BPS3102: Principles of Toxicology and Pharmacology

Pharmacokinetics III *cont.*:

Metabolism – Biotransformation Part 2

- ***Metabolism of xenobiotics***
  - ***Purpose, scope***
  - ***Kinetics***
  - ***Phase I metabolism***
  - ***Phase II metabolism***
  - ***Phase 0/III – transport in and out of cells***

Case Report

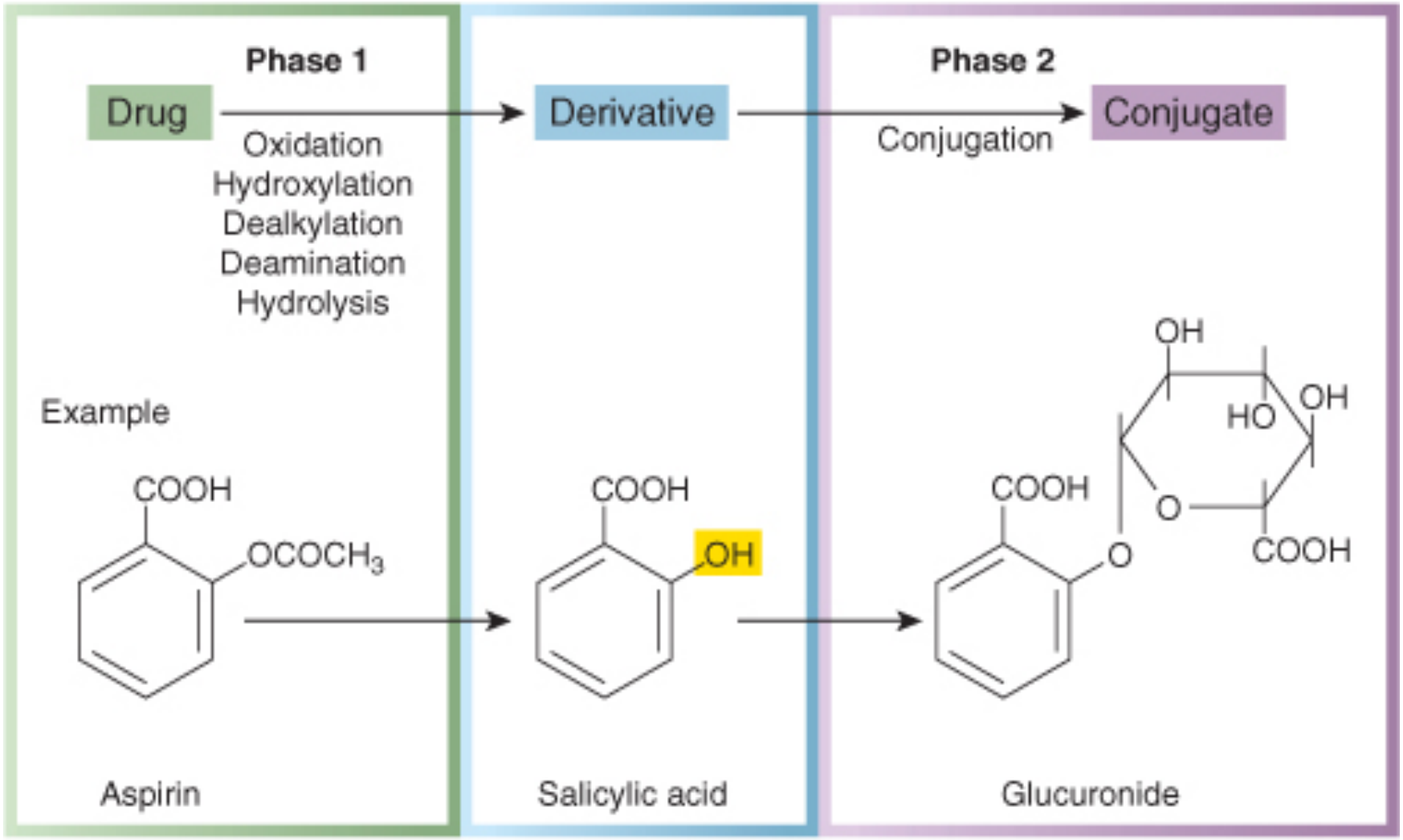
Patient: male, heavy smoker, probably middle aged, suffered back injury

- Treated with numerous pain medications, no relief; eventually moved to a high dose of methadone
- Finally decided to stop smoking
- Presented in emergency – why?

Case Report

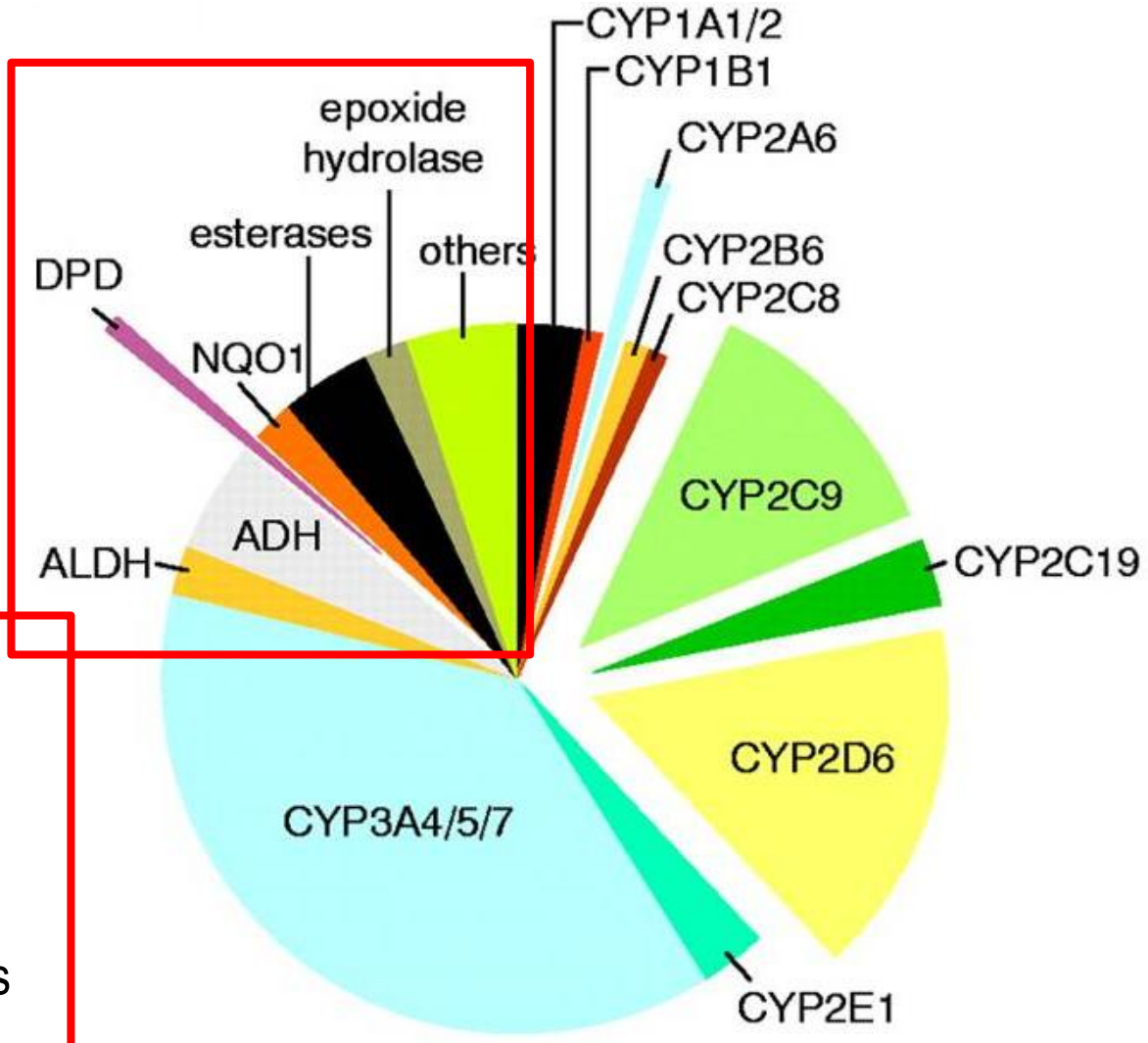
- **Antibiotic and Calcium-Channel Blocker: a Fatal Combination**
  - Garg A, Medscape Nov 18, 2013 reported first in Kidney Week 2013 and published online November 9 in *JAMA.*
- Clarithromycin can ↑ blood concentrations of calcium-channel blockers (such as verapamil, amlodipine, diltiazem) by as much as 500%.
  - an inhibitor of CYP3A4, the enzyme that metabolizes calcium-channel blockers

Drug metabolism



Phase I Enzymes

Add reactive groups
(e.g. -OH, -SH, -NH_2)

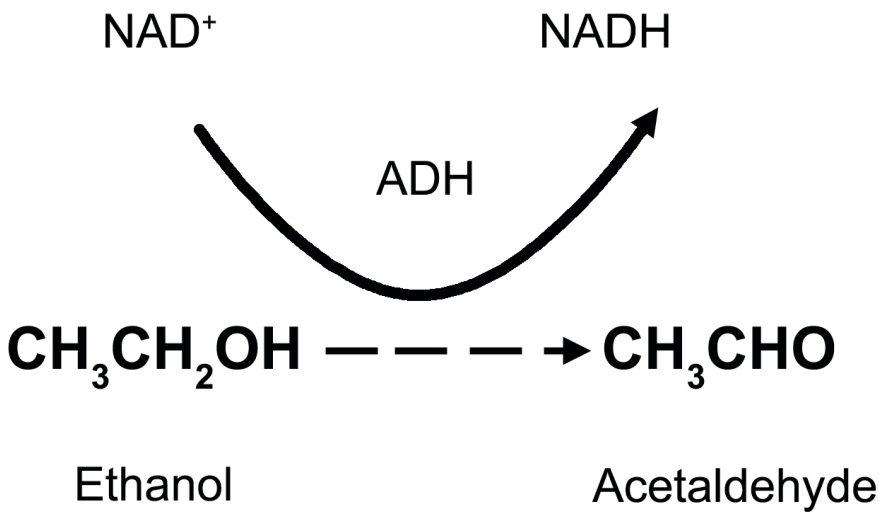


Other enzymes:

- Dehydrogenases
  - alcohol, aldehyde, NHDPH
- Epoxide hydrolases
- Esterases
- Flavin containing monooxygenases
- Monoamine oxidase
- S-oxidases
- aldehyde and xanthine oxidases

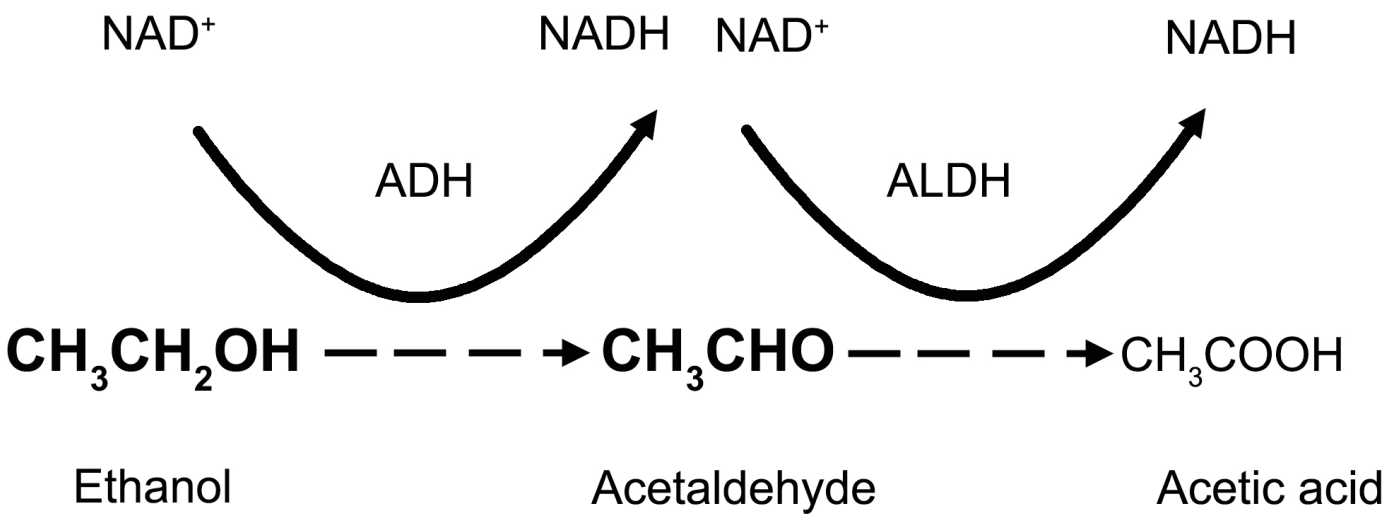
Alcohol dehydrogenases (ADH)

- **Reaction:** alcohol (RCH_2OH) to acetaldehyde (RCHO)
- **Probe drug:** ethanol
- **Substrates:** ethanol, choral hydrate
- **Genotype – Phenotype:** >6 genes contributing to 5 classes of dimeric ADH enzymes
  - hepatic form (class 1) primary in humans
  - Expression and activity vary between men and women, young and old, different populations



Aldehyde dehydrogenase 1 (ALDH)

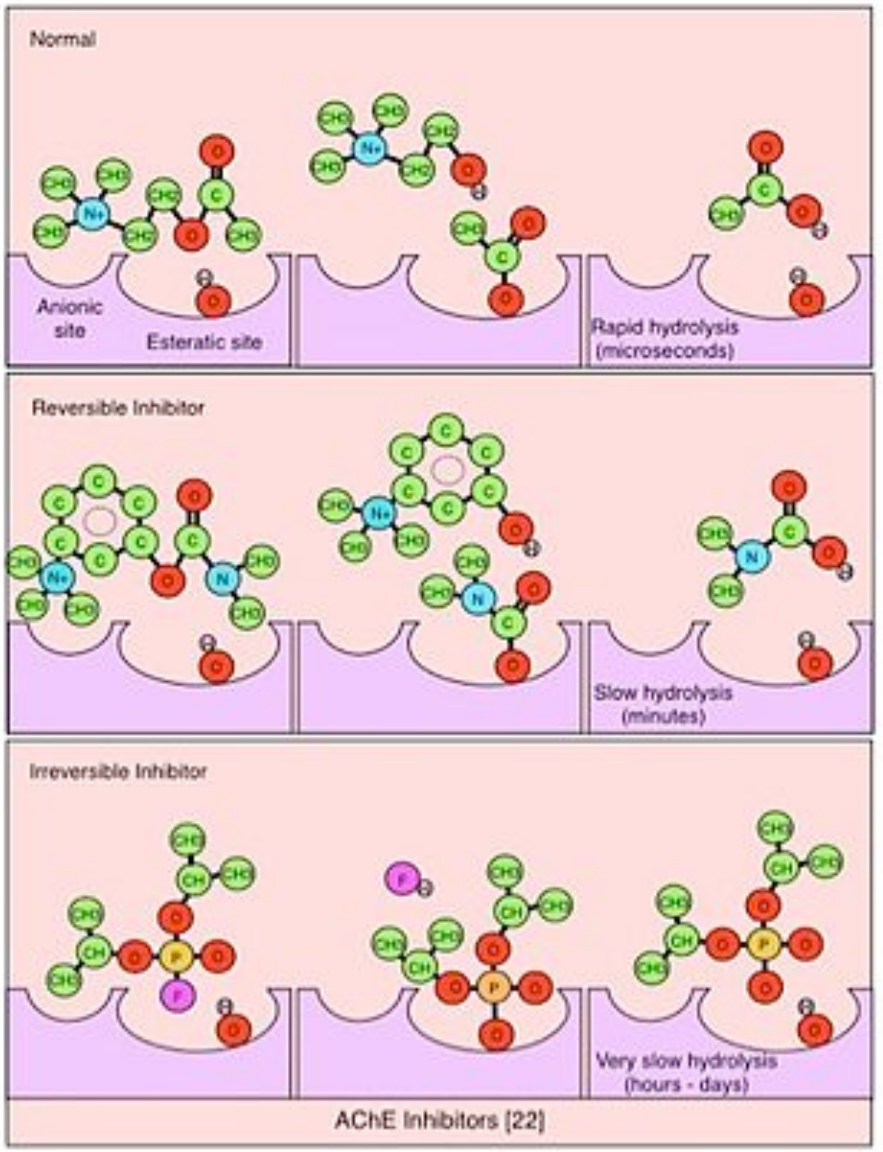
- **Reaction:** aldehyde (CHO) to acetate (COOH)
- **Substrates:** aldehyde, cyclophosphamide
- **Genotype – Phenotype:** 19 ALDH genes identified in human genome
  - Endogenous and exogenous aldehydes
  - ALDH1 & 2 most important; widely distributed but highest in liver
  - Deficiency results in the *Alcohol flush reaction*



Carboxylesterases

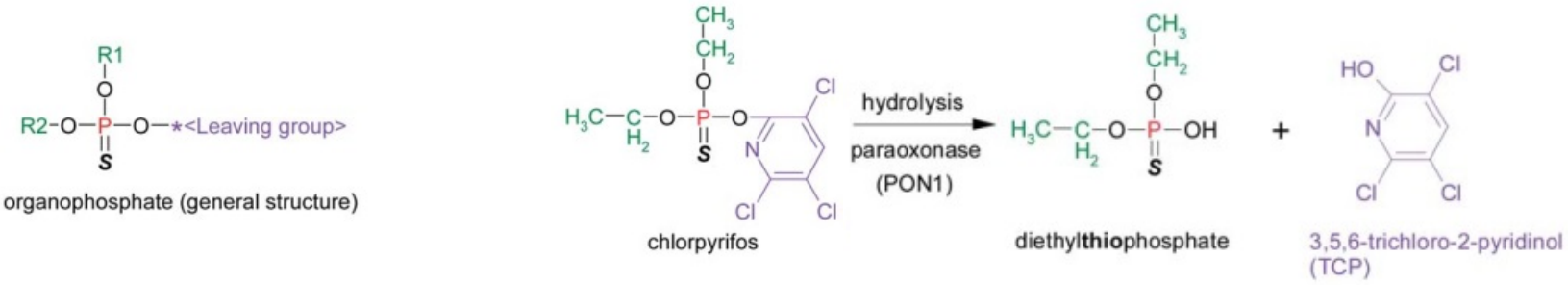
Acetylcholinesterase (AChE)

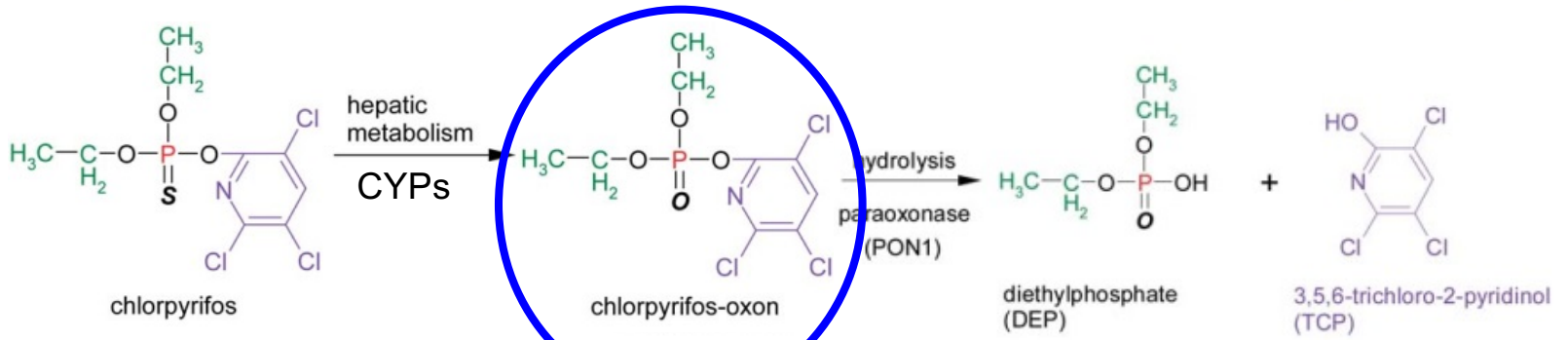
- **Reaction:** ester cleavage (RO--COR' to RO-H), scavenger role
- **Substrates:** anionic substrates; succinylcholine, substance P, diacetylmorphine, **acetlycholine**
- **Genotype – Phenotype:**
  - Single gene (mammals); alleleic variants give defective binding
  - Primary target of inhibition by organophosphorus xenobiotics (nerve gas - sarin, pesticides - malathion)
  - Target of Alzheimer`s Disease pharmacotherapy



Paraoxonase (PON1)

- carboxyl**esterase**; polymorphism common, 3 isoforms (PON1, PON2, PON3)
  - metabolize only a few drugs
  - prevents low-density lipoprotein oxidation
  - PON1 substrates: organophosphorus pesticides and nerve gas, carboxylic acid esters



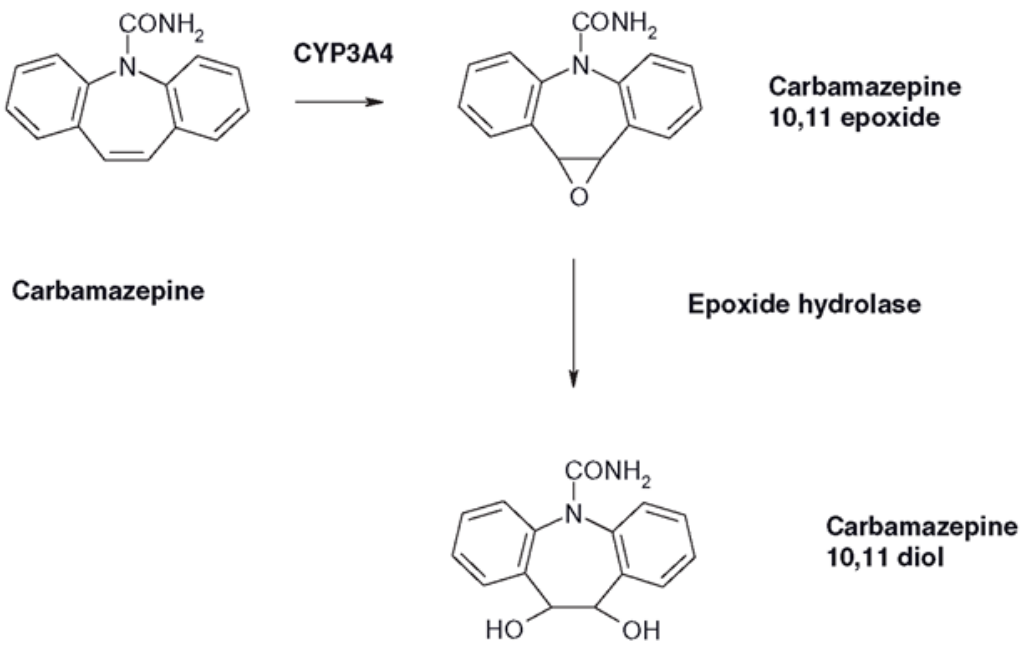


More potent inhibitor of AChE than chlorpyrifos

Epoxide hydratases (hydralases)

- **Reaction:** hydrolysis of epoxide (C2H2O) to trans-dihydrodiol (C2H2O2) intermediates;
- **Substrates:** phenytoin, carbamazepine, valproate, PAHs, aromatic amines, benzene
- **Genotype – Phenotype:** 4 isoforms: 2 metabolic roles, microsomal and cytoplasmic which hydrolyze range of substrates

- Detoxifies reactive primary metabolites



Flavin-dependent mono-oxygenase (FMO)

- Five families in mammals (FMO 1-5), 50-58% identity
  - subject to hormonal and developmental regulation with distinct patterns of gene expression in different tissues and species
- Not easily inducible nor readily inhibited, smooth endoplasmic reticulum membrane bound
- Catalyze the 4 electron reduction of dioxygen with 2e from NADPH and 2e from substrate
- Isoforms - pH and substrate dependent

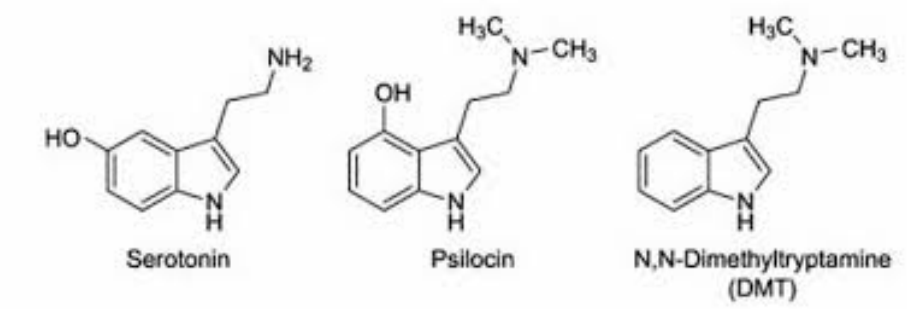
Cashman and Zhang, Annu Rev Pharmac Toxicol 46:65-100, 2006

Flavin-dependent mono-oxygenase (FMO)

- **Reaction:** oxidation at N, P, Se and S heteroatom centres
  - generally gives harmless, polar, readily excreted metabolites
- **Substrates:** wide range of secondary and tertiary amines, clozapine, chlorpromazine, methionine, morphine, nicotine, ranitidine, tamoxifen
- **Genotype – Phenotype:** >5 isoforms (humans), different structural properties and enzymatic reactions
  - Inhibited by thiourea
  - FMO1 – “hepatic” and highly stereoselective S-oxidation, main animal form
  - FMO 3 - most important human isoform; highest expression in human liver; not stereoselective; polymorphic

Monoamine oxidase (MAO)

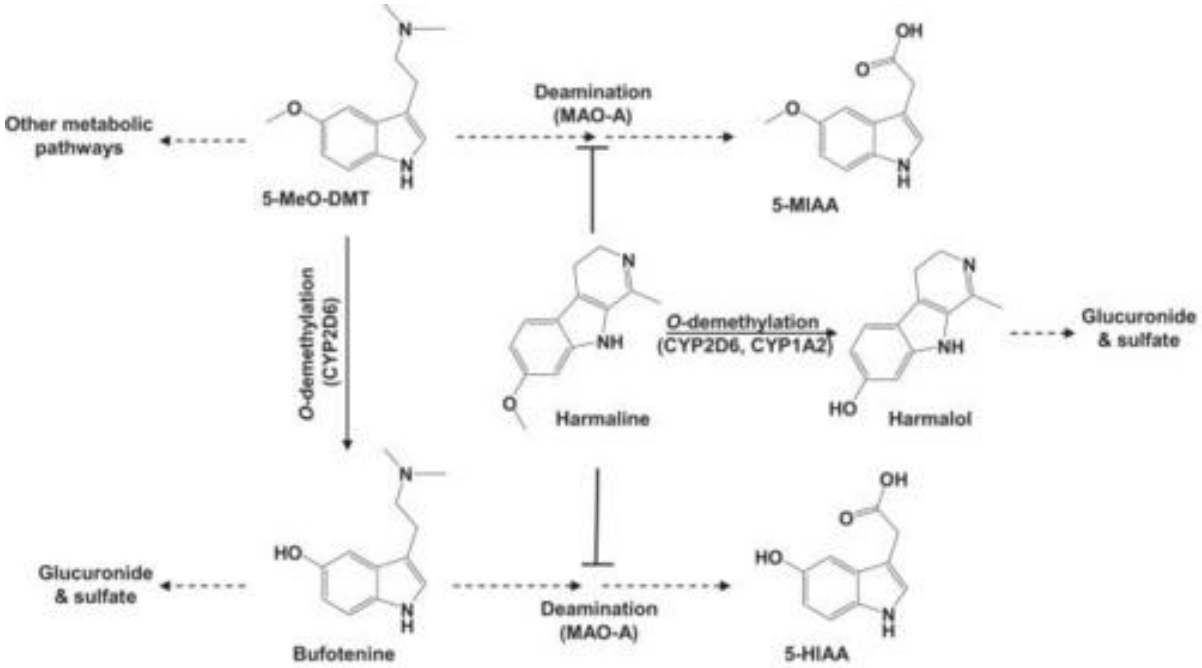
- **Reaction:** amine (RCH2NH2) oxidation leading to oxidative deamination (RCH2CHO)
- **Substrates:** monoamine drugs, sumitriptan, **monoamine neurotransmitters**
- **Genotype – Phenotype:** 2 types (humans): MAO-A & MAO-B (polymorphic)
- Both are found in neurons and astroglia
  - MAO-A is also found in the liver, pulmonary endothelial, gastrointestinal tract, and placenta
  - MAO-B is mostly found in blood platelets
- MAO-A: serotonin, melatonin, noradrenaline, adrenaline
- MAO-B: phenethylamine and benzylamine
- Both forms break down dopamine and tryptamine



Monoamine oxidase (MAO)

Ayahuasca (**yagé**), is a ceremonial and psychedelic plant mixture used in South America

- *Banisteriopsis caapi* vine alone or in combination with various plants.
- Usually mixed with the leaves of dimethyltryptamine (DMT)-containing species from the genus *Psychotria*

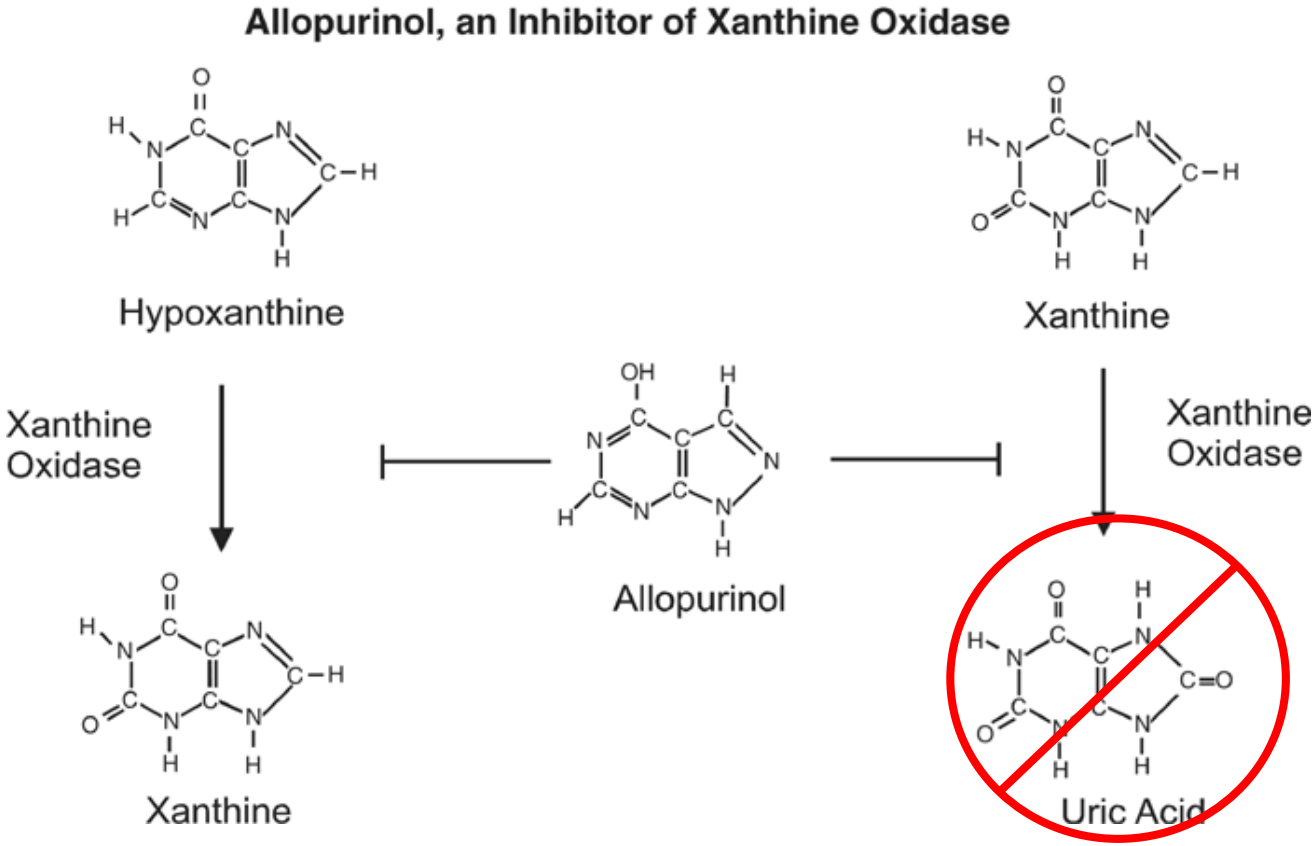


- indigenous Amazonian people received guidance directly from the plants and plant spirits.

Xanthine oxidase (XO)

- **Reaction:** oxidation of xanthines, nucleotide metabolism,
- **Substrates:** xanthines – caffeine, theophylline, theobromine
- **Genotype – Phenotype:** slow and ultra rapid metabolizers are rare.

- For gout and kidney stones



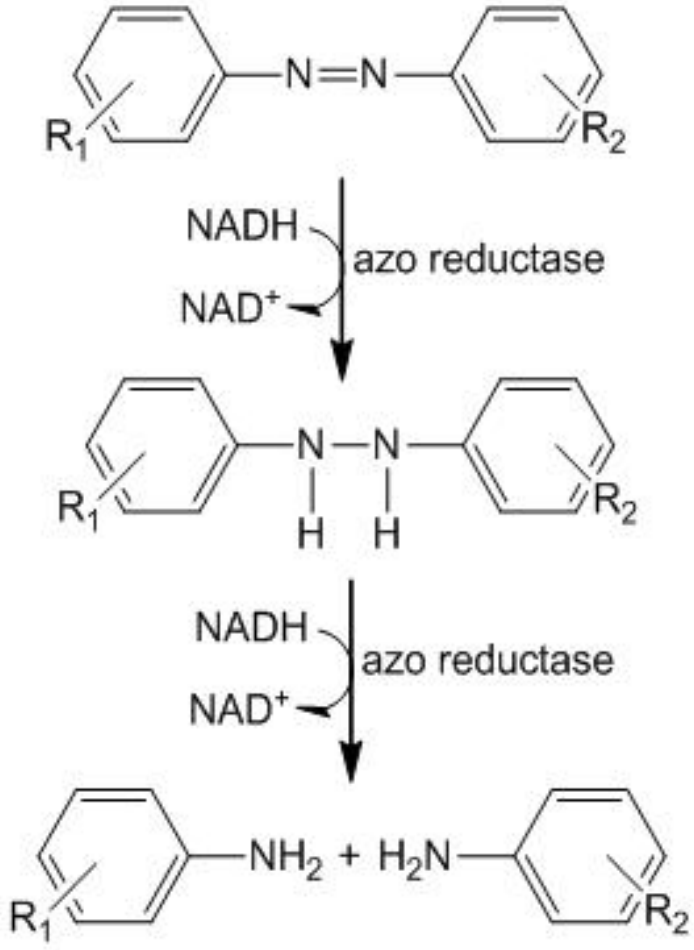
Others

Reductases: azo-, nitro-, N-oxide: act on nitrogenous compounds (donors) with NAD+ or NADP+ as acceptor

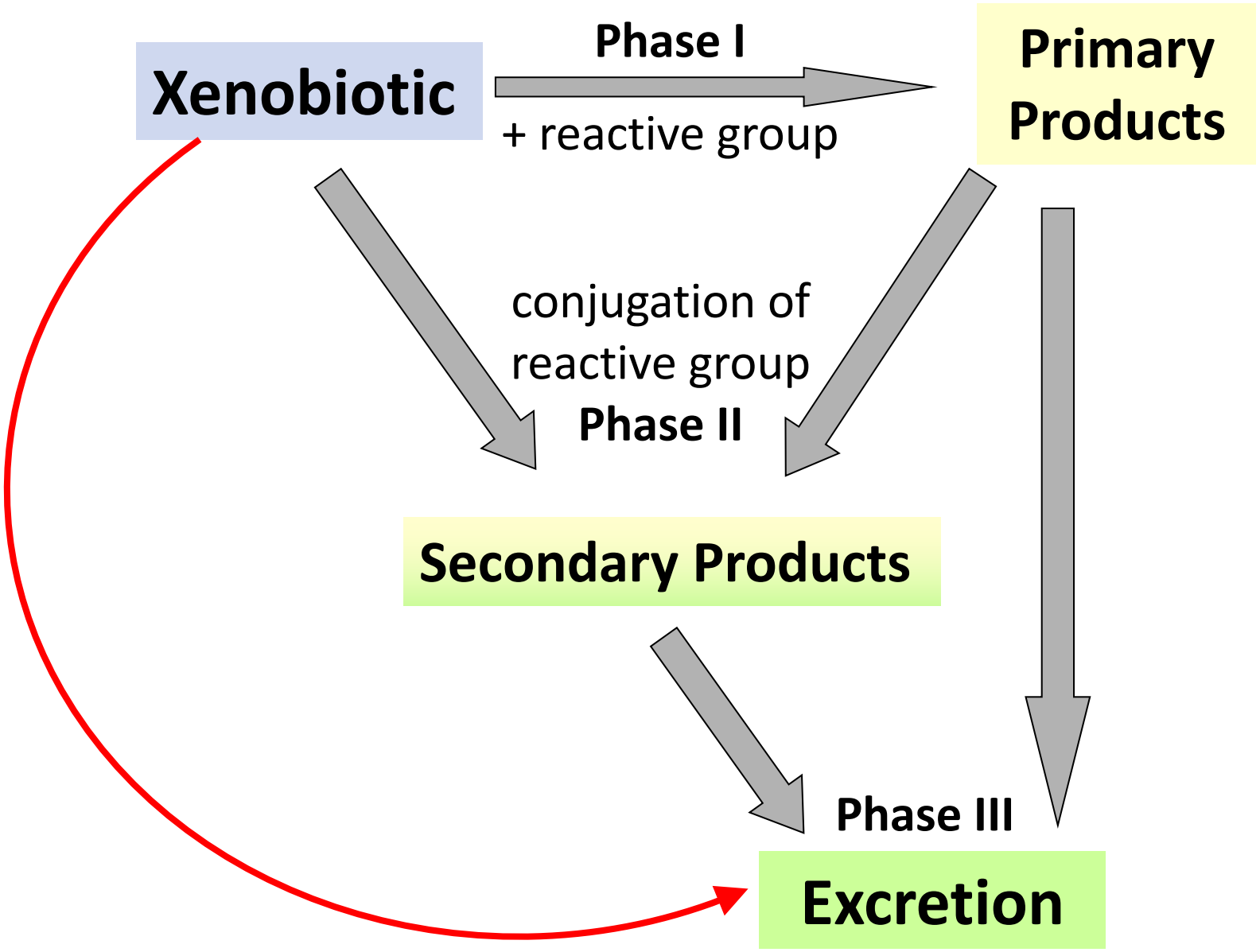
$$RN{=}NR_1 \rightarrow RNH_2 + R_1NH_2$$

$$RNO_2 \rightarrow RNH_2$$

$$RNO \rightarrow RNH_2$$



Phases of metabolism



Phase I & II metabolites

- **Phase I metabolites** are generally more polar and less-lipid soluble than parent
  - lower penetration into tissues
  - less tubular resorption

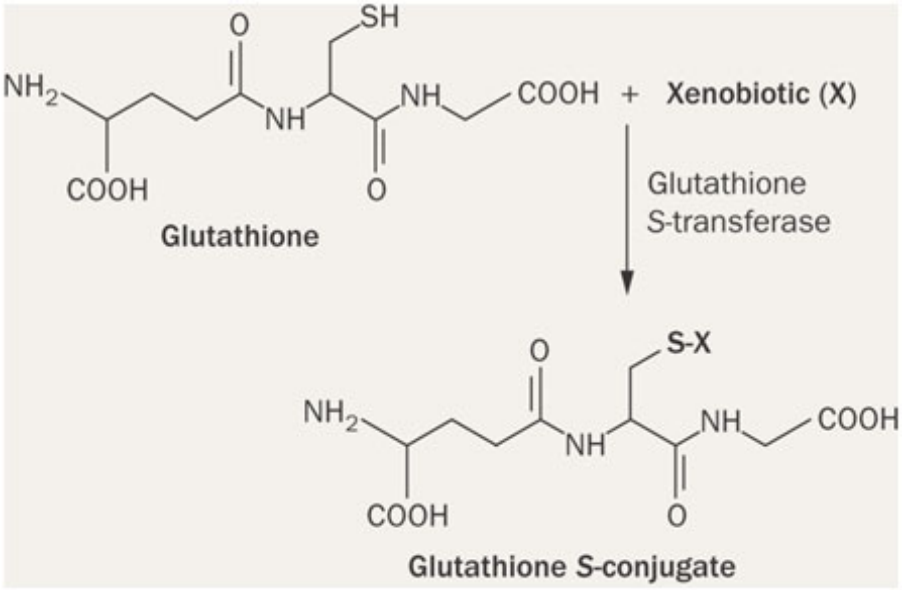
- **Phase II metabolites** are generally less active than parent
  - may retain some activity and toxicity
  - “inactive” at target site does not always mean inactive
  - Phase I metabolites are generally more active / toxic than Phase II metabolites

Phase II enzymes

- Glutathione S-transferases
- N-acetyltransferases
- N-acyltransferases (amino acids)
- N-,O-,S-methyltransferases
- Sulfotransferases
- UDP glucuronyl transferases

Glutathione S-transferase M1

- **Reaction:** conjugation of halocarbons and particularly bifunctional electrophiles, with reduced GSH.
  - **Detoxification but some substances are activated.**
- **Frequency – PM:** SNPs in M1: 50% Caucasians and Japanese are homozygous low, ~60% Chinese, ~33% Asian Indians.
- **Substrates:** electrophilic agents and many carcinogens

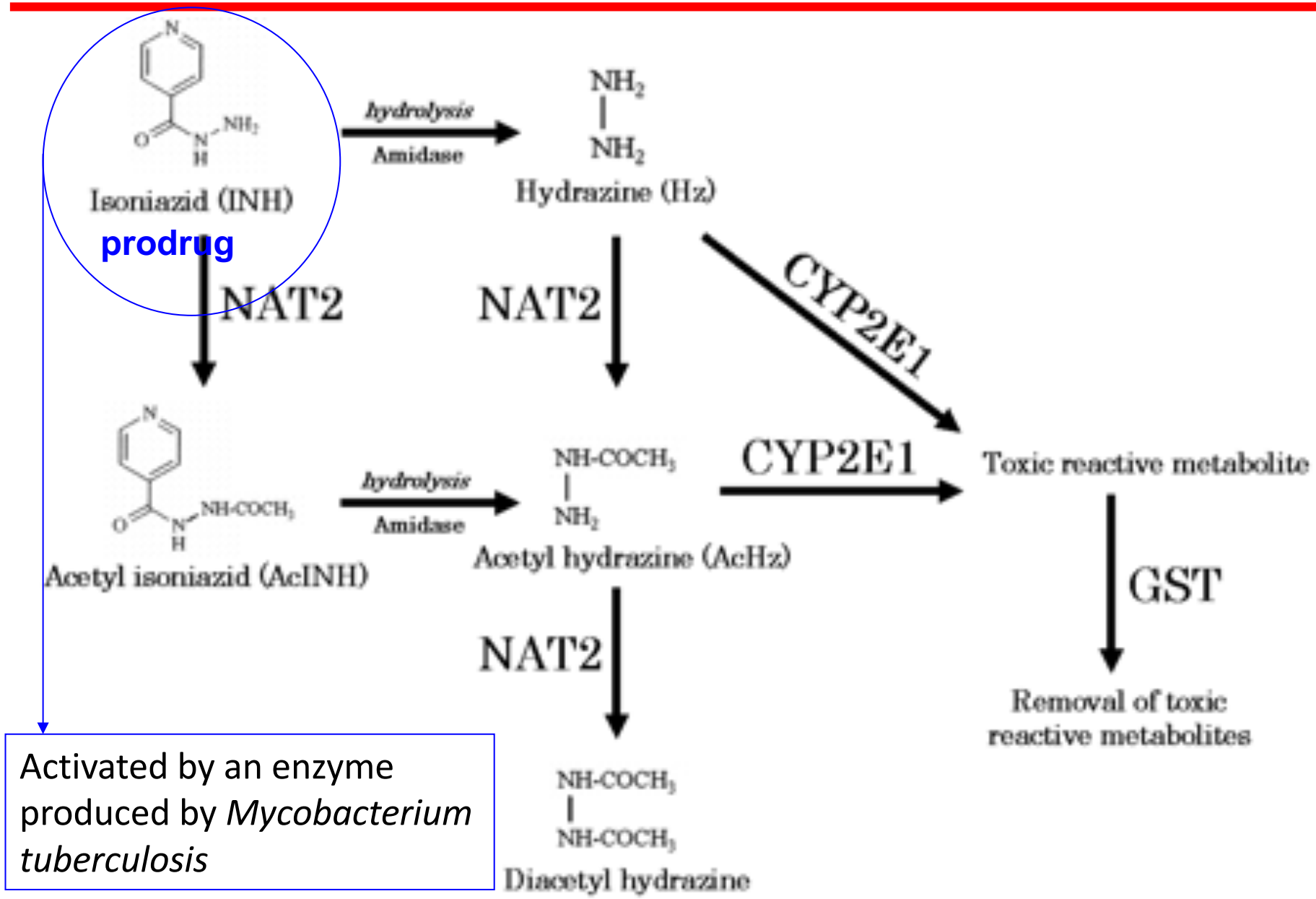


N-Acetyltransferases (NAT)

- **Reaction:** catalyzes the transfer of acetyl groups from acetyl-CoA to arylamines.
  - Two isozymes (NAT1, NAT2), polymorphic
  - wide specificity for aromatic amines (e.g. serotonin, folate)
  - reaction product is more ***hydrophobic***

- **Substrates:** arylamine drugs and environmental chemicals (e.g. cigarette smoke, exhaust fumes)
  - either detoxified and eliminated or bioactivated with potential to cause toxicity and/or cancer.

N-Acetyltransferases



Biopharmaceutics in action

- Nature Communications, Ueki N. *et al.*, 2013.
  - researchers deactivated the toxic agent puromycin to create a prodrug by adding an acetylated lysine:
    - inactive until it interacts with enzymes produced in the cancer cells it targets;
    - proposed activating enzymes include histone deacetylases (HDACs) and the protease cathepsin L (CTSL) that cleaves the lysine.
  - Puromycin is then active to kill any nearby cells by interrupting protein synthesis.

Catechol-O-methyltransferase (COMT)

- **Reaction:** O-methylation of catecholamines
- **Frequency – Population:** Caucasians 25% homozygous for each low and high (single gene)
- **Substrates:** L-dopa, methyldopa (dopamine, epinephrine, norepinephrine).
  - a cytoplasmic soluble form predominantly expressed in the human liver, intestine and kidney
  - a membrane–bound form more highly expressed throughout the human central nervous system

Catecholamine regulation is impaired in a number of medical conditions so several pharmaceutical drugs target COMT

S-methyltransferases

Thiol methyltransferase

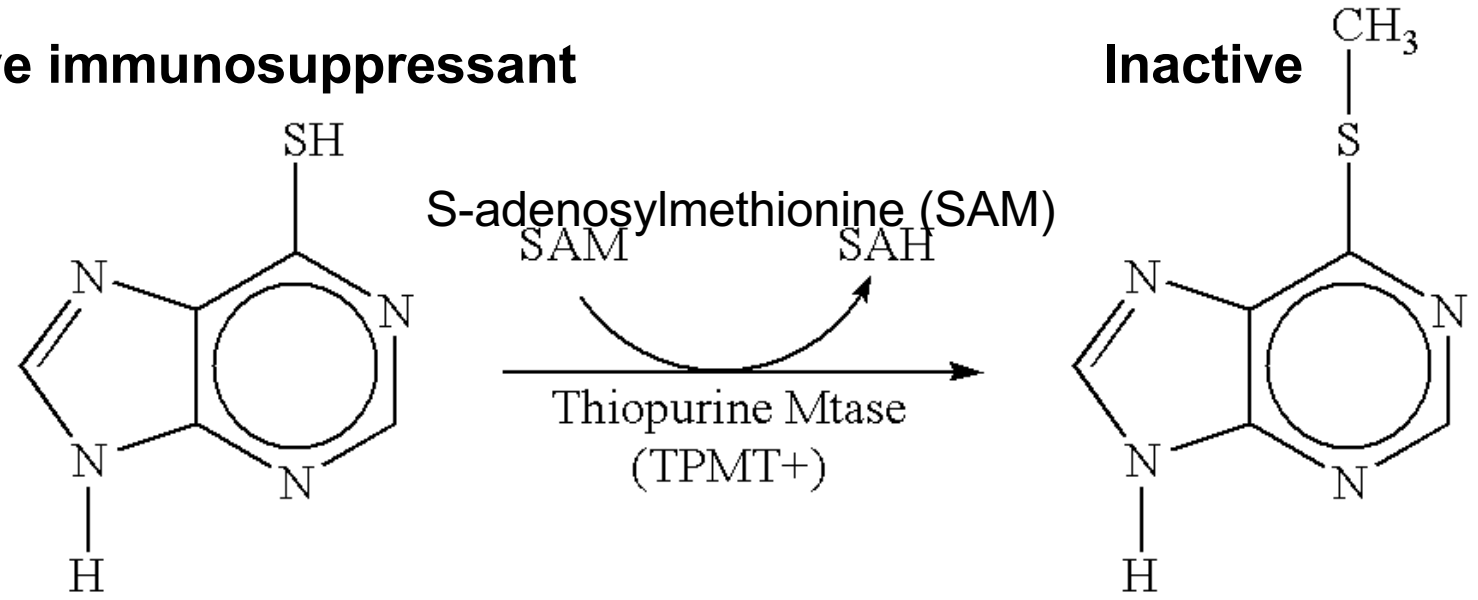
- **Reaction:** S-methylation of ***aliphatic sulfhydryls***
  - Membrane bound enzyme
- **Substrates:** captopril (antihypertensive), D-penicillamine (antirheumatic), xenobiotic thiols

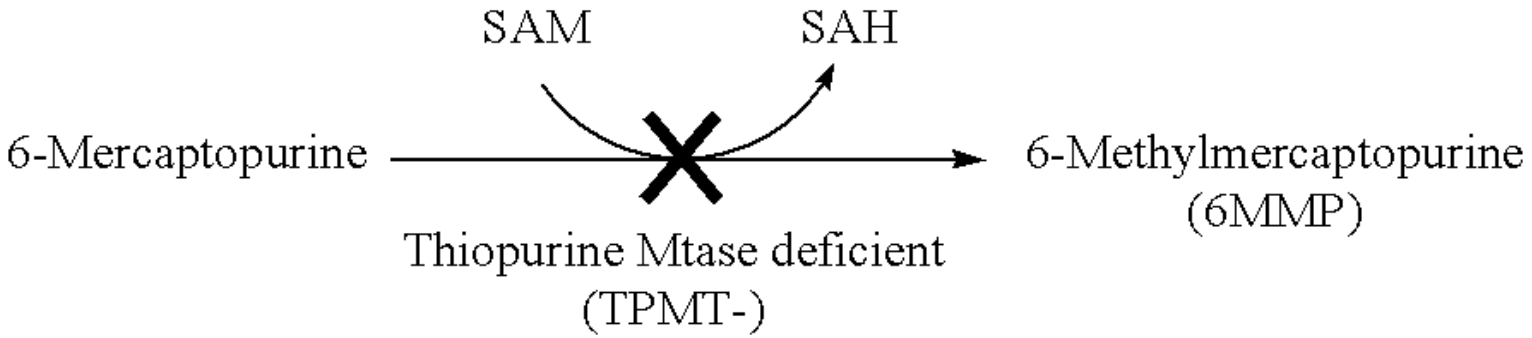
Thiopurine methyltransferase

- **Reaction:** S-methylation of ***aromatic & heterocyclic sulfhydryls*** (cytoplasmic)
- **Substrates:** anticancer and immunosuppressive thiopurines (narrow therapeutic index)

Thiopurine methyltransferase

Active immunosuppressant

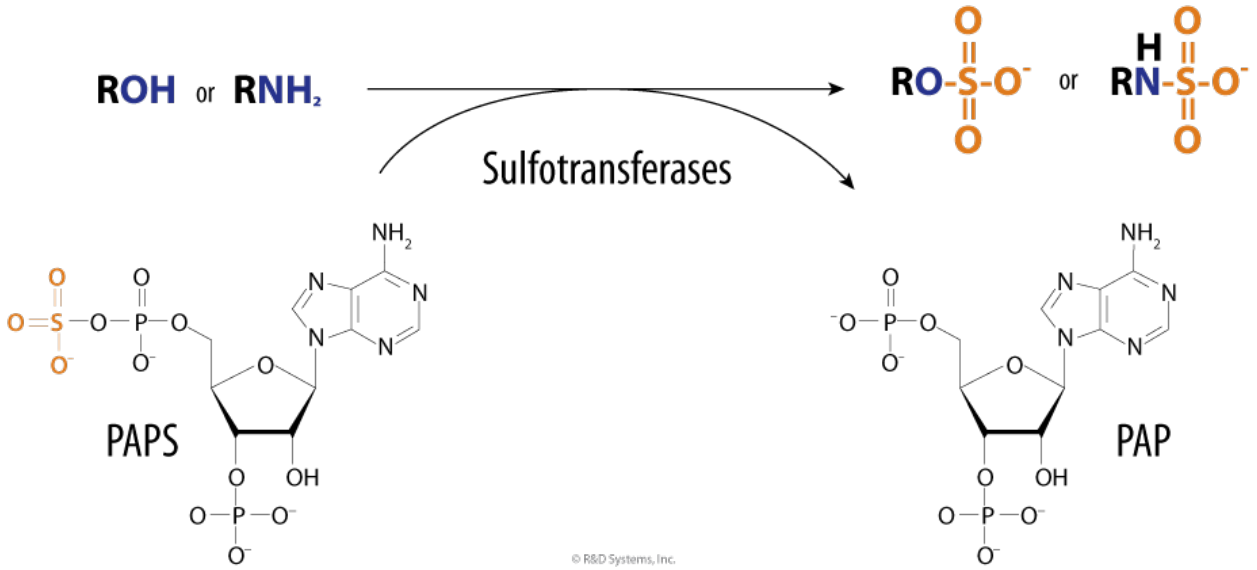




- Slow metabolizers (<1% of pop'n) treated with 6-10% of standard dose

Sulfotransferase (SULT)

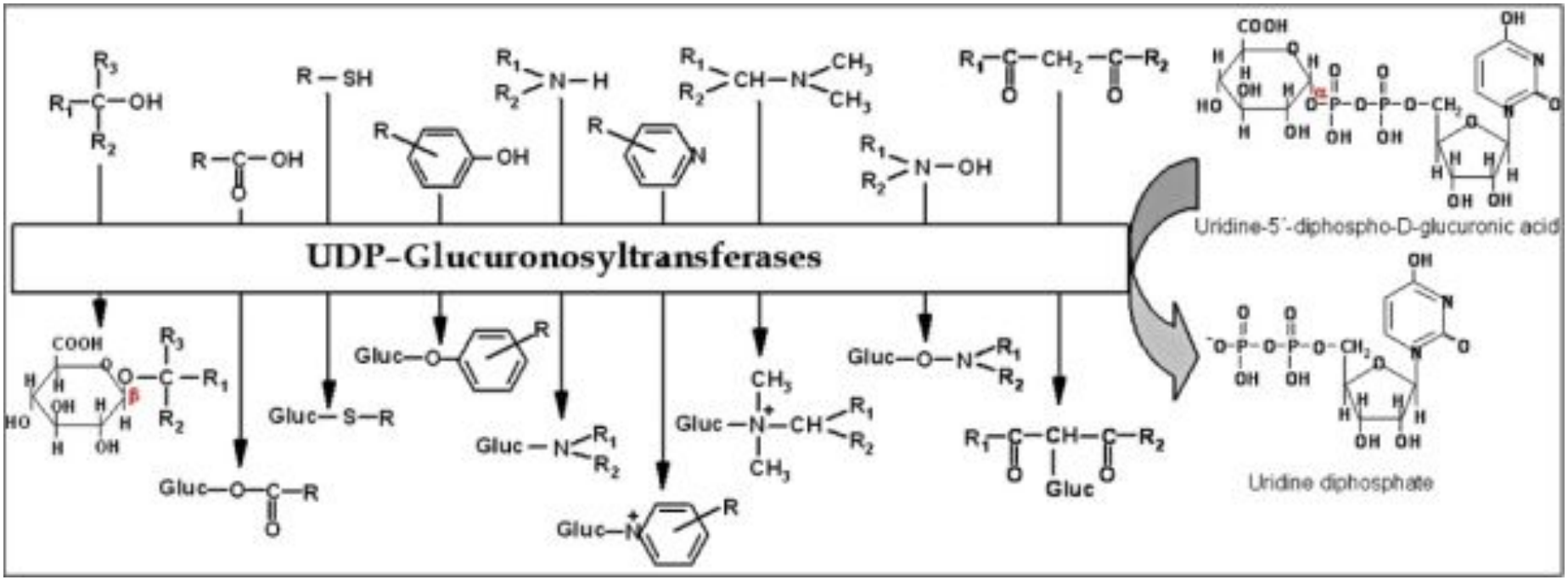
- **Reaction:** transfer sulfonate (SO_3) from donors to form -ROH, -SH or -NH_2 sulfate or sulfamate conjugates
  - more hydrophilic and less (or more) bioactive
- **Substrates:** non-peptide hormones (e.g. 17-estradiol) and many xenobiotics (acetaminophen)
- **Genotype – Phenotype:** at least 13 human genes in 3 families (SULT1, SULT2, SULT4), polymorphic, membrane bound and cytosolic forms



Uridine 5'-diphosphate-glucuronosyltransferase (UDPGT)

- **Reaction:** conjugation of small lipophilic molecules with D-glucuronic acid ($C_6H_{10}O_7$)
  - major pathway for removal of most drugs, dietary substances, toxins and endogenous substances
  - Increased polarity and solubility
- **Substrates:** isoforms have overlapping specificities, collectively conjugate 1000's of compounds
- **Genotype – Phenotype:** kidney, intestine, nasal mucosa, brain, liver
  - 2 families relevant to xenobiotic metabolsm (UGT1 and UGT2) with highly divergent and tissue specific activity

UDPGT


UDP-Glucuronosyltransferases
Uridine-5´-diphospho-D-glucuronic acid
Uridine diphosphate

Human Liver Microsome Catalytic Activity

21 samples (14 males, 6 females, 1?); nmol/min per mg protein

Phase I

	1A2	2A6	2C9	2C19	2D6	2E1	3A4	FMO
Mean	0.043	0.433	0.163	0.032	0.15	0.376	2.22	1.18
Range	3.0x	21x	7.9x	175x	17.7x	4.8x	17.9	2.5x

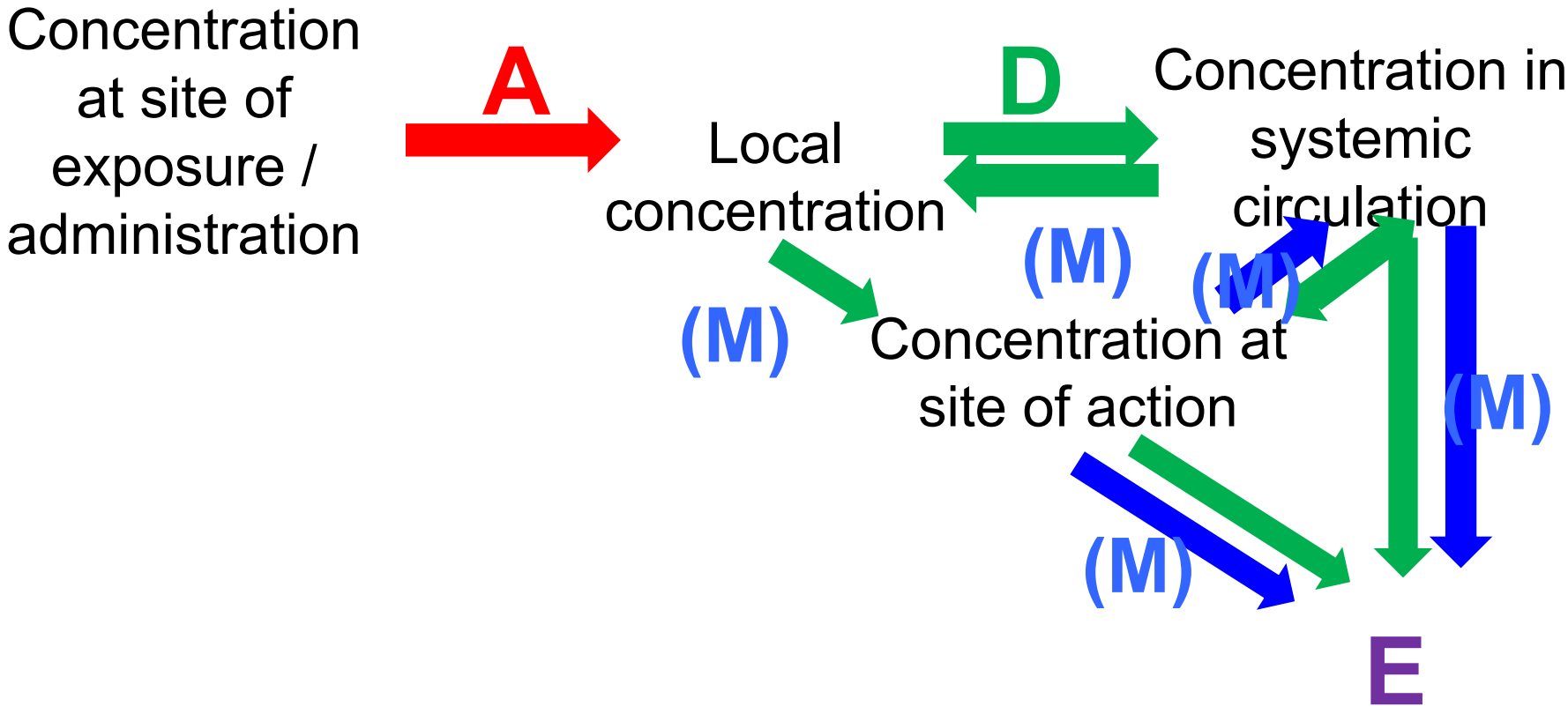
Phase II

	GST	UDPGT	NAT	ST	TPMT	COMT
Mean	2.46	17.8	0.628	0.308	0.04	0.246
Range	3.3x	3.8x	7.1x	4.8x	2.7x	4.7x

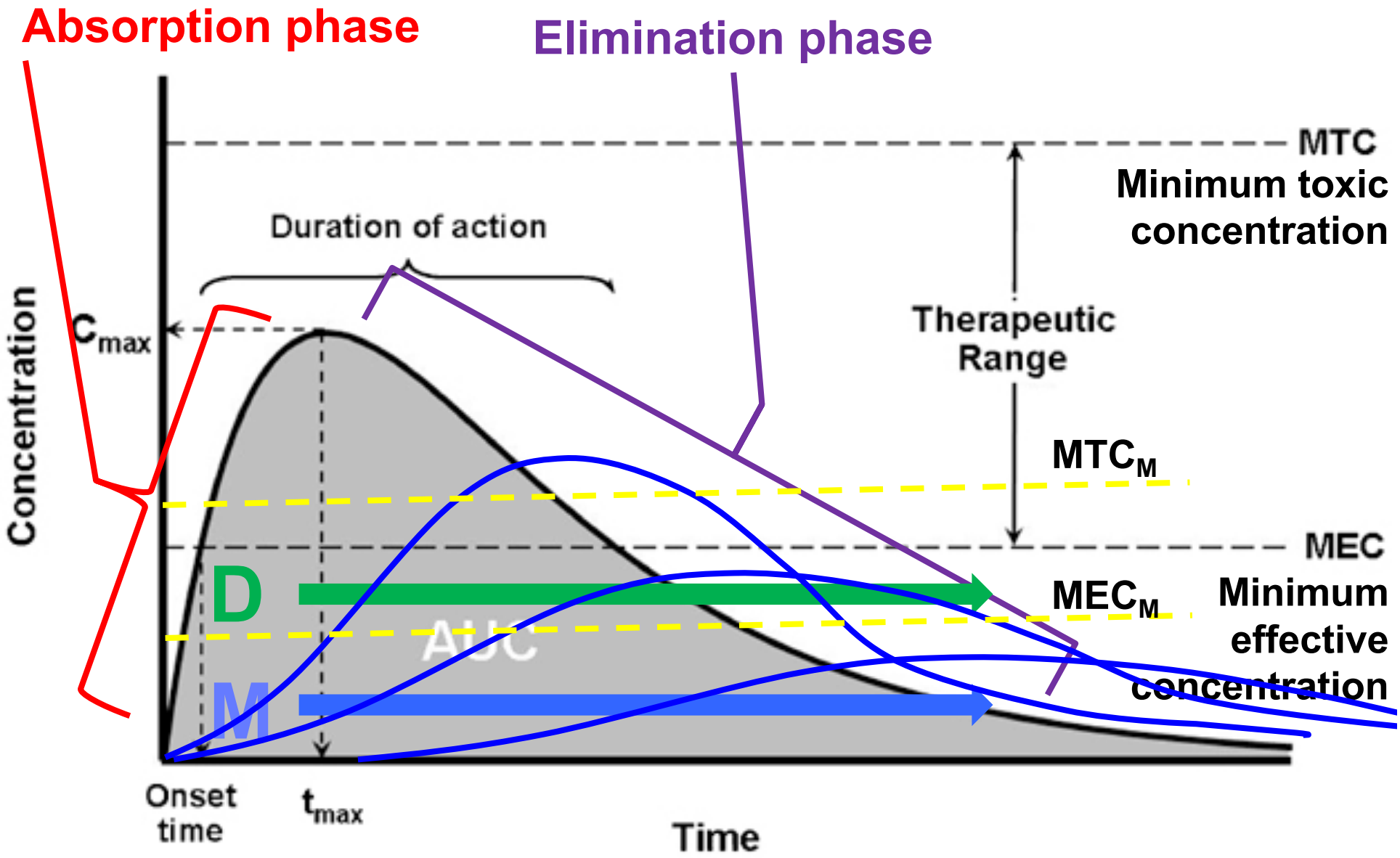
Phase II enzymes

Mechanism	Involved enzymes	Co-factor	Location
Methylation	Methyltransferases	S-adenosyl-L-methionine (SAM)	liver, kidney, lung, CNS
Sulphation	Sulfotransferases	3'-phosphoadenosine-5'-phosphosulfate (PAP)	liver, kidney, intestine
Acetylation	N-acetyltransferases	acetyl coenzyme A	liver, lung, spleen, gastric mucosa, RBCs, lymphocytes
Glucuronidation	UDP-glucuronosyltransferases	UDP-glucuronic acid	liver, kidney, intestine, lung, skin, prostate, brain
Glutathione conjugation	glutathione S-transferases	glutathione	liver, kidney

Pharmacokinetics



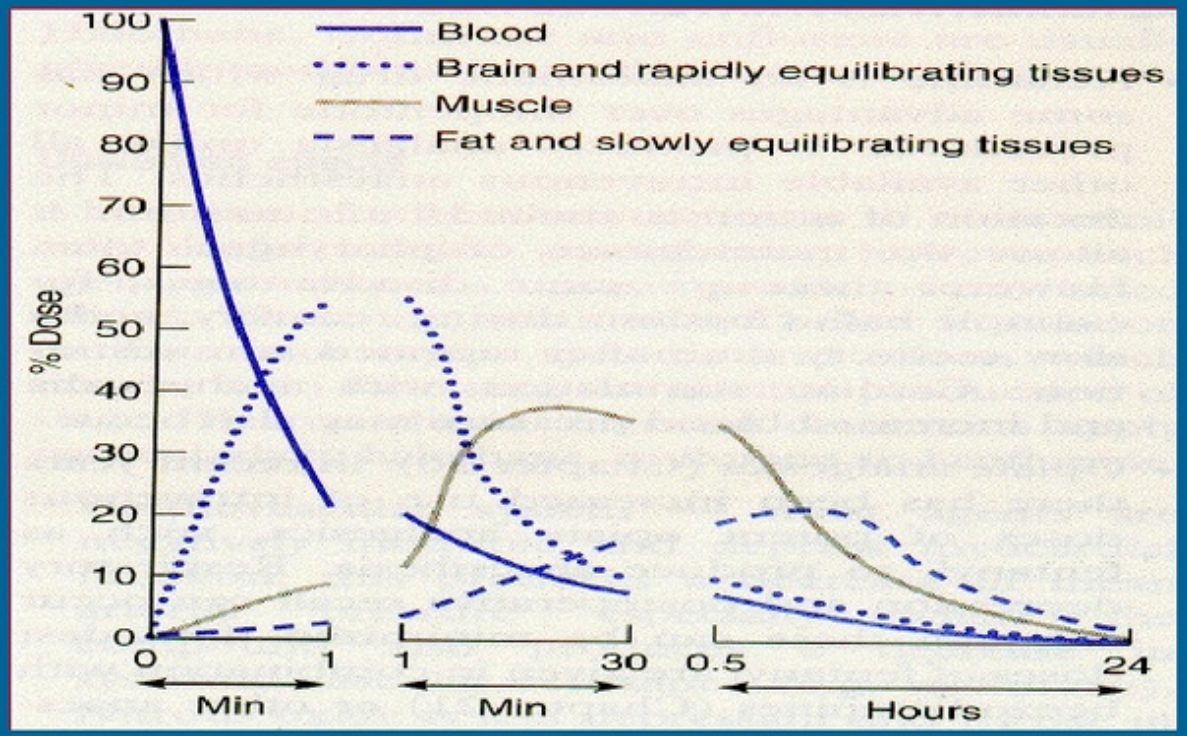
PK curve revisited



Distribution – Parent and metabolites

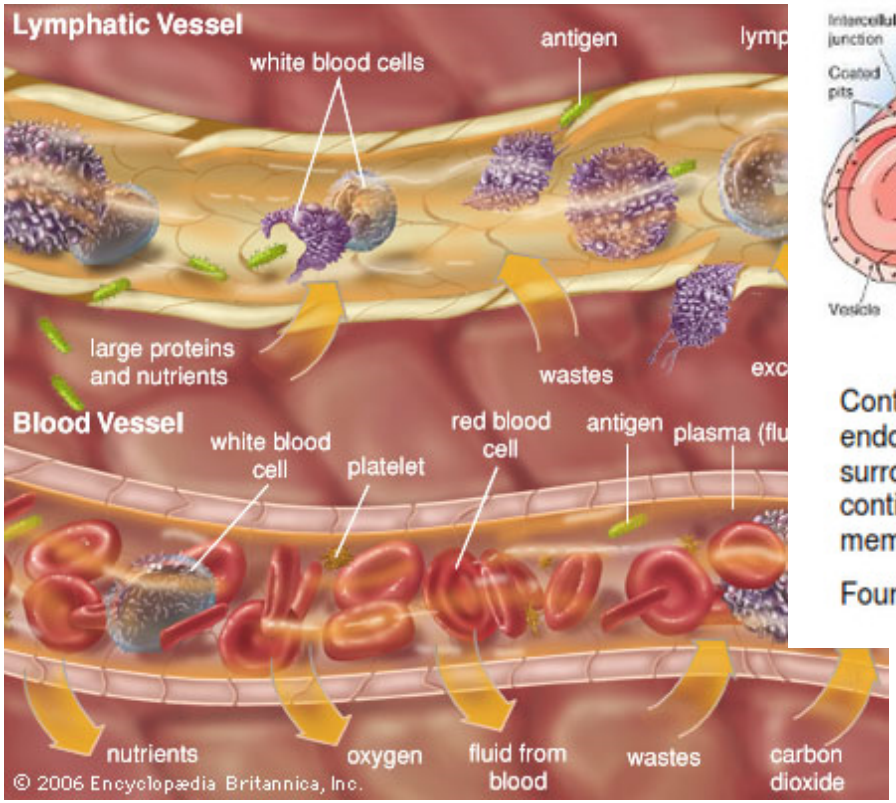
Redistribution

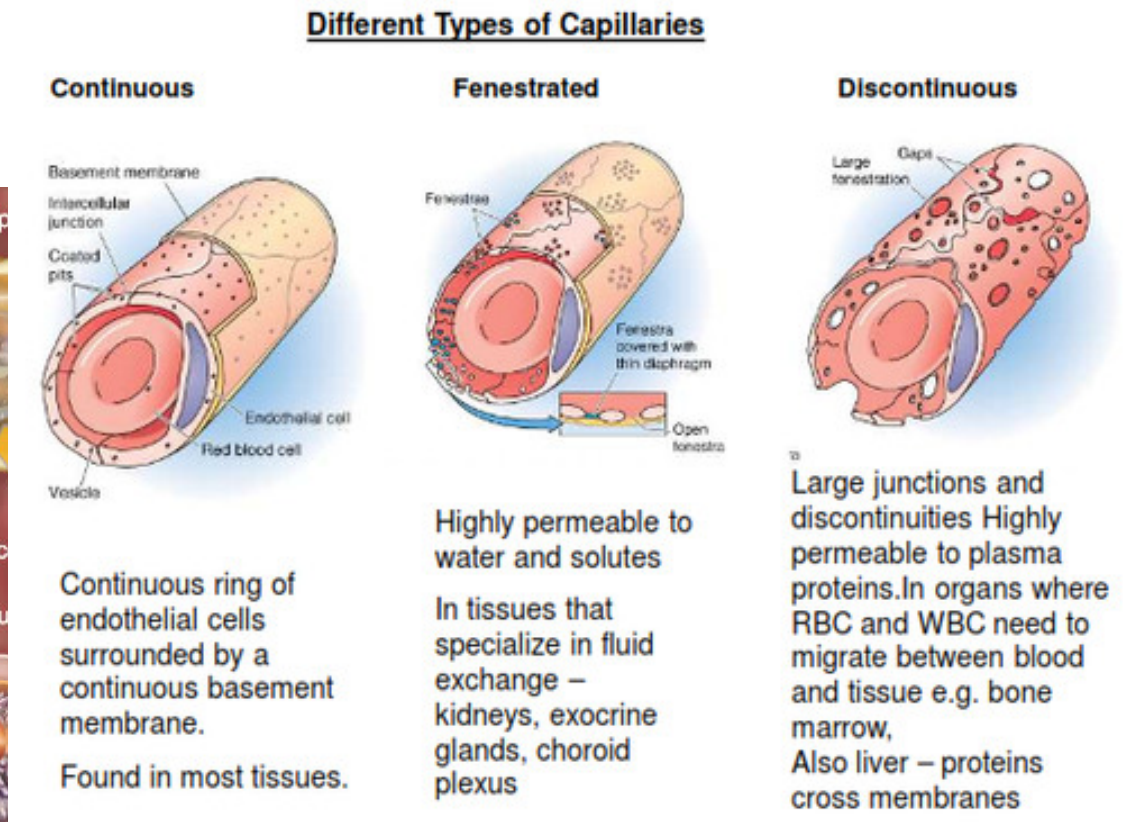
- Highly lipid soluble drugs – distribute to brain, heart and kidney etc. immediately followed by muscle and Fats



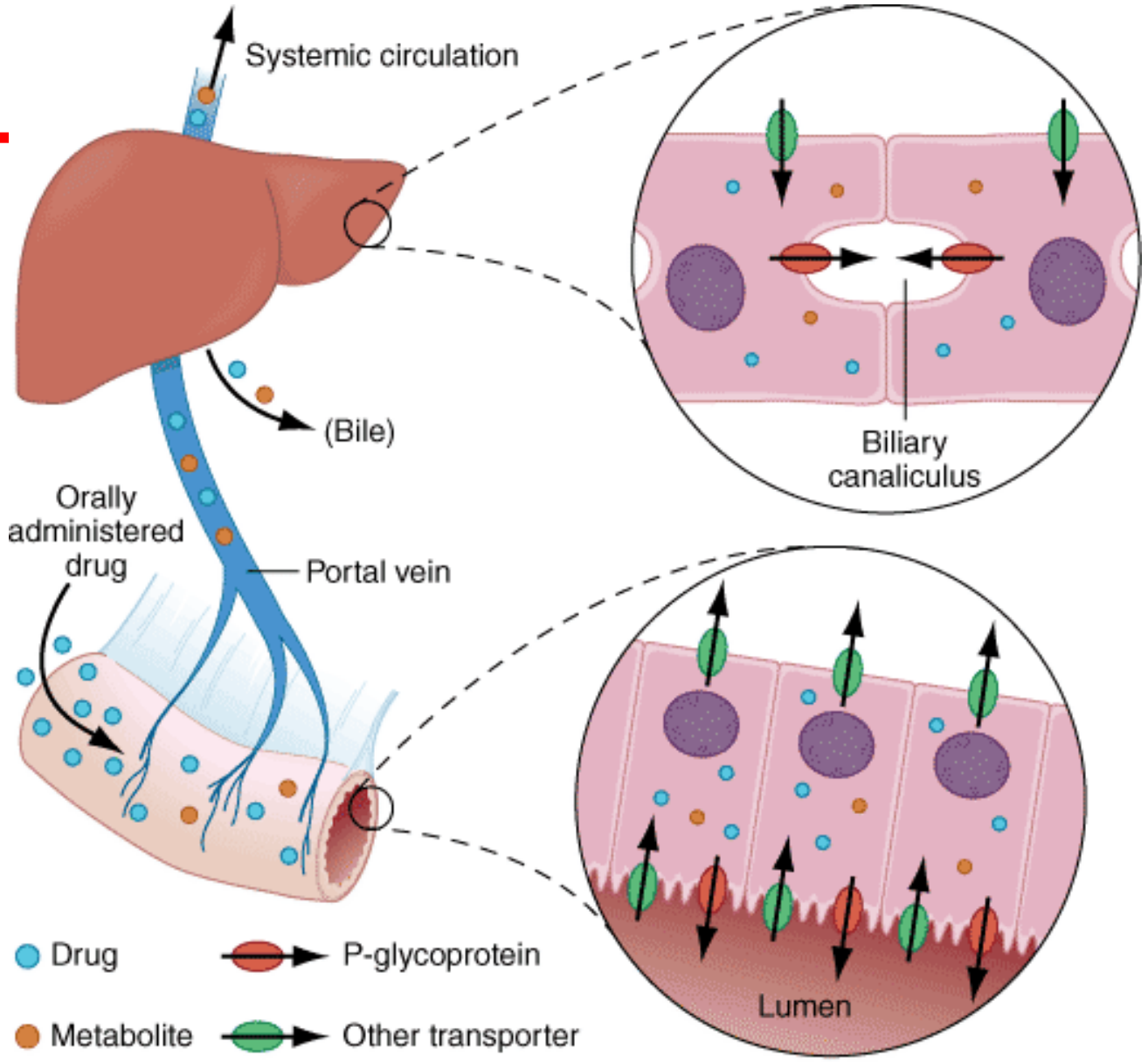
Metabolism to Elimination

Getting from site of action and/or **metabolism (liver)** back into the blood (or lymph, other) and out of the body





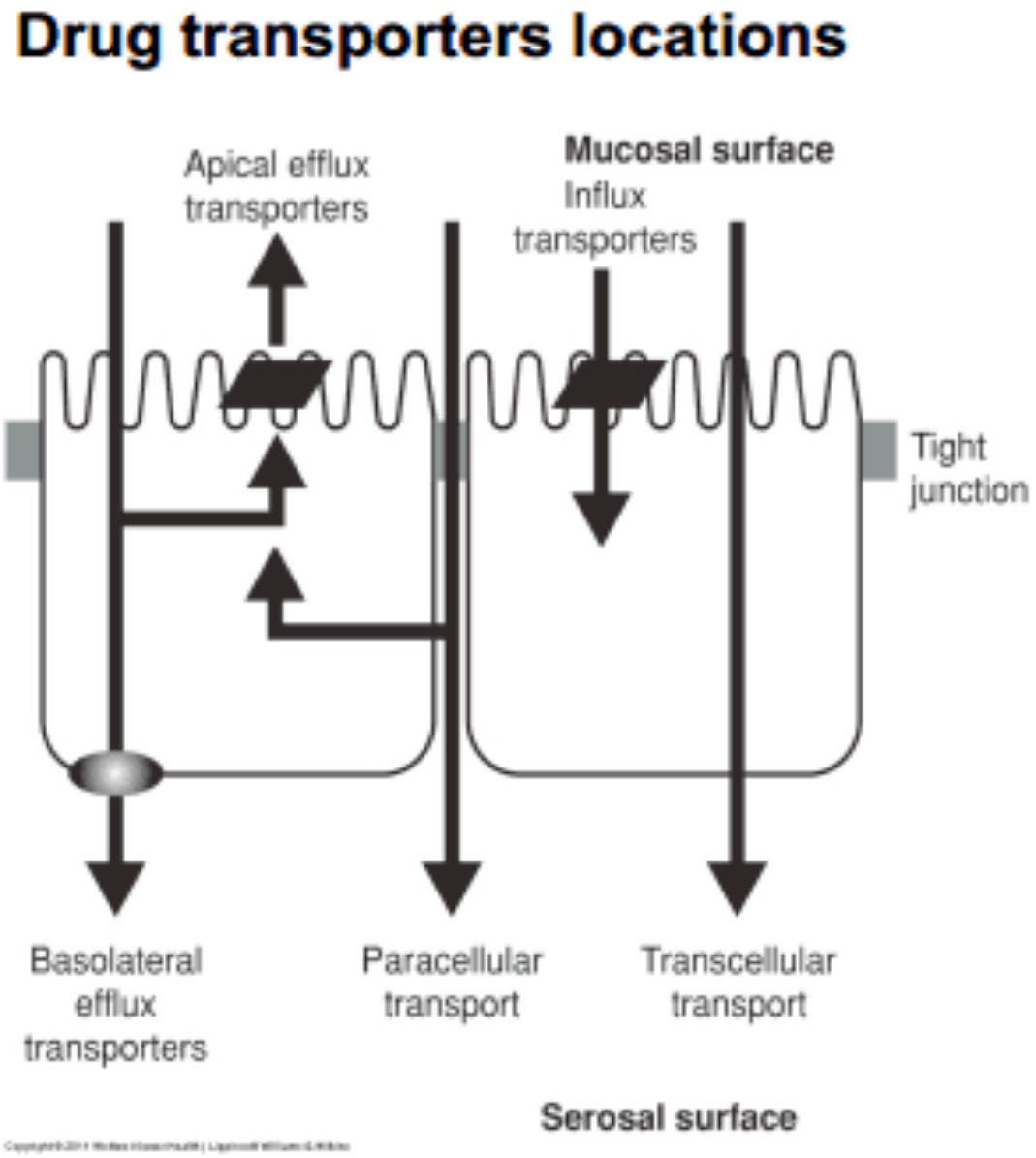
Metabolism to Elimination



Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J: *Harrison's Principles of Internal Medicine*, 17th Edition: http://www.accessmedicine.com

Transport Proteins

- About 4-10% of ALL proteins encoded in genome
  - Over 200 families
- **Channels** – diffusion (may be gated/regulated)
- **Carriers** – 1° & 2° active transport, facilitated
  - saturable



Phase 0, III - Transport Proteins

- 2 major families account for about 50% of xenobiotic transport across organismal membranes:
  - Major Facilitator Superfamily (MFS); transports many small solute molecules of biological importance;
    - 10 substrate classes; broad substrate specificity (from small oxyanions to large peptide fragments)
  - ATP-binding Cassette Superfamily (ABC); transports small, intermediate and macromolecules with both inwardly and outwardly directed polarity;
    - 13 classes

ABCC - Multiple Resistance Proteins

- MRP1 (P-glycoprotein) and MRP2 serve as a barrier to entry and as an effux pump for xenobiotics and cellular metabolites.
- MRP1, 2 & 3 - mediate transport of Phase II glutathione, glucuronide and sulfate conjugates into bile or urine.
- MRP1 - kidney, lung, intestine, brain, liver
- MRP2 - liver, colon, duodenum and kidney

ABCC - Multiple Resistance Proteins

- MRP3
  - Hepatic; efflux of organic ions into blood in presence of biliary obstruction
  - Inducible (liver-specific)
- MRP4
  - Transport nucleoside analogs; associated with antiretrovirals
- MRP6
  - Lipophilic anion pump with wide spectrum drug resistance

Hepatic and Renal Transporters

Solute carrier superfamily (SLC)

- **OATP - Organic Anion Transport Polypeptide:** important for hepatic uptake of large amphipathic organic anions, organic cations and uncharged substrates

- **OCTs and OATs - Organic anion/cation transporter:** mediate uptake of small organic cations and anions in liver and kidney.

- **PEPT1 - Peptide transporter 1 proton-dependant:** oligopeptide transporter; small intestine and kidney, brain
  - beta-lactams, peptide-like drugs

- Some overlap in substrate specificity
- Species divergence & polymorphism
- Some highly conserved

Organic Anion/Cation Transporters (OAT/OCT)

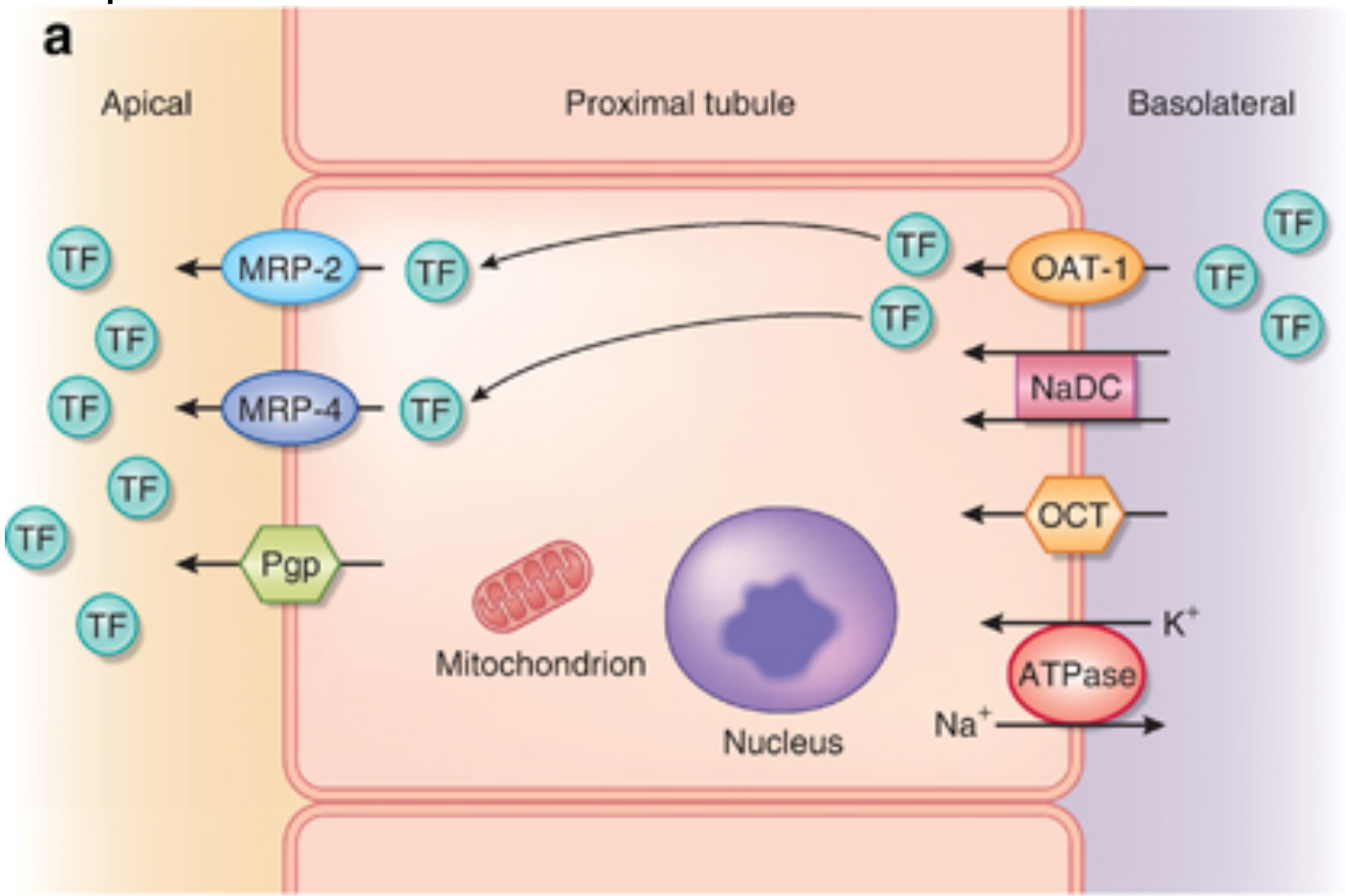
- organic cations (OCTs, SLC22A1-3) and organic anions (OATs, SLC22A6-11) mediate uptake of small hydrophilic organic cations and anions in liver and kidney.
  - substrates include anionic drugs such as beta-lactams, diuretics, NSAIDs, NRTS, NNRTS
- OAT1: kidney (basolateral) >> brain
- OAT2: liver >> kidney (basolateral), sinusoidal membrane
- OAT3: kidney >> skeletal muscle, brain >> liver
- OAT4: kidney (apical proximal tubule) >> placenta;

Organic Anion/Cation Transporters (OCT / OAT)

Active transport across proximal tubule cells (kidney)

urinary space

blood

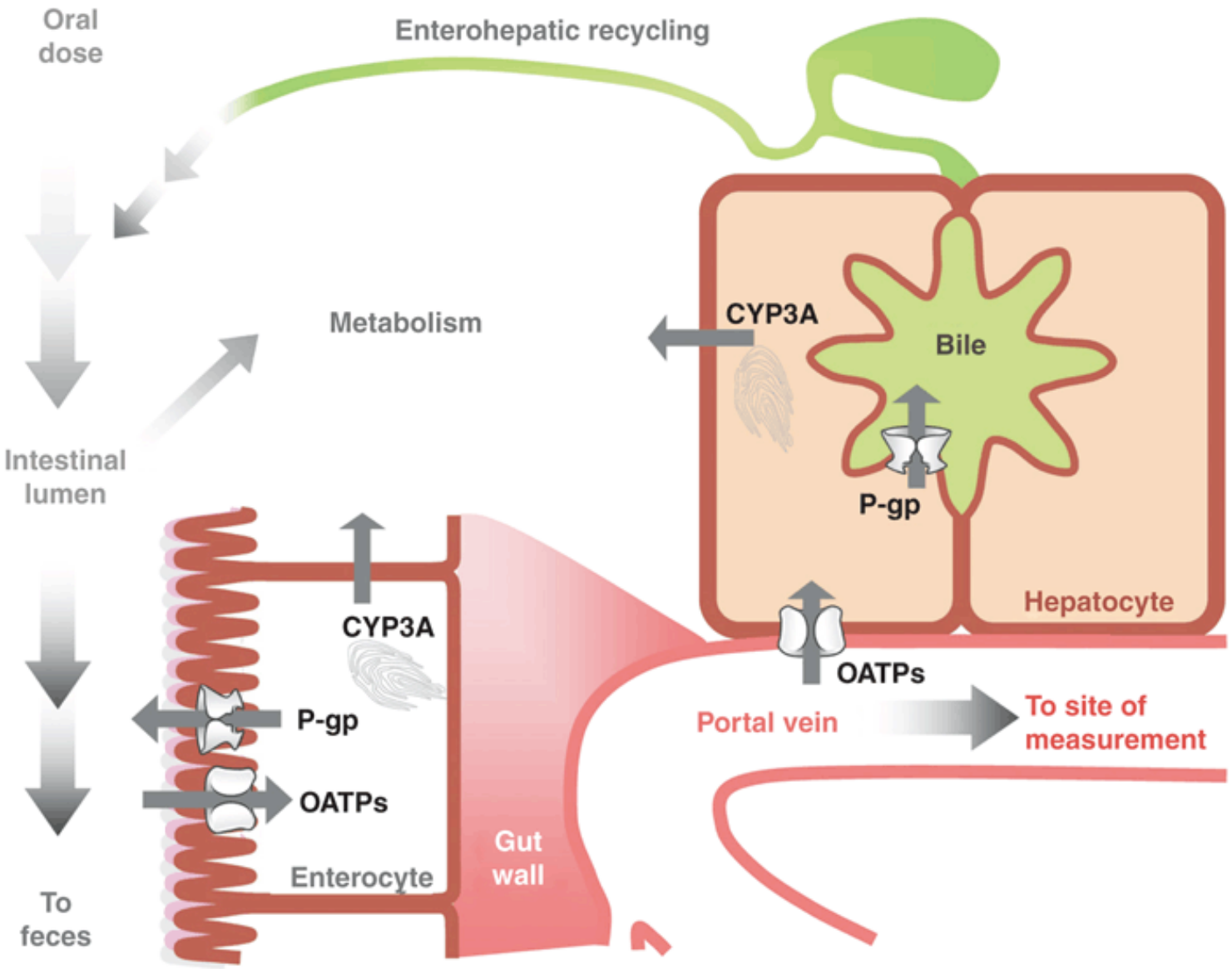


Organic Anion Transport Polypeptide (OATP)

- OATP [SLC01-5] regulate bioavailability, distribution and excretion of large amphipathic lipophilic organic anions, organic cations and uncharged substrates
  - also for organic solutes such as bile salts, neutral steroids & their conjugates, thyroid hormones, anionic oligopeptides, drugs, toxins and other xenobiotics

- expressed in multiple organs including the liver, blood-brain barrier (BBB), choroid plexus, lung, heart, intestine, kidney, placenta and testis
- narrow spectrum of transport substrates or selective, organ-specific expression
  - e.g. liver, expressed in basolateral membranes

OATPs – Enterohepatic recycling



Elimination

Elimination is an irreversible process by which a substance is removed from the body.

Major	**Liver** - Metabolism of xenobiotics - Transport of (more polar) xenobiotic metabolites into bloodstream	**Kidney** - Glomerular filtration - Tubular secretion & reabsorption
Minor	**Bile – Feces** - Lipophilic xenobiotics and metabolites - Sometimes major **Lungs (exhalation)**	**Others** - Sweat - Tears - Saliva - Mother's milk