# CHAPTER 7
# *Classification of Harmful Effects of Chemicals*

The large number of harmful effects that can result from chemical–biological reactions have been classified in the literature by systems (organs), by chemical groups, by types of chemical responses, and by direct and indirect effects of chemicals. Such classifications are frequently designed to consider undesirable effects associated with drug therapy. Drugs represent special types of biologically reactive chemicals which have been studied extensively under both laboratory and clinical conditions. They are intentionally administered, usually in specific doses to humans or at least animals, for the purpose of producing some desired (therapeutic) effect. The desired effect may be the normalizing of an abnormal physiological function, relief of a symptom, eradication of an infection, or destruction of a tumor. In order to achieve this purpose, a drug may produce at the same time undesirable (toxic) effects.

## DRUG-INDUCED TOXICITY

Drug toxicities in humans manifest themselves as functional, biochemical, and/or structural changes. Many times the same receptor systems are involved in both the therapeutic and toxic responses. Functional

toxicities are due to the pharmacologic effects that are not necessary for the achievement of the desired action of a drug. These funtional toxicities usually occur following conventional doses of the drug and are reversible on discontinuance of the drug. If such changes were not reversible the agent involved would have very restricted use as a drug. Examples of mild forms of these toxicities are the sedation that accompanies antihistamine drug therapy and the psychostimulation that accompanies such drugs as iproniazid. Whereas these changes are mild, functional effects and are reversible on discontinuance of the drug, others may be serious and necessitate withdrawal of the drug from the patient. Examples are the delusions or hallucinations associated with sedative drug use, the cardiac irregularities associated with quinidine therapy, asthma associated with beta adrenergic blocking agents, and edema associated with calcium blocking drugs.

Although all xenobiotic toxicities could be said to be due to fundamental biochemical changes, the functional classification given here defines biochemical toxicity as effects of the drug that do not produce gross or histologic evidence of damage; however, functional symptoms are usually associated with the biochemical damage. Examples are the shifts in hormonal balance accompanying anti-inflammatory hormonal therapy or the shift in acid–base balance associated with aspirin intoxication. Such changes are readily reversible on discontinuance of the drug, provided the normal homeostatic mechanisms are operative in the subjects.

Structural toxicities usually are produced indirectly by biochemical drug effects, but may be classified as toxicities that produce changes in the structure of an organ, tissue, or cell group. Again, such changes may be mild and reversible on discontinuance of the drug. An example is the fatty infiltration of the liver cells associated with chloroform anesthesia or consumption of alcohol. Such structural changes may also be very severe, such as depletion of white blood cells and the sloughing off of the lining of the intestine associated with anticancer drugs. Another good example is phenothiazine-induced cataracts.

All of the foregoing toxicities represent pharmacologic effects that are undesirable, but are known to result from therapeutic doses of the drugs involved. Some appear early in the course of drug therapy, whereas others appear only after continued use of the drug for weeks or months. Therefore, they are toxicities that are *side effects* in reference to the intended effect of the drug. Although side effects of drugs may be said to be dose-related and avoidable by decreasing the dosage of the drug, a decrease in dosage also may result in an unsatisfactory therapeutic effect.

## CHEMICALS INTENTIONALLY ADMINISTERED TO BIOLOGIC SPECIMENS

It has been pointed out that drugs belong to the group of *chemicals that are intentionally administered in specific amounts to biologic specimens.* There are many nondrug chemicals that are also intentionally administered to biologic specimens in specific amounts, some of which are not necessarily intended for administration to all species. The most obvious of such chemicals would be nutrient substances which consist of complex organic food substances such as the proteins, carbohydrates, and fats, and the accessory food substances such as inorganic salts and vitamins. The respiratory gases would also be included in this category. Less obvious members of this category are food additives (preservatives), drugs, and cosmetics, as well as pesticides and insecticides, exposure to which may be limited to certain species of biologic specimens. In the case of drugs, the dose is regulated to minimize undesirable effects from the drug, and in the case of insecticides the dose is regulated to be excessive only to the uneconomic species.

## CHEMICALS NOT INTENDED FOR ADMINISTRATION TO BIOLOGIC SPECIMENS

Chemicals that are intentionally administered to biologic specimens cannot be compared with a second category comprising *chemicals that are not intended for introduction into biologic specimens,* basically because there is no dose or concentration for use of the latter group in order to achieve a specific purpose. Thousands of chemicals are available in the form of pure agents or mixtures of agents and range from household products to industrial–occupational chemicals, or nonfood botanical specimens. The majority of these substances fundamentally are not intended for introduction into biologic systems. Exposure to these agents is frequently unavoidable, and when such compounds do gain access to biologic specimens through incidental, accidental, or intentional exposure, any dose of the chemical capable of producing an untoward effect would be considered an overdose.

## EXPECTED OR NORMAL EFFECTS OF CHEMICALS

With drugs as well as with all other chemicals, effects that can be repeatedly induced in biologic specimens become known as expected effects whether they are therapeutic or toxic; they might also be considered the

*normal effects* of the chemical. The incidence of occurrence of these effects is primarily dose-dependent, although the dose required to achieve these effects may be different between species or within members of a species. These normal effects do not require any form of preconditioning exposure to the chemical. Thus, if the dose is great enough, the effect occurs in virtually all animals exposed to the chemical. It should be recognized that a large number of factors play a part in the degree of hyper- or hyposensitivity of specific biologic specimens to any given chemical, and some of these factors are considered in other chapters in this book. Normal effects of xenobiotic chemicals on biologic tissue may be said to occur in any biologic specimen which inherently has the target system in the tissue which the foreign chemical is capable of influencing directly or indirectly. To clarify the foregoing statement, one would not expect a xenobiotic agent that reacted only with hemoglobin to produce the same effect on a biologic specimen that had no hemoglobin, but if the two biologic species had identical structures, a foreign chemical which directly affected that type of structure would be expected to produce a similar effect on the two species.

The intensity of effect of all chemicals that are capable of affecting biologic tissues is related directly to the concentration of the chemical that gains access to the target site in the tissue. Therefore, all normal toxic effects of chemicals are the result of overdose or excessive concentrations of the chemical. The toxic concentration necessary for one species may be greatly different from that for a second species, but since there is, for any chemical, a concentration sufficiently low to be harmless to the majority of the population, this concentration is generally considered an acceptable nontoxic concentration. If some members of the population respond excessively to the acceptable dose or concentration of the chemical, then that member is said to be hypersensitive, in a toxicologic sense, to the chemical. Hypersensitivity therefore is manifested as an excessive, normal, expected effect that is induced by a normal dose or concentration of a chemical in some members of a supposedly uniform population of biologic specimens. Therefore in a hypersensitive subject the normal dose, or even less than the normal dose, of a chemical represents an excessive dose.

## UNEXPECTED OR ABNORMAL EFFECTS OF CHEMICALS

A basic classification of types of toxicities from chemicals should not be limited to drugs, but rather should be applicable to all chemicals. Such a classification could be based on the fact that toxicity is essentially an unwanted effect that is produced on a biologic specimen by a chemical. All toxic effects can be conveniently divided into two principal types based on

whether or not the effect is one that involves preconditioning of the biologic specimen. The effects that involve no preconditioning of the specimen are the *expected* or *normal* effects described above, and the effects that involve preconditioning of the animal may be called the *unexpected* or *abnormal* toxic effects. Abnormal toxic effects are not pharmacologic effects or side effects that can be induced in the majority of members of a population of a species by simply increasing the dose of the chemical. Normal effects of chemicals, as described here, differ from the abnormal effects primarily in that the latter effects involve preconditioning (sensitization) of the biologic specimen to the chemical, in which case an immunologic mechanism is responsible for the toxicity.

The foregoing has indicated that for the purpose of developing a mechanistic classification of harmful effects of all chemicals (Table 7.1) there are four primary considerations that are pertinent: (1) all chemicals can be divided into two categories according to whether or not the chemical is intended for introduction into biologic specimens, (2) all effects of chemicals on biologic specimens can be divided into two categories depending upon whether the response is a normal or abnormal response, (3) all effects of chemicals are dose-dependent, and (4) there are biologic variations between species and within species in response to specific chemicals.

**TABLE 7.1 Classification of Mechanisms of Harmful Effects of Chemicals**

A. Chemicals *not* intended for introduction into biologic systems
  1. Normal effects depending on:
    a. Nonspecific caustic or corrosive actions
    b. Specific toxicologic actions
    c. Production of pathological sequelae
  2. Abnormal effects depending on:
    a. Immune mechanisms
B. Chemicals intended for introduction into biologic systems
  1. Normal effects associated with:
    a. Exposure to normal doses or concentrations and depending on:
      (1) Malfunction of mechanisms for terminating action of the agent
      (2) Actions on the wrong target system
      (3) Synergism with other chemicals
    b. Exposure to excessive doses or concentrations and depending on:
      (1) Nonspecific caustic or corrosive actions
      (2) Exaggerated pharmacologic effects
      (3) Specific toxicologic actions
      (4) Production of pathological sequelae
      (5) Sociologic complications
  2. Abnormal effects depending on:
    a. Immune mechanisms

It may be noted in Table 7.1 that harmful effects of chemicals that are intended for introduction into biologic systems may be associated with ordinary doses or excessive doses of the chemical. In the case of chemicals not intended for introduction into biologic systems, any dose that was capable of producing a harmful effect would be considered an excessive dose. Division of all chemicals into these two classes is proper only from the viewpoint of the toxicologist who is interested in harmful chemical–biologic reactions and interactions. The division of all chemicals into these two groups is not satisfactory to the chemist, neither is it a satisfactory division for the pharmacologist or biologist. The commonly encountered category of idiosyncratic reactions (reactions dependent on an individual example and for which no known mechanism is recognized) purposely has been omitted with the belief that as mechanisms of action become understood for any compound, they will fit into one or another of the categories listed in the classification. The classification presented does not attempt to categorize the "specific toxicologic effects," because such effects are numerous and varied and may be obtained for most compounds from the standard references listed in Chapter 15. Some examples of specific toxicologic effects constitute entire volumes. The following two chapters consist of a discussion of the normal and abnormal effects of chemicals and some examples of each type of effect.

# CHAPTER 8
# *Normal Toxic Effects of Chemicals*

## HARMFUL EFFECTS OF CHEMICALS NOT INTENDED FOR INTRODUCTION INTO BIOLOGIC SYSTEMS

Although there are many chemicals that are not intended for introduction into biologic systems, most such chemicals have been produced for use by humans. Proper labeling of solids, liquids, and gases and control of transportation of those agents that have a high order of potential toxicity are required by federal regulatory agencies so that ready recognition and due respect will be accorded these agents. The presence of foreign chemicals in contaminated atmospheric environments as well as in food substances and in potable water makes many such chemicals available to biologic tissue. Undue exposure of humans to such chemicals is usually incidental or accidental in nature, but may be intentional when such agents are used with suicidal intent, or for recreation.

### Nonspecific Caustic or Corrosive Actions

When toxicity in humans occurs on contact with xenobiotic chemicals, the toxicity may be manifested at the site of exposure, that is, on the skin

or in the respiratory tract. Frequently, such toxicity is the result of the caustic or corrosive nature of the chemical. Such agents are commonly referred to as "primary irritants" because they induce local, minor to severe inflammatory response or even extensive necrosis of the tissue cells in direct relation to the concentration available to the tissue. This action is nonspecific and occurs on all cells regardless of type. Strong inorganic or organic acids or bases are the most common examples of a substance which by definition is capable of producing a chemical "burn" at the site of contact. However, such effects may be induced by compounds that are not necessarily strong acids or bases. For example, the refrigerant methylbromide is an excellent methylating agent for organic synthesis; if applied to the skin or mucous membranes or inhaled in the respiratory system it will produce chemical burns of an intensity directly related to the exposure concentration. Gases such as sulfur dioxide and nitrogen dioxide, which are converted respectively to sulfurous and nitrous acid in the presence of water (at membranes or in the air), produce a primary irritant effect. Such agents may be encountered in smog (smoke–fog) conditions in urban areas where coal and fuel oil are used extensively. The aldehydes, such as acrolein (allyl aldehyde) and formaldehyde, are strong irritants to mucous membranes.

The primary irritants that are inhaled into the respiratory tract of animals have no greater action in the lungs than on the skin, but less severe effects on the alveolar tissue may lead to serious consequences because of edema in the lungs with loss of air space and impaired transfer of respiratory gases. In an animal with lung disease and only a borderline reserve of functional capacity, exposure to primary irritants in amounts that may be tolerated in the normal animal may lead to serious impairment of function.

### Specific Toxicologic Actions

In contrast to the nonspecific action of the primary irritants, other compounds may have a high degree of specificity, so that they will react at low dose levels only at certain receptor sites. One of the most potent agents known belongs to this group of substances. It is botulinus toxin, a high-molecular-weight globulin-like protein produced in the several groups of *Clostridium botulinum* bacteria. On the basis of lethal effect in the rat, the estimated lethal dose of botulinus toxin for an average adult human is approximately 0.01 $\mu$g. Botulinus toxin owes its toxicity to an action at certain nerve terminals whereby it prevents liberation of the neurohumor responsible for nerve to muscle transfer of impulses. Symptomatology is primarily that of paralysis of muscle function. The specific toxicologic effects of compounds in this group of chemicals may be either reversible or irrevers-

ible in nature, depending primarily on the dose. The effect of botulinus toxin is reversible in time if the animal has enough muscle function remaining to enable it to maintain its respiratory metabolic requirements throughout the symptom phase of the toxicity.

Chemicals in this group may have a high degree of specificity of action, which causes an initial effect on function of the organism and a subsequent pathological change in specific organs. Examples are the hepatotoxicity from the chlorinated hydrocarbons, such as carbon tetrachloride and dichlormethane, or the optic nerve damage that occurs in primates and is associated with methyl alcohol or a metabolite of methyl alcohol. In the above examples, exposure to greater concentrations of each agent would be expected to produce excessive organ damage. If the effect involves organic damage to tissue, then sequelae consistent with the degree of tissue damage would be expected to follow exposure to the chemical. The degree of permanent pathologic sequelae which would occur following intoxication with this group of chemicals is dependent on the efficiency of the repair mechanisms involved and on the type of tissue under consideration.

Certain tissues that have been structurally and functionally damaged by a toxic chemical may undergo healing by the process of regeneration whereby the dead cells are replaced with new cells, or if the cells were not sufficiently damaged, structure and function may return to normal in the same cells. In contrast to this, the damaged cells may be replaced with fibrous tissue (scar tissue), in which case the pathological sequelae involve the formation of a permanently altered tissue.

In contrast to chemicals that have nonspecific irritant actions and chemicals that have specific actions at receptor sites, certain additional agents are essentially biologically inactive. Examples of such agents are the inert gases and vapors such as neon, nitrous oxide, helium, methane and ethane. When such agents are present in sufficiently high concentrations in air to displace oxygen, the oxygen-dependent animals become hypoxic in direct proportion to the degree of oxygen deficit. Such gases are therefore properly termed asphyxiants. Unintentional, acute exposure of humans to high concentrations of these agents occurs without warning, since these agents are odorless and nonirritant. Toxicity from such agents is therefore indirectly induced, and acute as well as chronic pathological effects consequent to oxygen deprivation are again the result of a dose–response relationship.

Specific toxicologic effects that are irreversible, particularly when they are life threatening, include teratogenesis, mutagenesis, and carcinogenesis. Exposure to xenobiotics that produce any of these effects as an ordinary consequence of their actions must be limited. However, many compounds do not clearly fall in or out of these categories, particularly when the question concerns clinical toxicology. In general, in the absence of strongly

positive clinical experience with any compound, toxicologists rely on a battery of laboratory animal and *in vitro* tests to demonstrate whether any compound may or may not produce these toxicities. From these data an estimate is made regarding the risk involved when humans are exposed to the compound.

Although there is no generally recognized and accepted list of compounds that are known mutagens in humans, compounds that are carcinogenic via a direct action on the genetic mechanism could be classified as mutagens. That is, many carcinogenic compounds are also mutagens and there are recognized lists of carcinogenic compounds. The United States Department of Health and Human Services, through the National Toxicology Program, publishes annually a listing of carcinogenic substances. The 1994 report summary lists two categories of substances or groups of substances. One category consists of 24 substances for which evidence from human studies indicates that there is a causal relationship between exposure and human cancer. The second category consists of 156 substances which may reasonably be anticipated to be carcinogens, defined as those substances for which there is limited evidence of carcinogenicity in humans or sufficient evidence of carcinogenicity in experimental animals. Table 8.1 consists of selected substances from the above categories

**TABLE 8.1 A Selected List of Carcinogenic Substances (A) in Humans and (B) in Animals**

| A | B |
|---|---|
| Aflatoxins | Acetaldehyde |
| Asbestos | 2-Acetylaminofluorene |
| Arsenic | Butylated hydroxyanisole |
| Benzene | Carbon tetrachloride |
| Benzidine | Chloroform |
| Chromium | Dimethylsulfate |
| Conjugated estrogens | Estrogens (not conjugated) |
| Mustard gas | Formaldehyde |
| 2-Napthylamine | Lead acetate |
| Vinyl chloride | Phenacetin |
| | Polyaromatic hydrocarbons (PAHs) |
| | Saccharin |
| | Safrole |
| | Thiourea |
| | Urethane |

*Note.* From Seventh Annual Report on Carcinogens, 1994, Summary, U.S. Dept. Health and Human Services, National Toxicology Program, P.H.S. See text for definitions of A and B.

showing the diverse and unrelated nature of compounds that produce cancer.

A teratogen can be properly defined as a chemical that increases the occurrence of structural or functional abnormalities in offspring if administered to either parent before conception, to the female during pregnancy, or directly to the developing organism. The time of exposure of the fetus generally determines the nature of the teratogenic defect. A teratogen may be active in one species but not in another. For example, cortisone is commonly used to study cleft palate in experimental animals but it is not considered teratogenic in humans. Also, a biotransformation product of a xenobiotic agent may be responsible for a teratogenic action. A good example of this is the drug thalidomide, for which there is some evidence that a metabolic product of thalidomide is the responsible teratogen. It is also recognized that a teratogenic effect may be induced indirectly by a xenobiotic agent by creating a metabolic disturbance in the female during gestation. Ethyl alcohol is a possible example of this type of teratogenic agent. Table 8.2 is a selected list of agents known to be teratogenic in humans and the main teratogenic effects that are produced by each agent.

### The Concept of Pathologic Sequelae

Pathologic sequelae are those permanent malfunctions or malformations of tissue that remain after exposure to the chemical agent has been discontinued but which were initially caused by the chemical. An excellent example of a pathologic sequel is scar tissue, which is fibrous tissue that results from failure of normal cells to regenerate following laceration or burning of the skin. Severe chemical burns can result in the production of such scar

**TABLE 8.2 A Selected List of Teratogenic Agents in Humans and Their Principle Effects**

| Compound | Clinical effects |
|---|---|
| Thalidomide | Limb anomalies |
| Ethyl alcohol | Growth retardation, cranio-facial defects |
| Diethylstilbestrol | Clear cell carcinoma in young adult females |
| Cocaine | Limb and urinary tract defects, congenital heart disease |
| Organic mercury | Fetal "Minamata disease," cerebral palsy |
| Trimethadone | Cleft palate, cardiac anomalies |
| Aminopterin | Hydrocephalis, facial deformities |

*Note.* Modified from Shepard, T.H., *Catalog of Teratogenic Agents,* 7th ed., Johns Hopkins Press, 1992.

tissue. Chemically induced cancer is also a good example of a pathologic sequel that can become evident long after exposure to certain chemical carcinogens has occurred. However, more subtle forms of pathologic sequelae can occur following exposure to certain chemicals. For example, inhalation of excessive amounts of tricresyl phosphate or methyl butyl ketone as well as hexane has been reported to lead to the destruction of certain nerve cells, so that after exposure to the agents has been discontinued, residual muscle paralysis remains. In humans, ingestion of methylmercury as a contaminant of food is known to produce mental retardation which persists after the chemical is no longer in the body. In a similar manner, undue exposure of humans to the herbicide paraquat can lead to a change in lung tissue that continues to develop long after exposure to the agent has been discontinued. In each of the foregoing examples, it is almost certain that the initial effect of the chemical on the biologic tissue occurred at the time of exposure to the chemical and the subsequent pathologic sequel that developed was the result of failure of the tissue to regenerate normal cells. Such initial changes are biochemical in nature and they go undetected until the easily measurable structural changes become manifest, at which time the term "pathologic sequelae" is applicable.

## HARMFUL EFFECTS OF CHEMICALS INTENDED FOR INTRODUCTION INTO BIOLOGIC SYSTEMS

### Toxicity Associated with Normal Concentrations

Whenever a chemical is developed in the laboratory or extracted from a natural source and placed in the environment so that it becomes identified, some form of biologic specimen should be expected to come in contact with the chemical. However, many chemicals are developed expressly for use on biologic tissue, either for their desirable effects or for their harmful effects. Drugs are developed for the beneficial effects that may be achieved by their proper use. Pesticides and insecticides are presumed to be developed for the indirect beneficial effects on an economic species by virtue of their harmful effect on one or more uneconomic species. Ironically, some chemicals are even developed for war purposes with the objective of achieving either an incapacitating or lethal effect on an enemy. Cosmetics and food additives, such as antibacterial substances, antioxidants, flavors, and sweetening agents, are only a few of the many chemicals that are intended to be introduced into biologic systems.

Drugs are good examples of chemicals that are intended for use by humans. A characteristic of all drugs is that there is some established amount (or dose) as well as schedule of amounts (if the drug is one that is to be taken on repeated occasions) that represents an acceptable and recognized quantity that can be used with confidence that harmful effects will at least not occur frequently. However, even ordinary doses of drugs produce undesirable "side actions." The frequency of occurrence of such side actions is listed in the package inserts that come with the drug. Such side actions may become intolerable and even lethal.

All chemicals have specific limits in regard to the amount that can be used without producing undesired effects. These limits obviously must include wide margins of safety as far as the economic species is concerned. Even when the intended use of the chemical is to induce a toxic effect or death in an uneconomic species, precautions are observed by the user. Thus the toxicity of all chemicals in this group becomes important regardless of their intended use, especially when the economic species is the human. In earlier chapters, it has been pointed out that various biologic as well as chemical factors influence the biologic effect of all chemicals, and that variations in response to specific amounts of chemicals are ever prevalent in the biologic kingdom. Most conditions that give rise to toxic effects from amounts (or doses) of chemicals that are tolerated by the majority of a population can be ascribed to known mechanisms, or if enough information is available regarding such reactions, they would be expected to fit in one of the categories listed in Table 7.1.

It has already been mentioned that idiosyncratic toxic reactions to chemicals, that is, reactions supposedly due to unknown factors inherent in individuals, most certainly occur, but this terminology is purposely avoided here; as additional information becomes available such reactions seem to fit in the categories listed in Table 7.1.

Many of the toxic reactions that occur with this group of chemicals following normal doses involve drugs or pesticides, and the mechanisms have become clarified as a result of studies which were performed because of the occurrence and importance of the toxicities in humans.

***Toxicity Due to Malfunction of Mechanisms for Terminating Action of the Agent.*** The most obvious cause of toxicity from normal doses of a chemical is a malfunction of the mechanism responsible for terminating the action of the agent. Under this condition, normal doses would be present for a longer period of time in the animal, and repeated doses would be likely to result in accumulation of the chemical to toxic levels in the animal. An example of such an effect would be the accumulation, as a result of kidney disease, of drugs that are ordinarily terminated by excretion in the

urine. The antibiotic drug tetracycline, following conventional repeated dosage, rapidly accumulates in the human when kidney function is seriously impaired, and this accumulation has been known to cause death. Impairment of a biotransformation mechanism involved in detoxication of a chemical also would interfere with termination of action of the chemical. This may result from a genetic deviation in the subject (see Chapter 6) or from the presence of other chemicals that may inhibit the detoxication enzyme mechanism (see Chapter 4).

The presence of other drugs may influence the termination of action of a specific agent and thereby influence toxicity of the specific agent. An example is that of feeding tyramine (in cheese) to an animal that has been pretreated with a monoamine oxidase inhibitor. In this case the monoamine oxidase inhibitor inhibits the enzyme (monoamine oxidase) which is normally present in the intestinal wall and normally inactivates tyramine as it is being absorbed from the food in the gastrointestinal tract. The result is accumulation of tyramine in the circulation in sufficient amounts to produce undesirable cardiovascular effects. However, that tyramine is the sole agent responsible for the toxicity in such cases has not been fully established.

The adsorption of a drug on plasma proteins is basically a mechanism of inactivation or temporary removal of the drug from its active state in the animal. Displacement of one drug by another from protein carrier sites can lead to rapid increases in the free form of the first drug, thereby inducing toxicity from the first drug. A good example of this is the fact that the anticoagulant drug warfarin can be displaced from nonspecific binding sites on plasma proteins by phenylbutazone, to the extent that spontaneous hemorrhage may occur. Also, the anti-folic acid agent methotrexate is displaced by sulfonamides and salicylates, and since methotrexate is generally employed in amounts that approach seriously toxic tissue levels, such displacement could lead to toxicity due to methotrexate.

A malfunction of biotransformation mechanisms for terminating the action of several drugs exists in infants. Infants not only are immature in age, but are generally biochemically immature. Certain newborn rabbits, mice, and guinea pigs lack the microsomal systems that are present in the adult animals. Both the duration of sedative action and the lethal toxicity of hexobarbital have been shown to decrease with age in the animal, and to correlate well with the level of microsomal enzyme activity for detoxifying the drug. In contrast to the infant, the aged and debilitated subject presents a biologic specimen that is metabolically deficient. In these patients, a chemical that pharmacologically impairs physical or mental activity begins

its action on an already imperfect system, so that small amounts of drug effect may be excessive.

***Toxicity Due to Action on the Wrong Target System.*** Harmful effects of a drug may result from an effect of the drug on the wrong target organ or receptor system. All drugs are imperfect, and no drug is so selective in its action that it will have but one action on the biologic specimen. The oncolytic drugs and antibiotic drugs are good examples of this type of toxicity. The inhibitory effects of the oncolytic drugs on rapidly proliferating cells is the reason for their usefulness in the treatment of malignancies, but at present these agents are not sufficiently selective in action because they also inhibit rapidly proliferating cells that are desirable, such as cells in the intestinal mucosa, in the bone marrow, and in the testis. The effect of the oncolytic agents on these desirable cells is essentially an effect of the drug on the wrong target organ.

The high order of therapeutic efficiency of some of the antibiotic drugs when they are used in the treatment of bacterial infections not only leads to death of the invading bacteria but also leads to death of some desirable bacteria in the host, thereby promoting the development of superinfections. Such superinfections are infections caused by the growth of resistant organisms (bacteria, lichens, or fungi) that are normally held in check by the forms of bacteria normally present in the oral and intestinal tracts of the host animals. Therefore, superinfections that occur during antibiotic therapy result from effects of the antibiotic on the wrong target system.

Most side actions of drugs are essentially actions of the drugs on the wrong target organ. Usually, the side actions of a drug determine the allowable dose. If it were not for the side actions of morphine on the gut and the respiratory center of the brain, the normal doses of morphine could be increased indefinitely.

All drugs have side effects, and the occurrence of exaggerated side effects either directly or indirectly as a result of the use of a drug indicates toxicities of the drug. Skin and corneal pigmentation, development of Parkinson's tremors, and hepatic dysfunction with jaundice resulting from the use of chlorpromazine are side effects that can become excessive and necessitate discontinuance of the drug. Suppression of thyrotropic hormone is a side effect associated with adrenocorticoid therapy and can become serious and require discontinuance of the drug. Teratogenic actions of drugs are side effects that are actions of drugs on the wrong target organ, namely the fetus. Thus, thalidomide is a good sedative drug, even in the pregnant woman, but if it is administered to the pregnant woman at the improper interval after conception, it is likely to induce malformations in the developing fetus.

***Toxicity Due to Synergism with Other Chemicals.*** Harmful effects of a chemical associated with normal dosage of the chemical can occur as a result of the presence of other drugs that have similar pharmacologic actions. The total effect of two agents that have similar pharmacologic actions is a response that is either equal to the summation of the effects of the individual agents or greater than the summation of the independent effects of the two agents. The former condition is aptly termed "summation." The latter response is termed "potentiation" or "synergism" and represents the condition whereby one drug is made more potent in the presence of an amount of another drug which alone may produce minimal or no pharmacologic effect. The classic example of this is the potentiation of epinephrine response in the laboratory animal by the addition of cocaine.

Usually, the effect of two agents is the summation of the responses to each agent. An example of summation of action of two drugs is the enhancement of sedation as produced by an antihistaminic agent when barbiturates are administered. Summation of drug action is commonly encountered in regard to the use of anesthetic agents. Preanesthetic medication may consist of a sedative (such as a barbiturate), and when this is followed with general inhalation anesthesia (such as nitrous oxide, ether, or cyclopropane) the total dose of the inhaled anesthetic is less than that required in the absence of the preanesthetic medication. In these cases, the threshold required to produce a given intensity of pharmacologic effect by a second drug is lowered because of the presence of the initial drug. Attention has been directed toward the summation or potentiation that takes place among the various pesticides in regard to lethal response of mixtures of these agents. The hypokalemia that is associated with the clinical use of organomercurial diuretic agents may enhance the cardiac toxicity of the digitalis glycosides. Physiological imbalance in acid–base status, as well as dehydration of animals created by excessive vomiting or diarrhea that may be drug induced, increases the toxicity of improper ion therapy.

The list of direct and indirect effects that one chemical can have on the toxicity of a second chemical is almost limitless. Chemical-induced disease resulting in impairment of function of an organ can markedly influence the toxicity of a subsequently administered agent that has a similar action, and can influence the termination of action of a second agent that may have a completely different pharmacologic action. For example, in industry permanent injury to the respiratory airway, such as the pulmonary fibrosis and emphysema associated with chronic exposure to dusts or primary irritants, or damage to the liver and kidney associated with exposure to the chlorinated hydrocarbons, impairs the reserve capacity of the organ to carry on its function; if the organ is then exposed to additional agents that have a primary toxic effect on the same organ, small degrees of toxicities may be

sufficient to seriously impair the remaining function of the organ. Mercurial-induced disease in the kidney predisposes the organ to toxicity from subsequent exposures to arsenic. Industrial workers with various degrees of exposure to benzene may show a direct toxicity in hematopoietic tissue, manifested as diminution in erythrocytes; some subjects even show a reduction in hemoglobin. These subjects would be expected to be susceptible to methemoglobin-forming agents, such as nitrites, or oxygen-displacing agents, such as carbon monoxide.

### Toxicity Associated with Excessive Concentrations

***Nonspecific Caustic or Corrosive Actions.*** Exposure to excessive doses or concentrations of chemicals intended for administration to biologic specimens can induce toxic effects of the same type as those described for the chemicals not intended for introduction into biologic systems. Caustic or corrosive chemicals intended for introduction into biologic systems are usually agents used as bacteriocides or fungicides, e.g., cresol; mercurials such as thimerosal (Merthiolate) and merbromin (Mercurochrome); inorganic compounds such as silver nitrate, mercuric chloride, and iodine; the quaternary ammonia compounds such as benzalkonium and cetylpyridinium; and the acids such as boric acid. When these agents are used in proper concentrations on surfaces or skin, they serve a useful purpose as antiseptics or germicides, although it is generally agreed that when these substances are used on the human skin, they are not capable of sterilizing the skin without destroying it. Accidental or intentional consumption of these agents by animals can cause local effects ranging from primary irritation to gross necrosis of the tissue in the gastrointestinal tract as well as systemic effects.

***Exaggerated Pharmacologic or Toxicologic Actions.*** Excessive doses of drugs that affect physiological mechanisms can produce toxicity either by excessive pharmacologic effect or by specific toxic actions or side actions, which are ordinarily avoided by proper, intelligent use of drugs. An example of the former is the excessive and possibly lethal pharmacologic effect that occurs when barbiturates or tranquilizers are taken for suicidal purposes. Examples of the latter are the excessive toxicity produced by the cardiac glycosides on cardiac function and the hemorrhagic episodes associated with the use of anticoagulant drugs when therapy with these drugs is not properly conducted or followed. An additional important type of excessive exposure involves accidental or intentional exposure of the wrong target species, that is, the economic species, to agents such as the commonly available potent pesticides and insecticides which are used for their high order of toxicity to the uneconomic species.

***Pathologic Sequelae.*** In order for a chemical compound to be included in the group of chemicals intended for introduction into biologic systems, it should not produce serious pathologic sequelae at least in an economic species, except possibly under unwarranted conditions of abusive use of the chemical. However, there have been several instances in which serious pathologic sequelae occurred after chemical agents were already in use even by humans. In some of these cases, acceptable as well as excessive doses initiated the pathology. An example is the carcinogenic agent butter yellow, which had been used as a food coloring agent. Another example is the sedative drug thalidomide, which was found to be a potent teratogenic agent when administered at the proper time to pregnant women. Also, the drug triparanol was found to produce cataracts in man after it had been in use for several years. Such sequelae obviously result from the action of the chemical on the wrong target system; they are composed of toxicities that are essentially irreversible. Whenever serious pathologic sequelae such as teratogenic, mutagenic, or carcinogenic effects are discovered in an economic species, restrictions are placed on the use of the agent. Whenever a new chemical agent is intended for use as a food additive or a drug, it should be thoroughly tested through the use of preclinical toxicity tests for its ability to produce pathologic sequelae.

***Sociologic Factors in Toxicity.*** Certain drugs, such as those that influence the functions of the brain, acquire considerable importance more for sociologic reasons than for their therapeutic value. Notable among these are the narcotics, barbiturates, amphetamines, and alcohol. Their sociologic significance stems from the fact that certain people become addicted to their use, and society (in the United States) disapproves of addiction to such drugs. Pharmacologically, addiction evolves through the following conditions: first, habituation, which is the psychologic or emotional dependency on the drug; second, physical dependence, whereby an altered physiological state exists because of frequent exposure to the drug and withdrawal of the drug leads to a physical and emotional illness known as the abstinence or withdrawal syndrome; and third, tolerance to many of the pharmacologic effects of the drug.

It is unlikely that without tolerance, which leads to the use of greater than normal doses of the drug, a person could ever become addicted to these agents. This is because ordinary doses would fail to produce a desired pharmacologic effect, which is important in maintaining the compulsion to take the drug. It is for this reason that the toxic effect (in a sociologic sense) of addiction to drugs is placed in the category of harmful effects of excessive dosage of chemicals intended for exposure to biologic specimens. It may be argued that addiction should not be considered as a harmful

effect of such drugs because the addict who is able to obtain the drug may carry on normal activities and be physically normal. If direct organic or functional toxicity occurs in addicts, it may be said to occur only under conditions in which the chemical is withdrawn from the subject, thereby leading to the abstinence syndrome.

The *abnormal* effects of those chemicals that are intended for introduction into biologic systems are also associated with the development in the biologic system of immune mechanisms. These mechanisms, together with examples, are discussed in Chapter 9.

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# CHAPTER 9
# *Abnormal Response to Chemicals*

Physicians frequently use the terms "hypersensitivity reactions" and "sensitization reactions" interchangeably to describe chemical-induced effects that are attributable to the immune mechanism in the body. It would be preferable to reserve the term "hypersensitivity" to describe a response with the following characteristics: (1) it occurs in only a few individuals, (2) it consists of the normal, expected effects of the chemical, (3) it does not require preconditioning of the individual to the chemical, and (4) it occurs following the administration of a dose of a compound that is smaller than the usual dose. That is, a hypersensitive response also may be defined as a normal pharmacologic or toxicologic response of greater intensity than that which occurs in the majority of the population following a given dose of the chemical agent.

In contrast to this, a sensitization reaction to a chemical is the response involving the immune mechanism. It is an abnormal effect of the chemical in the sense that it is different from the pharmacologic effect associated with ordinary doses or the toxicologic effect resulting from excessive doses of the chemical.

## THE IMMUNE MECHANISM

The immune mechanism is a natural defense system present in mammals. It is a defense system in the sense that it protects the body from invading microbes and chemicals that have antigenic properties. An antigen by definition is any substance that activates the immune system leading to the production of proteins called antibodies. Antibodies can be unattached to cells or can be attached to cell membranes. Once formed by the initial exposure to the antigen the antibodies recognize any subsequent exposures to the antigen. A resultant antigen–antibody interaction takes place. If the antigen is a microbe (or part of one) the immune system destroys the microbe or facilitates destruction of the microbe via other cells (phagocytes) that the reaction recruits. Actually, one antibody can unite with different antigens and one antigen with many antibodies, in both cases with varying degrees of affinity. In such a manner the body develops "immunity," such as immunity to small pox by exposure to a small pox-like virus (cow pox virus).

A very important property of the normal immune system is that it is capable of recognizing "foreign" chemicals (such as xenobiotic agents) as being different from "self" chemicals. When the system fails in this regard self chemicals may initiate the immune mechanism. When this happens the subjects develop autoimmune disease, that is, immunity against their own cells. The immune system is therefore a two-step process, the first being formation of antibody associated with the initial exposure and the second being the antigen–antibody interaction associated with subsequent exposure to the antigen. Many xenobiotic chemicals are probably incomplete antigens (called haptenes) and bind to macromolecules in the body in order to become antigens.

The immune mechanism involves the following events: the initial exposure to a chemical substance, an induction period in the animal, and finally the production of a new protein called an antibody (Fig. 9.1). As illustrated in Fig. 9.1, the chemical or a metabolic product of the chemical acts as a *haptene* (or hapten), a substance that combines with an endogenous protein to form an *antigen.* An antigen is capable of eliciting the formation of cellular or humoral new proteins called *antibodies.* The initial exposures do not result in cellular damage but cause the animal to be "sensitized" to subsequent exposures to the chemical. Exposure of the animal to the chemical on a subsequent occasion will lead to the formation of the antigen, which reacts with the preformed antibodies leading to a cellular response or even cellular damage. This is the "immune response" or "allergic response."

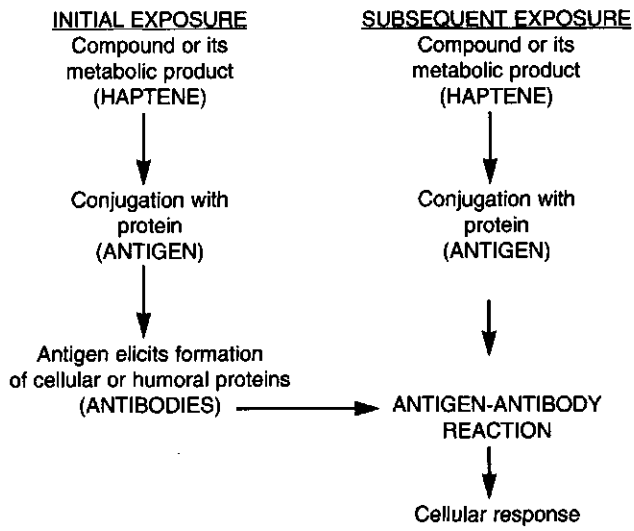



**FIGURE 9.1** The allergenic mechanism of sensitization responses to chemicals.

## INITIAL EXPOSURE TO HAPTENS—THE SENSITIZING SYSTEMS

The basic components of the immune system have been described in detail. A brief description of the system is helpful to understand the classification of what the "abnormal effects of chemicals" are and how they are produced by xenobiotic chemicals. Initially it is clear that not all but many chemicals are potential antigens. Those that are antigens (or fragments of which are antigens) when allowed to enter the body encounter specific cells (lymphocytes) in the blood that recognize any conceivable antigen. This process is aided by additional "accessory" cells which physically take the chemical antigen to sites in the body, mainly the spleen and lymph nodes, where there is an abundance of lymphocytes and where those lymphocytes are "activated" by recognizing the antigen. Activation causes the cell to produce antibodies and to collaborate with other nonantigen producing cells (i.e., phagocytes) and with proteins (termed "compliments") in an all-out attempt to inactivate the antigen. The immune system is under continuous self-regulation, enabling it to regulate the intensity of the immune response to the antigenic load that it encounters.

The activation of a lymphocyte leading to the production of an immune globulin by the cell involves the concept that the lymphocyte contains many

"mini-genes" that exist in an orderly manner on its chromosomes. The lymphocytes that participate in the immune system can be identified as B and T cells. The B cell is one that has maturated in the bursa or bone marrow and the T cell is one that maturated in the thymus gland. They play different roles in the immune mechanism, as shown in Fig. 9.2 and as described below.

T cells are the most abundant type of circulating lymphocytes in adults. In order for an antigen to be recognized by a T cell, the antigen must first combine with a surface receptor on a presenting cell, such as a monocyte-macrophage cell. Special receptors on the T cell then recognize the surface-bound antigen, thereby activating the cell to produce antibodies and to produce two types of regulatory T cells known as "helper" and "suppressor" cells. These helper and suppressor cells are important in the immune systems since they function, respectively, to increase or to decrease normal antibody production by B cells as well as T cells. In normal, healthy persons the relative numbers of these two types of cells are delicately balanced. Such T cell immunity can be passively transferred from an immune person to a naive person only through transfer of the blood cells.

In addition certain T cells possess cytotoxic properties and other T cells can be activated by bacterial antigens to form cytotoxic T cells. They are so named because they can directly destroy invaders such as bacteria, fungi, and viruses and even some tumor cells. An additional function of antigen-activated T cells is their ability to induce other cells to release several mediators, such as lymphokines, which attract a variety of cells to inflammation sites in the body.

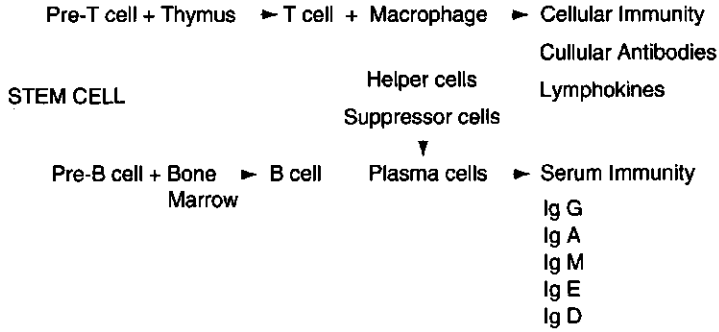



**FIGURE 9.2** Source and role of B and T lymphocytes in the immune system. Modified from *Allergy Theory and Practice*, 2nd ed. (P. E. Korenblat and H. J. Wedner, Eds.), W.B. Saunders, Philadelphia, 1992.

About 25% of all peripheral blood lymphocytes in adults are B cells. When a B cell encounters a chemical antigen that fits the cell receptor, even if the affinity is weak, activation of a minigene leads to the production of antibody. Once this process begins, the cell will undergo point mutation of the immunoglobulin genes resulting in additional lymphocyte clones with greater and greater affinity for the initial hapten. Even if there is no further exposure to the hapten the new clones of lymphocytes remain in the circulation in a resting state, alert to any further exposure to the hapten.

Following the initial recognition of an antigen by B cells, a cascade of helper (and suppressor) cells mentioned above assist (or inhibit) the activated B cells to enlarge, divide, and develop antibody-secreting capability. At this time they become known as plasma cells. The antibodies that are produced appear in the serum where they are recognized in the gamma globulin fraction as IgG, IgA, IgM, IgE, and IgD globulins. B cell immunity can be passively transferred to a naive person via blood plasma. The entire system can produce numerous variations in immunoglobulin molecules and it is self-controlled by feedback-inhibitory mechanisms. It has been said that a mouse weighing less than 1 oz can make over 100 million different antibodies.

## SUBSEQUENT EXPOSURE TO HAPTENS—THE ALLERGIC REACTION

After it was demonstrated that certain types of sensitization could be transferred to a naive person via the serum, it was found that the IgE protein was the responsible immunoglobulin. In the sensitized person reintroduction of the antigen allows for the reaction to take place between the antigen and the preformed circulating antibody. This complex then reacts with receptors on the mast cells and on basophils, causing them to release several "mediators" from their secretory granules into the extracellular environment. These mediators are pharmacologically active agents. Table 9.1 is a list of these mediators and their pharmacologic effects. The biologic effects of the mediators on the body constitute one form of allergic response or sensitization response.

## THE IMMUNE MECHANISM IN TOXICOLOGY

The immunogenic mechanisms play a significant role in all branches of human health. These mechanisms are of particular importance in toxicology because so many simple chemicals have varying degrees of haptenic activity.

**TABLE 9.1 Mediators and Biologic Effects of Immediate "Sensitization Reactions"[a]**

| Mediator | Effects |
|---|---|
| Intraglandular | |
| Histamine | Vasodilation, increased vasopermeability, pruritus, bronchoconstriction |
| Proteases (tryptase, chymase) | Degradation of blood vessel basement membranes, generation of vasoactive complement |
| Heparin | Complexes with proteases, inhibition of blood coagulation |
| ECF-A | Eosinophil chemotaxis |
| Neutrophil chemotactic factor | Neutrophil chemotaxis |
| Membrane-derived | |
| Prostaglandin D-2 | Vasopermeability, bronchoconstriction |
| Leukotrienes | Vasopermeability, bronchoconstriction |
| Platelet-activating factor | Platelet aggregation, vasopermeability, bronchoconstriction, chemotaxis |

[a] Modified from *Mediators of Immediate Hypersensitivity Reactions* (Serafin, W. E. and Austen, K. F.), *N. E. J. Med.* No. 1 1987. pp. 30–34.

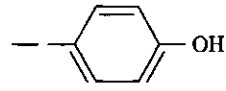
The fact that simple chemical compounds can activate the immune system was first demonstrated by Landsteiner and co-workers (Landsteiner, J., and Jacobs, J., *J. Exp. Med.* **61:** 643, 1935, and *J. Exp. Med.* **64:** 625, 1936). Their observations have been confirmed and extended. These workers showed that certain dinitrobenzene derivatives could be injected or applied at frequent intervals and in amounts that would not produce an undesirable effect on the animal, and that after a lapse of 7 to 14 days, a subsequent injection or application of the same or a closely related compound would induce a tissue response at the site of application or systematically in the animal. They showed that certain derivatives of the dinitrobenzene nucleus were highly effective in initiating this response, whereas other derivatives of the same nucleus were essentially ineffective.

Those compounds that were capable of initiating the sensitizing phenomena contained halogenated radicals. If the halogen in the dinitrobenzene nucleus was replaced with a hydrogen, hydroxyl, methane, or amino group, the resultant compound was essentially nonsensitizing and nonreactive. Landsteiner theorized that the presence of the halogen facilitated stable binding of the simple chemical with an endogenous protein carrier, and this complex was the antigen that was capable of eliciting the formation of antibodies in the animal.

Many simple chemicals have haptenic qualities, and all degrees of such activity are found. For example, phenol is rarely antigenic, even under laboratory experimental conditions, whereas the sedative drug phethenylate (phenylethylhydantoin) induces allergic symptoms in virtually every human who receives the drug on multiple occasions. Certain chemical groupings appear to have the quality of conferring haptenic properties on the chemical. In a similar manner, certain radicals on specific amino acids are probably involved in the formation of the hapten–protein complex. A series of chemical groupings on the hapten and on the protein which would be expected to confer such activity has been compiled by Davies with the help of O-Mant and is reproduced in Table 9.2. The hapten–protein complex involves a firm binding mechanism which is almost certainly a covalent bonding mechanism that causes the loss of the chemical characteristics of both the hapten and the protein. Chemicals that react with protein in a readily reversible reaction (as by ionic binding or by Van der Waal's forces) probably do not form antigens.

Many chemicals do not appear to react with protein *in vitro,* but are known to be sensitizers (or haptens) in the intact animal. Such chemicals are believed to undergo metabolic alteration *in vivo,* yielding products that have haptenic properties. If a biotransformation product of a chemical is highly reactive with protein, chemicals that undergo similar metabolic

**TABLE 9.2 Some Reactive Groups on Haptens and on Amino Acids Which Are Cabable of Reacting in the Formation of Antigens**[a]

| On hapten | | On amino acid | |
|---|---|---|---|
| Diazonium | $-\overset{+}{N}{=}N$ | Serine | $-OH$ |
| Thiol | $-SH$ | Lysine | $-NH_2$ |
| Sulfonic acid | $-SO_3H$ | Arginine | $-NHC(=NH)-NH_2$ |
| Aldehyde | $-CHO$ | | |
| | | Cysteine | $-SH$ |
| Quinone |  | | |
| Active halogen | | Cystine | $-S-S-$ |
| | | Tyrosine |  |

[a] Data from Davies, G. E., *Proc. Eur. Soc. Study Drug Tox.* **4**:198, 1964.

transformation may be responsible for the formation of a hapten common to all chemicals in that group. Regardless of whether the chemical is the hapten, closely related compounds may effectively substitute for each other in the role of acting as a specific hapten. It is now generally accepted that there are some specific reactive groups on haptens and on amino acids that are capable of interaction leading to the formation of antigens. Table 9.2 lists some of these groups.

The formation of the antibody is dose-dependent with regard to the antigen. When antibodies are demonstrated in the plasma, they appear only after a latent period following initial administration of the hapten or precursor of the hapten. This is followed in succession by a log phase period of exponential rise in the antibody concentration (or titre) of the blood, a secondary stationary phase when the antibody concentration remains constant, and a subsequent decay period in antibody concentration. If a challenging dose of a sensitizing chemical is administered at a time when the antibody concentration is high, the immune response would be expected to be more extensive than it would be if the sensitizing chemical were administered when the antibody titre was low. Therefore, a challenging dose of a sensitizing chemical may or may not elicit an immune response, depending on the time of the administration of the challenging agent and the level of the antibody titre at that time.

Various serologic methods have been used to demonstrate the presence of circulating antibodies. Such tests are only infrequently successful in evaluating the presence or level of chemical-induced antibodies, although challenging doses of the chemical administered to the intact animal will produce the characteristic immune reactions in sensitized animals. Although several serologic tests (passive hemagglutination, complement fixation, and passive cutaneous anaphylaxis) have low limits of sensitivity, it is probable that antibodies can be present, but the number may be below levels detectable by these testing procedures.

Penicillin preparations are commonly a cause of sensitization in humans, and a brief discussion of these preparations demonstrates some of the complexities of the sensitization process. The penicillin molecule is generally believed to be incapable of acting as a hapten because of its low order of reactivity and the reversible nature of its reactivity with proteins. Therefore, the hapten is assumed to be either a biotransformation product of penicillin or a contaminant that is common to all preparations of penicillin. The assumption that a breakdown product is the responsible agent has some experimental support.

Although several breakdown products from penicillin are possible haptens, a breakdown product that seems to be involved is penicillinic acid. It is a normal contaminant of all penicillin preparations, it appears as an

early degradation product of penicillin, and it is capable of reacting covalently with disulfide, sulfhydryl, or amide linkages. If penicillinic acid is reacted with an amino group at neutral pH, a penicilloyl linkage is formed. The reactions involved in the formation of the penicilloyl conjugate are shown in Fig. 9.3. Such a penicilloyl protein is highly antigenic in rabbits and guinea pigs. The antibodies that are formed can be detected in the serum and can be passively transferred to other animals. Furthermore, antipenicilloyl antibodies have been found in the serum of humans with a history of allergic reaction to penicillin. Although the penicilloyl group appears to be present in sensitization to penicillin, total allergy to penicillin probably involves multiple factors that characterize the genetic and immunologic status of the host.

In experimental toxicology, following the initial sensitizing dose of a chemical any subsequent doses are referred to as "challenge doses" which attempt to elicit the allergic or immune response. The antigen–antibody reaction is frequently a highly specific reaction in which only the specific antigen or hapten that was used for sensitization reacts with the antibody. Conversely, it may be stated that the antibody frequently will react only with the hapten or hapten–protein complex (antigen) that was used for sensitization. This mechanism is therefore utilized in protein immunochemistry for analytic purposes. However, the antigen–antibody reaction is not

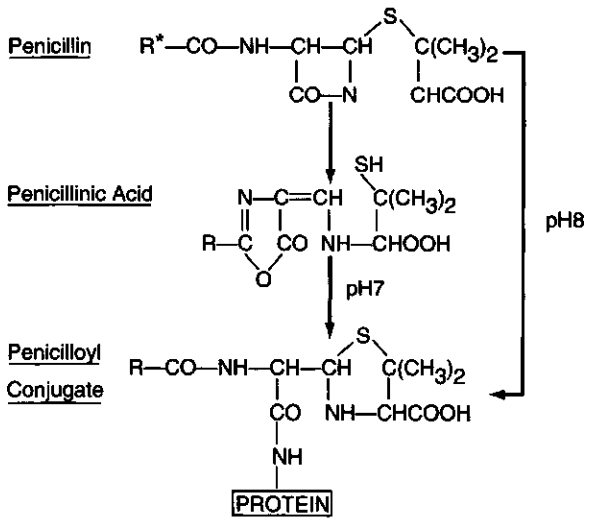



**FIGURE 9.3** *R varies with the type of penicillin. From DeWeck, A. L., *Proc. Eur. Soc. Study Drug Tox.* **4:** 206, 1964.

always entirely specific for the hapten when simple chemicals are used to induce the formation of the antibody. For example, when a certain dinitrophenyl derivative acts as the hapten, the antibody produced against that hapten also reacts when other dinitrophenyl derivatives are used as the challenge dose, although the reaction is of lesser intensity than that produced by the hapten used to induce the antibody.

Because of the lack of absolute specificity in hapten–antibody reactions, the term "cross-sensitization" has developed to indicate this lack of specificity for simple chemical haptens. Cross-sensitization essentially means that an induced antibody may react with antigens formed from haptens of similar chemical structures. This concept is of considerable importance in immune reactions to xenobiotic chemicals and particularly to drugs. Many series of drugs have been developed, such as the barbiturates, the synthetic narcotics, and the sulfonamides, in which members of the series have similar pharmacologic actions, have similar structures, and are similarly biotransformed by the animal. Thus one member of a series may serve to sensitize an animal so that the immune response will occur when the animal is challenged with other members of the series of drugs. The phenomenon of cross-sensitization has been observed clinically between benzoic acid, *o*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, and *p*-hydroxybenzoic acid esters. This is a particularly important cross-sensitization group of chemicals because of the extensive use of these esters as preservatives in nutrients, cosmetics, soaps, and drug preparations. Although cross-sensitization is generally considered to be limited to compounds having similar chemical structure, the similarity may not be obvious since cross-sensitization has been observed between the antibacterial agents neomycin and bacitracin, which represent distinctly different structures.

## IMMUNE MECHANISMS IN CLINICAL TOXICOLOGY

In clinical medicine four types of immunologic responses are recognized. These are:

Type 1 (immediate responses). Humoral serum antibodies release pharmacologic agents from mast cells and basophils. An example is allergic rhinitis (hay fever).

Type 2 (cytotoxic responses). Compliment is involved and the antibodies are on the surface of cell membranes. The response by which body cells are killed may be very specific. An example is penicillin-induced anemia.

Type 3 (immune complex sensitization responses). Soluble antigen combines with antibodies in the serum yielding an immune complex; serum

compliment is activated and the complex is deposited in specific tissues. An example is incompatible serum sickness.

Type 4 (delayed sensitization responses). Antigen-activated T cells suppress microbial infection mainly through macrophage activity and in the process enzymes may be released from the macrophages and lymphocytes. The enzymes destroy normal tissue cells. The process involves a 1- to 2-day delay before the tissue response is clinically manifested. Examples are contact dermatitis and the tuberculin reaction.

Types 1 through 3 can be transferred in the serum from sensitized to naive persons, whereas type 4 can only be transferred via the cells and the circulating antibody does not seem to be required.

The difficulties encountered in proving that a chemical is a cause of a toxic reaction, besides proving that the toxicity is due to a sensitization reaction in humans, are great unless the occurrence is so frequent that the cause–effect relationship becomes obvious. Current belief holds that whereas many of the adverse and abnormal reactions to chemicals are reported as sensitization reactions, proof of this is lacking. Compilations of adverse drug reactions, such as the Registry of Blood Dyscrasias of the American Medical Association, are useful to the practicing physician and lead to recognition of drugs commonly encountered in drug toxicities, but are of little value from the standpoint of shedding light on the mechanism involved in the production of the toxicity. However, by use of special methods, immune-mediated responses in the skin can be differentiated from simple irritant responses, thereby improving the evaluation of causation. Table 9.3 lists some classes and examples of materials that produce immunologic contact urticaria that have been seen in dermatology clinics.

A variety of chemicals are known to produce undesirable reactions in the skin of man when the skin is exposed to sunlight and, in some cases, to artificial light. These dermatologic lesions vary from sunburn-like responses to edematous, vesiculated, or bullous types of lesions. Since both exposure to light and the presence of the drug are involved, such reactions are called "photosensitivity" reactions. Two mechanisms are believed to be responsible for the reactions. The first is the phototoxic type, which is the result of a nonimmune mechanism and occurs on the first occasion of exposure to the drug plus light. By this mechanism the compound, which is present in the skin, absorbs energy (from the ultraviolet range of 2800 to 4000 Å) which converts the compound to a higher energy active state. The absorbed energy is reemitted as radiation energy, which becomes part of a photochemical reaction leading to cellular damage. Examples of compounds that are known to produce such phototoxic reactions are the

**TABLE 9.3 A Selected List of Classes and Examples of Agents Producing Immunologic Contact Urticaria**[a]

| Class | Examples |
|---|---|
| Animal products | Blood, hair, silk, wool |
| Food | |
| Dairy | Cheese, milk |
| Fruits | Apple, orange, peach |
| Grains | Flour, wheat bran |
| Nuts | Peanut butter, sunflower seeds |
| Meats | Beef, chicken, lamb |
| Seafood | Fish, shrimp |
| Vegetables | Cabbage, onion, tomato |
| Fragrances & flavorings | Menthol, vanillin |
| Medicaments | Aspirin, penicillin, local anesthetics |
| Metals | Copper, nickel |
| Plant products | Perfumes, spices, rubber latex |
| Preservatives | Benzoic acid, parabens |
| Resins | Epoxy resin, formaldehyde resin |

[a] Data from *Occupational and Industrial Dermatology* (H. I. Maiback, Ed.), 2nd ed., Year Book Publishers, Chicago, 1987.

coal tar derivatives pyridine and anthracene, the antibacterial agents dimethylchlortetracycline and sulfonamides, and the tranquilizer chlorpromazine.

The second type of photosensitive response to chemicals involves the haptene-allergic mechanism. The chemical or its metabolic product acts as the haptene, which combines with a tissue protein leading to the formation of an antigen which in turn elicits the formation of antibodies. On reexposure to the chemical plus exposure to light (electromagnetic energy of the proper wavelengths) the antigen–antibody response is manifested as cellular damage in the skin. There is probably a genetic basis for susceptibility to this mechanism, since only a small percentage of the population shows this form of photosensitivity to chemicals. Examples of agents that have been shown to induce this type of response are the thiazides, aminobenzoic acid, griseofulvin, promethazine, and chlorpropamide.

The cutaneous sensitization responses to chemicals have several common features which are summarized as follows: chemicals may be well tolerated for days, months, or years and suddenly give rise to sensitization reactions, and the sensitization response is different from the pharmacologic response. Chemicals that give different pharmacologic effects may give rise to identical allergic responses. The dosage necessary to produce the sensitization

reaction is less than that required to produce the expected pharmacologic effect. Once a reaction has occurred, it will recur regularly on administration of the chemical, even in small doses. A chemical may produce a different sensitization response in different persons as well as in the same person at different times.

The intensity and nature of an allergic reaction may be of such minor toxicologic importance that it is of little significance. In some cases, continued exposure to the offending chemical will be accompanied by gradual disappearance of the allergic response. However, all sensitization reactions are related to the dose of the challenging agent, to the antibody titre, and to the route of administration of the chemical. In the absence of knowledge of the antibody titre, administration of drugs or chemicals to known sensitized persons or animals may result in severe sensitization reactions. Such a procedure should be approached with extreme caution. The nature of the sensitization response is not always predictable. On one occasion or in one person, the response may be manifested as a cutaneous reaction, in another it may be a vascular response, and in a third it may be a blood cell suppression response.

## ACTIVATION AND SUPPRESSION OF THE IMMUNE SYSTEM

Failure of the immune system does occur clinically when there are deficient amounts or activity of various components of the system. Examples are diseases produced by compliment deficiency, phagocytic cell defects, lymphocyte deficiency, and particularly B or T cell deficiency. In recent years because of the worldwide awareness of Acquired Immunodeficiency Syndrome (AIDS), interest in methods of improving the overall activity of the immune systems has achieved a very high priority. Basically, AIDS is caused by infection with Human Immunodeficiency Virus (HIV) with a resulting decrease in the total number of lymphocytes and a disproportionate decrease in the absolute number of specific T cells. Currently there is no antiviral drug that will cure AIDS. In those immune deficiencies that involve antibody deficiency, antibodies can be replaced by treatment with intravenous serum globulin preparations, and purified IgG, IgA, and IgM are also available. In severe combined immunodeficiency disease, bone marrow transplantation has been used. As far as immune deficiency to xenobiotics is concerned, it might even be viewed as a beneficial condition were it not for the fact that the same system would fail to achieve its main function of protecting against microbial infection.

Interest in suppression of the immune mechanism has been widespread because of the role of this mechanism in allergy and autoimmune disease

in humans and in rejection of foreign grafts in transplantation experiments, and because it is basically involved with cell proliferation mechanisms in the animal. Whereas there is no agent that will selectively suppress only the antibody-forming mechanism, any procedure or drug that suppresses cell proliferation or interferes with protein or nucleic acid formation will, under proper experimental conditions, suppress the antibody-forming mechanism.

Clinical allergists commonly use an approach to treat allergy called Allergy Immunotherapy. The procedure has been used for many years in patients that have allergic rhinitis (hay fever). The procedure is to treat the patient once or twice weekly with subcutaneous injections of the antigen, using as the initial dose one that has been shown to be tolerated by skin tests. Subsequent doses may be increased depending on the occurrence of reactions to the lower dose. A precise explanation for the claimed effectiveness of allergy immunotherapy has not been demonstrated but the apparent goal of the procedure is twofold. One purpose is to attempt to produce a blocking antibody which can effectively compete with the existing antibody for the antigen. Second, the procedure is helpful in producing a tolerance type of response, presumably by decreasing the sensitivity of the target cells to the antigen–antibody response. It is known that when adult animals are given repeated subimmunogenic doses of an antigen, or when they are given excessively high doses of the purified antigen, this leads to the development of a tolerance that is characterized by suppression of the immune response to the particular antigen when it is subsequently administered in what is normally an immunogenic form. The mechanism of this tolerance induction is not known, but it is believed to be associated with an inhibition of lymphoid cell formation.

The effect of irradiation on the immune response has been shown to be either activating or depressing to the mechanism. Generally irradiation will be maximally depressing if the animals are irradiated a few hours to 2 days before sensitization with the antigen. However, the opposite effect of activation of mild sensitization in immune disease may follow whole-body radiation. Similar observations have been made in regard to the effects of chemical agents on the immune mechanism. Chemicals of interest in this regard are generally those that are powerful inhibitors of cellular proliferation and differentiation. Examples are alkylating agents such as mustards (methyl-bis-beta chlorethylamine or tris-beta chlorethylamine), purine and pyrimidine analogs such as 6-mercaptopurine and thioguanine, and antibiotics such as Actinomycin D. Whereas these agents are generally considered as nonspecific suppressive compounds, their effects on immune mechanisms largely depend on the experimental design used in the test, the species studied, and timing of administration.

The sensitization reaction represents an insidious form of chemical-induced toxicity. Persons who have a history of intolerance to certain foods or chemicals, especially drugs, or who have a history of allergic disease such as hay fever or asthma should be given drugs with caution. There is no definitive manner by which sensitization to a chemical can be predicted in specific individuals. A person should not be exposed to a known sensitizing chemical either in the form of a drug or in the process of occupational endeavor unless the benefits of such exposure outweigh the potential hazard associated with such exposure. Once the process of sensitization has occurred, it is more reasonable to expect that the person will continue to be sensitized for an indefinite period of time than to expect that desensitization can be accomplished or will spontaneously occur in time. It is not reasonable to use drugs that are known sensitizers (such as the antibiotics) in minor illness when such drugs may be desired and necessary for life-saving purposes on some subsequent occasion.

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