Chapter 5

Absorption, Distribution, Metabolism, and Elimination of Toxics
Biotransformation: Basic Concepts (1)

- Renal excretion of chemicals
• 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.
  – The transformation process may take place as a result of the interaction of the toxic substance with enzymes found primarily in the cell endoplasmic reticulum, cytoplasm, and mitochondria.
  – The liver is the primary organ where biotransformation occurs.
Biotransformation: Basic Concepts (3)
• Interaction with these enzymes may change the toxicant to either a less or a more toxic form.

• Generally, biotransformation occurs in two phases.
  – Phase I involves catabolic reactions that break down the toxicant into various components.
    • Catabolic reactions include oxidation, reduction, and hydrolysis.
      – Oxidation occurs when a molecule combines with oxygen, loses hydrogen, or loses one or more electrons.
      – Reduction occurs when a molecule combines with hydrogen, loses oxygen, or gains one or more electrons.
      – Hydrolysis is the process in which a chemical compound is split into smaller molecules by reacting with water.
    • In most cases these reactions make the chemical less toxic, more water soluble, and easier to excrete.
Phase II reactions involves the binding of molecules to either the original toxic molecule or the toxic molecule metabolite derived from the Phase I reactions. The final product is usually water soluble and, therefore, easier to excrete from the body.

- Phase II reactions include glucuronidation, sulfation, acetylation, methylation, conjugation with glutathione, and conjugation with amino acids (such as glycine, taurine, and glutamic acid).

- It is important to understand that these Phase I and II reactions may occur simultaneously or sequentially.
Biotransformation: Basic Concepts (6)

• Whether the more toxic intermediate produces an effect is dependent on
  – how rapidly the intermediate undergoes further metabolism to less toxic substances,
  – how much of it is produced and accumulated in cells,
  – what type of cellular damage is caused by the toxic intermediate, and
  – what factors may affect excretion of the toxic material.
Example: Result of Oxidation of Benzene

- Quinol
- Phenol (Major Product)
- Catechol
- Resorcinol
- 1,2,4-trihydroxybenzene
Phase I Reaction (1)

- Exposing a functional group on the starting compound
Phase I Reaction (2)

- Sequential metabolism of benzene
  - Adding a functional group
Phase I Reaction (3)

• Oxidation
  – Many toxicants are metabolized by the enzymes cytochrome P-450 reductase and cytochrome P-450 in association with NADPH (nicotinamide adenine dinucleotide phosphate).
    • NADPH is a co-enzyme present in most cells; it interacts with various substances during normal cell metabolism.
  – These two enzymes are found in abundance in the endoplasmic reticulum of liver cells.
  – When these enzymes interact with the toxic molecule, one atom of oxygen is attached to the toxic molecule and another oxygen atom interacts with hydrogen to form water.
Phase I Reaction (4)

- **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.
  - exists in multiple forms (determined by different genes)
  - levels can be increased by exposure to chemicals in the food, water, or air (induction)
During Phase II reactions the toxic substance or its metabolite is bound with a compound that generally renders the newly synthesized molecule less toxic. This type of reaction is referred to as conjugation.

Conjugation is the joining of two substances to form a single molecule, which can increase water solubility and excretion.
Phase II Reaction (2)

- There are several types of Phase II reactions, of which glucuronidation is probably the most important.

- Conjugation with glucuronidation
  - Glucuronidation occurs as a result of the conjugation of glucuronidation acid with either a metabolite from Phase I reactions or with the parent compound.
  - There are several chemical compounds that interact with glucuronic acid:
    - alcohols (R-OH)
    - carboxylic acids (R-COOH)
    - sulfhydryl compounds (R-SH)
    - amines (R-NH)
Phase II Reaction (3)

• Conjugation with glutathione
  – Conjugation with glutathione is an important Phase II reaction that renders highly toxic metabolites harmless.
  – Conjugation of these intermediates with glutathione prevents binding with the nucleic acids, therefore preventing the occurrence of mutations.
    • Metabolites of organic solvents such as benzene, chloroform, and carbon tetrachloride are conjugated with glutathione resulting in decreased toxicity.
Phase II Reaction (4)

- Illustration of glucuronidation

(A) Illustration of glucuronidation of phenol

(B) Illustration of glucuronidation of benzoic acid
Phase II Reaction (5)

- Sulfation
Phase II Reaction (6)

• Acetylation and methylation
  – Acetylation and methylation may increase toxicity by decreasing water solubility and, therefore, excretion.
  – Methylation plays only a minor role in the transformation of toxic substances.
    • Methylation of nicotine. A methyl group is added from SAM (S-adenosyl methionine) to nicotine.
Deactivation versus Bioactivation (1)

- In general, Phase I and Phase II reactions are designed to deactivate toxic substances by increasing water solubility and excretion of toxic substances. However, these reactions may also bioactivate some toxicants by transforming inactive non-toxic molecules to active toxic forms.
Deactivation versus Bioactivation (2)

- Four potential end results of any chemical undergoing metabolism.

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Chemical

Step 1

Nontoxic Metabolites

Step 2

Toxic Metabolites or Bioactivation

Step 3

Detoxified Metabolites

Step 4

Toxic Effect Produced
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Deactivation versus Bioactivation (3)

Deactivation:
- Ester Hydrolysis
  - Diethyl Phosphorothionate
- Deactivation
  - p-Nitrophenol

Bioactivation:
- Desulfuration
  - Diethyl Phosphate
- Ester Hydrolysis
  - p-Nitrophenol

Binding to and inactivation of acetylcholinesterase, resulting in neural stimulation, convulsions, and death
Deactivation versus Bioactivation (4)

- Metabolism of benzo(a)pyrene (BP)
Deactivation versus Bioactivation (5)

• Metabolism of benzene in the liver by cytochrome P450 enzymes.
Deactivation versus Bioactivation (6)

- Metabolism of dimethylnitrosamine.
Deactivation versus Bioactivation (7)

- Metabolic pathway for the bioactivation of bromobenzene
Factors Affecting Metabolism (1)

• The rate at which metabolism of toxic substances occurs is dependent on a variety of factors that can be categorized into two groups:
  – those factors that affect the metabolic processes directly, and
  – Those factors that affect the transport of toxic substance to tissues where metabolism occurs.

• Biotransformation is affected by the species of the test animal, age, sex, nutritional status, disease, enzyme induction or inhibition, and genetics.
  – Newborn babies and young infants are more susceptible to a variety of chemicals such as pesticides because the cytochrome p450 enzymes --- important in pesticide detoxification reaction --- are not well developed.
Factors Affecting Metabolism (2)

- A balanced diet will provide the necessary protein as well as essential metals and minerals such as copper, zinc, and calcium to assist normal cellular enzymatic activities associated with biotransformation.
- Protein-deficient diets can result in a decrease in protein synthesis, thus affecting the synthesis of enzymes involved in the metabolic reactions used in detoxification.
Factors Affecting Metabolism (3)

- Cirrhosis of the liver is often caused by excessive drinking of alcohol. During the disease process the liver cells are damaged and replaced by connective tissue. If enough cells are killed, the ability of the liver to metabolize toxic substances is dramatically reduced.

- Cirrhosis cal also be caused by repeated exposure to arsenic or to high levels of vitamin A. Exposure to chemicals such as carbon tetrachloride and vinyl chloride may result in liver cell damage and decrease metabolism of toxic substances. These two substances are also associated with the development of liver cancer.
Factors Affecting Metabolism (4)

- Kidneys are damaged by absorption and concentration of heavy metals (e.g., Hg, Cd, etc.) in the cells of the proximal convoluted tubules of the nephron.
Factors Affecting Transport and Absorption (1)

• Absorption rate, perfusion rate, plasma protein binding, and storage will affect the rate at which a toxic substance is delivered to the tissue where metabolism occurs.

• The perfusion rate of a given tissue is important in determining how quickly a toxic substance will be transformed. Organs such as the liver and kidneys have a high perfusion rate relative to other tissue types. These organs have the potential to extract and detoxify larger quantities of toxicants from the blood.
Factors Affecting Transport and Absorption (2)

• Toxic substances bound to proteins in the blood do not easily move across cell membranes. In many cases this slows the rate at which the toxicant is metabolized because the substance may not be readily absorbed by the tissue where detoxification occurs.
Excretion (1)

• The faster a substance is eliminated from the body, the more unlikely a biological effect will be.
• The primary organs involved in excretion are the kidneys, liver, and lung.
Excretion (2)

- Excretion by the liver
  - Many toxic substances are stored and detoxified in the liver. The toxic substances are then excreted into the bile. Bile is produced in the liver by the hepatic cells.
  - This mechanism is important in removing large protein-bound toxicants such as heavy metals.
  - Excretion of the toxicant from the liver to the intestinal tract will usually result in the substance being removed in the feces.
  - However, the intestinal bacteria are also capable of producing enzymes that cause the detoxified substance to become less water soluble.
Excretion by the liver

- As a result, the toxic substance may be reabsorbed from the digestive tract. The process of excreting toxic substances from the liver and their subsequent reabsorption from the digestive tract is referred to as enterohepatic circulation.
  - Gluconated polycyclic aromatic hydrocarbons and glutathione conjugates of trichloroethylene are reabsorbed by this mechanism and therefore retained.
  - Detoxification of toxic substances before they reach the other portions of the systemic circulation is referred to as the “first-pass effect.” This effect can decrease the systemic toxicity of those substances absorbed from the digestive tract.
Excretion (4)

• Excretion by the kidneys
  – The kidneys receive 25 percent of the cardiac output. The high perfusion rate not only results in significant exposure to circulating toxic substances, but also facilitate the excretion of the toxicants.
  – Toxic substances enter the kidneys as a result of active and passive transport mechanisms present in the glomerulus and the nephron tubules.
  – Several heavy metals --- such as cadmium, lead and mercury --- are excreted by the kidneys. These metals are bound to plasma protein. The protein-metal complex has a low molecular weight and is able to pass through the glomerulus to the nephron. However, this complex may be reabsorbed by active transport mechanisms in the proximal convoluted tubules.
Excretion (5)

• Excretion by the lungs
  – Excretion of volatile toxic gases, such as those associated with organic compounds, occurs in the lungs. The transfer of gases from the blood to the lungs is influenced by concentration gradients and by their solubility in water.
    • Ethylene --- which is only slightly soluble in water --- will readily diffuse from the blood into the lungs and will therefore be easily removed.
    • Chloroform however, is more water soluble and will not diffuse as easily from the blood into the lungs.
Cytochrome P450

- Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme cofactor and, therefore, are hemoproteins.
An Example of P450 Catalytic Cycle