Xenobiotic Biotransformation

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Section A

Biotransformation: Basic Concepts
Renal Excretion of Chemicals

- Afferent arteriole
- Glomerulus
- Bowman’s capsule
- Proximal tubule
- Filtered drug
- Efferent arteriole
- Unfiltered drug
- Active secretion
- Water soluble
- Lipid soluble

Filtered drug can undergo:
- Passive reabsorption
- Excretion and/or further passive reabsorption

Diagram illustrates the pathways for filtered drugs and unfiltered drugs through the kidney.
Biotransformation of Xenobiotics

*Biological basis for xenobiotic metabolism:*

- To convert lipid-soluble, non-polar, non-excretable forms of chemicals to water-soluble, polar forms that are excretable in bile and urine

Continued
Biotransformation of Xenobiotics

Adapted from Casarett & Doull’s Toxicology. 4th Edition.
Biotransformation Reactions

**Phase I Reactions**

- Enzymatic reactions that **add** or **expose** functional groups to xenobiotics such as -OH, -SH, -NH2 or –COOH
- Functional groups are analogous to having a trailer hitch on a vehicle
Biotransformation Reactions

Phase II Reactions

- Enzymatic reactions that result in the conjugation of large water-soluble, charged (polar)biomolecules to xenobiotics
- For these reactions to occur, a functional group must be present on either the parent compound or its Phase I product
The Truck-Hitch-Trailer Analogy to Xenobiotic Biotransformation

Foreign Chemical (xenobiotic)

TRUCK

- lipophilic
- not charged
- not water soluble
- poorly excretable
The Truck-Hitch-Trailer Analogy to Xenobiotic Biotransformation

- **Foreign Chemical (xenobiotic)**
  - lipophilic
  - not charged
  - not water soluble
  - poorly excretable

- **Phase 1 enzymes**
  - add or expose a functional group
  - still lipophilic
  - possibly reactive
  - poorly water soluble
  - poorly excretable
  - catalyzed by P450s
The Truck-Hitch-Trailer Analogy to Xenobiotic Biotransformation

- Foreign Chemical (xenobiotic)
  - lipophilic
  - not charged
  - not water soluble
  - poorly excretable

- Phase 1 enzymes
  - add or expose a functional group
  - still lipophilic
  - possibly reactive
  - poorly water soluble
  - poorly excretable
  - catalyzed by P450s

- Phase 2 enzymes
  - conjugate (transfer) endogenous molecules*
  - to the functional group
  - not lipophilic
  - usually not reactive
  - water soluble products
  - excretable
  - catalyzed by transferases

* sugars, amino acids, sulfates, acetyl groups

Photo by John Pittman. Creative Commons BY-NC-SA.
Section B

Biotransformation: Enzymes
Organ and Cellular Location of Biotransformation Enzymes

- Organs involved in biotransformation
  - Liver
  - Lung
  - Kidney
  - Intestine
    - Enterocytes
    - Gut flora (contribute to entero-hepatic circulation)
  - Skin
  - Gonads
## Biotransformation Enzyme-Containing Cells in Various Organs

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cell(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>Parenchymal cells (hepatocytes)</td>
</tr>
<tr>
<td>Kidney</td>
<td>Proximal tubular cells (S3 segment)</td>
</tr>
<tr>
<td>Lung</td>
<td>Clara cells, Type II alveolar cells</td>
</tr>
<tr>
<td>Intestine</td>
<td>Mucosa lining cells</td>
</tr>
<tr>
<td>Skin</td>
<td>Epithelial cells</td>
</tr>
<tr>
<td>Testes</td>
<td>Seminiferous tubules, Sertolli cells</td>
</tr>
</tbody>
</table>
Nature of the Xenobiotic Metabolizing Enzyme System

- **Phase I metabolism**
  - Small molecular weight changes like hydroxylation, reduction, hydrolysis, etc.
  - In general, Phase I metabolism prepares the xenobiotic for subsequent Phase II reactions
Cytochrome P450 Characteristics

- Can metabolize many xenobiotics (broad substrate specificity)
- Can catalyze many types of reactions
- Is widely distributed among tissues, and tissue distribution can be quite varied
Cytochrome P450 Characteristics

- Exists in multiple forms (determined by different genes)
- Levels can be increased by exposure to chemicals in the food, water, or air (induction)
## Multiple Forms of P450

**Major Mammalian Cytochrome P450 Gene Families**

<table>
<thead>
<tr>
<th>P450 Gene Subfamily</th>
<th>Characteristic Inducer</th>
<th>Characteristic Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>Polycyclic aromatic hydrocarbons</td>
<td>Benzo(a)pyrene hydroxylation</td>
</tr>
<tr>
<td>IIA</td>
<td>Phenobarbital</td>
<td>Steroid hydroxylation</td>
</tr>
<tr>
<td>IIB</td>
<td></td>
<td>Benzphetamine demethylation</td>
</tr>
<tr>
<td>IIC</td>
<td></td>
<td>Steroid hydroxilatation</td>
</tr>
<tr>
<td>IID</td>
<td></td>
<td>Debrisoquine hydroxylation</td>
</tr>
<tr>
<td>IIE</td>
<td>Ethanol</td>
<td>Ethanol hydroxylation</td>
</tr>
<tr>
<td>IIIA</td>
<td>Steroids</td>
<td>Steroid hydroxylation</td>
</tr>
</tbody>
</table>

*Continued*
## Multiple Forms of P450
### Major Mammalian Cytochrome P450 Gene Families

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<th>P450 Gene Subfamily</th>
<th>Characteristic Inducer</th>
<th>Characteristic Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVA</td>
<td>Hypolipidemic agents</td>
<td>Lauric acid hydroxylation</td>
</tr>
<tr>
<td>XIA</td>
<td></td>
<td>Cholesterol side chain cleavage</td>
</tr>
<tr>
<td>XIB</td>
<td>Deoxycortisol 11α—hydroxylation</td>
<td></td>
</tr>
<tr>
<td>XVII</td>
<td></td>
<td>Pregnenolone 17β—hydroxylation</td>
</tr>
<tr>
<td>XIX</td>
<td></td>
<td>Androgen conversion to estrogens</td>
</tr>
<tr>
<td>XXI</td>
<td></td>
<td>Progesterone 21-hydroxylation</td>
</tr>
</tbody>
</table>
Nature of the Xenobiotic Metabolizing Enzyme System

- **Phase II metabolism**
  - Involves the complexing or conjugation of xenobiotics with relatively large and highly water-soluble adducts to form glucuronides, sulfates, and glutathione adducts
Nature of Systems Involved in Phase II Metabolism

Four primary enzymes:
1. Glucuronosyltransferase—glucuronic acid
2. Sulfotransferase—sulfate
3. Glutathione-S-transferase—glutathione (GSH)
4. Acetyltransferase—acetyl
Phase II Reactions

- Many of the characteristics described earlier for cytochrome P450 also apply to these Phase II enzymes.
- However, cytochrome P450 is localized in cellular membraneness, whereas Phase II enzymes are, for the most part, in the cytoplasm (water portion) of cells.
Phase II Enzymes: Examples

Glucuronidation and Sulfation of a Hydroxyl Group

\[
\text{R-OH} \xrightarrow{\text{UDP-glucuronosyl transferase}} \text{UDP-GA} \xrightarrow{\text{Sulfotransferase}} \text{PAPS}
\]

Ethereal glucuronide

Ethereal Sulfate
Glutathione-S-Transferase

Structure of Reduced Glutathione (MW 307)

\[
\begin{align*}
&\text{O}^- \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{CH}_2 \quad \text{H} \\
&\text{C} \quad \text{C} \quad \text{N} \quad \text{C} \quad \text{C} \quad \text{N} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C} \quad \text{C} \\
&\text{O} \quad \text{H} \quad \text{O} \quad \text{H} \quad \text{O} \quad \text{H} \quad \text{H} \quad \text{H} \quad \text{H}_3\text{N} \quad \text{O}^- \\
\end{align*}
\]

- glycine (gly)
- cysteine (cys)
- \(\gamma\)-glutamatic acid (glu)
**Glutathione-S-Transferase**

- Glutathione adducts are further metabolized in the kidney to derivatives referred to as *mercapturic acids* of the associated xenobiotic. This occurs in the kidney. Mercapuric derivatives are then found in the urine.

- Glutathione adducts are excreted in the bile and feces un-changed.

- Some chemicals are reactive enough to form glutathione adduct without the assistance of GSH transferase.
Section C

Factors Affecting Biotransformation
Factors that Affect Xenobiotic Biotransformation

- Species, strain, and genetic variations
  - Risk assessment is often based on responses observed in animals
  - In this regard, there are often significant differences between species in their abilities to metabolize xenobiotics
Factors that Affect Xenobiotic Biotransformation

- Likewise, even within a species, including man, there are differences
- The basis of such differences is often genetic (polymorphisms)
Examples of Factors that Affect Xenobiotic Biotransformation

- Species, strain, and genetic variation
  - Hexobarbital
  - Aflatoxin B1
  - Benzo[a]pyrene 7,8-dihydriodiol
  - Isoniazid
- Age
- Diet
- Exposure to other chemicals
## Species Differences in the Duration of Action and Metabolism of Hexobarbital

<table>
<thead>
<tr>
<th>Species</th>
<th>Duration of Action (Sleeping time, min)</th>
<th>Relative Enzyme Activity (µg hexobarbitol metabolized/gm/liver/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>12 ± 8</td>
<td>598 ± 184</td>
</tr>
<tr>
<td>Rabbit</td>
<td>49 ± 12</td>
<td>196 ± 28</td>
</tr>
<tr>
<td>Rat</td>
<td>90 ± 15</td>
<td>134 ± 51</td>
</tr>
<tr>
<td>Dog</td>
<td>315 ± 105</td>
<td>36 ± 30</td>
</tr>
</tbody>
</table>

Species, Strain, and Genetic Variation

\[ \text{Aflatoxin } B_1 \quad \text{Aflatoxin } B_1\text{-2,3-oxide} \]

Metabolism of Aflatoxin \(B_1\) to a highly reactive epoxide

Continued
Species, Strain, and Genetic Variation

Benzo[a]pyrene 7,8-dihydrodiol

Benzo[a]pyrene 7, 8-dihydrodiol-9, 10-epoxide

Metabolism of benzo[a]pyrene 7, 8-dihydrodiol to a highly reactive epoxide
Metabolism of Aflatoxin B1 and Benzo[a]pyrene

Inter-individual differences in the metabolism of aflatoxin B1 (A) and benzo[a]pyrene 7,8-dihydrodiol (B) to mutagens (assessed by Ames test) by microsomes from samples of human liver obtained during abdominal surgery
The Bimodal Distribution of Patients into Those who Rapidly Inactivate Isoniazid and Those who Slowly Metabolize It
Age as Affecting Xenobiotic Biotransformation

*Schematic representation of the ontogeny of hepatic drug metabolic activity*
Diet as Affecting Xenobiotic Biotransformation

- Control hospital diet
- Charcoal broiled beef diet

Plasma concentration of Phenacetin (ng/ml)

Hours after phenacetin administration
Exposure to Other Chemicals

![Graph showing Antipyrine half-lives in controls and PCB workers](image)

Antipyrine half-lives in normal subjects and workers exposed to the PCBs mixture.

Controls

PCB Workers

**Antipyrine T 1/2 (hours)**

15.6±1.0 hrs

10.8±0.7 hrs
Section D

Induction of Biotransformation Enzymes
Induction of Xenobiotic Metabolizing Systems

1. Many chemicals can induce the synthesis of the enzymes involved in Phase I and II xenobiotic metabolism and include chemicals found in the environment, the diet, and cigarette smoke

2. Inducers often exhibit specificity for the enzymes which they induce
Induction of Xenobiotic Metabolizing Systems

3. Depending on the inducer, fairly high dose levels or repeated dosing may be required; on the other hand, TCDD (dioxin) is effective as an inducer at 1 microgram/kg in some species.
Induction of Xenobiotic Metabolizing Systems

4. Studies have demonstrated that a cluster of genes referred to as the *Ah locus* controls the induction of xenobiotic enzyme activities by polycyclic aromatic compounds and TCDD.
Induction of Xenobiotic Metabolizing Systems

5. Such toxic responses as cancer, chemical-induced cataracts, aplastic anemia, and fetal toxicity have been demonstrated to be affected by this cluster of genes.

6. Evidence exists for the *Ah locus* in man.
### Characteristics of the Hepatic Effects of Phenobarbital and Polycyclic Aromatic Hydrocarbons

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Phenobarbital</th>
<th>Polycyclic Hydrocarbons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzyme components</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytochrome P-450</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>Cytochrome P-448</td>
<td>No effect</td>
<td>Increase</td>
</tr>
<tr>
<td>NADPH-cytochrome c reductase</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td><strong>Substrate specificity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Demethylation</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>Aliphatic hydroxylation</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>Polycyclic hydrocarbon hydroxylation</td>
<td>Small increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Reductive dehalogenation</td>
<td>Increase</td>
<td>No effect</td>
</tr>
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<th>Phenobarbital</th>
<th>Polycyclic Hydrocarbons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of effects</td>
<td>8–12 hours</td>
<td>3–6 hours</td>
</tr>
<tr>
<td>Time of maximum effect</td>
<td>3–5 days</td>
<td>24–48 hours</td>
</tr>
<tr>
<td>Persistence of induction</td>
<td>5–7 days</td>
<td>5–12 days</td>
</tr>
<tr>
<td>Liver enlargement</td>
<td>Marked</td>
<td>Slight</td>
</tr>
<tr>
<td>Protein synthesis</td>
<td>Large increase</td>
<td>Small increase</td>
</tr>
<tr>
<td>Phospholipid synthesis</td>
<td>Marked increase</td>
<td>No effect</td>
</tr>
<tr>
<td>Liver blood flow</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>Biliary flow</td>
<td>Increase</td>
<td>No effect</td>
</tr>
<tr>
<td>Glucuronidation</td>
<td>Increase</td>
<td>Small increase</td>
</tr>
<tr>
<td>Glutathione conjugation</td>
<td>Small increase</td>
<td>Small increase</td>
</tr>
<tr>
<td>Epoxide hydrolase</td>
<td>Increase</td>
<td>Small increase</td>
</tr>
<tr>
<td>Cytosolic receptor</td>
<td>None identified</td>
<td>Identified</td>
</tr>
</tbody>
</table>

*Continued*
The *Ah* Receptor

- Ah receptor = **Arylhydrocarbon receptor**
- Examples = 3-methylcholanthrene, benzo[a]pyrene
- Also called *TCDD receptor* or *dioxin receptor*
Schematic Outline of the Function of the Ah Receptor as a Ligand-Activated Transcription Factor

TCDD

HSP 90

Ah receptor

TCDD

ARNT

AH receptor

TA/TGCGTG

AT/ACGCAC

nucleus

cytoplasm

HSP 90

ARNT

CYP1A1

Other transcription-activated proteins
Section E

Bioactivation and Toxicity
Bioactivation as a Basis for Chemical Toxicity

- One of the possible results of the interaction of a xenobiotic with enzyme systems is the biotransformation of that compound to a chemically reactive intermediate (i.e. **Bioactivation**)

- The reaction of either this initial reactive metabolite or secondary reactive products with target molecules brings about changes in cellular function (the **Molecular Targets Concept**
Proposed Relationship Between Biotransformation, Bioactivation, and Toxicity of a Xenobiotic

Xenobiotic → Metabolite → Elimination

Reactive Intermediate

Reactive Intermediate

Molecular Target Interaction

DNA or Protein

DNA

Injury

Cell Death

Mutation Cancer

Protein

Hapten Antigen

Adapted from Casarett & Doull’s Toxicology. 4th Edition.
Metabolism and Bioactivation of Benzo[a]pyrene

Ah Receptor

Benzo[a]pyrene

BP

P450

Phenols

Quinones

Expoxides

Mixed Function Oxidase (P450_{1A1})

Glutathione Conjugation

Epoxide Hydrolase

Glucuronide Conjugates

BP-7,8-epoxide

Reductase

7 OH BP (NIH Shift)

BP-7,8 Dihydrodiol

Sulfate Esters
BP-7,8-dihydrodiol

Mixed Function Oxidase (P450_{IIA4})

BP-7,8-diol 9,10-epoxide

Macromolecular Adducts

Tetrol Triol

Glutationone

Initiation Repair Mutation
Chemical Nature of Reactive Intermediates

- **Electrophiles**—Form covalent (irreversible) bonds with cellular nucleophiles such as GSH, proteins and DNA

- **Free Radicals**—Odd or unpaired electron
  - Can act as electrophiles
  - Can abstract hydrogen from target molecules, such as lipids or nucleic acids
  - Can activate molecular oxygen
Acetaminophen is a good example of a xenobiotic whose toxicity is due to bioactivation to an electrophile.
Acetaminophen

Activation by
Cytochrome p-450

NADPH
\( \text{O}_2 \)

N-Acetyl-p-Benzoylquinoneimine

GSH

Nucleophilic Cell
Macromolecules

Mercapturic Acid

UDP-GA
UDP-Glucuronosyl Transferase
Bioactivation of Acetaminophen

Relationship between hepatic glutathione levels and covalent binding of acetaminophen to target nucleophiles (proteins)

![Graph showing the relationship between initial glutathione in liver (%), dose of acetaminophen (mg/kg), and covalent binding (molecules/mg protein)]
Bioactivation to a Free Radical

Carbon Tetrachloride

\[
\text{CCl}_4 \xrightarrow{(+e) \text{ Cyt. P-450}} \text{CCl}_3^- + \text{Cl}^-
\]

Target Molecules
Human paraquat exposure can result in lung toxicity due to its accumulation in lung cells and redox cycling.

The structure of the herbicide paraquat (A) and the polyamines putrescine (B) and spermine (C).
Activation of Molecular Oxygen via Chemical Redox Cycling

Mechanism of paraquat (PQ) toxicity by “redox cycling.” PQ is reduced by an NADPH-dependent microsomal enzyme. The paraquat radical can auto-oxidize with regeneration of PQ and the production of superoxide.
Redox Cycling of Xenobiotics

- **Redox cycling** of xenobiotics initially results in the formation of a form of active oxygen called *superoxide* \( (O_2\cdot^-) \)

- Through a series of non-enzymatic often metal catalyzed, reactions other forms of reactive oxygen are formed
  - These include hydrogen peroxide \( (H_2O_2) \), the hydroxyl radical \( (\cdot OH) \) and singlet oxygen \( (^1O_2) \)
Redox Cycling of Xenobiotics

- This results in an **oxidative stress** in cells and the subsequent modification of critical biomolecules leading to cellular toxicity.
- In this situation, what is the active form that causes toxicity?