Chapter 2

Absorption, Distribution, and Excretion of Toxicants

INTRODUCTION

Apart from local effects at the site of contact, a toxicant can cause injury only after it is absorbed by the organism. Absorption can occur through the skin, the gastrointestinal tract, the lungs, and several minor routes. Furthermore, the nature and intensity of the effects of a chemical on an organism depend on its concentration in the target organs. The concentration depends not only on the administered dose but also on other factors, including absorption, distribution, binding, and excretion. For a chemical to be absorbed, distributed, and eventually excreted, a toxicant must pass through a number of cell membranes. A cell membrane generally consists of a biomolecular layer of lipid molecules with proteins scattered throughout the membrane (see Fig. 2-1).

There are four mechanisms by which a toxicant may pass through a cell membrane; the most important of them is passive diffusion through the membrane. The others are filtration through the membrane pores, carrier-mediated transport, and engulfing by the cell. The last two mechanisms are different in

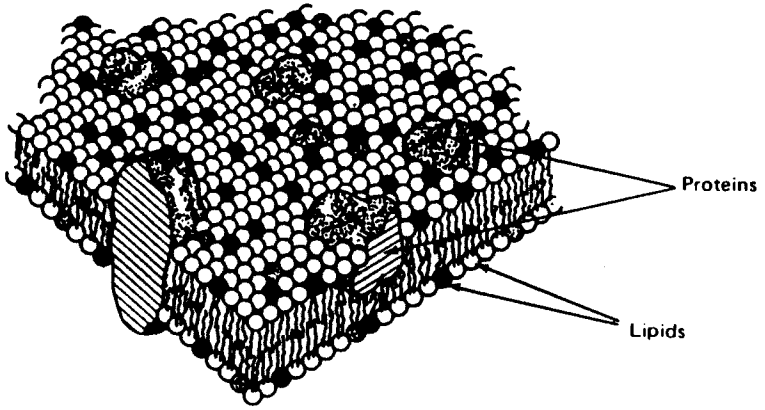

Figure 2-1 Schematic diagram of biologic membrane. Spheres represent head groups (phosphatidylcholine), and zig-zag lines indicate tail ends of lipids. Black, white, and stippled spheres indicate different kinds of lipids. Large bodies represent proteins; some are located on the surface, others within the membrane. (Modified from S. J. Singer and G. L. Nicholson, *Science*, 175:720. By permission of the author and the American Association for the Advancement of Science, copyright © 1972.)

that the cell takes an active part in the transfer of a toxicant across its membranes.

Passive Diffusion

Most toxicants cross cell membranes by simple, passive diffusion. The rate of passage is related directly to the concentration gradient across the membrane, and to the lipid solubility. For example, mannitol is hardly absorbed (<2%); acetylsalicylic acid is fairly well absorbed (21%); and thiopental is even more readily absorbed (67%). It is noteworthy that the chloroform : water partition of the nonionized forms of these chemicals are, respectively, <0.002, 2.0, and 100. For references to this and other examples see Timbrell (1982).

Many toxicants are ionizable. The ionized form is often unable to penetrate the cell membrane because of its low lipid solubility. On the other hand, the nonionized form is likely lipid-soluble enough to do so, and its rate of penetration is dependent on the lipid solubility. The extent of ionization of weak organic acids and bases depends on the pH of the medium. Thus, for the former, such as benzoic acid, diffusion is facilitated in acidic environment, where they exist mainly in the nonionized form; for the latter, such as aniline, diffusion is facilitated in basic environment (Fig. 2-2 and 2-3).

Filtration

The membranes of the capillaries and the glomeruli have relatively large pores (about 70 nm) and allow molecules smaller than albumin (molecular weight 60,000) to pass through. Bulk flow of water through these pores results from hydrostatic and/or osmotic pressure and can act as carrier of toxicants. The pores in most cells, however, are relatively small (about 4 nm) and allow chemicals only up to a molecular weight of 100–200 to pass through. Chemicals of larger molecules, therefore, can filter into and out of the capillaries. They can, therefore, establish equilibrium between the concentrations in the plasma and in the extracellular fluid, but they cannot do so by filtration between the extracellular and intracellular fluids.

Carrier-Mediated Transport

This involves the formation of a complex between the chemical and a macromolecular carrier on one side of the membrane. The complex then diffuses to the other side of the membrane, where the chemical is released. Thereafter the carrier returns to the original surface to repeat the transport process. The carrier has a limited capacity. When it is saturated, the rate of transport is no longer dependent on the concentration of the chemical and assumes zero order kinetics. Structure, conformation, size, and charge are important in determining the affinity of a chemical for a carrier site, and competitive inhibition can occur among chemicals with similar characteristics.

Active transport involves a carrier that moves molecules across a membrane against a concentration gradient, or, if the molecule is an ion, against an electrochemical gradient. It requires the expenditures of metabolic energy and can be inhibited by poisons that interfere with cell metabolism.

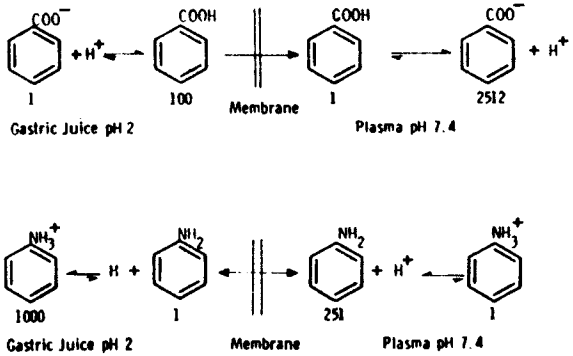

Figure 2-2 Disposition of benzoic acid and aniline in gastric juice and plasma. Figures immediately below the structural formulas represent proportions of ionized and nonionized forms (from Timbrell, 1982, p. 29).

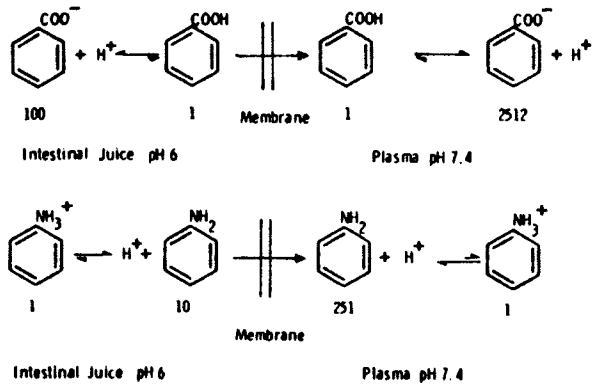

Figure 2-3 Disposition of benzoic acid and aniline in intestinal juice and plasma (from Timbrell, 1982, p. 30).

Facilitated diffusion is similar to active transport but does not move molecules against a concentration gradient. Furthermore, it is not energy-dependent, and metabolic poisons will not inhibit this process.

Engulfing by the Cell (Endocytosis)

Particles may be engulfed by cells. When the particles are solid, the process is called phagocytosis and when they are liquid, it is called pinocytosis. Such special transport systems are important for removal of particulate matter from the alveoli and of certain toxic substances from the blood by the reticuloendothelial system. Absorption of carrageenans (mol. wt. about 40,000) from the gut is also by this process.

ABSORPTION

The main routes by which toxicants are absorbed are the gastrointestinal tract, lungs, and skin. However, in toxicologic studies, such special routes as intraperitoneal, intramuscular, and subcutaneous injections are often used.

Gastrointestinal Tract

Many toxicants can enter the GI tract along with food and water or alone as drugs or other types of chemicals. With the exception of those that are caustic or very irritating to the mucosa, most toxicants do not exert any toxic effect unless they are absorbed. Absorption can take place along the entire GI tract. For example, certain drugs are administered as sublingual tablets and suppositories to be absorbed in the mouth and the rectum, respectively. However, the

mouth and rectum are, in general, insignificant sites of absorption of environmental chemicals.

The stomach is a significant site of absorption, especially for weak acids, which will exist in the diffusible, nonionized, lipid-soluble form. On the other hand, weak bases will be highly ionized in the acidic gastric juice and therefore not readily absorbable. The difference in absorption is further amplified by the circulating plasma. Weak acids will exist mainly in ionized form in plasma and be carried away, whereas weak bases will exist in nonionized form and can diffuse back to the stomach. The influence of these factors, using benzoic acid and aniline as examples, is shown in Fig. 2-2.

In the intestine, weak acids will exist mainly in the ionized form, hence less readily absorbable. However, upon entering the blood, they become ionized and thus will not readily diffuse back. On the other hand, weak bases will exist mainly in the nonionized form, hence more readily absorbable as shown in Fig. 2-3. It should be noted that intestinal absorption is further enhanced by the long contact time and the large surface area provided by the villi and microvilli.

In the intestine, there are special carrier-mediated transport systems that are responsible for the absorption of nutrients, such as monosaccharides, amino acids, and such elements as iron, calcium, and sodium. However, a few toxicants, such as 5-fluorouracil, thallium, and lead, are known to be absorbable from the intestine by active transport systems. In addition, particulate matters such as those of the azo dyes and polystyrene latex can enter the intestinal cell by pinocytosis.

Respiratory Tract

The main site of absorption in the respiratory tract is the alveoli in the lungs. This is especially true for gases such as carbon monoxide, nitrogen oxides, and sulfur dioxide and for vapors of volatile liquids such as benzene and carbon tetrachloride. Their ready absorption is related to the large alveolar area, high blood flow, and proximity of the blood to the alveolar air.

The rate of absorption is dependent on the solubility of the gas in the blood: the more soluble it is, the faster the absorption. However, equilibrium between the air and the blood is reached more slowly for more soluble chemicals, such as chloroform, compared to less soluble chemicals such as ethylene. This is because the more soluble a chemical, the more of it can be dissolved in the blood. Since the alveolar air can only carry a limited amount of the chemical, more respirations and hence a longer time will be required to attain equilibrium. It will take even longer if the chemical is also deposited in the fat tissue.

In addition to gases and vapors, liquid aerosols and airborne particles may also be absorbed. In general, large particles ($> 10\ \mu m$) do not enter the respira-

tory tract; when they do, they are deposited in the nose and disposed of by wiping, blowing, and sneezing. Very small particles (<0.01 μm) are likely to be exhaled. Those in the range of 0.01–10 μm are deposited in various parts of the respiratory tract. The larger ones are likely to be deposited in the nasopharynx and absorbed either through the epithelium of this region or through the gastrointestinal tract epithelium after they are swallowed along with the mucus. Smaller particles are deposited in the trachea, bronchi, and bronchioli and are then either aspirated onto the mucociliary escalator or engulfed by phagocytes. The particles carried up by the escalator will be coughed up or swallowed. The phagocytes with engulfed particles will be absorbed into the lymphatics. Some free particles can also migrate into the lymphatics. Soluble particles may be absorbed through the epithelium into the blood.

A detailed examination of the deposition of particles of various sizes in different parts of the respiratory tract is provided by a Task Group on Lung Dynamics (1966). However, as a rough estimate, 25% of inhaled particles are exhaled, 50% are deposited in the upper respiratory tract, and 25% are deposited in the lower respiratory tract (Morrow et al., 1966).

Skin

In general, the skin is relatively impermeable, and therefore it constitutes a good barrier, separating the organism from its environment. However, some chemicals can be absorbed through the skin in sufficient quantities to produce systemic effects.

A chemical may be absorbed via the hair follicles or through the cells of the sweat glands or those of the sebaceous glands. These are, however, minor routes for absorption since they constitute only a small surface area of the skin. Therefore, the percutaneous absorption of a chemical is essentially through the skin proper, which consists of the epidermis and dermis (see Fig. 14-1).

The first phase of percutaneous absorption is diffusion of the toxicant through the epidermis, which, especially its stratum corneum, is the most important barrier. The stratum corneum consists of several layers of thin, cohesive, dead cells that contain chemically resistant material (protein filament). Small amounts of polar substances appear to diffuse through the outer surface of the protein filaments of the hydrated stratum corneum; nonpolar substances dissolve in and diffuse through the lipid matrix between the protein filaments.

In the human stratum corneum, there are significant differences in structure and chemistry from one region of the body to another, which are reflected in the permeability to chemicals. For example, toxicants cross the scrotum readily, cross the abdominal skin less readily, and cross the sole and palm with great difficulty (Zbinden, 1976).

The second phase of percutaneous absorption is diffusion of the toxicant through the dermis, which contains a porous, nonselective, aqueous diffusion medium. Therefore it is much less effective as a barrier than the stratum corneum, and, as a consequence, abrasion or removal of the latter causes a marked increase in the percutaneous absorption. Acids, alkalis, and mustard gases will also increase the absorption by injuring this barrier. Some solvents, notably dimethyl sulfoxide (DMSO), also increase dermal permeability.

DISTRIBUTION

After a chemical enters the blood, it is distributed rapidly throughout the body. The rate of distribution to each organ is related to the blood flow through the organ, the ease with which the chemical crosses the local capillary wall and the cell membrane, and the affinity of components of the organ for the chemical.

Barriers

The *blood-brain barrier* is located at the capillary wall. The capillary endothelial cells there are tightly joined, leaving few or no pores between these cells (Bradbury, 1984). Thus, the toxicant has to pass through the capillary endothelium itself. A lack of vesicles in these cells further reduces their transport ability. Finally, the protein concentration of the interstitial fluid in the brain is low, in contrast to that in other organs; protein binding therefore does not serve as a mechanism for the transfer of toxicants from the blood to the brain. For these reasons, the penetration of toxicants into the brain is dependent on their lipid solubility. An outstanding example is the toxicant methyl mercury, which enters the brain readily and the main toxicity of which is on the central nervous system. In contrast, inorganic mercury compounds are not lipid-soluble, do not enter the brain readily, and exert their main adverse effects not on the brain but on the kidney.

The *placental barrier* differs anatomically among various animal species. There are six layers of cells between fetal and maternal blood in some species, whereas in others there is only one layer. Furthermore, the number of layers may change as the gestation progresses. Although the relationship of the number of layers of the placenta to its permeability needs quantitative determination, the placental barrier does impede the transfer of toxicants to the fetus, which is therefore protected to some extent. However, the concentration of a toxicant such as methyl mercury may be higher in certain fetal organs, such as the brain, because of the less effective fetal blood-brain barrier. On the other hand, the fetal concentration of the food coloring amaranth is only 0.03–0.06% of that of the mother (Munro and Willes, 1978).

Other barriers are also present in such organs as the eyes and testicles. In

addition, the erythrocyte plays an interesting role in the distribution of certain toxicants. For example, its membrane acts as a barrier against the penetration of inorganic mercury compounds but not that of alkyl mercury. Furthermore, there is affinity of the erythrocyte cytoplasm for alkyl mercury compounds. Because of these factors, the concentration of inorganic mercury compounds in the erythrocytes is only about half that in the plasma, whereas that of methyl mercury in the erythrocyte is about ten times that in the plasma (WHO, 1976).

Binding and Storage

As noted above, binding of a chemical in a tissue can result in a higher concentration in that tissue. There are two major types of binding. The covalent type of binding is irreversible and is, in general, associated with significant toxic effects. The noncovalent binding usually accounts for a major portion of the dose and is reversible. Therefore, this process plays an important role in the distribution of toxicants in various organs and tissues. There are several types of noncovalent binding as outlined by Guthrie (1980).

Plasma proteins can bind normal physiologic constituents in the body as well as many foreign compounds. Most of the latter are bound to the albumin and are therefore not immediately available for distribution to the extravascular space. However, since the binding is reversible, it permits the bound chemical to dissociate from the protein, thereby replenishing the level of unbound chemical, which may then cross the capillary endothelium. The toxicologic significance of the binding can be illustrated by the possible induction of coma by the administration of sulfonamide drugs to patients who are taking antidiabetic drugs. The antidiabetic drugs are bound to the plasma proteins but can be replaced by the sulfonamide drugs, which have a greater affinity for the plasma protein. The antidiabetic drugs thus released may precipitate a hypoglycemic coma.

The *liver* and *kidney* have a higher capacity for binding chemicals. This characteristic may be related to their metabolic and excretory functions. Certain proteins have been identified in these organs for their specific binding property, such as metallothionein, which is important for the binding of cadmium in the liver and kidney and possibly also for the transfer of the metal from the liver to the kidney. Binding of a substance can increase its concentration in an organ rapidly. For example, 30 minutes after a single administration of lead, its concentration in the liver is 50 times higher than that in the plasma.

The *adipose tissue* is an important storage depot for lipid-soluble substances such as DDT, dieldrin, and polychlorinated biphenyls (PCB). They appear to be stored in the adipose tissue by simple dissolution in the neutral fats. There exists the potential that the plasma concentration of the substances stored in the fat may rise sharply as a result of rapid mobilization of fat following starvation. Conjugation of fatty acid to toxicants such as DDT may also be a

mechanism by which these chemicals are retained in the lipid-containing tissues and cells of the body (Leighty et al., 1980).

Bone is a major site for storage for such toxicants as fluoride, lead, and strontium. The storage takes place by an exchange adsorption reaction between the toxicants in the interstitial fluid and the hydroxyapatite crystals of bone mineral. By virtue of similarities in size and charge, F^- may readily replace OH^-, and calcium may be replaced by lead or strontium. These stored substances can be released by ionic exchange and by dissolution of bone crystals through osteoclastic activity.

EXCRETION

After absorption and distribution in the organism, toxicants are excreted, rapidly or slowly. They are excreted as the parent chemicals, as their metabolites, and/or as conjugates of them. The principal route of excretion is the urine, but the liver and lungs are also important excretory organs for certain types of chemicals. In addition, there are a number of minor routes of excretion.

Urinary Excretion

The kidney removes toxicants from the body by the same mechanisms as those used in the removal of end-products of normal metabolism, namely, glomerular filtration, tubular diffusion, and tubular secretion.

The glomerular capillaries have large pores (70 nm); therefore, most toxicants will be filtered at the glomerulus, except those that are very large (greater than 60,000 mol. wt.) or are tightly bound to plasma protein. Once a toxicant enters the glomerular filtrate, it will either be passively reabsorbed across the tubular cells if it has a high lipid/water partition coefficient or remain in the tubular lumen and be excreted if it is a polar compound.

A toxicant can also be excreted through the tubules into the urine by passive diffusion. Since urine is normally acidic, this process plays a role in the excretion of organic bases. On the other hand, organic acids are unlikely to be excreted by passive diffusion through the tubular cells. However, weak acids are often metabolized to stronger acids, thereby increasing the percentage of the ionic forms that are not reabsorbed through the tubular cells and are thus excreted.

Certain toxicants can be secreted by the cells of the proximal tubules into the urine. There are two distinct secretory mechanisms, one for organic acids (e.g., glucuronide and sulfate conjugates) and the other for organic bases. Protein-bound toxicants can also be secreted, provided the binding is reversible. Furthermore, chemicals of similar characteristics compete for the same

transport system. For example, probenecid can increase the serum level of penicillin and prolong its activity by blocking its tubular excretion.

Biliary Excretion

The liver is also an important organ for the excretion of toxicants, especially for compounds with high polarity (anionic and cationic), conjugates of compounds bound to plasma proteins, and compounds with molecular weights greater than 300. In general, once these compounds are in the bile, they are not reabsorbed into the blood and are excreted via the feces. However, there are exceptions, such as the glucuronide conjugates, which can be hydrolyzed by intestinal flora, enabling the reabsorption of the free toxicants.

The importance of the biliary route of excretion for some chemicals has been well demonstrated in experiments that showed a severalfold increase of the acute toxicity in animals with ligated bile ducts. Such chemicals include digoxin, indocyanine green, ouabain, and, most dramatically, diethylstilbestrol (DES). The toxicity of DES is increased by a factor of 130 in rats with ligated bile ducts (Klaassen, 1973).

Lungs

Substances that exist in the gaseous phase at body temperature are excreted mainly by the lungs. Volatile liquids are also readily excreted via the expired air. Highly soluble liquids such as chloroform and halothane may be excreted very slowly because of their storage in the adipose tissue and the limited ventilation volume. Excretion of toxicants from the lung is accomplished by simple diffusion through cell membranes.

Other Routes

The *gastrointestinal tract* is not a major route of excretion of toxicants. However, because the human stomach and intestine each secretes about 3 liters of fluid per day, some toxicants are excreted along with the fluid. The excretion is mainly by diffusion and thus the rate depends on the pK_a of the toxicant and the pH of the stomach and intestine.

The excretion of toxicants in mother's *milk* is not important as far as the host organism is concerned. However, the presence of toxic substances in milk may be toxicologically significant because they can be passed in the milk from the mother to the nursing child and from cows to humans. The excretion is also via simple diffusion. Since milk is slightly acidic, basic compounds will reach a higher level in milk than in plasma, while the opposite is true with acidic compounds. Lipophilic compounds such as DDT and PCB also reach a higher level in milk because of its higher fat content.

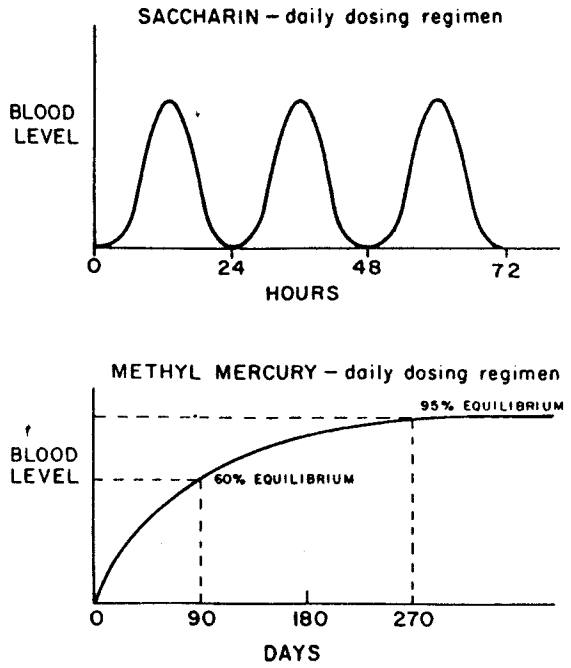

Figure 2-4 Comparative chemobiokinetics of saccharin and methyl mercury chloride (adapted from Munro and Willes, 1978, p. 138).

Sweat and *saliva* are also minor routes of excretion of toxicants. The excretion is also by diffusion; thus it is confined to the nonionized, lipid-soluble forms of the toxicants. Substances excreted in the saliva are usually swallowed and then become available for absorption in the GI tract.

LEVELS OF TOXICANTS IN THE BODY

As noted above, the nature and intensity of the effects of a chemical depend on its concentration at the site of action, namely, the effective dose rather than the administered dose. The level in the target organ is, in general, a function of the blood level. However, binding of a toxicant in a tissue will increase its level, whereas tissue barriers tend to reduce the level. Since the blood level is more readily determined, especially over a time period, it is the parameter often used in toxicokinetic studies.

While the toxicant is being absorbed, its blood level rises. In the meantime, the rates of its excretion, biotransformation (see Chapter 3), and the distribution to other organs and tissues also increase. The curve depicting the blood level

against time and the area under that curve (AUC) are useful tools in toxicokinetics. In a series of experimental studies, Smyth and Hottendorf (1980) demonstrated that the AUC for a solution of a chemical is, in general, greater than that for its suspension, and it is greater for acidic than basic chemicals. They also illustrated the effects of the route of administration, dose level, and dosing vehicle on the AUC.

The influence of the rate of excretion on the blood level is vividly shown in Fig. 2-4. Saccharin is rapidly excreted; hence its blood level drops rapidly, even after repeated administration. On the other hand, methyl mercury is excreted very slowly; its gradual accumulation culminates in a near plateau only after 270 days (Munro and Willes, 1978).

Methods for determining the rate and extent of absorption, distribution, binding, storage and excretion at various organs and tissues are readily found in the literature. A summary of some of them is given in a WHO publication (WHO, 1986).

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