Biotransformation

• The term *biotransformation* is the sum of all chemical processes of the body that modify endogenous or exogenous chemicals.

• Focus areas of toxicokinetics:
  – Biotransformation
  – Absorption
  – Distribution
  – Storage
  – Elimination
Biotransformation

• Biotransformation is affected by factors pertaining to the toxicant as well as the host.
• Host factors include:
  – age
  – sex
  – existing disease
  – genetic variability (toxicogenetics)
  – enzyme induction
  – nutritional status
Biotransformation

• The ability to metabolize a toxicant can vary greatly with age:
  – The developing fetus and the very young may have limited biotransformation capability primarily due to a lack of important enzymes.
  – These enzymes generally reach their optimal capacity for biotransformation by the time young adulthood is reached.
Biotransformation

• The ability to metabolize a toxicant can vary greatly with age:
  – Similarly, the elderly can also have difficulties with biotransformation due to functional loss with aging.
  – Enzyme fluctuations are at their lowest in early adulthood, which corresponds to the most efficient time in our lives for biotransformation (metabolism).
Biotransformation

• Differences in hormones account for gender-specific variability in the biotransformation of some toxicants.
Biotransformation and Nutritional Status

• Specific vitamin, mineral, and protein deficiencies can decrease the body’s ability to synthesize essential enzymes.

• Biotransforming enzymes cannot be synthesized or function efficiently in the absence of a dietary supply of important chemicals, such as amino acids; carbohydrates; and cofactors, such as essential vitamins and minerals.
Biotransformation

• Diseases that affect the liver can be particularly detrimental to biotransformation because the liver is the principal organ for these reactions.

  – Hepatitis can significantly reduce the biotransformation capacity of the liver, thus further contributing to a decline in the health of the affected individual.
Biotransformation

• Marked species differences must also be taken into consideration, especially because animals are used for toxicity studies that often form the basis for predicting human health effects.
Enzymes

• Enzymes are biological catalysts and high-molecular-weight proteins they allow for biotransformation reactions to proceed at rates that are consistent with life
Enzyme Defects Result in Altered Body Biochemistry

• This may result in injury to the body, especially if the enzyme is the catalyst for a biotransformation reaction that is essential to the body and for which no or less efficient alternative enzymatic pathways are available.

• Some individuals are born with a genetic condition in which the enzyme that converts the amino acid phenylalanine to another amino acid, tyrosine, is defective, resulting in a condition known as phenylketonuria.
Enzyme Defects Result in Altered Body Biochemistry

• These individuals must be maintained on a diet that restricts their intake of foods containing phenylalanine, including the use of some artificial sweeteners during infancy and childhood; otherwise, mental retardation may result.
Enzymes, cont.

- Enzymes provide the molecular surface for a chemical reaction to proceed for substrates with the correct molecular architecture to fit onto the anchoring and reaction sites of the enzyme.
  - This is sometimes referred to as enzyme specificity, or a “lock and key” arrangement.
  - In the absence of “proper fit,” biotransformation of the substrate(s) may not proceed.
  - The degree of enzyme specificity for substrates determines the extent of its involvement with different chemicals.
Figure 9-1 Enzyme (E) and substrate (S).
Enzymes, cont.

• The degree of specificity for an enzyme:
  – may be absolute and catalyze only one specific reaction
  – may be less restrictive and catalyze reactions of structurally similar chemicals such as those with a particular type of chemical bond or functional group.
Enzymes, cont.

• Consider the biotransformation of alcohols
  – share a common hydroxyl group
  – can be metabolized by the nonmicrosomal enzyme alcohol dehydrogenase
  – metabolites produced differ in their toxicity, depending on which alcohol is metabolized
Phenylketonuria (PKU) is an autosomal recessive condition in which an infant is born without phenylalanine hydroxylase (PAH), the enzyme required to break down phenylalanine to tryptophan. PAH-deficient children accumulate phenylalanine, as well as tetrahydrobiopterin and dihydrobiopterin. Plasma phenylalanine levels of 1,200 μmol/L or more are considered diagnostic of classic PKU. For comparison, the reference range for phenylalanine is 35 to 90 μmol/L.
Elevated levels of phenylalanine are associated with significant cognitive delay, although the mechanism has not been fully elucidated. Other symptoms include small cranial size, hyperactivity disorders, delayed social development, spasticity, and a mousy/musty odor. With an incidence of 350 cases per million live births, PKU is the most prevalent inborn amino acid metabolic error. The medical consequences of PKU are considered serious enough to warrant mandatory blood testing soon after birth in the United States and other countries.
 Phenylalanine is an essential amino acid found in many protein-rich foods. It is also found in the artificial sweetener aspartame. Packaged or processed foods high in phenylketonuria include warning labels for PKU sufferers. Children born with PKU should develop normally if they adhere strictly to low phenylalanine diets, particularly during periods of rapid growth. Low phenylalanine diets are not entirely benign, however.
Case in Point

PKU patients require lifetime dietary support with carnitine, fish oil, and low phenylalanine protein supplements to compensate for the loss of dietary phenylalanine. Because folate metabolism may be impacted, female PKU patients who become pregnant require close monitoring. Finally, insufficient phenylalanine is also associated with cognitive disability.
Enzymes and Biotransformation

• A number of enzymes are important for the biotransformation of toxicants.

• The resulting modification of the parent compound is a product that we refer to as the metabolite, and for any particular chemical it may be one that is used by the body to facilitate, improve, or impede physiological function, elimination, or storage.
Enzymes and Biotransformation, cont.

• For toxicants the “wisdom” of the process is essentially one whereby chemicals are ideally “detoxified” by:
  – Rendering them less harmful through enzymatic modifications
  – Rendering them more water soluble to facilitate their elimination from the body

• Unfortunately, depending on the chemical, biotransformation can result in the production of a metabolite(s) that may be more toxic than the parent compound. When this occurs, we refer to the process as bioactivation.
Figure 9-2 Bioactivation of chloroform to phosgene.

\[
\text{CHCl}_3 \xrightarrow{\text{P450}} \text{Cl} - \text{Cl} - \text{O}
\]

Chloroform \hspace{2cm} Phosgene

Less Toxic \quad More Toxic/Reactive
Figure 9-3 Metabolism of aniline by two different enzymes

Phenylhydroxylamine (more toxic metabolite)

N-Hydroxylase

Aniline

P450

p-Aminophenol (less toxic metabolite)
Case in Point

An individual was rushed to the hospital for severe chest pain and it was determined that his hemoglobin had reacted with carbon monoxide (CO). Appropriate supportive care was given, but it was later determined that the problem was actually a very large exposure to methylene chloride CH₂Cl₂, not CO. The mechanism was metabolism of CH₂Cl₂ to CO in the liver and subsequent binding of CO to hemoglobin in the blood, thus resulting in the formation of COHb, and decreased oxygen delivery to the heart.
Case in Point

The two compounds share an identical toxic intermediate (CO) with binding to the same target molecule, resulting in a reduced ability of the blood to carry oxygen. This could truly be a case of mistaken chemical identity but fortunately, the treatment option would be the same in either case.
Tissues Where Biotransformation Proceeds

• The enzymes for biotransformation reactions are found in many tissues of the body.
• The **liver** has the highest capacity for entering into reactions because of its high concentration of enzymes.
  – This makes it highly susceptible to toxicity from many chemicals that are bioactivated there.
  – This susceptibility is enhanced because the venous blood of the liver has a relatively high concentration of toxicants due to the “first-pass” effect.
Tissues Where Biotransformation Proceeds, cont.

• The **lungs and kidneys** have about a fifth of the biotransformation capacity of the liver.

• Other tissues of importance include:
  - lungs
  - Kidneys
  - Intestines
  - Placenta
  - skin
Phase 1 enzymes are found in the endoplasmic reticulum

- They are microsomal (membrane bound) and lipophilic.
- The term microsome refers to a mixture of fragmented endoplasmic reticulum vesicles present in a cell homogenate after mechanical breakage (homogenization) of tissues such as liver.
- Microsomes can be concentrated and separated from the other cellular components by means of differential centrifugation.
Phase 1 enzymes are found in the endoplasmic reticulum

• The P450 enzymes in microsomes are concentrated and collected for experimental use.
• Microsomes appear reddish brown in color due to the presence of heme in P450s and are most concentrated in liver tissue.
Tissues Where Biotransformation Proceeds, cont.

- Other enzymes of importance in the biotransformation of toxicants include:
  - hydrolases
  - reductases
  - carboxylesterases
Phase 1 Reactions & Cytochrome P450

- Phase 1 biotransformation reactions can be either microsomal or nonmicrosomal.
- The three main types of phase 1 reactions are oxidation, reduction, and hydrolysis.
Phase 1 Reactions & Cytochrome P450

• Oxidation reactions result in the loss of electrons from the parent compound (substrate) and can proceed via the removal of hydrogen from the molecule (dehydrogenation)
• The process of chemical reduction is one whereby the substrate gains electrons.
• Hydrolysis of toxicants is the common form of biotransformation that results in the splitting of the toxicant molecule into smaller molecules through the addition of water
Figure 9-8 Toxicant biotransformation in phase 1 by cytochrome P450
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Examples of Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoxidation, hydroxylation</td>
<td>Aldrin, nicotine, benzo(a)pyrene</td>
</tr>
<tr>
<td>S-Oxidation</td>
<td>Thiobenzamide, endosulfan methiocarb</td>
</tr>
<tr>
<td>N-Oxidation</td>
<td>2-Acetylaminofluorene</td>
</tr>
<tr>
<td>P-Oxidation</td>
<td>Diethylphenylphosphine</td>
</tr>
<tr>
<td>O-Dealkylation</td>
<td>p-Nitroanisole</td>
</tr>
<tr>
<td>S-Dealkylation</td>
<td>Methylmercaptan</td>
</tr>
<tr>
<td>N-Dealkylation</td>
<td>Ethylmorphine, atrazine</td>
</tr>
<tr>
<td>Desulfuration</td>
<td>Parathion, chlorpyrifos</td>
</tr>
<tr>
<td>Dehalogenation</td>
<td>CCl4, chloroform</td>
</tr>
<tr>
<td>Nitro reduction</td>
<td>Nitrobenzene</td>
</tr>
<tr>
<td>Azo reduction</td>
<td>O-Aminoazotoluene</td>
</tr>
</tbody>
</table>
Enzyme Induction

• The process of enzyme induction is one that results in an increased ability to metabolize toxicants.
Examples of Other Phase 1 Enzymes

- Epoxide hydrolases
- Flavin-containing monooxygenases
- Amidases and esterases
- Lipoxygenase
Enzymes and Oxidative Stress

• The metabolism of xenobiotics, particularly by the MFOs in phase 1 biotransformations, generates free radicals.
• This increases oxidative stress and can result in cellular damage.
Figure 9-9 Induction of P450 by a polycyclic aromatic hydrocarbon.
Phase 2 Reactions

• Xenobiotics that have undergone a phase 1 biotransformation reaction produce an intermediate metabolite.
• This metabolite now contains a “polar handle” such as a carboxyl (−COOH), amino (NH2), or hydroxyl (OH) functional group.
Phase 2 Reactions

- Although the metabolite is more hydrophilic in nature, it most often requires additional biotransformation to further increase hydrophilicity sufficient to permit significant elimination from the body. It is in these phase 2 reactions where this is accomplished.
Phase 2 Reactions: Conjugation Reactions

- Glutathione conjugation
  - (glutathione S-transferase)
- Glucuronide conjugation
  - (UDP-glucuronosyltransferase)
- Amino acid conjugation (aminotransferase)
- Sulfate conjugation (sulphotransferase)
- Acetylation (acetyltransferase)
- Methylation (methyltransferase)
Acetaminophen

• Acetaminophen toxicity can serve as a good example of the importance of a proper balance between phase 1 and phase 2 reactions.
• Consumption of clinically appropriate amounts generally of little toxicological significance to the liver – phase 2 reaction with the enzymes sulfotransferase and glucuronyl transferase to form the sulfate and glucuronide conjugation products that can be readily eliminated by the body.
Acetaminophen, cont.

- Large doses or doses taken too frequently can overwhelm the conjugating enzymes and result in toxicity
  - Phase 1 biotransformation mediated by cytochrome CYP2E1, producing a hepatotoxic metabolite, called $N$-acetyl-benzoquinoneimine (NAPQI)
Individual Response & Genetic Differences

• The drug isoniazid, for example, is used to treat TB and is detoxified through the addition of an acetyl group onto the molecule mediated via the enzyme $N$-acetyl-transferase.

• Individuals that have the normal form of this enzyme can eliminate a dose by 50% in approximately 1 hour. These individuals are referred to as “fast acetylators.”

• Individuals who possess a mutation that codes for this enzyme possess one that is less effective, requiring ~3 hours to eliminate half of the dose. These individuals are referred to as “slow acetylators.”
Individual Response and Genetic Differences, cont

• Some research has suggested that slow acetylators may be at greater risk for the development of certain types of cancers than fast acetylators, although no clear picture at this time has emerged.