Metabolism of Xenobiotics

Suggested Reading:
• Rose, RL, Hodgson E. Adaptation to Toxicants. Chemical and Environmental Factors Affecting Metabolism of Xenobiotics In: Hodgson E, Smart RC. INTRODUCTION TO BIOCHEMICAL TOXICOLOGY 3rd ed. Chapter 8 , pp. 163-198 (2001)

Optional Reading:
1. Xenobiotic Metabolism: Definition
Xenobiotic = "foreign"
Synonyms: Biotransformation; Drug Metabolism
Not: Detoxication Reactions

2. Phase I and Phase II Reactions: Classification

Phase I Reactions: Oxidation, Reduction, Hydrolysis
Introduce one of following groups into the initial compound: -OH, -COOH, -NH2, or –SH

Phase II Reactions: Conjugation
Introduces a highly hydrophilic group to promote excretion

Examples:

Phase I: Benzene -(oxidation)→ epoxide intermediate -(rearrangement)→ phenol with pKa=10 (<1% ionized at pH 7.4)

Phase II: Phenol -(glucuronidation)→ phenyl glucuroide with pHa=3.4 (very water soluble, greater than 99% ionized at pH 7.4)

3. Phase I Oxidations (at least in part) by microsomal system

3.1. Aromatic Hydroxylation (via epoxide)

Benzene -(aromatic hydroxylation)→ epoxide. Then either of 2 possible reactions:

(a) epoxide -(nonenzymatic rearrangement)→ phenol

(b) epoxide -(epoxide hydrolyase)→ 1,2-dihydro-1,2-diol
also 1,2-dihydro-1,2-diol -(cytosolic dehydrogenase)→ catechol
3. Phase I Oxidations (at least in part) by microsomal system

3.1. Aromatic Hydroxylation (via epoxide)

NIH Shift = intramolecular migration of substituent group at the site of oxidation that moves to an adjacent ring position.

If R group is not readily ionizable (-CH₃, -OCH₃, -phenyl, -halo, -nitro), then 40-65% migration of D

If R group is readily ionizable (-OH, -NH₂), then 0-6% migration of D

Groups capable of migration: D or deuterium, 3H or tritium, chloro, methyl

Other substrates subject to Aromatic Hydroxylation (via epoxidation):

Bromobenzene → bromobenzene epoxide

Chlorobenzene → chlorobenzene epoxide → ortho-chlorophenol, and para-chlorophenol
3.2. Aromatic Hydroxylation via O-insertion

Chlorobenzene $\rightarrow$ meta-chlorophenol

Aniline $\rightarrow$ para-hydroxyaniline

3.3. Aliphatic Hydroxylation

n-propyl-benzene $\rightarrow$ phenyl-CH$_2$-CH$_2$(CH$_2$OH) omega hydroxylation

and phenyl-CH$_2$(CHOH)CH$_3$ omega minus 1 hydroxylation

and phenyl(CHOH)CH$_2$-CH$_3$ alpha hydroxylation

The products from Omega and Omega-minus 1 hydroxylations are always the major metabolites.

If a substrate can be metabolized either by aliphatic hydroxylation or aromatic hydroxylation, aliphatic hydroxylation is always predominant.
3.4. N-Dealkylation (Oxidation of the Alkyl Group)

R-NH-CH₃ (oxidation of the C) → R-NH₂ + HCHO (methyl group is oxidized to formaldehyde)

R-NH-C(R₂) (oxidation of the carbon) → RNH₂ + ketone (e.g., acetone)

Also O-Dealkylation and S-Dealkylation (always oxidation of the carbon)

3.5 N-Oxidation (Oxidation of N)

Aromatic primary and secondary amines yield aromatic hydroxylamine
Products very reactive and toxic

Aliphatic tertiary amines yield N-Oxide
(CH₃)₃N → (CH₃)₃N=O

Aromatic tertiary amines → N-oxide

Two pathways leading to N-oxidation:
• Microsomal FAD-containing monoxygenase
  Not inhibited by CO
• Cytochrome P450
  Inhibited by CO
3.6. S-Oxidation

Both FMO and cytochrome P450 are active

\[
\begin{align*}
\text{sulfides,} & \quad \text{sulfoxide} & \quad \text{sulfone} \\
\text{thioethers} & \quad \longrightarrow & \quad \longrightarrow & \quad \longrightarrow \\
\text{(major product)} & \quad & \quad & \quad
\end{align*}
\]

4. Non-Microsomal Oxidations

4.1. Alcohol Oxidation

Aliphatic alcohol \(\rightarrow\) Aldehyde \(\rightarrow\) Carboxylic Acid

ADH = alcohol dehydrogenase
ALDH = aldehyde dehydrogenase

Rate of metabolism: primary alcohols > secondary alcohols >>> tertiary alcohols (latter not metabolized by alcohol dehydrogenase)
5. Phase I Reactions: Reductions

Favored by anaerobic conditions. Do occur in mammalian tissues where oxygen concentration is low.

In vitro conditions: replace air or oxygen with nitrogen

Nitro Reduction (Nitro Reductase)

Azo Reduction (Azo Reductase)

Nitro Reduction: 3 important enzyme systems

(a) Cytochrome P450 (e.g., in liver). Inhibited by CO
(b) DT-diaphorase: cytosolic flavoprotein (in liver) = NAD(P)H quinone oxidoreductase
(c) Bacterial intestinal enzymes
6. Phase I Reaction: Hydrolysis

Substrates:
(a) carboxylic acid ester, amides - same enzyme
(b) phosphate ester
(c) epoxide

Enzyme:
Epoxide hydrolase (former names: Epoxide hydratase, epoxide hydrase)

Most of enzyme in microsomes, but also in cytosol. Different gene products.
Product is always trans hydroxyls

7. Monooxygenase or Mixed Function Oxidase (MFO)

(a) Monooxygenase = 1 atom of molecular oxygen is added to substrate
(b) Mixed function oxidase = 1 atom of molecular oxygen is added to substrate, and 1 atom of oxygen is converted to water
(c) Terms in (a) and (b) have been used interchangeably, but term monooxygenase is probably the primary term now.

Overall reaction: \(-\text{SH} + \text{O}_2 + 2 \text{e}^- \rightarrow \text{SOH} + \text{H}_2\text{O}\)

reduced substrate + molecular oxygen + two one-electron transfer \(\rightarrow\) oxidized substrate + water

System also requires lipid. Lipid-soluble compounds are better substrates for cytochrome P450 than water-soluble substrates.
8. Cytochrome P450: Terminal Oxidase in Xenobiotic Metabolism

Cytochrome P450s family ("superfamily") of similar hemoproteins, and is critically important in xenobiotic metabolism.

The human genome encodes 57 P450 proteins: (Guengerich, 2003)
- 15 involved in metabolism of xenobiotic chemicals (i.e., chemicals, such as drugs, not normally found in the body)
- 14 primarily involved in the metabolism of sterols (including bile acids);
- 4 that oxidize fat-soluble vitamins; and
- 9 involved in the metabolism of fatty acids and eicosanoids.
Substrates are essentially unknown for the remaining 15 of the 57.

8. Cytochrome P450: Terminal Oxidase in Xenobiotic Metabolism

Substrates:

"The cytochrome P450 gene superfamily encodes many [isozymes] that are unusual in the variety of chemical reactions catalyzed and the number of substrates [metabolized]. The [substrates] include physiologically important substances such as steroids, eicosanoids, fatty acids, lipid hydroperoxides, retinoids, and other lipid metabolites, and xenobiotics such as drugs, alcohols, procarcinogens, antioxidants, organic solvents, anesthetics, dyes, [and] pesticides".

A limited number of the P450 isozymes, e.g., those P450s which metabolize steroids, are moderately specific in the nature of the substrates metabolized. However, "many of the P450s, [especially] those in the hepatic endoplasmic reticulum, catalyze a ... large number of chemical reactions with an almost unlimited number of biologically occurring and xenobiotic compounds. In the latter category are synthetic environmental chemicals, now estimated at about 250,000, most of which are potential P450 substrates if not inducers or inhibitors of the individual [P450s]"."
8.1. Nomenclature of Cytochromes P450

"The nomenclature system is based solely on the sequence similarity among P450s and does not indicate the properties or function of individual P450s"

In the current nomenclature system [], the cytochrome "P450s are named using the root symbol CYP ..., followed by an Arabic numeral designating the family number, a letter denoting the subfamily, and another aromatic numeral representing the [product of] the individual gene". Thus CYP2E1 [note: no space] is the cytochrome P450 in family 2, subfamily E, and gene product 1 in the subfamily.

"The P450 gene superfamily encodes numerous enzymes, of which more than 150 have so far been characterized. These vary from about 10% to over 90% in sequence identity." In the current nomenclature scheme:

[a] "Those P450 proteins from all sources with 40% or greater sequence identity are included in the same family, as designated by an Arabic number."

[b] "Those with greater than 55% identity are then included in the same subfamily, as designated by a capital letter."

[c] "The individual genes (and gene products) are then arbitrarily assigned numbers"

Names of the genes are written in italics, e.g., CYP1A1.

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P450 Gene Superfamily, Family, Subfamily, and gene designation (Nebert et al. 1987)

<table>
<thead>
<tr>
<th>Family 1 (polycyclic aromatic compound-inducible)</th>
<th>Family 2 (phenobarbital-inducible)</th>
<th>Family 3 (steroid-inducible)</th>
</tr>
</thead>
<tbody>
<tr>
<td>only one subfamily</td>
<td>2A subfamily</td>
<td>only one subfamily</td>
</tr>
<tr>
<td>1A1</td>
<td>2A1</td>
<td>3A1</td>
</tr>
<tr>
<td>1A2</td>
<td>2A2</td>
<td>3A2</td>
</tr>
<tr>
<td>Family 2</td>
<td>2B subfamily</td>
<td>3A3</td>
</tr>
<tr>
<td>2B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2B2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C subfamily</td>
<td>2C1</td>
<td>2A1 (40% identity)</td>
</tr>
<tr>
<td>2C1</td>
<td>2C2</td>
<td>3A1</td>
</tr>
<tr>
<td>2C2</td>
<td>2C3</td>
<td>3A2</td>
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<td>2C4</td>
<td>2C5</td>
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<tr>
<td>2C5</td>
<td>2C6</td>
<td>3A3</td>
</tr>
<tr>
<td>2C6</td>
<td>2C7</td>
<td></td>
</tr>
<tr>
<td>2C7</td>
<td>2C8</td>
<td></td>
</tr>
<tr>
<td>2C8</td>
<td>2C9</td>
<td></td>
</tr>
<tr>
<td>2C9</td>
<td>2C10</td>
<td></td>
</tr>
<tr>
<td>2D subfamily</td>
<td>2D1</td>
<td></td>
</tr>
<tr>
<td>2D1</td>
<td>2D2</td>
<td></td>
</tr>
<tr>
<td>2E subfamily (ethanol-inducible)</td>
<td>2E1</td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Family 4 (peroxisome proliferator-inducible)    only one subfamily 4A1

Family 17 (steroid 17-alpha-hydroxylase)          only one subfamily

Family 21 (steroid 21-hydroxylase)                only one subfamily

Family 11 (steroid 11-beta-hydroxylase)           only one subfamily

Family 22 (cholesterol side-chain cleavage)       only one subfamily

Family 51 (plant P450)                            1 gene

Cl (prokaryote P450)                              only one subfamily (1 gene; CIA1)
8.2. Cytochrome P450 Intracellular Localization

(a) Cellular: microsomes.
Technique: The endoplasmic reticulum (the intracellular site of Cytochrome P450) is disrupted into small sacs. The small sacs are collected by ultracentrifugation as a pellet referred to as microsomes. The microsomes do not occur as such within a cell.

(b) Outer nuclear membrane

(c) Mitochondria, in those tissues (e.g., adrenal cortex) that extensively hydroxylation

![Isolation of Microsomes by Differential Centrifugation](image)

**Importance of "S-9"**
Ames Salmonella typhimurium test for mutagenesis
Salmonella test for mutagenic activity of the starting compound or its metabolites
"S-9" contains both microsomes and cytosol to metabolism the starting to possible mutagens

8.3. Tissue Distribution of Cytochrome P450

Liver >> kidney, lung, small intestine >> heart, muscle, brain

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Aniline hydroxylation</th>
<th>Hexobarbital hydroxylation</th>
<th>Aminopyrine N-demethylation</th>
<th>NADPH-dehydrogenase</th>
<th>Amount of P-450</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>100 ± 9</td>
<td>100 ± 11</td>
<td>100 ± 9</td>
<td>100 ± 8</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>5 ± 2</td>
<td>9 ± 4</td>
<td>7 ± 5</td>
<td>13 ± 5</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Lung</td>
<td>14 ± 4</td>
<td>3 ± 2</td>
<td>4 ± 2</td>
<td>7 ± 3</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Heart</td>
<td>3 ± 1</td>
<td>1 ± 1</td>
<td>2 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Small Intestine</td>
<td>1 ± 1</td>
<td>0</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Muscle</td>
<td>1 ± 1</td>
<td>0</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Brain</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
</tr>
</tbody>
</table>
9. Mechanism of Cytochrome P-450 Dependent Oxidation

**step 1:** Association of the substrate with oxidized (or ferric) cytochrome P450

\[ P_{450}^{(Fe^{3+})} \rightarrow P_{450} - S \]

**step 2:** The next step is a one-electron transfer from NADPH to yield a complex between reduced (or ferrous) cytochrome P450 and the substrate. The reaction is catalyzed by NADPH-cytochrome P450 reductase.

\[ P_{450}^{(Fe^{3+})} \text{ from NADPH} \rightarrow P_{450}^{(Fe^{2+})} \text{ reduced (or ferrous)} \rightarrow P_{450} - \text{substrate} \]
9. Mechanism of Cytochrome P-450 Dependent Oxidation

**step 3:** The reduced cytochrome P450-substrate complex then reacts with molecular oxygen. Note that the product is oxygenated reduced P450-substrate complex. OXYGENATED, not oxidized.

**step 4:** The ternary complex undergoes a second one-electron reduction -- the reduction of the oxygenated reduced cytochrome P450-substrate complex.

The donor of the second one-electron transfer may differ with different substrates and/or the availability of the reduced pyridine nucleotides.

1. NADPH-cytochrome P450 reductase -- predominant
2. NADH cytochrome b5 reductase

The latter (2) appears to be important in microsomal desaturation of fatty acids.

**step 5:** There is a decomposition of the oxygenated cytochrome P450 with the release of water

**step 6:** The final step is the release of the hydroxylated substrate and the oxidized cytochrome P450:

The oxidized cytochrome can then recycle by binding to another molecule of substrate.
10. FAD-Containing Monooxygenase, or Flavin-Containing Monooxygenase

Liver, kidney, lung, others
Catalyze monoxygenation of nucleophilic S, N, P, Se
Substrates: tertiary and secondary amines
Tertiary amines yield N-oxides
Sulfides, thioethers, thiols, thiocarbamates, organophosphorus compounds
Many of these substrates are subject to both P450 metabolism and FMO metabolism: N, S, P Oxidations, desulfurations

11. Inhibitors of Cytochrome P450

(1) Competitive Inhibition
Omeprazole and deazepam
Both substrates are metabolized by CYP2C19
The competition for the same P450 decreases the clearance of diazepam and prolongs its plasma half life
Occurrence of this mechanism: many examples

(2) Competitive in nature but the inhibitor is not a substrate of the P450
CYP2D6 metabolizes dextromethorphan
Quinidine binds to and inhibits CYP2D6
And inhibits the metabolism of dextromethorphan
Occurrence of this mechanism: few or rare

(3) Mechanism-based (most commonly used term)
Metabolism-dependent (probably the best term)
Suicide inactivation
11. Inhibitors of Cytochrome P450 or agents that decrease activity of Cytochrome P450
Because of many isozymes, many different inhibitors. One inhibitor may affect different
isozymes to different extents.

(a) CO, most specific, binds to ferrous heme complex.

(b) Mechanism-based inhibitor definition.
A non-reactive inhibitor which is metabolized
to a reactive metabolite by cytochrome P450,
reacts with the cytochrome P450,
and thereby inhibits the Cytochrome P450.

(b1) SKF 525A.

(b2) Methylene dioxyphenyl compounds
(example: piperonyl butoxide)

(b3) Metyrapone
Initial, short-term effect: inhibition
Longer term exposure, induced synthesis of cytochrome P450.

(c) Inhibitors of heme synthesis

\[
glycine + \text{succinyl CoA} \rightarrow \text{ALA synthetase} \rightarrow \text{delta-aminolevulinic acid (ALA)} \rightarrow \text{ALA dehydratase} \rightarrow \text{PBG (porphobilinogen)} \rightarrow \text{protoporphyrin IX} \rightarrow \text{ferrochelatase or heme synthetase} \rightarrow \text{heme}
\]

\[\text{Pb}^+ \text{ inhibits ALA dehydratase and ferrochelatase (\text{heme synthetase})}\]

\[\text{Cobalt Co}^{2+} \text{ inhibits ALA synthetase, prevents normal association of heme to apocytochrome P450, inhibits heme synthetase, stimulates heme oxygenase (the enzyme involved in heme degradation)}\]

(d) Agents causing destruction of cytochrome P450
\[\text{AIA = allylisopropylacetamide} \text{ (mechanism based inhibitor, metabolite is the epoxide that reacts with the heme of cytochrome P450)}\]

\[\text{Cadmium Cd}^{2+} \text{ } \]

\[\text{Carbon tetrachloride } (.\text{CCl}_3 \text{ trichloromethyl free radical})\]
Phase II: Conjugation Reactions

Parent compounds or their Phase I metabolites that contain suitable chemical groups undergo conjugation reactions with endogenous substrates to yield conjugates. In general, conjugates are polar molecules that are readily excreted.

Summary

<table>
<thead>
<tr>
<th>Type of conjugation</th>
<th>Endogenous reactant</th>
<th>Transferase (cellular location)</th>
<th>Reactive sites</th>
<th>Types of substrates</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucuronidation</td>
<td>UDP glucuronic acid</td>
<td>UDP glucuronosyltransferase (microsomes)</td>
<td>OH, COOH, NH₂, SH, C-C</td>
<td>Phenols, alcohols, carboxylic acids, hydroxylamines, sulfinylamides</td>
<td>Acetanilidophen, nitronephol</td>
</tr>
<tr>
<td>Acetylation</td>
<td>Acetyl-CoA</td>
<td>N-acetyltransferase (cytosol)</td>
<td>NH₂, SO₂NH₂, OH</td>
<td>Amines</td>
<td>Isoniazid, sulfonamides</td>
</tr>
<tr>
<td>Glutathione conjugation</td>
<td>Glutathione</td>
<td>Glutathione S-transferase (cytosol)</td>
<td>Epoxide, organic halides, organic nitro compounds, unsaturated compounds</td>
<td>Epoxide, amines, nitro groups, hydroxylamines</td>
<td>Brucine/brucine</td>
</tr>
<tr>
<td>Glycine conjugation</td>
<td>Glycine</td>
<td>Acetyl-CoA</td>
<td>COOH</td>
<td>Acetyl-CoA derivatives of carboxylic acids</td>
<td></td>
</tr>
<tr>
<td>Sulfate conjugation</td>
<td>Phosphodiesterase phosphodiesterase (cytosol, microvesicles)</td>
<td>NH₂, OH</td>
<td>Phenols, alcohols, amines</td>
<td>Antipyrine, phenol, acetanilidophen</td>
<td></td>
</tr>
<tr>
<td>Methylation</td>
<td>S-adenosyl-</td>
<td>Transmethyllase (cytosol)</td>
<td>OH, NH₂, SH</td>
<td>Phenols, amines, catecholamines</td>
<td>Pyridine, histamine, epinephrine</td>
</tr>
</tbody>
</table>

15.A. Glucuronidation.

<table>
<thead>
<tr>
<th>Type of conjugation</th>
<th>Endogenous reactant</th>
<th>Transferase (cellular location)</th>
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<th>Types of substrates</th>
<th>Examples</th>
</tr>
</thead>
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<td>Phenols, alcohols, carboxylic acids, hydroxylamines, sulfinylamides</td>
<td>Acetanilidophen, nitronephol</td>
</tr>
</tbody>
</table>

**Glcuronidation**

![Glcuronidation](image)
Reactions and Products:
(a) O-glucuronides ethers
(from phenolic hydroxyls and aliphatic hydroxyls)
(b) O-glucuronides esters
(from carboxylic acids, -COOH)
(c) N-glucuronides
(d) S-glucuronides
(e) C-glucuronides

Enzymes:
UDP glucuronosyl transferases (UGT) are products of a multigene superfamily.

Rat liver UGTs are members of two gene families, UGT1 and UGT2. Each gene family consists of at least 4 distinct enzymes.

Humans produce at least 6 distinct UGT1 gene products.

In the rat, UGT1 family members are inducible by Ah-receptor ligands (TCDD, 3-methylcholanthrene), phenobarbital, and clofibrate

Reaction Characteristics:
Glucuronidation: low-affinity, high-capacity catalysis, and provides for efficient substrate conjugation at high substrate concentrations.

Reactions and Products: (page 3, Table 6.2)

Note upper hydroxylamine (replaces H on OH) and lower hydroxylamine (replaces H on the N)
Enzymes

The sulfotransferases comprise a superfamily of enzymes, Major subfamilies of cytosolic sulfotransferases are referred to as SULT 1, SULT 2, and SULT 3.

Reaction Characteristics:

Sulfation often provides for high-affinity, low capacity catalysis and provides for efficient substrate conjugation at low substrate condensations.

Sulfate is rate limiting component within cells. Total extent of sulfation in increased by including sulfate (or cysteine or methionine, which are degraded into inorganic sulfate.)

Reactions and Products:

Sulfate conjugation involves the transfer of sulfonyl (SO₂⁻), not sulfate (SO₄²⁻) from PAPS to the xenobiotic. "The commonly used terms sulfate and sulfate conjugation are used here, even though sulfonation and sulphonate are more appropriate descriptors."

### 15.D. Acetylation

<table>
<thead>
<tr>
<th>Type of conjugation</th>
<th>Endogenous reactant</th>
<th>Transferase (cellular location)</th>
<th>Reactive sites</th>
<th>Types of substrates</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation</td>
<td>Acetyl-CoA</td>
<td>N-acetyltransferase (cytosol)</td>
<td>NH₂, SO₂NH₂, CH</td>
<td>Amines</td>
<td>Isoniazid, sulfinamido</td>
</tr>
</tbody>
</table>

**Two types of acetylation reactions occur:**

One involves a activated conjugating intermediate, acetyl CoA, and the xenobiotic. The reaction is referred to as acetylation.

The second type involves the activation of the xenobiotic to an acyl CoA derivative, which then reacts with all amino acid to form an amino acid conjugate.

Acetylation typically results in the masking of the amine group with a non-ionizable acetyl moiety. As a result, the acetylated derivatives are generally less water soluble than the parent compound.

**Enzymes:**

Two cytoplasmic N-acetyltransferases, NAT1 and NAT2 have been identified in humans. A third enzyme, NAT3, has been identified in mice.

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**Importance to toxicity:**

Genetic polymorphisms in N-acetylation enzymes have been identified in humans and other species. The human population is segregated into slow acetylators and fast acetylators based on the rates of acetyl-CoA of the drug isoniazid. The slow acetylator phenotype is the result of polymorphisms in the NAT2 gene. Slow acetylators are predisposed to toxicity of drugs that are inactivated by acetylation such as isoniazid and dapsone. This enzyme also acetylates aromatic amine dyes to which workers have been exposed industrially such as benzidine dyes, 4-aminobiphenyl, and o-toluidine.

Workers in the acrylamide dye industry who are slow acetylators have been shown to have an increased risk of bladder cancer. The low activity of NAT2 in the liver of slow acetylators may make the aromatic amines more available for hydroxylation. The resulting hydroxylamines then accumulate in the bladder where they are acetylated by NAT1.
Endogenous substrate:
S-Adenosylmethionine

Reaction and Products:
Nitrogen
Oxygen
Sulfur

Methylation is a common but generally minor pathway of xenobiotic metabolism. Methylation phase II reactions generally decrease the water-solubility of xenobiotics and masks functional groups that might otherwise be conjugated by other phase II enzymes. However, methylation reactions that produce quaternary ammonium ions or methylation that produces positively charged sulfonium ions increase the solubility.
Glutathione Conjugation will be covered in detail in "Protective Systems" lectures
16. Metabolism of Carcinogens

Parent Compound → Proximate Carcinogen

→ Ultimate Carcinogen

Benzpyrene Metabolism

Inactivation of dihydrodiol polycyclic hydrocarbons
8-hydroxy importance

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**Figure 15.4** Examples of DNA-damaging carcinogens.
**Figure 2:** The enzymatic cleavage of breakdown is depicted in this figure.

<+i> <i>70% of DNA</i> 

contains a normally defined, hydrophobic region on the side of the substrate and is asymmetrically located above and below the enzyme to allow for a more significant steric hindrance between the substrate region and the enzyme active site. The active site regions of the enzyme molecule, which interact with a region about 1 Å wide and...