Drug Metabolism

- Most metabolic products are less pharmacologically active

  Important exceptions:

  - Where the metabolite is more active

    (Prodrugs, e.g. Erythromycin-succinate (less irritation of GI) --> Erythromycin)
  - Where the metabolite is toxic (acetaminophen)
  - Where the metabolite is carcinogenic

- Close relationship between the biotransformation of drugs and normal biochemical processes occurring in the body:
  - Metabolism of drugs involves many pathways associated with the synthesis of endogenous substrates such as steroid hormones, cholesterol and bile acids
  - Many of the enzymes involved in drug metabolism are principally designed for the metabolism of endogenous compounds
  - These enzymes metabolize drugs only because the drugs resemble the natural compound

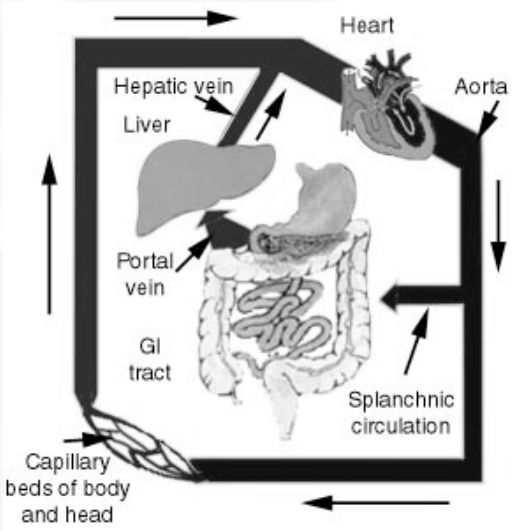
Phases of Drug Metabolism

- Phase I Reactions
  - Convert parent compound into a more polar (=hydrophilic) metabolite by adding or unmasking functional groups (-OH, -SH, -NH_2, -COOH, etc.)
  - Often these metabolites are inactive
  - May be sufficiently polar to be excreted readily

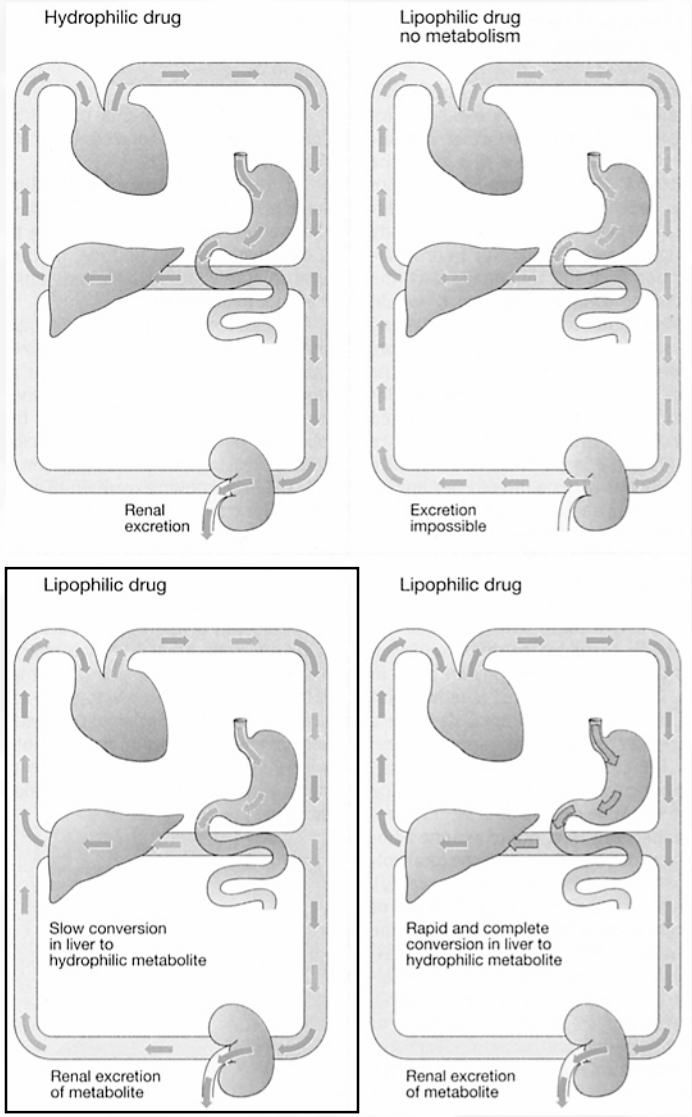
- Phase II Reactions
  - Conjugation with endogenous substrate to further increase aqueous solubility
  - Conjugation with glucoronide, sulfate, acetate, amino acid
  - Phase I usually precede phase II reactions

Liver is principal site of drug metabolism:

- Other sites include the gut, lungs, skin and kidneys
- For orally administered compounds, there is the "First Pass Effect"
  - Intestinal metabolism
  - Liver metabolism
  - Enterohepatic recycling
  - Gut microorganisms - glucuronidases



Drug Metabolism



Drug Metabolism - Phase I

- Phase I Reactions
  - Oxidation
  - Reduction
  - Hydrolytic cleavage
  - Alkylation (Methylation)
  - Dealkylation
  - Ring cyclization
  - N-carboxylation
  - Dimerization
  - Transamidation
  - Isomerization
  - Decarboxylation

Drug Metabolism - Oxidation

Two types of oxidation reactions:

- Oxygen is incorporated into the drug molecule (e.g. hydroxylation)
- Oxidation causes the loss of part of the drug molecule
  (e.g. oxidative deimination, dealkylation)

Microsomal Mixed Function Oxidases (MFOs)

- "Microsomes"
  form in vitro after cell homogenization and fractionation of ER
  - Rough microsomes are primarily associated with protein synthesis
  - Smooth microsomes contain a class of oxidative enzymes called

- "Mixed Function Oxidases" or "Monooxygenases"
  - These enzymes require a reducing agent (NADPH) and molecular oxygen
    (one oxygen atom appearing in the product and the other in the form of water)

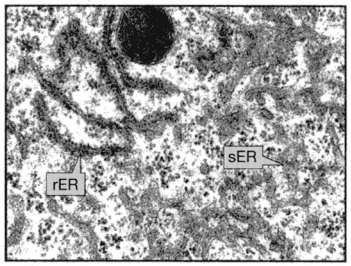
Drug Metabolism - Oxidation

- MFO consists of two enzymes:
  - Flavoprotein, NADPH-cytochrome c reductase
    - One mole of this enzyme contains one mole each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
    - Enzyme is also called NADPH-cytochrome P450 reductase
  - Cytochrome P450
    - named based on its light absorption at 450 nm when complexed with carbon monoxide
    - is a hemoprotein containing an iron atom which can alternate between the ferrous (Fe^{++}) and ferric (Fe^{+++}) states
    - Electron acceptor
    - Serves as terminal oxidase
    - its relative abundance compared to NADPH-cytochrome P450 reductase makes it the rate-limiting step in the oxidation reactions

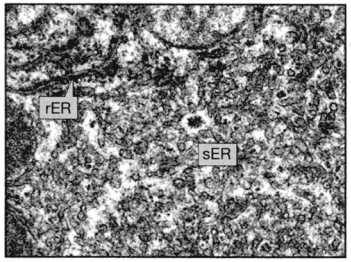
Drug Metabolism - Oxidation

- Humans have 18 families of cytochrome P450 genes and 43 subfamilies:
  - CYP1 drug metabolism (3 subfamilies, 3 genes, 1 pseudogene)
  - CYP2 drug and steroid metabolism (13 subfamilies, 16 genes, 16 pseudogenes)
  - **CYP3 drug metabolism** (1 subfamily, 4 genes, 2 pseudogenes)
  - CYP4 arachidonic acid or fatty acid metabolism (5 subfamilies, 11 genes, 10 pseudogenes)
  - CYP5 Thromboxane A2 synthase (1 subfamily, 1 gene)
  - CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus (1 subfamily member)
  - CYP7B brain specific form of 7-alpha hydroxylase (1 subfamily member)
  - CYP8A prostacyclin synthase (1 subfamily member)
  - CYP8B bile acid biosynthesis (1 subfamily member)
  - CYP11 steroid biosynthesis (2 subfamilies, 3 genes)
  - CYP17 steroid biosynthesis (1 subfamily, 1 gene) 17-alpha hydroxylase
  - CYP19 steroid biosynthesis (1 subfamily, 1 gene) aromatase forms estrogen
  - CYP20 Unknown function (1 subfamily, 1 gene)
  - CYP21 steroid biosynthesis (1 subfamily, 1 gene, 1 pseudogene)
  - CYP24 vitamin D degradation (1 subfamily, 1 gene)
  - CYP26A retinoic acid hydroxylase important in development (1 subfamily member)
  - CYP26B probable retinoic acid hydroxylase (1 subfamily member)
  - CYP26C probabvle retinoic acid hydroxylase (1 subfamily member)
  - CYP27A bile acid biosynthesis (1 subfamily member)
  - CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member)
  - CYP27C Unknown function (1 subfamily member)
  - CYP39 7 alpha hydroxylation of 24 hydroxy cholesterol (1 subfamily member)
  - CYP46 cholesterol 24-hydroxylase (1 subfamily member)
  - CYP51 cholesterol biosynthesis (1 subfamily, 1 gene, 3 pseudogenes) lanosterol 14-alpha demethylase

Drug Metabolism - Oxidation



Normal hepatocyte



Hepatocyte after phenobarbital administration

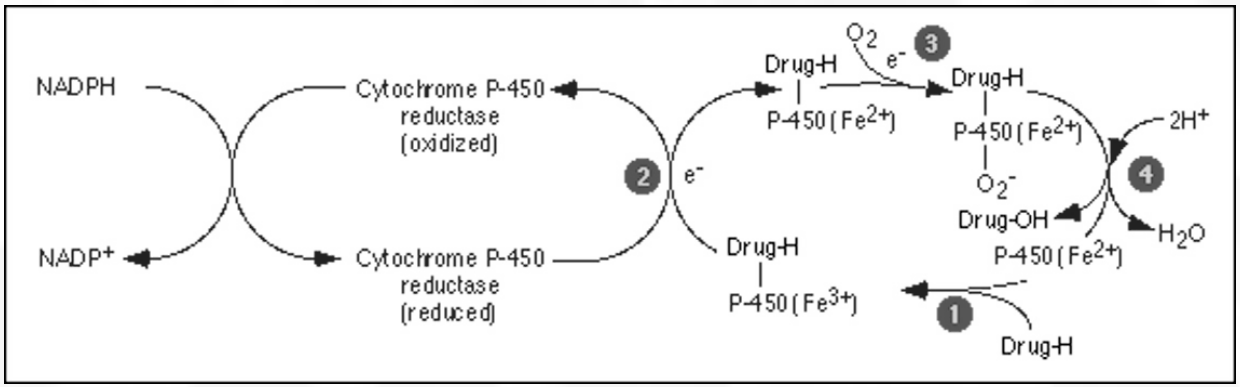
- Induction of P450 enzymes:
  - PPAR (peroxisome proliferator activated receptor) ligands (e.g.clofibrate)
  - CYP1 family are induced by aromatic hydrocarbons (cigarette smoke; charred food)
  - CYP2E enzymes induced by ethanol
  - CYP2B enzymes induced 40-50 fold by barbiturates
- Polymorphisms cause differences in drug metabolism:
  - CYP2C19 has a polymorphism that changes the enzyme's ability to metabolize mephenytoin (a marker drug). In Caucasians, the polymorphism for the poor metabolizer phenotype is only seen in 3% of the population. However, it is seen in 20% of the asian population.
    => It is important to be aware of a person's race when drugs are given that are metabolized differently by different populations
- P450s and drug interactions:
  - Barbiturates induce CYP2B => increased metabolism of other drugs
  - Antifungals (e.g. ketoconazole) inhibit fungal CYP51 and unintentionally also human CYP3A4 => reduced metabolism of other drugs
  - Grapefruit juice contains a CYP3A4 inhibitor =>12 fold increase in some drug concentrations

    CYP3A4 Substrates: • Acetominophen (Tylenol) • Codeine (narcotic) • Cyclosporin A (immunosuppressant), • Diazepam (Valium) • Erythromycin (Antibiotic) • Lidocaine (local anaesthetic), • Lovastatin (HMGCoA reductase inhibitor), • Taxol (cancer drug), • Warfarin (anticoagulant).

Drug Metabolism - Oxidation

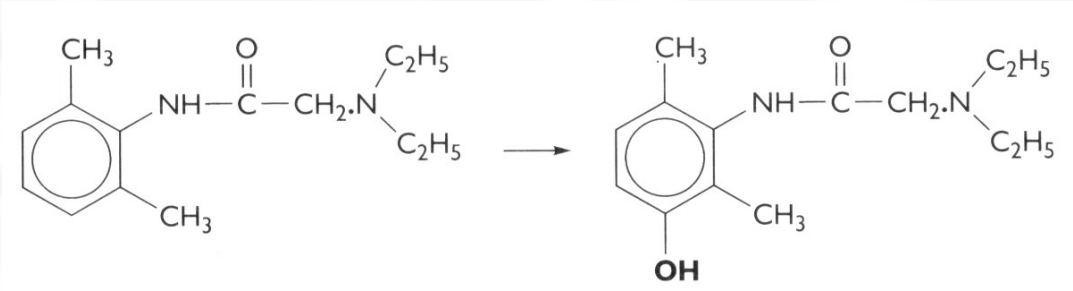
- <u>Drug oxidation requires:</u>
  - Cytochrome P450
  - Cytochrome P450 reductase
  - NADPH
  - Molecular oxygen

- <u>The cycle involves four steps:</u>
  1. Oxidized (Fe3+) cytochrome P-450 combines with a drug substrate to form a binary complex.
  2. NADPH donates an electron to the cytochrome P-450 reductase, which in turn reduces the oxidized cytochrome P-450-drug complex.
  3. A second electron is introduced from NADPH *via* the same cytochrome P-450 reductase, which serves to reduce molecular oxygen and form an "activated oxygen"-cytochrome P-450-substrate complex.
  4. This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product. The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates.



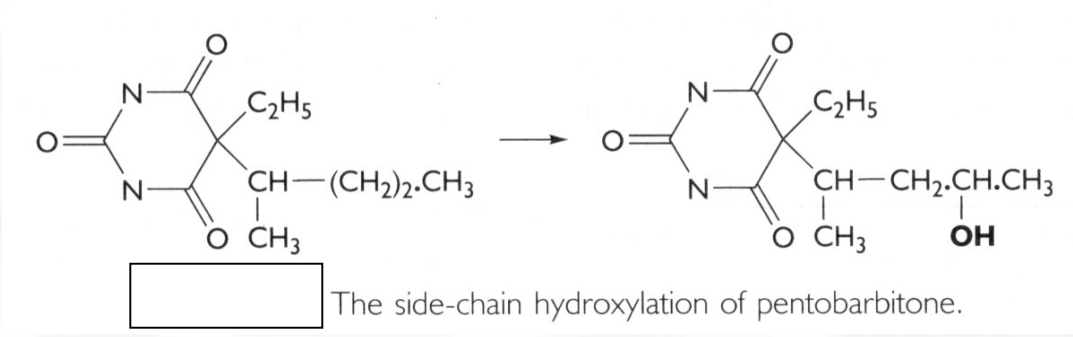
Drug Metabolism - Oxidation

Aromatic hydroxylation:



The 3-hydroxylation of lignocaine.

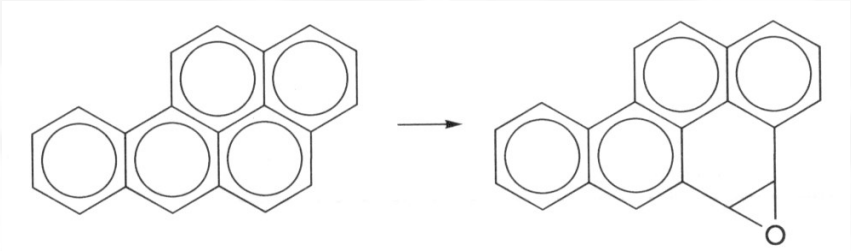
Aliphatic hydroxylation:



The side-chain hydroxylation of pentobarbitone.

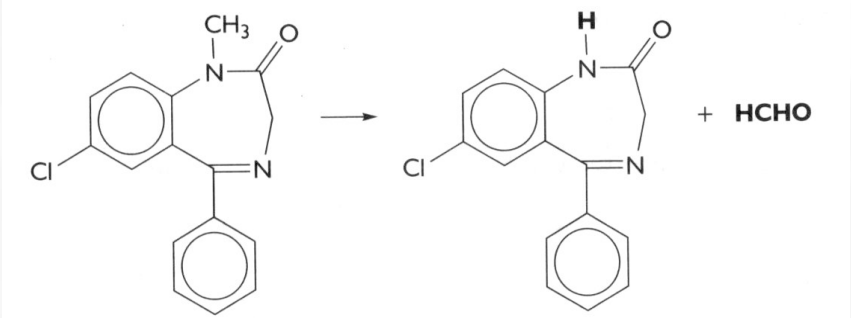
Drug Metabolism - Oxidation

Epoxidation:



The formation of benzo[*a*]pyrene-4,5-epoxide.

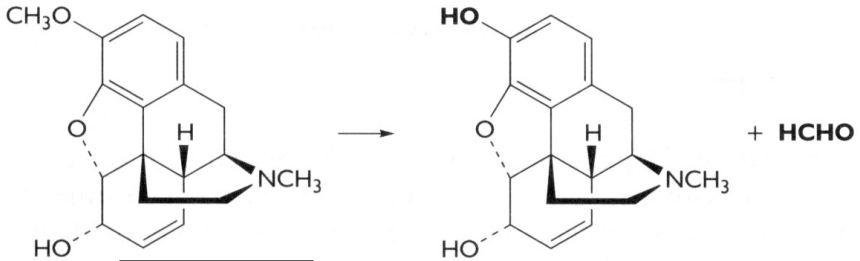
Dealkylation:



The *N*-demethylation of diazepam.

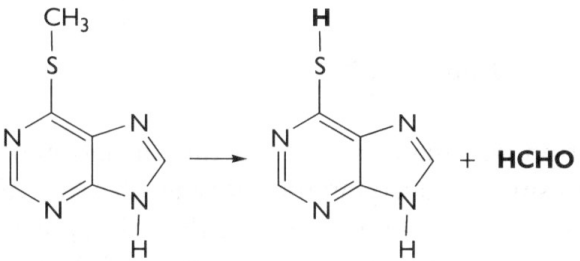
Drug Metabolism - Oxidation

O-demethylation:



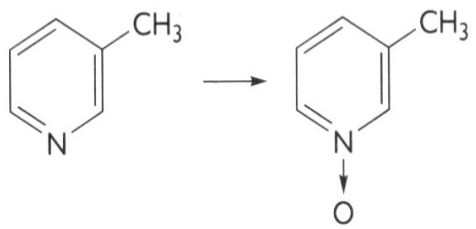
The *O*-demethylation of codeine.

S-demethylation:



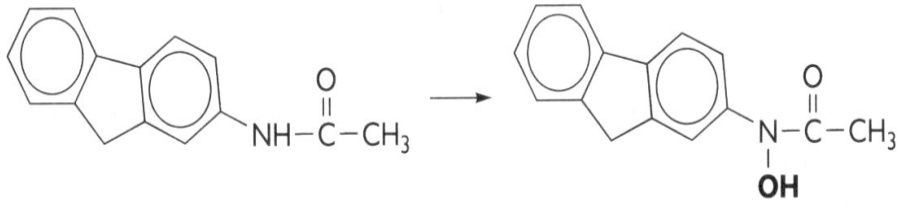
The *S*-demethylation of *S*-methylthiopurine.

N-oxidation:



The *N*-oxidation of 3-methylpyridine.

N-hydroxylation:



The *N*-hydroxylation of 2-acetylaminofluorene.

Drug Metabolism - Oxidation

Oxidation reactions NOT catalyzed by Cytochrome P450:

Flavin containing monoxygenase system

- Present mainly in liver but some is expressed in gut and lung
- Located in smooth endoplasmic reticulum
- Oxidizes compounds containing sulfur and nitrogen
- Uses NADH and NADPH as cofactors

- Alcohol dehydrogenase (cytosol)
- Aldehyde oxidation (cytosol)
- Xanthine oxidase
- Amine oxidases
  - Monoamine oxidase (nerve terminals, mitochondria)
  - Diamine oxidase found in liver microsomes
    - Primarily endogenous metabolism

Drug Metabolism - Oxidation

Monoamine Oxidases (MAO):

- Catalyze oxidative deamination of endogenous catecholamines (epinephrine)
- Located in nerve terminals and peripheral tissues
- Substrates for catecholamine metabolism found in foods (tyramine) can cause a drug/food interaction

- Inhibited by class of antidepressants called MAO inhibitors

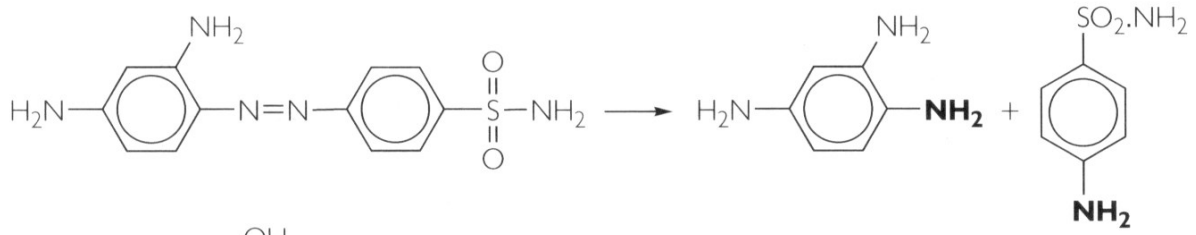
  (Inhibition of MAO isoforms in the CNS also effects levels of serotonin - Tranylcypromine)

  These drugs can cause severe or fatal drug/drug interactions with drugs that increase release of catecholamines or inhibit their reuptake in nerve terminals

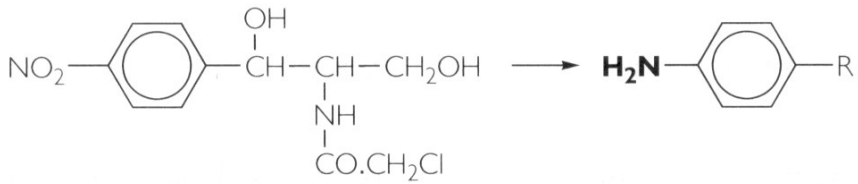
  (Meperidine, pentazocine, dextromethorphan, SSRI antidepressants)

Drug Metabolism - Reduction

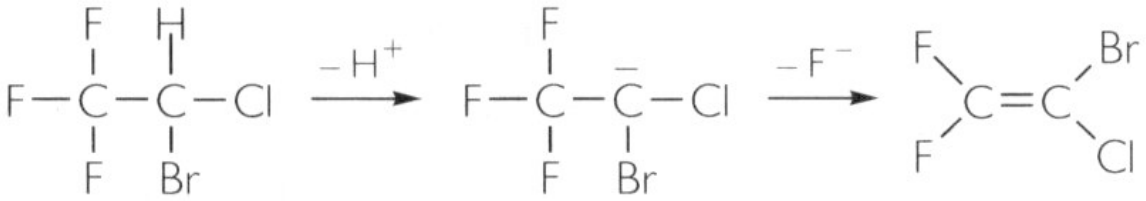
Azo-reduction:



Nitro-reduction:



Dehalogenation:

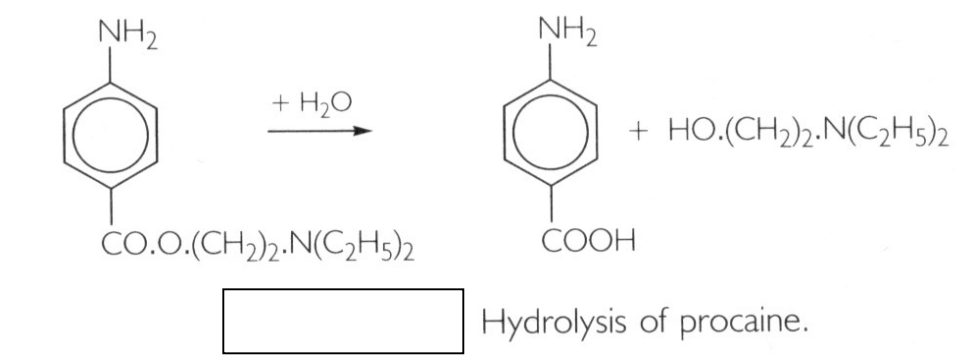


Reductive defluorination of halothane.

Drug Metabolism - Reduction

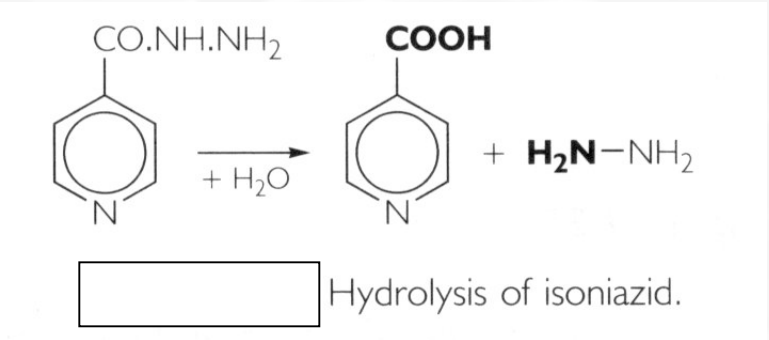
Hydrolysis reactions

Ester hydrolysis:



Hydrolysis of procaine.

Amide hydrolysis:



Hydrolysis of isoniazid.

Drug Metabolism - Phase I

- Almost any drug can undergo modifications by drug-metabolizing enzyme systems
- Drugs can be subject to several Phase I pathways
- These reactions create functional groups that place the drugs in a correct chemical state to be acted upon by Phase II conjugative mechanisms
- Main function of phase I reactions is to prepare chemicals for phase II metabolism and subsequent excretion
- Phase II is the true “detoxification” step in the metabolism process.

Drug Metabolism - Phase II

- Conjugation reactions
  - **Glucuronidation** by UDP-Glucuronosyltransferase:
    (on -OH, -COOH, -NH_2, -SH groups)
  - Sulfation by Sulfotransferase:
    (on -NH2, -SO_2NH_2, -OH groups)
  - Acetylation by acetyltransferase:
    (on -NH_2, -SO_2NH_2, -OH groups)
  - Amino acid conjugation
    (on -COOH groups)
  - Glutathione conjugation by Glutathione-S-transferase:
    (to epoxides or organic halides)
  - Fatty acid conjugation
    (on -OH groups)
  - Condensation reactions

Drug Metabolism - Glucuronidation

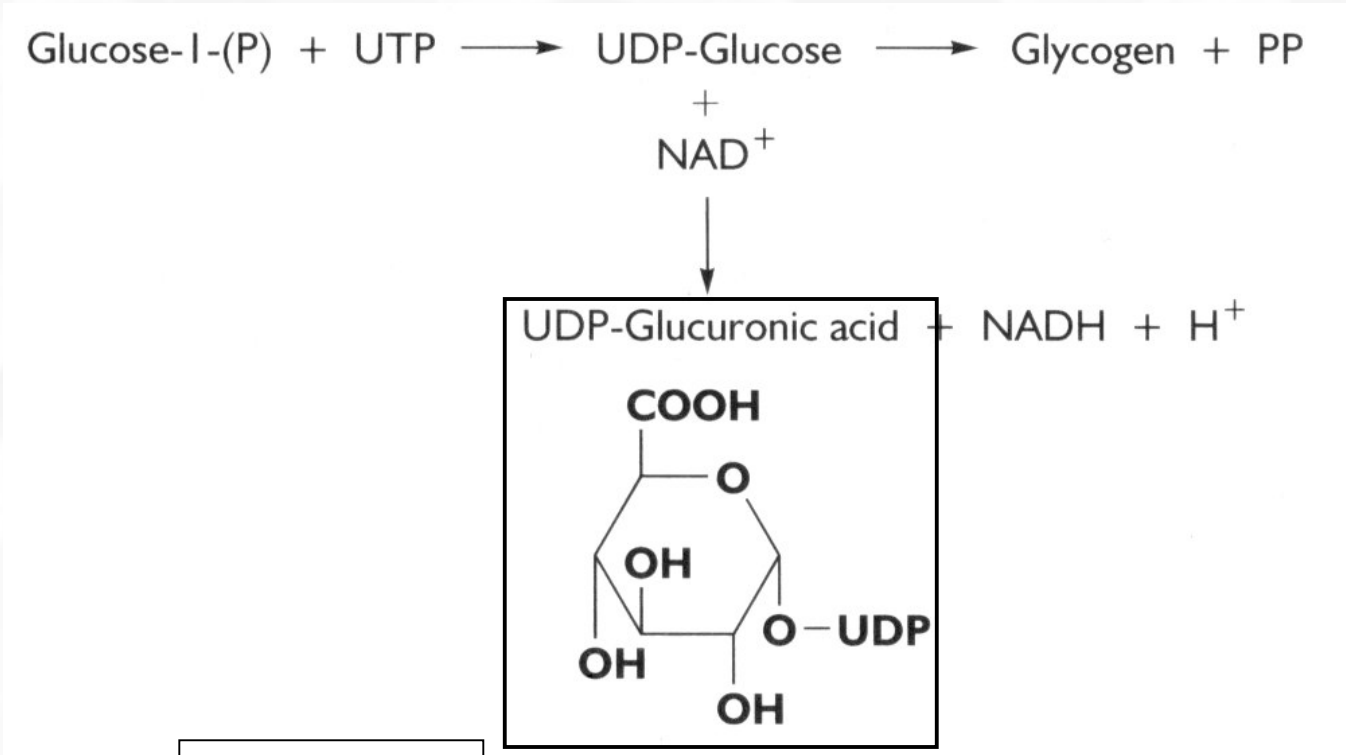
- Glucuronidation (= conjugation to α-d-glucuronic acid)
  - Quantitatively the most important phase II pathway for drugs and endogenous compounds
  - Products are often excreted in the bile.
  - Enterohepatic recycling may occur due to gut glucuronidases
  - Requires enzyme UDP-glucuronosyltransferase (UGT):
    - Genetic family of enzymes
      - Metabolizes a broad range of structurally diverse endogenous and exogenous compounds
      - Structurally related family with approximately 16 isoforms in man

	UGT activity[a]									
Substrate	**1A1**	**1A3**	**1A4**	**1A6**	**1A8**	**2A1**	**2B4**	**2B7**	**2B15**	**2B17**
Simple phenols	1900	239	30	2400	5300	735	0.4	5	167	38
Bilirubin	400	0	2	0	0	nd	0	0	0	0
Carboxylic acids	0	121	0	nd	170	68	0	2	0	nd
Primary amines	1	84	540	10600	1800	22	nd	3	0	nd
Opioids	0	130	0	0	0	73	0	3462	0	nd

[a] Data are maximum specific enzyme activities (pmol/min/mg protein). nd, not determined. Adapted from Tukey and Strassburg (2000) *Ann. Rev. Pharmacol. Toxicol.* **40**, 581–616. Note that these substrate specificities have yet to be further refined (see text).

Drug Metabolism - Glucuronidation

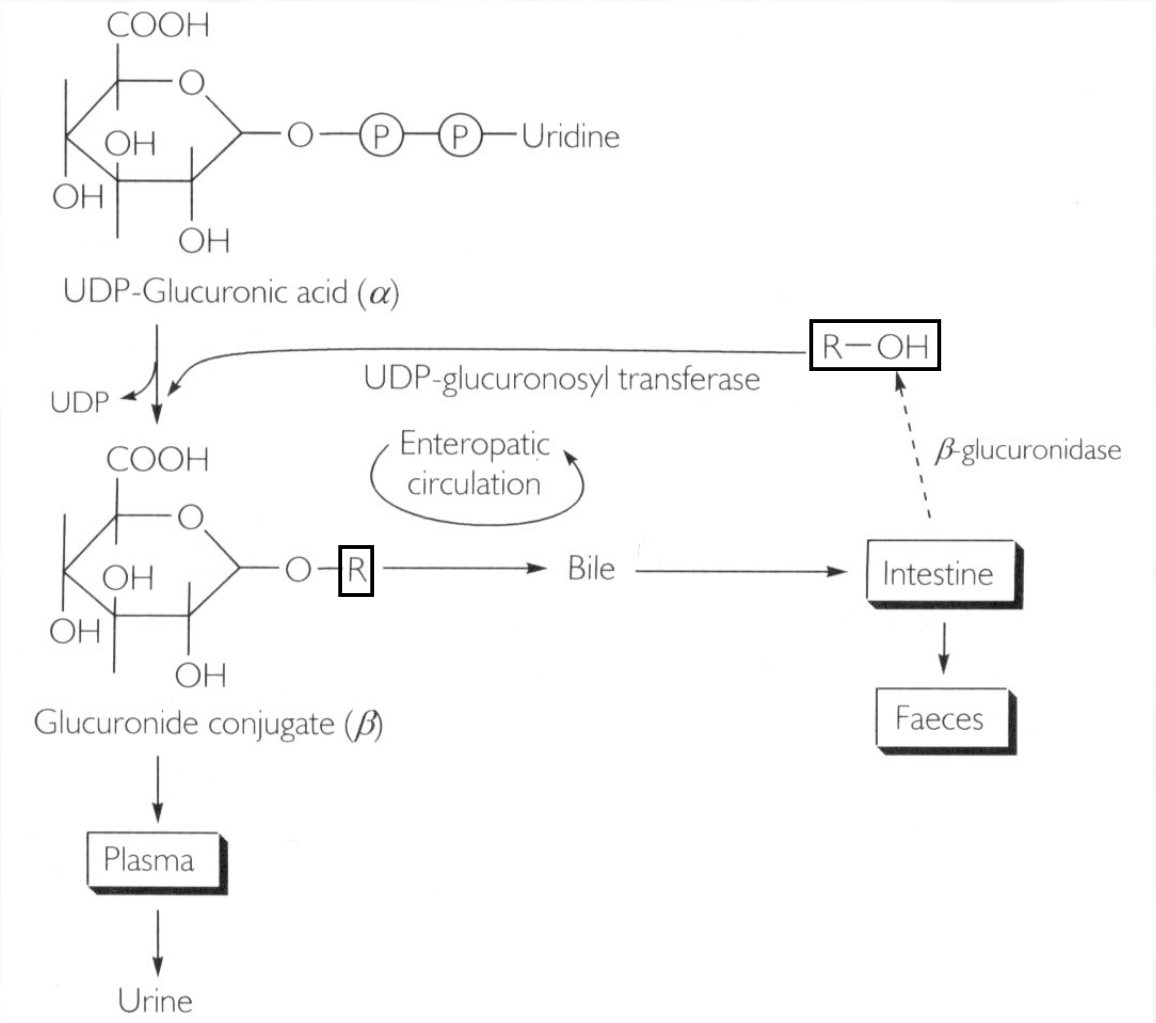
- Glucuronidation – requires creation of high energy intermediate: <u>UDP-Glucuronic Acid</u>:



Synthesis of UDP-glucuronic acid.

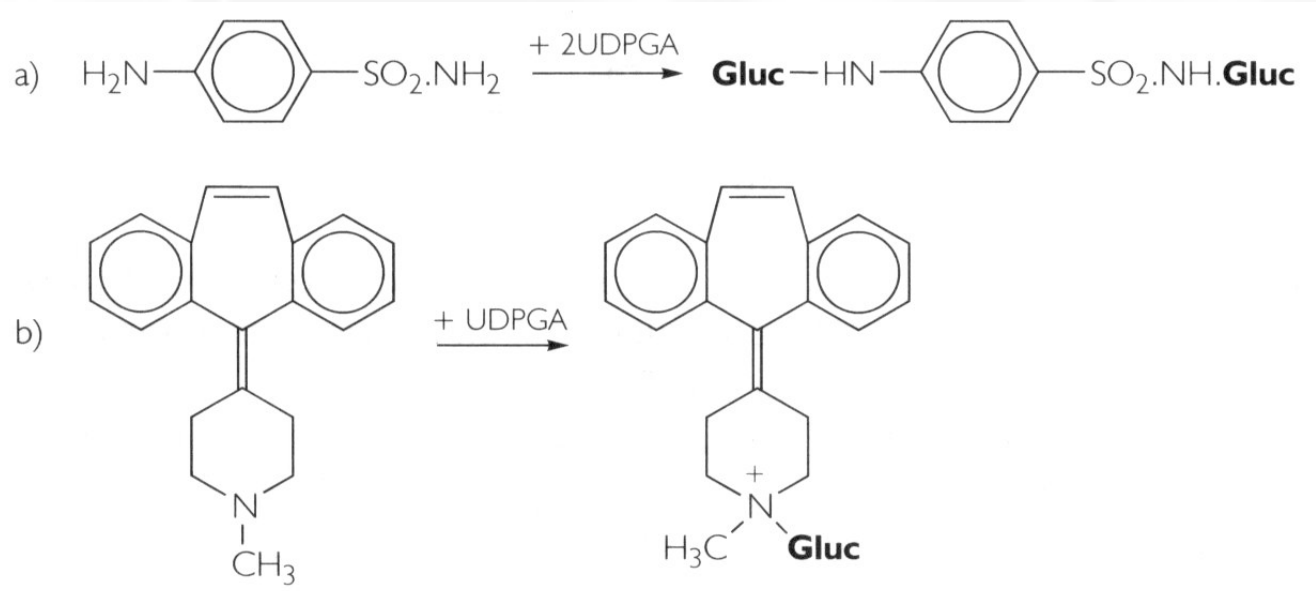
Drug Metabolism - Glucuronidation

- Glucuronidation Pathway and Enterohepatic Recirculation



Drug Metabolism - Glucuronidation

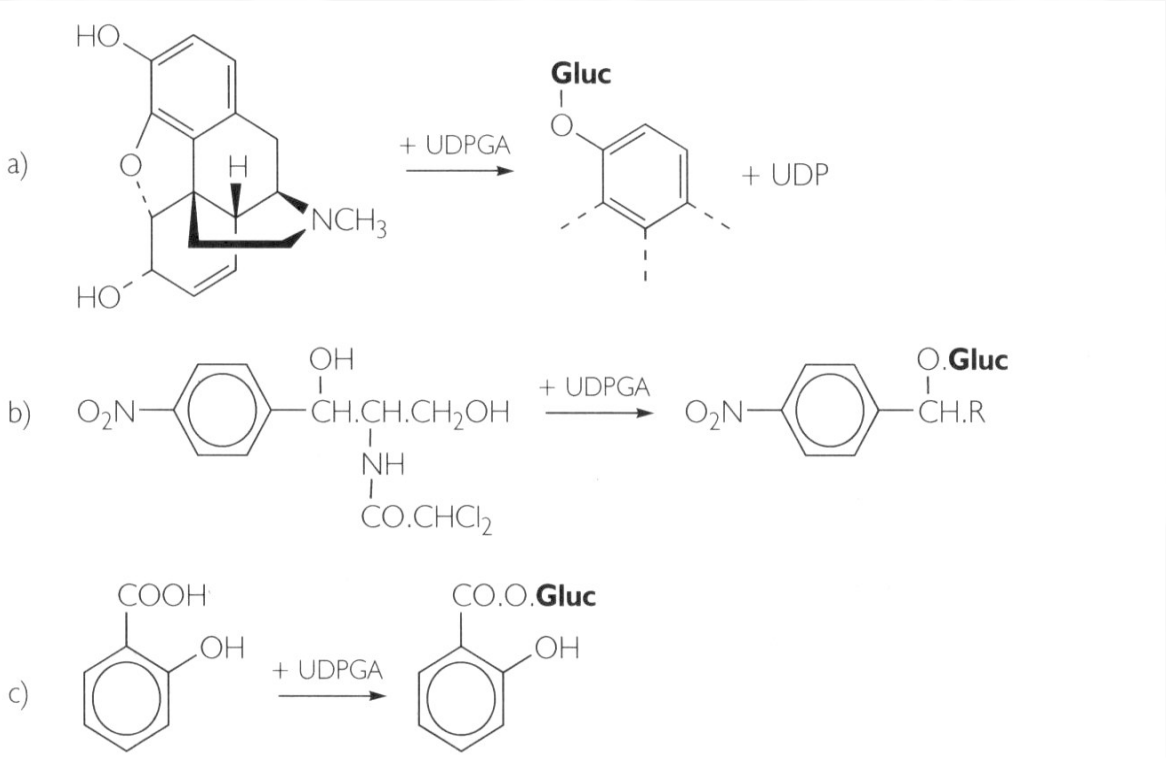
- N-glucuronidation:
  - Occurs with amines (mainly aromatic)
  - Occurs with amides and sulfonamides



The glucuronidation of (a) sulfanilamide and (b) cyproheptidine.

Drug Metabolism - Glucuronidation

- <u>O-glucuronidation:</u>
  - Occurs by ester linkages with carboxylic acids
  - Occurs by ether linkages with phenols and alcohols



The glucuronidation of (a) morphine, (b) chloramphenicol and (c) salicylic acid.

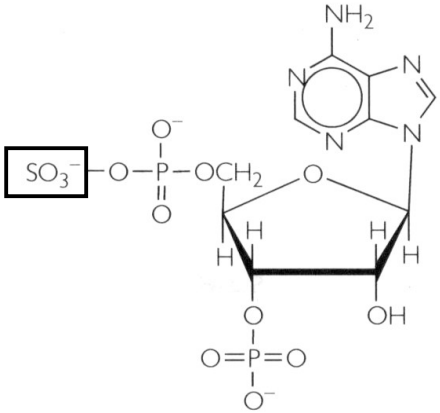
Drug Metabolism - Sulfation

Sulfation:

- Major pathway for phenols but also occurs for alcohols, amines and thiols
- Energy rich donor required:

PAPS (3'-Phosphoadenosine-5'-phosphosulfate)

- Sulfation and glucuronidation are competing pathways:
  - Sulfation predominates at low substrate concentrations
  - Glucuronidation predominates at higher concentrations
  - There is relatively less PAPS in cell cytosol compared to UDPGA



The structure of PAPS.

- Sulfotransferases (=SULTs) catalyze transfer of sulfate to substrates:
  - Phenol, alcohol and arylamine sulfotransferases are fairly non-specific
  - Steroid sulfotransferases are very specific

Drug Metabolism - Acylation

Acetylation:

- Common reaction for aromatic amines and sulfonamides
- Requires co-factor acetyl-CoA
- Responsible enzyme is N-acetyltransferase
- Takes place mainly in the liver
- Important in sulfonamide metabolism because acetyl-sulfonamides are less soluble than the parent compound and may cause renal toxicity due to precipitation in the kidney

Fatty Acid Conjugation:

- Stearic and palmitic acids are conjugated to drug by esterification reaction
- Occurs in liver microsomal fraction

  (Cannabinols are metabolized in this fashion => long half-life)

Drug Metabolism - Other conjugations

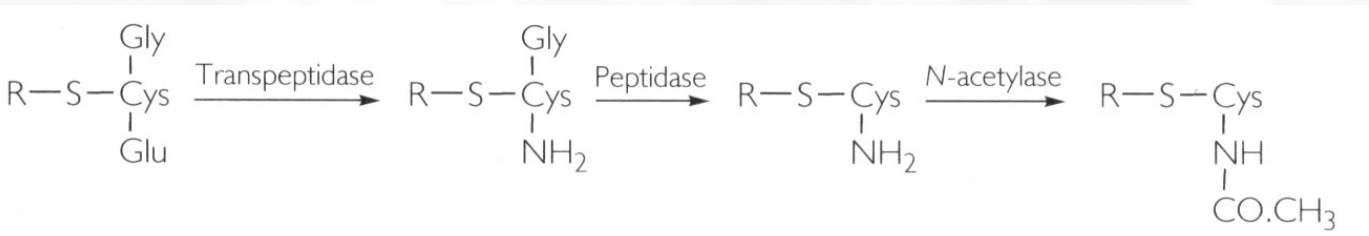
Amino Acid Conjugation:

- ATP-dependent acid:CoA ligase forms active CoA-amino acid conjugates which then react with drugs by N-Acetylation:
  - Usual amino acids involved are:
    - Glycine. Glutamine, Ornithine, Arginine

Glutathione Conjugation:

- Tripeptide Gly-Cys-Glu; conjugated by glutathione-S-transferase (GST)
- Glutathione is a protective factor for removal of potentially toxic compounds
- Conjugated compounds can subsequently be attacked by γ-glutamyltranspeptidase and a peptidase to yield the cysteine conjugate =>

  product can be further acetylated to N-acetylcysteine conjugate



The further metabolism of a glutathione conjugate.

Drug Metabolism - Phase I & II

Phase I and II - Summary:

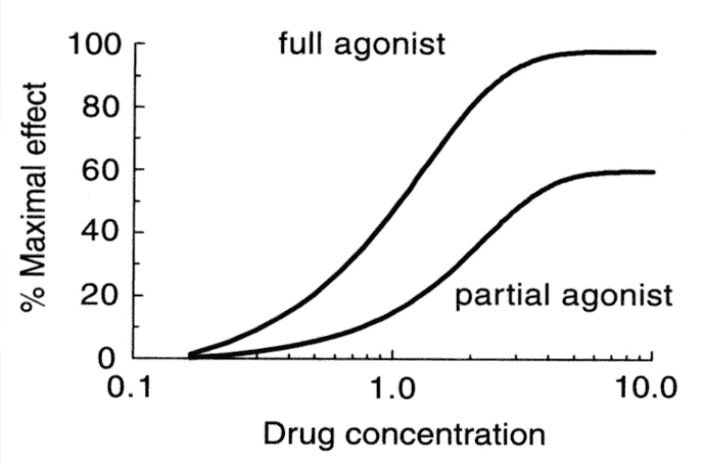
- Products are generally more water soluble
- These reactions products are ready for (renal) excretion
- There are many complementary, sequential and competing pathways
- Phase I and Phase II metabolism are a coupled interactive system interfacing with endogenous metabolic pathways

Drug Action: Receptor Theory

Many drugs act by binding to receptors (see Lecture 4) where they either provoke or inhibit a biological response.

Agonists:

- Can be drugs or endogenous ligands for the receptor
- Increasing concentrations of the agonist will produce an increase in the biological response:
  - Full Agonist: Evokes 100% of the maximum possible effect
  - Partial Agonist: Produces the same type of biological response, but cannot achieve 100% even at very high doses



Drug Action: Receptor Theory

Antagonists:

- Block or reverse the effects of agonists. They have no effects on their own
  - Competitive Antagonists: Compete with agonist for receptor binding => Agonist appears less potent, but can still achieve 100% effect (but at higher concentrations)
  - Non-competitive Antagonists: Bind to receptor at different site and either prevent agonist binding or the agonist effect => maximal achievable response reduced
  - Inverse Agonists: Not the same as antagonists! Inverse agonists trigger a negative response (= reduce baseline) (e.g. diazepam = full agonist = anticonvulsant BUT inverse agonists of benzodiazepin receptor are convulsants)