Introduction to Food Toxicology

Second Edition

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CHAPTER 1

Principles of Toxicology

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Toxicology is defined as the study of the adverse effects of chemicals on living organisms. Its origins may be traced to the time when our prehistoric ancestors first attempted to introduce substances into their diets that they had not encountered previously in their environments. By observing which substances could satisfy hunger without producing illness or death, ancient

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people developed dietary habits that improved survival and proliferation of the species in their traditional environment and allowed them to adapt to new environments. In its modern context, toxicology draws heavily on knowledge in chemical and biological fields and seeks a detailed understanding of toxic effects and means to prevent or reduce toxicity. In many instances, the original discoveries of toxins that caused devastating human illness and suffering have led to the development of the toxin as a probe of biological function that is used today to study basic mechanisms and to develop cures for human maladies as diverse as postpartum hemorrhage, psychosis, and cancer.

A brief history of documented uses of toxic agents serves to illustrate the importance of these substances since ancient cultures. The Ebers papyrus of about 1500 BCE, one of the oldest preserved medical documents, describes uses of many poisons such as hemlock, aconite arrow poison, opium, lead, and copper. By 399 BCE, death by hemlock poisoning was a well-established means of capital punishment in Greece, most notably in the forced suicide of Socrates. Around this same time, Hippocrates discussed bioavailability and overdosage of toxic agents, and intended poisonings—used mostly by aristocratic women as a means of dispatching unwanted husbands—were of common occurrence in Rome. By about 350 BCE, Theophrastus, a student of Aristotle, made many references to poisonous plants in his first *De Historia Plantarum*.

In about 75 BCE, King Mithridates VI of Pontus (in modern Turkey) was obsessed with poisons and, from a young age, took small amounts of as many as 50 poisons in the hopes of developing resistance to each of them. This practice apparently induced a considerable resistance to poisons, since according to legend, to avoid enemy capture, the standard poisonous mixture was not effective in a suicide attempt by the vanquished king and he had to fall on his sword instead. The term "mithridatic" refers to an antidotal or protective mixture of low but significant doses of toxins and has a firm scientific basis. However, the claim that vanishingly small doses of toxic agents also produce protective effects, which is the claimed basis for homeopathy, does not have scientific support.

In 82 BCE, *Lex Cornelia* (Law of Cornelius) was the first law to be enacted in Rome that included provisions against human poisonings. In approximately 60 CE, Dioscorides, a physician in the Roman armies of Emperors Nero, Caligula, and Claudius, authored a six to eight volume treatise that classified poisons on the basis of origin (plant, animal, mineral) and biological activity, while avoiding the common practice of classification based on fanciful theories of action that were considered important at the

time, such as the theory of humors, which posed that body function is regulated by the proper balance of fluids called black bile, yellow bile, phlegm, and blood. This treatise often suggested effective therapies for poisonings such as the use of emetics, and was the standard source of such information for the next 1500 years.

Paracelsus (1493–1541) is considered to be the founder of toxicology as an objective science. Paracelsus, who changed his name from Phillip von Hohenheim, was an energetic, irascible, and iconoclastic thinker (Figure 1.1). He was trained in Switzerland as a physician and traveled widely in Europe and the Middle East to learn alchemy and medicine in other traditions of the day. Although astrology remained an important part of his philosophy, he eschewed magic in his medical practice. His introduction of the practice of keeping wounds clean and allowing them to drain to allow them to heal won him considerable acclaim in Europe. Most notably for toxicology, Paracelsus was the first person who attributed adverse effects of certain substances to the substance itself and not to its association with an evil or angered spirit or god. Paracelsus is accredited with conceiving the basic concept of toxicology, which often is stated as follows:

> *All substances are poisons; there is none that is not a poison. The right dose differentiates the poison from a remedy.*

Although this and other concepts developed by Paracelsus were groundbreaking and major advances in thinking about disease for the time, they put him at odds with the major medical practitioners. As a result, he was forced to leave his home medical practice and spent several of his final years traveling. He was 48 when he died, and there are suspicions that his enemies caught up with him and ended his very fruitful life. How ironic it would be if the father of toxicology were murdered by poisoning!

FIGURE 1.1 *Paracelsus (1493–1541).*

It is useful to evaluate the significance of the Paracelsus axiom in our daily lives by considering examples of well-known substances with low and high toxicity. Water might be considered one of the least toxic of the substances that we commonly encounter. Can it be toxic? Indeed, there are many reports of water toxicity in the scientific literature. For example, in 2002 a student at California State University at Chico was undergoing a fraternity initiation ordeal in which he was required to drink up to five gallons

of water while engaged in rigorous calisthenics and being splashed with ice cold water. Consumption of this amount of water in a short span of time resulted in the dilution of the electrolytes in his blood to the point that normal neurological function was lost and tragically the young man died.

Let us now consider the converse concept that exposure to a small amount of a highly toxic agent can be of little consequence. For example, the bacterium that produces botulism, *Clostridium botulinum*, can produce deadly amounts of botulinum toxin in improperly sterilized canned goods. This bacterial toxin is one of the most toxic substances known. The same toxin, however, is used therapeutically, for example, to treat spastic colon and as a cosmetic to reduce wrinkles in skin.

BRANCHES OF TOXICOLOGY

The science of toxicology has flourished from its early origins in myth and superstition, and is of increasing importance to many aspects of modern life. Modern toxicology employs cutting-edge knowledge in chemistry, physiology, biochemistry and molecular biology, often aided by computational technology, to deal with problems of toxic agents in several fields of specialization.

The major traditional specialties of toxicology address several specific societal needs. Each specialty has its unique educational requirements, and employment in some areas may require professional certification. **Clinical toxicology** deals with the prevention, diagnosis, and management of poisoning, usually in a hospital or clinical environment. **Forensic toxicology** is the application of established techniques for the analysis of biological samples for the presence of drugs and other potentially toxic substances, and usually is practiced in association with law enforcement. **Occupational toxicology** seeks to identify the agents of concern in the workplace, define the conditions for their safe use, and prevent absorption of harmful amounts. **Environmental toxicology** deals with the potentially deleterious impact of man-made and natural environmental chemicals on living organisms, including wildlife and humans.

Regulatory toxicology encompasses the collection, processing, and evaluation of epidemiological and experimental toxicology data to permit scientifically based decisions directed toward the protection of humans from the harmful effects of chemical substances. Furthermore, this area of toxicology supports the development of standard protocols and new testing methods to continuously improve the scientific basis for decision-making processes. **Ecotoxicology** is concerned with the environmental distribution and toxic

effects of chemical and physical agents on populations and communities of living organisms within defined ecosystems. Whereas traditional environmental toxicology is concerned with toxic effects on individual organisms, ecotoxicology is concerned with the impact on populations of living organisms or on ecosystems.

Food toxicology focuses on the analysis and toxic effects of bioactive substances as they occur in foods. Food toxicology is a distinct field that evaluates the effects of components of the complex chemical matrix of the diet on the activities of toxic agents that may be natural endogenous products or may be introduced from contaminating organisms, or from food production, processing, and preparation.

DOSE-RESPONSE

Since there are both toxic and nontoxic doses for any substance, we may also inquire about the effects of intermediate doses. In fact, the intensity of a biological response is proportional to the concentration of the substance in the body fluids of the exposed organism. The concentration of the substance in the body fluids, in turn, is usually proportional to the dose of the substance to which the organism is subjected. As the dose of a substance is increased, the severity of the toxic response will increase until at a high enough dose the substance will be lethal. This so-called individual dose-response can be represented as a plot of degree of severity of any quantifiable response, such as an enzyme activity, blood pressure, or respiratory rate, as a function of dose. The resulting plot of response against the $\log_{10}$ of concentration will provide a sigmoidal curve (as illustrated in Figure 1.2) that will be nearly linear within a mid-concentration range and will be asymptotic to the zero response and maximum response levels. This response behavior is called a **graded dose-response** since the severity of the response increases over a range of concentrations of the test substance.

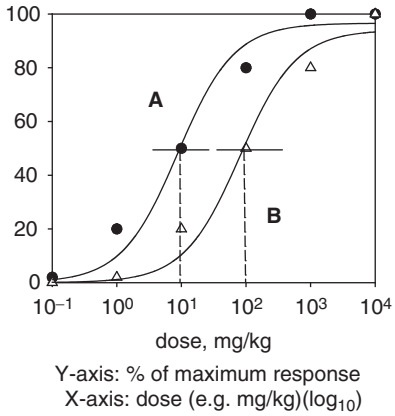

FIGURE 1.2 *Dose-response. The resulting plot of response against the $\log_{10}$ of concentration will provide a sigmoidal curve that will be nearly linear within a mid-concentration range and will be asymptotic to the zero response and maximum response levels.*

Toxicity evaluations with individual test organisms are not used often, however, because individual organisms, even inbred rodent species used in the laboratory, may vary from one another in their sensitivities to toxic agents. Indeed, in studies of groups of test organisms, as the dose is increased, there is not a dose at which all the organisms in the group will suddenly develop the same response.

Instead, there will be a range of doses over which the organisms respond in the same way to the test substance. In contrast to the graded individual dose-response, this type of evaluation of toxicity depends on whether or not the test subjects develop a specified response, and is called an **all-or-none** or **quantal population** response. To specify this group behavior, a plot of percent of individuals that respond in a specified manner against the log of the dose is generated.

Let us consider, for example, the generation of a dose-response curve for a hypothetical hypertensive agent. The test substance would be administered in increasing doses to groups of 10 subjects or test organisms. The percentage of individuals in each group that respond in a specific way to the substance (e.g., with blood pressure 140/100) then is determined. The data then are plotted as percent response in each group versus the log of the dose given to each group. Over a range of low doses, there will be no test subjects that develop the specified blood pressure. As the dose increases, there will be increased percentages of individuals in the groups that develop the required blood pressure, until a dose is reached for which a maximum number of individuals in the group respond with the specified blood pressure. This dose, determined statistically, is the mean dose for eliciting the defined response for the population. As the dose is further increased, the percentages of individuals that respond with the specified blood pressure will decrease, since the individuals that responded to the lower doses are now exhibiting blood pressures in excess of the specified level. Eventually, a dose will be reached at which all the test subjects develop blood pressures in excess of the specified level.

When the response has been properly defined, information from quantal dose-response experiments can be presented in several ways. A frequency-response plot (Figure 1.3) is generated by plotting the percentage of responding individuals in each dose group as a function of the dose.

The curve that is generated by these data has the form of the normal Gaussian distribution and, therefore, the data are subject to the statistical laws for such distributions. In this model, the numbers of individuals on either side of the mean are equal and the area under the curve represents the total population. The area under the curve bounded by the inflection points includes the number of individuals responding to the mean dose, plus or minus one standard deviation (SD) from the mean dose, or 95.5% of the population. This mean value is useful in specifying the dose range over which most individuals respond in the same way.

Frequency-response curves may be generated from any set of toxicological data where a quantifiable response is measured simply by recording the

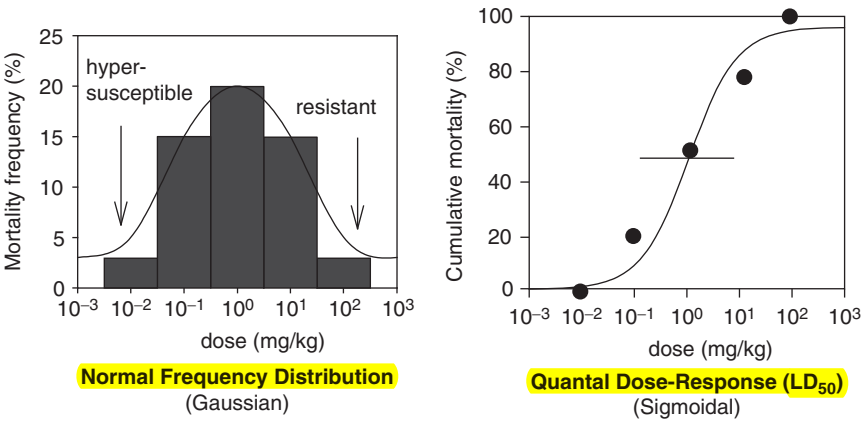

FIGURE 1.3

Comparison of shapes of the dose-response curves between Normal Frequency Distribution and Quantal Dose-Response.

percentage of subjects that respond at each dose minus the percentage that respond at the lower dose. Generally, the frequency-response curve obtained by experiment only approaches the shape of the true normal distribution. Such curves illustrate clearly, however, that there is a mean dose at which the greatest percentage of individuals will respond in a specific way. There will always be individuals who require either greater (hyposensitive) or smaller (hypersensitive) doses than the mean to elicit the same response.

Although the frequency-response distribution curves often are used for certain kinds of statistical analyses of dose-response data, the cumulative-response data presentation is employed more commonly, especially for representing lethal response data. The cumulative-response curve may be generated for nonlethal frequency-response data by plotting log dose versus percentage of individuals responding with at least a specified response. As illustrated in Figure 1.3, if the blood pressure responses used in the previous example are plotted as the percentage of individuals in each dosing group that respond with at least a level of 140/100, the resulting curve will be sigmoidal. Several important values used to characterize toxicity are obtained from this type of curve. The NOAEL (no observed adverse effect level) is the highest dose at which none of the specified toxicity was seen. The LOAEL (lowest observed adverse effect level) is the lowest dose at which toxicity was produced. The TD_{50} is the statistically determined dose that produced toxicity in 50% of the test organisms. If the toxic response of interest is lethality, then LD_{50} is the proper notation. At a high enough dose, 100% of the individuals will respond in the specified manner. Since

the LD and TD values are determined statistically and based on results of multiple experiments with multiple test organisms, the values should be accompanied by some means of estimating the variability of the value. The probability range (or *p* value), which is commonly used, generally is accepted to be less than 0.05. This value indicates that the same LD or TD value would be obtained in 95 out of a hypothetical 100 repetitions of the experiment.

The cumulative-response curves can facilitate comparisons of toxic potencies between compounds or between different test populations. For example, for two substances with nonoverlapping cumulative dose-response curves, the substance with the curve that covers the lower dose range is clearly the more toxic of the two. If prior treatment of a test population with substance A results in a shift of the dose-response curve to the right for toxin B, then substance A exerts a protective effect against substance B. In the case where the dose-response curves for different toxins overlap, the comparison becomes a bit more complex. This can occur when the slopes of the dose-response curves are not the same, as shown in Figure 1.5. These hypothetical compounds have the same LD_{80}, and are said to be equally toxic at this dose. Below this dose, however, compound A produced the higher percentage of toxicity than compound B and, therefore, compound A is more toxic. At doses above the LD_{80}, compound B produces the higher percentage of lethality and, thus, is the more toxic substance. Based on the LD_{50} values only, compound A is more toxic than compound B. Thus, in comparing the toxicities of two substances, the toxic response must be specified, the dose range of toxicity must be stated, and if the toxicities are similar, the slopes of the linear portions of the dose-response curves must be indicated.

POTENCY

Although all substances exhibit toxic and lethal dose-response behavior, there is a wide range of LD_{50} values for toxic substances. By convention, the toxic potencies fall into several categories. A list of LD_{50} values for several fairly common substances, along with a categorization of the toxicities from extreme to slight, are provided in Table 1.1.

Substances with LD_{50} values greater than about 2 g/kg body wt. generally are considered to be of slight toxicity and that relatively large amounts, in the range of at least one cup, are required to produce a lethal effect in an adult human and are easily avoided under most circumstances. However, exposure

Table 1.1 Potency of Common Toxins

Agent	LD_{50} (mg/kg)	Toxicity
Ethyl alcohol	9,000	
Sodium chloride	4,000	
BHA/BHT (antioxidants)	2,000	Slight
Morphine sulfate	900	
Caffeine	200	Moderate
Nicotine	1	High
Curare	0.5	
Shellfish toxin	0.01	
Dioxin	0.001	
Botulinum toxin	0.00001	Extreme

to substances in the extreme category with $LD_{50} < 1$ mg/kg requires only a few drops or less to be lethal and may be a considerable hazard.

HORMESIS

Hormesis is a dose-response phenomenon characterized by a low dose beneficial effect and a high dose toxic effect, resulting in either a J-shaped or an inverted U-shaped dose-response curve. A hormetic substance, therefore, instead of having no effect at low doses, as is the case for most toxins, produces a positive effect compared to the untreated subjects. A representative dose-response curve of such activity is presented in Figure 1.4.

Substances required for normal physiological function and survival exhibit hormetic dose-response behavior. At very low doses, there is an adverse effect (deficiency), and with increasing dose beneficial effects are produced (homeostasis). At very high doses, an adverse response appears from toxicity. For example, high doses of vitamin A can cause liver toxicity and birth defects while vitamin A deficiency contributes to blindness and increases the risk of disease and death from severe infections. Nonnutritional substances may also impart beneficial or stimulatory effects at low doses but produce toxicity at higher doses. Thus, chronic alcohol consumption at high doses causes esophageal and liver cancer, whereas low doses can reduce coronary heart disease. Another example is radiation, which at low levels induces beneficial adaptive responses and at high levels causes tissue destruction and cancer.

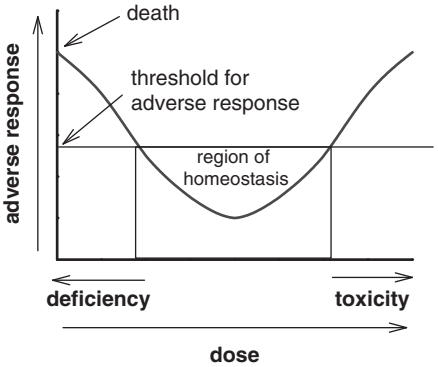

FIGURE 1.4 *Hormesis dose-response curve.*

MARGIN OF SAFETY

Safety is defined as freedom from danger, injury, or damage. Absolute safety of a substance cannot be proven since proof of safety is based on negative evidence, or the lack of harm or damage caused by the substance. A large number of experiments can be run that may build confidence that the substance will not cause an adverse effect, but these experiments will not prove the safety of the substance. There is always the chance that the next experiment might show that the substance produces an adverse effect in standard or new testing protocols. In addition, our concept of safety continues to evolve and we are now aware that even minute changes, for example, in the activity of an important enzyme, could portend a highly negative effect in the future. Indeed, our concept of safety in regard to toxic exposure continues to develop as our knowledge of biochemical and molecular effects of toxins, and our ability to measure them, grow.

Since absolute safety cannot be proven, we must evaluate relative safety, which requires a comparison of toxic effects between different substances or of the same substance under different conditions. When the experimental conditions for toxicity testing in a species have been carefully defined, and the slopes of the dose-response curves are nearly the same, the toxicities of two substances can often be calculated simply by determining the ratio of the TD_{50}s or LD_{50}s. Often, however, a more useful concept is the comparison of doses of a substance that elicit desired and undesired effects. The **margin of safety** of a substance is the range of doses between the toxic and beneficial effects; to allow for possible differences in the slopes of the effective and toxic dose-response curves, it is computed as follows:

$$\text{Margin of Safety (MS)} = LD_1/ED_{99}$$

LD_1 is the 1% lethal dose level and ED_{99} is the 99% effective dose level. A less desirable measure of the relative safety of a substance is the **Therapeutic Index**, which is defined as follows:

$$\text{Therapeutic Index (TI)} = LD_{50}/ED_{50}$$

TI may provide a misleading indication of the degree of safety of a substance because this computation does not take into account differences in the slopes of the LD and ED response curves. Nevertheless, this method has been used traditionally for estimations of relative safety. The dose-response data presented in Figure 1.5 serves to illustrate how the use of TI can provide misleading comparisons of the relative toxicities of substances.

In this example, drug A and drug B have the same LD_{50} = 100 mg/kg and ED_{50} = 2 mg/kg. The comparison of toxicities, therefore, provides the same TI = 100/2 = 50. Therapeutic index does not take into account the slope of the dose-response curves. Margin of safety, however, can overcome this deficiency by using ED_{99} for the desired effect and LD_1 for the undesired effect. Thus,

$$\begin{aligned} MS &= LD_1/ED_{99} = 10/10 = 1 \text{ for} \\ &\quad \text{drug A, and for drug B} \\ &= 0.002/10 = 0.0002 \end{aligned}$$

Therefore, according to the MS comparison, drug B is much less safe than drug A.

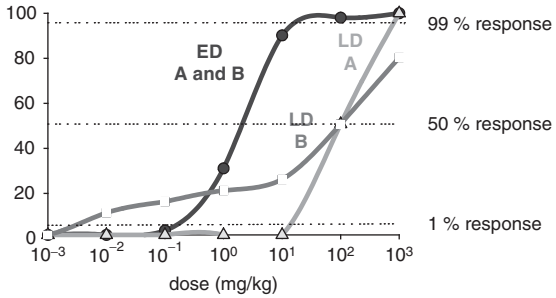

FIGURE 1.5 *The dose-response data serves to illustrate how the use of TI can provide misleading comparisons of the relative toxicities of substances.*

For substances without a relevant beneficial biological response, the concepts of MS and TI have little meaning. Many substances as diverse as environmental contaminants and food additives fall into this category. For these substances, safety of exposures is estimated based on the NOAEL adjusted by a series of population susceptibility factors to provide a value for the **Acceptable Daily Intake** (ADI). The ADI is an estimate of the level of daily exposure to an agent that is projected to be without adverse health impact on the human population. For pesticides and food additives, it is the daily intake of a chemical, which during an entire lifetime appears to be without appreciable risk on the basis of all known facts at the time, with the inclusion of additional safety factors. The ADI is computed as follows:

$$ADI = NOAEL/(UF \times MF)$$

where UF is the uncertainty factor and MF is the modifying factor.

UF and MF provide adjustments to ADI that are presumed to ensure safety by accounting for uncertainty in dose extrapolation, uncertainty in duration extrapolation, differential sensitivities between humans and animals, and differential sensitivities among humans (e.g., the presumed increased sensitivity for children compared to adults). The common default value for each uncertainty factor is 10, but the degree of safety provided by factors of 10 has not been quantified satisfactorily and is the subject of continuing experimentation and debate. Thus, for a substance that triggers all four of the uncertainty factors indicated previously, the calculation would be ADI = NOAEL/10,000. In some cases, for example, if the metabolism of the substance is known to provide greater sensitivity in the test organism compared to humans, an MF of less than 1 may be applied in the ADI calculation.

BIOLOGIC FACTORS THAT INFLUENCE TOXICITY

It is clear from the foregoing discussion that all substances can exhibit toxicity at sufficiently high doses and there will be a range of sensitivities among individuals to the toxic effects. We will now consider physiologic and anatomical factors that can influence this sensitivity. The scheme presented in Figure 1.6 summarizes biological processes that can modulate responses, both beneficial and adverse, to an administered chemical.

Tissue absorption generally is required for most substances to exhibit their toxic effects. This absorption, for example, can result in a limited distribution of the substance at or near the point of contact, or it can lead to the entry of the substance into the blood or lymph circulation and distribution into the entire body. When a substance enters the biologic fluid, it can exist in a free form or a form in which it is bound, most often to proteins in the blood. Also, while in the body fluid, the substance can be translocated in bound or free form to distant sites in the body. Storage sites are body compartments in which the compound is bound with sufficiently high affinity to reduce its free concentration in the general circulation. Bone, lipid tissue, and liver are common sites of storage of xenobiotics. The residence time for the substance in the storage site can be as long as decades and depends on the binding affinity for the site and the concentration of the substance in the circulating fluid. As the concentration of the substance in the body fluid drops due to a cessation in exposure, the substance will be released to the circulation at a rate that depends on its binding affinity for a component of the binding tissue.

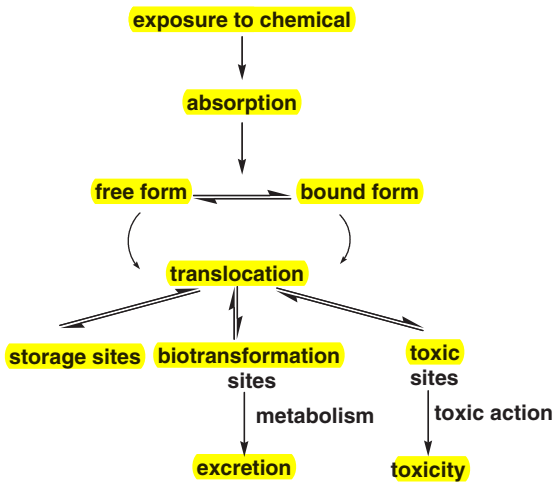

FIGURE 1.6 *Biological processes that can modulate responses, both beneficial and adverse, to an administered chemical.*

Biotransformation sites are locations in cells of certain organs that mediate the metabolism of xenobiotics. The most active tissues for this biotransformation are the portals of entry in the liver and small intestine. In most cases, biotransformation converts the substance to an oxidized and conjugated form that is water soluble and more readily excreted via the urine or the bile. In some cases, however, intermediates in the biotransformation process are responsible for the toxic effects of the administered substance.

Finally, the xenobiotic or its activated metabolite will encounter its site of action and toxicity. The molecular target is a component of a metabolic or signaling pathway that is important to the normal function or development of the organ.

Although a toxic agent may adversely affect the functions of many tissue macromolecules and the cells that contain them, these effects may not be important to the well being of the organ and the organism, and are not considered to be central sites of toxic action. Each of these factors that influence toxicity will be discussed in the following sections.

ABSORPTION

For a substance to gain access to a specific effector site within an organelle of a complex organism, the substance must often pass through a series of membranes. Although the membranes in various cells of the organism—such as the skin keratinocytes, intestinal enterocytes, vascular endothelial cells, liver hepatocytes, and the nuclear membrane—have certain characteristics that distinguish them from one another, the basic compositions of the membranes are very similar. An accepted general membrane model is illustrated in Figure 1.7.

In this model, the membrane is represented as a phospholipid bilayer with hydrophilic outer portions and a hydrophobic interior. Proteins are dispersed throughout the membrane with some proteins traversing the entire width and projecting beyond the surfaces of the membrane. The basic cell membrane is approximately 7.5 to 10 nanometers thick and is elastic. It is composed almost entirely of phospholipids and proteins with small quantities of carbohydrates on the surface.

A closer look at the chemical structure of the phospholipid component of the membrane provides insight into the effect of its composition on the function of the membrane. As represented in Figure 1.8, the polar head of the phospholipid is composed of a phosphate moiety bound to other small molecules such as choline, serine, ethanolamine, and inositol that can increase the polarity of the phospholipid or serve as sites for further modifications that control cell function, for example, by addition of carbohydrate or phosphate groups.

The composition of the lipid components of the phospholipid contributes to the fluidity of the membrane and, thereby, can affect cell function. For example, adequate fluidity of the membrane is maintained, in part, by incorporation of cis-unsaturated fatty acids. The cis-double bonds decrease the strength of interactions between adjacent lipid chains compared to lipids that are saturated or that contain trans-double bonds. Modification in fluidity can affect many cellular functions, including carrier-mediated transport, properties of certain membrane-bound enzymes and receptors, membrane transporters, immunological and chemotherapeutic cytotoxicity, and cell growth.

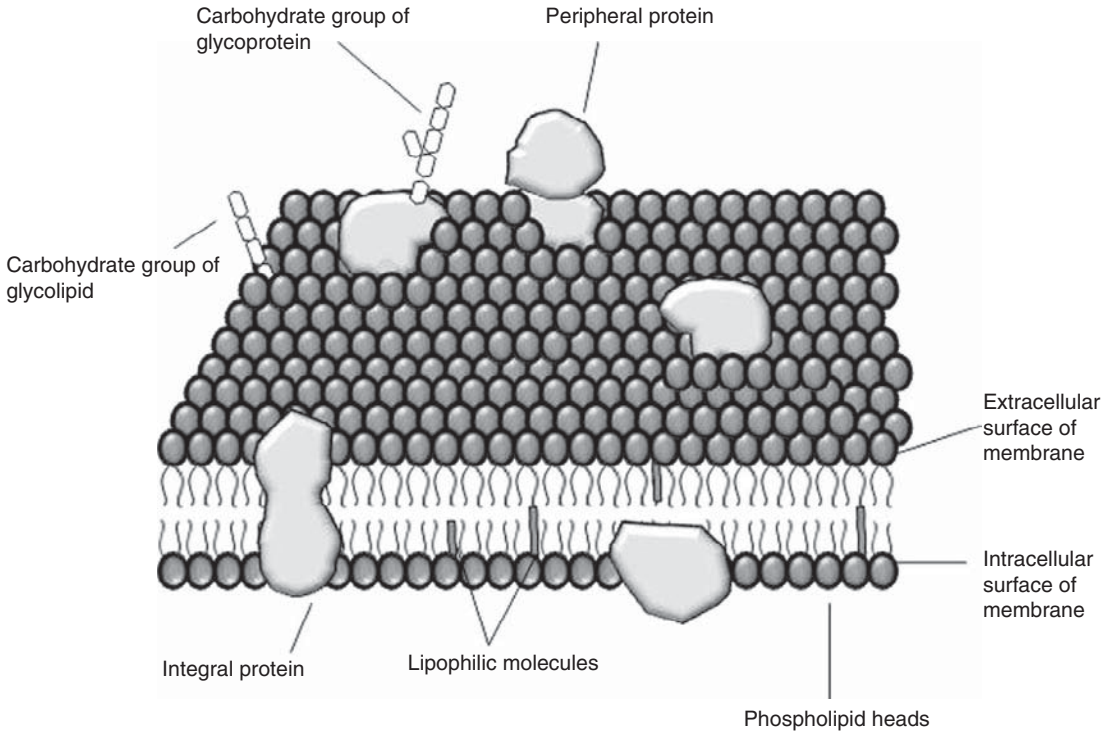

FIGURE 1.7 *General membrane model of animal cell.*

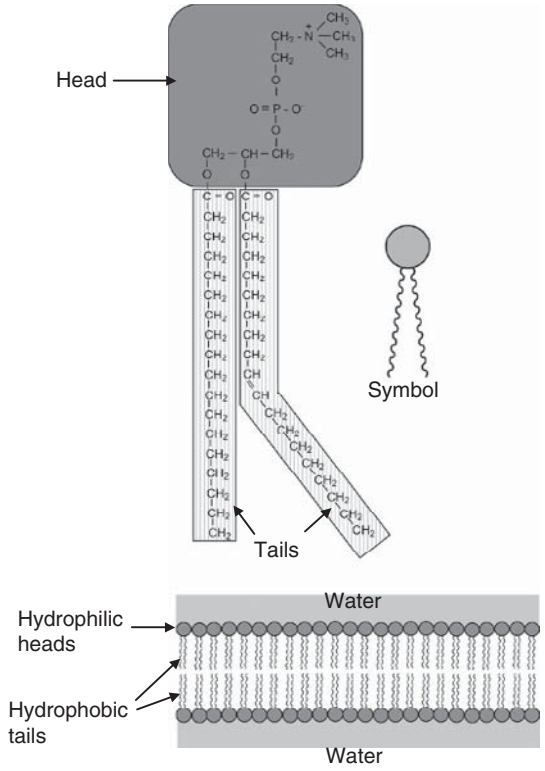

FIGURE 1.8

Chemical structure of the phospholipid component of the membrane.

Another important feature of the cell membranes is the presence of aqueous channels or pores. Although water can diffuse passively at a low rate through the continuous phospholipid bilayer of the membrane, some cell types exhibit much higher rates of water transport than others due to the presence of pores in the membrane. The transmembrane proteins that form these pores comprise a family of about 12 members called either **aquaporins**, if they allow passage of only water, or **aquaglyceroporins**, if they allow passage of glycerol and other small neutral solutes. The conformation of the most studied aquaporins, aquaporin 1 (AQP1) from red blood cells, is indicated in Figure 1.9. As visualized from the extracellular surface, AQP1 forms an elegant and highly symmetrical tetramer in the pore. Water passes through channels in each of the AQP1 molecules in the pore. The rates of passage of water and solutes through these pores depend on the size of the pore, which can be tissue specific and may be hormonally regulated. For example, channels in most cell types are less than 4 nm in diameter and allow passage of molecules with a molecular weight of only a few hundred Daltons. In contrast, the pores in the kidney glomerulus are much larger at approximately 70 nm, and allow passage of some small proteins (< 60,000 Da).

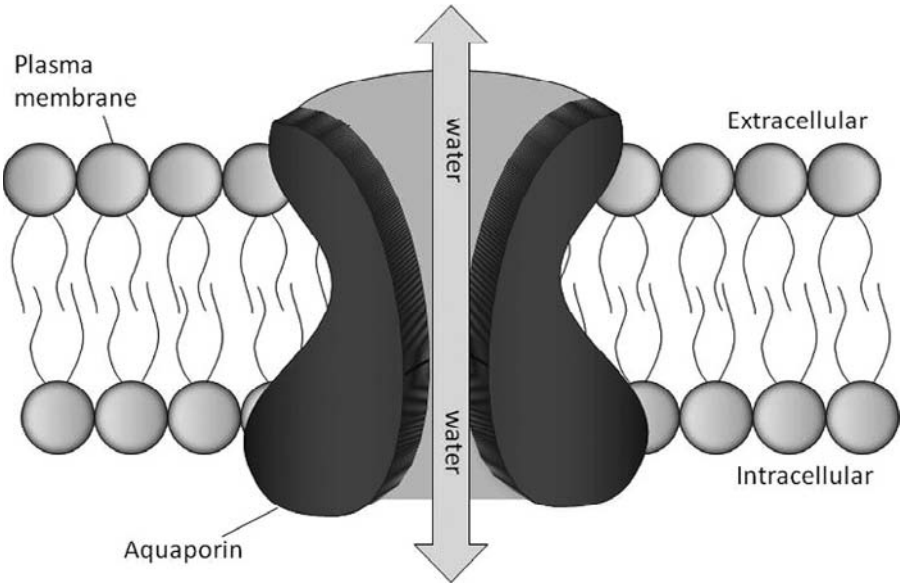

FIGURE 1.9 *Aquaporins, aquaporin 1 (AQP1) from red blood cells.*

TYPES OF MEMBRANE TRANSPORT

The processes of movement of substances across biological membranes are classified as passive diffusion and active transport. **Passive diffusion** processes, which include simple diffusion and facilitated diffusion, do not require energy in the form of ATP and are driven by concentration gradients.

Simple diffusion is characterized by the passive diffusion of hydrophobic molecules across the lipid membranes or of small hydrophilic molecules through aqueous pores. The rate of transport of lipophilic xenobiotics across the standard membrane depends on molecular size, hydrogen bonding, and polar surface area. Lipophilic xenobiotics with molecular weights of greater than about 500 Da tend not to pass readily across the lipid membrane. Such is also the case for substances that have very high hydrogen bonding characteristics. In general, the rate of passage of lipophilic xenobiotics across the membrane is proportional to the octanol/water partition coefficient or logP. Indeed, substances with a relatively high logP such as the pesticide DDT and environmental contaminant TCDD (logP approx. 7) are readily absorbed into the lipid membrane. Conversely, substances with relatively low logP values such as the pesticide paraquat, the antibiotic cephalosporin, and other charged molecules ($\log P < -4.5$) are not appreciably absorbed by a passive mechanism into the lipid membrane.

Facilitated diffusion is a carrier-mediated transport of water soluble substances that mimic the structures of endogenous substances that normally exist in the body. For example, glucose normally enters cells via special glucose transporters that do not require ATP. Certain drugs such as 2-deoxyglucose and related derivatives have been designed to enter the cell by competing with glucose for access to the glucose transporters. Other passive carriers exist for metal ions such as sodium, potassium, and calcium, some of which can be coopted by toxic metals such as cadmium and lead.

Although both types of passive diffusion processes are driven by substrate concentration gradients, under certain circumstances these processes can result in the differential accumulation of substances in the absence of such gradients. Such is the case, for example, in the diffusion of weak acids and weak bases across a membrane that separates compartments of different pH. Since ionized molecules are poorly absorbed into the lipid membrane compared to the unionized forms, the rate of transport depends on the degree of ionization of the molecule. Weak acids are protonated and unionized at low pH and are more lipophilic than at high pH. Similarly, weak bases are deprotonated and unionized at high pH and are more

lipophilic than at low pH. The degree of ionization, in turn, depends on the pK of the molecule and the pH of the medium as specified by the Henderson-Hasselbalch equation:

$$\text{For acids: } pKa - pH = \log[\text{nonionized}]/[\text{ionized}]$$

$$\text{For bases: } pKa - pH = \log[\text{ionized}]/[\text{nonionized}]$$

By these equations, the pH at which a weak organic acid or base is 50% ionized is the pKa. As the pH is changed by 2 units from the pK level, the degree of ionization of a weak acid or weak base either rises to 99% or declines to 1.0%. Thus, for a weak carboxylic acid such as benzoic with pKa = 4.2, on passage from the stomach, with a pH near 2, to the blood, with pH near 7, the degree of protonation falls from about 99% to about 0.1%. Thus, the weak acid accumulates in the blood compartment with the higher pH. By analogous reasoning, the absorption rates of weak bases such as aniline, with pKa = 5.0, are low because they exist as the less lipophilic conjugate acids in the low pH environment of the stomach.

An additional means by which substances are absorbed by passive diffusion involves the differential distribution of binding sites for the substance. If there are more binding sites on one side of the membrane compared to the other, the side of the membrane with the greater level of binding sites will have a greater amount of substance, regardless of whether both sides have equal concentrations of the free substance. For example, a substance that is introduced in the blood, which is rich in protein binding sites, will not accumulate in another fluid of another compartment such as the central nervous system where the level of protein binding sites is low. This differential distribution of binding sites for xenobiotics contributes to the barrier of accumulation of the substances in the central nervous system.

Although passive membrane transport processes primarily govern the access of most toxins to critical targets in the cell, **active transport** is critical in some important instances. In contrast to passive transport processes, active transport requires ATP for energy, works against substrate concentration gradients, and is saturable. Not surprisingly, the major active processes for transport of substances into cells are selective for nutrients and important endogenous substances. Included in this list are active transporters for sugars, neutral amino acids, basic amino acids, fatty acids, vitamin C, vitamin B_{12}, bile salts, and several metallic ions. As with the glucose facilitated diffusion transporter, toxic agents with structures similar to a nutrient can compete with the nutrient for active transport into the cell.

TOXIN ABSORPTION IN THE ALIMENTARY TRACT

In addition to molecular size and the lipophilic characteristics of the xenobiotic, the extent of absorption of xenobiotics in the alimentary tract depends on the physiological and anatomical features of each section (Figure 1.10), the residence time within each section, and the presence of food.

There is a reasonable correlation between logP value of certain well-known alkaloid drugs and their absorption following sublingual (oral) dosing. For cocaine, for example, a substance with relatively high lipid solubility, the ratio of sublingual effective dose to subcutaneous dose is approximately 2:1 (i.e., twice as much is required orally compared to direct injection) to produce the same response. In contrast, morphine, a substance with relatively poor lipid solubility, requires a sublingual dose roughly 10 times greater than the subcutaneous dose to give similar effects. These results are consistent with a primary role of the lipid-diffusion process in the absorption of these alkaloids.

The rate of absorption of xenobiotics in the stomach depends to a considerable extent on the acid/base properties of the substance. Weak acids are more lipid-soluble in their nonionized form in the highly acidic environment of the stomach, which, in humans, is normally in the range of pH 1 to 2 and rises to pH 4 during digestion. Since the pH of the blood is near neutral, the weak acids are deprotonated to the more polar conjugate base leading to the accumulation of the substance in the blood. Conversely, weak bases exist as protonated, conjugate acids in the low pH gastric juice and are not well absorbed.

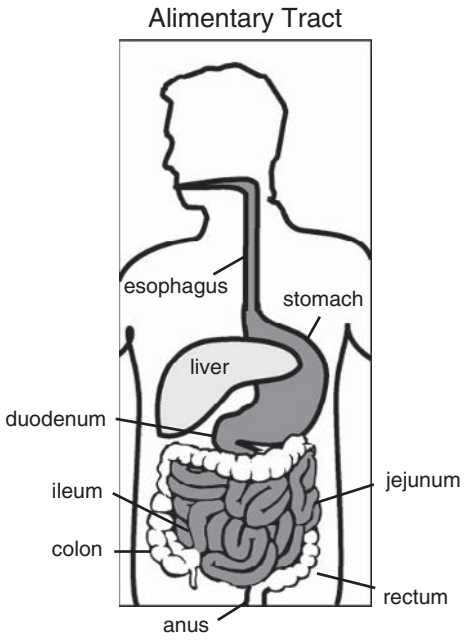

FIGURE 1.10 *General features of the human alimentary tract.*

The small intestine is the principal site of absorption of dietary xenobiotics. Since the pH of the contents of the small intestine ranges from pH 6 to 7 in the proximal small intestine to pH 7 to 8 in the distal ileum, acids and bases will be charged and relatively polar. According to the Henderson-Hasselbalch equation, however, unless the substances are strong acids or bases, a significant proportion of the xenobiotic will exist in the unionized, less polar form. Thus, since the small intestine is very long and is lined with highly absorptive enterocytes, absorption of weak acids and weak bases in this tissue can be

nearly complete even though the charge equilibrium lies heavily in favor of the charged species.

The colon is the final major absorption section of the alimentary tract. Although the pH of the colonic contents is similar to that of the distal small intestine (i.e., pH 7–8), and colonic epithelial cells express a wide range of transport proteins, xenobiotic absorption in the large intestine is relatively minor compared to the small intestine because of the considerably reduced length and surface area of the colon. However, the large intestine can serve as a site of absorption for certain substances, especially those produced by bacterial action within the lower gut, as will be discussed later in this chapter.

In addition to physiological and anatomical characteristics of the alimentary tract that influence xenobiotic absorption, the effect of food can also be significant. The majority of important food-drug interactions are caused by food-induced changes in the bioavailability of the drug. Indeed, the most common effect of food is to reduce the bioavailability of orally administered drugs, sometimes to the point of blocking the activity of the drug. Such interactions frequently are caused by chelation with components in food that occur with common antibiotics such as penicillamine and tetracycline. In addition, the physiological response to food intake, in particular gastric acid secretion, may reduce the bioavailability of certain drugs through destruction of the drug or the capsule in which it is delivered. In some cases, however, administration of the drug with food may result in an increase in drug bioavailability either because of a food-induced increase in drug solubility or because of the secretion of gastric acid or bile in response to food intake. Such increases in drug bioavailability may result in serious toxicity.

INTESTINAL MICROFLORA

An additional unique feature of the human digestive tract is that it normally harbors a large and diverse community of mostly anaerobic microorganisms. The conditions for bacterial growth in the various sections of the gastrointestinal tract differ considerably, leading to large differences in the concentrations of bacteria. Thus, there are normally low concentrations of bacteria in the stomach and duodenum, increasing concentrations in jejunum and ileum, and the highest concentration in the colon (10^9–10^{12} /mL). The composition of the intestinal microbiota is relatively simple in infants but becomes more complex with increasing age, reaching a high degree of complexity in adults. Diet strongly influences the type of

bacteria that are resident in the colon of an individual and of different human populations. Intestinal bacteria are important for the maturation and the maintenance of the immune system, and can influence colonic cell proliferation and contribute to the salvage of energy. In addition, the intestinal bacteria have a large metabolic capacity that can result in the conversion of dietary macromolecules to metabolites with beneficial or adverse health effects. For example, fermentation of dietary fiber can result in the formation of short chain fatty acids such as butyrate, and the fermentation of protein can result in the production of ammonia. Butyrate is thought to lower risk of colonic cancer, whereas high levels of ammonia can promote tumor development.

Intestinal bacteria also can mediate the metabolism of drugs and phytochemicals in the diet with consequences that may increase or decrease their biological activities. The gut microflora possess a diverse range of metabolic activities, including reductions, hydrolyses, and degradations. In many cases, these reactions can both complement and antagonize reactions of the liver, which are mainly oxidative and synthetic. For example, certain isoflavonoids, found for instance in soy, beer, or clover, have estrogenic properties and, depending on the developmental stage of growth at which they are administered, can either promote or inhibit mammary tumorigenesis in laboratory animals. In the human gastrointestinal tract, one of these isoflavones, daidzein, may undergo bacterial transformation to equol, which has a higher biological activity than daidzein. Differences in the gut microflora might account for the finding that approximately one of three individuals produces equol. Alternatively, daidzein may also be degraded by gut bacteria to the inactive metabolite, O-demethylangolensin. Thus, bacterial metabolism in the gut can strongly influence the activities of ingested xenobiotics and can account for a major proportion of individual variations in sensitivities to many orally administered substances.

THE BLOOD–BRAIN BARRIER

The blood–brain barrier (BBB) ensures an optimally controlled homeostasis of the brain's internal environment. The anatomical structure of the BBB is at the endothelial cell of arterioles, capillaries, veins, and at the epithelial cell surface of the choroid plexus, the area of the brain where cerebrospinal fluid (CSF) is produced. The endothelial cells allow a very selective transport of substances from blood to brain and brain to blood. In organs other than the brain, the extracellular concentrations of hormones, amino acids, and

ions such as potassium undergo frequent small fluctuations, particularly after meals or bouts of exercise. If the brain were exposed to such fluctuations, the result might be uncontrolled neurological activity. The BBB and the choroid plexus are required to protect the brain from these fluctuations.

Several anatomical and physiological features are the basis for the BBB. These features are (1) very small junctions between vascular endothelial cells, (2) the envelopment of the endothelial cells with astrocyte glial cells, (3) the low concentration of binding proteins in the CSF compared to the blood, and (4) the expression of several active transport systems that control the influx and efflux of many endogenous and exogenous substances. The net result of these features is to highly regulate the influx of hydrophilic chemicals into the CSF. These characteristics, however, do little to limit the influx of many lipophilic drugs and toxic substances into the CSF. For example, nicotine, heroin, and chloramphenicol have considerable lipophilicity and readily pass through the BBB to produce toxic effects.

XENOBIOTIC ABSORPTION INTO LYMPH

Absorption of xenobiotics in the intestinal lymph system can strongly influence their potency and organ selectivity. The lymphatic system is an extensive drainage network distributed throughout all areas of the body. The lymph system functions mainly to return fluid in the interstitial space back to the blood. The intestinal lymphatics are essential in the absorption of products from lipid digestion, such as long chain fatty acids and lipid soluble vitamins. Each of the gut villi is drained by a central lacteal, which conducts fluid via the lymphatic capillaries to the mesenteric lymph duct. In contrast to blood capillaries, the intercellular junctions between endothelial cells in lymphatic capillaries are more open. Chylomicrons, which are lipoproteins produced within the enterocyte from absorbed dietary lipids produced from digestion, have an average diameter of 200–800 nm. Following secretion by the enterocyte, these colloidal particles are too large to be absorbed by the blood capillaries and are consequently selectively taken up into the lymphatic capillaries. Since the volume of blood flow versus lymph flow is estimated to be 500:1, xenobiotics that are not highly lipophilic tend to be diverted preferentially toward the blood system. However, the great tendency for lipophilic xenobiotics to associate with dietary lipids and intestinal lipoproteins results in their selective absorption into the lymph system. Furthermore, the conditions of exposures that increase the association of xenobiotics with lipids can strongly influence their absorption into

the intestinal lymph system. For example, in a study with dogs, food was shown to markedly enhance lymphatic absorption in which the absorption of an orally administered lipophilic antimalaria drug, Hf, was increased from only about 1% in the fasted state to over 50% following a meal.

TRANSLOCATION

Translocation is the interorgan movement of substances in the body fluids and is responsible for the action of a toxin in a tissue that is not the site of exposure. The initial rate of translocation to distant organs and tissues is determined primarily by the volume of blood flow to that organ and the rate of diffusion of the chemical into the specific organ or tissue. As discussed previously, lymph can be an important vehicle for translocation of certain ingested lipophilic xenobiotics. Blood flow varies widely to the different organs and tissues in the body. Total blood flow (perfusion) is greatest in the liver, kidney, muscle, brain, and skin, and is much less in the fat and bone. Hence, the more highly perfused tissues receive the greater initial doses to xenobiotic.

The mammalian circulatory system has several features that can strongly influence the effects of the route of exposure on toxic action. A schematic representation of the major features of the circulatory system is presented in Figure 1.11.

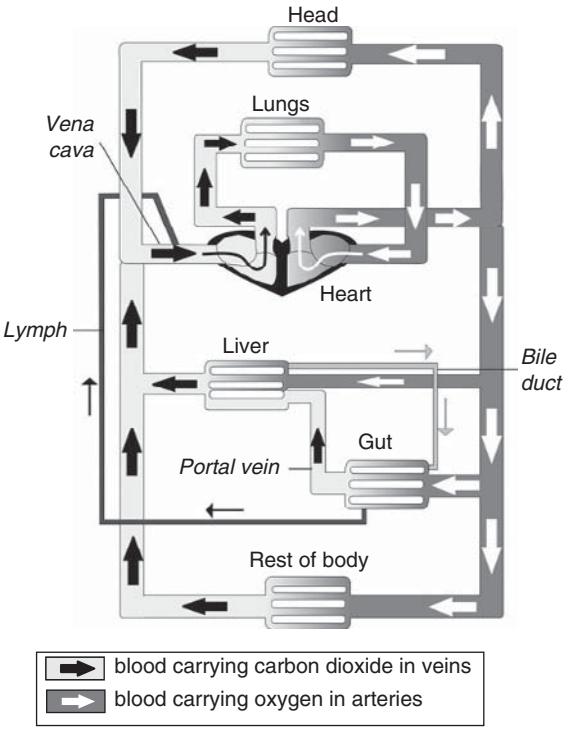

FIGURE 1.11 *General features of the mammalian circulatory system.*

Several features of the circulatory system that are important in toxin action are presented in the figure:

1. Venous blood is pumped by the heart to the lung for oxygenation. By this process, the first capillary system encountered by substances that enter the venous blood is in the lungs. This is true for substances that are absorbed in the mouth, or into the abdominal lymph system. This also occurs for substances that are administered by intravenous injection.

2. Arterial blood is pumped directly to the capillary systems of each of the organs, from which the blood is returned to the

heart through the venous blood. Thus, xenobiotics that are administered intra-arterially can go directly to all the organs.

3. Blood returning from the gut does not go directly to the heart but primarily enters the hepatic portal vein and flows to the liver. Thus, substances absorbed into the blood through the small intestine encounter the capillary system and metabolic capabilities of the liver before entering the general circulation. This is also true for substances administered by intraperitoneal injection, since the hepatic portal vein drains this cavity as well. Because of its high metabolic capability, the liver can convert a wide range of xenobiotics into products that are more easily excreted. This process of metabolic conversion of xenobiotics before they reach the general circulation is called **first-pass metabolism**.

4. Abdominal lymph flows into the thoracic duct, from which the lymph fluid is eventually reunited with the blood at the junction of the left jugular and the left subclavian veins immediately before it enters the heart. Thus, xenobiotics absorbed into the lymph from the small intestines will avoid first-pass metabolism in the liver and encounter the less efficient xenobiotic metabolic apparatus of the lung.

5. Bile produced in the liver is transported to the upper small intestine (duodenum) where bile acids assist in digestion of lipids in the diet. Xenobiotic metabolites that are secreted into the bile will return to the small intestine, which can expose them to the metabolic capabilities of enzymes in enterocytes in the upper intestine and to bacteria in the lower intestine. Conversion of a xenobiotic metabolite produced in the liver into a product that is then absorbed in the intestine can result in a recycling process called **enterohepatic circulation**, which can significantly increase the residence time of the xenobiotic in the body.

DISTRIBUTION

Although translocation is the interorgan movement of xenobiotics, distribution is the resulting apportioning of the xenobiotic in the body tissues and compartments. In the adult human, approximately 38 liters of body water is apportioned into three distinct compartments: interstitial fluid (11 liters), plasma fluid (3 liters), and intracellular fluid (24 liters). The concentration of a xenobiotic agent in blood following exposure depends largely on its apparent volume of distribution. The volume of distribution (V_d) is defined

as the ratio of the amount of compound in the body to the concentration of compound in plasma, or:

$$V_d = Dose_{iv}/C_p$$

where:

$Dose_{iv}$ is the amount of xenobiotic administered by intravenous injection

C_p is the concentration of xenobiotic in plasma

If the xenobiotic is distributed only in the plasma, a high concentration will be achieved within the vascular tissue and V_d will be small. In contrast, the concentration will be markedly lower if the same quantity of xenobiotic were distributed in a larger pool including the interstitial water and/or intracellular water. In this case the V_d will be large. For substances that distribute to both plasma and tissues, V_d values will be intermediate in magnitude.

Although for some substances the volume of distribution equates to a real physiologic volume, this is not always the case. For example, Evans Blue is a large, polar dye that does not pass through the capillary bed. As such, it is not able to pass out of the vascular system. The volume of distribution of Evans Blue, therefore, can be used as a measure of vascular volume. Bromide, moreover, is not able to cross cell membranes and distributes into extracellular space. Thus, bromide can be used to estimate the volume of extracellular water. In contrast, antipyrine and tritium labeled water are freely able to cross cell membranes and do not bind to cellular components. The apparent volume of distribution of these agents will equate to total body water. For other xenobiotics that extensively bind to intracellular components, however, the resulting apparent volume of distribution considerably exceeds the volume of total body water. The heart drug, digoxin, and the pesticide, DDT, and many other xenobiotics fall into this category. The volume of distribution of a drug that is much larger than total body water indicates that there is extensive tissue binding of the drug and that only a small fraction of the dose is in the vascular space.

The extent of binding to plasma proteins strongly influences the volume of distribution of many xenobiotic agents and their biological effects. Relatively few xenobiotics have sufficient solubility in blood for simple dissolution to regulate tissue distribution. Indeed, the distribution of most xenobiotics occurs in association with plasma proteins. Many organic and inorganic compounds of low molecular mass bind to lipoproteins, albumins, and other plasma proteins such as the iron binding protein transferrin. Since the protein-xenobiotic association is reversible, this binding can be

an efficient means for transport of xenobiotics to various tissues. However, since the xenobiotic-bound plasma protein cannot cross capillary walls due to its high molecular mass, the availability of these protein binding sites usually decreases the toxic effect of the xenobiotic. In many cases, the xenobiotic can compete with another drug or an endogenous compound for protein binding. In these cases, the xenobiotic can displace the other bound substance from the binding site and increase its activity. An example of this kind of interaction is the displacement of bilirubin from plasma albumin by sulfa drugs, which can produce neurotoxicity in infants as a result of the unbound bilirubin crossing the immature blood–brain barrier.

STORAGE

Many xenobiotics can accumulate in tissues at higher concentrations than those in the extracellular fluids and blood. Such accumulation may be a result of active transport or, more commonly, nonspecific binding. Tissue binding of xenobiotics usually occurs with cellular constituents such as proteins, phospholipids, or nuclear proteins and generally is reversible. The tendency for storage of xenobiotics in the body depends to a large extent on their polarity. The more polar organic substances tend to bind to proteins in the blood and soft tissues. Inorganic substances can bind to selective metal binding proteins in liver and kidney or to nonselective sites in bone. Lipophilic substances are stored primarily in lipid tissues. If a large fraction of xenobiotic in the body is bound in this fashion its biological activity may be strongly affected.

Organ Storage

Both the liver and the kidneys exhibit transport and storage capabilities that can strongly influence the activities of certain xenobiotics. The liver plays a critical role in regulating iron homeostasis by maintaining a reservoir of the essential metal bound to the storage protein, ferritin. Accumulation of iron, exceeding the binding capacity of ferritin, can result in liver toxicity. Metallothionein is a small molecular weight, cysteine-rich protein important in maintaining zinc homeostasis and controlling the toxicity of metals such as cadmium. Cadmium can accumulate as a nontoxic complex with metallothionein in liver and kidney. Indeed, as much as 50% of the cadmium in the body is found in complex with metallothionein in the kidney. Cadmium toxicity is produced in the kidney when the storage capacity of metallothionein is exceeded.

Lipid Storage

Many lipid-soluble drugs are stored by physical solution in the neutral fat. In obese persons, the fat content of the body may be as high as 50%, and in lean individuals, fat may constitute as low as 10% of body weight. Hence, fat may serve as a reservoir for lipid-soluble xenobiotics. For example, as much as 70% of highly lipid-soluble drugs such as sodium thiopental, or environmental contaminants such as DDT, may be found in body fat soon after exposure. Fat is a rather stable reservoir because it has a relatively low blood flow, which results in a relatively long residence time in the body for the absorbed xenobiotic. Rapid loss of fatty tissue through disease or dietary restriction can produce a rapid release of stored xenobiotic, possibly with toxic consequences.

Bone Storage

Although the rate of perfusion of bone by blood is relatively low compared to other organs, the binding affinity of bone tissue for certain xenobiotics is great. Thus, bone is an important storage site for certain substances. Divalent ions, including lead and strontium, can replace calcium in the bone matrix, whereas fluoride can displace hydroxyl ion. The tetracycline antibiotics and other calcium chelating xenobiotics can accumulate in bone by adsorption onto the bone crystal surface. Bone can also become a reservoir for the slow release of toxic agents into the blood such as lead or radium. However, the residence time for lead in the bone can be very long (decades) with no apparent toxic effect. In contrast, fluoride accumulation in bone can result in increased density and other abnormalities of bone development (skeletal fluorosis), and accumulation of radioactive strontium can lead to bone cancer.

The many possible effects of tissue storage on toxicity may be summarized as follows:

1. Storage at a site that is not the site of toxic action will reduce toxic potency.
2. Storage at the site of toxic action increases toxic potency.
3. Slow release from a safe storage site can result in chronic toxicity that may be different from the acute toxicity of the xenobiotic.
4. Displacement from storage of a substance with greater potency can result in toxicity from the previously administered substance.
5. Prior treatment with a substance that binds with greater affinity to a storage site may block the binding of the second substance and result in increased toxic potency of the second substance.

EXCRETION

Toxicants or their metabolites can be eliminated from the body by several routes. The main routes of excretion are via urine, feces, and exhaled air. Thus, the primary organ systems involved in excretion of nongaseous xenobiotics are the kidneys and the gastrointestinal tract. Other avenues for elimination include the expired air, saliva, perspiration, and milk, which is important in exceptional circumstances, such as the exposure of nursing infants to certain pesticides and lipophilic drugs encountered by the mother.

KIDNEY

Elimination of substances by the kidneys into the urine is the primary route of excretion of toxicants in terms of both the number of substances excreted by this pathway and the amount of each substance. The functional unit of the kidney responsible for excretion is the nephron, which consists of three primary regions, including the glomerulus, proximal convoluted tubule, and the distal convoluted tubule (Figure 1.12).

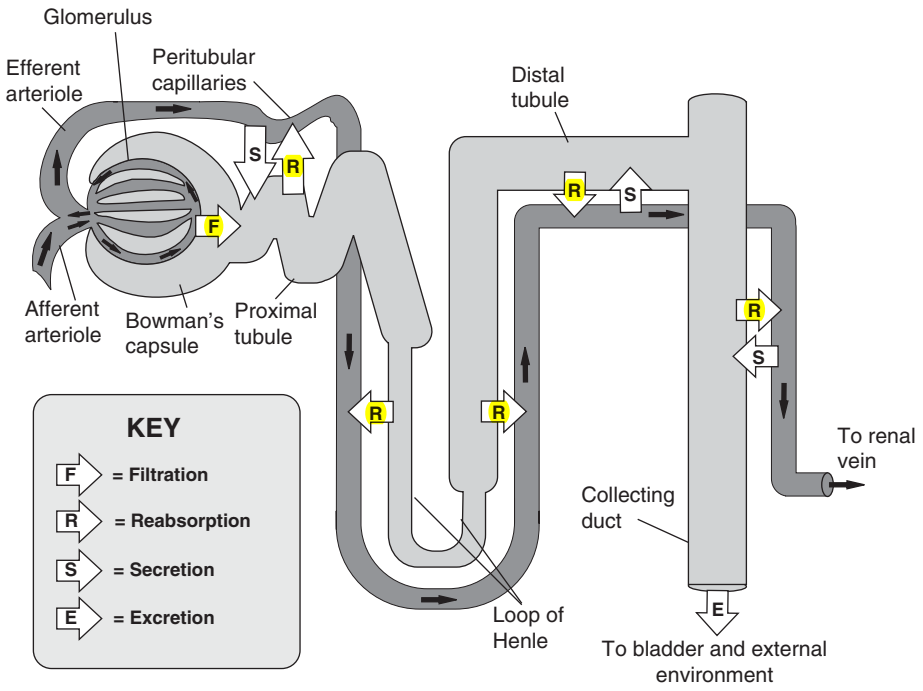

FIGURE 1.12 *General features of the nephron.*

Three physiological processes are involved in urinary excretion: filtration, secretion, and reabsorption. **Filtration** takes place in the glomerulus. A combination of characteristics lead to the production of approximately 45 gallons of filtrate each day for an adult person: a very large blood flow, a considerable hydrostatic pressure of the blood, and the large size pores in glomerular capillaries. The filtrate contains most of the lipid and water soluble substances in the blood, up to a molecular weight of approximately 60,000 Da. Thus, xenobiotics that are bound to albumen and other large proteins are not readily filtered from the blood, whereas substances that bind to small proteins such as metallothionein are filtered at the glomerulus to a large extent.

Secretion is an active process of transport of certain types of metabolites from the blood to the proximal tubule. Secreted substances include xenobiotics or their metabolites that are weak acids, including penicillin, uric acid, and many xenobiotic glucuronide conjugates, and weak bases, including histamine and many alkaloid drugs. The rate of active secretion of a xenobiotic can be reduced by treatment with a similar substance. For example, glucuronide conjugates secreted by organic acid active transporters can compete for excretion with uric acid and produce uric acid accumulation and toxicity in the form of gout. In another example, an organic acid of low toxicity called probenecid was developed during World War II to decrease the rate of excretion of penicillin by the organic acid active transporter.

Reabsorption takes place mainly in the proximal convoluted tubule of the nephron. Nearly all water, glucose, potassium, and amino acids lost during glomerular filtration reenter the blood from the renal tubules. Reabsorption occurs primarily by passive transfer based on concentration gradients, moving from a high concentration in the proximal tubule to the lower concentration in the capillaries surrounding the tubule. Lipophilic substances are passively reabsorbed to a large extent from the proximal convoluted tubule. Reabsorption of ionizable substances, especially weak organic acids and bases, is strongly influenced by the acidity of the urine. If the urine is alkaline, weak acids are highly ionized and thus are not efficiently reabsorbed and are excreted to a great extent in the urine. If the urine is acidic, the weak acids (such as glucuronide conjugates) are less ionized and undergo reabsorption with renal excretion reduced.

The urinary excretion rates of weak electrolytes are variable and depend on the pH of the tubular urine. Examples are phenobarbital and aspirin (acidic drugs), which are ionized in alkaline urine, and amphetamine (a basic drug), which is ionized in acidic urine. Treatment of barbiturate and aspirin poisoning may include changing the pH of the urine to facilitate excretion. The pH of the urine can be modified by several means. Acidosis

(decreased pH of urine and other body fluids) can result from conditions that lead to decreased loss of carbon dioxide in respiration, such as lung damage or obstruction; depression of the central nervous system (CNS) by drugs or damage; or conditions that result in altered metabolic status, such as diarrhea and diabetes or a high protein diet. Alkalosis (increased pH of body fluids) can result from increased loss of carbon dioxide through respiration as occurs, for example, with CNS stimulation or in high altitude; or altered metabolic conditions induced by alkaline drugs (e.g., bicarbonate), excessive vomiting, and high sodium or low potassium diets.

EFFECTS OF MATURATION ON KIDNEY EXCRETION

Renal organic anion (OAT) and cation (OCT) transporters protect against endogenous and exogenous toxins by secreting these ionic substances into the urine as discussed previously in this chapter. However, these transporters are not fully developed for several weeks following birth and can account for the differential toxicities of many xenobiotics in adults and infants. For example, the nephrotoxicity of cephalosporin antibiotics, which are weak organic acids, has been ascribed to effects of immature OATs. Cephalosporins produce proximal tubular necrosis, apparently by induction of oxidative stress, only after their transport into proximal tubule cells. A relative decrease in nephrotoxicity of cephalosporins is observed in the young that has been ascribed to decreased tubular transport. However, this OAT deficiency leads to a different manifestation of cephalosporin toxicity due to competition for transport of essential nutrients by cephalosporin, which can result in carnitine deficiency. This lack of full development of renal transporters also results in the reduced excretion in neonates of both endogenous organic acids, such as benzoic acid, and exogenous acids such as p-aminohippuric acid and penicillin. In addition to increasing the adverse or beneficial effects of these substances, the elevated levels of xenobiotics are thought to accelerate the maturation of the associated transporters.

Fecal Excretion of Xenobiotics

Although renal excretion is the primary route of elimination of most toxicants, the fecal route also is significant for many substances. Fecal elimination of absorbed xenobiotics can occur by two processes: excretion in bile and direct excretion into the lumen of the gastrointestinal tract. The biliary route is an especially important mechanism for fecal excretion of xenobiotics and their metabolites. This route generally involves distinct active

transport systems in liver for organic bases, organic acids, neutral substances, and metals. Whether a metabolite will be excreted into the urine or into the bile depends primarily on molecular size. Excretion via bile is the major excretory route for metabolites with molecular weights greater than approximately 350 Da. Similar metabolites with molecular weights smaller than about 350 Da are excreted preferentially in the urine. Examples of xenobiotics actively excreted in the bile include metal compounds such as dimethylmercury, lead, and arsenic, as well as metabolites of environmental toxins such as TCDD and drugs such as the estrogenic carcinogen diethylstilbestrol (DES).

DES provides an example of the major roles of biliary excretion and intestinal microflora in the activities of some xenobiotics. Research on experimental animals demonstrated that DES is excreted almost totally by the bile, and blockage of this pathway by bile duct cannulation resulted in a pronounced increase in the residence time of DES in the rodent body and an increase in the toxicity of DES by 130-fold! DES also is involved in the process of enterohepatic circulation. This process is initiated by the transport of the absorbed lipophilic xenobiotic to the liver via the portal vein where it undergoes a conjugation reaction to form a hydrophilic glucuronide or sulfate metabolite (Figure 1.13). If the conjugate is sufficiently large, it is then secreted into the bile. Compounds excreted in bile pass into the intestines where they may undergo deconjugation by the resident microorganisms. The deconjugated metabolite may be reabsorbed by the enterocytes and pass from the portal blood back to the liver. The processes of conjugation, biliary excretion, microbial deconjugation, and enterocyte absorption are repeated, thus comprising the enterohepatic cycle.

The efficiency of xenobiotic excretion in the bile and the effect of enterohepatic cycling can be influenced by several factors. The flow of bile in the liver usually is decreased with liver disease, whereas certain drugs such as phenobarbital can increase the rate of bile flow. Administration of phenobarbital has been shown to enhance the excretion of methylmercury by this mechanism, for example. The efficiency of enterohepatic circulation can be modified by conditions that reduce the intestinal microflora, as in antibiotic treatments, or decrease the reabsorption of xenobiotic metabolite such as by oral administration of binding agents.

A second manner by which xenobiotics can be eliminated via the feces is by direct intestinal excretion. Although this is not a major route of

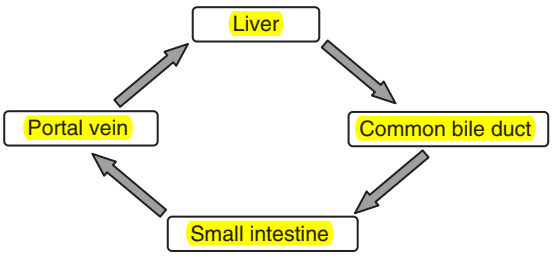

FIGURE 1.13 *General schematic of the enterohepatic cycle.*

elimination, a large number of substances can be excreted into the intestinal tract and eliminated via feces. Some substances, especially those poorly ionized in plasma (such as weak bases), may be eliminated in feces by passively diffusing through the walls of the capillaries and enterocytes and into the intestinal lumen. Other substances such as cholesterol, plant sterols, and other lipophilic substances, as well as certain conjugated metabolites, are actively transported from the apical portion of the enterocyte into the intestinal lumen by transporters for neutral substances and for organic anions. Moreover, increasing the lipid content of the intestinal tract can enhance intestinal excretion of some lipophilic substances. Indeed, active efflux in the small intestine is thought to contribute to the poor oral availability of many drugs. Intestinal excretion is a relatively slow process and is therefore an important elimination route only for those xenobiotics that have slow rates of metabolism or slow rates of excretion by other means.

ADDITIONAL READINGS

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