CHAPTER 10
The Basis of Selective Toxicity

Selective toxicity refers to the variability in toxicologic response of cells to xenobiotic agents. Although there are excellent examples of variation in response of different members of the plant kingdom to chemicals, the discussion here will be concerned only with variation between biologic cells, tissues, and organisms when they are exposed to foreign chemicals.

Biologic variation between cells is frequently observed as a variation between whole animals, and much of this text has been directed toward a description of some mechanisms that account for variation between animals in response to chemicals. Most chemicals that serve a useful purpose for man do so by influencing the function of only a very limited number or type of cells or systems. That is, the xenobiotic agent affects a normal biochemical system and interferes with the functions that are normally accomplished by that system. When a xenobiotic agent reacts at some specific biochemical site, that site is commonly referred to as the "target" or "receptor" for the agent(s). Cells that do not possess the proper receptor would not be affected in the same manner as those that do possess the receptor. Although there are many complex factors involved in the xenobiotic–receptor interaction, the system involves at least two basic requirements in order for a xenobiotic agent to be selectively active on one cell and not on another. One requirement is that it (or a biotransformation product) must gain access to the cell. Second, the cell must contain a receptor for the agent. Consequently the mechanisms involved in selective

toxicity are those that influence the concentration (i.e., translocation systems) and chemical properties of the agent and its products (i.e., biotransformation systems) and the existence of specific receptor systems in cells. These mechanisms are shown in Table 10.1. The following text presents some examples of each.

SELECTIVE TOXICITY DUE TO TRANSLOCATION MECHANISMS

In the intact animal, whenever a mechanism exists that is able to concentrate (bioaccumulate) a xenobiotic agent, the result is an elevated probability of occurrence of toxicity at that site. For example, in mammals the kidney functions as an excretory organ that is capable of excreting products from the blood via the urine at elevated concentrations. One example whereby this mechanism enables a compound to become selectively toxic to kidney cells is the case of uranium bicarbonate. This compound is water soluble and is readily excreted from the blood. The kidney returns the bicarbonate ions and water to the blood and retains the uranyl ions which are concentrated as the urine is being formed. This biocentration of uranyl ions takes place in the tubules of the excretory units of the kidney and concentrations are reached that produce selective renal tubular cell damage.

Barriers to absorption or translocation of a chemical may be present in some species of animals and not in others, thereby influencing concentrations of a chemical in different species. Studies by the author which were concerned with the toxicity of *N,N'*-Bis-(3 methanesulfonyloxypropionyl)-1,2-propanediamine, an anticancer agent, have shown that dogs that received 10 mg/kg of this compound per day, either by mouth, mixed with feed, or by the intravenous route, showed severe depression of white blood cell formation at the end of 5 days. Rats or monkeys tolerated 100 to 500 mg/kg per day by mouth, mixed with feed, for 1 to 3 months without effect on the white blood cells. It was subsequently shown that the drug was not destroyed by admixing it with the feed used for the rats or

TABLE 10.1 Mechanisms of Selective Toxicity

A. Mechanisms involving alteration of available effective concentration of the chemical at effector sites
1. Due to translocation factors
2. Due to biotransformation mechanisms
B. Mechanisms involving the presence or absence of target systems susceptible to the chemical

monkeys. (The same rats or rhesus monkeys subsequently received 10 mg/kg of the compound per day for 5 days by intravenous administration and showed the same toxic effect on blood cells as was shown in the dog.) Thus, selective toxicity to chemicals between species can be the result of variations in absorption or translocation processes.

The cyclic chlorinated insecticide chlorphenothane (DDT) owes its effectiveness on the insect at least partly to the fact that it is readily absorbed directly through the chitinous exoskeleton of the insect. DDT is only poorly absorbed through the skin of mammals. However, it has already been mentioned that because insects are small compared to mammals, they have a greater body surface area in relation to weight than do mammals. Therefore, in an atmosphere of DDT, insects receive a greater dose through the skin in relation to body weight than do mammals, thereby making an apparent selective action of DDT on insects the result of both high dose and ineffective barrier mechanisms in the insect.

Another interesting and important example of the effect of altered translocation of compounds as a mechanism of selective toxicity is demonstrated by fluoride and particularly fluoroacetate-induced toxicity. For many years it was believed that fluoroacetate produced its toxicity by competing with citrate for active sites on the enzyme aconitase, thereby blocking the citric acid cycle. As shown in Fig. 10.1 carbohydrate and fat serve as fuel, through conversion to acetyl-CoA, for the energy processes of the citric acid cycle. The cycle serves as a machine for the metabolism of acetyl-CoA. In the

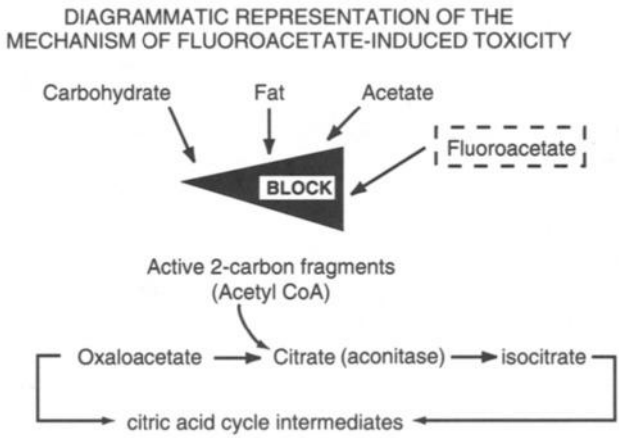

FIGURE 10.1 Transfer of acetate through membranes in the mitochondrial system of the cell is mediated by a carrier system which is blocked by fluoroacetate, thereby depleting the fuel source for the citric acid cycle.

cycle, citrate is converted to isocitrate by the enzyme aconitase. Blockage of this enzyme by fluoroacetate is one mechanism by which the citric acid cycle would be blocked. However, the enzymes of the citric acid cycle are located in membranous bound structures in the cell known as the mitochondria. There is now evidence that the action of fluoroacetate may be to complex with and inactivate a carrier substance that functions primarily to transfer acetate into the mitochondria. Therefore an important action of fluoroacetate may be to competitively block translocation of specific nutrients within the cell.

SELECTIVE TOXICITY DUE TO BIOTRANSFORMATION MECHANISMS

The processes of biotransformation supply a mechanism that accounts for many examples of selective toxicity. Since biotransformation may result in destruction of the biologically active form of a chemical or formation of a more toxic product, it is apparent that the presence or absence of such mechanisms within members of a species will effectively alter the concentration of the parent chemical in the biologic specimen. If the biotransformation process involves detoxication of the parent chemical, a failure or absence of the process may induce toxicity by failing to terminate the drug action. Examples of such a selective toxicity induced by genetic deviations between members of a species are described in Chapter 6.

In contrast to the examples of genetic deviations in biotransformation mechanisms within a species, such mechanisms may be present or absent in different species or strains, thereby accounting for strain or species selectivity in chemical-induced toxicity. In this manner certain strains of bacteria, particularly gram-negative bacilli, are resistant to penicillin at least partly because they elaborate an enzyme (penicillinase) which inactivates penicillin. Some strains of houseflies and mosquitoes are resistant to the insecticide malathion by virtue of their ability to produce a specific enzyme (esterase) that inactivates malathion.

The relative rates of activation and inactivation of a xenobiotic are of great importance in determining their overall biologic effect. The following example demonstrates how this can contribute to a selective toxic action of organophosphates on insects. The organophosphates produce toxicity primarily by their ability to inhibit the enzyme acetylcholinesterase (AChE). Two types of such organic phosphates are the direct inhibitors of AChE and the indirect inhibitors of AChE. The direct inhibitors, such as diisopropyl fluorophosphate and methylisopropyl phosphonofluoridate, do not require metabolic conversion (oxidation) to active forms, whereas the indirect in-

hibitors such as Parathion or Malathion are metabolically activated (oxidized by microsomal enzymes) to compounds that are able to inactivate AChE more rapidly. The activated derivatives are then hydrolyzed (by other esterases) and are thereby eventually inactivated. Basically, a species that could activate the indirect type of inhibitor but could not hydrolyze it would accumulate the active form, and thereby would be susceptible to the toxic effects of the compound. In contrast to this a species which could hydrolyze the indirectly acting organic phosphate instead of activating it would be expected to be resistant to the action of the compound. Different species of biologic specimens carry on these activation (oxidation) and inactivation (hydrolysis) reactions at different rates, thereby creating a condition in which the balance between these reaction rates determines the effective concentrations of the active forms and the extent of toxic effect of the organic phosphate in the species under study.

Figure 10.2 shows these reactions in the case of Malathion and the relative rates of the reactions in insects and mammals. Mammals hydrolyze and thereby rapidly inactivate Malathion, thus reducing the quantity available for conversion to Malaoxon, which is readily hydrolyzed to inactive products. Since insects do not as readily hydrolyze Malathion, more of it is available for oxidation to Malaoxon, which is more stable in the insect than in the mammal. With doses of Malathion being equal in the two species, therefore, enzyme activity would result in a greater accumulation of the active oxygen analog in the insect than in the mammal, thereby contributing to the selectively toxic action on the insect.

The foregoing examples collectively represent mechanisms that alter the available concentration of a chemical in an animal or at specific sites in

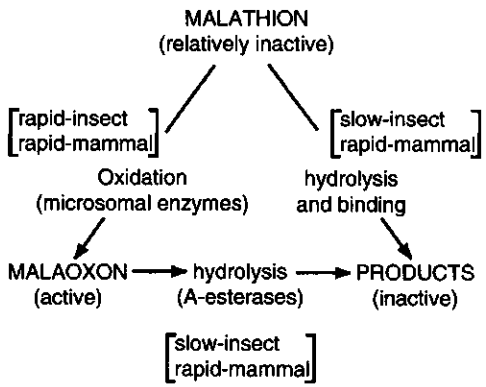

FIGURE 10.2 Pathways for biotransformation of the organic phosphate insecticide Malathion, and the relative rates of the reactions in insects and mammals.

the animal in comparison to the concentration of the compound that may be present in other tissues or other species of animals. In this sense, these mechanisms collectively represent a single type of selective toxicity of chemicals.

SELECTIVE TOXICITY DUE TO THE PRESENCE OR ABSENCE OF RECEPTORS

The second mechanism involved in selective pharmacologic or toxicologic action of chemicals on cells involves the existence or absence of specific targets or receptor systems in the exposed cells (Table 10.1). In this case, the concentration of the compound to which various cells are exposed is the same for all cells, but only certain cells are affected. The selective action of the normal neurohumors and endocrine substances, as well as that of many drugs, involves this mechanism. When administered to mammals, acetylcholine or norepinephrine, which are normal neurohumors liberated at the ends of nerves, selectively react only at specific sites on membranes that are presumed to contain specific receptors only for one or the other of these agents, but not for both. In fact, the specificity of the receptor may be so selective that only one isomeric form of a compound will react to any significant extent with that receptor. The adrenergic amines l-epinephrine and d-epinephrine are examples of this selective action of optical isomers; the levo isomer has 20 times the activity of the dextro isomer.

For our purposes it is not necessary to go into the details of receptor theory, but it is necessary to recognize that many of the most potent toxic agents known to man produce their biologic effects by reacting with chemicals (receptors) that are involved with normal essential biologic mechanism(s) with a resultant blockade of the mechanism(s). For example, cyanide and azide block oxidative cell metabolism, d-tubocurarine blocks transmission of nerve impulses to skeletal muscle, and botulinum toxin blocks release of the neurotransmitter at the nerve–muscle junction. Some rodenticides are useful because they show species-selective action due to the presence of receptors in rodents which are not present in man. An example is norbormide, which acts on receptors in smooth muscle in rats that are not present in man, dogs, or cats. The result is that doses of norbormide that are lethal to the rat are nontoxic to the other species.

Many biochemical enzymatic reactions are selectively specific with regard to the substrates that may be utilized in the reaction. In fact, such reactions are similar to the chemical-receptor types of reactions and also may be stereo-specific, that is, only one optical isomer is utilized or synthesized in

the reaction. An example is the action of arginase, which effects hydrolysis of only the levo form of arginine. Other enzymatic systems may have only group specificity and will utilize only substrates that are closely related chemically; an example is the enzyme acetylcholinesterase, which hydrolyzes not only acetylcholine, but also acetyl-β-methylcholine and triacetin. Still other enzyme systems in the mammal have a very low specificity of action, an example being the lipases, which hydrolyze most fats.

Many drugs have as the basis for their selectivity of action the fact that chemically they are sufficiently similar to normal enzyme substrates to permit them to compete with or displace the normal substrates for the active sites on enzyme systems in the animal. It is believed that if the drug has a sufficient affinity for reaction with the active groups of the enzyme, it occupies the active sites on the enzyme in much the same manner that the normal substrate occupies the same sites on the enzyme. Although the drug may act to occupy the active sites on the enzyme, usually the drug is at best a poor substrate and may simply occupy the active sites on the enzyme so that they are no longer available for the natural substrate. The result is inhibition of the enzyme. If the enzyme is part of an essential chain of enzymatic reactions necessary to produce a biologic response, that physiological mechanism is blocked. The blockade may be due to competition between the drug and the normal substrate for the active sites on the enzyme, in which case the drug can be displaced from the enzyme simply by increasing the concentration of the normal substrate.

A good example of a competitive inhibition mechanism which is responsible for selectivity of the action of a chemical is shown by the antibacterial sulfonamide drugs. Almost 60 years ago it was shown that *p*-aminobenzoic acid (PABA) would competitively prevent the lethal action of sulfonamide on susceptible strains of bacteria. Many subsequent investigations regarding the mechanism of this competitive action between PABA and sulfonamide drugs have led to the conclusion that PABA is essential for the formation of one or more coenzymes of which folic acid is an essential component. These coenzymes play an important role in conversion of amino acids and in the formation of purines. PABA therefore appears to be an essential "nutrient" in those bacteria that cannot utilize preformed folic acid in the media in which they are grown but must synthesize their own folic acid. PABA is not essential in those strains of bacteria that are capable of utilizing preformed folic acid from the media in which they are grown. This accounts for the selectivity of lethal action of sulfonamide drugs for specific strains of bacterial organisms that are not able to utilize preformed folic acid. The human uses performed folic acid and is therefore not susceptible to this action of the sulfonamide compounds.

An example of the competitive inhibition type of reaction in which the specific enzyme systems involved are not completely definable is the competition between vitamin K and Dicumarol. Vitamin K is essential for the formation of certain globulin proteins in the liver of most mammals. Dicumarol selectively competes with vitamin K in these reactions in the liver of mammals, thereby causing failure in the formation of the globulins. The action of Dicumarol on this system can be reversed if the concentration of available vitamin K is increased.

In contrast to the competitive enzymatic inhibition mechanism described above, the drug–substrate complex may involve strong covalent bonding forces so that the enzyme is virtually irreversibly inactivated. An example of an almost irreversible enzyme–drug complex is the reaction between the organic phosphates (such as methyl isopropyl phosphonofluoridate) and the enzyme acetylcholinesterase. The basis of action of these drugs is their selective effect on specific enzyme systems within a species, and their effect is predominantly on the cells or tissues containing that enzyme system. Basically, in these examples the enzyme (or active sites on the enzyme) act as receptors for the xenobiotic agent.

Similar mechanisms can account for selectivity between species. Certain antibiotic drugs owe their effectiveness on bacteria to the fact that they selectively act on a target system that is essential for either reproduction or maintenance of viability of the bacteria. The same target system is either nonexistent or nonessential in the physiology of mammals. For example, the lethal action of penicillin affects only actively multiplying bacterial cells. Penicillin interferes with the synthesis by the bacteria of mucopeptides and teichoic acid which are necessary, strength-supplying, structural components of the bacterial cell walls. Since the actively multiplying bacterial cells must maintain a high internal osmotic pressure compared to their external environment, penicillin-induced defective cell walls lead to bizarre and fragile forms and eventual death of the bacterial cells without affecting human cells.

The potential usefulness of selectively active chemicals on biologic mechanisms has progressed rapidly in the fields of agriculture and pharmacology, but is only in its infancy in the science of toxicology. It is evident that a prelude to the systematic development of selectively toxic substances is a better understanding of the biochemical systems that regulate and control the growth and viability of undesirable forms of cells. Such undesirable cells are manifested as cells in the liver that accumulate lipid or are replaced by fibrous tissue, cells that undergo degenerative changes without replacement, or cells that fail to maintain their state of differentiation and therefore become the malignant accumulations of undesirable cells. The evasive and complex nature of the biochemical mechanisms that initiate and control

reproduction, differentiation, maturation, degeneration, and regeneration of cells may preclude early absolute chemical identification of the subtle differences between cells that may be subject to attack by selectively toxic chemicals. Perhaps one "key" lead regarding the nature of such a point of attack will enable rational systematic development of selectively toxic agents without chemical identification of the receptor or receptors involved.

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CHAPTER 11
The Basis of Antidotal Therapy

In a situation in which a harmful effect of a chemical presents a threat to an economic species, it is necessary to be able either to reverse the harmful effect or at least to prevent further harm from the chemical. It is axiomatic that if a chemical causes death of living tissue, no procedure is successful in reversing such an effect on those cells, and new cells and tissues must be formed if the organ is to continue to function.

In contrast to this, a harmful effect of a chemical may consist of mild to severe impairment of function short of death of the biologic cells, tissues, or whole animal. Under this condition, procedures that are specifically directed toward limiting the intensity of the effect or reversing the effect are useful for preventing further harm. Such procedures are referred to as "antidotal procedures" or "antidotal therapy." When the use of a chemical is involved in such a procedure, the chemical is referred to as an "antidotal agent." Successful antidotal therapy becomes a necessity especially whenever humans are the economic species involved in harmful effects from chemicals. Therefore, most of the developments in antidotal therapy are concerned with protection of humans, or at least animals, from the toxic effects of chemicals.

GENERAL PRINCIPLES

All antidotal procedures are based on two concepts (see Chapters 2 and 3): first, that the intensity of all chemical–biologic reactions is related to the dose, or more accurately to the concentration, of the chemical at the effector sites in the tissue; second, following administration of a chemical to a biologic specimen, the concentration of the chemical within the tissues is dependent upon the ability of biologic barriers to prevent its translocation and on the chemical properties of the compound, which permit or prevent its translocation in the tissue.

Since translocation of a chemical is a time-dependent process, it may be said that the intensity of all chemical–biologic reactions is time-dependent. The following is an example of how these two concepts are concerned with antidotal procedures. In order for an animal to be exposed to a chemical, a route of administration must be involved. Any route of administration could be considered. If the intravenous or inhalation route is involved, the time intervals would be much shorter than for the oral route of administration, but the principle that time is involved in translocation of the chemical to effector sites would still be valid. For the purpose of presenting an example, the oral route is used here.

Following oral administration of an excessive dose of the chemical, depending on the chemical properties of the agent involved, it is absorbed or translocated to the blood from which it is in turn translocated to the various tissues of the animal. For practical purposes, the concentration of the chemical in the blood or in the tissues rises at a rate directly related to the dose and inversely related to the rate of termination (excretion, storage deposition, and metabolic transformation) of action of the chemical at the effector site in the animal. If it is assumed that the oral dose of the chemical is sufficient to produce a harmful effect, that effect will occur in relation to time when a toxic concentration of the chemical is present in the blood or at the effector site. Shown in Fig. 11.1A is the nature of the curve that would be achieved if an animal were given a selected compound in a dose that would produce a measurable toxic effect, but not death of the animal. It is apparent that if death of the animal occurred, the curve would terminate at that point in time. If death of the animal did not occur, at some point in time absorption of the chemical from the gastrointestinal tract would be complete, and the quantity of the chemical at the effector sites would decrease in direct relation to the rate of termination of action of the chemical at the effector sites. If the toxic effect consists of a reversible phenomenon, as the concentration of the chemical declines with the progression of time, recovery of the animal is complete. Any reversible tissue damage that may remain following termination of the existence of the

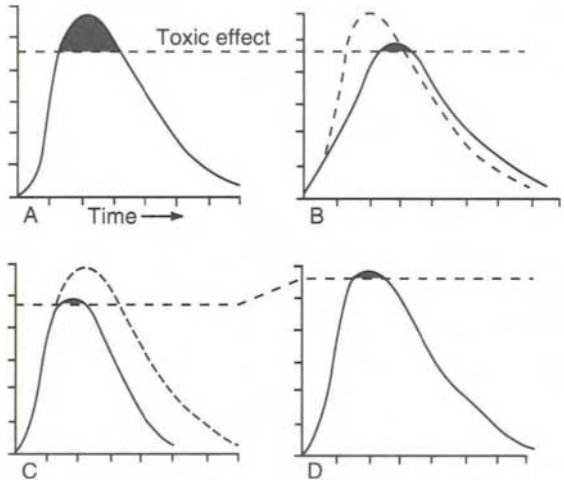


FIGURE 11.1 Schematic curves representing the relation between the effect in terms of concentration of a chemical at effector sites and time after oral ingestion of a hypothetical toxic chemical. See text for derivation of the curves.

chemical in the animal undergoes repair or regeneration in time, provided additional assault by the chemical is avoided. Tissue in which there is nonreversible, chemical-induced damage also undergoes replacement or fibrosis in time if the animal survives. Furthermore, the major difference between chemical-induced damage to tissue following acute exposure and that following chronic exposure to a chemical would be a difference in the time intervals involved for induction and recovery from the toxicity.

The curve in Fig. 11.1A contains a shaded portion which represents the area of time in relation to the intensity of effect when the toxic effect of the chemical is manifested in the animal. Since antidotal therapy is directed toward effectively abolishing the shaded area in the figure, there are three obvious procedures that would accomplish this objective: (1) decrease the slope of the rising portion of the curve, (2) increase the slope of the descending portion of the curve or displace the descending portion of the curve to the left, and (3) elevate the level or threshold at which the toxic range of effect occurs.

The effect of each of these three procedures on the shaded portion of the curve in Fig. 11.1A is shown, respectively, in Figs. 11.1B, 11.1C, and 11.1D. All three conditions constitute mechanisms that can be effectively utilized in antidotal procedures. The slope of the rising portion of the curve may be decreased (Fig. 11.1B) by actual or virtual removal of the unabsorbed portion of the chemical from the gastrointestinal tract or by

removal of the chemical before it is translocated within the animal to the effector site. The slope of the descending portion of the curve (Fig. 11.1C) can be increased and moved to the left by increasing the rate of termination of action at the effector sites by increasing the rate of excretion of the compound via the kidney or lungs or by other physiological excretory mechanisms, by promoting the inactivation of the compound by chemically binding it to nonspecific effector sites, and by mechanically translocating the chemical to the exterior of the animal. Finally, the threshold at which the toxic effect occurs can be elevated (Fig. 11.1D) by the administration of antidotal chemicals that either directly or indirectly pharmacologically antagonize the toxic effect of the chemical, or by mechanical procedures which substitute for, or carry out, the function that is impaired by the toxic effect.

In actual practice in human clinical toxicology, each of the three possible mechanisms for influencing toxicity is accomplished either by nonspecific or by specific methods. The nonspecific methods are those general methods which are applicable to large numbers of chemicals (that is, nonspecified chemicals). The specific methods are used when the specific compounds that are the probable or potential cause of the toxicity have been identified. Table 11.1 categorizes the various nonspecific antidotal procedures that are available, as well as the specific procedures as they are used for altering the time–response curves shown in Fig. 11.1.

PROCEDURES FOR DECREASING ABSORPTION OR TRANSLOCATION

The antidotal procedures for limiting absorption on exposure to topically applied offending chemicals are mechanical removal and the use of chemical agents that will combine with and detoxify the offending chemical. Following the ingestion of an amount of an offending chemical that is potentially adequate to produce a toxic or harmful effect, antidotal therapy may involve one or more of several procedures. Removal of the chemical from the stomach, either by gastric lavage or by the use of an emetic, represents the most direct procedure for preventing absorption of the chemical.

Gastric lavage is accomplished by inserting a tube into the stomach and washing the stomach with water or any suitable and relatively harmless solvent for the agent involved. Water is the lavage fluid preferred since it is the most innocuous of fluids; however, it would not be as effective as other solvents for substances that are only slightly soluble in water. In the case of lipid-soluble agents, liquid petrolatum would be a suitable lavage agent. If other solvents are used, the potential toxicity of the solvent should

be considered an added hazard to the procedure. Regardless of the solvent properties of water, the mechanical irrigation of the stomach with a water lavage would assist in removing particulate matter. The lavage procedure is practical only for removal of material that is in the stomach and does not remove material that has passed into the upper intestine.

An additional procedure for actual removal of the stomach contents is through the use of emetic agents. In humans, emesis can be induced by parenteral injection of apomorphine or by oral administration of syrup of ipecac. Apomorphine-induced emesis may be severe and the emesis does not lead to elimination of the apomorphine, which is in the circulation. In contrast to this, emesis that is induced by ipecac is primarily a consequence of a local action of the ipecac on the stomach, and the emesis results in the elimination of not only the potentially toxic agent, but also at least a portion of the ipecac. For this reason, ipecac would be the emetic of choice whenever an emetic agent is indicated in an antidotal procedure. Both drugs have a latent period of approximately 5 to 15 min between their administration and the induction of vomiting.

Properly performed lavage or the use of emetics seem to be of about equal efficiency in emptying the stomach. The time interval after the ingestion by mouth of amounts of a substance that would be potentially harmful is a very important factor to consider when emetics or lavage are to be used.

Animal studies suggest that when sedation is evident following ingestion of overdoses of sedative types of drugs, the production of emesis by emetic drugs cannot be relied on. That is, the sedative drug antagonizes the action of the emetic drug. If the intensity of sedation is so great that the subject is unconscious, the induction of vomiting is contraindicated unless particular caution is used to prevent aspiration of vomitus that may lodge in the subject's upper pharynx. In children who may have ingested petroleum products such as kerosene, it is a controversial subject regarding whether any attempts should be made to empty the stomach because of the high probability that some of the kerosene would be aspirated into the lungs with a resultant pneumonitis. On the other hand, it may be proper to use emetics or lavage if there is reason to believe that the child may suffer more serious toxicity than that of a pneumonitis if no stomach emptying procedure is used. Each case of potential clinical intoxication from ingestion of a chemical agent requires a general assessment of all factors involved before treatment is initiated. In any case, the treatment should not be more harmful to the patient than the absence of treatment. For example, strong salt (table salt, NaCl) solutions should not be used to induce vomiting because the procedure is not a reliable one and there is a great possibility of producing salt intoxication.

TABLE 11.1 Mechanisms and Examples in Antidotal Therapy

I. Decrease the ascending slope of the time–response curve
  A. By influencing the rate of absorption of material from the gastrointestinal tract
    1. Nonspecific methods
      a. Emetics (apomorphine, syrup of ipecac)
      b. Mechanical-induced emesis (finger in upper pharynx)
      c. Stomach lavage
      d. Chemical neutralization (acid–base neutralization)
      e. Adsorption (activated charcoal)
    2. Specific methods
      a. Formation of less toxic complex

Agent	Antidote	Product
Iron	Sod. bicarbonate	Ferrous carbonate
Iron	Desferroxamine	Chelated iron
Silver nitrate	Sod. Chloride	Silver Chloride
Strychnine	Pot. permanganate	Oxidation product
Nicotine	Pot. permanganate	Oxidation product
Fluoride	Calcium lactate	Calcium fluoride

  B. By influencing distribution or translocation of agent to receptor site
    1. Nonspecific methods
      a. Ion trapping by altering blood pH (may be used when therapy involves correcting acid–base balance)
      b. Substitute alternate binding sites (infusion of albumin)
    2. Specific methods
      a. Produce less toxic product, competitively block metabolic biotransformation

Agent	Antidote	Product or effect
Cyanide	Methemoglobin	Cyanmethemoglobin
Cyanide	Thiosulfate	Thiocyanate
Methanol	Ethanol	Competitive block
Fluoroacetate	Acetate or monoacetin	Competitive substitution
Heparin	Protamine	Complex formation

II. Increase the descending slope of the time–response curve
  A. By increasing the rate of elimination
    1. Nonspecific methods
      a. Hemodialysis
      b. Peritoneal dialysis
      c. Exchange transfusion
      d. Adjust pH and diuresis (alkalinize urine for weak organic acids and acidify urine for weak organic bases)
    2. Specific methods
      a. Enhance excretion or form less toxic product by chelation or complex formation

(*continues*)

TABLE 11.1 (*Continued*)

Agent	Antidote	Mechanism
Bromide ion	Chloride ion	Enhance renal excretion
Strontium, radium	Calcium	Enhance renal excretion
Lead, nickel, cobalt, copper	EDTA	Chelation
Mercury, arsenic, gold	BAL	Chelation
Copper	d-Penicillamine	Chelation
Botulinus toxin	Antitoxin	Complex
Organic phosphate	Pralidoxime	Nucleophilic enzyme reactivation
Acetaminophen	*n*-Acetylcysteine	Less toxic metabolite

III. Elevation of the threshold for toxicity in the time–response curve
  A. By clinical support of vital functions or the use of pharmacologic antagonistic agents
    1. Nonspecific methods
      a. Mechanical artificial respiration to maintain oxygenation of blood or hyperbaric oxygen
      b. Maintain circulation of blood (counter shock therapy, plasma expanders, vasoconstrictors)
      c. Maintain electrolyte balance
      d. Maintain renal function
    2. Specific methods
      a. Use of pharmacologic antagonists or alternate pathways

Agent	Antidote	Mechanism
Morphine	Naloxone	Antagonism
Carbon monoxide	Oxygen	Antagonism
Dicumarol, Warfarin	Vitamin K	Antagonism
Organophosphates	Atropine	Antagonism
Curare	Neostigmine	Antagonism
Methotrexate	Folinic acid	Alternate pathway
5-Fluorouracil	Thymidine	Alternate pathway
6-Mercaptopurine	Purine	Alternate pathway
Lysergic acid diethylamide	Phenothiazine	Antagonism

In addition to the actual physical removal of an offending chemical from the stomach, virtual removal may be accomplished by the use of substances that effectively react with, firmly bind with, or adsorb the offending chemical. When the identity of the offending chemical is known, specific agents

may be administered orally for this purpose. Examples are the administration of chelating agents such as calcium ethylenediaminetetraacetic acid ($CaNa_2$–EDTA) for chelation of metals, or the administration of activated charcoal to adsorb a variety of compounds. Chemical neutralization or chelation results in detoxication of the offending chemical. The adsorbent property of activated charcoal varies considerably depending upon the agent to be adsorbed (Table 11.2). Adsorption is not the same as chemical destruction, since adsorption may lead to release of the offending chemical as the pH of the environment changes during passage of the material through the gastrointestinal tract. Regardless of these factors adsorption *in* the stomach of material that otherwise might be absorbed *from* the stomach results in a delay of translocation of the offending chemical.

The "universal antidote" (one part magnesium oxide, one part tannic acid, and two parts activated charcoal) has been used as a combination of agents to perform several functions, since magnesium oxide neutralizes acids without gas formation and tannic acid forms insoluble salts with certain alkaloids and metals. However, it has been shown that tannic acid or magnesium oxide interferes with the adsorbent activity of activated charcoal. It is also known that if tannic acid is permitted to be absorbed,

TABLE 11.2 Amounts of Various Substances Adsorbed from Aqueous Solutions by 1 g of Activated Charcoal (Carbo. Med. Merck)

Substance	Maximal adsorption (mg)
Mercuric chloride	1800
Sulfanilamide	1000
Strychnine nitrate	950
Morphine hydrochloride	800
Atropine sulfate	700
Nicotine	700
Barbital	700
Salicylic acid	550
Phenol	400
Phenobarbital sodium	300–350
Aprobarbital sodium	300–350
5,5-Diallyl barbital sodium	300–350
Hexobarbital sodium	300–350
Cyclobarbital calcium	300–350
Alcohol	300
Potassium cyanide	35

Note. Data from Gosselin and Smith, *Clin. Pharmacol. Ther.* **7**:282, 1966.

it is capable of producing liver toxicity in some species of animals. Thus, it would seem that the mixture of agents used in the "universal antidote" defeats the purpose of attempting to achieve a universal effect by combining several antidotal agents in one preparation, and exposes the animal to at least one additional potentially toxic agent.

Obviously the use of procedures that either actually or virtually remove the offending chemical requires that profound emesis has not already occurred and unabsorbed amounts of the offending chemical are believed to be in the stomach. The interval of time between ingestion of the offending chemical and the use of these procedures should be reasonable in order to ensure that the agent is still in the stomach, since that portion of the chemical which has passed into the upper intestine would be beyond immediate reach by the procedure.

There is only a limited amount of clinical evidence to establish the effectiveness under practical conditions for most of the procedures that have been described. A rational approach to the use of these procedures would be that if there is good evidence, on the basis of history and physical examination of the subject, to support the belief that potentially lethal or seriously excessive quantities of an agent had been consumed and that some of the chemical was still in the stomach, one or more of the above procedures should be instituted to terminate further absorption of the offending chemical. This rationale would apply even in the presence of serious necrotic destruction of the esophagus or stomach. In the latter case, it would be more appropriate to avoid emetics. Orally administered chemical adsorbents and neutralizing agents would be preferred, to avoid the additional insult and possible perforation of the necrotized esophagus and stomach produced by gastric lavage.

Direct removal of unabsorbed amounts of a potentially toxic material from the intestinal tract is impractical. When unabsorbed material is present in the intestinal tract, procedures are used that are directed toward the prevention of absorption and the hastening of transport of the chemical through the intestinal tract so that it is eliminated in the excreta. Despite the lack of specific information on the effectiveness of such procedures, there is a rational basis for the use of cathartics and adsorbents in these cases. Poorly absorbable, innocuous materials, such as liquid petrolatum which is a solvent for fat-soluble agents and which also has a low-order cathartic activity, may effectively carry a fat-soluble offending agent through the intestinal tract, thereby limiting its absorption. The more rapid-acting cathartics, such as sodium sulfate or magnesium hydroxide, also function to hasten the transit of intestinal contents. When the insulting chemical is a slowly absorbed agent, such procedures may effectively remove the chemical from the body before absorption can be complete.

In addition to the measures used to prevent translocation of toxicants from the intestinal tract to the blood stream, there are antidotal mechanisms that influence translocation of the chemical from the blood stream to effector sites. An excellent example of such a mechanism is demonstrated in the prevention (and treatment) of cyanide intoxication, whereby the use of antidotal agents is directed toward preventing the cyanide from gaining access to the receptor sites. The brief description of cyanide toxicity which follows will help to clarify the concept that an antidotal agent can be more effective in preventing a toxic effect than in treating the toxic effect of a chemical intoxicant.

Although cyanide reacts with a number of metal-containing enzymes, it owes its toxicity primarily to its ability to react and form a stable complex with the iron in ferric cytochrome oxidase. This enzyme is thereby inhibited. Since aerobic metabolism is dependent on this enzyme system, the tissues can no longer utilize oxygen and the tissues suffer from hypoxia. Protection of the animal from the toxic effect of the cyanide is accomplished by diverting the cyanide before it reacts with the cytochrome enzyme. This diversion is accomplished by promoting the formation of additional sources of ferric iron which will react with cyanide, leading to the formation of less toxic cyanide products. The procedure is to administer nitrite, either as inhaled amyl nitrite or injected sodium nitrite, which causes the formation of methemoglobin by promoting the conversion of the ferrous iron in hemoglobin to ferric iron. The reactions are as follows.

$$\text{Hemoglobin } (Fe^{2+}) \xrightarrow{\text{nitrite}} \begin{array}{c}\text{Cyanide}\\ +\\ \text{Methemoglobin } (Fe^{3+})\\ \downarrow\\ \text{Cyanmethemoglobin}\end{array}$$

In addition to the use of nitrite, current therapy of potential cyanide intoxication also involves converting the cyanide to the less toxic thiocyanate. Normally, the body is capable of detoxifying cyanide by converting it to thiocyanate by the action of the liver enzyme rhodanase in the presence of sulfur. In the therapy of cyanide intoxication, the sulfur is made available by administration of sodium thiosulfate and the reaction is as follows.

$$\text{cyanide} + \text{thiosulfate} \rightarrow \text{thiocyanate}$$

Under experimental conditions, dogs can be protected from 20 LD_{50}'s of sodium cyanide by pretreatment with thiosulfate plus nitrite. Thiosulfate plus nitrite therapy effectively alters the translocation and detoxifies the cyanide radical before it can gain access to the receptor sites and thereby effectively decreases the slope of the ascending portion of the toxicity curve shown in Fig. 11.1B.

Whenever biotransformation of a compound results in the formation of a product that is more toxic than the initial compound, blockade or inhibition of the biotransforming system would theoretically reduce the availability of the toxic product. This is an additional system that would favorably influence the slope of the ascending portion of the toxicity curve shown in Fig. 11.1*B*. One example of this is the use of ethanol in the treatment of methanol intoxication. Methanol is prone to produce blindness in humans and other primates. The blindness is due to destruction of the retina and degeneration of the optic nerve. It appears that a metabolite of methanol and not the unchanged methanol is responsible for the blindness. It is also clear that in humans and other primates both ethanol and methanol are primarily oxidized by the same enzyme, namely alcohol dehydrogenase (ADH). ADH is localized most abundantly in the liver and it converts ethanol to acetaldehyde and methanol to formaldehyde with subsequent conversion of the formaldehyde to formic acid. The acidosis created by the presence of formic acid is a major problem, in addition to the blindness involved in the toxicity from ingestion of methanol. Ethanol is the preferred substrate for the enzyme ADH and is metabolized several times more rapidly than is methanol. When both alcohols are present at the same time, they compete for the enzyme and in this manner the rate of metabolism of methanol is suppressed so that the concentration of toxic metabolites is also diminished. Ethanol must be used with caution in any patient severely affected with methanol because both agents are depressant drugs and their depressant effects will summate in direct relation to the concentrations of the agents that are present.

PROCEDURES FOR ENHANCING TERMINATION OF ACTION OR ELIMINATION

In inhalation toxicology, the management of carbon monoxide intoxication in humans is an example of the principle of enhancing the termination of action of an agent as a means of treatment of the intoxication. Carbon monoxide produces toxicity when it is inhaled by virtue of its ability to compete with oxygen in binding with hemoglobin and certain respiratory enzymes, thereby resulting in a diminished availability of oxygen in the tissues. Since the combination of carbon monoxide with hemoglobin and the respiratory enzymes is readily reversible in the presence of an adequate amount of oxygen, treatment of carbon monoxide intoxication involves administration of oxygen. The affinity of oxygen for the hemoglobin receptor site is about 200 times less than the affinity of the same site for carbon monoxide. Therefore, the effectiveness of oxygen therapy is a function of

the concentration of oxygen that is used as the antidotal procedure. The time required to remove half of the carbon monoxide from the blood is about 300 min when the person is allowed to breathe atmospheric air (containing 20% oxygen) that is free of carbon monoxide, whereas by administration of pure oxygen it only takes approximately 80 min to eliminate half of the carbon monoxide from the blood; under hyperbaric conditions of three atmospheres of pure oxygen, 50% of the carbon monoxide will be cleared in 25 min. This same principle applies for any antidotal agent that acts by competitively displacing a toxicant from its receptor so that, in effect, the rate of elimination of the toxicant is increased.

Absorption of a toxic quantity of an agent from the gastrointestinal tract or from topical sites of administration and some degree of equilibrium with the various tissues in the animal occur concomitantly, resulting in the appearance of the subjective and objective harmful effects characteristic of that chemical. Antidotal procedures at this level of intoxication may consist of actual and virtual removal of the offending chemical from the effector sites. If the reaction between the chemical and the effector substance is a reversible one, removal of the chemical can be accomplished by lowering the concentration of the chemical in the extracellular compartments of the animal. Under these conditions actual removal is achieved by enhancement of excretion of the compound from the body.

Since the kidney is the predominant route of excretion of foreign chemicals or their metabolites (except gases, for which the lungs are the primary route of excretion), effective enhancement of excretion of chemicals by the kidneys is accomplished according to mechanisms outlined in Chapter 3. The kidney functions to form urine by the combined processes of ultrafiltration of the blood, active and passive reabsorption of the filtrate, and secretion from the blood to the filtrate. Many foreign chemicals are readily filtered and are reabsorbed by the blood from the filtrate.

The procedure of increasing urine formation by the induction of water diureses enhances the rate of excretion of many foreign compounds only to a minor degree. Diuresis produced by osmotic diuretics, such as urea or mannitol, is somewhat more effective. Urinary excretion of an organic electrolyte, however, can be significantly enhanced by adjusting the pH of the urine to favor ionization of the compound. Therefore, excretion in the urine of a weak organic acid, such as acetylsalicylic acid ($pK_a = 3.5$), is enhanced if the urine is alkalinized (see Fig. 4.1), and excretion of a weak organic base, such as quinine ($pK_a = 8.4$), is enhanced if the urine is acidified. If the pK_a of a compound is known, and provided the undissociated part of the substance is to some extent lipid-soluble, it is possible to predict the effect of altering the urinary pH on the excretion of the compound through calculations as described in Chapter 4. The effectiveness of this

technique has been well established for salicylate and barbiturate intoxication.

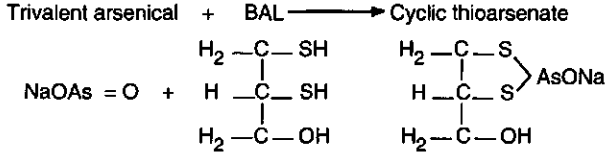
In addition to removal of a chemical from the circulation by enhancement of excretion by the kidney, many chemicals can be removed by the use of chemical–mechanical dialyzing devices, which substitute for or supplement the function of the kidney. Examples are the artificial kidney or peritoneal dialysis equipment. In acute, severe barbiturate intoxication, the artificial kidney is an effective device for the removal of barbiturate from the blood. Because of the limited availability of artificial kidneys, this procedure has limited emergency use. Peritoneal hemodialysis, a much simpler procedure, basically involves irrigating the peritoneal cavity with a dialyzing fluid.

Hemoperfusion is a direct method of lowering the blood concentration of several xenobiotic agents when they are the cause of intoxication. This procedure uses cartridges containing activated charcoal. Arterial blood from a patient is introduced directly into the cartridge so that it comes in contact with the charcoal. Xenobiotics as well as many of their biotransformation products may be absorbed on the charcoal, depending on their affinity for the charcoal. The blood then flows back into the patient's venous circulation. Arterial pressure maintains a continuous flow of blood through the cartridge, resulting in a gradual decrease in the body load of the intoxicant. This procedure has only a limited use in the clinic, but it has been used in the treatment of barbiturate and carbamazepine intoxication. Complications that occur are associated with concomitant removal of blood cells (lymphocytes and thrombocytes) from the blood by the charcoal.

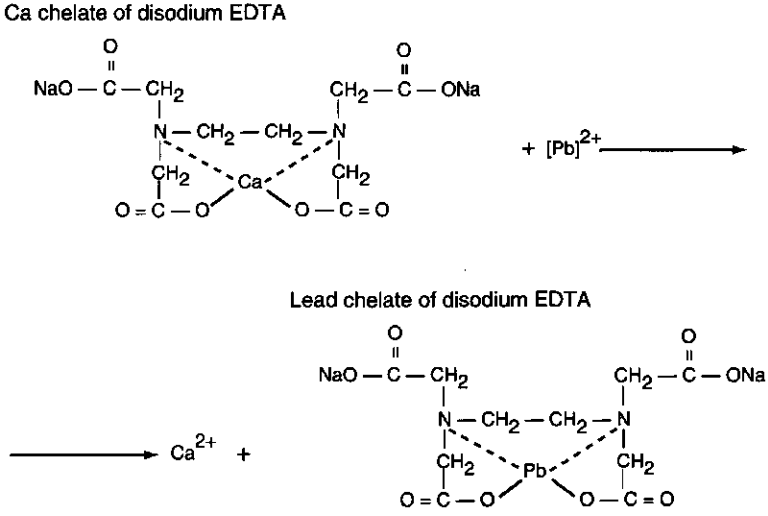
Since many chemicals of toxicologic interest have specific sites of action which are responsible for toxicity of the chemical in the biologic specimen, removal of the chemical from specific sites of action terminates the undesirable effect of the chemical. Removal of the chemical from other binding sites (nonspecific), together with detoxication of the compound by combining it with antidote, inactivates the compound in the animal. It is not necessary to enhance excretion of the chemical from the animal to effectively terminate the action of the compound at the effector site. This type of antidotal therapy, the most effective known, involves the use of antidotal chemicals that directly or indirectly produce less toxic complexes with the offending agent. Examples of such antidotal agents are "British Antilewisite" (BAL, or dimercaprol) and EDTA for the treatment of heavy metal intoxication, and the oximes for the treatment of organic phosphate intoxication.

The heavy metals most commonly encountered in clinical toxicology are mercury, lead, and arsenic. These metals owe their toxicity primarily to their ability to react with and inhibit sulfhydryl enzyme systems involved in several vital processes in the body, such as those involved in the production of cellular energy. Antidotal therapy of such intoxications is based on

either the administration of simple dithiol compounds as exemplified by BAL, or chelation as exemplified by EDTA. BAL and EDTA are capable of forming metal complexes more stable than the enzyme–metal complex. In the case of the arsenic enzyme complex, the product is a monothiol complex whereas the dithiol, BAL, causes the formation of a cyclic thioarsenate according to the following reaction.



In the case of lead intoxication, the use of the calcium chelate of disodium EDTA acts as an ion exchanger in which the calcium is exchanged for the heavy metal ion and a soluble and stable chelate of lead is formed. As a chelate, lead is nonionized, and this chelate is available for excretion by the kidney as a ring complex. This reaction is shown below.



In both examples, the combined form of the metal encompasses an unionized form of the metal. This complex is stable and is a less toxic molecule. Following the administration of EDTA, the rate of excretion of lead as the EDTA complex in the urine of patients intoxicated with lead may increase as much as 50-fold above the normal untreated excretion rate. Therefore, chelation therapy not only serves to detoxify the lead and withdraw it

from the effector sites, but also promotes elimination of the metal from the animal.

One of the most significant developments in antidotal therapy is the adoption of nucleophilic compounds in the treatment of organic phosphate intoxication. Early work in this field indicated that hydroxamic acids would displace phosphorus from the phosphorylated cholinesterase by reacting with the complexed enzyme, and the product would undergo hydrolysis resulting in release of the active enzyme. In 1955 reports appeared demonstrating the high order of effectiveness of pyridine-2-aldoxime methiodide as such a nucleophilic-reactivating agent for phosphorylated cholinesterase.

Several organic phosphate agents are used extensively as pesticides. These agents owe their high order of toxicity in intact biologic specimens to the fact that they readily react with and inactivate enzymes of the cholinesterase type by phosphorylating the enzyme. These enzymes, and particularly acetylcholinesterase, are important in regard to maintenance of impulse transfer between nerve cells as well as between nerve and skeletal muscle cells in most, if not all, species of biologic specimens that have nerve systems. Acetylcholinesterase functions to terminate the action of the neurohumor (acetylcholine) which is liberated at the end of the nerve. When acetylcholinesterase is inhibited by an organic phosphate, the neurohumor (acetylcholine) is believed to accumulate at the nerve ending so that transfer of nerve impulses across synapses at the autonomic ganglia and at the nerve-muscle junctions is prevented.

The sequence of reactions leading to phosphorylation and thus inactivation of cholinesterase by the organic phosphate diisopropylfluorophosphate (DFP), and the reaction involved in regeneration of the phosphorylated enzyme by the use of the oxime pyridine-2-aldoxime methiodide (2-PAM) are shown in Fig. 11.2. In the figure, the enzyme is depicted as having two active sites, one of which is the anionic site and the other an esteratic site. Reaction 1 leads to the formation of the phosphorylated (inhibited) enzyme in which the phosphate is attached to the esteratic site of the enzyme. When the inhibited enzyme reacts with the oxime (Reaction 2), the unoccupied anionic site on the enzyme serves to orient the oxime so that it combines with the phosphorus to form a product that undergoes hydrolysis (Reaction 3), resulting in liberation of a regenerated active enzyme. The oxime therefore functions as an antidotal agent by displacing the phosphorus from the enzyme. In the absence of additional organic phosphate, which could in turn rephosphorylate the enzyme, oxime therapy will return the cholinesterase-dependent physiological mechanism to normal.

The use of biologic antibodies (antitoxins) as antidotal agents is an important area in antidotal therapy but it has been generally applicable only in cases involving bacterial toxins. However, in the mid 1970s an

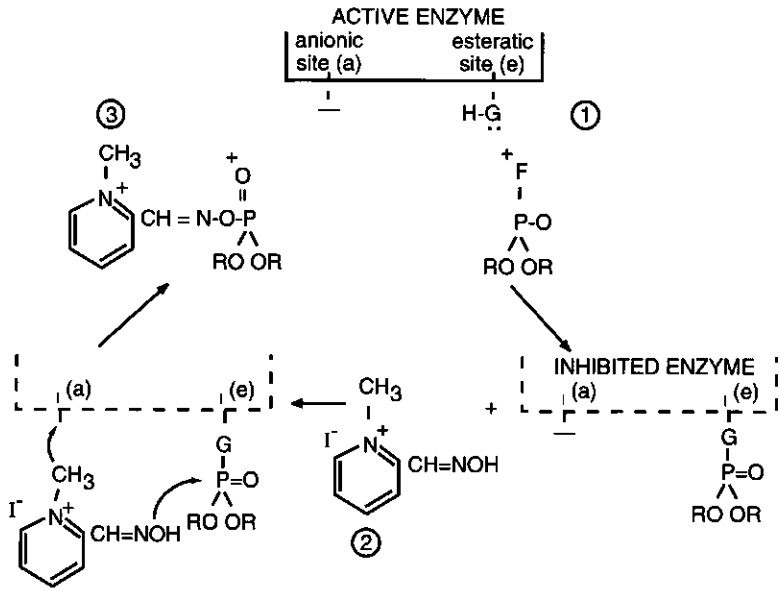


FIGURE 11.2 Diagrammatic illustration of inhibition of acetylcholinesterase (enzyme) with diisopropylfluorophosphate (Reaction 1) and reaction of the phosphorylated enzyme with 2-pyridine aldoxime methiodide (Reaction 2) and hydrolysis of the product of Reaction 2 which results in regeneration of active enzyme (Reaction 3). (Adapted from concepts of Wilson, I. B., *J. Am. Chem. Soc.* **77**:2385, 1955; and Jandorf, B. J., *J. Am. Chem. Soc.* **78**:3686, 1956.)

antibody fragment was prepared and successfully used in the treatment of digoxin intoxication. The antibody to digoxin was prepared by immunizing sheep with a digoxin–serum albumin conjugate. The immune globulin that was produced in the blood of the sheep was cleaved into fragments, one of which was found to have a high affinity for digoxin. The digoxin–antibody complex is excreted in the urine, thereby lowering the body load of digoxin. Currently it is the recommended antidote for digoxin intoxication. It is commonly known as the Fab antibody, that is, the Fragment a b antibody.

PROCEDURES FOR ELEVATING THE THRESHOLD OF TOXICITY

According to Fig. 11.1D, elevation of the threshold of toxicity without changing the concentration of the chemical at the effector site would abolish the toxicity of an offending chemical. Elevation of the threshold of toxicity is practically achieved with antidotal chemicals in two ways. One is to

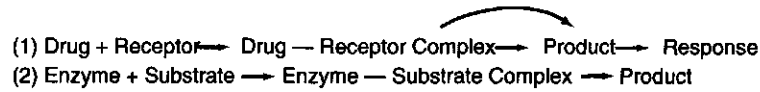
directly antagonize the system affected by the toxicity through enhancement of a physiologically opposing system. The other method is to use one of several drugs known to actually block the response produced by the presence of other drugs by an action on the same physiological mechanism. These mechanisms are clarified in the following paragraphs by use of examples.

The presence of physiologically antagonistic systems is common in most animals. Antagonistic muscle systems enable movement of structures and antagonistic nerve systems enable excitation or inhibition of organ systems. Drugs have been designed with the specific objective of having an effect on one or more of such antagonistic systems. The proper use of drugs that affect opposing physiological mechanisms for antidotal therapeutic purposes involves an extensive understanding of pharmacology. These drugs have known mechanisms of action and usually, when used as antidotes, they are used for the purpose of controlling harmful symptoms.

Generally the use of drugs that affect antagonistic physiological mechanisms is of greater academic interest than it is of practical value. This is probably because it is technically difficult to titrate a drug effect on one system against an existing effect of a chemical on an opposing system. Therefore, although there is considerable evidence that under highly controlled laboratory conditions, severe respiratory depression induced by barbiturates can be effectively antagonized by drugs such as picrotoxin or pentylene tetrazol, it has been demonstrated that under clinical conditions respiratory depression is more efficiently controlled by mechanical support of respiration.

In contrast to this, a simple fall in blood pressure that occurs following severe barbiturate intoxication can be effectively antagonized by intravenous administration of norepinephrine, both in the laboratory and in the clinic. Each of these examples involves the use of a drug that stimulates a physiological mechanism that is antagonistic to the system affected by the offending chemical. In each case, the offending chemical continues to exert its effect at its site of action, but the effect is overcome by the effect of the antidotal agent on an opposing physiological system.

In contrast to the pharmacologic antagonists that act on opposing physiological mechanisms, there are many drugs whose pharmacologic effect can be specifically blocked by an action of a second drug on the same mechanism. Insofar as antidotal concepts are concerned, the basis for drug action is that drugs that affect physiological mechanisms produce their effects by combining with "receptors." The drug–receptor combination either directly induces a response or initiates the formation of a product which causes a response. These reactions are analogous to conventional enzyme substrate reactions as follows.



Obviously the drug (or foreign chemical) action can be prevented if the drug is displaced from the receptor or if the same chemical sites on the receptor are occupied by an antidotal substance that does not initiate a physiological response.

An example of the competitive type of antidotal therapy is the use of naloxone as an antidote to the effects of morphine on respiration. In this case, morphine reacts with the receptor (respiratory center in the brain) and the product of this reaction produces the respiratory depression. Theoretically naloxone also reacts with and displaces morphine from the same receptor, but the product of this reaction has considerably less respiratory depressant effect. By a similar mechanism, morphine-induced narcosis can be reversed by the antidote and withdrawal symptoms can be induced in morphine addicts by the administration of naloxone.

Another example of displacement of one drug from a receptor by another is the use of vitamin K as an antagonist to the toxic effect of the anticoagulant drug Dicumarol. The receptor for Dicumarol in this instance is an unidentified enzyme system, which is present in the liver and for which vitamin K is the normal substrate. Vitamin K plus the enzyme yields an enzyme–substrate complex that is normally essential for the production of certain proteins, such as prothrombin and convertin, which are necessary for the normal coagulation of the blood. Dicumarol reacts with the same enzyme system, but this reaction fails to produce the proteins necessary for the coagulation of blood. Therefore, excessive doses of Dicumarol would create a tendency to hemorrhage from only minor injuries, or would even cause spontaneous hemorrhage.

A sufficient amount of vitamin K is an effective antidote to Dicumarol-induced hemorrhage, since it will compete with and displace Dicumarol from the enzyme complex and reestablish normal formation of the coagulation factors of the blood. Thus, vitamin K and Dicumarol appear to be mutually antagonistic on the receptor (enzyme) mechanism on the basis of a displacement by mass action and affinity for the enzyme system. In pharmacology, such antagonistic chemicals are referred to as competitive antagonists on an enzyme system.

In the foregoing two examples, the offending chemical is not detoxified. Rather, the concentration representing the threshold at which they are capable of producing a harmful effect is elevated by the presence of the antidotal agent.

An additional example of the use of the antidotal chemicals to elevate the threshold of toxicity is the use of atropine as an antidote to certain responses in intoxication with muscarine, which is partially responsible for one type of mushroom (*Aminata muscaria*) poisoning. One theory of the mechanism of induction of toxicity from the alkaloid muscarine is that muscarine reacts with the same receptor through which acetylcholine induces its effect. The muscarine–receptor complex initiates physiological responses that are similar to those induced by acetylcholine at the autonomic nerve endings, such as slowing of the heart, decrease in blood pressure, excessive salivation, and constriction of the airway in the lungs.

These effects of muscarine can be prevented by the administration of atropine which combines with the same receptor. Atropine either displaces the muscarine from the receptor or prevents the muscarine–receptor complex from producing a product that is in turn responsible for the physiological effects. Atropine does not directly react with, inactivate, or detoxify the muscarine molecule. Rather, the concentration of muscarine necessary to reach the threshold at which the toxic response occurs is greatly elevated by the presence of atropine. This concept is supported by experimental evidence obtained under laboratory conditions, which indicates that the pharmacologic effects of muscarine can be titrated against those of atropine.

A popular misconception is that concerned with the general use of chemical agents for specific antidotal therapy. There is no single agent or combination of agents that can be used as a universally effective chemical antidote. Some of the specific antidotal chemicals have been discussed in the foregoing examples. Such agents are used only when a diagnosis can be made in which the agent responsible for the toxicity can be absolutely identified. Even then, only in a relatively few cases is the toxicant one for which a specific antidotal agent is available for immediate use except in a few emergency clinics. In many cases of chemical intoxication in humans the causative toxicant can only be suspected on the basis of a history of possible exposure and the presence of certain symptoms. Of all cases of chemical intoxication encountered clinically, specific chemical antidotal therapy is possible for only relatively few.

No specific antidotal agents are known for the large majority of the commonly encountered harmful chemicals. In the absence of specific antidotal agents or definite knowledge of the nature of the chemical involved in a specific case, treatment of chemical intoxication is primarily directed toward limiting absorption of the chemical by either removing the chemical from the subject or removing the subject from the exposure to the chemical (as in the case of contaminated environments) and by supporting the vital functions of the body (such as blood pressure and respiration) as needed so that as time passes, normal detoxication and excretion mechanisms

terminate the presence of the offending chemical in the body. Furthermore, even under those conditions for which specific antidotal chemicals are known, improperly used antidotes are also capable of producing harmful effects. Therefore, employment of physiologically active antidotes is subject to their knowledgeable use, so that the objectives of therapy are achieved without induction of added chemical injury. The use of antidotal chemicals is therefore restricted to those cases in which irreparable damage or death is imminent and in which normal detoxication and excretion mechanisms are unable to cope with the excessive quantities of the harmful chemical.

CHAPTER 12
Principles of Biological Tests for Toxicity

GENERAL PRINCIPLES

Toxicology has been defined as the study of the effects of chemical agents on biological material with special emphasis on harmful effects. It basically involves an understanding of all effects of essentially all chemicals on all types of living matter. There is ample evidence to indicate that every chemical is capable, under some conditions, of producing some type of effect on every biological tissue. Toxicologic tests are therefore the tests that define the conditions that must be present when a biological cell is affected by a given chemical entity, and the nature of the effect which is produced. As far as the conditions that must be present are concerned, they may vary from being practically unattainable in ordinary circumstances to being so readily attained that simple exposure of living tissue to certain chemicals produces destruction of the cells. As far as the nature of any effect of a chemical on living tissue is concerned, effects may be of such minor significance that the tissue is able to carry on its ordinary function in a normal manner so that it is only under conditions of stress or critical tests that a chemical-induced effect is even detectable. Effects may result from small amounts of some chemicals whereas large amounts of other

compounds may be required to produce any positive untoward finding. Generally it is a simple matter to separate those relatively few chemicals that in small amounts produce prompt effects that are distinctly harmful to living cells from those that are practically harmless when exposure is over a short period of time, but it becomes difficult to demonstrate that small amounts of some compounds do not produce some types of toxicity when animals are exposed over a long period of time.

Most of the biological methods which have been developed in toxicology are the result of the practical need to obtain as much information as possible about the effects of chemicals insofar as they may be pertinent to man's continued physical well-being. The continuing economic progress of the human race has been accompanied by a continuing increase in the numbers of chemical entities to which man is either intentionally or unintentionally exposed. A person may be exposed through direct industrial or domestic occupational contact, through contact with the clothes or devices he wears, the food, liquid, and drugs he consumes, and the atmosphere he inhales. It is necessary not only to understand the toxicities that can occur but also to obtain assurance that exposure of man to large numbers of chemical entities will not lead to obvious direct or insidious indirect detrimental effects. Consequently it is essential that some toxicity data be acquired on all chemical agents. For those chemicals which are to be intentionally administered to man, such as food additives, food substitutes, or drugs, it is necessary to obtain as many toxicity data as is economically possible.

Because of the moral, ethical, and legal restrictions regarding the use of humans for experimental purposes in order to acquire toxicologic data, only limited amounts of such data are available. Information regarding the effects of chemicals on humans is obtainable only after a chemical is used by humans or from limited types of experimental procedures that may be conducted on humans. Biological methods in toxicology therefore generally involve the use of expendable species of animals on the hypothesis that toxicity studies in suitable species have an extrapolative value for man.

Several of the procedures involved in testing for toxicity involve the use of nonmammalian species and even cell cultures. It would be of great advantage to be able to utilize such species as bacteria, neurospora, daphnia, drosophila, the various echinoderms, or fish for evaluation of toxicity because of the economic advantage and abundance of such populations of living cells. Furthermore, some of these species lend themselves to accurate and simple procedures such as those that make use of their accurately defined and measurable genetic characteristics, reproductive processes, and enzymatic performance. The main drawbacks associated with the use of such species are the dissimilarities in translocation barriers as compared to man and differences in or the lack of biotransformation mechanisms that are present in man. These

factors preclude extrapolation of the data obtained on most nonmammalian species to man. Nevertheless such tests serve the purpose of alerting the investigator to potential toxic hazards which then can be further studied in mammalian species. When any chemical is used in massive quantities such as in agriculture and becomes available in the general environment, it is necessary to evaluate the toxicity of that agent in many species which may directly or indirectly influence the overall welfare of man.

It should be recognized that there are many variations in both short- and long-term chemical-induced toxicity among various mammalian species of animals; however, careful and complete evaluation of the effects of chemicals on animals has been shown to be the most rational, acceptable, and successful means of determining most types of toxicity for purposes of extrapolation to man.

PRINCIPLES OF EXPERIMENTAL TOXICOLOGIC METHODOLOGY

The principles of toxicologic methodology are based on the premise that all effects of chemicals on living tissues are the result of a reaction with or interaction between any given chemical entity and some component of the living biologic system. This initial reaction may not be evident. The result of this reaction is manifested as an effect on the function, and in many cases the structure, of the biologic system. The effect on function may not necessarily be accompanied by a detectable change in structure of the biologic system, that is, it may be only a biochemical lesion. The effect may be reversible in the absence of continued exposure to the chemical or it may result in death of the cell. The study of toxicological methods is centered on the detection and evaluation of the nature of the chemical-induced changes in function and structure and the significance of these effects on living cells. Since all effects of chemicals on living systems are not necessarily harmful effects, a principal function of the science of toxicology is to identify clearly those chemicals capable of producing serious harm to living systems. As a science, toxicology has developed a methodology to detect chemical-induced alterations in function and structure of living systems, to investigate many of the factors that determine how chemicals gain access to biological cells, to establish the parameters of the conditions under which various chemicals do or do not produce biologic effects, and to define the mechanisms by which chemicals interact with the various components of living systems in order to directly or indirectly produce toxic effects.

As a result of the development of this toxicologic methodology, certain general principles have become apparent. These principles apply to many, and perhaps all, toxicologic test procedures. They are as follows:

1. In order for a chemical to produce a biologic effect, it must come into immediate contact with the biological cells (or receptors) under consideration.
2. For each chemical there exists a quantity below which it produces no detectable effect on all biologic systems, and a quantity at which it produces a significant effect on all biologic systems. Between these extremes lies the range of quantities at which each chemical will exert a significant effect on some types of biologic systems.
3. Cells having similar functions and similar metabolic pathways in various species generally will be affected similarly by a given chemical entity.
4. Small changes in the structure of a chemical agent may greatly influence the biological action of that agent.

These principles have been introduced in previous chapters and will be considered here only in regard to how they contribute to an understanding of the various test procedures that are used in toxicology.

TRANSLOCATION FACTORS IN TOXICOLOGIC TESTS

The discussion in Chapter 3 has defined the main factors that are involved in the transfer of a chemical to various compartments within an animal. The principle which states that chemicals must come in contact with the biologic system in order to have an effect on the system would be a simple and obvious one if all biologic systems consisted only of a solution of living material in some universal solvent. Such is not the case, since even the simplest single living cell consists of both suspended and dissolved material in a highly selective solvent, all of which are encased in a membrane. The total complex is characterized by its ability to carry on a series of functions that make it a "living cell." In progressing from the single-celled organism to the multicelled organism and on to the many tissue and multiorgan type of biologic specimen, particles within cells become encased in membranes so the membranes increase in number, cells become encased in organs and the organs encased in other tissue so that the final biologic form becomes a protected unit that lives within its own internal and usually closely regulated environment. In order for a foreign chemical to exert an effect other than at its site of application, it must gain access to the various parts of this internal environment. Therefore it must necessarily be translocated to the various parts of the animal, tissue, or cell.

In a complex biologic system such as man, those chemicals which are absorbed neither through the skin nor from the gastrointestinal tract would be innocuous if applied to the skin or consumed by mouth except for

whatever action they may exert on the skin or in the gastrointestinal lining. In contrast to this, if such a chemical were injected through the skin or into the bloodstream, or inhaled into the lungs, and if it were translocated to all cells in the internal environment, it could potentially exert an effect at essentially any site within the body. In the case of those chemical agents that are used as drugs for therapeutic purposes, the biologic barriers to translocation of the agent may be so effective that in order to get a therapeutic effect from the drug, it must be administered by injection methods. Therefore, the methods used for a reasonably complete evaluation of the toxicity of any chemical agent necessarily involve administering the agent to the experimental animal by a variety of routes. The route by which the agent would be expected to come in contact with the biologic specimen under the conditions of practical use of the chemical would necessarily be included in the test procedure. That is, experimental programs using animals involving the route of intended use of any chemical would be expected to provide the most acceptable data for practical extrapolative use to man if the compound is intended for use by man. When new chemical entities are not intended for use by humans, the experiments of greatest practical value are those that simulate the routes by which accidental exposure to the chemical could occur if the intention of the experiments is to obtain data to evaluate toxicity or ensure safety to man.

Certain compounds commonly are referred to as being biologically inert, but such compounds are inert only in the sense that under ordinary conditions they do not gain access to the cells. Usually this is because they are insoluble in biological fluids. In such cases, these chemicals, if present in sufficient concentration, still could affect cells in a mechanical traumatic manner. Examples of such agents are the metals, natural minerals, and highly nonreactive plastic polymers. However, agents which are insoluble in the biological fluids, if implanted in a biologic specimen, will elicit some response in the cells even if it is no more than a response to a "foreign" body. The principle under consideration is intended to be valid only under the condition that the chemical entity is soluble to some degree in biological fluids. Its action on cells may be basically nonspecific, that is, changing the total ion strength or pH, or simply occupying space. Obviously, in a multicelled organism, any effect of a chemical on one type of cell may indirectly influence other cells in the organism since the organism must maintain all cells in a viable and normally functioning state if absolute, total, normal function is to be maintained.

The chemical agents that are of significant toxicologic interest are usually compounds which are soluble in the fluid phases of biologic systems and are therefore potentially available to the cells. The solubility of such compounds in body water may be very small. Although in all biologic systems

the fluid phase is mainly water, the systems also contain protein and lipid or lipid-like material as well as a number of inorganic ions. The protein may loosely bind to the chemical and the lipid may serve as a significant solvent for the transport of the chemical agent involved. In fact, most drugs are weak organic acids or bases, and as such are frequently soluble in lipids. Furthermore, in many animals such lipid-soluble agents frequently are biotransformed by oxidative enzyme systems to water-soluble derivatives which may have either more or less toxic potential than the parent compound. The study of the toxicity of any chemical entity is necessarily a study of any products which may arise as a result of changes (biotransformation) in the chemical which are induced by the test animal. In this manner, although some degree of solubility of any chemical agent in body fluids is essential if it is to be translocated, the compound does not necessarily have to be soluble in water. Such agents may be carried on protein, dissolved in lipids, or biotransformed to different derivatives which have different solubility characteristics. The general principle under consideration stipulates that under some conditions all cells will be affected by chemicals which come in contact with them. The effect which is manifested may be different in different cells, just as the concentration of the compound which is necessary to produce an effect may be different in different types of cells.

CONCENTRATION–RESPONSE FACTORS IN TOXICOLOGIC TESTS

The second general principle is concerned with the fact that there is some minimal amount of each chemical agent below which there is no effect on biologic systems and that there is a greater amount of each chemical at which a significant effect will be present on essentially all living systems.

Whenever the effect of a chemical is determined on a single experimental animal, two types of data may be obtained. One of these is the all-or-none type of data in which an effect is either present or absent (e.g., the animal either lives or dies). The second type of data is the graded response type in which an effect is present but may be of a specific intensity, such as impairment of some type of performance, decrease in heart rate, or even an increase in the number of tumors. The latter type of effects must always be quantitated. This is frequently done for any given effect in terms of percentage of normal or in terms of incidence of occurrence in a given group of test animals. The more sophisticated the experiment the more the graded effect may be quantitated. In either type of experimental data, the information is quantitative in nature.

Toxicologic experiments are generally not conducted on a single animal. Whenever an experiment is conducted, it usually involves the use of a selected number of experimental animals (or biologic preparations) rather than a single animal. Also, the quantity of the chemical agent under consideration which is administered to the animals will be varied in different groups of the animals. Therefore, all experimental toxicologic data are obtained by administering a series of progressively increasing amounts of a chemical to different groups in which each group contains specific numbers of experimental biological preparations or whole animals. When this is done under actual laboratory conditions and data regarding effects of the chemical are obtained, the data consist of measurable responses in each group from each selected quantity of the chemical. Regardless of the chemical under consideration, if an all-or-none type of response, such as lethality, is one datum which is obtained, there will be some minimal quantity below which none of the animals in the group will die. At some intermediate quantity, a portion of the group of animals receiving that dose will die, but not all of them will die. Therefore, although this latter quantity produces an all-or-none response, in each test the data represent a graded response when groups of animals compose the test population. This variation in response between biologic test specimens within a given species is well established. It is generally referred to as "biological variation" and is to be recognized as being different from "species variation" in response to chemicals.

Toxicologists are accustomed to administering chemicals to experimental animals in specific quantities at specific intervals of time, and the quantity is referred to as the "dose" of the chemical. The terminology used to identify doses varies with the type of chemical. If the chemical is a solid, it is administered to the test animals in terms of weight, or it may be dissolved in a solvent such as water whereby the quantity is then defined as given volume of a known concentration of the chemical in solution. If it is a liquid, it may be in terms of weight or volume of the chemical. If it is a gas, it is usually in terms of volume or concentration of the gas in a mixture of gases, such as air. Furthermore, the quantity is further defined in terms of quantity per unit weight or per unit of body surface area of the test animal. Occasionally the interval of time over which the dose is administered is necessarily part of the dose terminology. Conventional examples of doses are grams or milligrams per kilogram of body weight or per square meter of body surface area, milliliters or cubic centimeters of a solution per kilogram of body weight or per square meter of body surface area, or parts per million (ppm) or millimoles per liter of a gas in air or other gases.

When comparative dose–response relations are made between species of animals and man, there is some evidence that the best correlation is obtained by comparing doses on the basis of body surface area rather than on the basis of animal weight. Table 12.1 compares some of the more common experimental laboratory animals with man in regard to the effect of their relative weights and body surface areas on the dose of certain chemical agents of toxicologic interest.

If there were no biological barriers or other factors which would affect the equal distribution of a chemical throughout a biological specimen, then the quantity of the chemical represented by the dose would be related directly to the concentration of the chemical in most compartments of the specimen. Although this is true for some chemicals it is more frequently not true for the majority of chemicals, particularly when the test specimen consists of a whole animal. The factors that influence distribution of a dose of a chemical in the animal are:

1. Membranous barriers to translocation.
2. Selective storage depots such as binding to proteins or selective partitioning into lipids.
3. Concentration-dependent metabolic inactivation.
4. Excretion.

These factors are responsible for a continuous variation in the distribution of a chemical in the various compartments of an animal so that at any given time there may be great differences in concentration of the chemical

TABLE 12.1 Relative Sensitivity of Man as Compared to Some Common Laboratory Animals[a]

Dose on the basis of body weight (mg/kg)		Dose on the basis of body surface area (mg/m^2)
MTD in man = 1/12 LD_{10}	or	3/4 LD_{10} mice
MTD in man = 1/9 LD_{10}	or	5/7 LD_{10} in hamsters
MTD in man = 1/7 LD_{10}	or	6/7 LD_{10} in rats
MTD in man = 1/3 MTD	or	1 MTD in rhesus monkeys
MTD in man = 1/2 MTD	or	1 MTD in dogs

Note. Toxic dose levels of anticancer drugs when the dose is calculated according to body weight or according to body surface area. Data compiled from retrospective studies reported by Pinkel *et al., Clin. Pharmacol. Ther.* **11**:33, 1970, and from Paget, G. E., *Clinical Testing of New Drugs,* Herrick and Cattell, Eds., p. 33, Revere Publishing Company, New York, 1965. MTD, maximum tolerated dose. LD_{10}, lethal dose for 10% of the animals.

between the various body organs and compartments. However, when a chemical is administered to several members of the same species, the various listed factors may be assumed to be similarly operative in each member of the group. Therefore, if the same dose is administered to more than one member of a single species of animals, that dose may be assumed to distribute itself similarly throughout each compartment within each member of the species. (Unequal distribution between the compartments of two apparently identical members of the same species is probably the principal mechanism which is involved in the phenomenon of biological variation.) The result is that although any biologic effect that is induced by a chemical is related directly to the concentration of the chemical at the site where it can produce its effect, in a given species the concentration is related directly to the dose and therefore there will be a direct relation between dose and effect.

Common usage involves correlation of the effects of chemicals in terms of dose rather than in terms of concentration of the chemical in the animal. In many animal toxicologic studies, the concentration of the chemical at the site where it produces its effect is not known. Therefore, conventional data consist of dose versus effect types of data, that is, dose is plotted versus intensity or incidence of response in the same manner that a concentration–response curve is derived.

At this point it is important to point out that there are some examples of chemical agents that represent exceptions to the concept which has been stated as follows: "It can be readily demonstrated that there is a direct relationship between the frequency of occurrence or intensity of any measurable effect of the chemical on the biological system and the dose or concentration of the chemical agent which is present." The exceptions are uniformly those chemicals that are normally present in the animal. Since the internal environment of all living cells is a rather specific chemical environment involving the presence of specific concentrations of ions and nutrients essential to the life processes of the cells, it follows that depletion of the cells of these substances will lead to harmful consequences or toxicity to the cells. It also follows that there will be a direct relationship between the concentration of the chemical present and the harmful effect on the cell only when the concentration of the chemical is greater than that normally present in the cell. As an example, sufficient depletion of potassium in an animal will produce cardiac arrest and death of the animal and elevation of the depleted potassium concentration to normal will abolish the effect on the heart and prevent death. In contrast to this, if the concentration of potassium is sufficiently elevated above normal, cardiac arrest and consequently death of the animal will occur. In fact, some of the metals that are essential for normal function of animals become known primarily for their toxicity. A good example is selenium which occurs in abundance

in the environment and is a cause of illness in animals that eat forage contaminated with salts of this metal. The point to be made is that the response to endogenous chemicals is related directly to the concentration of the chemicals when the concentrations are above normal and indirectly related to the concentrations of the chemicals when the concentrations are below the normal concentration present in the healthy animals. Thus the principle as initially stated is intended to apply to all exogenous (foreign) compounds and to endogenous compounds only when the concentration is greater than normal. As analytical capabilities improve and pharmacodynamics of chemicals and biological systems are better understood, concentration versus effect types of data will become more prevalent, particularly as mechanistic studies are initiated as part of, or complementary to, classical testing protocols.

The dose–response relationship is plotted conventionally by statistical methods as dose of the chemical versus the incidence of occurrence or intensity of an effect. Separate plots are made for experimental data obtained on different species or strains within a species. Figure 12.1 is an example of a log dose–probability plot such as that which would be obtained when lethality of a compound is obtained experimentally following administration of various doses of a chemical to a series of groups of mice. The significance of the slopes of concentration–response relationships is considered elsewhere (see Chapter 2). Regardless of the biological function which is measurably influenced by the chemical, under the practical conditions of an experiment, the proximate intersect of the concentration–response curve of the abscissa (concentration) does not establish the absolute absence of the effect. Obviously the number of animals involved in the test and the degree of experimental perfection in detecting a biologic effect will determine the confidence of the test and therefore the statistical point of intersection of the dose–response curve with the abscissa. However, since the plot is derived statistically, the intersect with the abscissa is a virtual intersect obtained by extrapolation to a proximate zero-effect.

The experimental estimation of the zero-effect or no-effect dose (more properly stated as the no-apparent-effect dose) is the information needed for estimation of the order of safety of any compound under investigation. The experimental demonstration of safety based on demonstration of absence of toxicity is in practice dependent on the nature and type of toxicity under investigation.

TOXICITY VERSUS SAFETY—EXPERIMENTAL CONCEPTS

The principle which states that there is some concentration below which all chemicals are without toxicologic effect, achieves considerable impor-

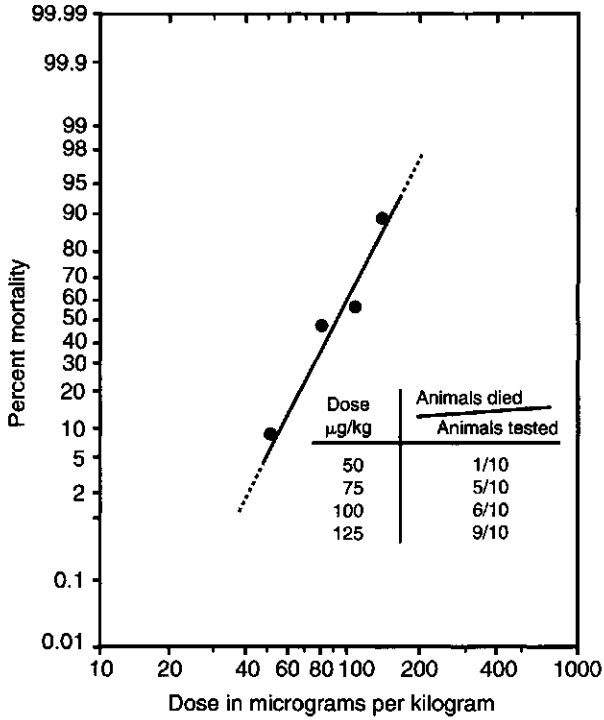


FIGURE 12.1 Lethality in male mice plotted on dose probability coordinates. The compound is hemicholinium-3, a neuromuscular paralyzing drug. The compound was administered intravenously in a tail vein. The animals were followed for 24 hr after the injection. Data were obtained in one of the author's laboratory.

tance in the design of biological tests for evaluation of safety. In fact, the existence of a threshold toxicologic dose or in other words a no-effect level or concentration of a chemical is a continuing argument as far as some forms of toxic effect are concerned although the argument, in the authors' opinion, is valid for all forms of toxicity. The controversy exists primarily because toxicity tests are conducted not only for the purpose of demonstrating the existence of toxic effects but also to estimate the limits of safety associated with the use of a compound. The problem then centers on the need to establish absence of effect beyond the level of confidence that can be achieved by ordinary experimental techniques which always involve the use of limited numbers of experimental animals.

In any attempt to establish a no-effect level or concentration of a chemical in any toxicologic test procedure, it is important to define what is meant by "effect" and the confidence that can be placed on the result of the test

procedure. Basically, in order to have any toxicologic action from a chemical, there must occur some measurable phenomenon that is detrimental to the experimental animal, and it must occur within some meaningful period of time after the animal is administered the chemical. If the test animals are mice and the effect to be measured is death or any other form of toxicity of the animals, then it must be stipulated that the effect must occur within a certain period of time after administration of the chemical. The time interval could necessarily be any interval up to and including the life span of the mouse. If the effect under consideration is that of determining the influence of the chemical on the longevity of mice, the experimental time interval must necessarily be extended to the life span of the mouse. Therefore, every toxicologic experiment must necessarily define what effects are to be studied and the time interval over which they will be studied.

Since all chemicals under some conditions of use will produce toxic effects in biological sample material, it is evident that experiments usually can be designed that will show such toxic effects. In fact it is stated frequently that a good experiment is one which does demonstrate some kind of toxicity at least in the group of test animals that received the highest dose concentrations. However, even in the experiment which demonstrates a positive toxic effect the data must include adequate *negative control* information if the toxicity is to be conclusively considered as being produced by the test chemical. This means that an identical (negative control) group of animals as large or larger than the experimental group should be treated similarly to the experimental group in all respects except for omission of the chemical under test. All procedures that are performed on the test group must be performed on the control group. If a solvent is used to administer the chemical to the test group, the same solvent without the chemical should be administered to the control group. Control groups from prior experiments should not be used. These procedures should be standardized in all laboratories. In very long-term toxicity tests it also is important that a single adequate supply of the test chemical be tested and identified for purity and any presence of contaminating chemicals. The chemical should be available in sufficient quantity and properly stored for use during the entire test period. The numbers of animals used in the experimental test should be the numbers needed to yield statistically reliable results. In this manner the data from the experimental group can be ultimately compared with the data from the control group. The actual number of animals necessary to establish a cause–effect relationship is dependent on the incidence of occurrence of the toxic effect in the controls as compared to the test animals. Table 12.2 summarizes the number of animals needed in each group when there is no effect on the controls.

TABLE 12.2 Minimum Incidence of a Toxic Effect in a Series of Different Sized Groups of Animals That Can Be Considered Significant at a Probability Level of 0.025 When the Effect Is Absent in Controls and When Equal Numbers of Experimental and Control Animals Are Used[a]

Number of animals in each group	Minimum percentage incidence in experimental groups that can be regarded as significant
3	100.0
5	60.0
10	40.0
20	20.0
50	8.0
100	4.0

[a] Data from *Problems in the Evaluation of Carcinogenic Hazard from Use of Food Additives,* National Academy of Sciences—National Research Council, Publication 749, Washington, DC, 1960.

Some forms of toxicity may have also a normal occurrence in controls. In this situation in order to have an acceptable level of confidence that any positive findings in the test animals were due to the chemical, there must always be at least a specific number of examples of the toxicity in the test group greater than the number that occur in the controls. Table 12.3 lists the differences between the two groups necessary for the experimenter to have confidence that a positive finding was not due to chance in at least 95 of 100 such experiments.

In contrast to this, the good series of toxicological experiments on a chemical will demonstrate that at some lower dose no toxicity was produced. The controversial problem is whether the experiment which shows no measurable positive effect does in actuality represent a no-effect result or, in other words, a "safe" concentration or dose of the chemical. By using the same statistical concepts that were applied in the case where toxicity is demonstrated, it is apparent that, for the experiment which was conducted, the no-effect dose did indeed represent a safe dose. However, if the experiment which was conducted was performed on a relatively few animals then it would not be correct to state that the same dose was "safe" for all animals of that species unless the test included all animals. It is

TABLE 12.3 Percentage Incidence of a Normally Occurring Type of Toxicity in Control and Test Groups of Various Sizes That Can Be Considered as Significant at a Probability Level of 0.05[a]

10 Animals per group		20 Animals per group		50 Animals per group	
% Affected, control group	% Affected, test group	% Affected, control group	% Affected, test group	% Affected, control group	% Affected, test group
10	70	10	45	10	26
20	80	20	55	20	38
30	90	30	65	30	50
50	100	50	85	50	70

[a] Data from *Problems in the Evaluation of Carcinogenic Hazard from Use of Food Additives,* National Academy of Sciences—National Research Council, Publication 749, Washington, DC, 1960.

practically not feasible to test all or even a small percentage of the total number of animals in any species.

In the properly conducted test whereby negative results are obtained, it is desirable that the same test include a group of animals that show positive results for the type of toxicity under investigation. Otherwise the species that was investigated may not be fundamentally capable of showing the type of toxicity under investigation. In this case it is necessary to add to the experimental protocol a *positive control.* The positive control consists of groups of animals identical to the test group and treated in an identical manner except that a compound known to produce the type of toxicity under investigation is substituted for the test compound. Through the use of the positive control not only is the test species proven to be capable of showing the toxicity but also data will be obtained regarding the relative potency of the test compound as compared to the positive control compound.

If the objective of an experiment is to conclude that a toxic effect would not occur on more than one of a thousand test animals, then the experiment must be designed so that the confidence limits of the experimental protocol are adequate to reach such a conclusion. In any group of animals that are exposed to a given dose of a chemical and show a negative response, the limits of confidence regarding whether the experimental dose did, indeed, represent a no-effect dose must be determined by statistical methods. The limits of confidence of the experimental results are related to the number of animals in the test and control groups and the sensitivity of the experi-

ment to demonstrate minimal amounts of toxicity. If the number of animals was high and the technical expertise was high, the confidence in the results is then high. However, elementary statistical concepts have established that even by using as many as 1000 test animals in a single test group in which no toxicity was observed, if the experimenter uses 90% confidence limits, the experiment shows that not more than 2.3 animals of the 1000 animals tested could have shown the toxic effect (Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation Report, 1971). Therefore, it is generally economically infeasible to conduct toxicity tests that would establish absolute safety of many chemical entities.

The problem becomes even more complicated when the nature of the toxicity under consideration is a tremendously harmful one, such as tumorigenicity or mutagenicity, primarily because these toxicities frequently have a normal incidence of occurrence in untreated control animals. For example in the case of tests for tumorigenicity even with 1000 treated and 1000 control animals each developing 10 tumors, the experimenter can only be 95% confident that the elevation in incidence rate due to the chemical does not exceed 1%, that is, the agent could still have produced 1 additional tumor per 100 test animals. Again the demonstration of absolute safety in biologic testing methods generally is not obtainable. Extrapolation of dose–response curves outside of the range of experimental results is hazardous, and extrapolation of animal data to man in a quantitative manner is subject to great inaccuracies because of the known lack of quantitative or uniform correspondence in the toxicity of a substance for different mammalian species. When safety to man is the objective, the only rational approach to the problem is through a consideration of the nature of the toxicity in question and the application of an acceptable interpretation of benefits versus potential hazards under the conditions of intended use of the compound.

In any experimental study, the criteria which can be considered as indicative of a toxic effect in an animal as well as the duration of the experimental study can be stipulated in the design of the study. The schedule and route of exposure to the chemical can be precisely stipulated and a sufficient number of animals can be included in the experimental and control groups to ensure statistical acceptability of the study at any level of significance which the experimenter wishes to set. When these conditions are incorporated into the experiment, the results which are obtained generally will show a dose- or concentration-dependent graded response from any chemical under consideration. Such results will be obtained whether the study involves a population of single cells, such as bacteria, or a population from a species of higher animals.

TOXICITY VERSUS SAFETY—THEORETICAL CONCEPTS

Even though there are statistical and technical difficulties in establishing a no-effect dose of a chemical which would be applicable to large populations of animals, there is a theoretical basis for the existence of such a dose for all chemical agents.

Paul Ehrlich (1854–1915) initially developed the concept that in order for a chemical to produce an effect on a biological system, the chemical must react with or become "affixed" to some component of the biological system. Since the site at which the chemical compound becomes affixed was, and in most cases still is, unknown, Langley referred to this site as a "receptive substance." Currently, such sites are simply called "receptors." In those cases where receptors have been identified, the structural interaction between the drug and the receptor can be specifically defined in chemical terminology. The kinetics of the chemical receptor interaction is a simple bimolecular equilibrium reaction which is conventionally depicted as

$$C + R \rightleftarrows CR,$$

where C is the chemical and R is the receptor. This reaction obeys the Mass Law which stipulates that the concentration of the free and combined chemical is in equilibrium so that the equilibrium constant, K, for the reaction is,

$$K = \frac{[C] + [R]}{[CR]}$$

If one assumes that the concentration of the complex [CR] constitutes the stimulus that is responsible for any biologic effect, then the quantity of the complex determines the quantity of effect on the biologic system. The quantity of receptor R may be presumed to be constant in any normal biologic system. Therefore, the concentration of the chemical compound C is related directly to the concentration of CR. Hence, there must necessarily be a concentration of the chemical below which very few receptors would be occupied so that no effect is practically determinable. There must also be a range of concentrations of the chemical at which all receptors will not be occupied. Furthermore, there must be a concentration of the chemical at which for practical purposes essentially all receptors will be occupied. The theory that the quantity of the complex CR which is present at any time constitutes the stimulus that is responsible for some types of biologic effect has been questioned by the postulate that the stimulus for any biological response is produced whenever an agent molecule combines with a receptor molecule and that the rate of this combination determines

the intensity of the stimulus. However, the basic concept of a chemical receptor interaction is still valid. If the affinity for any given chemical entity for a receptor is very high, then the condition for equilibrium highly favors the formation of the chemical–receptor complex which must necessarily be the case when very small quantities of toxic agents are capable of producing profound effects on a biological specimen. However, even if the affinity of a chemical for a receptor were as great as possible, it would still take a finite number of molecules of the chemical entity to occupy a significant number of receptors and therefore be capable of producing a measurable effect. The conclusion that must be reached is that there must necessarily be a quantity of chemical agent below which no biologic effect would be achieved.

CHEMICAL–BIOLOGICAL REACTIONS IN DIFFERENT SPECIES

The third principle states that cells which have similar metabolic pathways will usually be similarly affected by a given chemical entity. This concept applies not only to cells in the same species but also to similarly functioning cells in different species when appropriate pairs of species are under consideration. That is, a nerve or muscle cell in the mouse may differ in size from corresponding cells in man, but may have similar metabolic systems and functional purpose. Compounds that react with receptors which are common to either the nerve or muscle cells in one species frequently react in a similar manner with the corresponding nerve or muscle cell in the other species. This concept applies whenever the compound under test is the compound responsible for the biologic effect. However, if the compound under test is biotransformed selectively to an active compound in one species but not in another, then it is apparent that the active compound would not be present in the latter species.

When two or more mammalian species possessing functionally identical cell types, such as nerve or muscle cells, are exposed to a chemical agent, the various possibilities of achieving the same effect in the various species would be dependent on the following factors: (1) whether the receptor is present or absent, (2) whether the compound is or is not activated or inactivated in the animals, (3) whether the compound is or is not translocated to the receptor site, and (4) whether secondary substitute mechanisms are or are not available in certain species in which the affected receptor system is bypassed. Table 12.4 summarizes these possibilities and the mechanisms responsible for the occurrence or lack of occurrence of any specific

TABLE 12.4 Possible Mechanisms Responsible for the Occurrence or Lack of Occurrence of Any Specific Toxicologic Effect from Any Single Chemical Agent in Five Species Having Functionally Identical Cell Types

Functionally identical cell types	Biochemical receptor site	Toxicologic effect
Species A	Present	Present
Species A_1	Present	Present
Species A_2	Present	Absent
Species A_3	Absent	Absent
Species A_4	Absent	Present

Note. If a specific toxicologic effect can be demonstrated in a single species (Species A), then it will occur (Species A_1) or be absent (Species A_2) in other species that have the same receptor site. If the effect is absent (Species A_2), the agent has been inactivated by a biotransformation mechanism, a substitute function has taken over for the affected function, or translocation of the agent to the receptor site is blocked. In Species A_3 and A_4 the receptor site is absent but the toxicologic effect may be absent (Species A_3) or present (Species A_4). If the effect is present as in Species A_4, then either the agent has been activated by a biotransformation mechanism so that a different receptor is involved, or a secondary mechanism capable of producing the same toxicity is involved.

toxicological effect from any single agent in five species having functionally identical cell types.

Compounds having great biological interest frequently are selective in regard to their ability to affect given types of cells. When such is the case, the compound usually reacts with and inactivates some normal component (receptor) of the cell which is involved in the function of the cell. As already indicated the receptor mechanism involved frequently is not clearly definable, but if cells from two different species are affected similarly by a given chemical entity then it is probable that the same type or at least a similar type of receptor is involved in each species. Examples of systems involved in the function of cells which may be readily affected by foreign chemicals are the energy transfer systems and the systems involved in maintaining inorganic ion gradients across the cell membranes. Therefore, if two different cells are identical from a functioning standpoint, and have common biochemical regulatory mechanisms and a common receptor for the chemical agent under investigation, both cells would be similarly affected by any chemical under consideration.

There are many examples which verify this concept. An example at the molecular level is that of the reaction of arsenic with sulfhydryl groups. Such sulfhydryl groups are present in most living systems and the arsenic–sulfhydryl complex will be formed regardless of the source of the chemical group. An example at the physiological level is that certain compounds such as the barbiturates produce anesthesia equally well by an action on the central nervous system in dogs, rats, and most warm-blooded animals including man. The local anesthetic agents also block conduction of nerves whether the nerve is in the dog, cat, rat, frog, or, again, man. At a toxicologic level, carbon tetrachloride will produce similar damage to the liver in essentially all animals which have this organ. In the case of the latter examples, the receptor is not known in a chemical sense, but rather is identified as being common to the various species by virtue of the common effect produced by the chemical under consideration.

This concept achieves considerable importance when it is necessary to design an animal experiment for evaluating the toxic effects of a chemical when the data are to be extrapolated to another species such as man. It is clearly evident that the best method for the determination of the toxic effects of a chemical in man is to use man himself as the test species, or to use a species which possesses the same functional systems as man. The state of the art in toxicology at the present time is not sufficiently developed to the extent that it would be possible to predict which species is most similar to man as far as response to many chemical entities is concerned. It is very evident that there are species which are similar to man and other species which differ from man with respect to the toxic effects of individual compounds.

To carry this concept further, cells with different functions and different biochemical regulatory mechanisms may or may not be similarly affected by any given chemical entity if the action of the compound is that of affecting a biochemical regulatory mechanism. If two types of cells with no functional similarity are affected by similar amounts of a foreign chemical in a similar manner, this would be good evidence that the cellular mechanism involved in the action of the chemical was common to both types of cells. When greatly different amounts of a given chemical are necessary to produce effects on different types of cells, this would be good evidence that the mechanism of action of the chemical is different between the two examples of cells. The fact that two types of cells are not necessarily similarly affected by similar amounts of a given chemical entity is the basis of the concept of selective toxicity of chemicals. Some degree of selectivity of action of chemicals on cells is a necessary requirement for those chemical agents which are used as drug or pesticides.

The action of a chemical on a cell may be that of reacting with a component which is specific and necessary for continued function of the cell, and if the product of the reaction is not capable of functionally substituting for the role of the original component of the cell, that function of the cell is altered and/or destroyed. Whether the cell is or is not capable of continuing to survive is primarily dependent on whether other mechanisms within the cell are capable of taking over and substituting for the loss of the one or more affected mechanisms.

It is because of the selectivity of biologic action of many compounds on biochemical or physiological mechanisms that are common to cells in different species that toxicologic data obtained on animals are of value in predicting effects of the compounds in man.

In an attempt to assess the predictable value for the human of results obtained on animals, the incidence of 89 different effects caused by six drugs in three species (rats, dogs, and man) has been evaluated. From the data pertaining to the rat and dog, the occurrence or absence of each effect in man was predicted by following two rules; first, if a sign was found in both rats and dogs, it was predicted to occur in man, and second, if a sign was found in either the dog or the rat, but not in both, it was predicted that the sign would not occur in man. Since the effects of the drugs in man were already known, the number of items that were predicted correctly or incorrectly could be tabulated. Of 86 predictions for the six drugs, 26 of 38 positive predictions were correct and 38 of 48 negative predictions were correct. The average correct prediction was 74%. Predictions made on the basis of flipping a coin would be expected to yield 50% correct results, but the probability of obtaining 74% correct predictions by the coin flipping method is very small.

In this example, therefore, animal studies permitted, to some degree, prediction of drug effects in man based only on the theory that a drug action that is seen in both the rat and the dog probably involves a common physiologic mechanism that is likely to be present in the human, whereas an effect seen in only one of the two species indicates that the effect is peculiar to that species and is less likely to be present in the third species. Such evidence shows the importance of using two species in animal tests and the value of animal tests for predicting the effects of chemicals in man.

STRUCTURE–ACTIVITY FACTORS IN TOXICOLOGIC TESTS

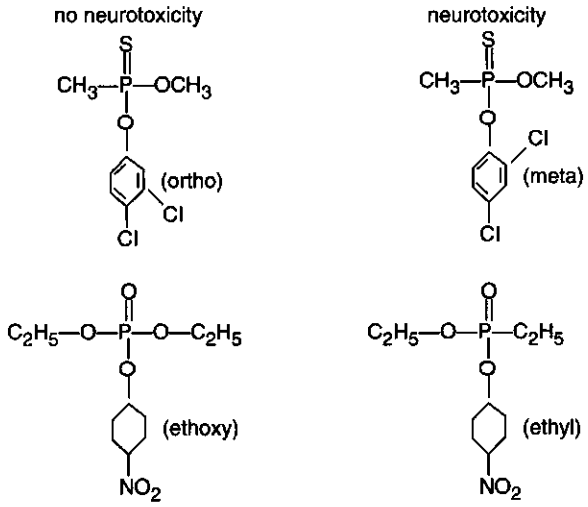
The fourth principle states that small changes in the structure of a chemical may greatly influence the biologic action of the chemical. This principle is basically an extension of the concept that all chemical–biologic effects

are the result of a physical–chemical reaction or interaction between the chemical and some component of the living system. The study of the type of chemical structure of a foreign chemical that will react with such a component is commonly referred to as the study of structure–activity relationships. Such studies have been productive in the development of drugs and pesticides. The first discovery of an example of a new chemical entity that induces biologic effects will initiate the synthesis of additional related analogs of the compound in the hopes of finding more useful or at least more effective agents capable of producing the same biologic effect. In some cases the possibilities for the synthesis of chemical analogs of effective compounds are unlimited. For example, thousands of derivatives of barbituric acid, phenothiazine, and sulfonamide-type compounds have been synthesized and tested for action on animals. Such investigations have resulted in the use of several structurally related agents as drugs to achieve a common type of biologic effect. The objective of structure–activity relationship studies is to define as precisely as possible the limits of variation in structure of a chemical nucleus which are consistent with the production of a specific biologic effect. Presumably if enough of this type of data becomes available, a hypothesis can be developed regarding the most likely structures of the receptor involved. An example of such a hypothetical formulation is the description of the active sites on the enzyme acetylcholinesterase when this enzyme is inhibited by organic phosphates or carbamates. Furthermore, the synthesis and testing of closely related homologs of known biologically active compounds occasionally result in compounds that are essentially biologically inactive on the biologic system under investigation; such information is also equally helpful in regard to the formulation of hypotheses concerning the structure of the receptor.

That small differences in chemical structures can significantly influence biologic effects of chemicals is evident in a number of examples. In pharmacology and toxicology there are several examples whereby optical isomers of single compounds or different valences on single elements have different degrees of action or even different actions. An example of the effect of optical isomerism in regard to biologic action is that shown by the drug amphetamine (racemic, B-phenylisopropylamine). This compound has several well known effects in mammals. These effects are central nervous system stimulation and stimulation of receptors which are normally innervated by the sympathetic nervous system. The d-isomer, however, is three to four times more potent than the l-isomer in regard to the action on the central nervous system, whereas the l-isomer is about two times more potent than the d-isomer in regard to its action on the heart. An example of the effect of valence on toxicity is shown by arsenic. Although the difference in the lethal toxicity of trivalent arsenic as compared to the pentavalent

arsenic is not great in many mammals, the difference is considerable for lower animals and plants. The trivalent arsenites are much more lethal for protozoa, bacteria, and yeast than are the pentavalent arsenates. Chromium in the trivalent form is required for normal glucose metabolism, as an insulin cofactor; however, hexavalent chromium is carcinogenic to most animal species.

Several examples that demonstrate the fact that small changes in structure can greatly affect toxic potency have been described in regard to delayed neurotoxicity. Toxicity to the nervous system produced by several of the organophosphate and triaryl phosphate types of pesticides has been known for years. The neurotoxic action has an onset following repeated administration of low doses of the compounds. In the early stages of toxicity, animals and man show a weakness in the limbs which can progress to a state of complete paralysis. The biochemical processes that are responsible for the signs appear to be similar for all of the causative compounds; however, small changes in the structure of the compounds greatly influence the ability of the compounds to produce the neurotoxicity as shown for the following agents.



The demonstration that small changes in chemical structure may significantly influence the biologic activity of closely related chemical entities is of importance in the design of methods in toxicology. The experimental toxicologist has reservations about his confidence in being able to predict the toxicologic effect of a series of similar compounds. Rather, each compound of a series under investigation must usually be tested for all measur-

able effects. Also, since small changes in chemical structure can produce variations in effects, it becomes apparent that studies performed with compounds of questionable purity are valid only for that example of the test mixture. It is, indeed, rare that newly synthesized complex organic chemical agents can be said to be more than 95–98% free of impurities. Such impurities frequently are intermediates used in the synthesis procedure and therefore may be related chemically to the compound of interest, but may be solely responsible for toxicologic effects obtained in animal experimental procedures. A good example of this is the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5T). It has been demonstrated that in the manufacture of 2,4,5T a toxic impurity was formed. The impurity was 2,3,6,7-tetrachlorodibenzodioxin (TCDD) and although it was present in the technical grade 2,4,5T only in amounts of 20 to 30 parts per million, it was found to be primarily responsible for production of birth defects in animal experiments as well as chloracne, a disfiguring skin ailment, in humans. Because of this it is necessary to eliminate TCDD in the herbicide.

DEVELOPMENT OF THE CATEGORIES OF TOXICOLOGIC TESTS

The extent to which a chemical compound is studied in the toxicology laboratory is largely dependent on the intended use of the compound. Those compounds that are intended for introduction into the human, such as drugs or food additives, obviously require extensive toxicological testing. In the case of drugs, if the compound is to be used for only short periods, i.e., a few doses, the extent of toxicological testing is different than that for drugs which are to be used over long periods of time. Another factor that determines the extent of toxicological testing is the economic importance of the chemical. Any chemical which is to be incorporated into hundreds of household preparations requires extensive toxicological testing even though it may not be intended for direct consumption by humans.

Extensive toxicological testing means that the compound is subjected to a series of individual short-term tests that are designed to detect specific types of toxicity, plus the exposure of at least two different species of animals to the compound for at least a major portion of the lifetime of the animals. If the compound eventually is to become an environmental agent, the extent of the tests involves investigations utilizing insects, fish, wildfowl, or any animal.

The generally accepted philosophy in connection with the design and conduct of toxicity tests in animals is to maintain flexibility of the protocols; however, in contrast to this philosophy in conventional practice rather rigid

protocols are routinely used. The use of "fixed design" or rigid protocols in any given laboratory for each type of toxicity test has certain merits which perpetuate their use.

A rigid protocol for a toxicity test is one in which the outline of the procedure for conduct of the test is prepared so that it includes specific types and numbers of animals as test subjects, and specific routes of administration as well as dose schedules and duration of administration of the test chemical. Also, specific functional and pathologic procedures to be followed are stipulated in the test. The principal advantage of standardization of the test is that the laboratory becomes proficient in conducting the test and direct comparisons of effects produced by different compounds on essentially the same type of test can be made. Furthermore, such tests provide specific answers frequently required by governmental regulation agencies. It would, of course, be a disadvantage if the toxicologist failed to make additional observations for toxic effects which may occur during the conducting of a toxicity test but may have been unforeseen at the time the test is designed.

Over the years certain types of toxicity testing procedures have been designed, modified, and improved so that they are generally acceptable by most toxicologists. The modern toxicologist, therefore, has available a series of rather specific types of toxicological tests for the determination of the various types of toxicity. Regardless of the nature of the experimental protocol, all such tests fall into one of three major categories dependent primarily on the duration of the test. These categories are the *acute* test, the *prolonged,* test and the *chronic* test. In general, acute tests involve administration of the test chemical on one occasion. In a rare instance administration may occur on two or three closely spaced occasions. The period of observation of the test animals may be as short as a few hours, although it is usually at least 24 hr and in some cases it may be as long as a week or more. In general, prolonged tests involve administration of the test chemical on multiple occasions. The test chemical may be administered one or more times each day, irregularly as when it is incorporated in the diet, at specific times such as during pregnancy, or in some cases regularly but only at weekly intervals. Also, in the prolonged tests the experiment is usually conducted for not less than 90 days in the rat or mouse or a year in the dog. In some literature, the prolonged test is called a "subchronic toxicity test."

In contrast to the acute and prolonged types of test, the chronic toxicity tests are those in which the test chemical is administered for a substantial portion of the lifetime of the test animal. In the case of the mouse and rat, this is a period of 2 to 3 years. In the case of the dog, it is for 5 to 7 years.

These three types of toxicity tests have developed because of necessity. With all chemicals it is desirable to acquire information regarding just how much of the chemical can be given to a test animal on one or a few occasions in order to produce some minimal detectable effect, as compared to the amount necessary to produce death in the animal. The acute toxicity test serves this purpose; however, there is no assurance that the single minimal effective dose (MED) can be given to the same animal on repeated occasions without producing more intense or additional effects or in fact no measurable effect.

Table 12.5 is a general outline of the principal categories of tests commonly conducted for toxicological purposes. The table indicates that the major difference between the categories of tests is the duration of the tests. Even in the category of "special tests" the interval of time over which the animals receive the compound is frequently of considerable importance. Therefore an understanding of the factors that influence toxicity of a chemical when it is administered on a single occasion as compared to multiple occasions is fundamental to the conducting of toxicity tests.

TIME–EFFECT RELATIONSHIPS IN TOXICITY TESTS

There are examples in which a single dose of a compound given early in the life of an animal can result in considerably delayed appearance of toxicity which cannot be said to be associated with persistence of the presence of the chemical in the animal. An example is the induction of cancer appearing late in the life of the animal following administration of single doses of 3-methyl cholanthrene or 3,4-benzpyrene to the young animal. However, with the exception of carcinogenicity, chemical-induced toxicity appears to be related to the time over which exposure occurs to given concentrations of the agent.

When a single dose of a chemical alters some function of an animal, if the chemical is not given to the animal on a second occasion, any altered biochemical mechanism, function, or structure usually returns to normal after the chemical is permitted time to leave the animal by excretion or detoxication. Such an effect would be called a reversible response. If the chemical-induced damage to the tissue is so great that it is not normally repaired simply by removing the chemical, then most tissues undergo replacement of the injured tissue with new normal cells or by the development of fibrous tissue. When replacement by normal cells occurs there is no permanent injury, although such a response produced an irreversible response on the original cells. Whereas small degrees of tissue damage may not necessarily result in altered function, repeated, sufficiently frequent

TABLE 12.5 An Outline of Types of Animal Toxicologic Tests

I. Acute tests (single dose)
  A. LD_{50} determination (24-hr test and survivors followed for 7 days)
    1. Two species (usually rats and mice)
    2. Two routes of administration (one by intended route of use)
  B. Topical effects on rabbit skin (if intended route of use is topical; evaluated at 24 hr and at 7 days)

II. Prolonged tests (daily doses)
  A. Duration—3 months
  B. Two species (usually rats and dogs)
  C. Three dose levels
  D. Route of administration according to intended route of use
  E. Evaluation of state of health
    1. All animals weighed weekly
    2. Complete physical examination weekly
    3. Blood chemistry, urinalysis, hematology, and function tests performed on all ill animals
  F. All animals subjected to complete necropsy including histology of all organ systems

III. Chronic tests (daily doses)
  A. Duration—2 to 7 years depending on species
  B. Species—Selected from results of prior prolonged tests, pharmacodynamic studies on several species of animals, possible single dose human trial studies. Otherwise use two species.
  C. Minimum of two dose levels
  D. Route of administration according to intended route of use
  E. Evaluation of state of health
    1. All animals weighed weekly
    2. Complete physical examination weekly
    3. Blood chemistry, urinalysis, hematologic examination, and function tests on all animals at 3- to 6-month intervals and on all ill or abnormal animals
  F. All animals subjected to complete necropsy including histologic examination of all organ systems

IV. Special tests
  A. For potentiation with other chemicals
  B. For effects on reproduction
  C. For teratogenicity
  D. For carcinogenicity
  E. For mutagenicity
  F. For skin and eye effects
  G. For behavioral effects
  H. For immune effects

chemical-induced assault on a receptor system will generally eventually lead to altered function and grossly observable damage in that tissue. In this case the initially reversible effect can lead to irreversible changes. Generally this may be thought of as a situation whereby minimally effective doses lead to small amounts of tissue damage, and if a second dose of the chemical is given to the animal before the tissue repairs itself, the second and each subsequent dose leads to the development of added damage to the tissue. In time, the response to each dose therefore summated and resulted in intensification of the toxic effect. In such a case the repeated chemical insult to the tissue causes the toxicity to accumulate. An example of an experiment that demonstrates this accumulative concept is as follows. The local dermal toxicity of acetic acid can be determined by applying 0.1 ml of a 5% aqueous solution of acetic acid to a gauze pad and holding the pad in contact with a specific area of the shaved skin of the back of rabbits for 30 min, followed by thorough rinsing of the area with water. Separate groups of animals were treated at either daily or weekly intervals until each animal received a total of eight applications. The animals were killed 1 week following the last application of acetic acid solution. Those animals that were dosed at weekly intervals showed no gross or histologic evidence of irritant effect to the skin, whereas those animals that received the daily dose showed marked gross and histologic evidence of skin damage.

Whether a chemical compound is given on only one occasion, on many occasions, or for the lifetime of an experimental animal, the concentration–time relationship generally determines the extent of toxicity. The discussion of antidotal therapy (Chapter 11) shows that the amount of the chemical in a test specimen at any time is dependent on the rate at which the chemical enters the specimen (that is, by absorption mechanisms) versus the rate at which the chemical is eliminated from the test specimen (that is, by excretion or inactivation including storage which together constitute the mechanisms of elimination of the chemical). At any point in time, the concentration of the chemical will increase when the rate of absorption is greater than the rate of elimination of the chemical. The concentration of the chemical will be unchanged during any time interval when the absorption and elimination rates are equal. The concentration of the chemical will decline when the elimination rate exceeds the absorption rate. When repeated doses of a chemical are given to an animal at a frequency that exceeds the rate of elimination of the compound from the animal, a definite possibility exists that the concentration of the chemical can increase in the animal with time. When this occurs and the total body load of the chemical increases, the chemical is described as being "accumulative." Thus, in a sense any chemical is accumulative under the condition that it is administered to the animal

at a rate which exceeds the rate of elimination of the chemical from the animal. Sufficient accumulation of the chemical in the body of an animal can lead to toxicity. This type of accumulation should be recognized as being different from the type of "accumulation of effect" previously described. The rate at which a chemical gains access to the animal is related to the dosage, the route by which it is administered, and the physical–chemical characteristics of the agent. Agents which are given by mouth or injection are, to various degrees, absorbed from the gastrointestinal tract or from the site of injection and are to various degrees distributed or translocated throughout the body. The physical–chemical factors that influence this rate of absorption and distribution (or translocation) have been discussed. Termination of action of chemicals in the body may occur simply by excretion of the compound in the expired air or via the kidneys, by biotransformation, or by selective disposition in storage (nonreceptor) areas.

THE BIOLOGICAL LIFE AND HALF-LIFE OF COMPOUNDS IN RELATION TO TOXICITY TESTS

The duration of the presence of a compound in a biological specimen is frequently referred to as the "biological life" of the compound. The conditions that influence this "biological life" may be different between species as well as between members of a species. In any experimental procedure in which only a single dose of a compound is administered to an animal, that animal will eventually elminate the compound. The process of elimination may be different between species or within different members of the same species of test animals. In the case of the compound that is eliminated solely by excretion in the urine or in the expired air, it is considerably less likely that there would be extensive differences between normal members of the species than when a compound is terminated by metabolic biotransformation processes. This is because it is highly improbable that a great difference in kidney or respiratory function can exist in normal individual animals without this condition leading to general and obvious debilitation of the animal or even the failure of the animal to survive. In contrast to this, several of the enzyme systems involved in biotransformation can be altered through genetic defects or by enzyme induction or inhibition and go unnoticed until the animal is challenged in an experiment with a suitable chemical agent.

The biological life of the compound determines the frequency of administration of the chemical in a prolonged or chronic toxicity test if accumulation of the chemical is to be avoided. From an academic point of view the ideal test would be one in which the compound under investigation was

administered by a method which would enable a preselected tissue concentration to be rapidly obtained and then maintained for the duration of the study. In any prolonged toxicity study such a condition is technically and economically difficult to accomplish. From a practical point of view, it may be argued that such a condition is not even desirable. The basis of such an argument is that the usual toxicologic experiment should be performed in a manner which simulates the conditions of use of the compound by man, and it would be, indeed, rare that a human would be continuously exposed to most chemicals at a constant concentration. Even the agents which are identified as atmospheric pollutants vary in concentration from time to time depending on atmospheric conditions.

Most toxicological tests are conducted by administering the "dose" of the chemical mixed with the feed, added to the drinking water, applied to the skin as an ointment or cream or in a solvent, introduced into the breathing air, or injected by use of a hypodermic needle at periodic intervals. When the agent is added to the drinking water, the frequency of intake of the agent will vary, dependent on the animal's need and desire for water. When a compound is introduced into the atmosphere, the amount of activity and respiratory function of the animal will result in variation in the exposure and dose from time to time. When the agent is applied to the skin or injected, these conditions always involve the administration of the compound at specific intervals. Regardless of the route or method of administering a test chemical in a toxicological test, a uniform, stable, constant concentration of the chemical in the animal for the duration of the study is only rarely achieved. Since the frequency of administration is an interrupted procedure there are three possible consequences in regard to the tissue concentration of the agent.

1. If the dose is constant and the frequency of administration is sufficiently infrequent so that it is longer than the biological life of the compound, the result is a series of maximum and minimum tissue concentrations with interspaced intervals in which there will be essentially zero concentration of the agent in the tissue.

2. If the rate of administration and dose are sufficiently frequent to be shorter than the biological life, then the result is again a sequence of maximum and minimum tissue concentrations, but there will be no intervals of zero tissue concentrations. In this case if the dose and frequency of administration are not varied, as the experiment progresses each subsequent dose would be expected to produce a maximum tissue concentration which is greater than that produced by the previous dose. This is because the maximum tissue concentration is a result of the summation of the concentration of the residual chemical from the previous dose, plus the concentration

that is created by each subsequent dose. It is now apparent that in time, unless the quantity of each dose is decreased or the interval between administration of the doses of the compound is lengthened, the chemical will in time progressively accumulate in the test animal. In actual practice the amount of accumulated chemical in the animal is self-limiting because of mechanisms which will be described. However, in this manner, an animal eventually can accumulate a sufficient concentration of the chemical to result in toxicity.

3. If the rate of administration and dose of the agent are modified during a prolonged or chronic experiment so that the dose administered at any interval is just adequate to replace that portion of the previous dose which was eliminated from the animal during the interval between doses, the result in regard to tissue concentration of the agent is a fluctuating maximal and minimal concentration which is synchronized with the frequency of dosing. At this time an average overall steady state of the body load of the chemical is maintained. Because the rate of administration equals the rate of elimination, the maximal and minimal tissue concentrations would not vary and the agent would not accumulate in the animal. This situation can be accomplished in an experimental animal by periodic analytical monitoring of the tissue concentrations. It also can be approximated when sufficient information on the biological life of the compound becomes available.

It is common practice to determine the biologic "half-life" of a compound whenever an analytical method for the compound is available. The biologic half-life of a compound in an animal is that interval of time in which one-half of the compound present in the body is eliminated from the body. In actual practice the biologic half-life is determined by measurement of the interval of time during which the concentration of the compound in the body following a single dose of the compound decreases to half of any given concentration. This is done at a time when absorption of the compound is complete.

The concept of the existence of the biologic half-life of a compound in the body is generally valid because most of the elimination mechanisms result in an elimination of a constant fraction of the total amount of the chemical in the body with each equal interval of time. In other words, as the concentration of the compound in the body increases its rate of elimination from the body increases. This is basically the condition developed by first order kinetics, that is, at any particular moment the rate of elimination is a finite figure, but as the concentration of the chemical in the body changes, the rate of elimination changes. Although the biologic half-life is obtained by administering a single dose of the compound to an animal and measuring the time required for a tissue concentration to decline to 50%

of its initial concentration, the result obtained is valid only if the measurement is made at a time when absorption of the chemical from its site of administration is complete. However, some compounds are eliminated at a rate which is independent of the concentration of the compound in the body at least until low concentrations are involved. In this case, and according to the definition of a biologic half-life as stated above, the half-life would be different when different concentrations of the chemical were involved.

Additional factors can influence the biologic half-life of certain compounds when they are given on multiple occasions. For example, if a compound is detoxified in the animal by enzymatic biotransformation it is not uncommon to find that the compound "induces" the enzyme responsible for its own detoxification. When this occurs the rate of termination of action of the compound is changed and consequently the half-life of the compound would be changed.

The pharmacologist and the physician, in the course of using drugs, are primarily interested in establishing a fixed dose and a dosage schedule that will create a specifically optimal concentration of a drug in the human. Such a concentration of the drug would presumably be that which would achieve the therapeutic objective and avoid any covert toxic effect. Such a concentration is referred to as the "effective drug concentration." With many, if not most, drugs, the biologic half-life and the rate constant for elimination of the drug from the body are known. When such information is available, if it is assumed that the absorption rate of the drug follows zero order kinetics (that is, there is a constant rate of absorption and that absorption is complete during the time interval between doses) and that the rate of elimination is according to first order kinetics (that is, it is an exponential rate of elimination), it then is possible to predict by the use of suitable mathematical formulas the optimal dose, the number of doses, and the interval between doses that would be required to achieve the effective drug concentration. The equations for this purpose can be found in the textbook references cited in Chapter 15.

Some prolonged and chronic toxicity studies are performed on compounds under conditions for which there is no known method for analytical measurement of the concentration of the compound in the tissue. Furthermore, even when chemical analytical methods are available, distribution of a test compound would have to be known or simple monitoring of blood would not reflect the extent of accumulation of the compound in other compartments of the animal.

In contrast to the need of the pharmacologist to achieve a "therapeutic" and presumably constant concentration of a drug in the body, the toxicologist seeks to ascertain how to produce, as well as how to prevent, the occurrence of toxicity. The biologic half-life of a compound does not supply

information regarding either a therapeutic or a toxicologic effect. However, since toxicologic effect is fundamentally related to concentration of the chemical at the effector sites, knowledge of the half-life could be used under some conditions to determine whether accumulation of the compound would occur. If accumulation of the compound did occur and the plateau that was finally reached was above the threshold for the occurrence of toxicity, then one would expect toxicity to occur at a time that could be predicted from the dose, the frequency of administration, and the half-life of the compound. However, if a single dose of a compound was great enough to produce some form of toxicity which outlasts the biological life of the compound, then toxicity would accumulate from repeated doses of the compound even though the doses were separated by a period of time greater than the biological life of the compound in the animal. That is, at least from a theoretical standpoint toxicity could accumulate without accumulation of the chemical at the receptor sites in the animal. Because of the importance in toxicology of the relationships between the biologic half-life when it is used as a measure of accumulation of a chemical and the toxicologic effects as they are manifested because of accumulation of the chemical, this subject is considered in greater detail in the following paragraphs.

The biologic half-life of a compound has been shown to be a major factor that determines whether a compound will accumulate in an experimental subject upon repeated exposure to the chemical. Since accumulation of a chemical is one mechanism that leads to elevated concentrations of a chemical in a biologic system, and since all toxicities are concentration related, the rate of onset and development of toxicity will be related directly to the rate of accumulation, and thus the half-life, of the compound in an experimental animal. In contrast, the rate of recovery of a biologic system from a chemical-induced toxicity may or may not parallel the rate of elimination of the chemical from the animal. In other words, the "half-life of recovery from a toxicologic effect" does not necessarily parallel the biologic half-life, which is a measure of the rate of disappearance of the chemical from the animal. Rather, the half-life of a toxicologic effect is a function of the degree to which the effect is or is not reversible. This concept applies to all forms of toxicity, including carcinogenesis, teratogenesis, and mutagenesis, and not just various types of degenerative organ toxicity, since in every type of toxic effect the initial chemical insult must involve some finite degree of damage to a cell. Such damage can be sufficient to be permanent and self-perpetuating, as in mutagenicity and/or carcinogenicity, or insufficient so that regeneration of the cell to normal takes place.

If it is assumed that all reversible toxicologic effects result from derangement, short of death, of a biologic system as a result of an unfavorable

exposure to a chemical, then once the chemical has been removed from the deranged system, it will return to its normal function state either (1) immediately, because the effect is readily reversible and dependent only on the presence and concentration of the chemical, or (2) delayed, because the effect is either slowly reversible or nonreversible so that it persists beyond the time when the chemical can be detected in the biologic system. In the situation for which recovery from an effect is immediate (or at least rapid), the direct relation between a declining concentration and recovery is easily comprehended. In the situation for which recovery is delayed, several mechanisms may be involved and these mechanisms are collectively referred to in pathology as the "regeneration process." Whenever a biologic cell is damaged short of death, regardless of the cause of the damage, the cell may undergo the process of "regeneration," which only means that it returns to normal both functionally and structurally.

The mechanisms responsible for regeneration of damaged cells are not clearly definable, but for purposes of this discussion, the mechanisms are time dependent and can be demonstrated by the following example. When ethyl alcohol is administered to animals or humans in an adequately large dose, one effect of the alcohol is that of causing the deposition of abnormal amounts of fat in the liver cells. The fat can be seen in histologic sections of the liver, and can be determined analytically from a sample of the liver. The alcohol disappears from the body (by metabolic and excretory mechanisms), and in time the accumulated fat disappears from the liver; however, this latter process involves considerably more time than the time involved in losing the alcohol. If additional exposure to alcohol occurs before the cells have regenerated to normal, additional fat will be deposited in the liver. Thus the toxicity, manifested as fat deposition, can accumulate under conditions in which the alcohol does not accumulate. Furthermore, if the rate of regeneration of the cells was measured analytically in the absence of additional exposure to alcohol, it would be possible to determine quantitatively the time required for the tissue to regenerate to a degree equal to 50% improvement. This figure would represent the "half-life for recovery from the toxic effect" (hereafter referred to as the "half-life of the toxic effect"). If the rate of regeneration was exponential, that is, related directly to the total amount of fat present, the half-life of the toxic effect would be a fixed figure, regardless of the degree of accumulation of fat in the liver. However, if the rate of regeneration was linear and independent of the amount of fat in the liver, then the half-life of the toxic effect would vary with variations in the degree of liver damage.

Thus a general concept appears to be valid whenever exposure to a chemical is repetitive. The concept is that if the initial exposure to a chemical is sufficient to reach or exceed a threshold of some form of toxicity, and

if the half-life of the toxic effect exceeds the biologic half-life of the chemical, then it is not necessary for the chemical to accumulate in the animal in order for the toxic effect to accumulate. However, if the animal is exposed to a chemical on repeated occasions such that the interval between exposures is sufficiently less than the biologic half-life of the compound and accumulation of the chemical occurs, as the accumulated concentrations of the chemical approach the threshold of producing a toxicity, that toxicity would become evident. Then, if the half-life of the toxic effect is greater than the biologic half-life of the chemical, the amount of toxicity would progressively approach a plateau.

Figure 12.2 diagrammatically presents an example of the relationship between accumulation of a chemical and accumulation of its toxic effect. In the figure, a readily absorbed compound is administered to an animal

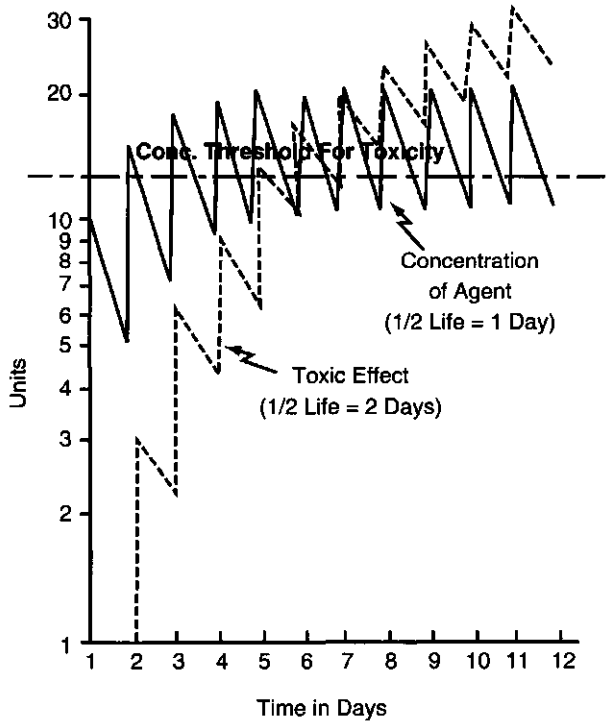


FIGURE 12.2 Diagrammatic representation of relationship between the rate of accumulation of a chemical and the rate of accumulation of a toxic effect from the chemical. The chemical is administered daily at a uniform dose schedule and its biologic half-life is 1 day. The half-life of the toxic effect is 2 days. Both the concentration and the toxicity are in arbitrary units.

at a uniform daily dosage rate which is adequate to produce a whole-body maximum concentration of 10 units. The biologic half-life of the compound is 1 day, and the threshold concentration for the occurrence of toxicity is 12 units. Thus the toxic effect occurs whenever the concentration of the agent exceeds 12 units. The half-life of the toxic effect is 2 days. Elevating the threshold of toxicity would shift the cumulative toxicity curve to the right. Lowering the threshold of toxicity would shift the cumulative toxicity curve to the left. Also, increasing the biologic half-life of the chemical or increasing the half-life of the toxic effect would shift the toxic effect curve in the figure to the left, whereas decreasing the biologic half-life of the chemical or the half-life of the toxic effect would shift the toxic effect curve in the figure to the right.

Under the practical conditions of exposure of humans to chemicals in their occupations or in their environment, chemicals that have short biologic half-lives are less likely to accumulate than chemicals that have longer biologic half-lives. In a similar manner, chemicals that produce toxicities with short half-lives are less likely to produce accumulated toxicity than chemicals that produce toxicities with long half-lives. A relatively safe chemical would be one that had a short biologic half-life and produced toxicities that were readily reversible, i.e., it would produce only those toxicities that have short half-lives.

A classical experimental method for measuring the cumulative effect of compounds orally administered to animals is described below. The method involves comparing the 1-dose LD_{50} with the 90-dose LD_{50}. The 90-dose LD_{50} was selected because there is experimental evidence which shows that 90-day studies in rats and dogs involving daily dosing of the animals showed results that were similar to corresponding lifetime studies in these species for a wide range of compounds (except for cancer). The procedure determines the 1-dose LD_{50} when the compound was given by stomach tube to groups of rats. The 90-dose LD_{50} was determined by addition of appropriate concentrations of the compound in the feed for the animals. Groups of rats were fed different concentrations of the test compound for 90 days. The range of doses was sufficiently large so that some of the animals died during the 90-day interval. All surviving animals were followed for a minimum of 2 weeks after the 90-day period. The dose in the 90-dose study was expressed as mg/kg/day as calculated from measured feed consumption. The ratio of the 1-dose LD_{50} to the 90-dose LD_{50} is a measure of cumulative effect of the compound. This ratio expressed as a quotient is called the "chronicity factor" for the compound. By definition, therefore, a chronicity factor of 90 would mean that the compound was absolutely cumulative; that is, the 90-day LD_{50} in mg/kg/day equals 1/90th of the 1-dose LD_{50}. The chronicity factor is greater than 2.0 for compounds that are relatively

cumulative in their effects and less than 2.0 for compounds that show little cumulative effect. Warfarin (3-α-phenyl-β-acetylethyl-4-hydroxycoumarin), an antiocoagulant drug, was found to have a chronicity factor of 2.0. The chronicity factor for potassium cyanide (mixed with the feed) was 0.04, suggesting that the presence of feed along with each dose markedly protected the animal from the effect of potassium cyanide. The test is not applicable to all chemicals since some compounds mixed with feed result in poor acceptance of the feed by the animals.

In the process of designing prolonged and chronic biological tests for detection of toxicity, it is therefore highly desirable to be able to obtain some information regarding cumulative effects of the compound and on the biologic half-life and the effect of repeated dosing on the half-life of the compound which is under investigation. When prolonged 90-day experiments are conducted at a fixed dose schedule, unless that dose schedule is adjustly appropriately to accommodate for the biologic half-life of the compound, the experiment will eventually result either in progressive accumulation of the compound in the animal until a steady state is reached or in repeated challenges to the animals with intervals of absence of exposure to the chemical that is under investigation. Since toxicity in general is always related to the concentration of the chemical at the effector sites, the foregoing discussion indicates that some compounds studied under the conditions of two different dose frequencies could lead to different conclusions about the quantity of the chemical necessary to produce any type of toxicity, as well as the quantity of the chemical which would be considered as "safe."

Some prolonged and chronic toxicity studies are performed utilizing so-called "maximally tolerated doses" in animals. The highest dose which permits the animal to survive without effect is the dose which is called the maximally tolerated dose. The determination of the maximally tolerated dose is usually experimentally derived from short-term studies. This involves exposing groups of animals to various fixed doses and fixed frequency (unless the dose is incorporated in the feed of the animal) schedules from 10 days to a few weeks. The animals are observed for the interval of the test and are killed and examined for evidence of abnormal or toxicological changes which may be the result of administering the chemical to the animal. From this experiment a dose and a method of administration of the dose are obtained which permit the animal to survive in an apparently normal condition. The animals in that group which received the next highest dose of the chemical must of course have shown toxicity as a result of administration of the chemical if the objective of the experiment is to determine the maximally tolerated dose.

SUMMARY OF PRINCIPLES INVOLVED IN TOXICITY TESTS

The foregoing discussion demonstrates that certain prerequisite information is desirable before the toxicologist can develop an intelligent program for testing the toxicity of any compound. This consists of at least the following three types of information. The first concerns a knowledge of the nature and chemical purity as well as the physical–chemical characteristics of the compound. On many occasions, the compound of interest is that which would be available commercially rather than the absolutely pure compound, merely because the pure agent would only rarely be available for general use. Hence most toxicity studies consist of the toxicological evaluation of a mixture of chemicals of which one constitutes the majority of the mixture. From this information, the toxicologist can estimate, on the basis of prior knowledge and from the published literature, the possible nature of action of the compound and even perhaps the degree of expected potency of the compound. The physical–chemical characteristics of the compound, such as solubility in various solvents, dissociation constants, and stability, will aid the toxicologist in determining the route by which the compound can be administered to experimental animals and will indicate how the compound will be distributed in the various tissues of the animals as well as how the compound will be eliminated from the animal.

The second type of prerequisite information which is highly desirable concerns an analytical method for quantitative estimation of the chemical in biological sample material. When such a method is available, the rate of absorption from various routes of administration can be determined. The biologic half-life of the compound can be determined. Dosage schedules can be designed to preclude excessive accumulation of the compound in the animal in prolonged or chronic tests. Effects observed in the animal can be related to tissue concentrations of the compound. The mechanism of elimination can be determined and predictions can be developed regarding the most suitable test animals species for the subsequent toxicological tests. If the compound is one which is biotransformed in the animal, the rate and products of biotransformation can be estimated.

The third type of information desirable before toxicological tests are conducted concerns any prior biologic studies on the compound or closely related compounds. Such information will give the toxicologist an estimate of the nature of toxicologic effects that may be anticipated in his studies. Compounds that produce specific organ damage, such as damage to the liver, kidney, or intestine, will suggest that certain biologic and chemical function tests should be incorporated early in the toxicological testing protocols. The duration of action of single as well as multiple doses of structurally related compounds may be of value as an indication of the degree of reversibility or irreversibility of the effects of the compound.

The objective of toxicological testing is to evaluate the relative potential of a compound for producing harm to biological tissue. The procedures therefore necessarily involve the use of species of animals in which the amount of the compound that is given to some of the animals is sufficiently great so that distinct toxicity is produced. The procedures involved also necessitate the administration of the compound in sufficiently small amounts so that no detectable effect results. In the most strict sense, even then, the data that are obtained have some extrapolative value to estimate the potential toxicity of the compound in other species of animals that are at least in a similar category in the biological kingdom. Although relative harmfulness may be thought of as a reciprocal of safeness of a compound, the science of toxicology has not reached the degree of perfection so that any compound can be proven to be absolutely safe under all conditions of use in essentially any population. Safeness of a compound is contingent always on the condition of use of the compound and can be expressed only in terms of the probabilities that harm would not occur. For every condition of use of a compound, an evaluation must be eventually made which stipulates the frequency, the degree, and the type of harm that will be acceptable if a compound is to be used.

After completion of animal toxicity tests, only the use of the compound by man will reveal any subtle and unforeseen toxicity. Therefore, toxicological testing is not complete until the compound has stood the test of time and use in the hands of man. Even then, when the compound is used by man, unless there is adequate follow-up and recording of untoward effects from the compound, it may become generally recognized as safe without adequate data to support that contention.

The procedures for animal toxicity testing have proven to be a successful means of evaluating the harmfulness of compounds for man. When various types of toxicity occur in man that were not observed in proper animal tests, a retrospective review of the animal data reveals that the studies performed or the species tested was not appropriate for the type of toxicity which was revealed in man. Usually when a toxicity becomes evident in man and the compound then is taken back to the laboratory and tested in the proper species of animals, positive results are seen in the experimental animal. There is no doubt that the early and adequate toxicological evaluation of chemicals in animals clearly warns about the harmfulness associated with the use of a compound and thereby prevents distinctly harmful compounds from becoming generally available. Thus in modern society no compound should be made available for general human use without appropriate toxicological evaluation by accepted methodology.

CHAPTER 13
Toxicologic Testing Methods

In general all toxicity testing methods can be divided into two categories. The first category consists of tests that are designed to evaluate the overall effects of compounds on experimental animals. The individual tests in this category differ from each other basically in regard to the duration of the test and the extent to which the animals are evaluated critically for general toxicity. The tests are identified as acute, prolonged, and chronic toxicity tests.

The second category of tests consists of those tests that are designed to evaluate in detail specific types of toxicity. The prolonged and chronic tests do not detect all forms of toxicity but they may reveal some of the specific toxicities and indicate the need for more detailed studies. Also, the intended use of a compound may require that an estimate of the order of safety from certain specific toxicities be investigated. The second category of toxicity tests has been developed to fill these needs. Examples of specific toxicity tests are: (1) those that determine the effects on the fetus in a pregnant animal, that is, teratogenic tests, (2) tests to determine effects on reproductive capacity of the animals, that is, reproduction tests, (3) tests to determine effects on the genetic code system, that is, mutagenic tests, (4) tests to determine the ability of agents to produce tumors, that is, tests for tumorigenicity and carcinogenicity, (5) tests to determine local effects of agents when they are applied directly to the skin and eyes, (6) tests to determine the effect of agents on various behavior pattern of animals, that