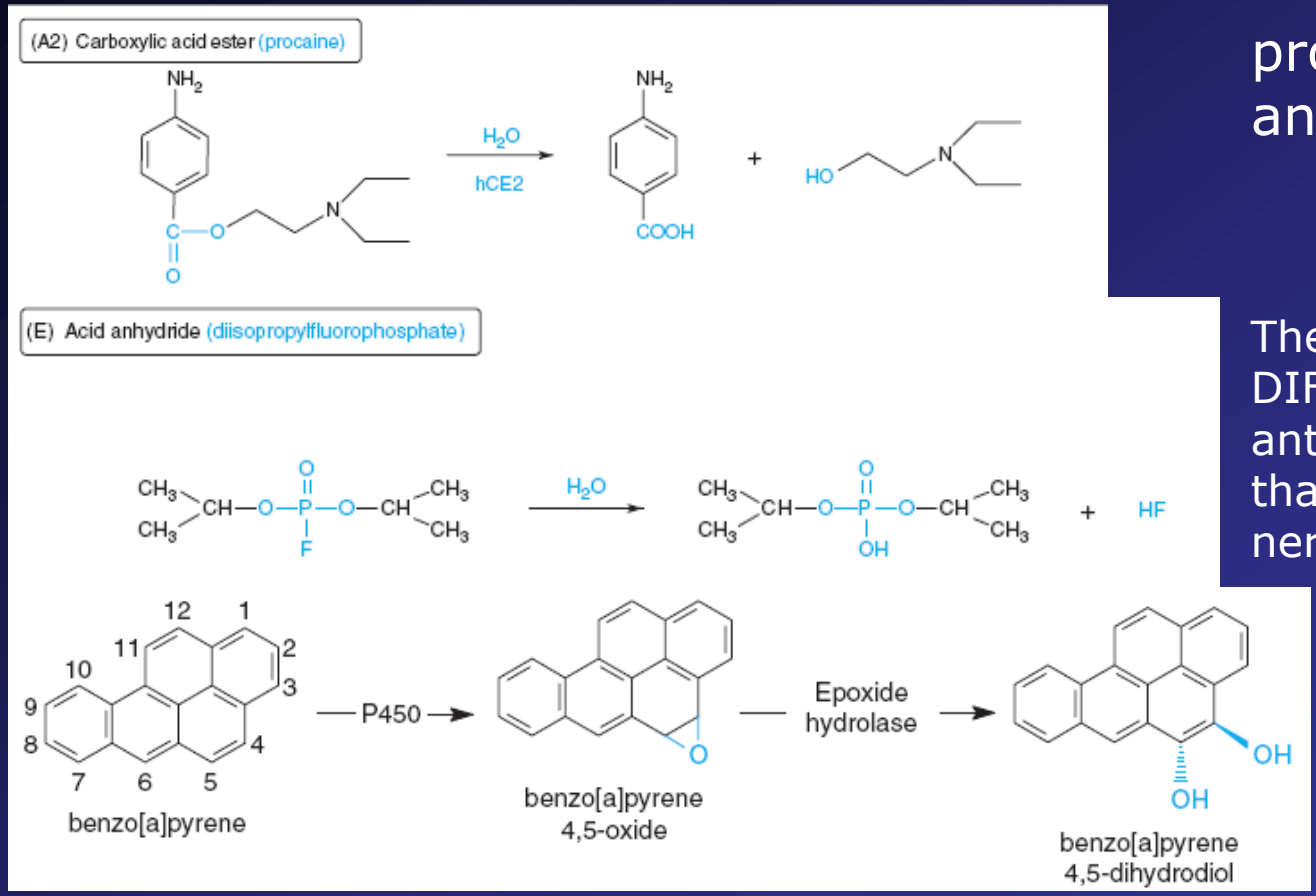


Note H_2O molecule shown in reactions

hCE1 is name of hydrolytic enzyme

Here's a trans-esterification (ROH not HOH)

Specific Hydrolytic Reactions



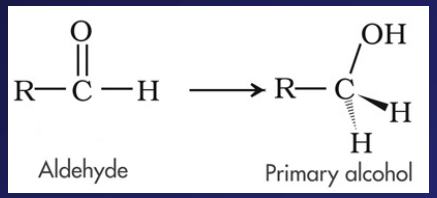
procaine is local anesthetic

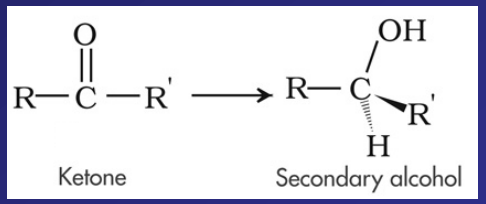
The organophosphate DIFP is a powerful anti-cholinesterase that was studied as a nerve warfare agent

The epoxide hydrolase follows the epoxidation by a CYP enzymes

Reductions

- When a molecule has electrons transferred to it (often being protonated or acquiring hydrogen atoms too), this is a reduction
- These reactions are generally about reducing carbonyl groups in aldehydes & ketones





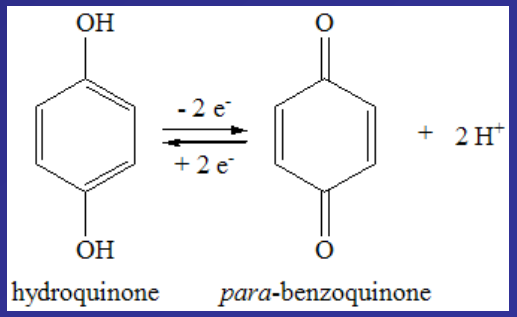
- Reduction transformations can occur in three ways
  1. Enzymatically by host NAD(P)H-dependent oxidoreductases (found in many cells, but chiefly done by liver microsomes)
  2. Nonenzymatically perhaps by direct interaction of an reductant (NAD(P)H, FAD, FMN, glutathione) with toxicant
  3. Intestinal bacteria which have many such enzymes

Reduction Enzymes

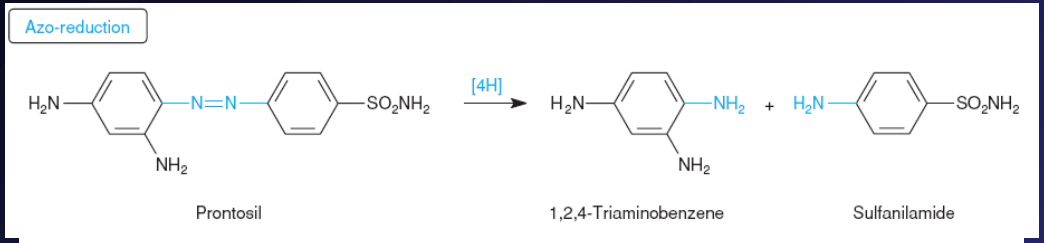
- Many host reductases have been identified, and fall into 4 superfamilies
- A table for these reductases is on the next slide

These 4 classes of reductases are

1. Aldo-keto reductases (AKR)
   specific for carbonyl groups (C=O) on aldehydes and ketones
2. Short-chain dehydrogenase/reductases (SDR)
3. Medium-chain dehydrogenase/reductases (MDR)
4. Quinone reductases (NQO)

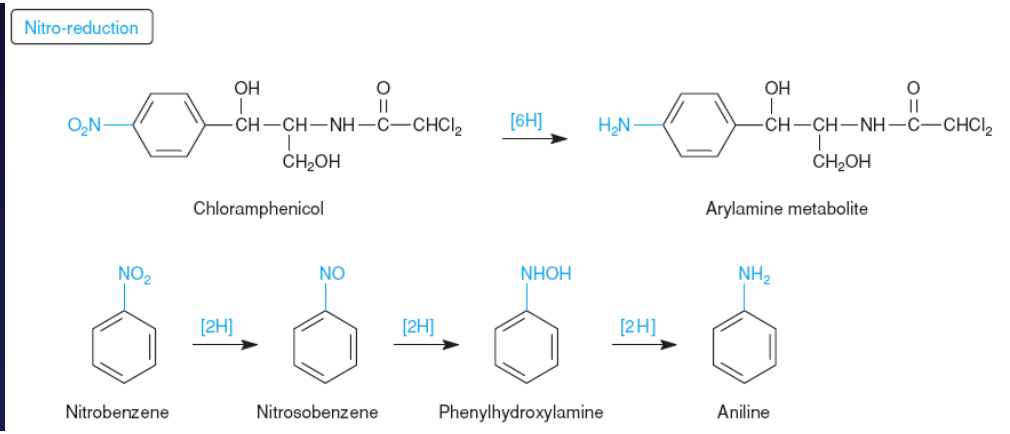


Specific Reduction Reactions

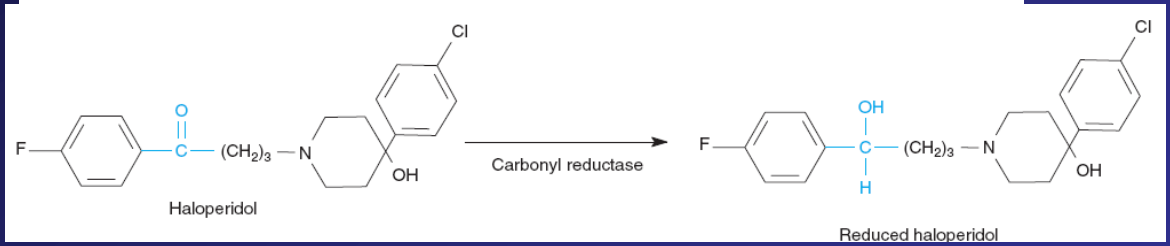


The azo group is R-N=N-R

The double bond was hydrogenated twice to split into amines



Conditions used in the laboratory to reduce aryl (aromatic ring) nitro ($-NO_2$) groups use toxic chemicals and catalysts



Haloperidol is an anti-psychotic used to treat schizophrenia, choreas, severe anxiety, tics in Tourette's, hiccups not responsive to other care. Used in adults and children

Table 6-5

Human Aldo–Keto Reductases (AKRs), Short-Chain Dehydrogenases/Reductases (SDRs), Medium-Chain Dehydrogenases/Reductases (MDRs), and Quinone Reductases (NQOs)

ENZYME SUPERFAMILY	EXAMPLE ENZYMES	ALTERNATIVE NAMES	SUBCELLULAR LOCALIZATION
Aldo–keto reductase (AKR) 13 enzymes	AKR1A1	Aldehyde reductase	Cytosol
	AKR1B1	Aldose reductase	Cytosol
	AKR1B10	Small intestine reductase	Cytosol
	AKR1C1	20α-Hydroxysteroid dehydrogenase	Cytosol
	AKR1C1, 1C2, 1C4	Dihydrodiol dehydrogenases (DD1, DD2, DD4)	Cytosol
	AKR1C1, 1C2, 1C3, 1C4	3α-Hydroxysteroid dehydrogenase	Cytosol
	AKR1C3	17β-Hydroxysteroid dehydrogenase type V	Cytosol
	AKR1C4	Chlordecone reductase	Cytosol
	AKR1D1	Δ^4-3-Ketosteroid-5β-reductase	Cytosol
	AKR6A3, 6A5, 6A9	Shaker-channel subunit Kvb1, Kvb2, Kvb3	Cytosol
	AKR7A2	Aflatoxin B_1 aldehyde reductase 2	Golgi
	AKR7A3	Aflatoxin B_1 aldehyde reductase 3	Cytosol
Short chain dehydrogenase/ reductases (SDR) 39 enzymes	Cytosolic carbonyl reductase	Xenobiotic ketone reductase with pH 6.0 activity; Prostaglandin 9-ketoreductase; Human placental NADP-linked 15-hydroprostaglandin dehydrogenase	Cytosol
	Microsomal carbonyl reductase	11β-Hydroxysteroid dehydrogenase; 11β-reductase, 11-oxidoreductase	Microsomes
Medium chain dehydrogenase/ reductases (MDR) 7 ADH enzymes	ADH1A	Class I ADH; ADH1, α, β, γ; *hADH1,2,3*	Cytosol
	ADH1B	Class I ADH; ADH2, β, γ; ADHII; *hADH4*	Cytosol
	ADH1C	Class I ADH; ADH3, γ	Cytosol
	ADH4	Class II ADH; π, *hADH7*	Cytosol
	ADH5	Class III ADH; χ	Cytosol
	ADH6	Class V ADH	Cytosol
	ADH7	Class IV ADH; μ or σ	Cytosol
NQO 2 enzymes	NQO1	DT diaphorase, menadione reductase	Cytosol
	NQO2	*N*-Ribosyldihydronicotinamide dehydrogenase	Cytosol

Oxidations

- When a molecule has electrons removed from it, this is an oxidation, because much of the time, oxygen (O) atoms are taking the electrons, the O atoms becoming part of the molecule
- Dehydrogenations are also oxidations because electrons are taken, although O atoms may not necessarily be bonded products nor involved in the reaction

Oxidation Enzymes

- Alcohol, Aldehyde Dehydrogenases

  NAD-based enzymes producing aldehydes and carboxylates
- Mo^{2+}-based Hydroxylases
- Aldehyde Oxidase

  An Mo^{2+}-centered enzyme also
- Flavin Mono-oxygenases

  creates a reactive hydroperoxide that puts an O atom on the substrate
- Cytochrome P450 Enzymes

  oxidize a wide variety of substrates

Cytochrome P450 (CYP) Enzymes

- These enzymes (at least 57 types) are present in all tissues, but the primary location for detoxifying done in microsomes of liver cells
- The catalytic center is a iron-centered heme
- In Phase I biotransformations, CYP enzymes perform oxidations
- CYP enzymes are mono-oxygenases

$$\text{Substrate (RH)} + O_2 + \text{NADPH} + H^+ \rightarrow \text{Product (ROH)} + H_2O + NADP^+$$

- The enzymes binds the substrate & O_2 molecule, and gets electrons from another enzyme handling NADPH reductant

Specific CYP Enzymes

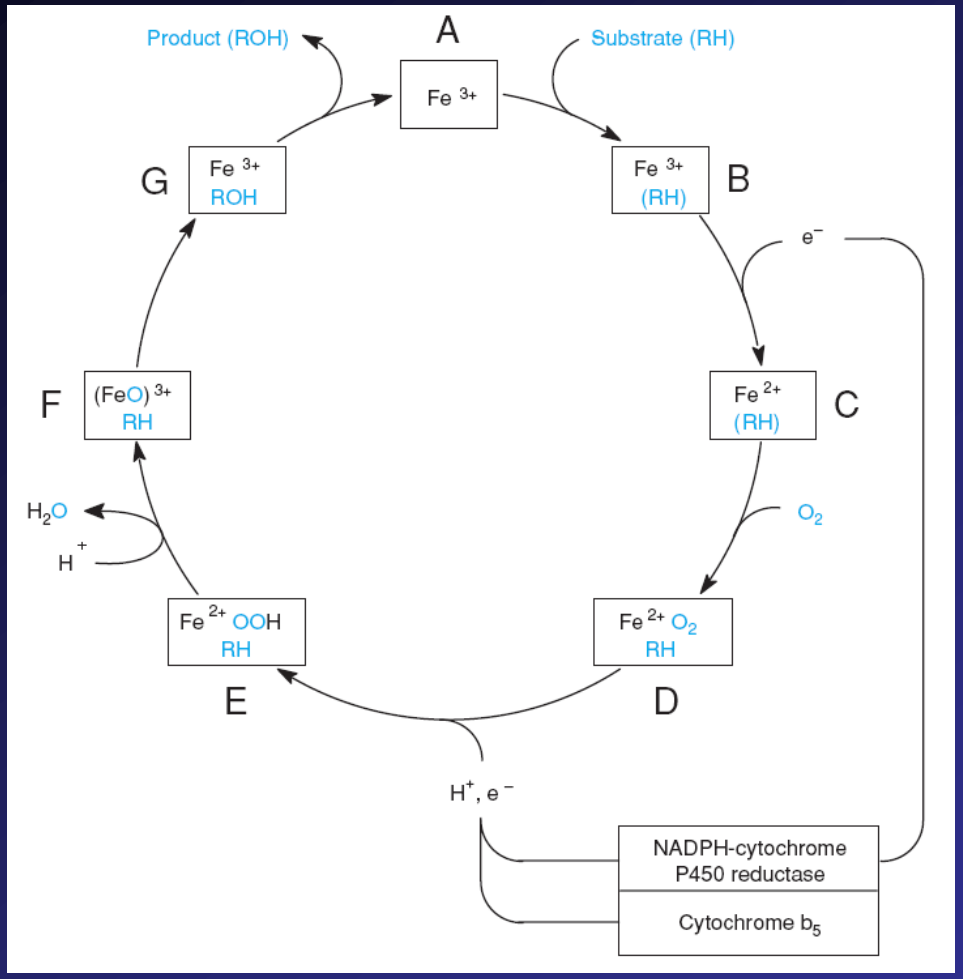
- Families of CYP enzymes (isoforms) show differences in the variety of substrates bound
- CYP3A4/CYP3A5, CYP2D6, CYP2C8/CYP2C9 and CYP1A2 have been identified as carrying out most modifications compared to other CYP isoforms
- Intestinal mucosal cell CYP3A4 will metabolize drugs such as chlorpromazine & clonazepam

Induction of CYP Gene Expression

- Aside from the chemical reactions & products formed by these oxidizing CYP enzymes, a crucial feature is that the expression of their genes is induced by toxicants
- This induction is a sword with many edges

1. It can render all drugs (other drugs) metabolized by the CYP ineffective or less effective, since their metabolic deactivation is increased.

CYP Mechanism



- The cycle starts with substrate binding to heme
- Fe^{3+} is reduced to Fe^{2+} in a 1-electron reaction
- O_2 then binds to the enzyme
- O_2 is then "activated" in a 1-electron reduction
- A single O atom is then dissociated with an oxidation of Fe^{2+}, and protonation of complex involves H_2O leaving
- The remaining O atom is highly energetic and reacts with the substrate, which then leaves

Recent Understanding

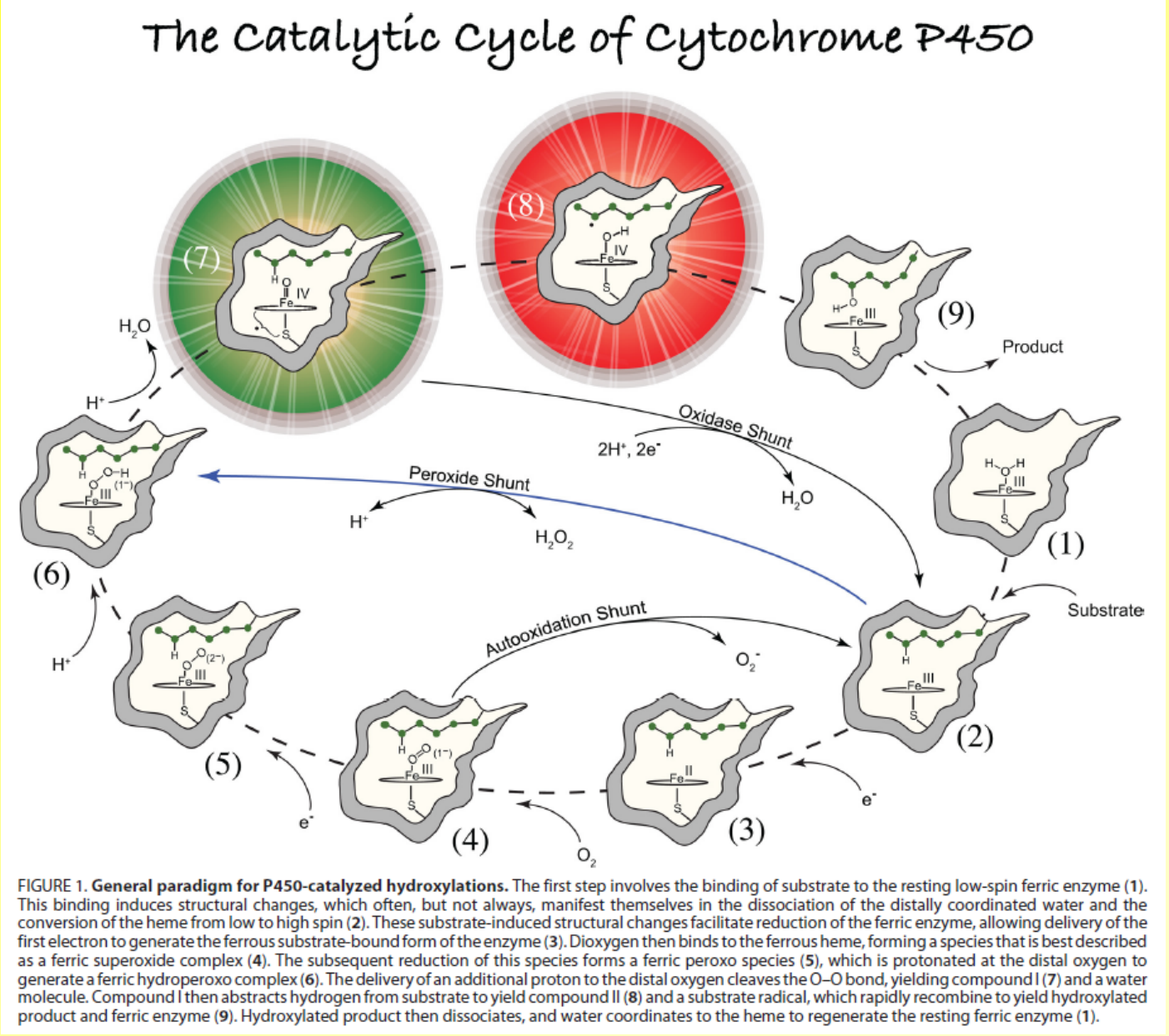
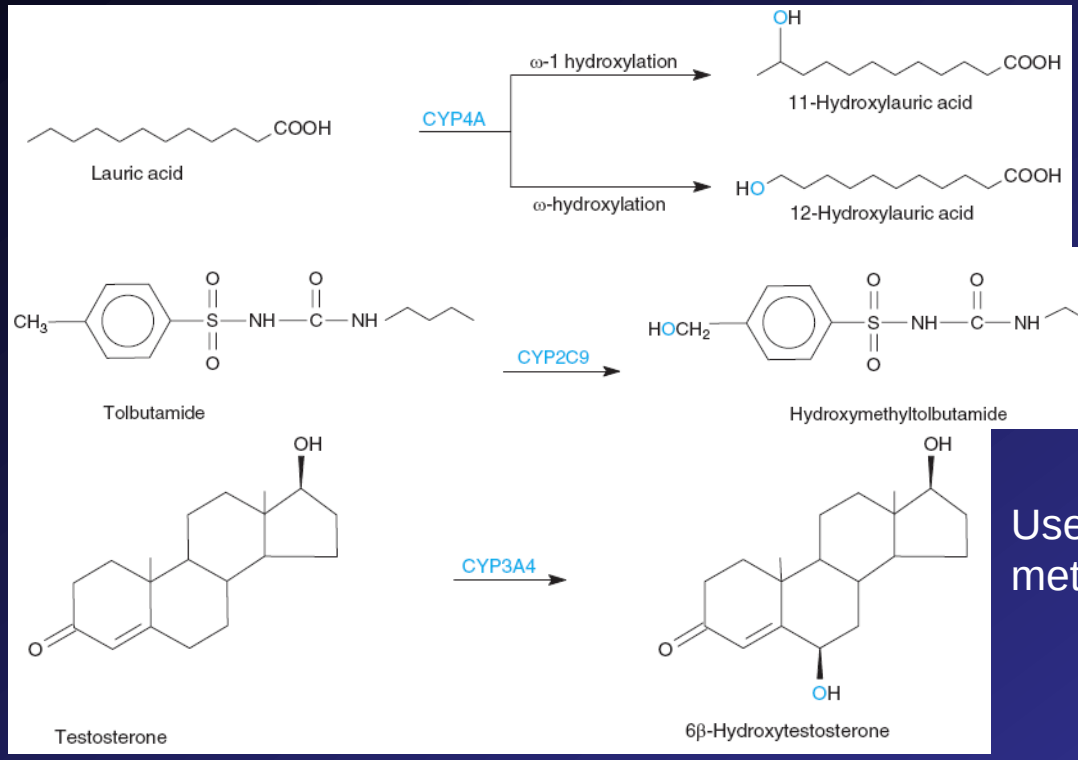


FIGURE 1. **General paradigm for P450-catalyzed hydroxylations.** The first step involves the binding of substrate to the resting low-spin ferric enzyme (**1**). This binding induces structural changes, which often, but not always, manifest themselves in the dissociation of the distally coordinated water and the conversion of the heme from low to high spin (**2**). These substrate-induced structural changes facilitate reduction of the ferric enzyme, allowing delivery of the first electron to generate the ferrous substrate-bound form of the enzyme (**3**). Dioxygen then binds to the ferrous heme, forming a species that is best described as a ferric superoxide complex (**4**). The subsequent reduction of this species forms a ferric peroxo species (**5**), which is protonated at the distal oxygen to generate a ferric hydroperoxo complex (**6**). The delivery of an additional proton to the distal oxygen cleaves the O–O bond, yielding compound I (**7**) and a water molecule. Compound I then abstracts hydrogen from substrate to yield compound II (**8**) and a substrate radical, which rapidly recombine to yield hydroxylated product and ferric enzyme (**9**). Hydroxylated product then dissociates, and water coordinates to the heme to regenerate the resting ferric enzyme (**1**).

Specific CYP Reactions



Mono-oxygenation (hydroxylation) of the tail-end of long-chain alkyl groups is important

Tolbutamide is used to treat type 2 diabetes

Used in the catabolism of natural metabolites, steroids

There are far too many **chemical reaction types** catalyzed by CYP enzymes to show in this course: oxygenation of N and S atoms; dealkylation of O, N, S, Si; deamination, desulfuration by oxidation; epoxidation

Conjugation (Phase II Reactions)

- Conjugation is the biochemical reaction of adding a functional group (often a large molecule) to the molecule that is either the toxicant or its product(s) that underwent Phase I reactions
- Molecules that are conjugated to toxicants will give the xenobiotic more polar (multiple –OH groups) or electrically charged (particularly anionic or negatively charged) groups

Purposes

1. Primarily to increase the water solubility for excretion
2. Ipso facto, reduce the lipophilic character that allows xenobiotics to traverse membranes into other compartments
3. Put a "tag" on a xenobiotic that marks it for excretion
4. Potentially reduce its toxic nature (even further)

Conjugation Reactions

- Glucuronidation

  attaches a large anionic sugar acid (glucuronic acid) using UDPGlcA, to O atoms on ROH/RCOOH, N atoms on amines, S atoms on thiols
- Sulfation

  attaches anionic $-SO_3^-$ to –OH using PAPS
- Acetylation

  modifies usually amine groups with an acetyl (CH_3COO-) group using acetyl-coenzyme A

Conjugation Reactions

- Methylation

  Methyl group transferred to O, N, and S atoms using S-adenosylmethionine (SAM)

- Glutathione conjugation

  the thiol (–SH) group of glutathione can be involved in aromatic substitutions, C=C additions, oxidations,

  Note that glutathione is a 3-amino acid compound: γ-Glu-Cys-Gly

- Amino acid conjugation
  - the amine ($-NH_2$) group of the amino acid transfers to an acyl carbon atom in a nucleophilic acyl substitution
  - the carboxyl group can transfer from a tRNA synthetase to O atom of hydroxylamines
  - amino acids such as glycine, glutamine, serine, taurine

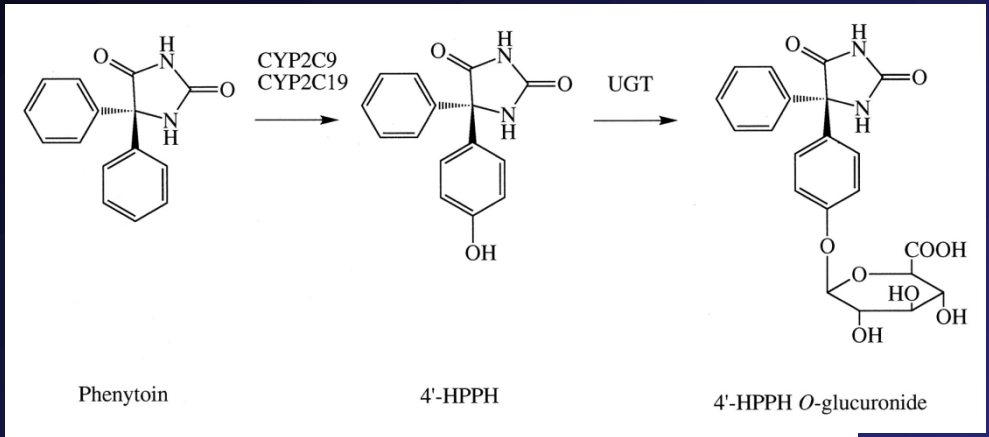
Activated Conjugation Reactant

It is important to note that all groups transferred to the target toxicant in the conjugation reaction are activated

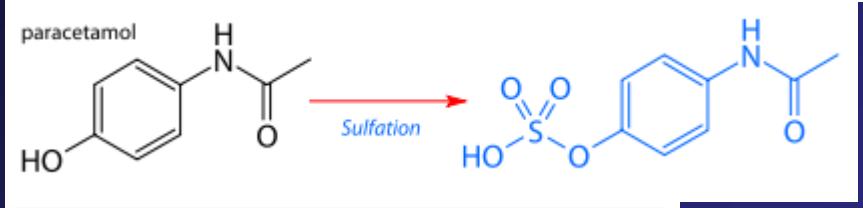
- Activated means "brought to a higher energy state"

- UDPGlcA is formed from UTP
- acetyl-Coenzyme A is produced by two high energy phosphate ester bonds from ATP
- S-adenosylmethionine is formed from ATP
- PAPS formation is driven by use of two ATP

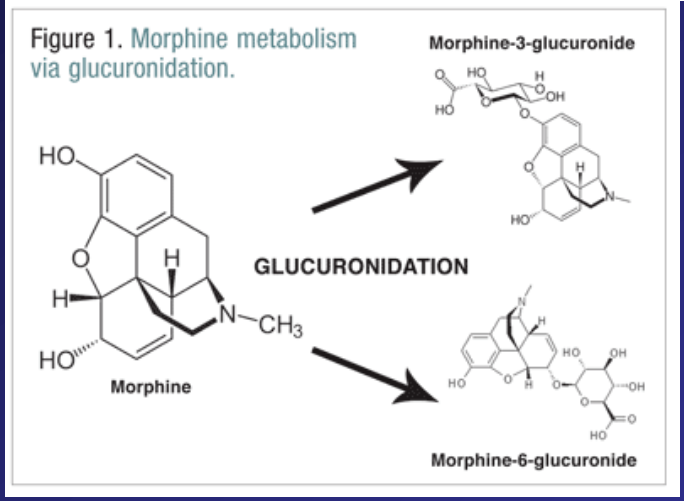
Specific Conjugation Reactions



The anticonvulsant phenytoin is hydroxylated on the ring by the P450 enzyme and then conjugated with glucuronic acid

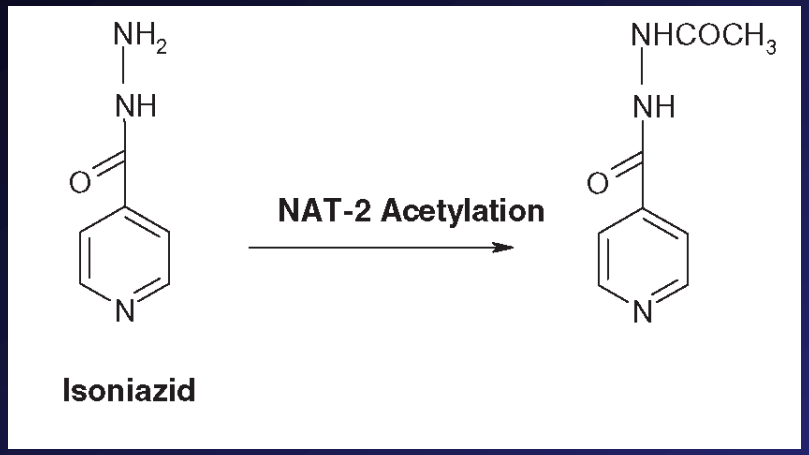


Sulfation of acetominophen



The opiate morphine glucuronidated in several locations

Specific Conjugation Reactions



Acetylation of the anti-tuberculosis drug isoniazid

Sulfation of acetominophen

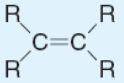
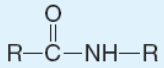
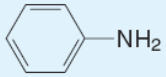
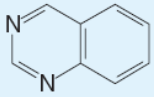
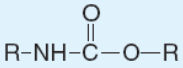
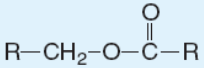
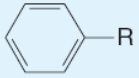
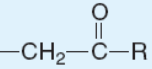
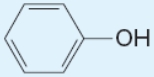
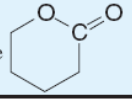
The opiate morphine glucuronidated in several locations

- In the very useful table on biotransformation reactions on the next slide, 3 columns are shown
- The 1st column shows the chemical functional group on the toxicant to be modified or transformed
- The 2nd column shows the enzyme (class) that performs the reaction/modification/transformation
- The 3rd column shows the type of reaction, indicating the product to be obtained

- Note that the table has two columns of the 3-column

Table 6-2

Common Chemical Groups and Enzymes Possibly Involved in Their Metabolism

CHEMICAL GROUP	ENZYME(S)	REACTION(S)	CHEMICAL GROUP	ENZYME(S)	REACTION(S)
Alkane $R-CH_2-R$	CYP	Hydroxylation, dehydrogenation	Aldehyde 	CYP, ALDH	Oxidative de-formylation, oxidation to carboxylic acid
Alkene 	CYP, GST	Epoxidation, glutathione adduct formation	Amide 	Amidase (esterase)	Hydrolysis
Alkyne $R-C\equiv C-R$	CYP	Oxidation to carboxylic acid	Aniline 	CYP, NAT, UGT, peroxidase, SULT	N-Hydroxylation, N-acetylation, N-glucuronidation, N-oxidation, N-sulfonation
Aliphatic alcohol $R-CH_2-OH$	CYP, ADH, catalase, UGT, SULT	Oxidation, glucuronidation, sulfonation	Aromatic azaheterocycles 	UGT, CYP, aldehyde oxidase	N-Glucuronidation, hydroxylation, N-oxidation, ring cleavage, oxidation
Aliphatic amine $R-NH_2$	CYP, FMO, MAO, UGT, SULT, MT, NAT, peroxidase	N-Dealkylation, N-oxidation, deamination, N-glucuronidation, N-carbamoyl glucuronidation, N-sulfonation, N-methylation, N-acetylation	Carbamate 	CYP, esterase	Oxidative cleavage, hydrolysis
Amidine $HN=CR-NH_2$	CYP	N-Oxidation	Ester 	CYP, esterase	Oxidative cleavage, hydrolysis
Arene 	CYP	Hydroxylation and epoxidation	Ether $R-CH_2-O-CH_2-R$	CYP	O-Dealkylation
Carboxylic acid $R-COOH$	UGT, amino acid transferases	Glucuronidation, amino acylation	Ketone 	CYP, SDR, AKR	Baeyer–Villiger oxidation, reduction
Epoxide 	Epoxide hydrolase, GST	Hydrolysis, glutathione adduct formation	Phenol 	CYP, UGT, SULT, MT	Ipso-substitution, glucuronidation, sulfonation, methylation
Lactone 	Lactonase (paraoxonase)	Hydrolysis (ring opening)	Thioether $R-CH_2-S-CH_2-R$	CYP, FMO	S-Dealkylation, S-oxidation

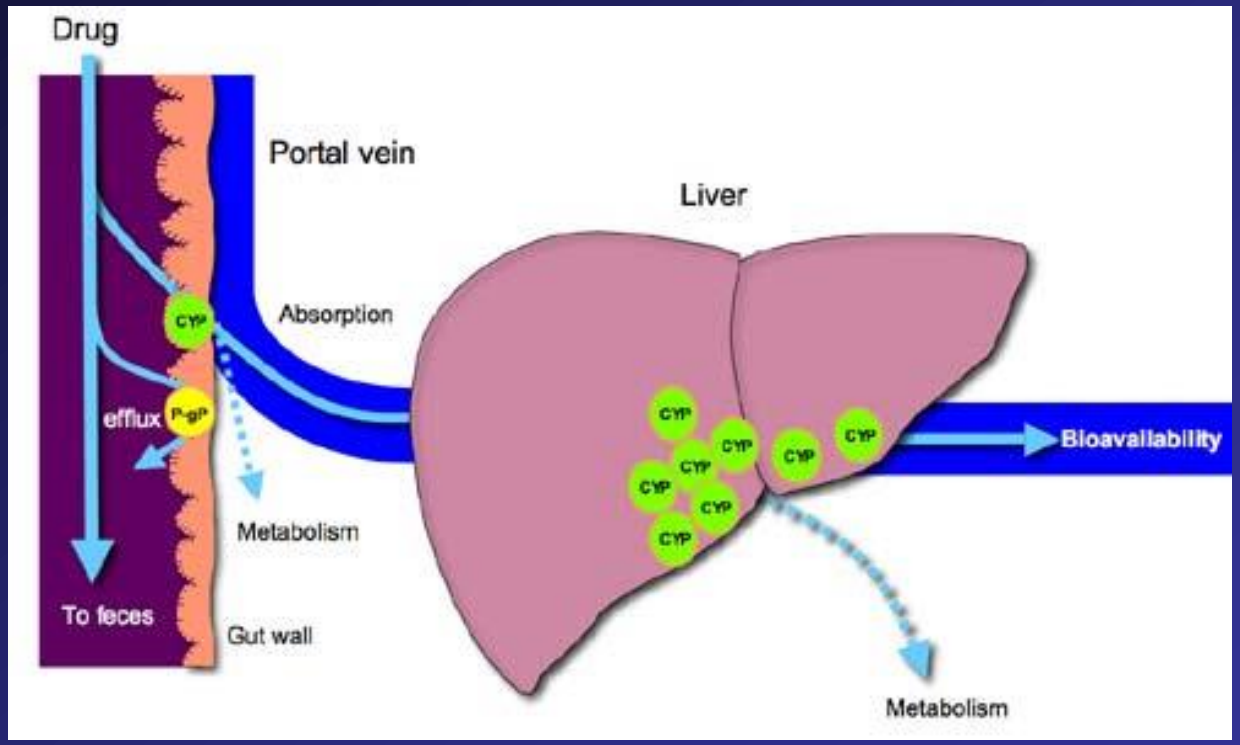
ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AKR, aldo-keto reductases; FMO, flavin monoxygenase; GST, glutathione transferase; MAO, monoamine oxidase; MT, methyltransferase; SDR, short-chain dehydrogenases/reductases; NAT, N-acetyltransferase; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

SOURCE: Data adapted from Williams JA, Hurst SI, Bauman J, *et al.*: Reaction phenotyping in drug discovery: moving forward with confidence? *Curr Drug Metab* 4:527–534, 2003b.

Phases?

- There is criticism of the use of the Phase I and Phase II to describe biotransformations, as it implies a temporal sequence of reactions
- There are cases where conjugation ("Phase II") reactions precede oxidation ("Phase I") for example
- Aside from the debates, do not think of Phase I or Phase II as the temporal relationships, but rather as categorization of biotransformation processes

This visually summarizes previous slides, showing how a drug or toxicant can get absorbed or not, and how it will be potentially metabolized in the liver before getting a chance to be distributed in the body

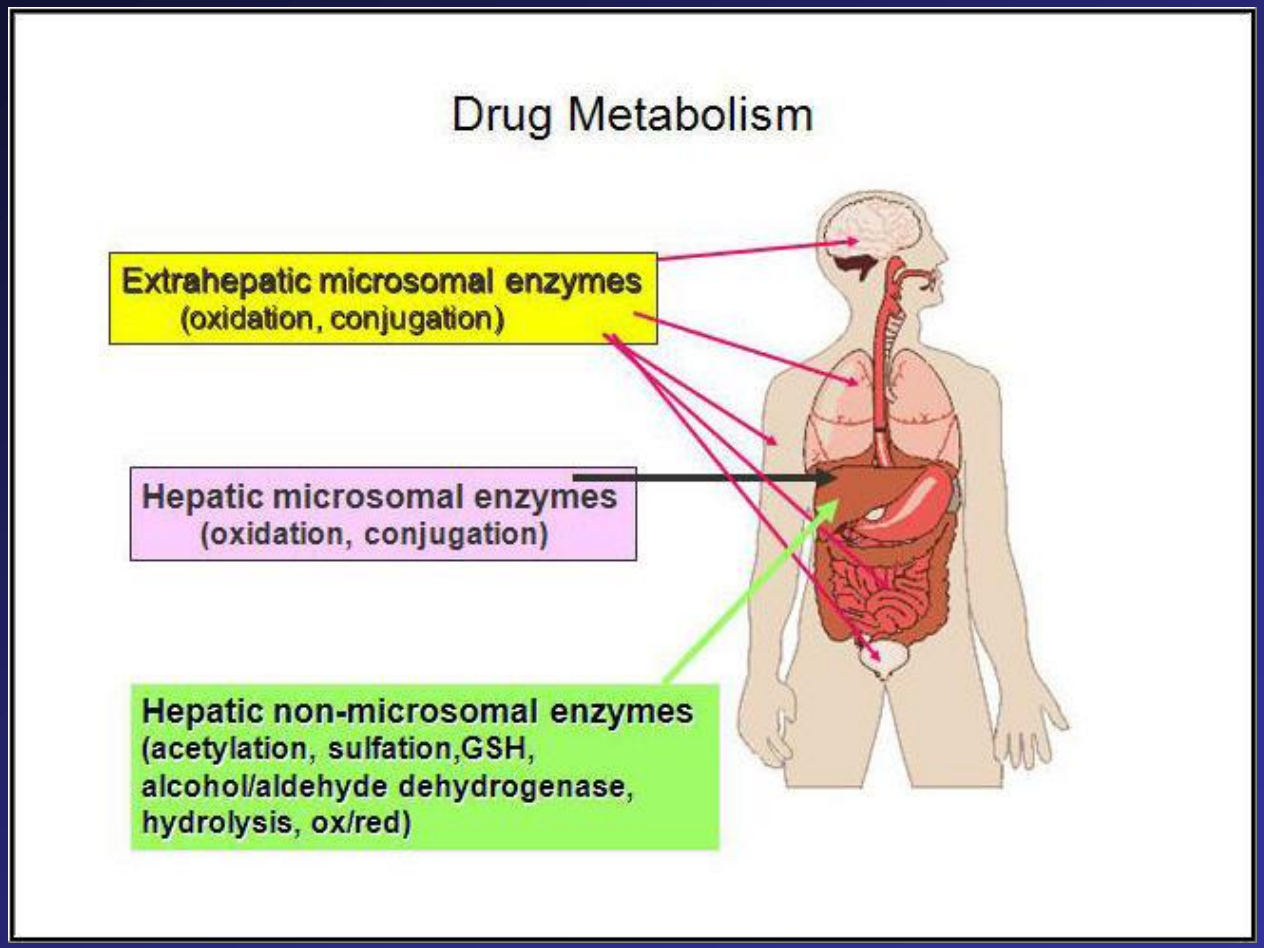


http://www.doctorfungus.org/thedrugs/images/antifung_1.jpg

CYP = cytochrome P_{450} enzymes

P-gp = P-glycoprotein representing itself and other ATP-binding cassette (ABC) efflux transporting membrane proteins involved in multi-drug resistance

This is to summarize what toxicant/drug metabolism (biotransformation) is about, and generally where it happens in the body



http://intranet.tdmu.edu.ua/data/kafedra/internal/magistr/classes_stud/English/First%20year/Clinical%20Pharmacology/01_Clinical%20pharmacokynetic_pharmacodynamic.files/image017.jpg

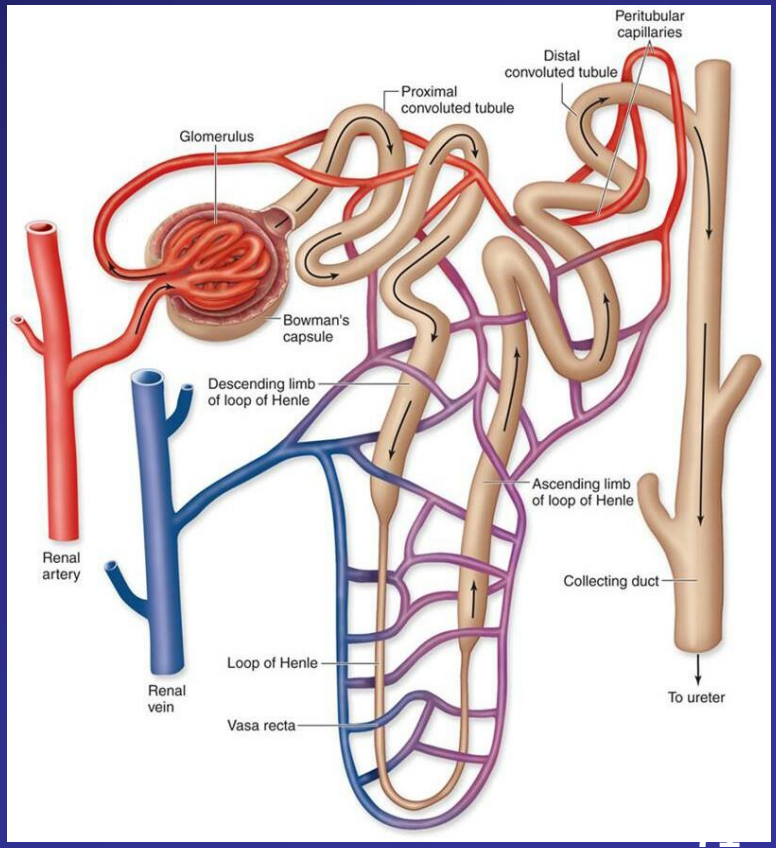
Excretion

- The process of eliminating the drug or its (de-toxified) products
- Routes of elimination
  - Urine (kidney)
  - Feces (GI tract and biliary elimination)
  - Exhalation (lungs)
  - Sweat (skin)
  - Saliva (oral)
  - Breast Milk

Major routes are through urine and feces, so we concentrate on these

Kidney

- More xenobiotics excreted by kidney than any other route
- The nephron is functional unit of excretion
  - Glomerulus
  - Proximal convoluted tubules
  - Distal convoluted tubules
  - Collecting ducts
- Water-soluble compounds of low MW (< 350 Da)



Urinary Excretion

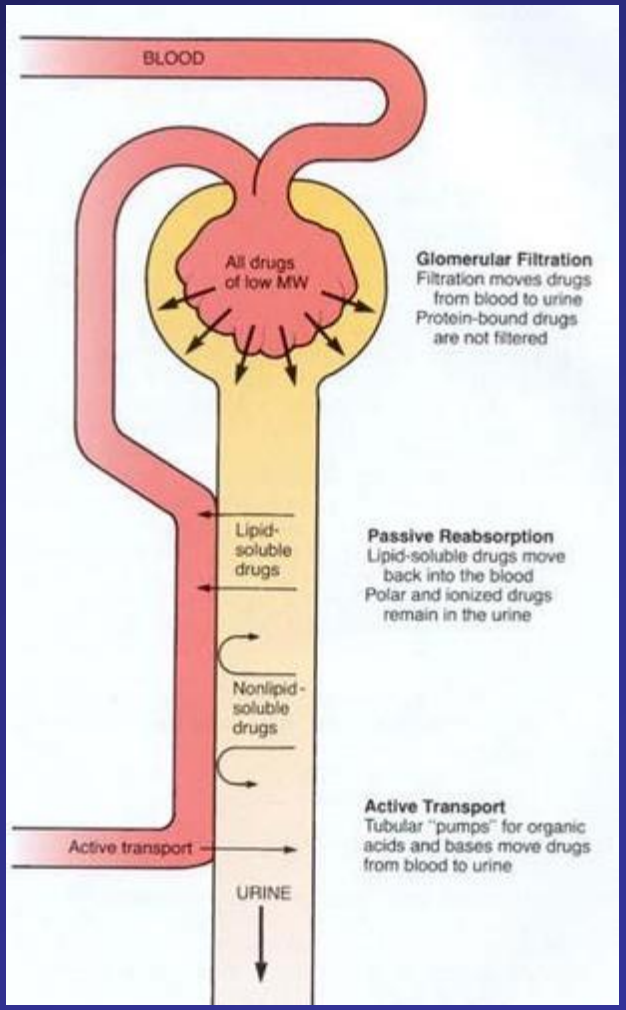
- Glomerular filtration
  - water + small molecules pass through net from plasma into proximal convoluted tubules (PCT)
  - compounds of < 60 kDa pass through large pores
  - albumin-bound xenobiotics retained in blood
  - polar and ionized toxicants filtered here may be excreted
- Tubular Reabsorption

  diffuse from tubule back into peritubular capillaries
- Active secretion into tubules

  Most drugs excreted by this process

Tubular Reabsorption/Secretion

- Lipophilic drugs will be re-absorbed in the proximal convoluted tubule (PCT), while ionized toxicants will pass out to the urine
- Organic anions and cations can be actively secreted into the PCT from the adjoining blood vessels



https://o.quizlet.com/i/ZfKbIDUW0AbVVEH4_97uOg.jpg

Characteristics of Bile

Bile is a yellow-colored mix of bile acids, glutathione, phospholipids, cholesterol, bilirubin (heme metabolite), organic anions, proteins, metals, inorganic ions, xenobiotics

Formation of Bile in Liver

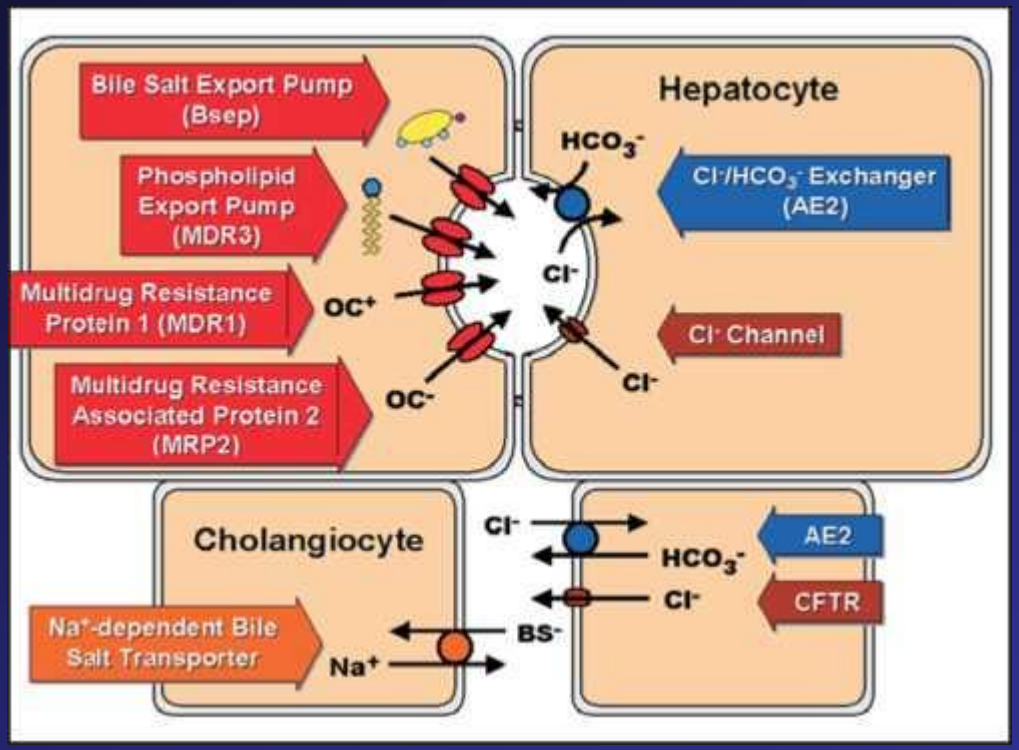
- More xenobiotics excreted by kidney than any other route
- The liver plays its part
- Hepatocytes form channels (canaliculi) between adjacent cells with tight junctions to make intercellular access impermeable
- Proteins on the membrane forming canaliculi contain efflux transporters for organic anions and for lipophilic molecules. These become part of bile.

Bile is a yellow-colored mix of bile acids, glutathione, phospholipids, cholesterol, bilirubin (heme metabolite), organic anions, proteins, metals, inorganic ions, xenobiotics

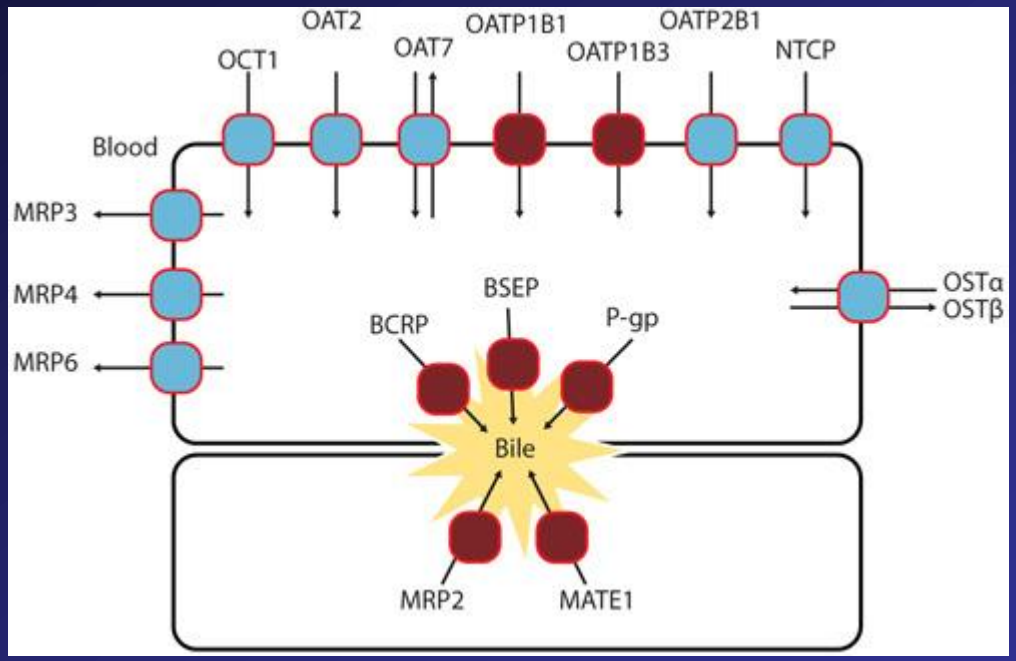
Biliary-Fecal Excretion

- Bile is transported from liver and delivered by a duct into the duodenum of the small intestine
  - it may be temporarily stored in the gall bladder
- Components of bile relevant to toxicology—conjugated and/or biotransormed toxicants/xenobiotics may then be excreted in feces
- Intestinal bacteria could however alter the chemistry of the byproducts, making it possible a toxicant or its metabolite is re-absorbed, thus re-exerting an effect

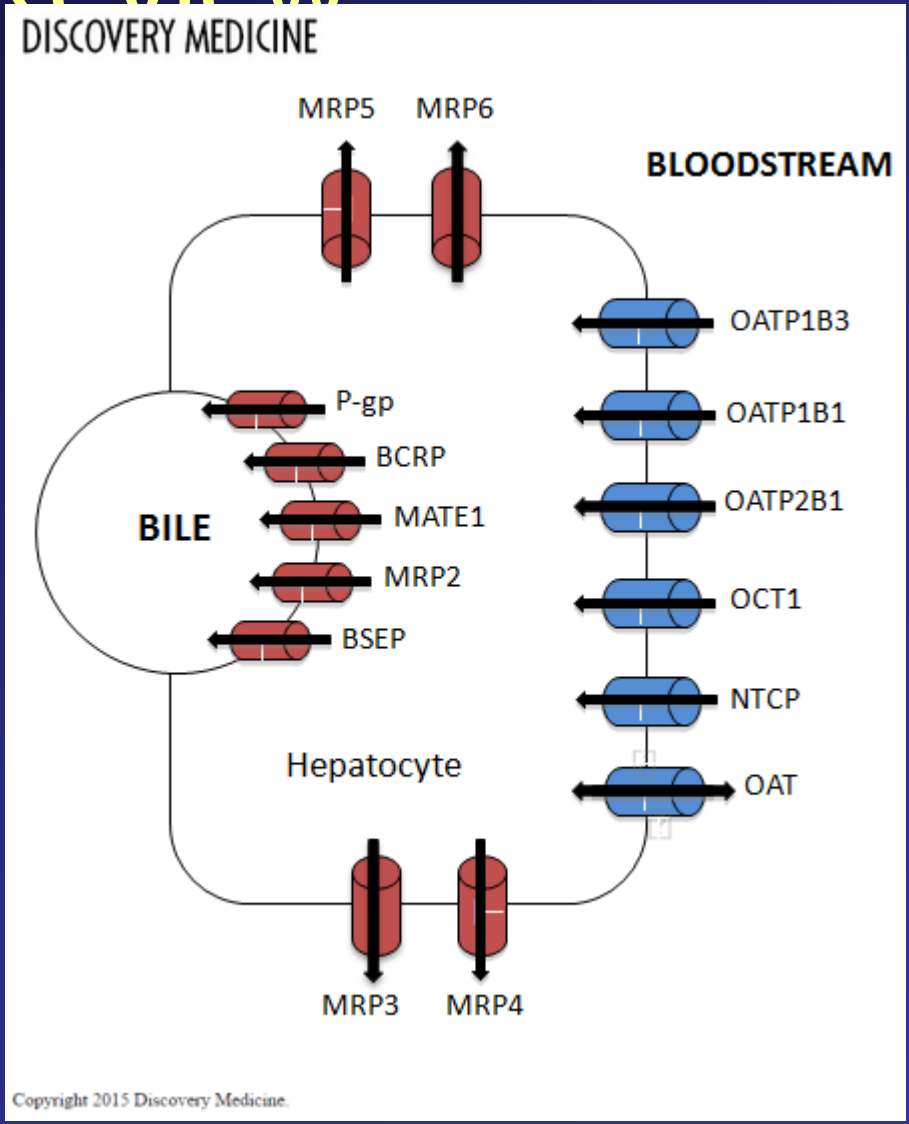
Bile Formation Review



Bile Formation Review



Bile Formation Review



Select transporters expressed on the canalicular and sinusoidal membrane of hepatocytesl. Influx transporters (blue) transport drugs from the blood into hepatocytes where they are metabolized. Efflux transporters (red) then efflux drugs and their metabolites into bile or back into the blood. MATE1, multidrug and toxin extrusion 1 protein; MRP, multidrug resistance protein; NTCP, sodium/ltaurocholate cotransporting polypeptide; OCT, organic cation transportier

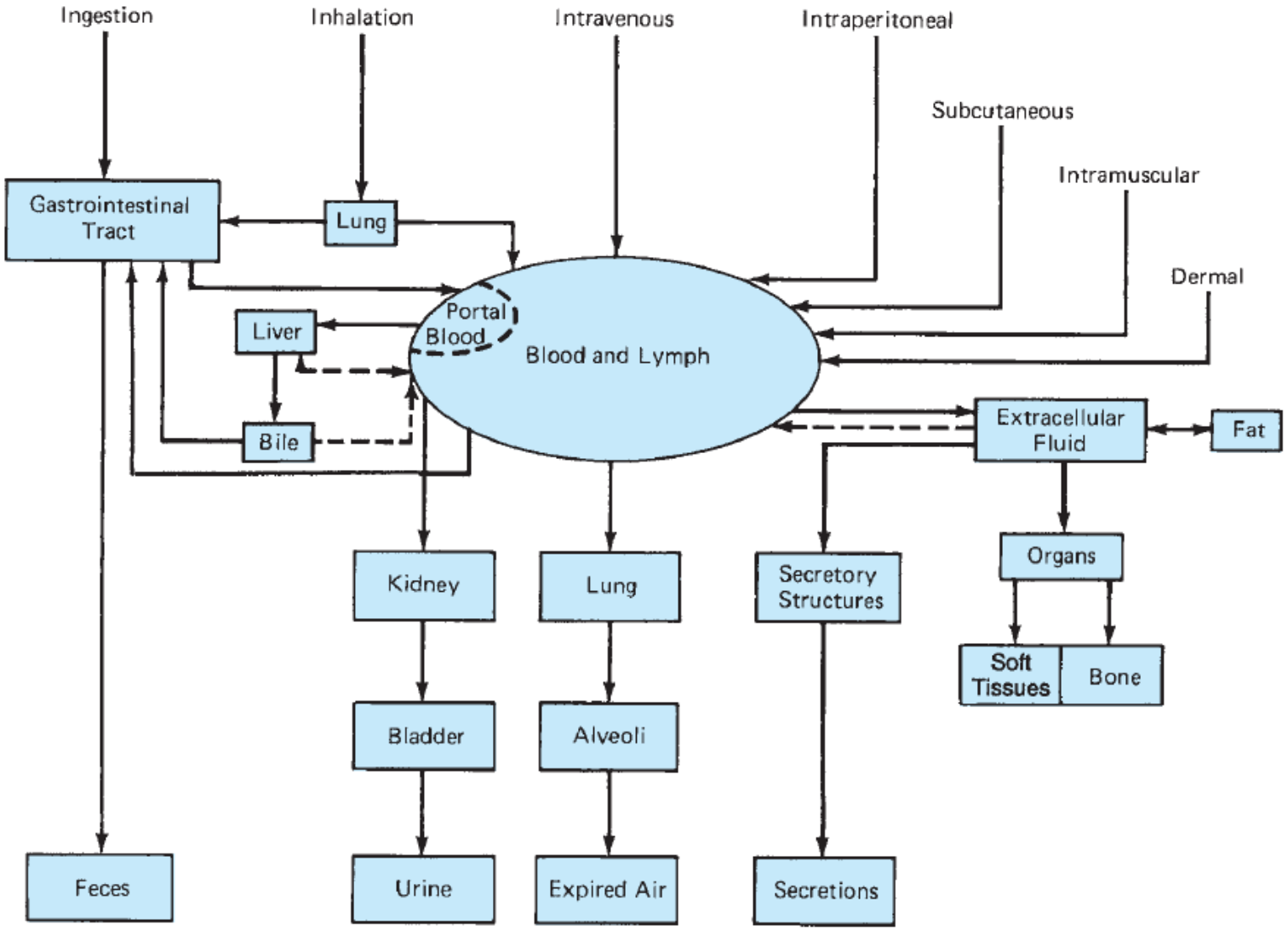
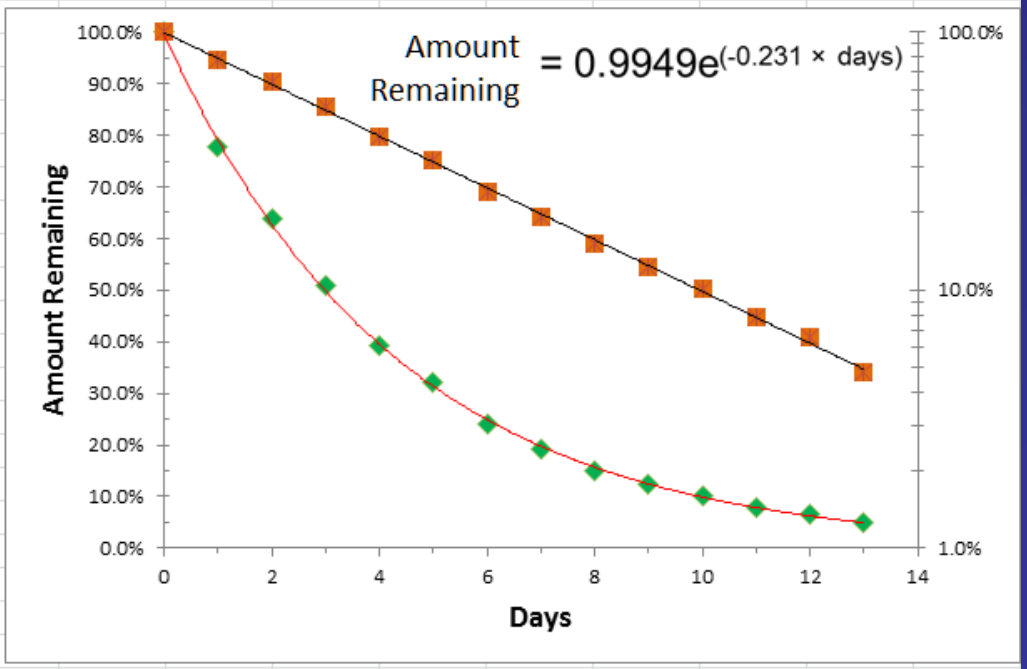


Figure 5-1. Summary of the disposition of toxicants as determined by absorption, distribution, and excretion in the body.

Half-Life

- The time period between an initial amount of something (a substance) to the point where half of the initial amount is present

time	amount	
0	100.0%	100.0%
1	77.9%	77.9%
2	64.0%	64.0%
3	51.0%	51.0%
4	39.4%	39.4%
5	32.0%	32.0%
6	24.0%	24.0%
7	19.2%	19.2%
8	15.1%	15.1%
9	12.3%	12.3%
10	10.1%	10.1%
11	7.9%	7.9%
12	6.6%	6.6%
13	4.8%	4.8%
ln(2)/0.231 =	3.000637	days



Drug/Toxicant Half-Life

- The half-life of a drug depends on its volume of distribution and clearance
- For patients taking therapeutic drugs, the half-life is used to determine the dosing interval in order to maintain a steady-state level (concentration) to maintain the therapy/effect
- For drugs with a narrow therapeutic index (TI), the time required to reach a steady-state level could be much longer since patient body fluids (serum) must be assayed to monitor dose with serum levels
- This value can be computed as

$$t_{1/2} = \frac{\ln 2}{k_{\text{el}}}$$

where k_{el} is the rate of elimination of the drug from the blood

Group Project

1. Find partner
2. Decide an area of interest
3. Develop clear, concise research question in the area of interest
4. Clarify the specific aims in answering the question
5. Do literature search to establish knowledgebase (heavy note taking)
6. Before start of Week 3, submit following
   - research question (item 3)
   - proper bibliography along with question before start of Week 3