Elimination of Toxicants

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10.1 INTRODUCTION

The ability to efficiently eliminate toxic materials is critical to the survival of a species. The complexity of toxicant elimination processes has increased commensurate with the increased complexity associated with animal form. For unicellular organisms, passive diffusion can suffice for the elimination of toxic metabolic wastes produced by the organism. Similarly, as exogenous toxic materials derived from the environment diffuse into a unicellular organism, they can also readily diffuse out of the organism. The large surface area to mass ratio of these organisms ensures that a toxic chemical within the cell is never significantly distanced from a surface membrane across which it can diffuse.

As organisms evolved in complexity, several consequences of increased complexity compromised the efficiency of the passive diffusion of toxic chemicals:

1. They increased in size.
2. Their surface area to body mass decreased.
3. Their bodies compartmentalized (i.e., cells, tissues, organs).
4. They generally increased in lipid content.
5. They developed barriers to the external environment.

Size. With increased size of an organism, a toxic chemical has greater distance to traverse before reaching a membrane across which it can diffuse to the external environment. Thus overall retention of the chemical will increase as will propensity for the chemical to elicit toxicity.

Surface Area to Body Mass Ratio. Increased size of an organism is associated with a decrease in the surface area to body mass ratio. Accordingly, the availability of surface membranes across which a chemical can passively diffuse to the external environment decreases and propensity for retention of the chemical increases.

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Compartmentalization. With increased complexity comes increased compartmentalization. Cells associate to form tissues and tissues associate to form organs. Compartmentalization increases the number of barriers across which chemicals must traverse before sites of elimination are reached. As different compartments often have different physicochemical characteristics (i.e., adipose tissue is largely fat while blood is largely aqueous), chemicals are faced with the challenge to be mobile in these various environments.

Lipid Content. As a general but not universal rule, the ability of organisms to store energy as fat increases with increased size of the organism. Thus large organisms tend to have significant lipid stores into which lipophilic chemicals can be stored for extended periods of time. These stored chemicals tend to be largely immobile and difficult to release from the adipose tissue.

Barriers to the Environment. Through evolution, increased complexity of organisms led to increased exploitation of various environments. In order to survive in these environments, organisms developed barriers such as skin and scales that protect from harsh conditions on the outside and minimize loss of vital constituents such as water on the inside. Likewise these barriers impede the elimination of toxic constituents by the organisms, requiring the development of specialized membranes and organs through which toxic materials can be eliminated.

A consequence of this hindrance to elimination of toxic materials by complex organisms was the development of specialized routes of elimination. These routes generally evolved in concert (i.e., co-evolved) with biotransformation processes that render chemicals amenable to these modes of elimination (see Chapters 7–9).

Three major routes of elimination culminate in the specialized organs of elimination, the liver, kidneys, and lungs. The liver serves as a major organ at which lipophilic materials are collected from the blood, biotransformed to generally less toxic and more polar derivatives, then eliminated into the bile. The kidneys complement the liver in that these organs collect wastes and other chemicals in the blood through a filtration process and eliminate these wastes in the urine. The respiratory membranes of the lungs are ideal for the removal of volatile materials from the blood into expired air. In addition to these major routes of elimination, several quantitatively minor routes exist through which toxic materials can be eliminated from the body. These include the following:

1. *Skin.* Skin constitutes the largest organ in the human body, and it spans the interface between the body and the external environment. While the skin's epidermis constitutes a relatively impervious membrane across which chemical elimination is difficult, the shear surface area involved requires consideration of this organ as a route of elimination. Volatile chemicals are particularly adept at traversing the skin and exiting the body through this route.
2. *Sweat.* Humans lose an average of 0.7 L of water per day due to sweating. This loss of fluid provides a route for the elimination of water-soluble chemicals.
3. *Milk.* Mother's milk is rich in lipids and lipoproteins. Milk thus serves as an ideal route for the elimination of both water-soluble and fat-soluble chemicals from the mother's body. For example, the DDT metabolite DDE, the flame retardant mirex, and the polychlorinated biphenyls (PCBs) often have been detected in mother's

milk. While lactation may provide a benefit to the mother by the elimination of toxic chemicals, transfer of these toxicants to the suckling infant can have dire consequences.

4. *Hair.* Growing hair can serve as a limited route through which chemicals can escape the body. Pollutants such as mercury and drugs such as cocaine have been measured in human hair, and hair analyses is often used as a marker of exposure to such materials.

10.2 TRANSPORT

For a chemical to be eliminated from the body at a site of elimination (i.e., kidney) that is distant from the site of storage (i.e., adipose tissue) or toxicity (i.e., brain), the chemical must be transported from the site of origin to the site of elimination. Chemicals are transported to the site of elimination largely via the circulatory system. Sufficiently water-soluble chemicals can freely dissolve into the aqueous component of blood and be transported by both diffusion and blood circulation to sites of elimination. With decreasing water solubility and increasing lipid solubility, chemicals are less likely to freely diffuse into blood and extraction of these chemicals from sites of toxicity or storage can be more challenging. These materials generally associate with transport proteins in the blood, which either contain binding sites for chemical attachment or lipophilic cores (lipoproteins) into which lipophilic chemicals can diffuse. The blood contains various transport proteins that are typically suited for the transport of specific endogenous chemicals. These include albumin, sex steroid-binding globulin, and lipoproteins. Often xenobiotics can utilize these proteins, particularly the nonspecific transporters, to facilitate mobilization and transport in the aqueous environment of the blood. At the site of elimination, xenobiotics may diffuse from the transport protein to the membranes of the excretory organ, or the transport protein may bind to surface receptors on the excretory organ, undergo endocytosis and intracellular processing, where the xenobiotic is released and undergoes processing leading to elimination.

10.3 RENAL ELIMINATION

The kidneys are the sites of elimination of water-soluble chemicals that are removed from the blood by the process of reverse filtration. Two characteristics are primarily responsible for determining whether a chemical will be eliminated by the kidneys: size and water solubility.

Size. The reverse filtration process requires that chemicals to be removed from the blood are able to pass through 70 to 100 A pores. As a rule, chemicals having a molecular mass of less than 65,000 are sufficiently small to be subject to reverse filtration.

Water Solubility. Non-water-soluble chemicals will be transported to the kidneys in association with transport proteins. Thus, in association with these proteins, the chemicals will not be able to pass through the pores during reverse filtration. Lipophilic chemicals are generally subject to renal elimination after they

have undergone hydroxylation or conjugation reactions (Chapter 7) in the liver or elsewhere.

Blood is delivered to the human kidney by the renal artery. Blood flows to the kidneys of the adult human at a rate of roughly 1 L/min. The adult human kidney contains approximately 1 million functional units, called nephrons, to which the blood is delivered for removal of solutes. Collected materials are excreted from the body in the urine.

Blood entering the nephron passes through a network of specialized capillaries called the glomerulus (Figure 10.1). These capillaries contain the pores through which materials to be eliminated from the blood pass. Blood in the capillaries is maintained under high positive pressure from the heart coupled with the small diameter of the vessels. As a result these sufficiently small solutes and water are forced through the pores of the glomerulus. This filtrate is collected in the glomerular (or Bowman's) capsule in which the glomerulus is located (Figure 10.1). Included in this filtrate are water, ions, small molecules such as glucose, amino acids, urate, and foreign chemicals. Large molecules such as proteins and cells are not filtered and are retained in the blood.

Following glomerular filtration, molecules important to the body are re-absorbed from the filtrate and returned to the blood. Much of this re-absorption occurs in the proximal tubules (Figure 10.1). Cells lining the proximal tubules contain fingerlike projections that extent into the lumen of the tubule. This provides an expanse of cell surface area across which water and ions can diffuse back into the cells and, ultimately, be returned to the blood. The proximal tubules also contain active transport proteins that recover small molecules such as glucose and amino acids from the filtrate. From the proximal tubules, the filtrate passes through the Loop of Henle. Significant water

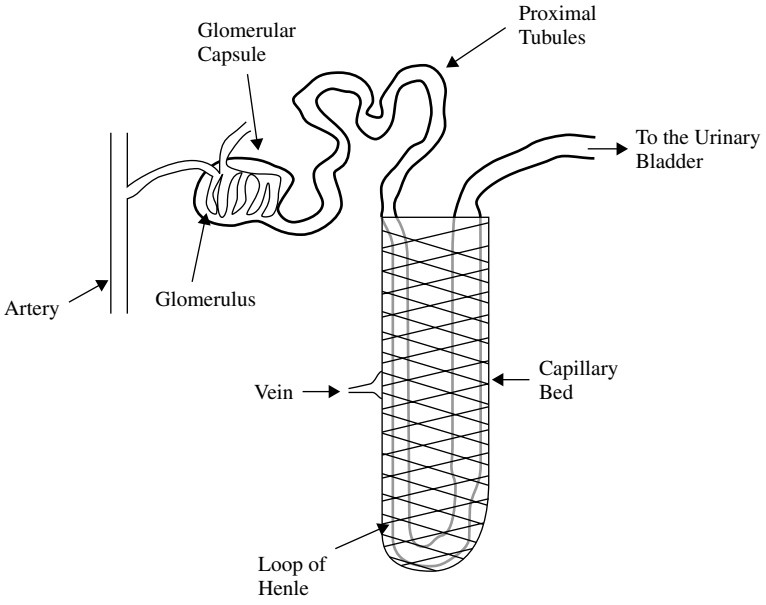

Figure 10.1 The nephron of the kidney. The nephron is the functional unit of the kidney that is responsible for the removal of water-soluble wastes and foreign compounds from the blood.

re-absorption occurs in the descending portion of the loop, resulting in concentration of the filtrate. Water re-absorption does not occur in the ascending portion of the loop. Rather, the remaining, concentrated ions such as sodium, chloride, and potassium are re-absorbed. Those materials retained in the filtrate during passage through the nephron constitute the urine. The urine is transported through the ureters to the bladder and retained until excretion occurs.

The kidneys are a common site of chemical toxicity since the nephron functions to concentrate the toxicant and thus increase levels of exposure to the materials. This increased exposure can result from the concentration of the toxicant in the tubules. It also can occur by concentration within the cells of the nephrons when a chemical is capable of utilizing one of the active transport proteins and is shuttled from the lumen of the tubules into the renal cells.

10.4 HEPATIC ELIMINATION

The liver serves many vital functions to the body. It has a large capacity to hold blood and thus serves as a blood storage site. The liver synthesizes and secretes many substances that are necessary for normal bodily function. It cleanses the blood of various endogenous and foreign molecules. It biotransforms both endogenous and exogenous materials, reducing their bioreactivity and preparing them for elimination. It eliminates wastes and foreign chemicals through biliary excretion. Three of these functions occur coordinately in a manner that makes the liver a major organ of chemical elimination: chemical uptake from blood, chemical biotransformation, and biliary elimination of chemicals.

Blood is delivered to the liver from two sources. Oxygen-rich blood is delivered through the hepatic artery. In addition blood is shunted from the capillaries that service the intestines and spleen to the liver by the hepatic portal vein. These two vessels converge, and the entire hepatic blood supply is passaged through sinusoids (Figure 10.2). Sinusoids are cavernous spaces among the hepatocytes that are the functional units of the liver. Hepatocytes are bathed in blood as the blood passes through the sinusoids, as 70% of the hepatocyte surface membrane contacts the blood in the sinusoid. This provides for a tremendous surface area across which chemicals can diffuse to gain entry into the hepatocytes. Chemicals may passively diffuse across the sinusoidal

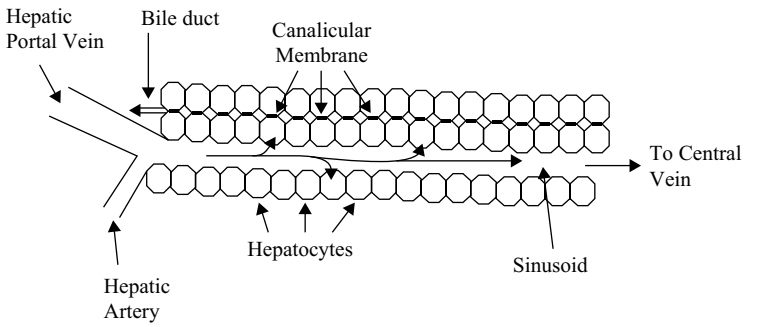

Figure 10.2 Diagrammatic representation of the basic architecture of the liver.

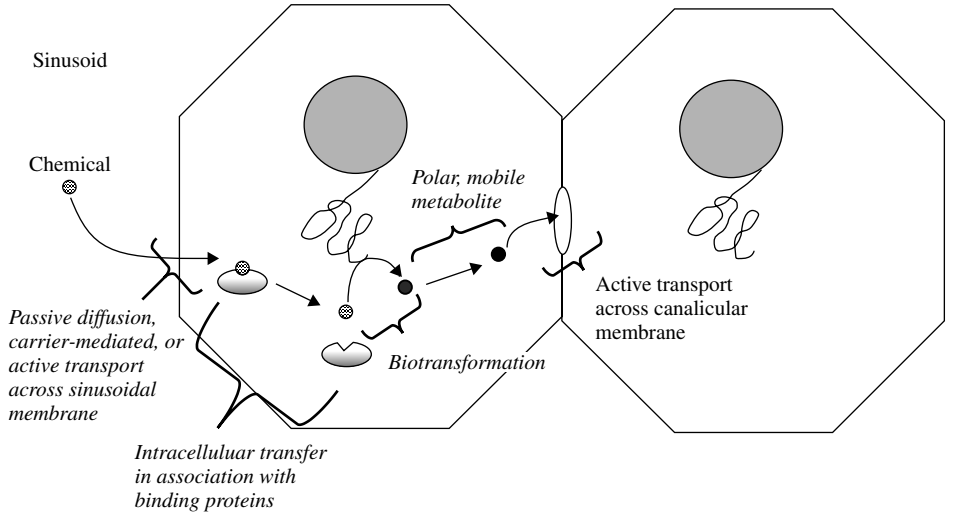

Figure 10.3 Vectorial transport of a chemical from the liver sinusoid, through the hepatocyte, to the canalicular space.

membrane of the hepatocytes, they may be exchanged between blood transport proteins and the sinusoidal membranes, or their carrier proteins may bind to sinusoidal membrane receptors and then undergo endocytosis (Figure 10.3).

Lipophilic materials require intracellular carrier proteins to be optimally mobilized, just as they required transport proteins in the blood (Figure 10.3). Several intracellular carrier proteins that mobilize specific endogenous chemical have been characterized, although less is known of which proteins typically mobilize xenobiotics. Some of the cytosolic glutathione *S*-transferase proteins have been shown to noncatalytically bind xenobiotics and to be coordinately induced along with xenobiotic biotransformation enzymes and efflux transporters, suggesting that these proteins may function to mobilize xenobiotics.

Once mobilized in the hepatocyte, chemicals can contact and interact with biotransformation enzymes (Chapter 7). These enzymes generally increase the polarity of the chemical, thus reducing its ability to passively diffuse across the sinusoidal membrane back into the blood. Biotransformation reactions also typically render the xenobiotics susceptible to active transport across the canalicular membrane into the bile canaliculus and, ultimately, the bile duct (Figure 10.3). The bile duct delivers the chemicals, along with other constituents of bile, to the gall bladder that excretes the bile into the intestines for fecal elimination.

10.4.1 Entero-hepatic Circulation

Once in the gastrointestinal tract, chemicals that have undergone conjugation reactions in the liver may be subject to the action of hydrolytic enzymes that de-conjugate the molecule. De-conjugation results in increased lipophilicity of the molecule and renders them once again subject to passive uptake. Re-absorbed chemicals enter the circulation via the hepatic portal vein, which shunts the chemical back to the liver where

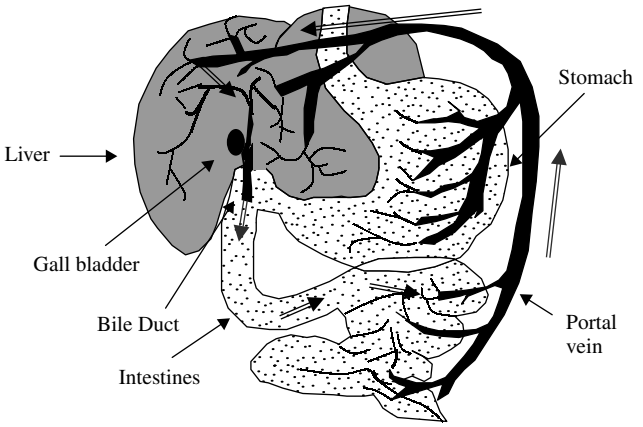

Figure 10.4 Enterohepatic circulation (as indicated by ⟹). Polar xenobiotic conjugates are secreted into the intestine via the bile duct and gall bladder. Conjugates are hydrolyzed in the intestines, released xenobiotics are reabsorbed, and transported back to the liver via the portal vein.

the chemical can be reprocessed (i.e., biotransformed) and eliminated. This process is called entero-hepatic circulation (Figure 10.4). A chemical may undergo several cycles of entero-hepatic circulation resulting in a significant increase in the retention time for the chemical in the body and increased toxicity.

The liver functions to collect chemicals and other wastes from the body. Accordingly, high levels of chemicals may be attained in the liver, resulting in toxicity to this organ. Biotransformation of chemicals that occur in the liver sometimes results in the generation of reactive compounds that are more toxic than the parent compound resulting in damage to the liver. Chemical toxicity to the liver is discussed elsewhere (Chapter 14).

10.4.2 Active Transporters of the Bile Canaliculus

The bile canaliculus constitutes only about 13% of the contiguous surface membrane of the hepatocyte but must function in the efficient transfer of chemical from the hepatocyte to the bile duct. Active transport proteins located on the canalicular membrane are responsible for the efficient shuttling of chemicals across this membrane. These active transporters are members of a multi-gene superfamily of proteins known as the ATP-binding cassette transporters. Two subfamilies are currently recognized as having major roles in the hepatic elimination of xenobiotics, as well as endogenous materials. The *P*-glycoprotein (ABC B) subfamily is responsible for the elimination of a variety of structurally diverse compounds. *P*-glycoprotein substrates typically have one or more cyclic structures, a molecular weight of 400 or greater, moderate to low lipophilicity ($\log K_{ow} < 2$), and high hydrogen (donor)-bonding potential. Parent xenobiotics that meet these criteria and hydroxylated derivatives of more lipophilic compounds are typically transported by *P*-glycoproteins.

The multidrug-resistance associated protein (ABC C) subfamily of proteins largely recognizes anionic chemicals. ABC C substrates are commonly conjugates of xenobiotics (i.e., glutathione, glucuronic acid, and sulfate conjugates). Thus conjugation

not only restricts passive diffusion of a lipophilic chemical but actually targets the xenobiotic for active transport across the canalicular membrane.

10.5 RESPIRATORY ELIMINATION

The lungs are highly specialized organs that function in the uptake and elimination of volatile materials (i.e., gasses). Accordingly, the lungs can serve as a primary site for the elimination of chemicals that have a high vapor pressure. The functional unit of the lung is the alveolus. These small, highly vascularized, membraneous sacs serve to exchange oxygen from the air to the blood (uptake), and conversely, exchange carbon dioxide from the blood to the air (elimination). This exchange occurs through passive diffusion. Chemicals that are sufficiently volatile also may diffuse across the alveolar membrane, resulting in removal of the chemical from the blood and elimination into the air.

10.6 CONCLUSION

Many processes function coordinately to ensure that chemicals distributed throughout the body are efficiently eliminated at distinct and highly specialized locations. This unidirectional transfer of chemicals from the site of origin (storage, toxicity, etc.) to the site of elimination is a form of vectorial transport (Figure 10.5). The coordinate action of blood binding proteins, active transport proteins, blood filtration units, intracellular binding proteins, and biotransformation enzymes ensures the unidirectional flow of chemicals, ultimately resulting in their elimination. The evolution of this complex interplay of processes results in the efficient clearance of toxicants and has provided the way for the co-evolution of complexity in form from unicellular to multi-organ organisms.

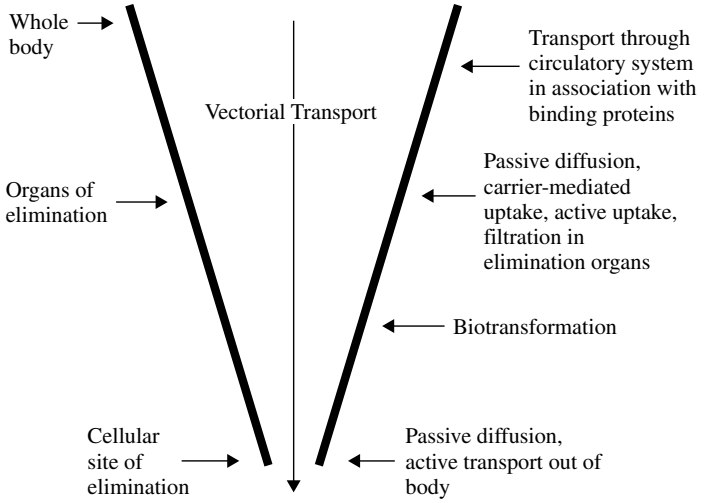

Figure 10.5 Processes involved in the vectorial transport of xenobiotics from the whole body point of origin to the specific site of elimination.

SUGGESTED READING

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