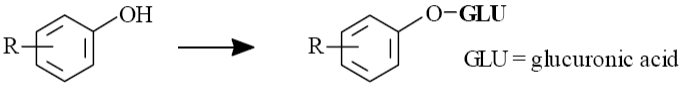
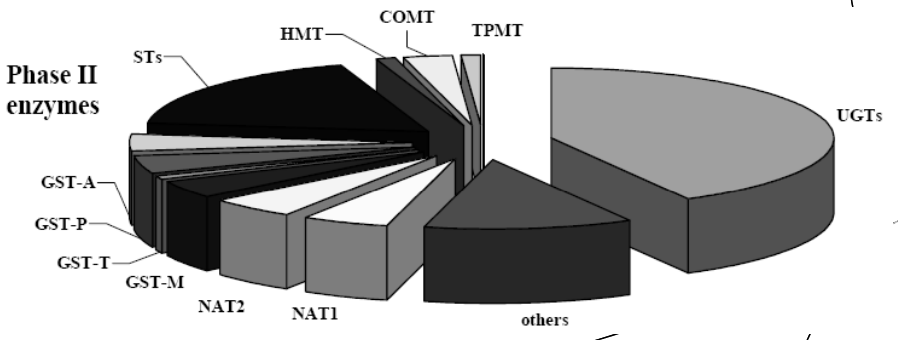
Phase II DME

- In phase II biotransformation reactions 'conjugation reactions' the drug becomes linked to an endogenous moiety through one or more functional groups.
- Conjugation reactions start with the replacement of a hydrogen atom present in a hydroxyl, amino or carboxyl group, by the conjugating agent.
- In general, the resulting conjugated metabolites have no pharmacological activity, are highly water-soluble and therefore subsequently readily excreted in the urine.





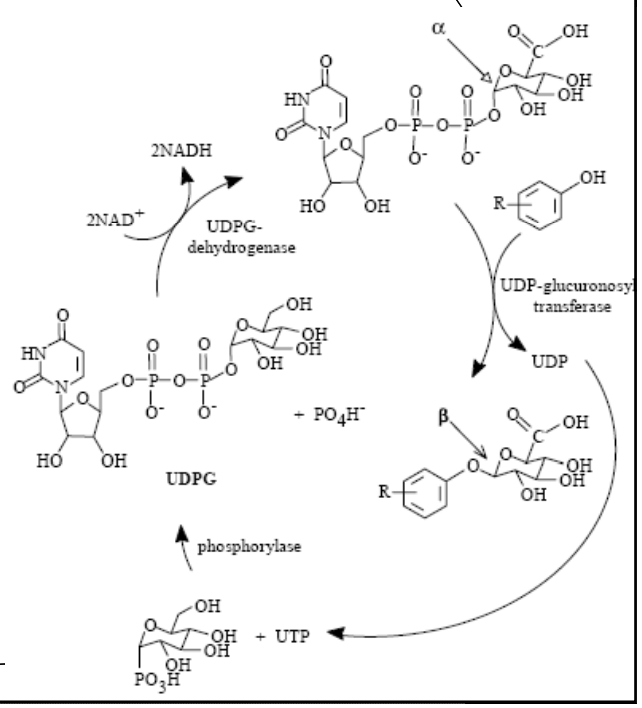
Glucuronidation

- glucuronide formation is the most important form of conjugation both for drugs and endogenous compounds.
- Glucuronidation is conjugation with D-glucuronic acid and is indeed the most widespread of the conjugation reactions, probably due to the relative abundance of the cofactor for the reaction, UDP-glucuronic acid.
- The transfer of glucuronic acid from UDP- glucuronic acid (UDPGA) is catalysed by a family of enzymes generally designated as UDP- glucuronosyl transferases (UGTs).

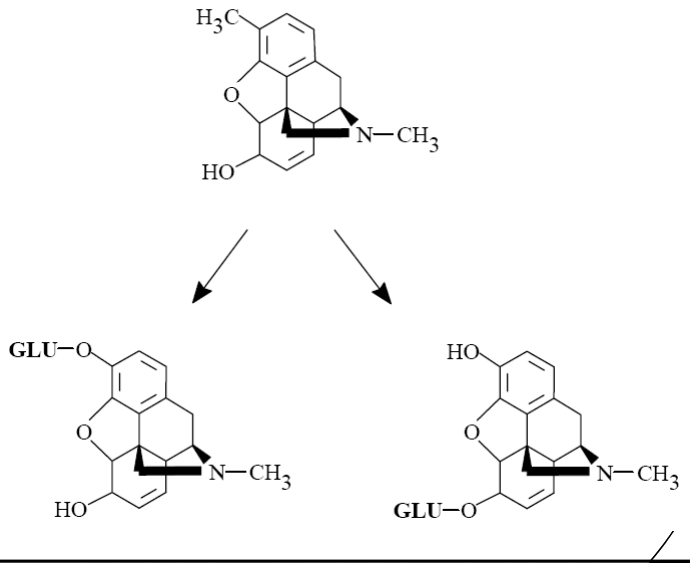
Glucuronidation

- The different isoenzymes of the UGT family have high organ specificity locations: for example, bilirubin UGT is highly expressed in human liver, but is absent in human kidney, whereas phenol UGT is highly expressed in both organs.
- Individual UGTs are subject to differential induction by hormones, leading to tissue-specific regulated expression. In addition, the spectrum of UGTs in different tissues can be differentially altered by exposure to drugs and other xenobiotics.

Examples

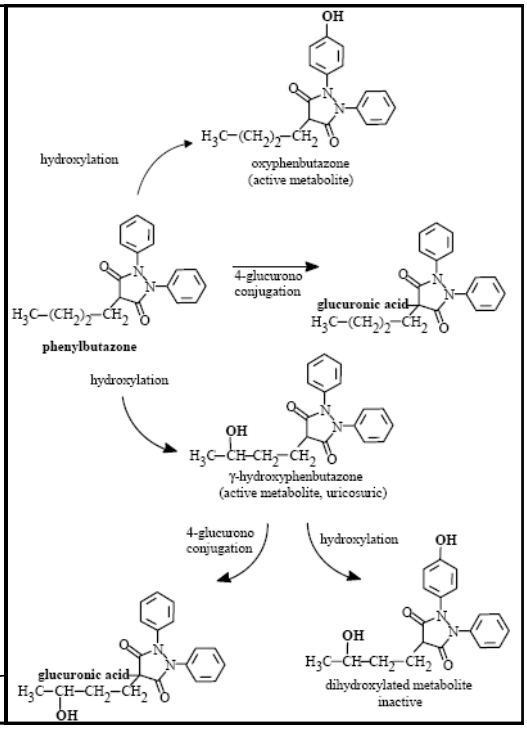


Glucuronidation

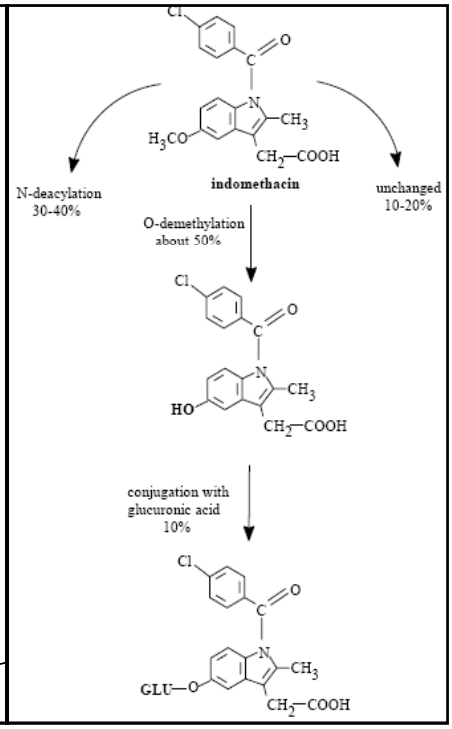


Examples for combined Phase I and II DME

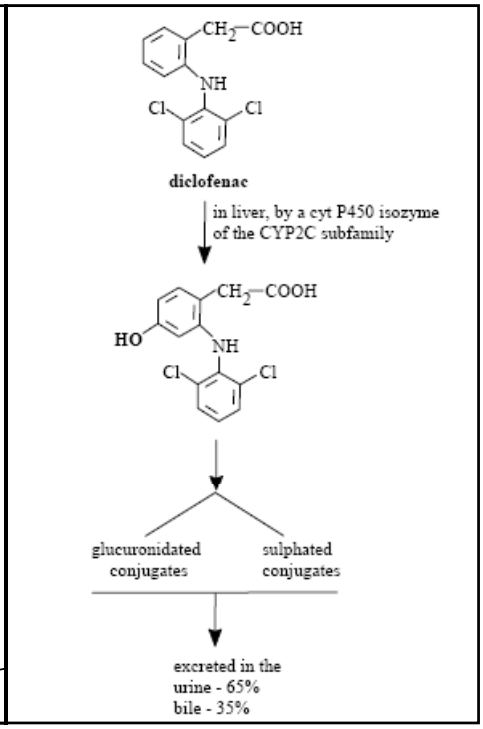
Phenylbutazone



Indomethacin



Diclofenac



Toxicological Aspects of Drug Metabolism

- We interact with an environment that introduces us to thousands of unique compounds. (~8g/day of food additives alone).
- Humans have responded to these chemicals with phase I and phase II metabolic enzymes that process xenobiotics and encourage their elimination.
- This was originally termed detoxication after it was assumed that a xenobiotic was transformed into a metabolite less toxic than the parent molecule.

Metabolic Conversions Leading to Toxic Metabolites

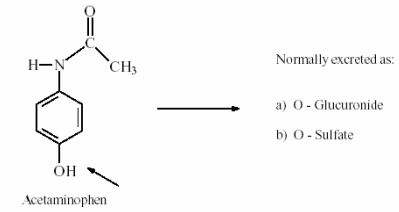
- This is called activation or bioactivation, Therefore, xenobiotics can be activated by the phase I or phase II metabolic pathways into:
  - a) Active metabolites.
  - b) Reactive metabolites (electrophiles).
- These electron deficient molecules can covalently attach to DNA, protein, and lipids.

I. Toxicity of Acetaminophen

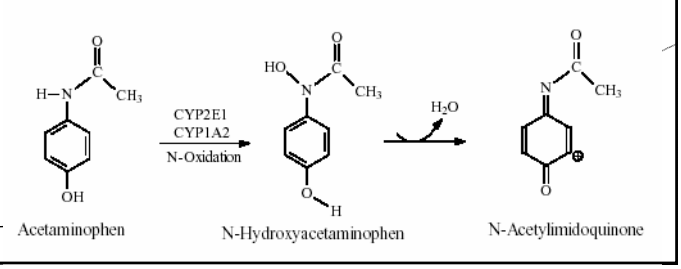
- Acetaminophen was identifies as a safe drug with analgesic and antipyretic properties more than 100 years ago.
- It is widely used in many countries as an alternative to aspirin.
- Even with widespread use over many years, reports of poisoning did not appear in the literature until 1966.
- How can Acetaminophen cause toxicity?
  - a) Metabolism - Major Metabolites.
  - b) Metabolism - Minor Metabolites.

How can Acetaminophen cause toxicity?

- Major Metabolites.

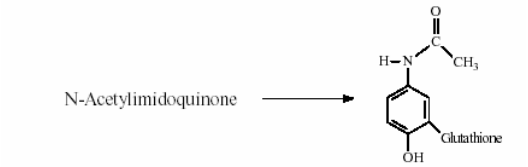


- Minor Metabolits



How can Acetaminophen cause toxicity?

- At therapeutic doses, glutathione in the liver combines with the reactive metabolite to form the glutathione conjugate.

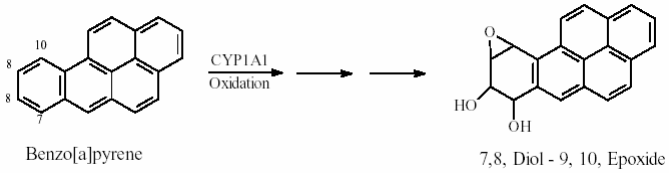


- Following an overdose, glutathione becomes depleted and the reactive metabolite binds to hepatic proteins.
- After a massive overdose, metabolism by oxidations will increase because the glucuronidation and sulfation pathways become saturated.

II. Carcinogenicity

1) The Polycyclic Aromatic Hydrocarbon (PAH)

- PAH are ubiquitous environmental contaminants formed from: auto emissions, Cigarette smoke, and BBQ.
- It is the electrophilic diol epoxide metabolite that readily
- reacts with DNA to form covalently bound adducts.



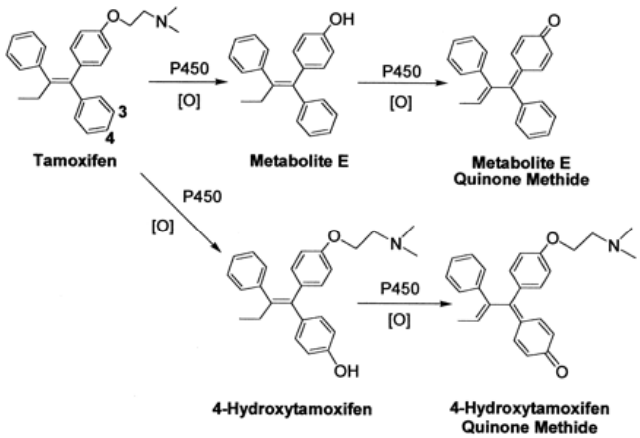
II. Carcinogenicity

2) Tamoxifen (antiestrogen)

- Inhibits the binding of estradiol to estrogen receptors.
- Is the current agent of choice for treating all stages of breast cancer.
- Of concern are the findings that:
  - a) Tamoxifen increases the incidence of human endometrial cancer.
  - b) Tamoxifen causes hepatocellular carcinoma in rats

II. Carcinogenicity

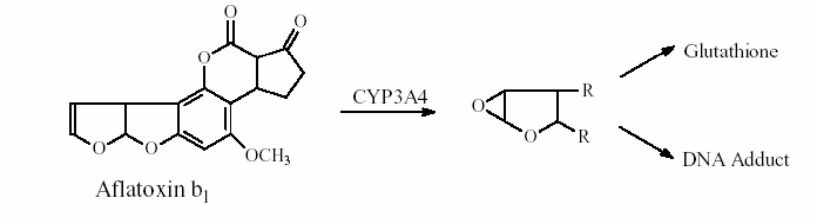
3) Tamoxifen (antiestrogen)



II. Carcinogenicity

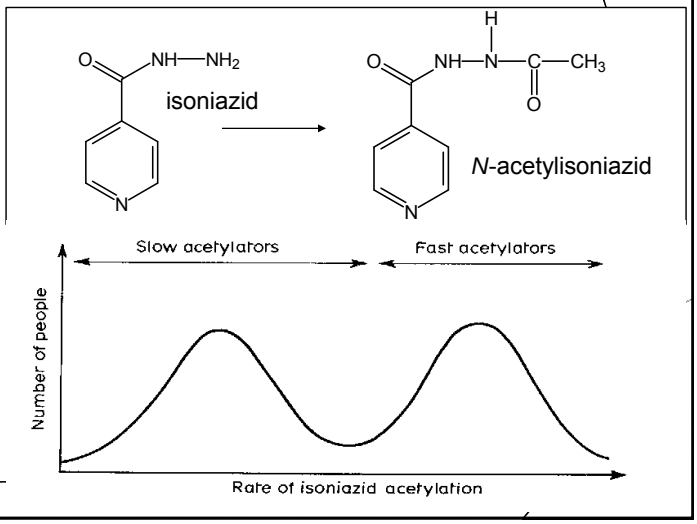
3) Aflatoxin b1

Aflatoxin is a secondary metabolite secreted into the environment by the fungus *Aspergillus flavus.*

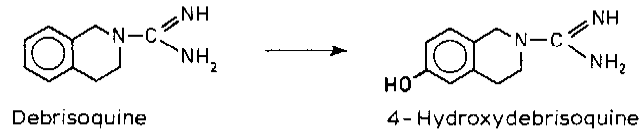


Genetic Differences in General Metabolism

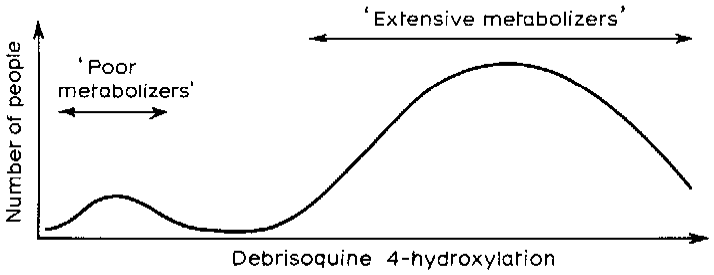
General population has a distribution of people with varying levels of activity in specific metabolic pathways



Genetic Differences in Metabolism of drugs



Debrisoquine 4-Hydroxydebrisoquine



Methods used for studying drug metabolism

1. *In Vivo* Method:

- One of the major methods for studying drug metabolism.
- Based on the determination of the drug concentration in various body fluids such as blood, urine, saliva.
- Substrate used for in vivo clearance: antipyrine, phenacetin, caffeine, and theophylline.

Methods used for studying drug metabolism

2. Breath analysis method:

- Based on hepatic breakdowns of certain drugs via demethylation to yield carbon dioxide which excreted via the lungs.
- Radiolabelling with 14C of the substrate leads to 14CO2 being excreted.
- Examples: aminopyrine, caffeine, antipyrine, diazepam and eryrthromycin.

Methods used for studying drug metabolism

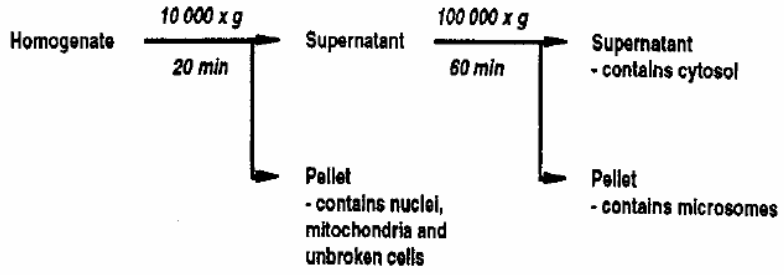
3. *In Vitro* method:

- Subcellular Fractions of Human Liver Tissue
  - Microsomes
  - The S9 subcellularfraction
- Whole Cell Models
  - Liver slices.
  - Isolated hepatocytes
  - Immortalized cell lines3.
- Heterologous Expressed and Purified Human Drug-Metabolizing Enzymes

Subcellular Fractions of Human Liver Tissue: *A Microsomes*

- Hepatic microsomes are a subcellular tissue fraction obtained by differential high-speed centrifugation of homogenized liver.
- Important drug metabolizing enzymes present within the microsomal fraction include the CYPP450, flavin-monooxygenases, epoxidehydrolases, and a variety of transferases (e.g. the UDP-glucuronosyl transferases).
- Microsomal preparations require the addition of exogenous cofactors, including a source of NADPH.
- Transferase activity can be studied by supplementing microsomal preparations with conjugating moieties.

Subcellular Fractions of Human Liver Tissue: *A Microsomes*



Subcellular Fractions of Human Liver Tissue: *A Microsomes*

Spectral Assay of microsomal CYP450 content

- The reduced (Fe+2) form of P450 binds with CO to form a complex having an absorption maximum at 450 nm.
- Method:
  - Combine the following components in a cuvette
  - 1 mg of microsomalprotein-200 ul of potassium phosphate buffer
  - Sufficient distilled water to give 1 ml final volume
  - few mg of sodium dithionite.
  - Bubble the sample with CO for 2 min.
  - Spectrophotometricallyfrom 400 – 500 nm.

Subcellular Fractions of Human Liver Tissue: *A Microsomes*

Advantages

- relatively easy to prepare and commercially available.
- relatively inexpensive technique.
- If properly handled, liver tissue from surgery or organ donors can be frozen (in liquid N2 and held at -80 °C) essentially indefinitely without apparent loss of P450 enzyme activity.
- All of the P450s are present in the material.
- can also be stored for long periods.
- The availability of human microsome preparations does allow for insight into the variability among the population.

Subcellular Fractions of Human Liver Tissue: *A Microsomes*

Disadvantages

- predictions of drug interactions from cell-free systems such as microsome smay be irrelevant if marked in vivo differences between plasma concentrations and intracellular hepatocyte concentrations are not taken into account.
- contains only phase I DMEs and UDPGT.
- are generally not an appropriate system in which to study sequential metabolic reactions (i.e. the coupling of phaseI and II reactions) owing to the disruption of the natural orientation between subcellularcomponents.
- requires strictly specific substrates and inhibitors or antibodies for individual DMEs.

Subcellular Fractions of Human Liver Tissue: *B S9 Subcellular F*

The S9 subcellular fraction (supernatant of liver homogenate resulting from precipitation of the nuclei and mitochondria by centrifugation at 9,000-20,000g) is a useful preparation in which to study drug metabolism involving both microsomal and cytosolic enzymes.

Whole Cell Model: *Liver Slices*

- Precision-cut highly reproducible tissue slices from various organs and different species, especially when kept in dynamic organ culture on the interface of medium and gas phases.
- In the adult mammalian liver, 65-70% of the cells are hepatocytes and about 30-35% nonparenchymal cells including sinusoidal endothelial cells, kupffer cells, and fat storing cells.
- Most of these cell types can biotransform compounds in that they contain CYP450 proteins and peroxidases.
- The cells also release a variety of mediators which regulate the function of other nonparenchymal cells or hepatocytes.

Whole Cell Model: *Liver Slices*

- The organ slice system provides the opportunity to explore the uptake of compounds by various cell types for a particular organ, routes and rates of biotransformation.

Whole Cell Model: *Liver Slices*

Advantages:

- A maintenance of a higher level of biological organization which may better reflect the response of the target organ.
- Maintenance of a differentiated state which is favored in tissue slices based on cell-cell and cell-matrix interaction.
- The functional heterogeneity of the cultured tissue which may be better preserved in tissue slices.
- The lack of a requirement for proteolytic enzymes normally employed in cell isolation which avoids digestion of important cell surface proteins.
- Maintenance of intermediary metabolic control over xenobiotic metabolism which may better reflect in vivo.

Whole Cell Model: *Liver Slices*

Disadvantages:

- Requires specific techniques and well established procedures.
- Clearance predictions from liver slices may be lower than those from hepatocytes or microsomes if, due to slice thickness, equilibration is not achieved between the cells of the slice and the incubation medium.
- Short-term stability of enzymatic activities represents the major problem with liver slices (<24 h).
- The viability of these preparations varies depending on culture conditions and the relative stability of the different enzymes under investigation.