CHAPTER 1
Introduction, Scope, and Principles

Early in the days of the development of civilization, man in his quest for food must have attempted to eat a variety of materials of both botanical and animal origin. Through this experience, it is likely that he found that certain substances, principally of plant origin, if taken into the body produced varying degrees of illness or caused death. Other materials served as a desirable form of food. Therefore, it would seem reasonable to believe that man soon recognized that there were harmful as well as beneficial consequences associated with taking materials into his body. All materials could be placed in two classes, one of which was safe and the other harmful. The word "poison" would be the term used to describe those materials or chemicals that were distinctly harmful to the body, and "food" would be the term used for those materials that were beneficial and necessary for the body to function.

This concept involving the division of chemicals into two categories has persisted to the present day and, as such, serves a useful purpose in society. It readily places certain biologic substances, and in fact all distinctly harmful chemicals, into a category which is accorded due respect. However, in a strictly scientific sense, such a classification is not warranted. Today, we certainly recognize that it is not possible to describe a strict line of demarcation, on one side of which may be placed the beneficial chemicals, and on

the other side of which may be placed the harmful chemicals. Rather, it is much more reasonable, as experience has shown, to recognize that there are degrees of harmfulness and degrees of safeness for any chemical. Even the most innocuous of substances, when taken into the body in sufficient amounts, may lead to undesirable, if not distinctly harmful, effects. In contrast to this, the most harmful of all chemical agents can be taken into the body in sufficiently small amounts so that there will be no untoward effect from such chemicals. It is apparent that the harmfulness or safeness of a chemical compound is related primarily to the amount of that compound that is present in the body.

The single factor that determines the amount of harm that a chemical compound produces is the quantity of the compound that comes in contact with a biologic system. This quantity of the compound is commonly called the "dose." (Dose may be expressed using a variety of terminologies and is further discussed in the next chapter.) If a sufficient dose is taken into the body or comes in contact with a biologic mechanism, a harmful effect will be the consequence in the sense that the ability of that biologic mechanism to carry on a function is destroyed or seriously impaired. As the dose is increased from minimal to maximal levels, there is no sudden appearance of undesirable effects from any chemical agent. Rather, the response, whether it be beneficial or harmful, is a graded response and is related to progressive changes in dose. One of the most fundamental observations which may be made with respect to any biologic effect of a chemical agent is the relationship between the dose (or concentration) and the response that is obtained.

Thus, toxicology has developed into the study of the quantitative effects of chemicals on biologic tissue. Its focus is on the harmful actions of chemicals on biologic tissue, but in the quest for information regarding the harmful actions of chemicals, the toxicologist also acquires information which is relevant to the degree of safeness of the compound.

The word "toxic" may be considered synonymous with harmful in regard to the effects of chemicals. Many chemicals are so nonselective in their action on tissues or cells that they may be said to exert an undesirable or harmful effect on all living matter. Furthermore, such chemicals may be effective in rather small concentrations. In contrast to this, a given chemical may be sufficiently selective in its ability to produce harm that it acts only on specific cells. A chemical may be harmful to essential systems in several species of organisms, but capable of exerting its harmful effect only in a few of these species because of protective devices present in the resistant species.

When a chemical is said to be toxic, the average person interprets this to mean that it would have a harmful or undesirable effect on humans.

This may not be true when the toxicologist uses the word "toxic" and "toxicity," because it is evident that what may be considered harmful to one biologic specimen may be relatively harmless to another specimen; in fact, a chemical that is toxic to some organisms may be desirable as far as man is concerned. For example, a chemical could be harmful or even lethal to the mosquito, but relatively harmless, and therefore indirectly beneficial and desirable, to mankind. For this reason, man can make use of chemicals to his advantage solely because they may be toxic or harmful to some biologic mechanism. Therefore, if the term toxic or toxicity is used, it is necessary to identify the biologic mechanism on which the harmful effect is produced. Toxicity is a relative property of a chemical and may be directly or indirectly desirable or undesirable as far as man is concerned, but toxicity always refers to a harmful effect on some biologic mechanism.

Toxicity is a relative term commonly used in comparing one chemical with another. It is common to say that one chemical is more toxic than another chemical. Such a comparison between chemicals is most uninformative unless the statement includes information regarding the biologic mechanism under consideration as well as the conditions under which it is harmful. Therefore, toxicology is approached as the study of the effects of chemicals on biologic systems, with emphasis on the mechanisms of harmful effects of chemicals and the conditions under which harmful effects occur.

HISTORY

Modern toxicology is a multidisciplinary science and as such had to await the development of many of the natural sciences before it could become a quantitative field. Although many descriptions regarding the actions of poisons and antidotes were published prior to the nineteenth century, little of this information was based upon scientific studies.

The father of modern toxicology was M. J. B. Orfila, a Spaniard born on the island of Minorca, who lived from 1787 to 1853. Early in his career he studied chemistry and mathematics, and subsequently he studied medicine in Paris. He is said to be the father of modern toxicology because his interests centered on harmful (as well as therapeutic) effects of chemicals, and because he introduced quantitative methodology into the study of the actions of chemicals on animals. He was the author of the first book devoted entirely to studies of the harmful effects of chemicals. (Orfila, M. J. B.: *Traité des Poisons Tirés des Règnes Minéral, Végétal et Animal, ou, Toxicologie Générale Considérée sous les Rapports de la Physiologie, de la Pathologie et de la Médecine Légale.* Crochard, Paris, 1814-1815.) He was the first to point out the valuable use of chemical analyses as proof that existing

symptomatology was related to the presence of the chemical in the body. He criticized and demonstrated the inefficiency of many of the antidotes that were recommended for therapy in those days. Many of his concepts regarding the treatment of poisoning by chemicals remain valid today, for he recognized the value of such procedures as artificial respiration, and he understood some of the principles involved in elimination of poison from the body. Like many of his immediate followers, he was concerned primarily with naturally occurring substances whose harmfulness was the focus of considerable folklore.

Although Orfila is considered the father of modern toxicology, Philippus Aureolus Theophratus Bombastus von Hohenhein, more commonly known as Paracelsus, also was a significant figure in the history of toxicology. Paracelsus (born in 1492 near Einsiedeln, Switzerland; died in Salzburg on September 24, 1541) formulated many then-revolutionary views that remain part of present day toxicology. He believed in the value of experimentation, a break with earlier tradition. Paracelsus, however, is best remembered as establishing the dose response when he stated, "All substances are poison; there is none that is not a poison. The right dose differentiates a poison and a remedy."

Modern toxicology borrows freely from several of the basic sciences. A knowledge of, and an ability to study, the interaction between chemicals and biologic mechanisms is predicated on a background in all of the basic physical, chemical, and biologic subjects. Toxicology borrows freely from the principles of chemistry, and more particularly biochemistry. It is dependent upon a knowledge and understanding of physiology. Familiarity with statistics and public health is fundamental to the study of toxicology. Pathology is a major part of toxicology, for a harmful effect from a chemical on a cell, tissue, or organism must necessarily manifest itself in the form of gross, microscopic, or submicroscopic deviations from the normal. The field most closely related to toxicology is pharmacology, for the pharmacologist must understand not only the beneficial effects of chemicals, but also the harmful effects of those chemicals that may be put to therapeutic use.

SCOPE OF MODERN TOXICOLOGY

In the United States in the past half century the teaching of toxicology has developed from a few incidental lectures given in courses presented in the Health Sciences and Public Health fields to the complete programs currently given in specific areas of the science. Occasionally one finds general introductory courses that are designed and presented in senior undergraduate programs. However, toxicology does not currently enjoy

the status of an independent department within a college or university. Hence, the student of toxicology continues to study the subject in a fragmented fashion from a variety of sources. In medical schools, the teaching of toxicology is usually allocated to the pharmacology division of the school, where the subject is taught primarily as a description of the harmful effects on man of therapeutic agents and a few highly toxic substances. The diagnosis and treatment of chemical intoxication is taught in the clinics, and the clinical chemical methodology is taught in the departments of laboratory medicine. These sources of training are usually designed for the medical student but also are available to the student of toxicology. Courses in public health and preventive medicine generally include the statistical methodology and problems associated with exposure to chemicals in a working or domestic environment. Veterinary schools have excellent facilities for the study of the harmful effects of chemicals on livestock and pets. These schools instruct in the absorption, distribution, excretion, and metabolism of foreign chemicals and in the treatment of chemical poisoning in animals. Departments of fisheries and oceanography study and instruct in the effects of chemicals on marine forms of biologic tissue.

Thus, modern toxicologists are a collection of scientists from multiple disciplines who have a common interest in the harmful biologic effects of chemicals. There are the engineers and geologists who study the distribution of chemicals in the air, soil, and water. There are the chemists whose interest and ability rest in the detection and quantification of chemicals in biologic tissue. There is the pharmacologist whose interest is in the harmful effects of chemicals that are used as drugs. There are the industrial health physicians and public health officers who specialize in the control of pollution and the effects of pollutants on populations. There is the pathologist whose studies are concerned with the gross and microscopic effects of foreign chemicals. There are the veterinarians who are concerned with the effects of chemicals and plants, as well as feed additives, on livestock and pets. There are the marine biologists who are concerned with the adverse effects of foreign chemicals on marine life. Immunologists, geneticists, oncologists, and mutagenicists not only evaluate new agents for activity in their special areas of expertise but also use compounds that produce effects on these systems as tools in their studies.

The multidisciplinary nature of toxicology is one of its greatest strengths, for it brings the capabilities and techniques of experts in those sciences into the field of toxicology. It also allows for the practical and logical division of the subject into sections on the basis of the disciplines involved. Figure 1.1 shows three such divisions: Environmental, Economic, and Medical. The Environmental division includes the roles that engineering, environmental, and chemical specialists play in the identification and quantifica-

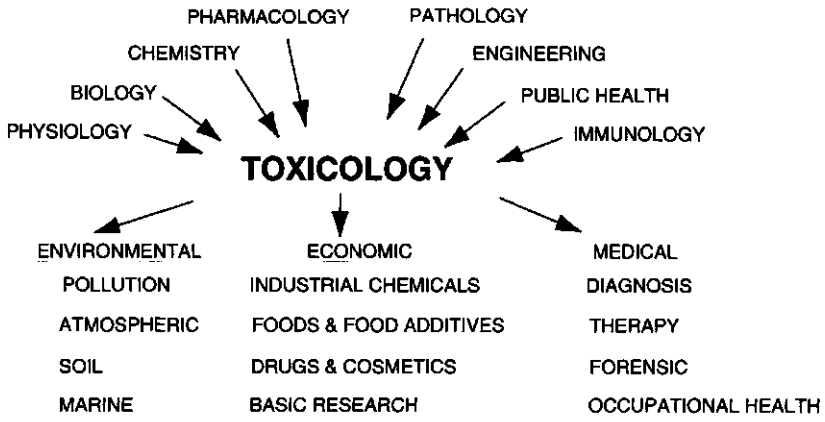

FIGURE 1.1

tion of natural as well as unnatural agents responsible for contamination (pollution) as well as transfer of chemicals between and within air, soil, and water. The Economic division involves the biologists, chemists, and basic medical scientists who identify and quantify the chemicals responsible for toxicologic problems in industry, in foods, and in drugs. It also includes the basic laboratory research programs that elucidate the chemical–biologic mechanisms responsible for harmful effects of chemicals on biologic tissue. The Medical division utilizes the capabilities of physicians and veterinarians for the diagnosis and therapy of chemical intoxication. As such it involves the forensic aspects of clinical toxicology, the pathology involved, and the public health consequences of chemically induced adverse health effects.

ENVIRONMENTAL TOXICOLOGY

The industrial revolution together with population growth has produced a complicated array of patterns by which chemicals are transferred from their sources into and within the environment. A simplified overview of these patterns is shown in Fig. 1.2. It shows that all chemicals eventually become waste and are translocated either as the original agent or as a transformed product of the original agent. Regardless of whether the original agent was man-made, was a product from biologic sources, or preexisted in the soil, it can eventually reach the environment. The environment, composed of air, water, and soil, serves as both a supply source and a dump site for chemicals and their derivatives and translocates each agent

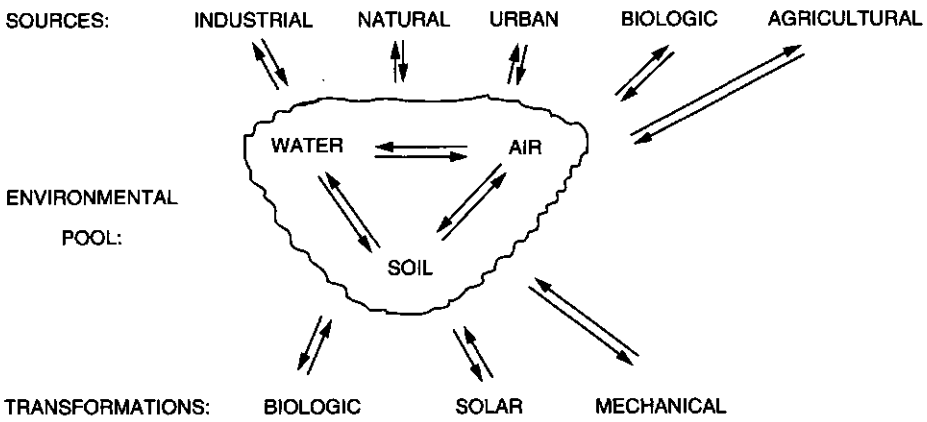

FIGURE 1.2 Waste chemicals and the environmental cycles.

within the environmental pool. Transformation products may be relatively more or less toxic as compared to the untransformed agent. The pool serves as an efficient system for diluting agents generally without showing partiality regarding the order of toxicity of various compounds. Hence dilution by the massive environment is a major mechanism by which chemicals supposedly disappear from existence and from importance in toxicology. Only when the pool is overwhelmed and the patterns of distribution fail, thereby leading to accumulation of agents at sites important to man's existence, does the system result in adverse chemical-induced effects on man. Some examples are the localized creation of smog over highly industrialized urban areas and the bioaccumulation of industrial waste products (e.g., methyl mercury) in fish consumed by humans.

Exposure to chemicals in the environment and the public health consequences are a continuing source of concern in toxicology. Because direct, reliable data regarding what ultimately happens to the large number of chemicals produced and used by humans are fragmented, there is concern about their persistence and possible accumulation in the environment. Some such agents do, in fact, accumulate, particularly in animals and aquatic forms of life which ingest them with food or water. Others undergo bacterial or biologic transformation to new chemical entities which also eventually reach the environment. Still others appear in the groundwater or soil where solar energy converts them to additional products. Others simply persist because of their high degree of stability. Although it is tempting to believe that the environment is sufficiently massive that it will ultimately dilute

such agents to concentrations that have no biologic effect, this is clearly not true. Furthermore, the environment is not always a detoxication medium; for example, the chlorine which is added to drinking water will appear in wastewater, where it can react with organic material to produce chlorinated hydrocarbons, some of which are suspected carcinogens. This is only one of many reasons that nationwide monitoring of drinking water supplies has been conducted routinely since the early 1970s. Various water supplies have been shown to collectively contain minute quantities of more than 1000 chemical entities. The National Academy of Sciences (Safe Drinking Water Committee) has published 10 volumes on the subject of pollutants in drinking water, covering aspects ranging from the basic principles of water contamination to the potential health effects of contaminated water.

Although contamination of air and water presents major problems, contamination of soil by natural as well as man-made chemicals is considerably more complicated because of the complexity of the chemistry of soils compared to air and water. In addition, all soils contain microorganisms and their nutrients and by-products, as well as air and water; this allows for the transfer of contaminants between these environments. Every individual physical–chemical property of each agent is involved in facilitating or inhibiting the translocation of the agent within the environment. Ascertaining the final depot of waste chemicals and their products is central to understanding and controlling the potential for the occurrence of adverse effects on humans.

In spite of the large number of chemicals that appear as waste in the environment, the exposures that have occurred (with the exception of certain catastrophes listed in Table 1.1 and some examples of occupationally related tumorigenicity) have not been shown to be responsible for significant mortality in humans. There are reports of a positive statistical relationship between water quality and tumorigenicity in humans, but the risks appear to be at the minimum reliable levels of the procedures involved.

The sublethal effects associated with exposures of large populations to chemical wastes in the environment present a complicated diagnostic medical problem. This subject is considered further in Chapter 14. The uncertainties regarding potential hazards associated with environmental contamination necessitate continued scientific vigilance in order to safeguard the public health.

ECONOMIC TOXICOLOGY

Although toxicology is a very diverse discipline, its central concern lies in the evaluation of chemicals with the potential for harmful effects on

TABLE 1.1 Some Disasters Resulting in Chemically Induced Illness and/or Death in Humans

Date	Toxicity	Location	Compound	Source	Illness	Death
1930	Paralysis	U.S.	Tri-*o*-cresyl phosphate	Beverage contaminent	50,000	?
1937	Kidney failure	U.S.	Diethylene glycol	Solvent in sulfanilamide elixir	353	105
1951–74	Minimata disease	Japan	Methyl mercury	Containment in food (fish)	520	80
1956–60	Teratogenesis	Europe	Thalidomide	Sedative drug	8,000	?
1971–72	Coma	Iraq	Methyl mercury	Pesticide-treated grain	6,000	459
1976	Chloracne, liver damage	Italy	Dioxin	Factory explosion	700–22,000	?
1984	Lung damage	India	Methyl Isocyanate	Factory explosion	60,000	1700

Note. Data adapted from *General and Applied Toxicology* (Ballantyne, B., *et al.*, Eds.), Stockton Press, New York, 1993; and *Goldfrank's Toxicologic Emergencies* (Goldfrank, L. R., *et al.*, Eds.), Appleton & Lang, Connecticut, 1990.

man. Since it is a rare occasion that toxicity data are obtained initially on man, most information is derived from experimental animal studies, on the basis that animal data, properly qualified, are applicable to man.

Industry recognizes that it is essential for some toxicologic data to be obtained on every new chemical that is to be released to society; consequently, a company either has their own elaborate facilities and research programs or hires outside laboratories to estimate the toxicity of their products. Federal regulation agencies set requirements regarding the nature of toxicologic data necessary for chemicals that are added to foods. In a similar manner, data must be made available on all new (as well as existing) drugs to ensure that such therapeutic agents are not only effective but also safe.

Academia, as well as industry, expends considerable effort in determining the mechanisms of chemical–biological interactions. History has shown that an understanding of the mechanism of action of some highly toxic compounds can suggest concepts for the development of new drugs and newer, safer industrial chemicals. Although most toxicologic data are obtained via animal experimentation, considerable progress has been made in recent years in the development of *in vitro* toxicologic protocols.

The industries involved in production of chemicals, the agencies involved in the regulation and control of distribution of chemical products, and the laboratories involved in studying chemical–biological interactions all contribute to the acquisition of knowledge about the harmful effects of chemicals. These activities collectively compose the branch of toxicology that is identified in Fig. 1.1 as "economic toxicology."

MEDICAL TOXICOLOGY

Currently there are more than 100,000 chemical entities to which the general human population could be exposed. A student of toxicology could not be expected to be knowledgeable about the toxicity of even a small fraction of these. However, in spite of the multitude of chemicals that are potentially harmful, only a few have been adequately documented as causative of serious health problems in humans.

Periodically, catastrophic accidents expose large numbers of people to specific, known chemical agents. Examples of such accidents are given in Table 1.1. In these instances the consequences consist of not only deaths but also sublethal clinical effects. These incidents (and the publicity they receive) have made the general public justifiably concerned about insidious, sublethal, delayed, and harmful effects of chemicals to which they are exposed. In regard to sublethal illness from chemical agents, except for

those catastrophic accidents, adequate documentation regarding causation is frequently lacking. Also, whereas some of the adverse drug or industrial chemical reactions have been well documented, others appear in the literature with inadequate documentation to support the role of the drug or chemical as the causative agent. Verification of possible chemical-induced illness presents a difficult problem to the clinical toxicologist (see Chapter 14).

In the disasters cited above, the chemical agents involved and the clinical consequences are clearly delineated. In the emergency rooms of modern hospitals as well as in the facilities of medical examiners the chemical involved in most lethal cases is determined by direct analysis. However, many deaths may be associated with the presence of specific chemical agents while the cause of death may not be reported in a form that allows it to be included in the statistical data on chemical-induced deaths. For example, an automobile driver intoxicated with alcohol may die in a collision, yet such a death would not be recorded as due to alcohol since the presence of the drug may be only coincidental. Similarly the death of a cigarette smoker may be due to lung cancer, but to record such a death as being due to cigarette smoke would be speculative. Conversely, a death that occurs in a residential fire may show that the death was due to carbon monoxide, but such a death would not be recorded as a chemical-induced death. Statistics that specify their data sources and limit their conclusions to the boundaries of the methodology used are the best sources of information on chemical-induced illness and death.

Regardless of the problems associated with the accuracy and completeness of data on chemical-induced morbidity and mortality, only through the acquisition of statistically evaluated data on these subjects can the magnitude of chemical-induced clinical problems be demonstrated.

Table 1.2 lists data showing that in 1970 and 1990 in the United States there were, respectively, 5299 and 5803 chemical-induced accidental deaths, or 2.6 and 2.3 per 100,000 population, and a similar number of chemical-induced suicidal deaths. Drugs and medicines were deemed to be responsible for about 3 out of 4 accidental deaths.

There are many more incidents in which chemicals cause sublethal poisoning rather than death. Table 1.3 indicates that in the United States in 1992 there were a total of 1.8 million inquiries regarding potential poisonings, from which 705 or 0.04% resulted in deaths. Approximately 50,000 or 2.7% of the total inquiries resulted in moderate or major consequences. Intentional exposure (that is, suicidal or abusive use) was involved in almost 11% of the exposures.

Table 1.4 summarizes a more detailed examination of the suspected causative agents responsible for the 705 deaths. It indicates that a variety

TABLE 1.2 Annual Deaths from Chemicals in the United States in 1970 and 1990

	Number of deaths		Rate/100,000 population	
	1970	1990	1970	1990
Deaths from accidents				
Drugs and medicines	2505	4506	1.2	1.8
Other solids/liquids	1174	549	0.6	0.2
Gases and vapors	1620	748	0.8	0.3
Total	5299	5803	2.6	2.3
Deaths from suicide poisoning (includes liquids and gases)	6584	5224	(3.2)	(2.1)

Note. From *Statistical Abstracts of the United States,* 113th edition, 1993, p. 98, except for numbers in parentheses which were calculated by the author (T.A.L.).

of chemical agents were responsible for the observed lethality. Drugs were responsible for 541 or 76.7% of the 705 deaths. Also, of all drug deaths, 391 or 72.3% were due to only 17 different drugs. Non-drugs were responsible for 164 or 23.3% of the 705 deaths, and about half of these deaths were due to only 10 chemical entities. Hence these data suggest that only a few chemicals or drugs (27) were responsible for a very high percentage of chemical-induced deaths.

Medical toxicology involves those disciplines that are concerned principally with the chemical identification, clinical effects, diagnosis, and treatment of chemical intoxication in human populations. In addition, medical toxicology involves the acquisition of information and the estimation of human risk associated with exposures of individuals as well as populations to chemical entities. This latter function includes the production of statistics on chemical-induced morbidity and mortality in humans.

FUNDAMENTAL PRINCIPLES IN TOXICOLOGY

From a fundamental and practical standpoint, the consequences of toxicologic effects on humans can be divided into two categories. One includes those consequences that are generally considered "irreversible," such as mutagenicity, carcinogenicity, teratogenicity, and of course death. The second category includes consequences that are "reversible," providing the initial damage is not overwhelming. Among these effects are organ damage,

TABLE 1.3 Poisonings Reported by Poison Control Centers in the United States in 1992

	Exposures		Deaths	
	Number	% of total	Number	% of total
Total	1,864,188	—	705	—
Consequence				
Unknown	873,718	46.9	—	—
None or minor	887,659	47.6	—	—
Moderate or major	49,693	2.7	—	—
Death	705	0.04		
Site of exposure				
Residence	1,716,917	92.1	—	—
School	18,641	1.0	—	—
Workplace	46,602	2.5	—	—
Age group (years)				
<6	1,092,568	58.6	29	4.1
>50	7,270	3.9	201	28.5
Exposure				
Accidental[a]	1,624,424	87.1	107	15.2
Intentional[b]	199,950	10.7	541	76.7
Adverse reaction[c]	7,790	0.4	5	0.007

Note. Data are from 68 reporting centers serving a population of 196.6 million persons and are estimated to represent 78% of human exposures leading to poison center contacts in the United States in 1992. The data include telephone reports and treated patients and were condensed by the author (T.A.L.) from *Annual Report of Poison Control Centers Toxic Exposure Surveillance System* (Litovitz, T. L., Holm, K. C., *et al.*), *Am. J. Emer. Med.* **2,** No. 5, pp. 494–555, Sept. 1993.

[a] Misuse, occupational, environmental.

[b] Suicidal, abuse.

[c] Effects from drugs, foods.

such as damage to liver, kidney, or skin, and functional damage, such as respiratory depression, loss of consciousness, or convulsive effects.

Regardless of the category of any specific toxicologic effect, there are at least four basic principles that are generally applicable to all chemical-induced biologic effects of toxicologic interest.

1. The chemical must get to the effector site in a biologic system in order to produce a biologic effect. Although this may seem obvious, it is frequently overlooked in discussions of toxicity. For example, alcohol can produce harmful effects on humans, but a liter of whiskey in a bottle can have no effect (other than a psychologic effect) if it is not consumed; a liter of whiskey consumed in a short interval, however, contains enough alcohol to be lethal to the average adult. This concept can be extended to such scenarios as the asbestos in a building or the polychlorinated biphenyls in

TABLE 1.4 Some Suspected Causative Agents Involved in 705 Deaths Reported by Poison Control Centers in the United States in 1992

Drugs			Nondrugs		
Category/agent	Number of deaths		Category/agent	Number of deaths	
Antidepressants	166		Fumes, gases, and vapors	36	
Amitriptyline		50	Carbon monoxide		24
Desipramine		22	Hydrogen sulfide		5
Doxepin		21	Chemicals (general)	19	
Imipramine		16	Cyanide		7
Nortriptyline		21	Strychnine		3
Analgesics	122		Insecticides	18	
Acetaminophen		63	Diazinon		5
Aspirin		35	Household cleaners	17	
Codeine		3	Hydrochloric acid		6
Morphine		7	Hydrocarbons	16	
Propoxyphene		7	Butane		9
Cardiovasculars	61		Alcohols	16	
Digoxin		16	Ethanol		10
Verapamil		28	Automotive Products	13	
Street drugs	61		Ethylene glycol		5
Cocaine		42	Methanol		5
Heroin		11	Miscellaneous[a]	29	
Methamphetamine		6			
Sedatives and hypnotics	49		Totals	164	79
Phenobarbital		9			
Antiasthmatics	36				
Theophylline		34			
Miscellaneous[a]	46				
Totals	541	391			

Note. Data in the Table were condensed by the author (T.A.L.) from the extensive data reported by Litovitz, Holm, *et al.* Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System, *Am. J. Emer. Med.* **11,** No. 5, pp. 494–555, 1993. The data include telephone reports and treated patients from 68 reporting centers.

[a] Miscellaneous includes categories with less than 10 deaths. For drugs this includes such categories as antihistamines, antimicrobials, anticoagulants, and topical preparations; for nondrugs, this includes adhesives, venoms, cosmetics, mushrooms, herbicides, heavy metals, etc.

the soil at a dump site. The physical–chemical properties, translocation, absorption, biotransformation, distribution, and elimination of chemicals are vital to an understanding of how an agent gets to a biological effector site.

2. Not all chemical-induced biologic effects are harmful. All therapeutic drug effects support this concept. This concept may be controversial in examples such as the increase in the amount (induction) of certain liver enzymes produced in experimental animals by many different chemicals and which may be either harmful or beneficial.

3. The occurrence and intensity of chemical-induced biologic effects are dose related; there is some dose below which no effect can be demonstrated, and there is a dose above which the agent is lethal. This principle is stated here because it is scientifically supportable. There is no conflict regarding the lethality of all compounds; however, the low dose, no effect clause is controversial since it is popular (but becoming less so) to describe those chemicals that induce irreversible effects such as cancer as creating a finite statistical risk, regardless of how small the dose may be.

4. Effects of chemicals on animals, if properly qualified, are applicable to humans. In order for this concept to be generally acceptable it should be recognized that there are quantitative differences in the effects of chemicals both between and within species. Also, the converse of this principle may be applicable in certain situations; that is, an effect may eventually be found to appear in humans which did not occur in animals. Usually such a finding is attributed either to differences between species in biotransformation systems or in biologic receptor sites, or to simple failure to examine for the effect in animal experiments. Consequently animal data must be "qualified" for it to be applicable to humans. Generally, whenever two species have similar biotransformation systems and physiological functions, those two species will respond similarly to chemicals.

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CHAPTER 2
Numbers in Toxicology

It has been proposed that no chemical agent is entirely safe and likewise no chemical agent should be considered as being entirely harmful. This concept is based on the premise that any chemical can be permitted to come in contact with a biologic mechanism without producing an effect on that mechanism, provided the concentration of the chemical agent is below a minimal effective level. An implication of the concept is that all chemical agents produce a significant degree of undersirable effects if a sufficiently great concentration of the chemical is allowed to come in contact with the biologic mechanism. Thus, the single most important factor that determines the potential harmfulness or safeness of a compound is the relationship between the concentration of the chemical and the effect that is produced upon the biologic mechanism.

If one considers that the ultimate effect is manifested as an all-or-none response such as death of the biologic mechanism, and that a minimal concentration produces no effect, then there must be a range of concentrations of the chemical which would give a graded effect somewhere between the two extremes. The experimental determination of this range of doses is the basis of the dose–response relationship.

DOSE

The word "dose" as it is most commonly used refers to the quantity of a chemical involved or introduced into a biologic system in a unit period

of time. Although the word dose has already been used several times in this text it should be recognized that it may appear in a variety of forms, the most common of which is weight of the chemical per unit weight of the experimental animal given on a single occasion (g/kg) or repeated daily (g/kg/day). A total daily dose may be divided into several doses administered at specific intervals (g/kg every 6 hr). As the need arises, dose may also be expressed as weight per unit body surface area, i.e., grams per square meter of body surface area per day.

In order for the word dose to be meaningful the route of administration or exposure also should be indicated. In animal experiments, the preferable route is by mouth (oral), in which case the chemical may be administered by stomach tube or may be dissolved or mixed with the animal's feed or water. However, several other routes are commonly used, such as intramuscular (IM), intraperitoneal (IP), topical, and intravenous (IV). When gases or vapors are involved the route of exposure is inhalation, in which case it is expressed as the concentration of the agent in the inspired air and the duration of exposure to that concentration. If an adverse effect is the objective of the experiment then the observation may be for a fixed time after the exposure. If death of the animal is the adverse effect that is measured, and exposure is for one continuous 8-hr interval with observation of the animals for 24 hr, then the dose is expressed as the 8-hr lethal concentration (LC-8 hr or LCt). In this latter example the quantity of chemical within the body of the animal is not known or implied in the term LCt; hence, it is an improper expression of "dose" but it is used for convenience. Also, when lethal aquatic experiments are conducted using fish, exposure is via the environment (water) and the procedure involves determination of the LCt, although exposure may be continuous for the duration of the experiment. Even in most *in vitro* studies in which cultured cells or tissues are exposed to chemicals, the lethal concentration (LC) in the nutrient solution represents the dose. In studies involving exposure to the chemical via a skin patch, the concentration of the chemical in the patch and the duration of exposure again determine the unit used to express the dose.

Some dosage forms carry specific meanings. One of the most frequent terms used in animal toxicology is the term NOEL for "no observable effect level." By definition this is the maximum dose used in an experimental protocol which produces no observable effect of any kind. It is always accompanied by the route of administration and the species involved (or type of experimental protocol). A NOEL may be misleading since it commonly represents only the highest dose from a series of widely spaced doses used in the experiment. If the sequence of doses had been more closely spaced, the experimenter may have found a different NOEL.

In clinical toxicology additional forms of dosage are common. One highly useful dosage form is that which identifies the amount or concentration of a substance in the atmosphere to which humans may be exposed without adverse health effects. Such a dose is called the threshold limit value (TLV) and is expressed as weight per cubic meter of air or as parts of chemical per million parts of air (PPM). TLVs are the clinical equivalent of the animal inhalation NOELs. The data for TLVs may be obtained from human experience or experiment and animal studies, and represent the opinion of an expert committee who are supplied with the animal and clinical data. Further modification of TLVs with the addition of "safety factors" by expert committees has led to the appearance of dosage terms used for regulatory and legal purposes. Such terms are PELs (permissable exposure levels), which represent the concentration of an agent in the atmosphere to which a person may be exposed without risk, and ADIs (allowable daily intakes), which represent, in the case of food additives, the amount that can be taken daily in the diet, even for a lifetime, without risk. These latter values may have little relation to any actual experimental data.

DOSE–RESPONSE RELATIONSHIPS

Under practical conditions, the biology experimenter finds that differences exist among the individual members of a supposedly homogeneous population of cells, tissues, or animals. The nature of these differences is seldom obvious and becomes evident only when the biologic mechanism is challenged, such as by exposure to a chemical agent. For example, a group of single cells such as bacteria or a group of whole animals such as inbred mice may be considered as uniform populations of biologic mechanisms, and as such may be exposed to a suitably selected concentration or dose of a specific chemical agent.

If the chemical agent is capable of producing an observable effect, such as death of the organism, or an effect from which the cells or animals completely recover in a period of time, then the dose or concentration of the chemical could be selected so that it would produce that effect. Furthermore, if the effect could be quantified, then the experiment would show that not all members of the group respond to the same dose or concentration of the chemical in a quantitatively identical manner. Rather, some of the animals would show an intense response, whereas others would show a minimal response to the same dose of the agent. Or, if the dose were properly selected, some of the animals or cells would die and others survive. Thus, what has been considered as an all-or-none response applies only to a single member of the test group and is found to be actually a

graded response when viewed in regard to the entire group of members in the test. Such deviations in the response of apparently uniform populations of cells or animals to a given concentration of the chemical may be generally ascribed to biologic variation, a subject that is dealt with in detail in Chapter 6.

Frequency Response

Experience has shown that biologic variation in response to chemicals within members of a species is generally small as compared to biologic variation between species. Since one of the criteria of our experiment is that the response can be quantified regardless of the effect that is measured, then by further experiment each animal in a series of supposedly uniform members of a particular species may be given an adequate dose of the chemical to produce an identical response. The data obtained from such an experiment may be plotted in the form of a distribution or frequency–response curve. Such a representation for a hypothetical chemical agent is shown in Fig. 2.1.

The plot shown in Fig. 2.1 is frequently referred to as a quantal response curve because it represents the range of doses required to produce a quantitatively identical response in a large population of test subjects. The curve indicates that a large percentage of the animals that receive a given dose (Dose X) will respond in a quantitatively identical manner. As the dose varies in either direction from Dose X, some animals will show the same response to a lower dose, and others require a higher dose. Such a curve follows the laws represented by the normal Gaussian distribution pattern,

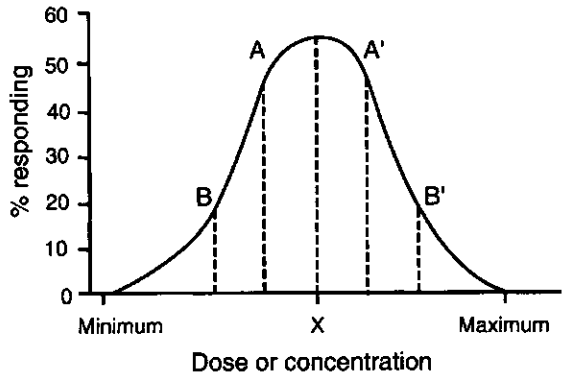

FIGURE 2.1 Hypothetical frequency–response plot following administration of a chemical agent to a uniform population of biologic specimens.

and is of considerable interest because it permits the use of statistical procedures applicable to such curves. The curve will be noted to have two main inflection points (A, A^1, B, B^1) on each side of the maximal frequency range. The dose identified as X is the *mean* dose, and the sum of all the animals responding to doses higher than the mean dose is equal to the sum of all the animals responding to doses of less than the mean dose. By definition, the area under the curve that is bounded by lines vertical to the abscissa from points A and A^1 encloses the total population corresponding to the mean dose plus or minus 1 standard deviation from the mean dose. Also, by definition, the area under the curve bounded by the vertical lines from B and B^1 to the abscissa includes the total population responding to the mean dose plus or minus 2 standard deviations of the mean dose. In actual practice, the true Gaussian curve is rarely, if ever, obtainable. Rather, a skewed variation of the curve is usually obtained as the best fitting curve for the experimental data.

Cumulative Response

In toxicology, frequency–response curves are not commonly used. Rather, it is conventional to plot the data in the form of a curve relating the dose of the chemical to the cumulative percentage of animals showing the response (such as death). Such curves are commonly known as dose–response curves. Figure 2.2 represents the dose–response relationship for two hypothetical compounds, the data of which may be obtained experimentally as follows: Groups of a homogeneous species, such as mice, are given

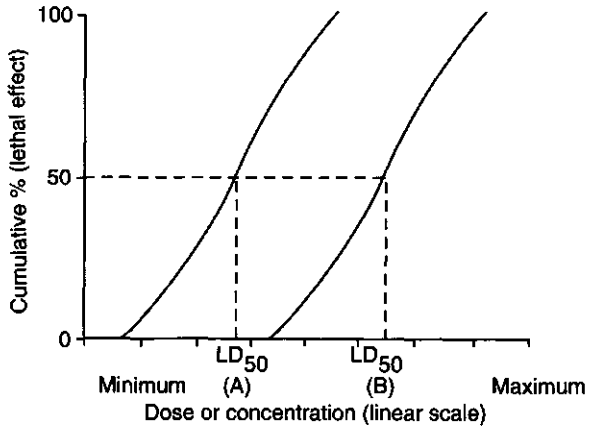

FIGURE 2.2 Hypothetical dose–response curves for two chemical agents (A and B) administered to a uniform population of biologic specimens.

the solution of the chemical by a specific route of administration. Only by experimentation can a dose be selected so that not all the animals die, nor do all of them survive. The initial dose may well be a dose so small that no effect is manifested by the animals. In subsequent groups of animals the dose would be increased by a constant multiple such as 2, or on a logarithmic basis, until ultimately a sufficiently high dose of the compound would be administered so that all of the animals in the group would die as a consequence of the exposure to the chemical. Thus, the only observation made in the experiment is that of death or survival of the animals. Under these conditions data would be obtained which may be plotted to give the results shown in graph form in Fig. 2.2. Figure 2.2 fulfills the implications of the concept presented in the initial paragraph of this chapter, that is, the dose of the compound may be sufficiently small so that no deaths occur. However, as the dose is increased, the typical S-shaped curve is obtained so that at sufficiently high doses, 100% of the animals die as a consequence of the exposure to the chemical.

Biologic experiments may be designed with the objective of determining the dose of a chemical required to produce any specific effect. When the experiments are done and a frequency distribution curve of the data is plotted, that curve is a visual representation of the differences among members of a test group of experimental subjects, a condition frequently referred to as "biological variation." Biological variation exists not only within species but also between species. When the data from the above experiment are plotted as a cumulative dose–response curve, the slope of the curve then becomes a visual and mathematical index of the differences among members of the test group. The measured effect need not be death of the animal but may be any type of biologic effect that can be quantified. The experiment need not be conducted on whole animals but may be conducted on a single cell system (such as a bacterial cell) or an isolated organ, tissue, or cell from any biologic system.

STATISTICAL CONCEPTS AND LD_{50}'s

Note that the major portion of the dose–response curve approaches linearity, and that insofar as this portion of the curve is concerned, the incidence of death is directly related to the concentration of the compound present. There is no question that the compound may be considered harmful or safe, depending upon the dose given.

The curve represents the concept by which the lethal dose for 50% of the animals is obtained. The lethal dose for 50% of the animals, which is commonly known as the LD_{50}, is that dose of the compound that will

produce death in 50% of the animals. The LD_{50} is a statistically obtained virtual value. It is a calculated value which represents the best estimation of the dose required to produce death in 50% of the animals and is therefore always accompanied by some means of estimation of the error of the value, such as the probability range of the value. The limits of the probability range are arbitrarily selected by the experimenter to indicate that similar results would be obtained in 90 or 95 of 100 tests performed in a manner identical to that described. Several methods are available for the performance of such a calculation.

In the example given here, which is simplified for illustrative purposes, the LD_{50} is obtained from the curve by drawing a horizontal line from the 50% mortality point on the ordinate to where it intersects the curve. At the point of intersection, a vertical line is drawn and this line intersects the abscissa at the LD_{50} point. It is evident from the curve that information with respect to the lethal dose for 95% of the animals or for 5% of the animals also may be derived by a similar procedure. The LD_{84} (lethal dose for 84% of the animals) represents +1 S.D. (standard deviation) from the LD_{50}, and the LD_{16} (lethal dose for 16% of the animals) represents −1 S.D. from the LD_{50}. The percentage mortality may be converted to probits, which are numbers assigned to percentages so that 50% mortality equals a probit of 5, 50% mortality ±1 S.D. equals a probit of 6 or 4, respectively, 50% mortality ±2 S.D. equals a probit of 7 or 3 etc.

The statistical procedures that are commonly involved in toxicology are the same procedures that are used in the other biological sciences. The progressive improvements in the statistical methods and the development of computer technology as well as the lowering of the cost of the software and hardware has made it readily accessible to students and indispensable to scientists. The modern toxicologist needs mainly to understand which statistical procedures are applicable to his specific data and how to use modern computer technology to perform the mathematical chores involved.

POTENCY VERSUS TOXICITY

When data are obtained for two compounds, identified as compounds A and B, the curves representing the relationship between the dose and the incidence of death may be plotted as shown in Fig. 2.2. If the LD_{50} for compound B is greater than that of compound A, compound B may be said to be less potent than compound A. Furthermore, if dose and lethality are the only considerations, compound A can be said to be more toxic (harmful) than compound B. This would indicate that potency (in terms of quantity of chemical involved) and toxicity (in terms of harmfulness)

are relative terms that can be used only with reference to another chemical. Therefore, one of the criteria that may be used to describe relative toxicities of two compounds is that of the relation of the doses required to produce an equal effect. However, as far as evaluation of the toxicity of a single compound is concerned, the absolute value of the LD_{50} may be in terms of a few micrograms or as much as several grams of a particular compound. If the LD_{50} is only a few micrograms for one compound, and is several grams for a second compound, the difference between the two compounds becomes highly significant. It is common practice to use the term "potent" for a chemical if the dose required to produce any effect is small, i.e., at most a few milligrams. Table 2.1 lists the LD_{50} values of a series of selected types of compounds and illustrates the range over which lethal effects can be induced in animals.

Because of the fact that some chemicals will produce death in microgram doses, such chemicals are commonly thought of as being extremely toxic (or poisonous). Other chemicals may be relatively harmless following doses in excess of several grams. Since a great range of concentrations or doses

TABLE 2.1 Approximate LD_{50} of a Selected Variety of Chemical Agents

Agent	Animal	Route	LD_{50} in mg/kg
Ethyl alcohol	Mouse	Oral	10,000
Sodium chloride	Mouse	IP	4,000
Ferrous sulfate	Rat	Oral	1,500
Morphine sulfate	Rat	Oral	900
Phenobarbital, sodium	Rat	Oral	150
DDT[a]	Rat	Oral	100
Picrotoxin	Rat	SC	5
Strychnine sulfate	Rat	IP	2
Nicotine	Rat	IV	1
d-Tubocurarine	Rat	IV	0.5
Hemicholinium-3	Rat	IV	0.2
Tetrodotoxin	Rat	IV	0.10
Dioxin (TCDBD)[b]	Guinea pig	IV	0.001
Botulinus toxin	Rat	IV	0.00001

Note. IP, intraperitoneal; IV, intravenous; SC, subcutaneous. LD_{50}'s are listed according to averages of nearest round figures from many sources. The principal sources are: Barnes, C. D., and Eltherington, L. G., *Drug Dosage in Laboratory Animals—A Handbook,* University of Calif. Press, Berkeley, 1964; *Handbook of Toxicology,* Vol. 1 (Spector, W. S., Ed.), W. B. Saunders Co., Philadelphia, 1956; Goldenthal, E. I. Compilation of LD_{50} values in newborn and adult animals, *Toxicol. Appl. Pharmacol.* **18:** 185, 1971.

[a] DDT, P,P[1] dichlorodiphenyl trichloroethane.

[b] TCDBD, 2,3,6,7-tetrachlorodibenzodioxin.

of various chemicals may be involved in the production of harm, categories of toxicity have been devised on the basis of amounts of the chemicals necessary to produce harm. An example of such a categorization, along with the respective lethal doses, is given below.

Extremely toxic	(1 mg/kg or less)
Highly toxic	(1 to 50 mg/kg)
Moderately toxic	(50 to 500 mg/kg)
Slightly toxic	(0.5 to 5 g/kg)
Practically nontoxic	(5 to 15 g/kg)
Relatively harmless	(more than 15 g/kg)

This classification serves a practical and useful purpose, but if the basis for ascribing the property of being "highly toxic" is because the lethal dose is small, then the question arises as to just where the line is to be placed to separate toxic from nontoxic chemicals.

Basically it is apparent that toxicity is relative and must be described as a relative dose–effect relationship between compounds. However, it is also apparent that the concept of toxicity as a relative phenomenon is true only if the slopes of the curves of the dose–response relationship for the compounds are essentially identical. It is possible that the slopes of the dose–response curves for any two compounds could be distinctly different, as are those that are shown for compounds C and D in Fig. 2.3. The LD_{50} of compound C is less than the LD_{50} of compound D. However, the reverse

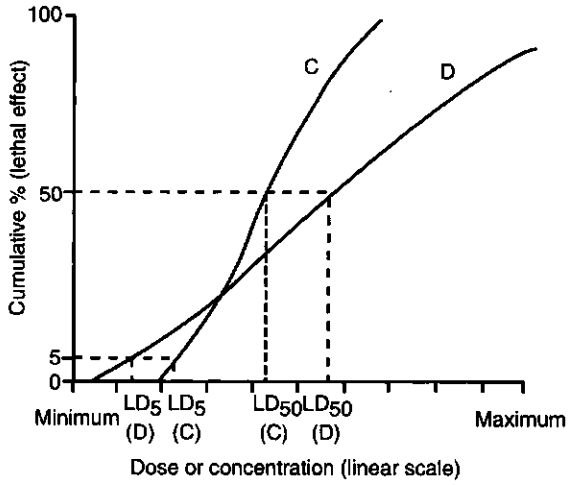

FIGURE 2.3 Hypothetical dose–response curves for two chemical agents (C and D) administered to a uniform population of biologic specimens.

is true for the LD_5's for the two compounds. If dose is the only consideration, it is apparent that compound C is less toxic than compound D because compound C has a higher LD_5 than compound D. On the other hand, compound C is more toxic than compound D when the comparison is made between the LD_{95}'s. It is therefore apparent that the slope of the dose–response curve may be most significant with respect to comparing relative toxicities of two compounds.

SAFETY VERSUS TOXICITY

Although the ultimate extreme in toxicity resulting from a chemical agent is manifested as a lethal effect, it is apparent that sublethal or reversible effects of chemicals may be harmful or undesirable, and therefore should be considered in any evaluation of chemicals with regard to their degree of harmfulness or safeness. Some of the commonly used drugs are the best examples of chemicals that can produce undesirable effects. Drugs that have as the basis of their action an ability to interfere with biologic processes are potentially harmful agents. This is particularly true if the primary action of the drug is concerned with a vital process. With such a drug, the therapeutic use of the agent is based upon obtaining a graded response from given doses which would result only in a desirable effect. If the desirable effect is exceeded, then the vital process would be sufficiently influenced so that a significant, harmful effect may result.

Many drugs have actions (side actions or side effects) in addition to the basic action of the drug. The side actions may or may not be undesirable, and as a rule a chemical becomes a drug only if the undesirable actions are not significant in comparison to the desirable actions of the drug. When morphine is given to produce analgesia, it also produces respiratory depression. When the anticholinergic agents are given for their effect on gastric motility, they also produce dryness of the mouth. Application of the antihistamine compounds or penicillin to the skin may initiate immunologic mechanisms resulting in sensitization phenomena which can be so severe that they result in death.

Undesirable effects of drugs are believed to be related to the dose of the drug. In the case of side effects of drugs, e.g., the morphine-induced respiratory depression or anticholinergic-induced dryness of the mouth, a relationship between intensity of the action and the dose of the drug is usually evident. That is, as the dose is increased, the intensity of the undesirable side effect also increases. In the case of sensitization to the drug applied to the skin, there may be little, if any, relationship between the dose necessary to produce a therapeutic effect and the dose necessary to

induce sensitization, but there is usually a direct relationship between the dose, however small it may be, and the intensity of the sensitization response. The phenomenon of sensitization to a chemical involves an abnormal response to the chemical; this phenomenon is discussed in greater detail in Chapter 9. Therefore, toxicity or harmful effects from certain drugs necessitate separate consideration, for it is common practice to refer to one drug as being more or less "toxic" than another drug. However, toxicity from drugs is also a relative term, for it is also common practice to speak of one drug as being less toxic than another because the incidence or severity of side effects is less than for similarly useful drugs. The hope of the pharmacologist is to develop drugs which would be safe in all circumstances, but this is rarely, if ever, accomplished.

To a pharmacologist, the term "potency" means the relative dose of the drug that is required to produce an effect equal to that produced by a similarly acting drug. Thus, if two drugs are capable of producing a quantitatively identical effect, the drug that produces the effect with the lower dose is said to be the more potent of the two drugs. If the slopes of the dose–response curves for the two drugs are parallel, then the margin of safety between the two drugs may not be different.

The margin of safety to a pharmacologist is the dosage range between the dose producing a lethal effect and the dose producing the desired effect. This margin of safety is referred to as the therapeutic index and is obtained experimentally as follows: Two dose–response curves are obtained on a suitable biologic system such as mice or rats. One of the curves represents the data obtained for the therapeutic effect of the drug and the second curve represents the data obtained for the lethal effect of the drug. Figure 2.4 represents the data which may be obtained for a hypothetical drug in which curve A represents the cumulative therapeutic response and curve B the cumulative lethal response. The therapeutically effective dose for 50% of the animals (ED_{50}) is calculated from curve A, and the lethal dose for 50% of the animals (LD_{50}) is calculated from curve B. The margin of safety (therapeutic index) is represented by the ratio LD_{50}/ED_{50}. This is a useful concept in considering the margin of safety for practical use of the drug. Several authors have validly proposed that a more significant value would be derived from the ratio LD_1/ED_{99} as the most critical evaluation of safety of the compound. It is evident from Fig. 2.4 that if the lethality curve is shifted to the left so that it approaches the effective curve, the therapeutic index becomes a smaller ratio, the margin of safety is decreased, and the compound may be said to increase in toxicity.

When several drugs have similar actions and are used for similar therapeutic purposes, the drug with the greatest potency (in terms of therapeutic dose) is not necessarily either the safest or the most desirable drug. If no

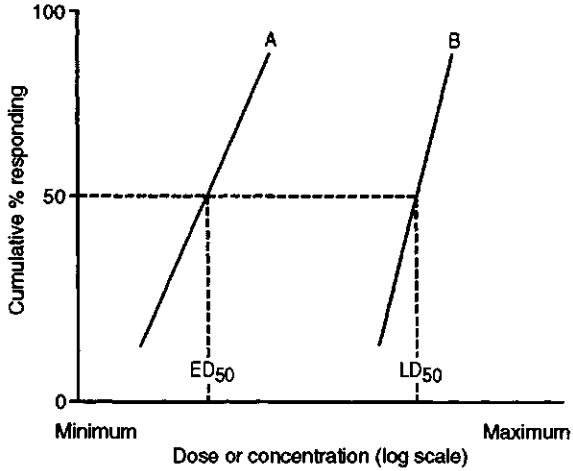

FIGURE 2.4 Hypothetical dose–response curves for a drug administered to a uniform population of animals. Curve A represents the therapeutic effect (for example, anesthesia) and curve B represents lethal effect.

other factors were involved, the drug with the highest therapeutic index would be the safest or least toxic drug, since therapeutic doses of it would be less likely to produce lethal effects. However, additional factors are always involved because, as already indicated, few, if any, drugs have but a single action. For example, since a margin of safety (or therapeutic index) is used to relate the therapeutic effect to the lethal effect, a similar margin of safety could also be calculated for the relationship between undesirable side actions and therapeutic actions. Such information is the aim of the drug toxicologist, who would like to develop drugs that have not only a high therapeutic index but also a high index in regard to freedom from all undesirable effects of the chemical.

The word safety basically implies the reciprocal of harmfulness. All chemical-induced biologic effects, some of which can be labeled as harmful, can be produced in the experimental laboratory. Harmful effects may be either reversible (sublethal) or irreversible (lethal). Each of these effects, including the absence of any effect, can be expressed in the form of a dose–response curve. Thus the overall picture of the safety as well as harmfulness of any chemical is dose related and can be graphically demonstrated as shown in Fig. 2.5. The figure shows that each effect would be represented by a curve with a specific slope and the range of doses applicable would be defined. If one first defines the nature of the harmful effect, then

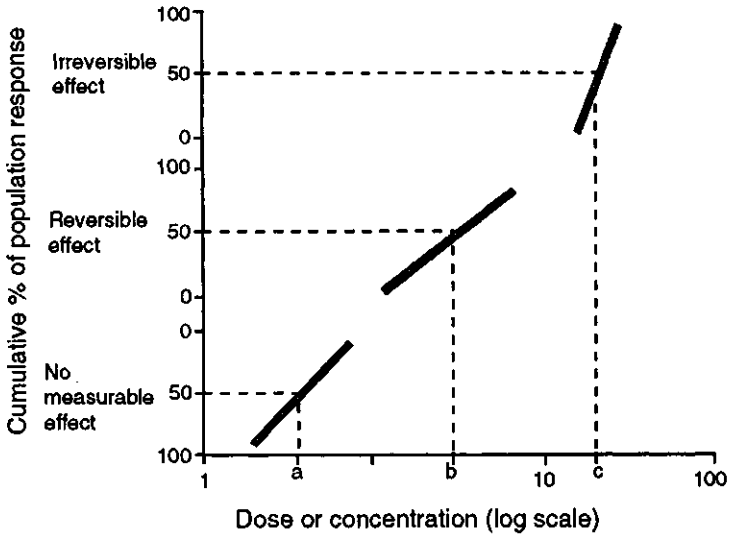

FIGURE 2.5 Hypothetical dose–response curves for "no measurable effect," "reversible effect," and "irreversible effect" from a single chemical substance. For illustrative purposes each curve is shown to have a different slope. The letters a, b, and c on the abscissa identify, respectively, that dose or concentration of the chemical at which 50% of the test population responds to each test parameter.

the order of safety or freedom from that effect in terms of dose becomes evident.

The numbers that are used in toxicology are derived from appropriately conducted experimental studies. These numbers are then subjected to statistical procedures that produce acceptable although virtual numbers. The virtual numbers are simply a concise form for expression of the results of an experiment and should be recognized as being applicable only to the specific initial experiment. However, in clinical toxicology it is acceptable to conclude that data from studies on animals, properly qualified, are applicable to humans. The final extrapolation of data from animal to man takes various forms, one of which is to simply add a safety factor of from 10- to 100-fold. Such a procedure results in numbers that are entirely virtual.

HYPERSENSITIVITY AND HYPOSENSITIVITY

In biology the application of statistical procedures to data that are experimentally obtained is a necessity, because only by such procedures can the range of the data be identified and validity of differences be established.

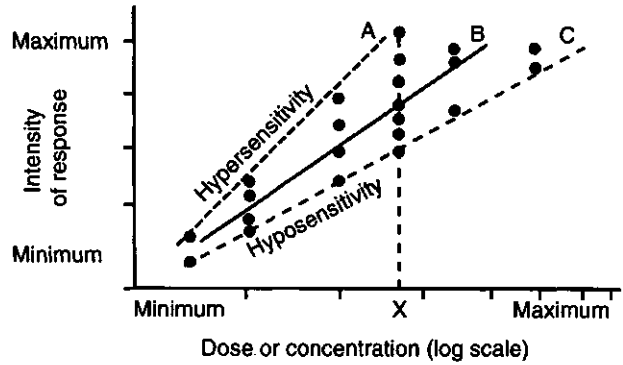

FIGURE 2.6 Hypothetical dose–response relationships for a drug administered to a uniform population of animals. Each point respresents response of a single animal.

However, the virtual nature of a statistically obtained value, such as the LD_{50} or ED_{50} of a chemical, tends to obscure an important concept in toxicology, the concept that there is no fixed dose or concentration of a chemical that can be relied upon to produce a given biologic effect in a population. This is evident from the frequency distribution curve shown in Fig. 2.1. It is common practice to speak of normals, hypersensitive members, and hyposensitive members of a population. The conditions that determine these categories of response are readily seen when the crude data for each contributing member are plotted on dose–response coordinates.

Figure 2.6 is a plot of a theoretical compound in which each point represents one item of contributing data. The figure shows that the mean dose–response relation exists (line B) for the normal subjects, and in order to include 100% of the contributions to the data, lines A and C represent the extremes of the data. Those subjects that deviate from the mean (line B) in the direction of line A are said to be hypersensitive to the chemical. Those subjects that deviate from line B toward line C are said to be hyposensitive to the chemical. The graph also indicates that following a given dose (or concentration), such as Dose X, the response may be below average or average in intensity, or the response may be maximum, *i.e.,* death of the biologic mechanism. The factors responsible for hypersensitivity of biologic systems to chemicals are an important part of the study of toxicology.

RESPONSE CONCEPTS FOR COMPOUNDS ESSENTIAL TO THE BIOLOGIC SYSTEM

Although the foregoing description of the direct relationship between the concentration of a chemical and any given response is correct for all

compounds that are not normally present in the biologic system (such compounds are frequently called "xenobiotics," taken from the word "xeno," meaning foreign or strange), the concept does not hold for compounds that are normally present in the biologic system (that is, normal endogenous compounds). For example, the normal human will be in a state of health only as long as the body is supplied with nutrients and water as well as essential minerals and accessory food substances such as the vitamins. In the absence of these compounds as well as in the presence of an excess of the compounds, the human will develop undesirable effects. An example is shown in Fig. 2.7, which is a graph of the relationship between the concentration of total calcium in the serum and the response manifested in a human. According to the figure, there is a concentration range for calcium between 9 and 10.5 mg of calcium per 100 ml of serum which is necessary for normal function. This normal concentration of calcium as well as comparable normal concentrations of numerous other substances (i.e., glucose, hormones, sodium, potassium) is finely regulated by various homeostatic mechanisms. When there is a decrease in the level of the calcium, such as can occur when the body is not supplied adequate vitamin D or sources of calcium, the subject encounters muscle cramping due to the hypocalcemia. Conversely, when the calcium concentration is elevated above normal, the subject suffers from hypercalcemic malfunction of the kidney. Death can occur because of exceptionally low or high calcium concentrations in the serum of humans. In general, depletion of essential endogenous substances as well as excess of any essential endogenous substance results in toxicity to the subject. The specific nature of the endogenous substances that are essential to different biologic species will vary between species. This simple fact constitutes a mechanism that is extensively utilized as a means of producing death in an undesirable species by the use of chemicals. Some of the most widely used antibacterial drugs owe their action to an ability of the drug to prevent the bacteria from utilizing

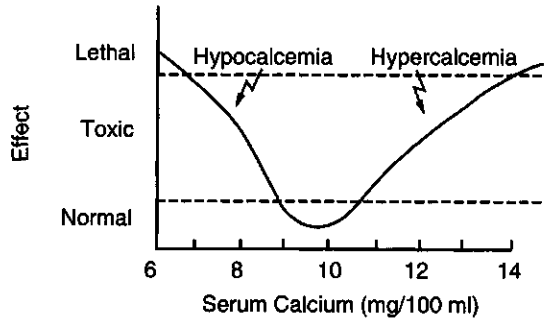

FIGURE 2.7 Relationship between serum calcium concentration and response in humans.

some essential nutrient; if that nutrient is essential only in certain species of bacteria, then only those species of bacteria will be affected by the antibacterial drug.

The conclusion that is reached is that xenobiotic compounds show simple, essentially linear concentration–response relationships, whereas essential endogenous compounds show biphasic concentration–response relationships.

Chemicals that possess selectively harmful mechanisms of action represent the greatest contribution of toxicology to science. Based on the theory that a chemical could be designed to be selectively toxic (lethal to a biologic mechanism that is present only in that species which it is desirable to eliminate) and provided the chemical is free of additional effects, such a compound would possess an infinitely high margin of safety for the biologic mechanisms which it is desirable to maintain. The development of chemicals as antibacterial agents, as pesticides, as herbicides, as anticarcinogenic agents, and as insecticides is rapidly approaching this ideal relationship between safeness and harmfulness of chemicals.

CHAPTER 3

Biologic Factors That Influence Toxicity

In order for a chemical to produce an effect on a biologic mechanism, a reaction (chemical or physical–chemical in nature) usually occurs between the chemical and some reactant chemical in the biologic system under study. It is also possible that an effect could be achieved solely on the basis of physical phenomena, such as a change in osmotic pressure, but such events are uncommon in toxicology. In all cases, the chemical agent under consideration must come in contact with some "reactant" chemical which is normally present at some location in the biologic system if a chemical reaction or interaction is going to occur. The reactant chemical is commonly referred to as the "receptor."

Even under nonbiologic conditions, a reaction between two chemicals takes place readily only if certain criteria are met by the reacting substances. First, the chemicals must be suitably selected and placed in physical contact with each other so that a reaction will take place. Second, the chemicals must be mutually soluble to some degree in some vehicle, because the addition of two substances in dry form usually does not permit sufficient contact between the reactants for a reasonable rate of reaction to occur. Third, unless a product of the reaction is removed, the reaction does not go to completion; rather, an equilibrium is obtained between the reactants and the products of the reaction. These criteria also must be met for a chemical reaction to take place in a biologic system.

Whenever a chemical is applied or administered to a complex system such as the human, unless the receptor is located at the site of application, the chemical must be translocated within the human to a receptor site if it is eventually going to produce an effect. For example, if a chemical is given by mouth it may be carried into the gastrointestinal tract, from there into the blood, and subsequently into the tissues and cells. Simultaneously while the processes of translocation are taking place, the chemical will be reaching incidental sites where it may be "chemically bound" (or "adsorbed") or "biotransformed" to new chemical entities. Furthermore, the initial chemical and its products will reach sites that serve to "eliminate" or "excrete" them from the body. Consequently chemicals that are taken into the body are eventually eliminated from the body. Different chemical entities will be present in the body for different periods of time, and since the mechanisms responsible for elimination collectively are usually dependent on the total body load, each chemical will show a specific half-life (time for the concentration of a compound in the body to fall by 50%).

The processes of absorption from some site of administration, translocation, and elimination occur simultaneously and regulate the presence and concentration of each chemical in the various compartments of the body. Although it is common to discuss absorption of an agent from the site of administration separately from translocation, the mechanisms responsible for absorption and translocation are similar since both basically involve passage of the agent across biologic membranes. The kinetics of these processes can be mathematically described and have developed into a branch of toxicology known as "toxicokinetics" (for non-drug chemicals) and "pharmacokinetics" (for drugs). These kinetics are discussed in detail in treatises devoted entirely to that subject. For the purpose of the current discussion, the kinetics involved in the translocation and elimination of a chemical agent supply the details of a fundamental concept in toxicology. This concept is that whenever a chemical is administered on multiple occasions over a period of time so that its rate of administration exceeds the rate of elimination, the agent will accumulate in the animal and thereby influence the degree of toxicity that is produced.

The biologic factors that influence the processes of translocation, elimination, and accumulation of chemicals in the body are considered in this chapter and the chemical factors are presented in the following chapter.

ABSORPTION AND TRANSLOCATION OF CHEMICALS

The complex biologic organism as exemplified by man is composed of a collection of organs, tissues, cells, and subcellular organelles, each of

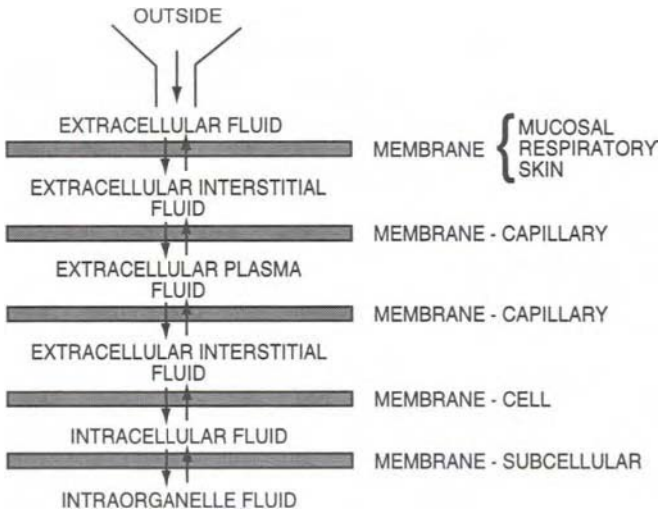

FIGURE 3.1 Schematic representation of membranous barriers involved in translocation of a foreign chemical from outside the mammalian organism to intracellular fluid in organelles.

which is protected from its environment by specialized coverings. These coverings give the structures their physical form and serve not only to protect their contents from temperature extremes, fluid loss, or mechanical insult but also to influence efficiently the free transfer of chemicals from access to their contents. In order for a chemical to gain access to a receptor that lies on or in an intracellular organelle, the chemical must effectively pass through a host of protective membranes.

Figure 3.1 is a schematic presentation of the membranous barriers that influence translocation of any chemical from the exterior of an animal to the intracellular compartments, that is, the organelles.[1] The process involves a series of translocation steps and increases the possibility of exposure of the administered chemical to large endogenous molecules, such as proteins, which may effectively bind and therefore functionally alter and remove the offending chemical from the animal.[2] Such a process also exposes the chemical to all parts of the body so that it is subject to excretion by the

[1] Organelles are subcellular compartments such as the mitochondrium, nucleus, and endoplasmic reticulum.

[2] In mammals, nonspecific binding of chemicals to proteins, such as albumin in the blood, effectively removes the chemical from the body by making it unavailable to react with a specific receptor. However, nonspecific binding is only temporary since it involves a readily reversible reaction; that is, as the concentration of the free drug diminishes with time there is a corresponding decrease in the concentration of the bound form. This reaction is shown in Fig. 3.2.

kidneys, by the respiratory tract, or even by the sweat glands. The molecule may be altered (biotransformed) by specific or nonspecific enzymatic systems present in the various organs. It may be deposited in storage tissues. Almost any reactive chemical that is administered to the organism is almost immediately subjected to mechanisms that may confine its translocation within the organism or terminate its existence as a free chemical (Fig. 3.2).

Since many foreign chemicals are capable of producing a specific effect on some biologic system or systems, such systems may be said to be the site or locus of action of the chemical. The locus may be strictly confined in one anatomic location within the animal, or it may be diffusely located throughout the animal. Since the effect of a chemical is a function of its concentration at the locus of action, it would be desirable to determine the concentration of the chemical at this site of action. In the intact animal, a limited amount of such information may be obtained by the use of agents tagged with isotopes which emit radiation energy or with heavy isotopes. More commonly the organ, tissue, or cells are prepared and analyzed by suitable chemical or physicochemical methods.

Once the existence and amount of the chemical at the effector site are verified, this information may be compared to the blood concentration of the agent at the same time. The blood is selected only because it is a readily available source of biologic sample material and the blood circulation represents the principal mechanism by which a chemical is carried to all parts

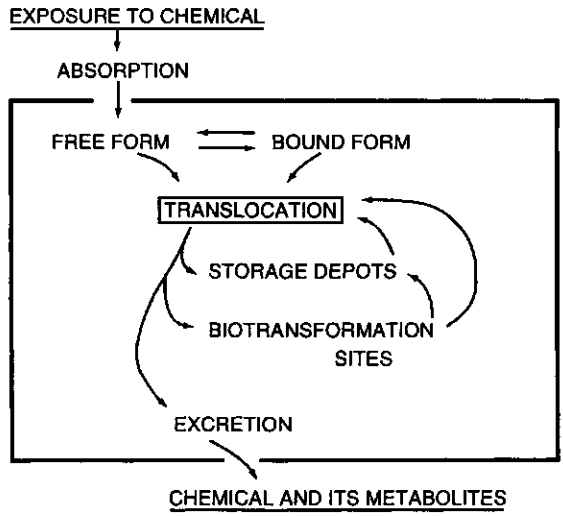

FIGURE 3.2 Schematic representation of the pathways through which a chemical agent may pass during its presence in the mammalian organism.

of the body. The ratio of the concentration of the agent in the tissue to the concentration of the agent in the blood at any given time would represent an index of the effectiveness or lack of effectiveness of the membranes to influence translocation of the compound. This technique may be used to demonstrate the translocation of a chemical between any of the systems of the animal. It is not uncommon that a chemical may pass readily from the gastrointestinal tract to the blood or from the lungs to all parts of the body except to the brain. Therefore, evidence indicating that a chemical agent readily passes through one membranous system in the organism does not necessarily mean that it will readily pass through all membranous systems in the organism.

Current concepts indicate that any chemical that does pass through membranes must do so by one or more of three possible mechanisms: (1) filtration through the spaces or pores in the membrane, (2) passive diffusion through the membrane through the spaces or pores or by dissolving in the lipoid material of the membrane, or (3) specialized transport systems which carry water-soluble substances across the membrane by a lipoid soluble "carrier" molecule, which effectively complexes with the chemical.

Fluid and its solutes are translocated between organs in the mammalian organism primarily by the blood and lymph circulation systems. The larger vessels of these systems serve only as conduits for transport of their contents to the various organs. It is only at the capillary division of the circulation system that fluid passes into and out of the blood. Capillary walls structurally consist of a single layer of flat epithelial cells held together by an "intercellular cement substance," through which it is currently believed that water and its solutes may be passively filtered. The process of filtration through a biologic membrane is not only a function of the hydrostatic pressure differential across the membrane, but also a function of the osmotic pressure differential on the two sides of the membrane. Shown in Fig. 3.3 are the forces that influence the transfer of water and water-soluble substances through the capillary membrane. At the arteriolar end of the capillaries, the hydrostatic pressure minus the plasma protein osmotic pressure within the capillary would have to be greater than the tissue hydrostatic pressure minus the interstitial protein osmotic pressure outside the capillary to permit the filtration of fluid from the lumen of the capillary to the extracellular interstitial fluid.

In contrast to this, at the venous end of the capillary the reverse condition would have to prevail, so that the quantity of fluid lost from the plasma at one end of the capillary is returned to the plasma at the other end of the capillary if the tissue is to maintain a constant fluid volume or weight. These conditions are met in the normal mammalian organism, and maintenance

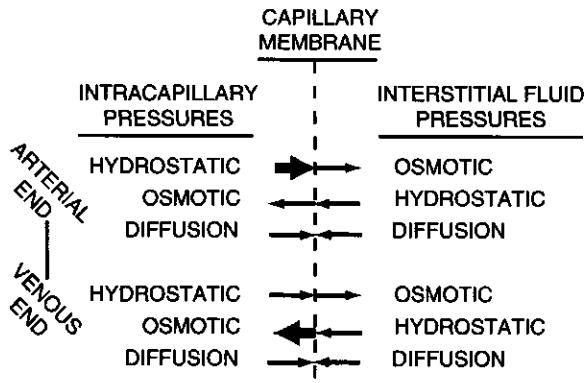

FIGURE 3.3 Schematic representation of the forces influencing transfer of water and water-soluble molecules through the capillary membrane in mammals.

of a constant body water volume is the result of the proper balance between filtration, hydrostatic pressure, diffusion, and colloid osmotic pressure on the two sides of the membranous barriers. If a filterable foreign chemical is present in the plasma and is only passively carried along with the filtered water, simple filtration would permit an equilibration to occur only between the plasma and the extracellular spaces in the tissue, but the filtration process could not account for the passage of foreign chemicals across the final cell membrane.

The mammalian cell membrane, which separates the contents of the cell from its environment, is composed of a thin layer of lipid or lipid-like material which is bounded on both sides by a protein layer, and the resultant membrane contains multiple pores. Most such membranes in mammals are of the order of approximately 100 Å in thickness, and the extremely small pores vary from 2 to 4 Å in diameter. Such a membrane is an effective barrier to the transfer of some chemicals into (and/or out of) the interior of the cell, and is an ineffective barrier to the transfer of other chemicals into (and/or out of) the interior of the cell.

Transfer of water-soluble foreign chemicals from extracellular tissue fluid to intracellular fluid must involve either the process of diffusion across the membrane in the direction of a concentration gradient or a transport mechanism which would permit transfer of the chemical through the lipoid portion of the membrane. Organic solutes of low molecular weight (such as urea, creatinine, and organic acids) pass freely across most cell membranes by simple diffusion, whereas ions such as sodium and potassium are selectively partitioned by the cellular membrane. In the latter case, the ions are selectively partitioned by an active transport mechanism involving

energy expenditure to maintain electrochemical gradients on the opposing sides of the membrane.

The transfer of water across cell membranes probably occurs via the small-diameter water-filled pores in the membrane. Also, molecules that are sufficiently small may pass through the pores in the membrane. The pores of the membrane are relatively few in number per unit area of membrane. Therefore, lipid-insoluble substances that gain access to the interior of the cell have necessitated the development of a concept involving a special lipid-soluble carrier molecule, which is present in the membrane and is capable of combining with water-soluble molecules and carrying them across the lipid membrane to the interior surface of the cell, where the complex dissociates in the intracellular fluid. Thus, whereas some degree of water solubility appears to be desirable for the effective translocation of a chemical to the various organs in an organism, the cell membranes are only poorly permeable to such water-soluble substances. A water- and lipid-insoluble foreign chemical therefore would have difficulty gaining access to the intracellular fluid in an organism.

In normal humans, approximately two-thirds of all body water is intracellular. Therefore, if a compound is water-soluble and is carried freely across membranes, approximately two-thirds of the total amount of the foreign chemical in the body would be expected to be in the intracellular water. In contrast to this, the compound that could not pass the cellular membrane but was soluble in water would be partitioned in the extracellular water of the body. Thus, the addition of a foreign chemical, that is, a chemical not normally present in the cell, to an intact biologic system in its normal environment differs from the simple mixture of solutions of two or more chemicals. It is commonplace to observe that the amount of the chemical that produces inhibition of an enzyme system when tested on the isolated purified enzyme may bear no relation to the amount of the chemical necessary to produce a similar effect on that same enzyme when administered to the intact biologic cell; in fact, there may be a several hundred-fold difference between the two concentrations.

Some xenobiotic chemicals are so reactive with membranes that they are "captured" at the site of exposure to the chemical and little or no translocation takes place. The importance of this in toxicology is demonstrated by two highly reactive compounds, formaldehyde and methyl isocyanate, which are volatile at ordinary temperatures, thereby making inhalation the route of administration. Animal experiments have shown that these compounds can be efficiently removed from the air by the nasal mucous membranes during inspiration. Hence it is questionable whether even small amounts of these compounds are translocated to other parts of the body by inhalation.

RESERVE FUNCTIONAL CAPACITY

Organs basically have a reserve capacity to carry on their overall function. For example, approximately 50% of the liver of the dog can be chemically damaged or even surgically removed, and the remaining intact liver will carry on adequately to support at least the minimal requirements of the animal. Likewise, half the lung tissue in the rat may be removed without seriously endangering the life of the rat. Certainly, one kidney can be removed from any animal without harming the animal as long as a remaining kidney is in good functioning condition. Demonstration of chemical-induced damage to a living organ usually involves the use of one or more forms of tests designed to measure the function of the organ. Since it has been indicated that a large portion of the organ may be damaged before the reserve capacity of the organ is sufficiently diminished to render it functionally impaired, it is quite likely that function tests would not show small amounts of chemical-induced damage. In actual practice, this has been repeatedly demonstrated so that one must continue to rely on histologic or histochemical evidence to demonstrate the initial degrees of chemical-induced damage to an organ. Much research is currently directed at developing more specific and more sensitive tests of organ function in an attempt to detect small degrees of chemical-induced damage to organs. It is apparent that as long as the organ maintains a reserve (excess) capacity to carry on its overall function, such research attempts are ill directed unless a function of the organ is selected for which the organ maintains no reserve capacity, or unless the organ can be so stressed that it carries on the function at the maximal level.

The final concentration of the added chemical at various areas throughout the organ varies depending upon the ability of the membranes to be immaterial or to enhance or inhibit transfer of the chemical through the organ. In the process of the transfer of the chemical to a specific cell within the organ, the chemical must necessarily come in contact with nonspecific binding sites which functionally remove it from the system. Since it is postulated that a specific concentration of a chemical or its metabolites must be present if such a preparation is going to lead to toxicity of the organ, it is reasonable to conclude that some cells will be more or less affected than other cells for no reason other than the physical and biologic limitations placed on free transfer of the chemical uniformly throughout the organ. Such limitations may be practically insignificant if the chemical has the property of being freely transferable through the membranes. On the other hand, such limitations may be most significant and not easily overcome with a poorly translocated chemical, for it would mean that a constant concentration of a chemical would have to be maintained in contact

with the organ for a sufficient time for some degree of uniform distribution of the agent to take place. Thus, an organ that has been insulted by minimal toxic concentrations of a foreign chemical agent on one occasion would not be expected to show the overall toxicity that would occur as a result of continuous insult by the same concentration of the chemical for an extended period of time.

ACCUMULATION AND STORAGE OF CHEMICALS IN THE ORGANISM

Exposure of a living biologic cell to a sufficient amount of a foreign chemical on a single occasion will lead to the presence of the chemical on and within the cell, provided the cell membranes allow its translocation within the cell. If the cell is then removed from the environment of the chemical, in time the organism will be free of the foreign chemical. The same mechanisms that are involved in the uptake of the chemical agent would be involved in the elimination of the agent from the cell, regardless of whether the cell is a single bacterial cell or one of a host of similar cells within an organ. In the case of the single bacterial cell, the act of removing the cell from the chemical-containing medium to a medium that is free of the chemical reverses the concentration gradient of the chemical, so that diffusion of the chemical could accomplish its elimination from the cell. In the case of the experimental animal, additional mechanisms of elimination are involved.

If the chemical passes into the circulatory system, it must then be eliminated from the circulatory system before the animal is free of it. If the chemical is in solution as a gas at body temperature, it will appear in the expired air from the animal; if it is a nonvolatile substance, it may involve excretion by the kidney via the urinary system, or it may be chemically altered by the animal and excreted via any of the mechanisms available to the animal, such as excretion in the urine, in the sweat, or in the saliva. The rate of elimination depends on the nature of the chemical and the mechanisms that are used to terminate the presence of the chemical in the organism. Generally chemicals that are metabolically converted by the animal to derivatives will have short lives within the body. An example is ethyl alcohol, which is metabolized at an approximate rate of 200 mg/kg/hr in man or the dog.

A chemical that is both metabolized and deposited in fat has a short life span in the blood and the nonfatty tissues; thiopental, for example, has a brief anesthetic action (15 min or less) following conventional single doses in man. This is because that portion of the drug which is in the blood rapidly

undergoes conversion to nonanesthetic forms of the drug; the remainder of the drug is deposited in fat and muscle. Then as rapidly as the drug diffuses from the fat back to the blood, it is converted to inactive forms so that the blood remains essentially free of effective concentrations of the drug (see Fig. 3.4).

Many chemicals are selectively adsorbed on or combined with proteins or enzymes or even components of bone. Such chemicals may remain in the animal for years following a single dose. The drug Teridax (2,4,6 triiodo-3-hydroxyphenyl propionic acid) is so tightly bound to plasma protein that its half-life in the body is 2 1/2 years. Ninety-eight percent of the drug dicumarol is carried in the blood in combined form with albumin, where it is not available to produce an effect on cells or for metabolic attack by the animal. Atabrine is distributed in the dog so that the liver concentration of the drug after a single dose may be as high as 2000 times greater than the concentration in the plasma. The tetracycline drugs combine with components of newly formed bone in most laboratory animals so that reabsorp-

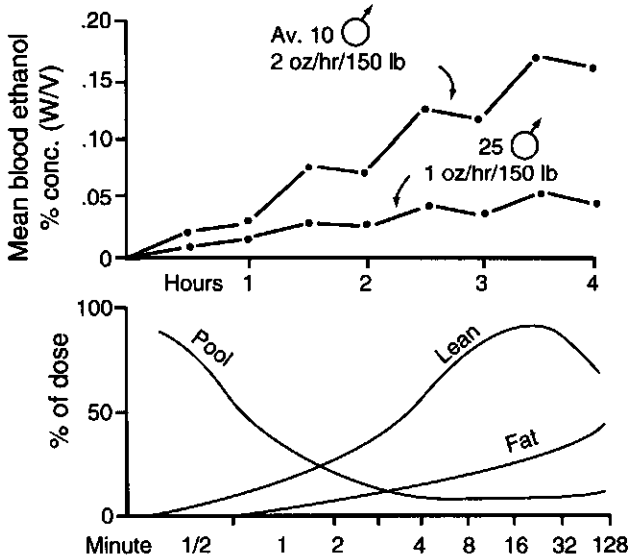

FIGURE 3.4 Upper graph represents, in the blood of human subjects, accumulation of ethanol following consumption of 1 or 2 oz of 100 proof whiskey per hour. First drink at Time 0 and one drink each hour thereafter. (Data from Forney and Hughes, *Clin. Pharmacol. Ther.* **4:** 619, 1963.) Lower graph represents translocation of a single dose of intravenously administered thiopental, a compound which is rapidly redistributed in the body and accumulated in fat. The figure shows initial translocation to the lean tissue and subsequent transfer to the fat. Data according to Price *et al.*, as predicted mathematically and compared with direct measurement in human blood and fat. (Price *et al., Clin. Pharmacol. Ther.* **1:** 16, 1960.)

tion of bone must take place before the drug can be eliminated from the animal. The insecticide DDT (dichloro-diphenyl-trichloroethane) is stored in fat and remains in the animal for months. The organophosphate agents as exemplified by diisopropylfluorophosphate are effectively bound to and inhibit the enzyme cholinesterase, and hydrolysis of the phosphorylated enzyme is so slow that the enzyme remains inhibited for weeks.

Such sites of deposition, adsorption, or reaction of chemicals within the body limit the ability of the body to excrete them from the body. Such sites therefore act as effective storage depots for chemicals that otherwise may be so effectively metabolized or excreted that they would have only a transient existence in the body. Such sites of storage also effectively prevent the occurrence of high concentrations of the free chemical so that toxic concentrations of the chemical are not normally achieved until the storage site become saturated with the chemical. Thus, it would be reasonable to state that the potential toxicity of a chemical is influenced by the availability in the animal of an abundance of efficient nonspecific binding sites, or the presence or absence of efficient biotransformation mechanisms. Animals lacking such mechanisms would be very susceptible to rapid rises in concentration of the active chemical in the body, and since toxicity is directly related to the available active concentration of a chemical, such animals would be expected to be highly susceptible to the toxic action of these chemicals.

In general, single exposures of an experimental organism to a chemical result in uptake of the chemical by the organism and subsequent elimination of the chemical from the organism. The rate of elimination of the chemical is under the influence of the binding and storage mechanisms available for that chemical within the organism. Some gases may have half-lives within the body as short as a few minutes, whereas some strongly bound chemicals may exist in the body for years. Therefore, repeated exposures to a chemical in which the interval between exposure is less than the life of the chemical within the organism would lead to accumulation of that chemical in the organism.

Figure 3.4 consists of examples of accumulation of chemicals due to repeated dose (upper graph) and to translocation mechanisms (lower graph).

Chemicals that are bound to protein or to sites on the cell not involved in the pharmacologic or toxicologic response to the chemical represent large storehouses for such chemicals within the body. These bound forms of the chemical are generally not available for reaction with effector sites on the cells. However, it has been demonstrated that another chemical agent may displace the first chemical from the binding sites, making the first agent available in the free form. In this way the administration of a

second drug could induce toxicity from the first drug; for example, the strongly bound sulfonamide drugs can induce hypoglycemic coma by replacing antidiabetic drugs which are bound to protein. Also, when patients who have received quinacrine (Atabrine) for the treatment of malaria are given primaquine, the primaquine displaces the quinacrine, which rapidly reaches toxic concentrations in the blood.

Compounds that accumulate in the body because of repeated frequent ingestion as contaminants of food have occasionally caused insidious harm to sizable populations of humans. Among the various chemicals involved in this type of clinical toxicology, methyl mercury is an outstanding agent that is responsible for at least eight separate episodes of tragedy directly affecting a total of over 8000 humans. Intoxication from this compound has resulted either from misuse, as food, of mercury-treated grain that was intended for seed purposes only, or because of industrial discharge of the compound (or related mercury compounds that are subsequently converted to the alkyl mercury by the action of bacteria) whereby it bioaccumulated in edible fish. The toxic manifestations of alkyl mercury (methyl or ethyl) intoxication in humans are loss of sensation, that is, paresthesia in the hands, feet, and the oral area, impairment of speech, and impairment of vision eventually leading to blindness. These effects are probably related to the effect of the alkyl mercury on brain cells. As far as the paresthesia is concerned, there appears to be a direct relation between the concentration of methyl mercury in the blood and the incidence and severity of the paresthesia. When a compound such as methyl mercury is taken by mouth as a contaminant of food, and when the particular food constitutes a staple item of the diet so that it is consumed daily, the compound has a good chance of accumulating in the body. Under these conditions of repeated intake by the oral route, methyl mercury is particularly prone to accumulate since it is very slowly eliminated from the body and it is very readily absorbed from the gastrointestinal tract. The half-life of methyl mercury is approximately 2 months.

Since it has already been demonstrated that toxicity is related to concentration of a chemical at effector sites and since accumulation of a chemical is a mechanism of increasing the concentration of a chemical in the body, compounds that tend to accumulate are prone to produce toxicity.

TOLERANCE

A poorly understood phenomenon in toxicology is that of "tolerance" to chemicals. Tolerance is the ability of an organism to show less response to a specific dose of a chemical than was shown on a prior occasion from

the same dose. It is as if the organism becomes partially refractory to the effect of the chemical by virtue of previous exposures to the chemical. Tolerance could be due to any of at least three possible mechanisms. These are failure in translocation of the chemical to receptor sites, enhanced excretion or biotransformation systems, and elevation of the threshold of response at the receptor site. Currently there is little experimental evidence for or against the first mechanism. Biotransformation mechanisms are known to be altered by prior use of the same or related chemicals; this is discussed in the next chapter. The third possibility involves the creation of an altered receptor system through a change in the numbers or types of receptors involved.

In the toxicologic literature the term "tolerance" is frequently used incorrectly. For example, tolerance is different from resistance, as in the case of certain strains of rabbits which are known to be resistant to the effects of atropine because they have an enzyme in their gastrointestinal tract that inactivates atropine. Likewise, immunity that is developed to certain chemicals, such as the protein and polysaccharide toxins of bacteria, involves development within the animal of an antibody; this antibody then reacts directly with and inactivates the toxin on subsequent exposure so that the animal develops a resistance (immunity) and not a tolerance, to the toxin.

A significant component of the phenomenon of tolerance is that if a sufficient concentration of the chemical is presented to the organism, it responds by showing the conventional pharmacologic and toxicologic effects of the chemical. Tolerance is usually acquired over a period of time by exposure to an agent, but resistance to an agent may be acquired rapidly following only a few doses of the agent over a period of a few hours. In pharmacology this phenomenon is known as "tachyphylaxis." A good example of tachyphylaxis is the effect produced by the drug ephedrine. When ephedrine is administered to a cat or dog by the intravenous route it promptly produces a rise in blood pressure. With proper doses, this effect subsides within a half-hour, at which point a second dose will produce less effect. Each subsequent dose produces less and less effect until no response occurs, even if the dose is increased to virtually lethal levels. Hence, tachyphylaxis is not synonymous with tolerance to chemicals.

Tolerance occurs with most habit-forming drugs and in this respect acquires considerable significance in toxicology. In man as well as other animals repeated administration of morphine and related drugs results in tolerance to these drugs; a highly tolerant subject may require 20 to 40 times the usual dose of morphine in order to achieve the same degree of pharmacologic effect. However, even subjects with extensive tolerance to these drugs not infrequently take lethal overdoses of the drugs. Minor

changes in absorption and translocation of morphine in the tolerant subject do not account for the tolerance. Rather, altered receptor sites in the brain, which have been described, appear to be responsible for the tolerance to morphine and related compounds.

Tolerance to many of the drugs that affect the human brain is accompanied by a compulsion to take the drug. If the compulsion is sufficiently strong so that the drug is taken repeatedly, a cycle of events leading to progressive increase in tolerance is established. Withdrawal of the drug may then lead to severe physical incapacitation and even death of such subjects. Such subjects are said to be addicted to the drug. Tolerance is a constant factor in addiction. The withdrawal effects that occur obviously constitute a type of harmful effect that is an exception to the basic concept that harmful effects of chemicals are directly related to concentration of the chemical present at the effector site. Withdrawal effects, as exemplified by delirium tremens in alcoholics or the hallucinating and convulsive effects in narcotic addicts, appear to be inversely related to the concentration of the drug in the tissue. In fact, administration of the drug relieves these effects. This represents perhaps the only condition whereby a type of harmful effect results from withdrawal of a xenobiotic chemical and is therefore inversely related to the concentration of the chemical at effector sites in the animal.