Curtis D. Klaassen
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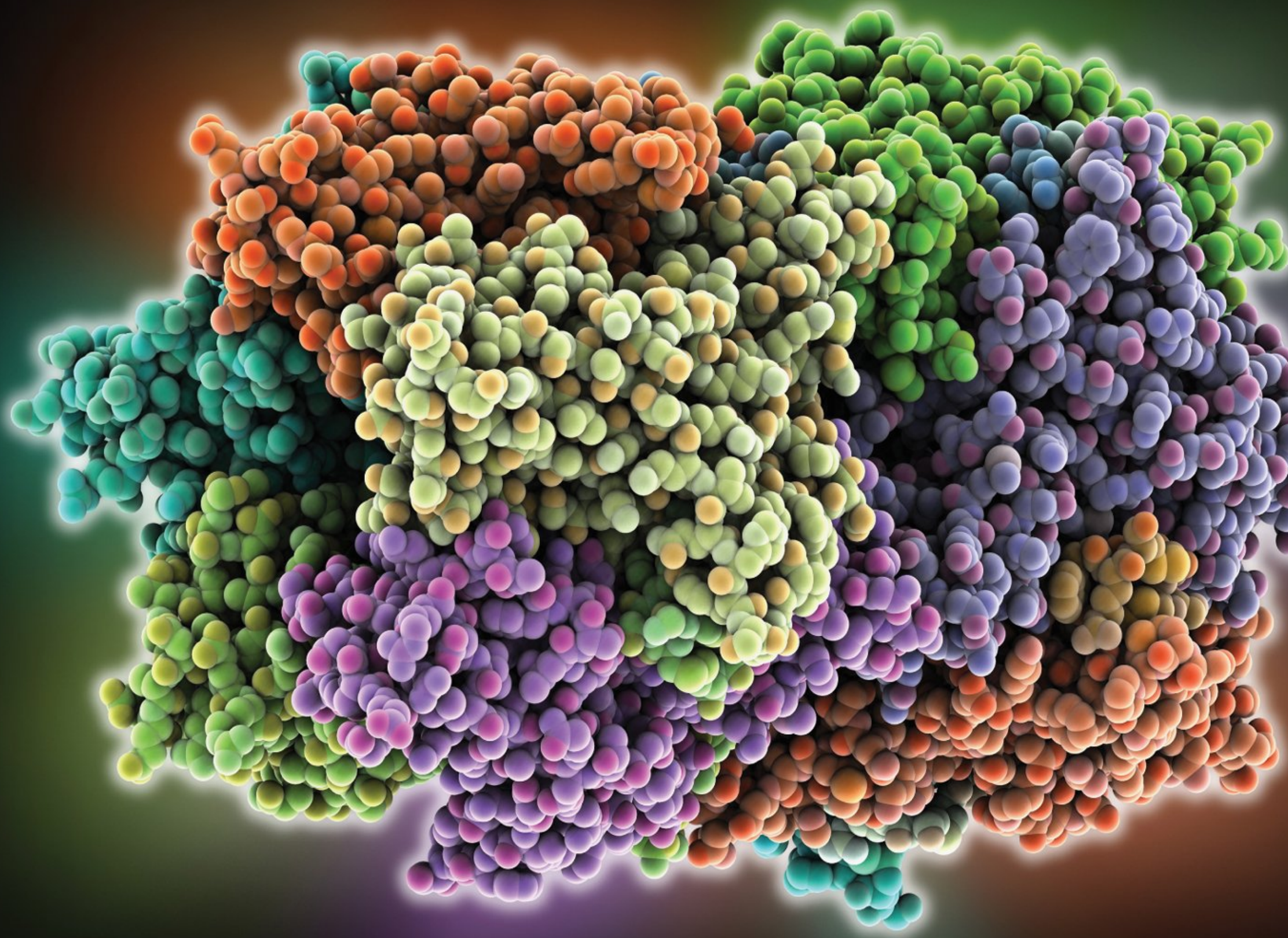

CASARETT & DOULL'S

ESSENTIALS of TOXICOLOGY

Third Edition

Mc Graw Hill Education
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INTERNATIONAL EDITION

Casarett & Doull's Essentials of Toxicology

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Casarett & Doull's Essentials of Toxicology

Third Edition

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Preface

This updated full-color edition of Essentials of *T*oxicology distills the major principles and concepts of toxicology that were described in detail in the eighth edition of Casarett & Doull's *T*oxicology: The Basic Science of Poisons. We are grateful to the authors who contributed to the eighth edition of Casarett & Doull's *T*oxicology: The Basic Science of Poisons; their chapters in the parent text provided the foundation for the chapters in this edition of Essentials of *T*oxicology.

Essentials of *T*oxicology concisely describes the expansive science of toxicology, and includes important concepts from anatomy, physiology, and biochemistry to facilitate the understanding of the principles and mechanisms of toxicant action on specific organ systems. We trust that this book will assist students in undergraduate and graduate courses in toxicology, as well as students from other disciplines, to develop a strong foundation in the concepts and principles of toxicology.

The book is organized into seven units: (1) General Principles of Toxicology; (2) Disposition of Toxicants; (3) Nonorgan-directed Toxicity; (4) Target Organ Toxicity; (5) Toxic Agents; (6) Environmental Toxicology; and (7) Applications of Toxicology. A summary of key points is included at the beginning of each chapter, and a set of review questions is provided at the end of each chapter. We invite readers to send us suggestions of ways to improve this text and we appreciate the thoughtful recommendations that we received on the last edition.

We would like to acknowledge all individuals who were involved in this project. We particularly give a heartfelt and sincere thanks to our families for their love, patience, and support during the preparation of this book. We especially appreciate Richard J. Batka and Alyssa Shapiro who provided invaluable assistance on this project. The capable advice, guidance, and assistance of the McGraw-Hill staff is gratefully acknowledged. Finally, we thank our students for their enthusiasm for learning and what they have taught us during their time with us.

Curtis D. Klaassen
John B. Watkins III

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UNIT 1 GENERAL PRINCIPLES OF TOXICOLOGY

CHAPTER

History and Scope of Toxicology

1

Michael A. Gallo

HISTORY OF TOXICOLOGY

Antiquity
Middle Ages
Renaissance
Age of Enlightenment

20TH CENTURY TOXICOLOGY: THE AWAKENING OF UNDERSTANDING

AFTER WORLD WAR II

21ST CENTURY TOXICOLOGY

KEY POINTS

- Toxicology is the study of the adverse effects of xenobiotics on living systems.
- Toxicology assimilates knowledge and techniques from biochemistry, biology, chemistry, genetics, mathematics, medicine, pharmacology, physiology, and physics.
- Toxicology applies safety evaluation and risk assessment to the discipline.

HISTORY OF TOXICOLOGY

Modern toxicology goes beyond the study of the adverse effects of exogenous agents by assimilating knowledge and techniques from most branches of biochemistry, biology, chemistry, genetics, mathematics, medicine, pharmacology, physiology, and physics and applies safety evaluation and risk assessment to the discipline. In all branches of toxicology, scientists explore the mechanisms by which chemicals produce adverse effects in biological systems. Activities in these broad subjects complement toxicologic research.

Antiquity

Knowledge of animal venoms and plant extracts for hunting, warfare, and assassination presumably predate recorded history. One of the oldest known writings, the Ebers Papyrus (circa 1500 b.c.), contains information pertaining to many recognized poisons, including hemlock, aconite, opium, and metals such as lead, copper, and antimony. The Book of Job (circa 1400 b.c.) speaks of poison arrows (Job 6:4) and Hippocrates (circa 400 b.c.) added a number of poisons and clinical toxicology principles pertaining to bioavailability in therapy and

overdosage. T eophrastus (370–286 b.c.), a student of Aristotle, included numerous references to poisonous plants in De Historia Plantarum. Dioscorides, a Greek physician in the court of the Roman emperor Nero, made the first attempt at classifying poisons as plant, animal, and mineral in his book De Materia Medica, which contains reference to some 600 plants.

One legend tells of Roman King Mithridates VI of Pontus, who was so fearful of poisons that he regularly ingested a mixture of 36 ingredients as protection against assassination. On the occasion of his imminent capture by enemies, his attempts to kill himself with poison failed because of his successful antidote concoction. T is tale leads to use of the word mithridatic as an antidote or protective mixture. Because poisonings in politics became so extensive, Sulla issued the Lex Cornelia (circa 82 b.c.), which appears to be the first law against poisoning and later became a regulatory statute directed at careless dispensers of drugs.

Middle Ages

T e writings of Maimonides (Moses ben Maimon, a.d. 1135–1204) included a treatise on the treatment of poisonings from insects, snakes, and mad dogs (*T*reatise on Poisons and T eir Antidotes, 1198). Maimonides described the subject of bioavailability, noting that milk, butter, and cream could delay intestinal absorption. In the early Renaissance and under the guise of delivering provender to the sick and the poor, Catherine de Medici tested toxic concoctions, carefully noting the rapidity of the toxic response (onset of action), the effectiveness of the compound (potency), the degree of response of the parts of the body (specificity and site of action), and the complaints of the victim (clinical signs and symptoms).

Renaissance

All substances are poisons; there is none that is not a poison. T e right dose differentiates a poison from a remedy.

Paracelsus

Philippus Aureolus T eophrastus Bombastus von Hohenheim-Paracelsus (1493–1541) was pivotal, standing between the philosophy and magic of classic antiquity and the philosophy and science willed to us by figures of the seventeenth and eighteenth centuries. Paracelsus, a physician-alchemist, formulated many revolutionary views that remain integral to the structure of toxicology, pharmacology, and therapeutics today. He focused on the primary toxic agent as a chemical entity, and held that (1) experimentation is essential in the examination of responses to chemicals, (2) one should make a distinction between the therapeutic and toxic properties of chemicals, (3) these properties are sometimes but not always indistinguishable except by dose, and (4) one can ascertain a degree of specificity of chemicals and their therapeutic or toxic effects. T ese principles led Paracelsus to articulate the dose–response relation as a bulwark of toxicology.

Come bitter pilot, now at once run on
T e dashing rocks thy seasick weary bark!
Here's to my love! O true apothecary!
T y drugs are quick. T us with a kiss I die.

Romeo and Juliet, act 5, scene 3

Although Ellenbog (circa 1480) warned of the toxicity of mercury and lead from goldsmithing and Agricola published a short treatise on mining diseases in 1556, the major work on the subject, On the Miners' Sickness and Other Diseases of Miners (1567), was published by Paracelsus. T is treatise addressed the etiology of miners' disease, along with treatment and prevention strategies. Occupational toxicology was further advanced by the work of Bernardino Ramazzini when he published in 1700 his Discourse on the Diseases of Workers, which discussed occupations ranging from miners to midwives and including printers, weavers, and potters. Percival Pott's (1775) recognition of the role of soot in scrotal cancer among chimney sweeps was the first report of polyaromatic hydrocarbon carcinogenicity. T ese findings led to improved medical practices, particularly in prevention.

Age of Enlightenment

Experimental toxicology accompanied the growth of organic chemistry and developed rapidly during the nineteenth century. Magendie (1783–1885), Orfila (1787–1853), and Bernard (1813–1878) laid the groundwork for pharmacology, experimental therapeutics, and occupational toxicology.

Orfila, a Spanish physician in the French court, used autopsy material and chemical analysis systematically as legal proof of poisoning. His introduction of this detailed type of analysis survives as the underpinning of forensic toxicology. Orfila published a major work devoted expressly to the toxicity of natural agents in 1815. Magendie, a physician and experimental physiologist, studied the mechanisms of action of emetine and strychnine. His research determined the absorption and distribution of these compounds in the body. One of Magendie's more famous students, Claude Bernard, contributed the classic treatise, An Introduction to the Study of Experimental Medicine.

German scientists Oswald Schmiedeberg (1838–1921) and Louis Lewin (1850–1929) made many contributions to the science of toxicology. Schmeideberg trained approximately 120 students who later populated the most important laboratories of pharmacology and toxicology throughout the world. Lewin published much of the early work on the toxicity of narcotics, methanol, glycerol, acrolein, and chloroform.

20TH CENTURY TOXICOLOGY: THE AWAKENING OF UNDERSTANDING

Toxicology has drawn its strength and diversity from its proclivity to borrowing from almost all the basic sciences to test its hypotheses. T is fact, coupled with the health and occupational

regulations that have driven toxicology research since 1900, has made this discipline exceptional in the history of science.

With the advent of anesthetics and disinfectants in the late 1850s, toxicology as it is currently understood began. T e prevalent use of "patent" medicines led to several incidents of poisonings from these medicaments, which, when coupled with the response to Upton Sinclair's exposé of the meatpacking industry in T e Jungle, culminated in the passage of the Wiley Bill in 1906, the first of many U.S. pure food and drug laws.

During the 1890s and early 1900s, the discovery of radioactivity and the vitamins, or "vital amines," led to the use of the first large-scale bioassays (multiple animal studies) to determine whether these "new" chemicals were beneficial or harmful to laboratory animals.

One of the first journals expressly dedicated to experimental toxicology, Archiv für *T*oxikologie, began publication in Europe in 1930. T at same year the National Institutes of Health (NIH) was established in the United States. As a response to the tragic consequences of acute kidney failure after taking sulfanilamide in glycol solutions, the Copeland bill was passed in 1938. T is was the second major bill involving the formation of the U.S. Food and Drug Administration (FDA). T e first major U.S. pesticide act was signed into law in 1947. T e significance of the initial Federal Insecticide, Fungicide, and Rodenticide Act was that for the first time in U.S. history a substance that was neither a drug nor a food had to be shown to be safe and efficacious for approval.

AFTER WORLD WAR II

You too can be a toxicologist in two easy lessons, each of ten years.
Arnold Lehman (circa 1955)

T e mid-1950s witnessed the strengthening of the U.S. FDA's commitment to toxicology. T e U.S. Congress passed and the president of the United States signed the additives amendments to the Food, Drug, and Cosmetic Act. T e Delaney clause (1958) of these amendments stated broadly that any chemical found to be carcinogenic in laboratory animals or humans could not be added to the U.S. food supply. Delaney became a battle cry for many groups and resulted in the inclusion at a new level of biostatisticians and mathematical modelers in the field of toxicology. Shortly after the Delaney amendment, the first American journal dedicated to toxicology, *T*oxicology and Applied Pharmacology, was launched. T e founding of the Society of Toxicology followed shortly afterward.

T e 1960s started with the tragic thalidomide incident, in which several thousand children were born with serious birth defects, and the publication of Rachel Carson's Silent Spring (1962). Attempts to understand the effects of chemicals on the embryo and fetus and on the environment as a whole gained momentum. New legislation was passed, and new journals were founded. Cellular and molecular toxicology developed as a subdiscipline, and risk assessment became a major product of toxicologic investigations.

Currently, many dozens of professional, governmental, and other scientific organizations with thousands of members and over 120 journals are dedicated to toxicology and related disciplines. In addition, the International Congress of Toxicology is composed of toxicology societies from Europe, South America, Asia, Africa, and Australia, which brings together the broadest representation of toxicologists.

21 ST CENTURY TOXICOLOGY

T e sequencing of the human genome and that of several other organisms has markedly affected all biological sciences, including toxicology. Genetically modifying organisms is now commonplace and those possessing orthologs of human genes (e.g., zebrafish [Danio rerio], roundworms [Caenorhabditis elegans], and fruit flys [Drosophila melanogaster]) are widely used in toxicology. Deeper understanding of epigenetics has provided novel approaches to studying the fetal origin of adult diseases including cancers, diabetes, and neurodegenerative diseases and disorders.

Toxicology has an interesting and varied history. Perhaps as a science that has grown and prospered by borrowing from many disciplines, it has suffered from the absence of a single goal, but its diversification has allowed for the interspersion of ideas and concepts from higher education, industry, and government. T is has resulted in an exciting, innovative, and diversified field that is serving science and the community at large. Few disciplines can point to both basic sciences and direct applications at the same time. Toxicology—the study of the adverse effects of xenobiotics—may be unique in this regard.

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QUESTIONS

1. Which one of the following statements regarding toxicology is true?
   - **a.** Modern toxicology is concerned with the study of the adverse effects of chemicals on ancient forms of life.
   - **b.** Modern toxicology studies embrace principles from such disciplines as biochemistry, botany, chemistry, physiology, and physics.
   - **c.** Modern toxicology has its roots in the knowledge of plant and animal poisons, which predates recorded history and has been used to promote peace.
   - **d.** Modern toxicology studies the mechanisms by which inorganic chemicals produce advantageous as well as deleterious effects.
   - **e.** Modern toxicology is concerned with the study of chemicals in mammalian species.

2. Knowledge of the toxicology of poisonous agents was published earliest in the:
   - **a.** Ebers papyrus.
   - **b.** De Historia Plantarum.
   - **c.** De Materia Medica.
   - **d.** Lex Cornelia.
   - **e.** *T*reatise on Poisons and T eir Antidotes.

3. Paracelsus, a physician-alchemist, formulated many revolutionary views that remain integral to the structure of toxicology, pharmacology, and therapeutics today. He focused on the primary toxic agent as a chemical entity and articulated the dose–response relation. Which one of the following statements is not attributable to Paracelsus?
   - **a.** Natural poisons are quick in their onset of actions.
   - **b.** Experimentation is essential in the examination of responses to chemicals.
   - **c.** One should make a distinction between the therapeutic and toxic properties of chemicals.
   - **d.** T ese properties are sometimes but not always indistinguishable except by dose.
   - **e.** One can ascertain a degree of specificity of chemicals and their therapeutic or toxic effects.

4. T e art of toxicology requires years of experience to acquire, even though the knowledge base of facts may be learned more quickly. Which modern toxicologist is credited with saying that "you can be a toxicologist in two easy lesions, each of 10 years?"
   - **a.** Claude Bernard.
   - **b.** Rachel Carson.
   - **c.** Upton Sinclair.
   - **d.** Arnold Lehman.
   - **e.** Oswald Schmiedeberg.

5. Which of the following statements is correct?
   - **a.** Claude Bernard was a prolific scientist who trained over 120 students and published numerous contributions to the scientific literature.
   - **b.** Louis Lewin trained under Oswald Schmiedeberg and published much of the early work on the toxicity of narcotics, methanol, and chloroform.
   - **c.** An Introduction to the Study of Experimental Medicine was written by the Spanish physician Orfila.
   - **d.** Magendie used autopsy material and chemical analysis systematically as legal proof of poisoning.
   - **e.** Percival Potts was instrumental in demonstrating the chemical complexity of snake venoms.

CHAPTER

2

Principles of Toxicology

David L. Eaton and Steven G. Gilbert

INTRODUCTION TO TOXICOLOGY

- Different Areas of Toxicology
- Toxicology and Society
- General Characteristics of the Toxic Response

CLASSIFICATION OF TOXIC AGENTS

SPECTRUM OF UNDESIRED EFFECTS

- Allergic Reactions
- Idiosyncratic Reactions
- Immediate versus Delayed Toxicity
- Reversible versus Irreversible Toxic Effects
- Local versus Systemic Toxicity
- Interaction of Chemicals
- Tolerance

CHARACTERISTICS OF EXPOSURE

- Route and Site of Exposure
- Duration and Frequency of Exposure

DOSE–RESPONSE RELATIONSHIP

- Individual, or Graded, Dose–Response Relationships
- Quantal Dose–Response Relationships
- Shape of the Dose–Response Curve
  - Essential Nutrients
  - Hormesis
  - Threshold
  - Nonmonotonic Dose–Response Curves
- Assumptions in Deriving the Dose–Response Relationship
- Evaluating the Dose–Response Relationship
  - Comparison of Dose–Responses
  - Therapeutic Index
  - Margins of Safety and Exposure
  - Potency versus Efficacy

VARIATION IN TOXIC RESPONSES

- Selective Toxicity
- Species Differences
- Individual Differences in Response

DESCRIPTIVE ANIMAL TOXICITY TESTS

- Acute Toxicity Testing
- Skin and Eye Irritations
- Sensitization
- Subacute (Repeated-dose Study)
- Subchronic
- Chronic
- Other Tests

TOXICOGENOMICS

- Genomics
- Epigenetics
- Transcriptomics and Proteomics

KEY POINTS

- A poison is any agent capable of producing a deleterious response in a biological system.
- A mechanistic toxicologist identifies the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms.
- Toxicogenomics permits mechanistic toxicologists to identify and protect genetically susceptible individuals from harmful environmental exposures, and to customize drug therapies based on their individual genetic makeup.
- A descriptive toxicologist is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements.
- A regulatory toxicologist both determines from available data whether a chemical poses a sufficiently low risk to be marketed for a stated purpose and establishes standards for the amount of chemicals permitted in ambient air, industrial atmospheres, and drinking water.
- Selective toxicity means that a chemical produces injury to one kind of living matter without harming another form of life even though the two may exist in intimate contact.
- T e individual or "graded" dose–response relationship describes the response of an individual organism to varying doses of a chemical.
- A quantal dose–response relationship characterizes the distribution of responses to different doses in a population of individual organisms.
- Hormesis, a "U-shaped" dose–response curve, results with some xenobiotics that impart beneficial or stimulatory effects at low doses but adverse effects at higher doses.
- Descriptive animal toxicity testing assumes that the effects produced by a compound in laboratory animals, when properly qualified, are applicable to humans, and that exposure of experimental animals to toxic agents in high doses is a necessary and valid method of discovering possible hazards in humans.

INTRODUCTION TO TOXICOLOGY

Toxicology is the study of the adverse effects of chemicals on living organisms. A toxicologist is trained to examine the nature of those effects (including their cellular, biochemical, and molecular mechanisms of action) and assess the probability of their occurrence. Fundamental to this process is characterizing the relation of exposure (or dose) to the response. T e variety of potential adverse effects from the abundant diversity of chemicals upon which our society depends often demands specialization in one area of toxicology.

Different Areas of Toxicology

A mechanistic toxicologist identifies the cellular, biochemical, and molecular mechanisms by which chemicals exert toxic effects on living organisms (see Chapter 3 for a detailed discussion of mechanisms of toxicity). Mechanistic data may be useful in the design and production of safer chemicals and in rational therapy for chemical poisoning and treatment of disease. In risk assessment, mechanistic data may be very useful in demonstrating that an adverse outcome observed in laboratory animals is directly relevant to humans. Toxicogenomics permits the application of genomic, transcriptomic, proteomic, and metabolomic technologies to identify descriptive and mechanistic information that can protect genetically susceptible individuals from harmful environmental exposures, and to customize drug therapies based on their individual genetic makeup. Numerous genetic tests can identify susceptible individuals in advance of pharmacological treatment.

A descriptive toxicologist is concerned directly with toxicity testing, which provides information for safety evaluation and regulatory requirements. Toxicity tests (described later in this chapter) in experimental animals are designed to yield information that can be used to evaluate risks posed to humans and the environment by exposure to specific chemicals.

A regulatory toxicologist has the responsibility for deciding, on the basis of data provided by descriptive and mechanistic toxicologists, whether a drug or another chemical poses a sufficiently low risk to be marketed for a stated purpose. Regulatory toxicologists are involved in the establishment of standards for the amount of chemicals permitted in foods, drugs, ambient air, industrial atmospheres, and drinking water (see Chapter 4).

Forensic toxicology is a hybrid of analytic chemistry and fundamental toxicologic principles that focuses primarily on the medicolegal aspects of the harmful effects of chemicals on humans and animals (see Chapter 31).

Clinical toxicology is concerned with disease caused by or uniquely associated with toxic substances (see Chapter 32).

Environmental toxicology focuses on the impacts of chemical pollutants in the environment on biological organisms,

specifically studying the impacts of chemicals on nonhuman organisms such as fish, birds, terrestrial animals, and plants. Ecotoxicology, a specialized area within environmental toxicology, focuses specifically on the impacts of toxic substances on population dynamics in an ecosystem (see Chapter 29).

Developmental toxicology is the study of adverse effects on the developing organism that may result from exposure to chemical or physical agents before conception (either parent), during prenatal development, or postnatally until the time of puberty. Teratology is the study of defects induced during development between conception and birth (see Chapter 10).

Reproductive toxicology is the study of the occurrence of adverse effects on the male or female reproductive system that may result from exposure to chemical or physical agents (see Chapter 20).

Toxicology and Society

Knowledge about the toxicologic effect of a compound affects consumer products, drugs, manufacturing processes, waste cleanup, regulatory action, civil disputes, and broad policy decisions. T e expanding inf uence of toxicology on societal issues is accompanied by the responsibility to be increasingly sensitive to the ethical, legal, and social implications of toxicologic research and testing.

T ere are several ethical dilemmas in toxicology. First, experience and new discoveries in the biological sciences have emphasized the need for well-articulated visions of human, animal, and environmental health. Second, experience with the health consequences of exposure to such things as lead, asbestos, and tobacco has precipitated many regulatory and legal actions and public policy decisions. T ird, we have an increasingly well-defined framework for discussing our social and ethical responsibilities. Fourth, all research involving humans or animals must be conducted in a responsible and ethical manner. Fifth, the uncertainty and biological variability inherent in the biological sciences requires decision making with limited or uncertain information.

General Characteristics of the Toxic Response

Virtually every known chemical has the potential to produce injury or death if it is present in a sufficient amount. Table 2–1 shows the wide spectrum of dosages needed to produce death in 50% of treated animals (lethal dose 50, LD_{50}). Chemicals producing death in microgram doses are often considered extremely poisonous. Note that measures of acute lethality such as LD_{50} may not accurately ref ect the full spectrum of toxicity, or hazard, associated with exposure to a chemical. For example, some chemicals with low acute toxicity may have carcinogenic or teratogenic effects at doses that produce no evidence of acute toxicity. For a given chemical, each of the various effects that may occur in a given organism will have their own dose–response relationship.

TABLE 2–1 Approximate acute LD_{50} of some representative chemical agents.

Agent	LD_{50}, mg/kg*
Ethyl alcohol	10 000
Sodium chloride	4 000
Ferrous sulfate	1 500
Morphine sulfate	900
Phenobarbital sodium	150
Picrotoxin	5
Strychnine sulfate	2
Nicotine	1
Tubocurarine	0.5
Hemicholinium-3	0.2
Tetrodotoxin	0.10
Dioxin (TCDD)	0.001
Botulinum toxin	0.00001

*LD_{50} is the dosage (mg/kg body weight) causing death in 50% of exposed animals.

CLASSIFICATION OF TOXIC AGENTS

Toxic agents are classified depending on the interests and needs of the classifier. T ese agents may be discussed in terms of their target organs, use, source, and effects. T e term toxin generally refers to toxic substances that are produced by biological systems such as plants, animals, fungi, or bacteria. T e term toxicant is used in speaking of toxic substances that are produced by or are a by-product of human activities. Toxic agents may be classified in terms of their physical state, chemical stability or reactivity, general chemical structure, or poisoning potential. No single classification is applicable to the entire spectrum of toxic agents and, therefore, a combination of classifications is needed to provide the best characterization of a toxic substance.

SPECTRUM OF UNDESIRED EFFECTS

T e spectrum of undesired effects of chemicals is broad. In therapeutics, e.g., each drug produces a number of effects, but usually only one effect is associated with the primary objective of the therapy; all the other effects are referred to as undesirable or side ef ects. However, some of these side effects may be desired for another therapeutic indication. Some side effects of drugs are always deleterious to the well-being of humans. T ese are referred to as the adverse, deleterious, or toxic effects of the drug.

Allergic Reactions

Chemical allergy is an immunologically mediated adverse reaction to a chemical resulting from previous sensitization to that chemical or to a structurally similar one. The terms hypersensitivity, allergic reaction, and sensitization reaction are used to describe this situation (see Chapter 12). Once sensitization has occurred, allergic reactions may result from exposure to relatively very low doses of chemicals. Importantly, for a given allergic individual, allergic reactions are dose-related. Sensitization reactions are sometimes very severe and may be fatal.

Most chemicals and their metabolic products are not sufficiently large to be recognized by the immune system as a foreign substance and thus must first combine with an endogenous protein to form an antigen (or immunogen). Such a molecule is called a hapten. The hapten–protein complex (antigen) is then capable of eliciting the formation of antibodies. Subsequent exposure to the chemical results in an antigen–antibody interaction, which provokes the typical manifestations of an allergy that range in severity from minor skin disturbance to fatal anaphylactic shock.

Idiosyncratic Reactions

Chemical idiosyncrasy refers to a genetically determined abnormal reactivity to a chemical. The response observed is usually qualitatively similar to that observed in all individuals but may take the form of extreme sensitivity to low doses or extreme insensitivity to high doses of the chemical. For example, some individuals are abnormally sensitive to nitrites and other substances capable of oxidizing the iron in hemoglobin. This produces methemoglobin, which is incapable of binding and transporting oxygen to tissues. Consequently, they may suffer from tissue hypoxia after exposure to doses of methemoglobin-producing chemicals, whereas normal individuals would be unaffected. It is now recognized that many idiosyncratic drug reactions are due to the interplay between an individual's ability to form a reactive intermediate, detoxify that intermediate, and/or mount an immune response to adducted proteins. Specific genetic polymorphisms in drug-metabolizing enzymes, transporters, or receptors are responsible for many of these observed differences.

Immediate versus Delayed Toxicity

Immediate toxic effects occur or develop rapidly after a single administration of a substance, whereas delayed toxic effects occur after the lapse of some time. Most substances produce immediate toxic effects. However, carcinogenic effects of chemicals usually have long latency periods, often 20 to 30 years after the initial exposure, before tumors are observed in humans.

Reversible versus Irreversible Toxic Effects

Some toxic effects of chemicals are reversible, and others are irreversible. If a chemical produces pathological injury to a tissue, the ability of that tissue to regenerate largely determines whether the effect is reversible or irreversible. Liver tissue has high regeneration ability and most injuries are, therefore, reversible. However, CNS injury is largely irreversible because its cells are differentiated and cannot be replaced. Carcinogenic and teratogenic effects of chemicals, once they occur, are usually considered irreversible toxic effects.

Local versus Systemic Toxicity

Another distinction between types of effects is made on the basis of the general site of action. Local effects occur at the site of first contact between the biological system and the toxicant. In contrast, systemic effects require absorption and distribution of a toxicant from its entry point to a distant site, at which deleterious effects are produced. Most substances, except for highly reactive materials, produce systemic effects. Some materials can produce both effects.

Most chemicals that produce systemic toxicity usually elicit their major toxicity in only one or two organs, which are referred to as the target organs of toxicity of a particular chemical. Paradoxically, the target organ of toxicity is often not the site of the highest concentration of the chemical.

Target organs in order of frequency of involvement in systemic toxicity are the CNS; the circulatory system; the blood and hematopoietic system; visceral organs such as the liver, kidney, and lung; and the skin. Muscle and bone are seldom target tissues for systemic effects.

Interaction of Chemicals

Chemical interactions can occur via various mechanisms, such as alterations in absorption, protein binding, and the biotransformation and excretion of one or both of the interacting toxicants. In addition to these modes of interaction, the response of the organism to combinations of toxicants may be increased or decreased because of toxicologic responses at the site of action.

An additive effect, most commonly observed when two chemicals are given together, occurs when the combined effect of two chemicals is equal to the sum of the effects of each agent given alone (e.g.: $2 + 3 = 5$). A synergistic effect occurs when the combined effects of two chemicals are much greater than the sum of the effects of each agent given alone (e.g.: $2 + 2 = 20$). Potentiation occurs when one substance does not have a toxic effect on a certain organ or system but when added to another chemical makes that chemical much more toxic (e.g.: $0 + 2 = 10$). Isopropanol, e.g., is not hepatotoxic, but when it is administered in addition to carbon tetrachloride, the hepatotoxicity of carbon tetrachloride is much greater than that when it is given alone.

Antagonism occurs when two chemicals administered together interfere with each other's actions or one interferes with the action of the other (e.g.: $4 + 6 = 8$; $4 + (-4) = 0$; $4 + 0 = 1$). There are four major types of antagonism: functional, chemical, dispositional, and receptor. Functional antagonism occurs when two chemicals counterbalance each other by producing opposite effects on the same physiologic function.

For example, the marked fall in blood pressure during severe barbiturate intoxication can be effectively antagonized by the intravenous administration of a vasopressor agent such as norepinephrine or metaraminol. Chemical antagonism or inactivation is simply a chemical reaction between two compounds that produces a less toxic product. For example, chelators of metal ions decrease metal toxicity and antitoxins antagonize the action of various animal toxins. Dispositional antagonism occurs when the absorption, biotransformation, distribution, or excretion of a chemical is altered so that the concentration and/or duration of the chemical at the target organ are diminished. Receptor antagonism occurs when two chemicals that bind to the same receptor produce less of an effect when given together than the addition of their separate effects (e.g.: 4 + 6 = 8) or when one chemical antagonizes the effect of the second chemical (e.g.: 0 + 4 = 1). Receptor antagonists are often termed blockers.

Tolerance

Tolerance is a state of decreased responsiveness to a toxic effect of a chemical resulting from prior exposure to that chemical or to a structurally related chemical. Two major mechanisms are responsible for tolerance: one is due to a decreased amount of toxicant reaching the site where the toxic effect is produced (dispositional tolerance) and the other is due to a reduced responsiveness of a tissue to the chemical.

CHARACTERISTICS OF EXPOSURE

Toxic effects in a biological system are not produced by a chemical agent unless that agent or its metabolic breakdown (biotransformation) products reach appropriate sites in the body at a concentration and for a length of time sufficient to produce a toxic manifestation. Whether a toxic response occurs is dependent on the chemical and physical properties of the agent, the exposure situation, how the agent is metabolized by the system, and the overall susceptibility of the biological system or subject.

Route and Site of Exposure

T e major routes (pathways) by which toxic agents gain access to the body are the gastrointestinal tract (ingestion), lungs (inhalation), skin (topical, percutaneous, or dermal), and other parenteral (other than intestinal canal) routes. Toxic agents generally produce the greatest effect and the most rapid response when given directly into the bloodstream (the intravenous route). An approximate descending order of effectiveness for the other routes would be inhalation, intraperitoneal, subcutaneous, intramuscular, intradermal, oral, and dermal. T e "vehicle" (the material in which the chemical is dissolved) and other formulation factors can markedly alter absorption. In addition, the route of administration can inf uence the toxicity of agents. For example, an agent that acts on the CNS, but is efficiently detoxified in the liver, would be expected to be less toxic when given orally than when inhaled, because the oral route requires that nearly all of the dose pass through the liver before reaching the systemic circulation and then the CNS.

Duration and Frequency of Exposure

Toxicologists usually divide the exposure of experimental animals to chemicals into four categories: acute, subacute, subchronic, and chronic. Acute exposure is defined as exposure to a chemical for less than 24 h. While acute exposure usually refers to a single administration, repeated exposures may be given within a 24-h period for some slightly toxic or practically nontoxic chemicals. Acute exposure by inhalation refers to continuous exposure for less than 24 h, most frequently for 4 h. Repeated exposure is divided into three categories: subacute, subchronic, and chronic. Subacute exposure refers to repeated exposure to a chemical for 1 month or less, subchronic for 1 to 3 months, and chronic for more than 3 months.

In human exposure situations, the frequency and duration of exposure are usually not as clearly defined as in controlled animal studies, but many of the same terms are used to describe general exposure situations. T us, workplace or environmental exposures may be described as acute (occurring from a single incident or episode), subchronic (occurring repeatedly over several weeks or months), or chronic (occurring repeatedly for many months or years).

For many agents, the toxic effects that follow a single exposure are quite different from those produced by repeated exposure. Acute exposure to agents that are rapidly absorbed is likely to produce immediate toxic effects but also can produce delayed toxicity that may or may not be similar to the toxic effects of chronic exposure. Conversely, chronic exposure to a toxic agent may produce some immediate (acute) effects after each administration in addition to the long-term, low-level, or chronic effects of the toxic substance. T e other time-related factor that is important in the temporal characterization of repeated exposures is the frequency of exposure. T e relationship between elimination rate and frequency of exposure is shown in Figure 2–1. A chemical that produces severe effects with a single dose may have no effect if the same total dose is given in several intervals. For the chemical depicted by line B in Figure 2–1, in which the half-life for elimination (time necessary for 50% of the chemical to be removed from the bloodstream) is approximately equal to the dosing frequency, a theoretical toxic concentration of 2 U is not reached until the fourth dose, whereas that toxic concentration is nearly reached with only two doses for chemical A, which has an elimination rate much slower than the dosing interval (time between each repeated dose). Conversely, for chemical C, where the elimination rate is much shorter than the dosing interval, a toxic concentration at the site of toxic effect will never be reached regardless of how many doses are administered. Of course, it is possible that residual cell or tissue damage occurs with each dose even though the chemical itself is not accumulating. T e important consideration, then, is whether the interval between

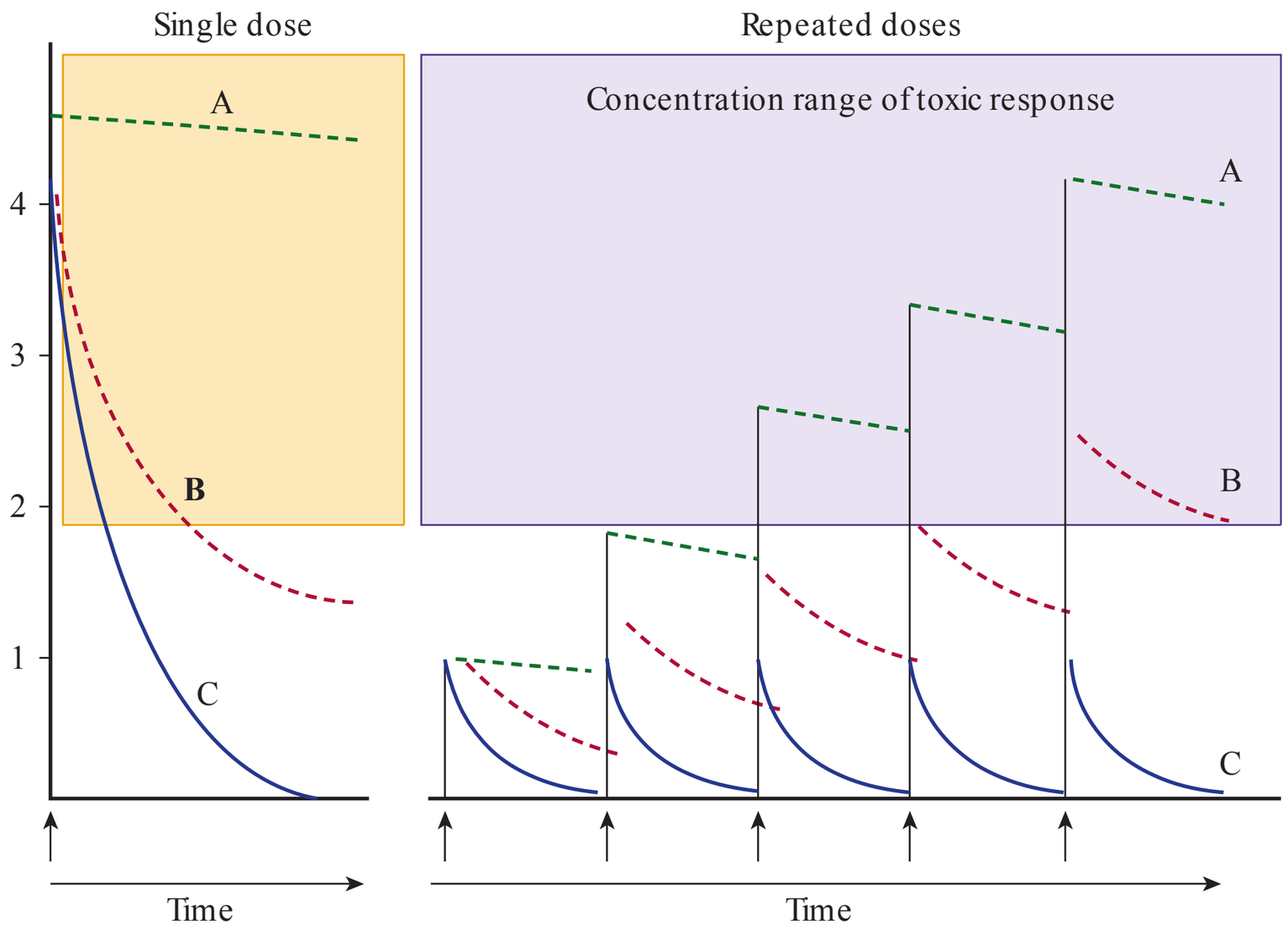

FIGURE 2–1 **Diagrammatic view of the relationship between dose and concentration at the target site under different conditions of dose frequency and elimination rate.** Line A. A chemical with very slow elimination (e.g., half-life of 1 year). Line B. A chemical with a rate of elimination equal to frequency of dosing (e.g., 1 day). Line C. Rate of elimination faster than the dosing frequency (e.g., 5 h). Purple shaded area is representative of the concentration of chemical at the target site necessary to elicit a toxic response.

doses is sufficient to allow for complete repair of tissue damage. Chronic toxic effects may occur, therefore, if the chemical accumulates in the biological system (rate of absorption exceeds the rate of biotransformation and/or excretion), if it produces irreversible toxic effects, or if there is insufficient time for the system to recover from the toxic damage within the exposure frequency interval. For additional discussion of these relationships, consult Chapters 5 and 7.

DOSE–RESPONSE RELATIONSHIP

T e characteristics of exposure and the spectrum of effects come together in a correlative relationship customarily referred to as the dose–response relationship. Whatever response is selected for measurement, the relationship between the degree of response of the biological system and the amount of toxicant administered assumes a form that occurs so consistently as to be considered the most fundamental and pervasive concept in toxicology.

From a practical perspective, there are two types of dose–response relationships: (1) the individual dose–response relationship, which describes the response of an individual organism to varying doses of a chemical, often referred to as a "graded" response because the measured effect is continuous over a range of doses, and (2) a quantal dose–response relationship, which characterizes the distribution of responses to different doses in a population of individual organisms.

Individual, or Graded, Dose–Response Relationships

Individual dose–response relationships are characterized by a dose-related increase in the severity of the response. For example, Figure 2–2 shows the dose–response relationship between different dietary doses of the organophosphate insecticide chlorpyrifos and the extent of inhibition of two different enzymes in the brain and liver: acetylcholinesterase and carboxylesterase. In the brain, the degree of inhibition of both enzymes is clearly dose-related and spans a wide range, although the amount of inhibition per unit dose is different for the two enzymes. From the shapes of these two dose–response curves, it is evident that, in the brain, cholinesterase is more easily inhibited than carboxylesterase. T e toxicologic response that results is directly related to the degree of cholinesterase enzyme inhibition in the brain. It should be noted that most toxic substances have multiple sites or mechanisms of toxicity, each with its own "dose–response" relationship and subsequent adverse effect. When these dose–response data are plotted using a logarithmic scale for the dose, the data "fit" a straight line.

Quantal Dose–Response Relationships

In contrast to the "graded" or continuous-scale dose–response relationship that occurs in individuals, the dose–response relationships in a population are by definition quantal—or "all or none"—in nature; that is, at any given dose, an individual in the

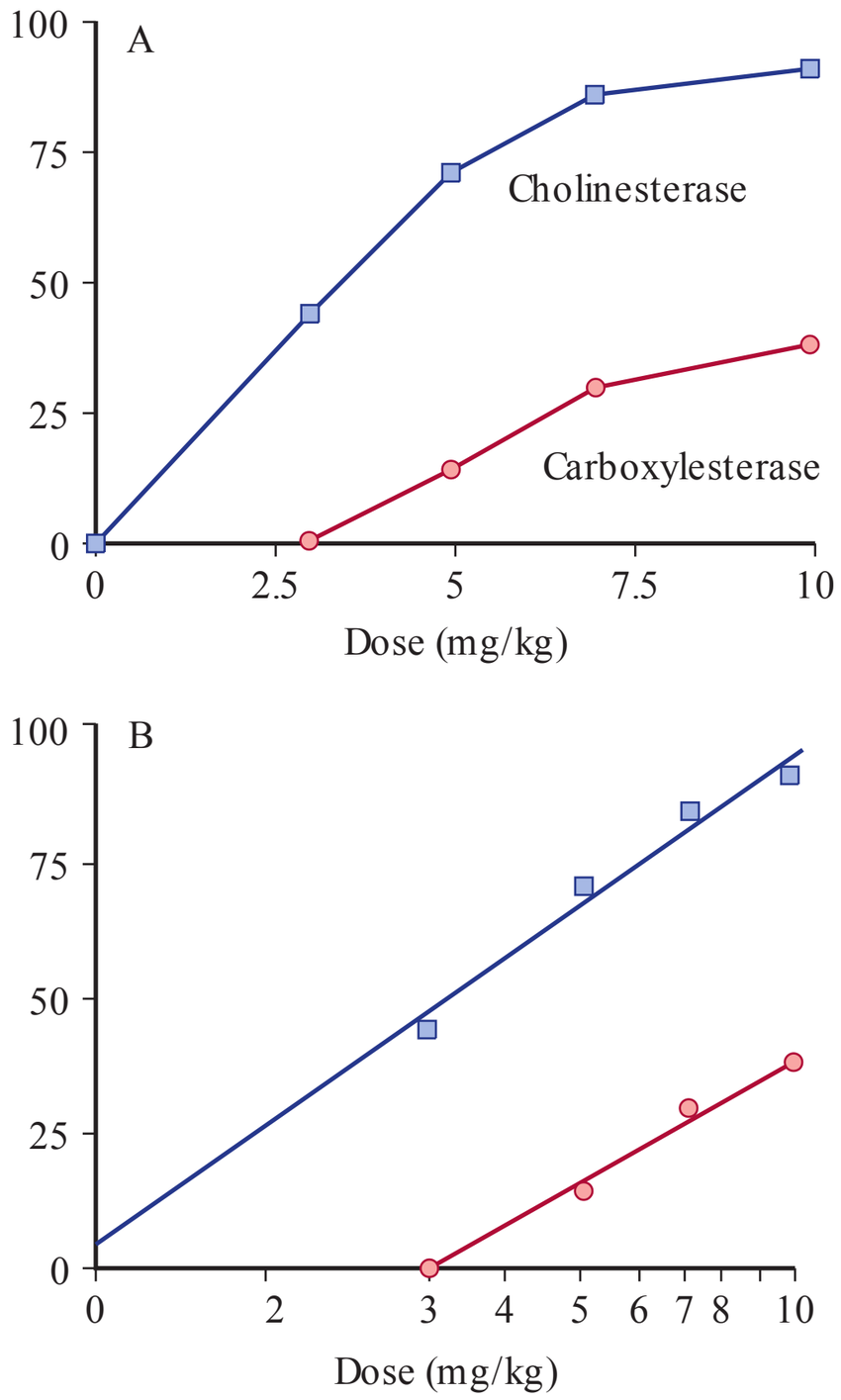

FIGURE 2–2 **Dose–response relationship between different doses of the organophosphate insecticide chlorpyrifos and esterase enzyme inhibition in the brain.** Open circles and blue lines represent acetylcholinesterase activity and closed circles represent carboxylesterase activity in the brains of pregnant female Long–Evans rats given 5 daily doses of chlorpyrifos. A. Dose–response curve plotted on an arithmetic scale. B. Same data plotted on a semi-log scale. (Data from Lassiter TL, et al.: Gestational exposure to chlorpyrifos: dose response profiles for cholinesterase and carboxylesterase activity, Toxicol Sci, 1999 Nov;52(1):92–100.)

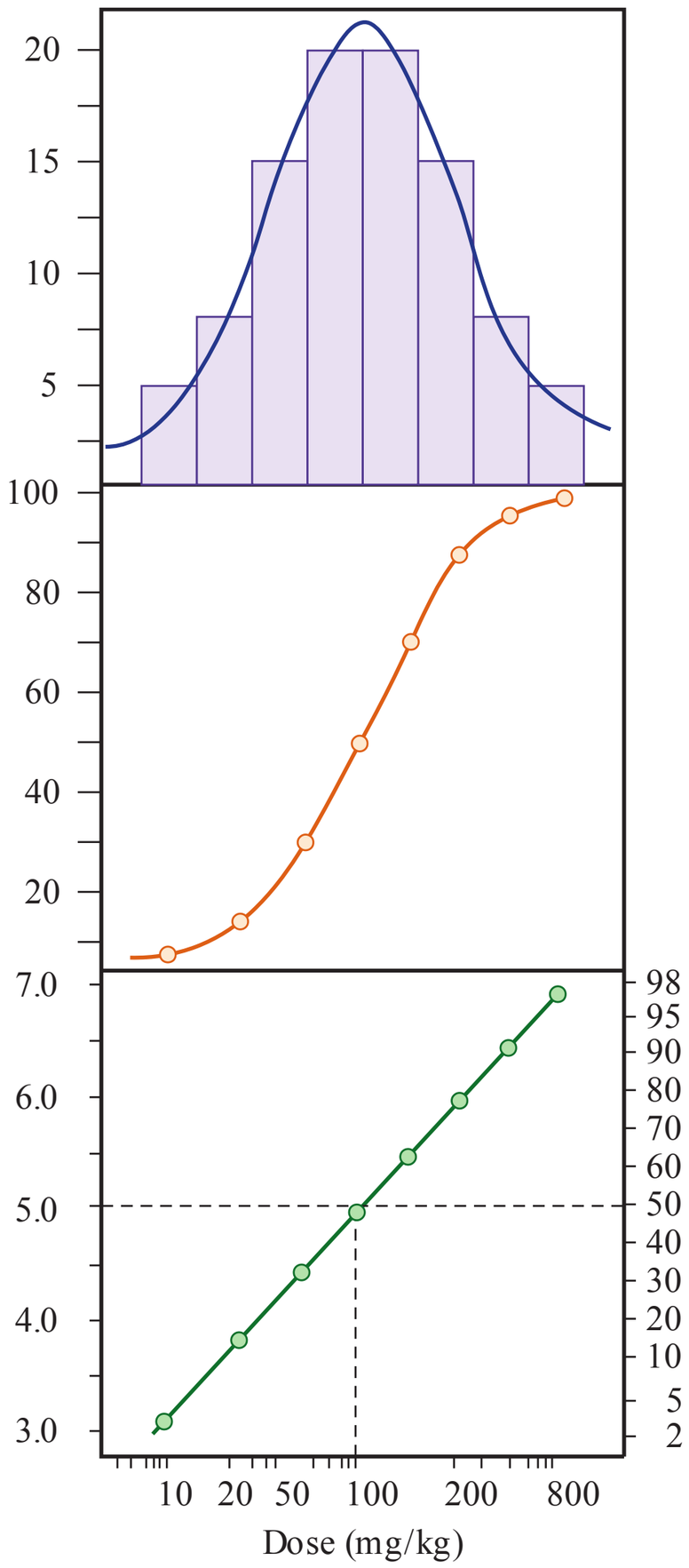

FIGURE 2–3 **Diagram of a quantal dose–response relationship.** The abscissa is a log dosage of the chemical. In the top panel the ordinate is response frequency, in the middle panel the ordinate is percent response, and in the bottom panel the response is in probit units (see text).

population is classified as either a "responder" or a "nonresponder." Although these distinctions of "quantal population" and "graded individual" dose–response relationships are useful, the two types of responses are conceptually identical. T e ordinate in both cases is simply labeled the response, which may be the degree of response in an individual or system or the fraction of a population responding, and the abscissa is the administered dose range.

T e effective dose (ED) is a widely used statistical approach for estimating the response of a population to a toxic exposure. Generally, the 50% response level is used (ED_{50}), although any response level, such as an ED_{01}, ED_{10}, or ED_{30}, could be chosen.

T e top panel of Figure 2–3 shows that quantal dose–responses exhibit a normal or Gaussian distribution. T e frequency histogram in this panel also shows the relationship between dose and effect. T e bars represent the percentage of animals that responded at each dose minus the percentage that responded at the immediately lower dose. One can clearly see that only a few animals responded to the lowest dose and the highest dose. Larger numbers of animals responded to doses intermediate between these two extremes, and the maximum frequency of response occurred in the middle portion of the dose range. T us, we have a bell-shaped curve known as a normal *f*requency distribution. T e reason for this normal distribution is that there are differences in susceptibility to chemicals among individuals. Animals responding at the left end of the curve are referred to as hypersusceptible, and those at the right end of the curve are called resistant. If the numbers of individuals responding at each consecutive dose are added together, a cumulative, quantal dose–response relationship is obtained. When sufficient doses are used with a large number of animals per

dose, a sigmoid dose–response curve is observed, as depicted in the middle panel of Figure 2–3. With the lowest dose (6 mg/kg), 1% of the animals respond. A normally distributed sigmoid curve such as this one approaches a response of 0% as the dose is decreased and approaches 100% as the dose is increased, but—theoretically—it never passes through 0% and 100%. However, the minimally ED of any chemical that evokes a stated all-or-none response is called the threshold dose even though it cannot be determined experimentally.

T e sigmoid curve has a relatively linear portion between 16% and 84%. T ese values represent the limits of 1 standard deviation (SD) of the mean (and the median) in a population with truly normal distribution. T us, the mean ± 1 SD represents 68.3% of the population, the mean ± 2 SD represents 95.5% of the population, and the mean ± 3 SD equals 99.7% of the population. One can convert the percent response to units of deviation from the mean or normal equivalent deviations (NEDs). T us, the NED for a 50% response is 0; an NED of + 1 is equated with an 84.1% response. Units of NED can be converted by the addition of 5 to the value to avoid negative numbers and be called probit units (from the contraction of probability unit). In this transformation, a 50% response becomes a probit of 5, a + 1 deviation becomes a probit of 6, and a − 1 deviation is a probit of 4.

T e data given in the top two panels of Figure 2–3 are replotted in the bottom panel with the mortality plotted in probit units to form a straight line. In essence, what is accomplished in a probit transformation is an adjustment of quantal data to an assumed normal population distribution, resulting in a straight line. T e ED_{50} is obtained by drawing a horizontal line from the probit unit 5, which is the 50% response point, to the dose–effect line. At the point of intersection, a vertical line is drawn, and this line intersects the abscissa at the ED_{50} point. In addition to the ED_{50}, the slope of the dose–response curve can also be obtained. Figure 2–4 demonstrates the dose–response curves of two compounds. Compound A exhibits a "f at" dose–response curve, showing that a large change in dosage is required before a significant change in response will be observed. However, compound B exhibits a "steep" dose–response curve, where a relatively small change in dosage will cause a large change in response. T e ED_{50} for both compounds is the same (8 mg/kg); however, the slopes of the dose–response curves are quite different. At one-half of ED_{50} of the compounds (4 mg/kg), less than 1% of the animals exposed to compound B would respond but 20% of the animals given compound A would respond.

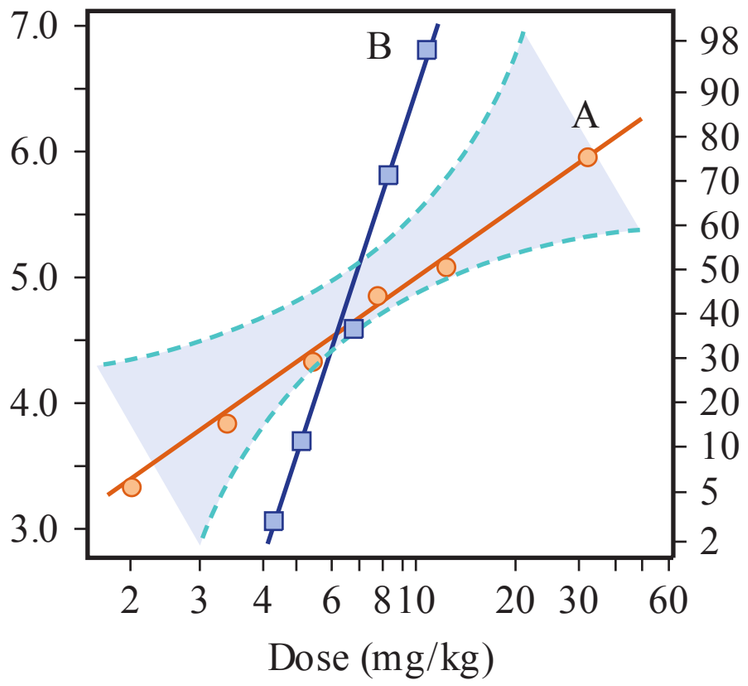

FIGURE 2–4 **Comparison of dose–response relationship for two different chemicals, plotted on a log dose–probit scale.** Note that the slope of the dose–response relationship is steeper for chemical B than for chemical A. Dotted lines represent the confidence limits for chemical A.

Allometry studies the relationship of body size to shape, and allometry is often expressed as a scaling exponent based on body mass or body length. If allometric principles are considered in dosage determination, then viewing dosage on the basis of body weight would be considered less appropriate than if based on surface area, which is approximately proportional to 10.5 × (body weight)^x, where x = 2/3 or 3/4. In Table 2–2, selected values are given to compare the differences in dosage

TABLE 2–2 Allometric scaling of dose across different species.

Species	Weight (kg)	Surface Area (cm^2)*	Fold Difference, Relative to Humans, Normalized by Body Weight		
			mg/kg	$(BW)^{2/3}$	$(BW)^{3/4}$
Mouse	0.02	103	1	13.0	7.0
Rat	0.2	365	1	6.9	4.3
Guinea pig	0.4	582	1	5.5	3.6
Rabbit	1.5	1 410	1	3.5	2.6
Cat	2	1 710	1	3.2	2.4
Monkey	4	2 720	1	2.6	2.0
Dog	12	5 680	1	1.8	1.5
Human	70	18 500	1	1.0	1.0

*Surface area of animals is closely approximated by the formula SA = 10.5 × (body weight [in grams])$^{2/3}$.

by the two alternatives. If a scaling factor of (body weight)$^{2/3}$ is used, then the dose would be approximately 13 times greater in mice than if that dosage were expressed per surface area (mg/cm^2). However, not all toxic responses will necessarily scale across species in the same way.

Shape of the Dose–Response Curve

Essential Nutrients—The shape of the dose–response relationship has many important implications in toxicity assessment, e.g., for substances that are required for normal physiologic function and survival (e.g., vitamins and essential trace elements such as chromium, cobalt, and selenium), the shape of the "graded" dose–response relationship in an individual over the entire dose range is actually U-shaped (Figure 2–5). That is, at very low doses (or deficiency), there is a high level of adverse effect, which decreases with an increasing dose. As the dose is increased to a point where the deficiency no longer exists, no adverse response is detected and the organism is in a state of homeostasis. However, as the dose is increased to abnormally high levels, an adverse response (usually qualitatively different from that observed at deficient doses) appears and increases in magnitude with increasing dose.

Hormesis—Some nonnutritional toxic substances may also impart beneficial or stimulatory effects at low doses but, at higher doses, they produce adverse effects. This is concept of "hormesis" may also result in a U-shaped dose–response curve. For example, chronic alcohol consumption is well recognized to increase the risk of esophageal cancer, liver cancer, and cirrhosis of the liver at relatively high doses, and this response is dose-related (curve A, Figure 2–6). However, there is substantial clinical and epidemiologic evidence that low to moderate consumption of alcohol reduces the incidence of coronary heart disease and stroke (curve B, Figure 2–6). Thus, when all responses are plotted on the ordinate, a U-shaped dose–response curve is obtained (curve C, Figure 2–6).

Threshold—Another important aspect of the dose–response relationship at low doses is the concept of the threshold, that is some dose below which the probability of an individual responding is zero. For the individual dose–response relationship, thresholds for most toxic effects certainly exist, although interindividual variability in response and qualitative changes in response pattern with dose make it difficult to establish a true "no effects" threshold for any chemical. In the identification of

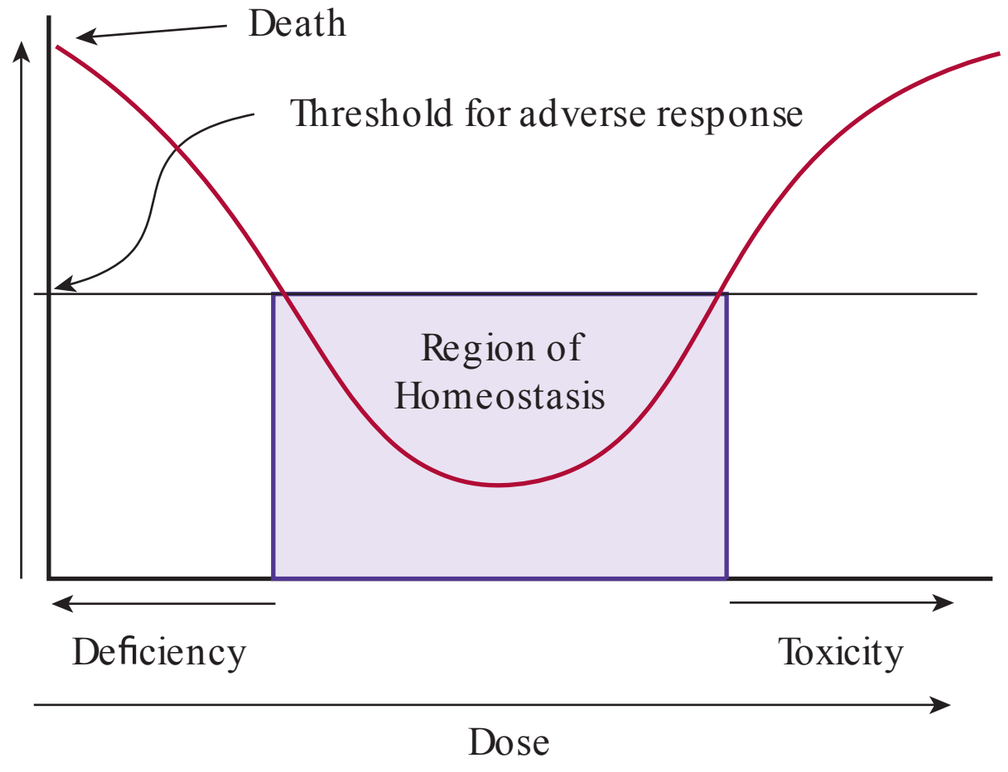

FIGURE 2–5 **Individual dose–response relationship for an essential substance such as a vitamin or trace element.** It is generally recognized that, for most types of toxic responses, a threshold exists such that at doses below the threshold, no toxicity is evident. For essential substances, doses below the minimum daily requirement, as well as those above the threshold for safety, may be associated with toxic effects. The purple-shaded region represents the "region of homeostasis"—the dose range that results in neither deficiency nor toxicity.

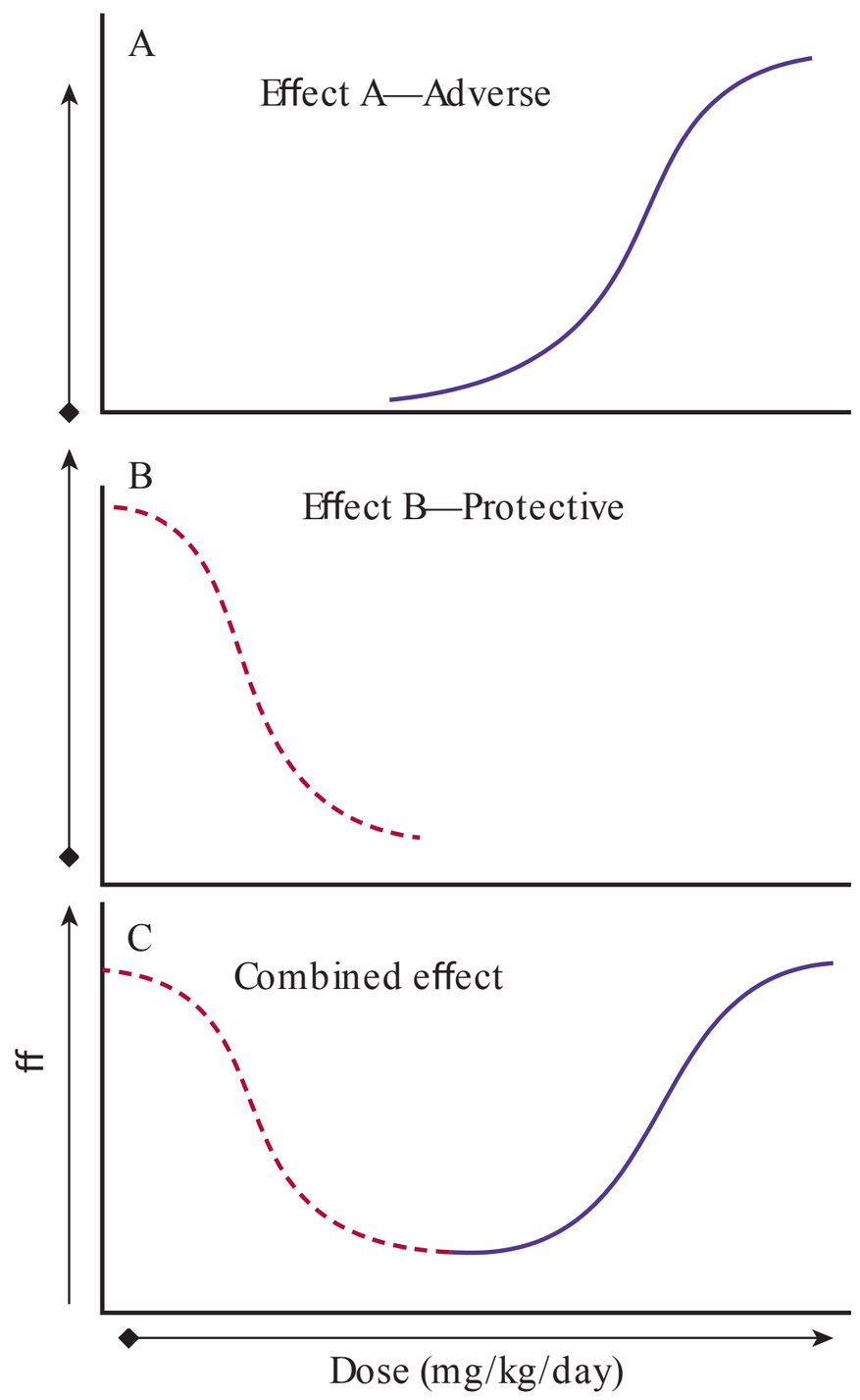

FIGURE 2–6 **Hypothetical dose–response relationship depicting characteristics of hormesis.** Hormetic effects of a substance are hypothesized to occur when relatively low doses result in the stimulation of a beneficial or protective response (B), such as induction of enzymatic pathways that protect against oxidative stress. Although low doses provide a potential beneficial effect, a threshold is exceeded as the dose increases and the net effects will be detrimental (A), resulting in a typical dose-related increase in toxicity. The complete dose–response curve (C) is conceptually similar to the individual dose–response relationship for essential nutrients shown in Figure 2–5.

"safe" levels of exposure to a substance, it is important to determine the absence or presence of a threshold.

In evaluating the shape of the dose–response relationship in populations, it is realistic to consider inf ections in the shape of the dose–response curve rather than absolute thresholds. T at is, the slope of the dose–response relationship at high doses may be substantially different from the slope at low doses, usually because of dispositional differences in the chemical. Saturation of biotransformation pathways, protein-binding sites or receptors, and depletion of intracellular cofactors represent some reasons why sharp inf ections in the dose–response relationship may occur.

Nonmonotonic Dose–Response Curves—Some chemicals, especially the endocrine disruptors, may exert effects at low doses that are not evident at high doses. T ese agents produce the so-called nonmonotonic dose–response curves. T ese curves may result from upregulation of some receptors at low doses with downregulation of those receptors at higher doses. T e chemical may also act on different molecular pathways with common endpoints but opposite effects. Bisphenol A is one chemical that shows nonmonotonic dose response curves.

Assumptions in Deriving the Dose–Response Relationship

A number of assumptions must be considered before dose–response relationships can be used appropriately. T e first is that the response is due to the chemical administered, a cause-and-effect relationship.

T e second assumption is that the magnitude of the response is in fact related to the dose. T is assumes that there is a molecular target site (or sites) with which the chemical interacts to initiate the response, which is related to the concentration of the agent at the target site, which, in turn, is related to the dose administered.

T e third assumption in using the dose–response relationship is that there exists both a quantifiable method of measuring and a precise means of expressing the toxicity. A given chemical may have a family of dose–response relationships, one for each toxic endpoint. For example, a chemical that produces cancer through genotoxic effects, liver damage through inhibition of a specific enzyme, and CNS effects via a different mechanism may have three distinct dose–response relationships, one for each endpoint.

With a new substance, the customary starting point is a single dose acute toxicity test designed to provide preliminary identification of target organ toxicity. Studies specifically designed with lethality as an endpoint are no longer recommended by U.S. or international agencies. Data from acute studies provide essential information for choosing doses for repeated dosing studies, as well as choosing specific toxicologic endpoints for further study. From these studies, clues as to the direction of further studies come about in two important ways. Detailed physiologic measurements and behavioral observations are collected from onset of exposure to the toxicant to the end of the observation period. An acute toxicity study ordinarily is supported by histologic examination of major tissues and organs for abnormalities. From these observations, one can usually obtain more specific information about the events leading to the lethal effect, the target organs involved, and often a suggestion about the possible mechanism of toxicity.

Evaluating the Dose–Response Relationship

Comparison of Dose–Responses—Figure 2–7 illustrates a hypothetical quantal dose–response curve for a desirable effect of a chemical ED such as anesthesia, a toxic dose (TD) effect such as liver injury, and the lethal dose (LD). Even though the curves for ED and LD are parallel, the mechanism by which the drug works is not necessarily that by which the lethal effects are caused. T e same admonition applies to any pair of parallel "effect" curves or any other pair of toxicity or lethality curves.

Therapeutic Index—T e hypothetical curves in Figure 2–7 illustrate two other interrelated points: the importance of the selection of the toxic criterion and the interpretation of comparative effect. T e therapeutic index (TI) is defined as the ratio of the dose required to produce a toxic effect and the dose needed to elicit the desired therapeutic response. Similarly, an index of comparative toxicity is obtained by the ratio of doses of two different materials to produce an identical response or the ratio of doses of the same material necessary to yield different toxic effects.

T e most commonly used index of effect, whether beneficial or toxic, is the median dose—that is, the dose required to result in a response in 50% of a population (or to produce 50% of a

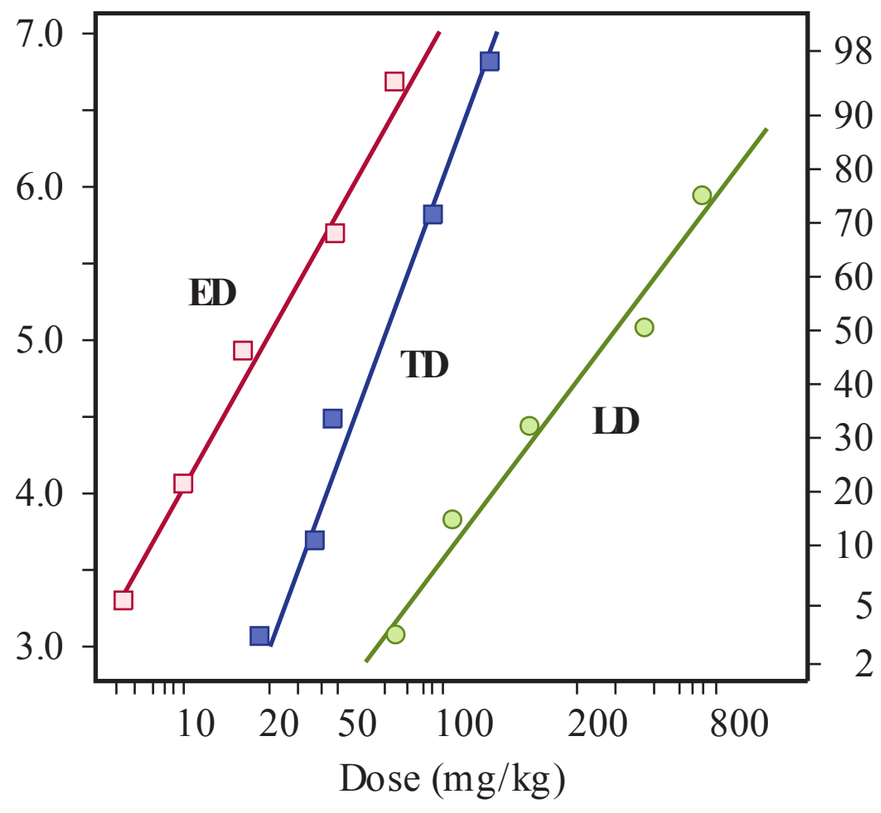

FIGURE 2–7 **Comparison of effective dose (ED), toxic dose (TD), and lethal dose (LD).** The plot is of log dosage versus percentage of population responding in probit units.

maximal response). The TI of a drug is an approximate statement about the relative safety of a drug expressed as the ratio of the TD (historically the LD) to the therapeutic dose:

$$TI = \frac{TD_{50}}{ED_{50}}$$

From Figure 2–7, one can approximate a TI by using these median doses. The larger the ratio is, the greater the relative safety. The ED_{50} is approximately 20, and the TD_{50} is about 60; thus, the TI is 3, a number indicating that reasonable care in exposure to the drug is necessary to avoid toxicity. However, median doses tell nothing about the slopes of the dose–response curves for therapeutic and toxic effects.

Margins of Safety and Exposure—One way to overcome this deficiency is to use the ED_{99} for the desired effect and the LD_1 for the undesired effect. These parameters are used to calculate the margin of safety:

$$\text{Margin of safety} = \frac{LD_1}{ED_{99}}$$

For nondrug chemicals, the term margin *of safety* is an indicator of the magnitude of the difference between an estimated "exposed dose" to a human population and the no observable adverse effect level (NOAEL) determined in experimental animals.

Potency versus Efficacy—To compare the toxic effects of two or more chemicals, the dose–response to the toxic effects of each chemical must be established. The potency and maximal efficacy of the two chemicals to produce a toxic effect can be explained by reference to Figure 2–8. Chemical A is said to be more potent than chemical B, and C is more potent than D, because of their relative positions along the dosage axis. Potency thus refers to the range of doses over which a chemical produces increasing responses. Maximal efficacy reflects the limit of the dose–response relationship on the response axis to a certain chemical. Chemicals A and B have equal maximal efficacy, whereas the maximal efficacy of C is less than that of D.

VARIATION IN TOXIC RESPONSES

Selective Toxicity

Selective toxicity means that a chemical produces injury to one kind of living matter without harming another form of life even though the two may exist in intimate contact. By taking advantage of biological diversity, it is possible to develop agents that are lethal for an undesired species and harmless for other species. Such selective toxicity can be due to differences in distribution (absorption, biotransformation, or excretion) or to differing biochemical processing of the toxicant by different organisms.

Species Differences

Although a basic tenet of toxicology is that "experimental results in animals, when properly qualified, are applicable to humans," it is important to recognize that both quantitative and qualitative differences in response to toxic substances may occur among different species. Identifying the mechanistic basis for species differences in response to chemicals establishes the relevance of animal data to human response.

Individual Differences in Response

Even within a species, large interindividual differences in response to a chemical can occur because of subtle genetic differences referred to as genetic polymorphisms. These may be responsible for idiosyncratic reactions to chemicals and for interindividual differences in toxic responses.

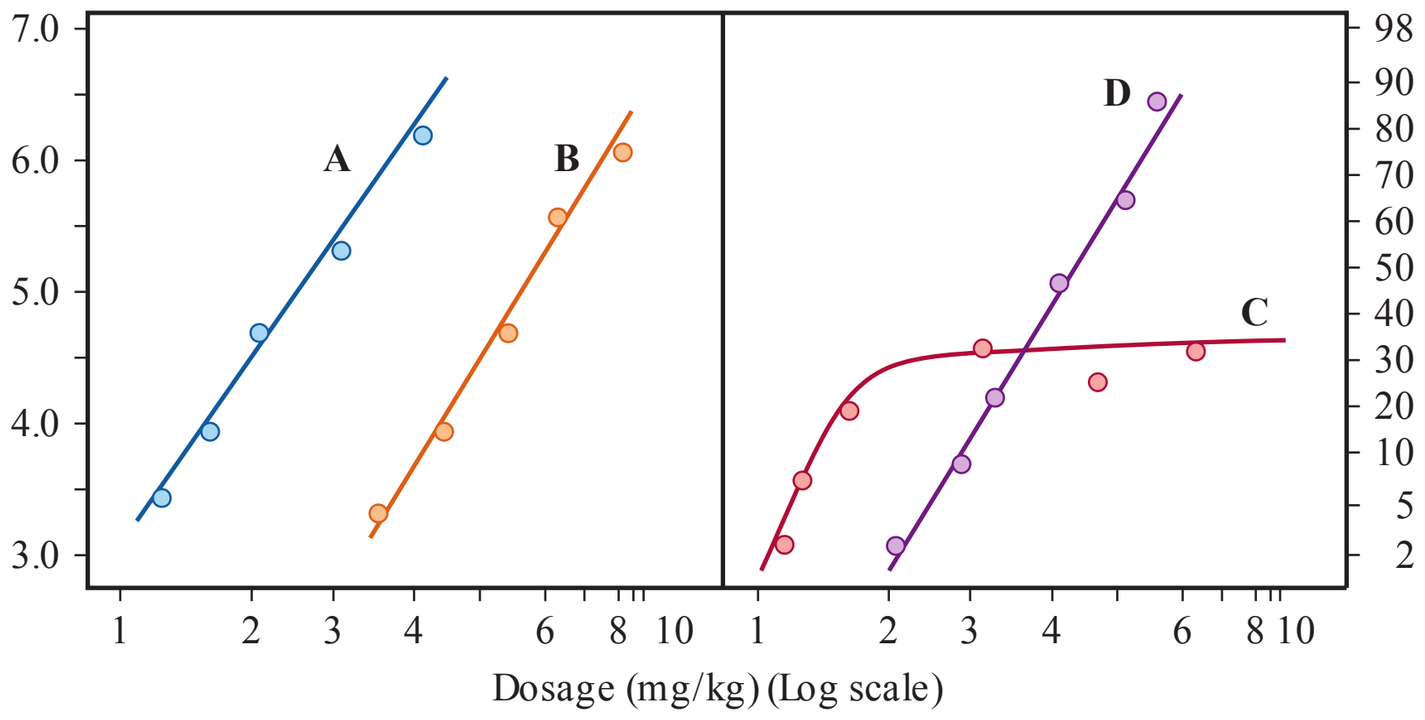

FIGURE 2–8 **Schematic representation of the difference in the dose–response curves for four chemicals (A–D), illustrating the difference between potency and efficacy (see text).**

DESCRIPTIVE ANIMAL TOXICITY TESTS

Two main principles underlie all descriptive animal toxicity testing. The first is that the effects produced by a compound in laboratory animals, when properly qualified, are applicable to humans. The second principle is that exposure of experimental animals to toxic agents in high doses is a necessary and valid method of discovering possible hazards in humans because the incidence of an effect in a population is greater as the dose or exposure increases. Obtaining statistically valid results from the small groups of animals used in toxicity testing requires the use of relatively large doses so that the effect will occur frequently enough to be detected. However, the use of high doses can create problems in interpretation if the response(s) obtained at high doses does not occur at low doses.

Toxicity tests are not designed to demonstrate that a chemical is safe but to characterize the toxic effects a chemical can produce. There are no set toxicology tests that have to be performed on every chemical intended for commerce. Depending on the eventual use of the chemical, the toxic effects produced by structural analogs of the chemical, as well as the toxic effects produced by the chemical itself, contribute to the determination of the toxicology tests that should be performed.

Acute Toxicity Testing

The first toxicity test performed on a new chemical is acute toxicity, which is determined from the administration of a single exposure. The LD_{50} and other acute toxic effects are determined after one or more routes of administration (one route being oral or the intended route of exposure) in one or more species, usually the mouse and rat, but sometimes the rabbit and dog. Daily examination of the animals for signs of intoxication, lethargy, behavioral modifications, food consumption, etc., and tabulation of the number of animals that die in a 14-day period after a single dosage occurs. Acute toxicity tests (1) give a quantitative estimate of acute toxicity (LD_{50}), (2) identify target organs and other clinical manifestations of acute toxicity, (3) identify species differences and susceptible species, (4) establish the reversibility of the toxic response, and (5) provide dose-ranging guidance for other studies.

Determination of the LD_{50} has become a public issue because of increasing concern for the welfare and protection of laboratory animals. Because LD_{50} is not a constant and many variables influence its estimation, for most purposes it is only necessary to characterize the LD_{50} within an order of magnitude range (e.g., 5 to 50 and 50 to 500 mg/kg).

If there is a reasonable likelihood of substantial exposure to the material by dermal or inhalation exposure, acute dermal and acute inhalation studies are performed. When animals are exposed acutely to chemicals in the air they breathe or the water they (fish) live in, the lethal concentration 50 (LC_{50}) is usually determined for a known time of exposure, that is, the concentration of chemical in the air or water that causes death to 50% of the animals. The acute dermal toxicity test is usually performed in rabbits. The site of application is shaved, and the substance is applied and covered for 24 h, and then removed. The skin is cleaned and the animals observed for 14 days to calculate LD_{50}. Acute inhalation studies are performed that are similar to other acute toxicity studies except that the route of exposure is inhalation for 4 h.

Acute lethality studies are essential for characterizing the toxic effects of chemicals and their hazard to humans. The most meaningful scientific information derived from acute lethality tests comes from clinical observations and postmortem examination of animals rather than from the specific LD_{50} value.

Skin and Eye Irritations

For the dermal irritation test (Draize test), the skin of rabbits is shaved, the chemical applied to one intact and two abraded sites and covered for 4 h. The degree of skin irritation is scored for erythema (redness), eschar (scab), edema (swelling) formation, and corrosive action. These dermal irritation observations are repeated at various intervals after the covered patch has been removed. To determine the degree of ocular irritation, the chemical is instilled into one eye of each test rabbit. The contralateral eye is used as the control. The eyes of the rabbits are then examined at various times after application.

Alternative in vitro models, including epidermal keratinocyte and corneal epithelial cell culture models, have been developed for evaluating cutaneous and ocular toxicity of substances.

Sensitization

Information about the potential of a chemical to sensitize skin is needed in addition to irritation testing for all materials that may repeatedly come into contact with the skin. In general, the test chemical is administered to the shaved skin of guinea pigs topically, intradermally, or both, over a period of 2 to 4 weeks. About 2 to 3 weeks after the last treatment, the animals are challenged with a nonirritating concentration of the test substance and the development of erythema is evaluated.

Subacute (Repeated-dose Study)

Subacute toxicity tests are performed to obtain information on the toxicity of a chemical after repeated administration for typically 14 days and as an aid to establish doses for subchronic studies.

Subchronic

Subchronic exposure usually lasts for 90 days. The principal goals of the subchronic study are to establish a "lowest observed adverse effect level" (LOAEL) and a NOAEL, and to further identify and characterize the specific organ or organs affected by the test compound after repeated administration.

A subchronic study is usually conducted in two species (rat and dog for FDA; mouse and rat for EPA) by the route of intended exposure. At least three doses are employed (a high

dose that produces toxicity but less than 10% fatalities, a low dose that produces no apparent toxic effects, and an intermediate dose). Animals should be observed once or twice daily for signs of toxicity. All premature deaths should be recorded and necropsied. Severely moribund animals should be terminated immediately to preserve tissues and reduce unnecessary suffering. At the end of the 90-day study, all the remaining animals should be terminated and blood and tissues should be collected for further analysis. T e gross and microscopic conditions of the organs and tissues are recorded and evaluated. Hematology, blood chemistry, and urinalysis measurements are usually done before, in the middle of, and at the termination of exposure. Hematology measurements usually include hemoglobin concentration, hematocrit, erythrocyte counts, total and differential leukocyte counts, platelet count, clotting time, and prothrombin time. Clinical chemistry determinations commonly include glucose, calcium, potassium, urea nitrogen, alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), gamma-glutamyltranspeptidase (GGT), sorbitol dehydrogenase, lactic dehydrogenase, alkaline phosphatase, creatinine, bilirubin, triglycerides, cholesterol, albumin, globulin, and total protein. Urinalysis includes determination of specific gravity or osmolarity, pH, proteins, glucose, ketones, bilirubin, and urobilinogen as well as microscopic examination of formed elements. If humans are likely to have significant exposure to the chemical by dermal contact or inhalation, subchronic dermal and/or inhalation experiments may also be required.

Chronic

Long-term or chronic exposure studies are performed similarly to subchronic studies except that the period of exposure is usually for 6 months to 2 years. Chronic toxicity tests are often designed to assess both the cumulative toxicity and the carcinogenic potential of chemicals. Both gross and microscopic pathological examinations are made not only on animals that survive the chronic exposure, but also on those that die prematurely.

Dose selection is critical to ensure that premature mortality from chronic toxicity does not limit the number of animals that survive to a normal life expectancy. Most regulatory guidelines require that the highest administered dose be the estimated maximum tolerable dose (MTD), that is, the dose that suppresses body weight gain slightly in a 90-day subchronic study. Generally, one or two additional doses, usually one-half and one-quarter MTD, and a control group are tested.

Chronic toxicity assays commonly evaluate the potential oncogenicity of test substances. Both benign and malignant tumors must be reported. Properly designed chronic oncogenicity studies require a concurrent control group matched for variables such as age, diet, and housing conditions.

Other Tests

T e effects of chemicals on reproduction and development are discussed in Chapters 10 and 20. Oncogenicity bioassays are introduced in Chapter 8. Mutagenicity is discussed in detail in Chapter 9. Information on methods, concepts, and problems associated with inhalation toxicology is provided in Chapters 15 and 28. A discussion of neurotoxicity and behavioral toxicology can be found in Chapter 16. Immunotoxicity assessment is mentioned in Chapter 12.

TOXICOGENOMICS

Toxicogenomics defines the interaction between genes and toxicants in toxicity etiology. Transcript, protein, and metabolite profiling is combined with conventional toxicology. T e human genome consists of approximately 3 billion base pairs of deoxyribonucleotides. T e differential expression of genes in a given cell is largely responsible for the diverse function of the thousands of different cells, tissues, and organs that constitute an individual organism. Experimental data on how a toxicant affects gene expression (transcriptomics), protein production (proteomics), and small molecule metabolism and function (metabolomics) from a test species (rat/mouse, etc.) can be combined with those of humans and analyzed with the computational tools of bioinformatics to ascertain unique or predictive patterns of toxicity.

Genomics

T e identification and characterization of various genetic variants will aid understanding of interindividual differences in susceptibility to chemicals or other environmental factors and the complex interactions between the human genome and the environment. How chemicals affect genomic DNA, mRNA, small interfering RNA (siRNA), etc. is of particular importance to toxicogenomics.

Epigenetics

Toxicants may also act on areas "above or in addition" to genes. Epigenetics concerns a mitotically or meiotically heritable change in gene expression that occurs independently of an alteration in DNA sequence. Changes in DNA methylation or histone acetylation may suppress, silence, or activate gene expression without altering the DNA sequence. T ere is evidence in some animal models that epigenetic changes may be transgenerational thereby inf uencing toxicological assessment. Subtle epigenetic changes resulting from environmental exposures may not produce cytotoxicity or mutation or may lead to cancer, neurodevelopment disorders, autoimmune diseases, metabolic disorders, asthma, or neurologic/behavioral disorders (Figure 2–9).

Transcriptomics and Proteomics

T e transcriptome contains all mature mRNA species in the cell at a given time. It is dynamic and represents the steady state

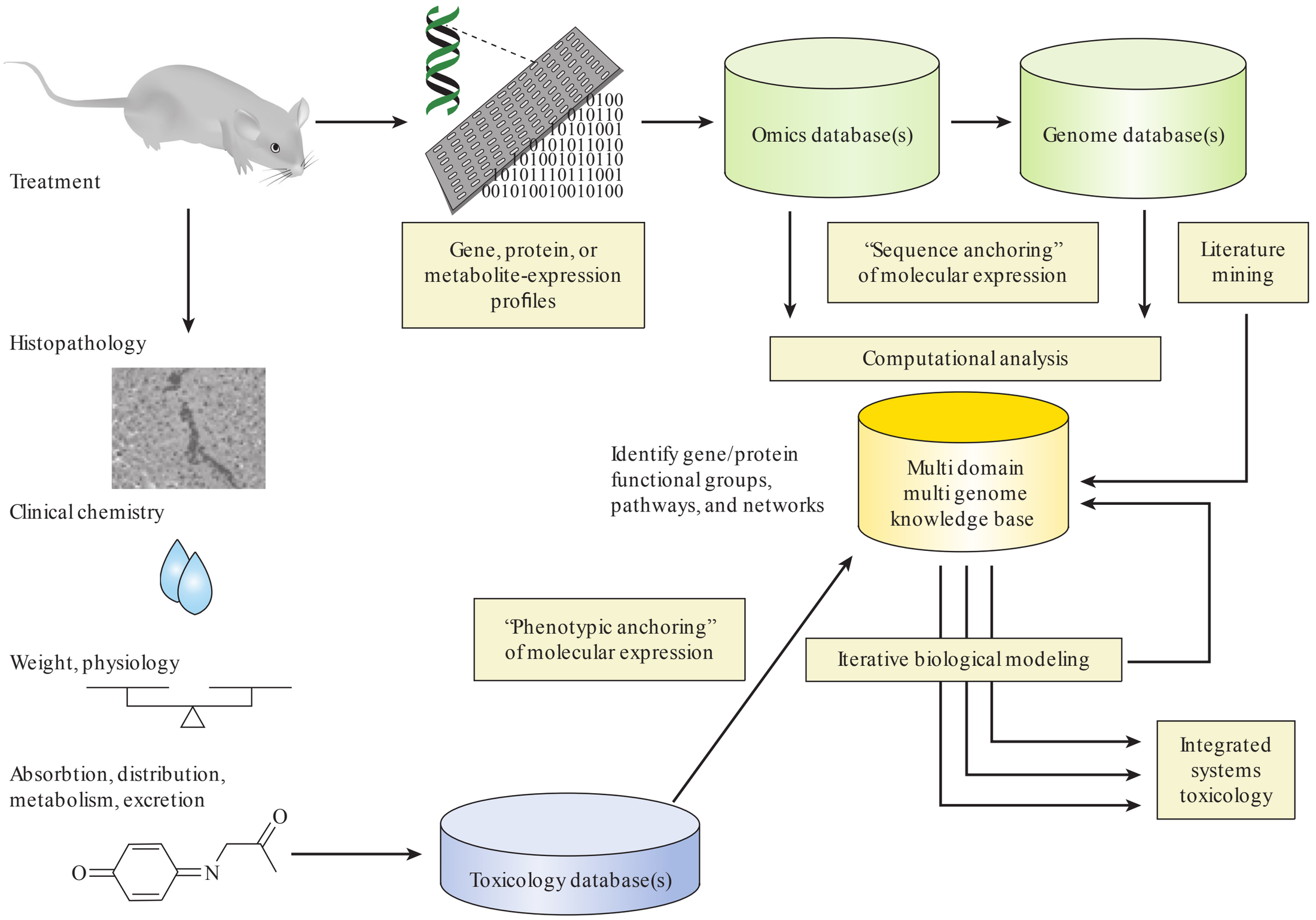

FIGURE 2–9 **Conceptual approach for incorporating "omics" technologies and resulting large databases into toxicological evaluation.** Data from experiments that evaluate the ef ects of a chemical on global patterns of gene expression (transcriptomics), protein content (proteomics), and small molecules/metabolites (metabonomics/metabolomics), combined with genomic information from both the test species (e.g., rats, mice) and the target species of interest (e.g., humans), are analyzed by computational tools (bioinformatics) for unique or potentially predictive patterns of toxicity. Essential to the use of omics data for predictive toxicology/safety assessment is the ability to reliably tie observed omics patterns to traditional measures of toxicity, such as histopathology and clinical chemistry (phenotypic anchoring). (Reproduced with permission from Waters MD and Fostel JM. Toxicogenomics and systems toxicology: aims and prospects. Nat Rev Genet, 2004 Dec;5(12):936–948.)

between synthesis (transcription) and degradation of mRNA. Northern blots, reverse transcriptase polymerase chain reaction, and microarray technologies permit determination of effects of chemical exposure on gene expression. One of the major challenges in toxicogenomics is the recognition that transcriptional regulation is highly dynamic as gene expression profiles can change dramatically with both dose and time. Alterations in gene expression often contribute to phenotypic changes that occur, but the transcriptome is somewhat removed from the ultimate biochemical functions that dictate the actual biologic function of the cell.

T e proteome is the entire complement of proteins that are present in a cell or tissue at a specific time point. Unambiguous protein identification is difficult and generally requires separation techniques (gel electrophoresis or high pressure liquid chromatography) followed by tandem mass spectrometry. Proteomics can potentially identify unique patterns of protein expression that may be predictive of early toxicity or subsequent disease development.

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QUESTIONS

1. Five identical experimental animals are treated with 1 mg of one of the following toxins. T e animal treated with which toxin is most likely to die?
   a. ethyl alcohol (LD_{50} = 10,000 mg/kg).
   b. botulinum toxin (LD_{50} = 0.00001 mg/kg).
   c. nicotine (LD_{50} = 1 mg/kg).
   d. ferrous sulfate (LD_{50} = 1500 mg/kg).
   e. picrotoxin (LD_{50} = 5 mg/kg).

2. Place the following mechanisms of toxin delivery in order from most effective to least effective—1: intravenous; 2: subcutaneous; 3: oral; 4: inhalation; 5: dermal.
   a. 1, 5, 2, 4, 3.
   b. 4, 1, 2, 3, 5.
   c. 1, 4, 2, 3, 5.
   d. 4, 2, 1, 5, 3.
   e. 1, 4, 3, 2, 5.

3. A toxin with a half-life of 12 h is administered every 12 h. Which of the following is true?
   a. T e chemical is eliminated from the body before the next dose is administered.
   b. T e concentration of the chemical in the body will slowly increase until the toxic concentration is attained.
   c. A toxic level will not be reached, regardless of how many doses are administered.
   d. Acute exposure to the chemical will produce immediate toxic effects.
   e. T e elimination rate of the toxin is much shorter than the dosing interval.

4. Urushiol is the toxin found in poison ivy. It must first react and combine with proteins in the skin in order for the immune system to recognize and mount a response against it. Urushiol is an example of which of the following?
   a. antigen.
   b. auto-antibody.
   c. superantigen.
   d. hapten.
   e. cytokine.

5. Toxic chemicals are most likely to be biotransformed in which of the following organs?
   a. central nervous system.
   b. heart.
   c. lung.
   d. pancreas.
   e. liver.

6. When chemicals A and B are administered simultaneously, their combined effects are far greater than the sum of their effects when given alone. T e chemical interaction between chemicals A and B can be described as which of the following?
   a. potentiative.
   b. additive.
   c. antagonistic.
   d. functionally antagonistic.
   e. synergistic.

7. With respect to dose–response relationships, which of the following is true?
   a. Graded dose–response relationships are often referred to as "all or nothing" responses.
   b. Quantal dose–response relationships allow for the analysis of a population's response to varying dosage.
   c. Quantal relationships characterize the response of an individual to varying dosages.
   d. A quantal dose–response describes the response of an individual organism to varying doses of a chemical.
   e. T e dose–response always increases as the dosage is increased.

8. When considering the dose–response relationship for an essential substance:
   a. there are rarely negative effects of ingesting too much.
   b. the curve is the same for all people.
   c. adverse responses increase in severity with increasing or decreasing dosages outside of the homeostatic range.
   d. the relationship is linear.
   e. deficiency will never cause more harm than over-ingestion.

9. T e therapeutic index of a drug:
   - **a.** is the amount of a drug needed to cure an illness.
   - **b.** is lower in drugs that are relatively safer.
   - **c.** describes the potency of a chemical in eliciting a desired response.
   - **d.** describes the ratio of the toxic dose to the therapeutic dose of a drug.
   - **e.** explains the change in response to a drug as the dose is increased.

10. Penicillin interferes with the formation of peptidoglycan cross-links in bacterial cell walls, thus weakening the cell wall and eventually causing osmotic death of the bacterium. Which of the following is true?
    - **a.** Treatment with penicillin is a good example of selective toxicity.
    - **b.** Penicillin interferes with human plasma membrane structure.
    - **c.** Penicillin is a good example of a drug with a low therapeutic index.
    - **d.** Penicillin is also effective in treating viral infections.
    - **e.** Penicillin is completely harmless to humans.

CHAPTER 3

Mechanisms of Toxicity

Zoltán Gregus

STEP 1—DELIVERY: FROM THE SITE OF EXPOSURE TO THE TARGET

Absorption versus Presystemic Elimination
Absorption
Presystemic Elimination
Distribution to and away from the Target
Mechanisms Facilitating Distribution to a Target
Mechanisms Opposing Distribution to a Target
Excretion versus Reabsorption
Excretion
Reabsorption
Toxication versus Detoxication
Toxication
Detoxication

STEP 2—REACTION OF THE ULTIMATE TOXICANT WITH THE TARGET MOLECULE

Attributes of Target Molecules
Types of Reactions
Noncovalent Binding
Covalent Binding
Hydrogen Abstraction
Electron Transfer
Enzymatic Reactions
Effects of Toxicants on Target Molecules
Dysfunction of Target Molecules
Destruction of Target Molecules
Neoantigen Formation
Toxicity Not Initiated by Reaction with Target Molecules

STEP 3—CELLULAR DYSFUNCTION AND RESULTANT TOXICITIES

Toxicant-induced Cellular Dysregulation
Dysregulation of Gene Expression
Dysregulation of Ongoing Cellular Activity
Toxic Alteration of Cellular Maintenance
Impairment of Internal Cellular Maintenance: Mechanisms of Toxic Cell Death
Depletion of ATP
Sustained Rise of Intracellular Ca^{2+}
Interplay between the Primary Metabolic Disorders Spells Cellular Disaster
Mitochondrial Permeability Transition (MPT) and the Worst Outcome: Necrosis
An Alternative Outcome of MPT: Apoptosis
What Determines the Form of Cell Death?
Induction of Death by Unknown Mechanisms
Impairment of External Cellular Maintenance

STEP 4—REPAIR OR DYSREPAIR

Molecular Repair
Repair of Proteins
Repair of Lipids
Repair of DNA
Cellular Repair: A Strategy in Peripheral Neurons
Autophagy of Damaged Cell Organelles
Regeneration of Damaged Axons
Tissue Repair
Apoptosis: An Active Deletion of Damaged Cells
Proliferation: Regeneration of Tissue
Side Reactions to Tissue Injury
Mechanisms of Adaptation
When Repair and Adaptation Fail
Adaptation
Toxicity Resulting from Inappropriate Repair and Adaptation
Tissue Necrosis
Fibrosis
Carcinogenesis

CONCLUSIONS

KEY POINTS

- Toxicity involves toxicant delivery to its target or targets and interactions with endogenous target molecules that may trigger perturbations in cell function and/or structure or that may initiate repair mechanisms at the molecular, cellular, and/or tissue levels.
- Biotransformation to harmful products is called toxication or metabolic activation.
- Biotransformations that eliminate the ultimate toxicant or prevent its formation are called detoxications.
- Apoptosis, or programmed cell death, is a tightly controlled, organized process whereby individual cells break into small fragments that are phagocytosed by adjacent cells or macrophages without producing an inflammatory response.
- Sustained elevation of intracellular Ca^{2+} is harmful because it can result in (1) depletion of energy reserves by inhibiting the ATPase used in oxidative phosphorylation, (2) dysfunction of microfilaments, (3) activation of hydrolytic enzymes, and (4) generation of reactive oxygen and nitrogen species (ROS and RNS).
- Cell injury progresses toward cell necrosis (death) if molecular repair mechanisms are inefficient or the molecular damage is not readily reversible.
- Chemical carcinogenesis involves insufficient function of various repair mechanisms, including (1) failure of DNA repair, (2) failure of apoptosis (programmed cell death), and (3) failure to terminate cell proliferation.

An understanding of the mechanisms of toxicity provides a rational basis for interpreting descriptive toxicity data. The cellular mechanisms that contribute to the manifestation of toxicities are overviewed by relating a series of events that begins with exposure, involves a multitude of interactions between the invading toxicant and the organism, and culminates in a toxic effect.

As a result of the huge number of potential toxicants and the multitude of biological structures and processes that can be impaired, there are a tremendous number of possible pathways that may lead to toxicity (Figure 3–1). Commonly, a toxicant is delivered to its target, reacts with it, and the resultant cellular dysfunction manifests itself in toxicity. Sometimes a xenobiotic does not react with a specific target molecule but rather adversely influences the biological environment, causing molecular, organellar, cellular, or organ dysfunction leading to deleterious effects.

The most complex path to toxicity involves more steps (Figure 3–1). First, the toxicant is delivered to its target or targets (step 1), interacting with endogenous target molecules (step 2a) or altering the environment (step 2b), triggering perturbations in cell function and/or structure (step 3), which initiate repair mechanisms at the molecular, cellular, and/or tissue levels (step 4). When the perturbations induced by the toxicant exceed repair capacity or when repair becomes malfunctional, toxicity occurs. Tissue necrosis, cancer, and fibrosis are examples of chemically induced toxicities that follow this four-step course.

STEP 1—DELIVERY: FROM THE SITE OF EXPOSURE TO THE TARGET

Theoretically, the intensity of a toxic effect depends on the concentration and persistence of the ultimate toxicant at its site of action. The ultimate toxicant is the chemical species that reacts with the endogenous target molecule or critically alters the biological environment, initiating structural and/or functional alterations that result in toxicity. The ultimate toxicant can be the original chemical to which the organism is exposed (parent compound), a metabolite, or a reactive oxygen or nitrogen species (ROS or RNS) generated during the biotransformation of the toxicant, or an endogenous molecule.

The concentration of the ultimate toxicant at the target molecule depends on the relative effectiveness of the processes that increase or decrease its concentration at the target site (Figure 3–2). Increased concentration is facilitated by absorption, distribution to the site of action, reabsorption, and toxication, while presystemic elimination, distribution away from the site of action, excretion, and detoxication will decrease the toxicant concentration at its target.

Absorption versus Presystemic Elimination

Absorption—Transfer of a chemical from the site of exposure, usually an external or internal body surface, into the systemic circulation is called absorption. Transporters contribute to gastrointestinal (GI) absorption of some chemicals; however, the vast majority of toxicants traverse epithelial barriers via diffusion. Factors that influence absorption include concentration, surface area of exposure, characteristics of the epithelial layer through which the toxicant is being absorbed, and, usually most important, lipid solubility because lipid-soluble molecules are absorbed most easily into cells.

Presystemic Elimination—During transfer from the site of exposure to the systemic circulation, toxicants may be eliminated. This is common for chemicals absorbed from the gastrointestinal (GI) tract because they must first pass through the GI mucosal cells, into the liver (enterohepatic circulation), and then

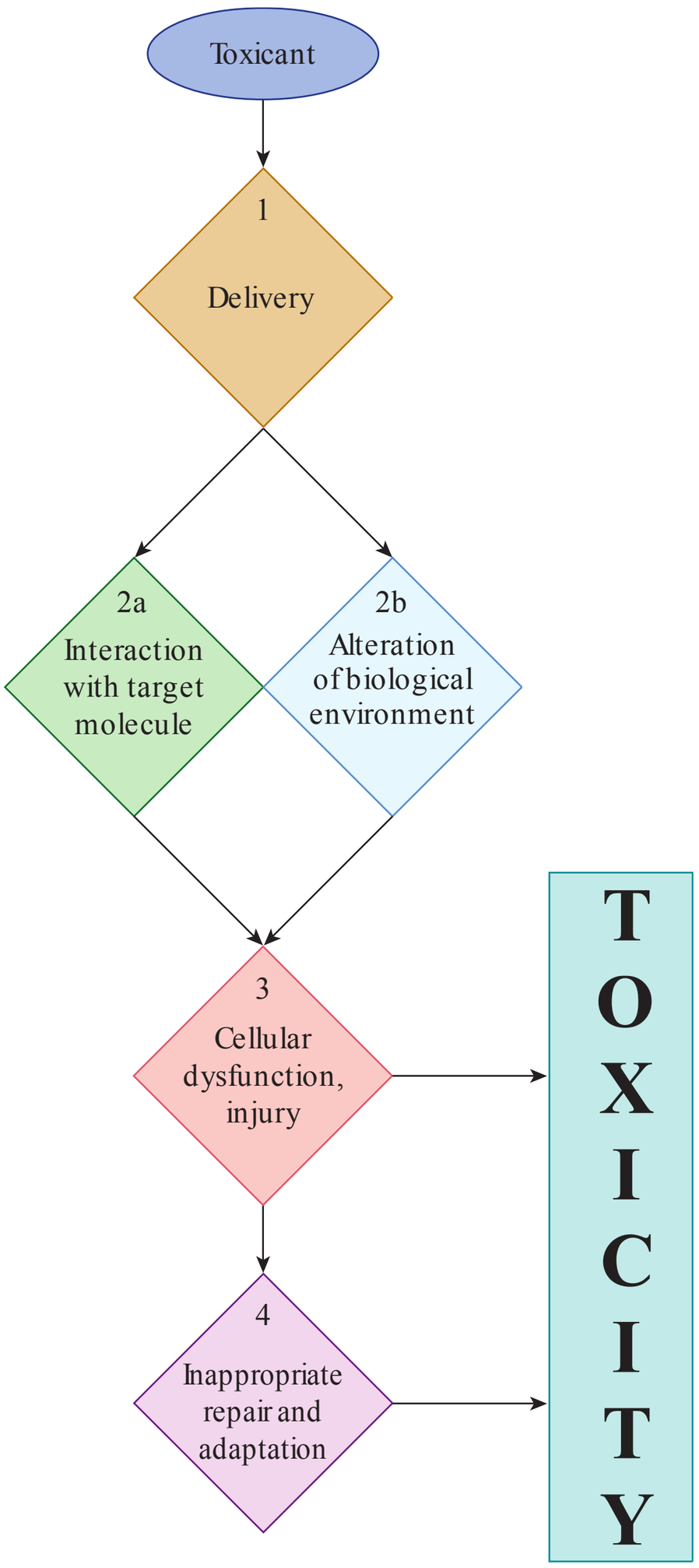

FIGURE 3–1 **Potential stages in the development of toxicity after chemical exposure.**

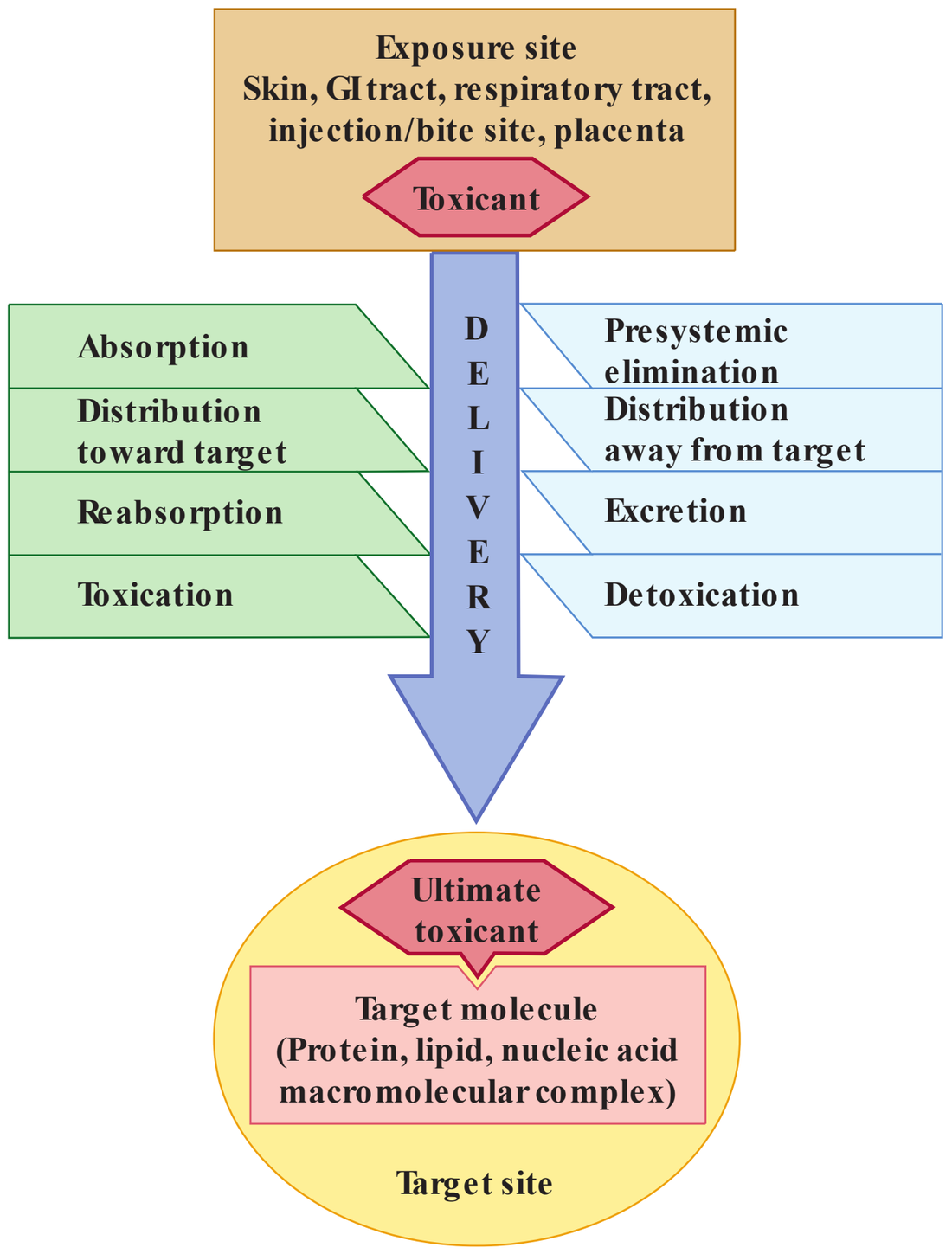

FIGURE 3–2 **The process of toxicant delivery is the first step in the development of toxicity.** Delivery—that is, movement of the toxicant from the site of exposure to the site of its action in an active form—is promoted by the processes listed on the left and opposed by the events indicated on the right.

lung (pulmonary circulation) before being distributed to the rest of the body (systemic circulation). The GI mucosa and the liver may eliminate a significant fraction of a toxicant during its passage through these tissues. Presystemic or first-pass elimination generally reduces the toxic effects of chemicals that reach their target sites by way of the systemic circulation, but may contribute to injury of the digestive mucosa, the liver, and the lungs because these processes necessitate toxicant delivery to those sites.

Distribution to and away from the Target

Toxicants exit the blood during the distribution phase, enter the extracellular space, and reach their site or sites of action, usually a macromolecule on either the surface or the interior of a particular type of cell. Chemicals also may be distributed to the site or sites of toxication, usually an intracellular enzyme, where the ultimate toxicant is formed through biotransformation.

Mechanisms Facilitating Distribution to a Target

Porosity of the Capillary Endothelium—There are three types of capillaries (continuous, fenestrated, and sinusoidal), each with varying degrees of porosity. Endothelial cells in the hepatic sinusoids and in the renal peritubular capillaries have large fenestrae (50 to 150 nm in diameter) that permit passage of even protein-bound xenobiotics. This relatively free filtration promotes the accumulation of chemicals in the liver and kidneys.

Specialized Transport across the Plasma Membrane—Specialized ion channels and membrane transporters can contribute to the intracellular delivery of toxicants, making those cells targets. Na^+,K^+-ATPase, voltage-gated Ca^{2+} channels, carrier-mediated uptake, endocytosis, and membrane recycling are some examples of methods that facilitate the entry of toxicants into specific cells. Further, endocytosis of some toxicant–protein complexes also occurs in some cells.

Accumulation in Cell Organelles—Amphipathic xenobiotics with a protonatable amine group and lipophilic character accumulate in lysosomes as well as mitochondria. Lysosomal accumulation occurs by pH trapping, that is, diffusion of the amine in unprotonated form into the acidic interior of the organelle, where the amine is protonated, preventing its efflux, so that it impairs phospholipid degradation. Mitochondrial accumulation takes place electrophoretically. The amine is protonated in the intermembrane space and then sucked into the matrix space by the strong negative potential (−220 mV), where it may impair β-oxidation of fatty acids and oxidative phosphorylation.

Reversible Intracellular Binding—Chemicals such as organic and inorganic cations and polycyclic aromatic hydrocarbons accumulate in melanin-containing cells by binding to melanin.

Mechanisms Opposing Distribution to a Target

Binding to Plasma Proteins—Hydrophobic xenobiotics generally bind proteins or lipoproteins in the plasma. In order to leave the blood and enter cells, these xenobiotics must dissociate from these proteins. Therefore, strong binding to plasma proteins delays xenobiotics movement across membranes and prolongs their effects and elimination.

Specialized Barriers—Brain capillaries lack fenestrae and are joined by extremely tight junctions, preventing the access of hydrophilic chemicals to the brain except by active transport. The spermatogenic cells are supported by Sertoli cells that are tightly joined to form the blood–testis barrier. Transfer of hydrophilic toxicants across the placenta is also restricted. However, none of these barriers are effective against lipophilic substances.

Distribution to Storage Sites—Some chemicals accumulate in tissues (i.e., storage sites) where they do not exert significant effects. Such storage decreases toxicant availability for their target sites.

Association with Intracellular Binding Proteins—Binding to nontarget intracellular sites, such as metallothionein, temporarily reduces the concentration of toxicants at the target site.

Export *f*rom Cells—Intracellular toxicants may be transported back into the extracellular space. Some ATP-dependent membrane transporters, also known as the multidrug-resistance (mdr) proteins, extrude chemicals from cells.

Excretion versus Reabsorption

Excretion—Excretion is the removal of xenobiotics from blood and their return to the external environment. Excretion is a physical mechanism, whereas biotransformation is a chemical mechanism for eliminating the toxicant.

The route and speed of excretion depend largely on the physicochemical properties of the toxicant. The major excretory organs—the kidney and the liver—efficiently remove highly hydrophilic chemicals such as organic acids and bases.

There are no efficient elimination mechanisms for nonvolatile, highly lipophilic chemicals. If they are resistant to biotransformation, such chemicals are eliminated very slowly and tend to accumulate in the body on repeated exposure. Three rather inefficient processes are available for the elimination of such chemicals: (1) excretion from the mammary gland in breast milk, (2) excretion in bile, and (3) excretion into the intestinal lumen from blood. Volatile, nonreactive toxicants such as gases and volatile liquids diffuse from pulmonary capillaries into the alveoli and are exhaled.

Reabsorption—Toxicants in the blood are filtered at the glomerulus into the renal tubules. These filtered toxicants may reenter the blood by diffusing through peritubular capillaries. This reentry is facilitated by tubular fluid reabsorption which increases intratubular fluid concentration and residence time of nonreabsorbed chemical by slowing urine flow.

Reabsorption by diffusion is dependent on the lipid solubility of the chemical and inversely related to the extent of ionization, because the nonionized molecule is more lipid soluble. Therefore, pH of the tubular fluid affects reabsorption such that acidification favors excretion of weak organic bases and alkalinization favors the elimination of weak organic acids.

Toxicants delivered to the GI tract by biliary, gastric, and intestinal excretion and secretion by salivary glands and exocrine pancreas may be reabsorbed by diffusion across the intestinal mucosa. Reabsorption of compounds excreted into bile is possible only if they are sufficiently lipophilic or are converted to more lipid-soluble forms in the intestinal lumen.

Toxication versus Detoxication

Toxication—**A number of xenobiotics are directly toxic, whereas other xenobiotics exert a toxic effect through their metabolites.** Biotransformation to harmful products is called toxication or metabolic activation. With some xenobiotics, toxication confers physicochemical properties that adversely alter the microenvironment of biological processes or structures. Occasionally, chemicals acquire structural features and reactivity by biotransformation that allows for a more efficient interaction with specific receptors or enzymes. Most often, however, toxication renders xenobiotics and occasionally other molecules in the body, such as nitric oxide, indiscriminately reactive toward endogenous molecules with susceptible functional groups. This increased reactivity may be due to conversion into (1) electrophiles, (2) free radicals, (3) nucleophiles, or (4) redox-active reactants.

Electrophiles are molecules that contain an electron-deficient atom with a partial or full positive charge that allows it to react by sharing electron pairs with the electron-rich atoms in nucleophiles. A free radical is a molecule or molecular fragment that contains one or more unpaired electrons. One of the more biologically relevant free radicals is superoxide anion ($O_2^{\bullet -}$), which is formed both endogenously and exogenously. The immune system produces $O_2^{\bullet -}$ and transforms it into hypochorous acid (aka bleach, HOCl) through a

series of reactions in order to combat pathogens. The most reactive metabolites are electron-deficient molecules and molecular fragments such as electrophiles and neutral or cationic free radicals. Some nucleophiles are inherently reactive (e.g., HCN and CO); however, many are activated by conversion into electrophiles.

Detoxication—Biotransformations that eliminate the ultimate toxicant or prevent its formation are called detoxications. In some cases, detoxication may compete with toxication.

Detoxication *of* Toxicants with No Functional Groups—In general, chemicals without functional groups, such as benzene and toluene, are detoxicated in two phases. Initially, a functional group such as hydroxyl or carboxyl is introduced into the molecule, most often by cytochrome P450 enzymes. Next, an endogenous acid, such as glucuronic acid, sulfuric acid, or an amino acid, is added to the functional group by a transferase. With some exceptions, the final products are inactive, highly hydrophilic organic acids that are readily excreted.

Detoxication *of* Nucleophiles—Nucleophiles generally are detoxicated by conjugation of a functional group to the nucleophilic atom. Sulfonation, glucuronidation, methylation, and acetylation are common reactions. Conjugation prevents peroxidase-catalyzed conversion of the nucleophiles to free radicals and biotransformation of phenols, aminophenols, catechols, and hydroquinones to electrophilic quinines and quinoneimines. Alternative mechanisms of nucleophile detoxication exist, including oxidation by flavin-containing monooxygenases and oxidation to carboxylic acids, as is the case with ethanol.

Detoxication *of* Electrophiles—Generally, detoxication of electrophilic toxicants involves conjugation with the nucleophile, glutathione. This reaction may occur spontaneously or can be facilitated by glutathione S-transferases. Covalent binding of electrophiles to proteins can be regarded as detoxification, provided that the protein has no critical function and does not become a neoantigen or otherwise harmful.

Detoxication *of* Free Radicals—Detoxication and elimination of $O_2^{\bullet -}$ is important because it can be converted into much more reactive compounds (Figure 3–3) such as the hydroxyl radical ($HO^{\bullet}$), nitrogen dioxide ($^{\bullet}NO_2$), and the carbonate anion radical ($CO_3^{\bullet -}$). Superoxide dismutases (SODs), located in the cytosol (Cu, Zn-SOD) and the mitochondria (Mn-SOD), convert $O_2^{\bullet -}$ to hydrogen peroxide (HOOH) (Figure 3–4). Subsequently, HOOH is reduced to water by cytosolic glutathione peroxidase or peroxisomal catalase (Figure 3–4). No enzyme eliminates $HO^{\bullet}$ owing to its extremely short half-life (10^{-9} s). The only effective protection against $HO^{\bullet}$ is to prevent its formation by converting its precursor, HOOH, to water (Figure 3–4).

Peroxynitrite ($ONOO^-$), like HOOH, is an intermediate of $O_2^{\bullet -}$ toxication and is not a free radical oxidant itself. It is significantly more stable than $HO^{\bullet}$, and rapidly reacts with CO_2 to form the reactive free radicals, $^{\bullet}NO_2$ and $CO_3^{\bullet -}$ (Figure 3–3). Glutathione peroxidase can reduce $ONOO^-$ to nitrite (ONO^-), thereby preventing free radical production. In addition,

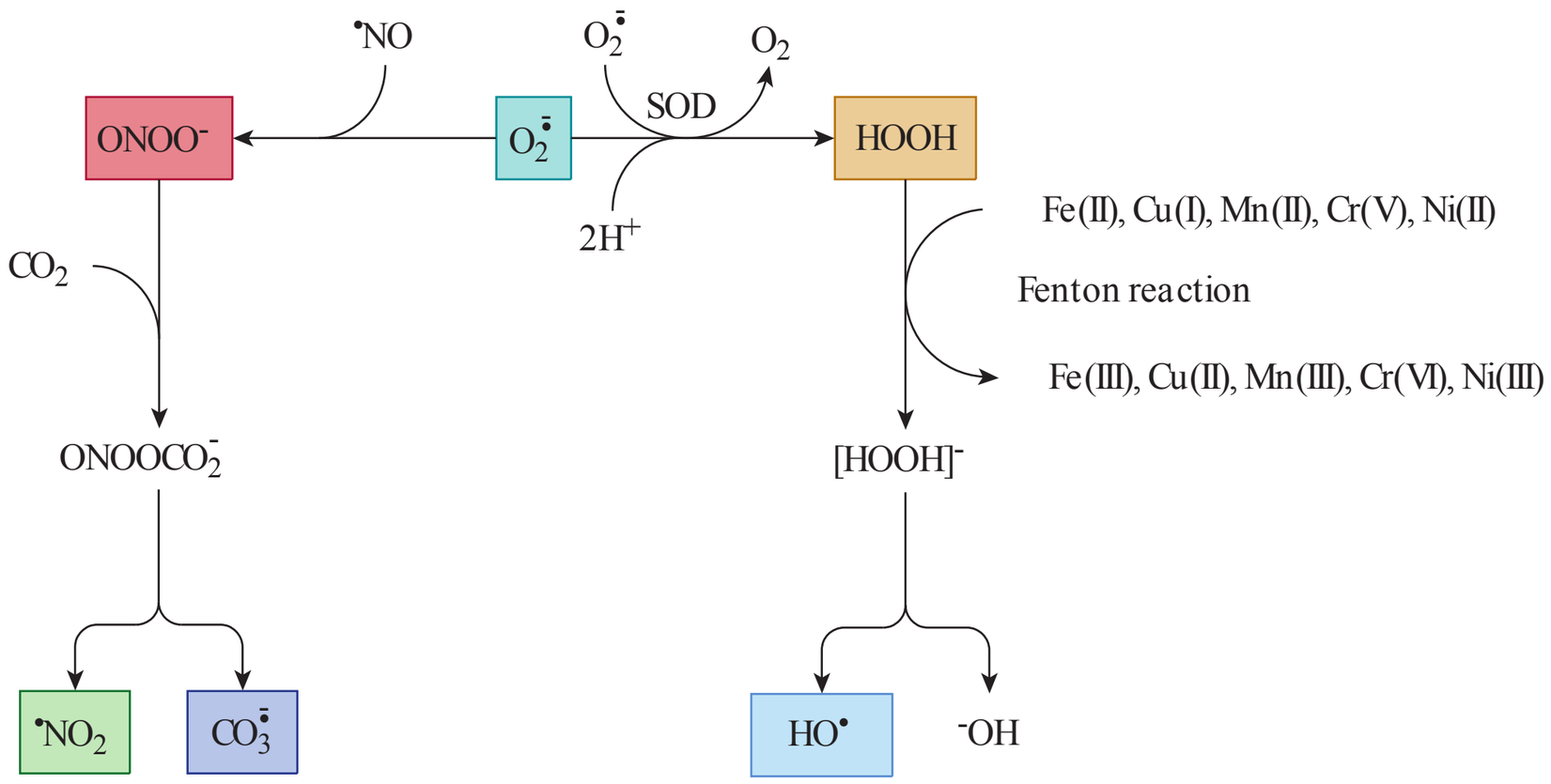

FIGURE 3–3 **Two pathways for toxication of superoxide anion radical ($O_2^{\bullet -}$) via nonradical products (ONOO– and HOOH) to radical products ($^{\bullet}NO_2$, $CO_3^{\bullet -}$, and $HO^{\bullet}$).** In one pathway, conversion of ($O_2^{\bullet -}$) to HOOH is spontaneous or is catalyzed by SOD. Homolytic cleavage of HOOH to hydroxyl radical and hydroxyl ion is called the Fenton reaction and is catalyzed by the transition metal ions shown. Hydroxyl radical formation is the ultimate toxication for xenobiotics that form $O_2^{\bullet -}$ or for HOOH, the transition metal ions listed, and some chemicals that form complexes with these transition metal ions. In the other pathway, $O_2^{\bullet -}$ reacts avidly with nitric oxide ($^{\bullet}NO$), the product of $^{\bullet}NO$ synthase (NOS), forming peroxynitrite ($ONOO^-$). Spontaneous reaction of $ONOO^-$ with carbon dioxide (CO_2) yields nitrosoperoxy carbonate ($ONOOCO_2^-$) that is homolytically cleaved to nitrogen dioxide ($^{\bullet}NO_2$) and carbonate anion radical ($CO_3^{\bullet -}$). All three radical products indicated in this figure are oxidants, whereas $^{\bullet}NO_2$ is also a nitrating agent.

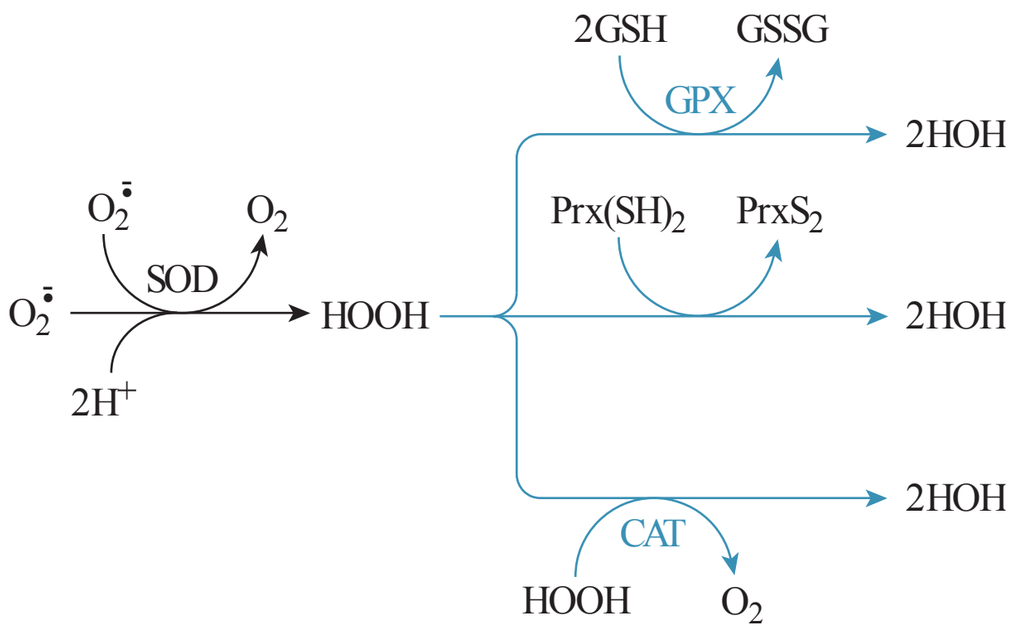

FIGURE 3–4 **Detoxication of superoxide anion radical ($O_2^{\bullet-}$) by superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT).**

$ONOO^-$ reacts with oxyhemoglobin, heme-containing peroxidases, and albumin, all of which could be important binding sites for $ONOO^-$. Furthermore, elimination of the two $ONOO^-$ precursors—that is, $^{\bullet}NO$ by reaction with oxyhemoglobin and $O_2^{\bullet-}$ by SODs—is a significant mechanism in preventing $ONOO^-$ buildup.

Peroxidase-generated free radicals are eliminated by electron transfer from glutathione. This results in the oxidation of glutathione, which is reversed by NADPH-dependent glutathione reductase (Figure 3–5). Thus, glutathione plays an important role in the detoxication of both electrophiles and free radicals.

Detoxication of Protein Toxins—Extra- and intracellular proteases are involved in the inactivation of toxic polypeptides. Several toxins found in venoms, such as α- and β-bungarotoxin, erabutoxin b, and phospholipase, contain intramolecular disulfide bonds that are required for their activity. These proteins become inactivated by the enzyme thioredoxin, which reduces the essential disulfide bond.

When Detoxication Fails—Detoxication may be insufficient for several reasons: (1) the toxicant overwhelms the detoxication processes, (2) a reactive toxicant inactivates a detoxicating enzyme, (3) the detoxication is reversed after transfer to other tissues, or (4) harmful by-products are produced by the detoxication process.

STEP 2—REACTION OF THE ULTIMATE TOXICANT WITH THE TARGET MOLECULE

Toxicity is typically mediated by a reaction of the ultimate toxicant with a target molecule (step 2a in Figure 3–1). Subsequently, a series of secondary biochemical events occur, leading to dysfunction or injury that is manifest at various levels of biological organization, such as at the target molecule itself, cell organelles, cells, tissues and organs, and even the whole organism.

Attributes of Target Molecules

Practically all endogenous compounds are potential targets for toxicants. The most prevalent and toxicologically relevant targets are nucleic acids (especially DNA), proteins, and membranes. The first target for reactive metabolites is often the enzyme responsible for their production or the adjacent intracellular structures. Not all targets for chemicals contribute harmful effects. Covalent binding to proteins without adverse consequences may even represent a form of detoxication by sparing toxicologically relevant targets. Thus, to conclusively identify a target molecule as being responsible for toxicity, it should be demonstrated that the ultimate toxicant (1) reacts with the target and adversely affects its function, (2) reaches an effective concentration at the target site, and (3) alters the target in a way that is mechanistically related to the observed toxicity.

Types of Reactions

The ultimate toxicant may bind to the target molecules noncovalently or covalently and may alter it by hydrogen abstraction, electron transfer, or enzymatically.

Noncovalent Binding—Hydrophobic interactions, hydrogen bonding, and ionic bonding are forms of noncovalent binding through which a toxicant can interact with targets such as membrane receptors, intracellular receptors, ion channels, and certain enzymes. Noncovalent binding usually is reversible because of the comparatively low bonding energy.

Covalent Binding—Being practically irreversible, covalent binding permanently alters endogenous molecules. Covalent adduct formation is common with electrophilic toxicants such

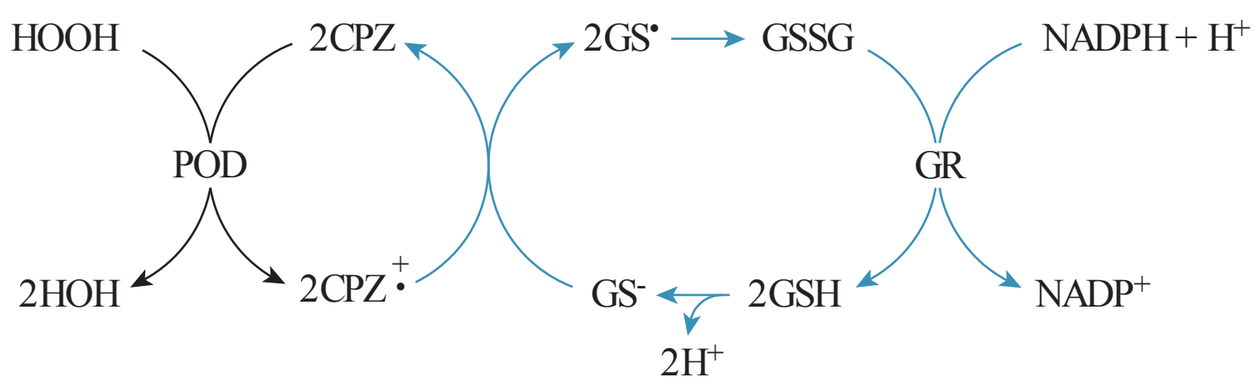

FIGURE 3–5 **Detoxication of peroxidase (POD)-generated free radicals such as chlorpromazine free radical ($CPZ^{\bullet+}$) by glutathione (GSH).** The by-products are glutathione thiyl radical ($GS^{\bullet}$) and glutathione disulfide (GSSG), from which GSH is regenerated by glutathione reductase (GR).

as nonionic and cationic electrophiles and radical cations. These toxicants react with nucleophilic atoms that are abundant in biological macromolecules, such as proteins and nucleic acids. Neutral free radicals such as $HO^{\bullet}$, $^{\bullet}NO_2$, and $Cl_3C^{\bullet}$ also can bind covalently to biomolecules. Nucleophilic toxicants are, in principle, reactive toward electrophilic endogenous compounds. However, such reactions are infrequent due to the rarity of electrophilic biomolecules. Carbon monoxide, cyanide, hydrogen sulfide, and azide are examples of nucleophiles that form coordinate covalent bonds with iron in various heme proteins.

Hydrogen Abstraction—Neutral free radicals can readily abstract H atoms from endogenous compounds, subsequently converting those compounds into radicals. Radicals can also remove hydrogen from methylene groups (CH_2) of free amino acids or from amino acid residues in proteins and convert them to carbonyls (C= O), forming cross-links with DNA or other proteins.

Electron Transfer—Chemicals can exchange electrons to oxidize or reduce other molecules, leading to formation of harmful by-products. For example, chemicals can oxidize Fe(II) in hemoglobin to Fe(III), producing methemoglobinemia.

Enzymatic Reactions—A few toxins act enzymatically on specific target proteins. For example, diphtheria toxin blocks the function of elongation factor 2 in protein synthesis and cholera toxin activates a G protein through such a mechanism.

In summary, most ultimate toxicants act on endogenous molecules on the basis of their chemical reactivity. Those with more than one type of reactivity may react by different mechanisms with various target molecules.

Effects of Toxicants on Target Molecules

Dysfunction of Target Molecules—Some toxicants activate protein target molecules, mimicking endogenous ligands. More commonly, chemicals inhibit the function of target molecules by blocking neurotransmitter receptors or ion channels, inhibiting enzymes, and interfering with cytoskeleton dynamics.

Protein function is impaired when conformation or structure is altered by interaction with the toxicant. Many proteins possess critical moieties that are essential for catalytic activity or assembly to macromolecular complexes. Covalent and/or oxidative modification of these moieties by xenobiotics can cause aberrant signal transduction and/or impaired maintenance of the cell's energy and metabolic homeostasis. Toxicants may also interfere with the template function of DNA. The covalent binding of chemicals to DNA causes nucleotide mispairing during replication.

Destruction of Target Molecules—In addition to adduct formation, toxicants alter the primary structure of endogenous molecules by means of cross-linking and fragmentation. Cross-linking imposes both structural and functional constraints on the linked molecules.

Other target molecules are susceptible to spontaneous degradation after chemical attack. Free radicals such as $Cl_3COO^{\bullet}$ and $HO^{\bullet}$ can initiate peroxidative degradation of lipids by hydrogen abstraction from fatty acids. This is not only destroys lipids in cellular membranes but also generates endogenous toxicants, free radicals, and electrophiles, which can go on to harm adjacent molecules (e.g., membrane proteins) or more distant molecules (e.g., DNA). Several forms of DNA fragmentation can be caused by toxicants, including imidazole ring-opening on purines, imidazole ring-contraction on pyrimidines, single-strand breaks (SSBs), phosphodiester bond cleavage, and double-strand breaks (DSBs).

Neoantigen Formation—Covalent binding of xenobiotics or their metabolites to proteins may evoke an immune response (Chapter 12). Some chemicals (e.g., dinitrochlorobenzene, penicillin, and nickel) bind to proteins spontaneously. Others may obtain reactivity by autooxidation to quinones (e.g., urushiols, the allergens in poison ivy) or by enzymatic biotransformation.

Toxicity Not Initiated by Reaction with Target Molecules

Some xenobiotics alter the biological microenvironment (see step 2b in Figure 3–1), leading to a toxic response. Included here are (1) chemicals that alter H^+ ion concentrations in the aqueous biophase, (2) solvents and detergents that physicochemically alter the lipid phase of cell membranes and destroy transmembrane solute gradients, and (3) xenobiotics that cause harm merely by occupying a site or space.

STEP 3—CELLULAR DYSFUNCTION AND RESULTANT TOXICITIES

Reaction of toxicants with a target molecule may result in impaired cellular function as the third step in the development of toxicity (Figures 3–1). Each cell in a multicellular organism carries out defined programs, some of which determine whether cells undergo division, differentiation, or apoptosis. Other programs control the ongoing (momentary) activity of differentiated cells, determining whether they secrete more or less of a substance, whether they contract or relax, and whether they transport and metabolize nutrients at higher or lower rates. For regulation of these cellular programs, cells possess signaling networks that can be activated and inactivated by external signaling molecules.

As outlined in Figure 3–6, the nature of the primary cellular dysfunction caused by toxicants, but not necessarily the ultimate outcome, depends on the role of the target molecule affected. The reaction of a toxicant with targets serving external functions can influence the operation of other cells and integrated organ systems. However, if the target molecule is involved predominantly in the cell's internal maintenance, the resultant dysfunction can ultimately compromise survival of the cell.

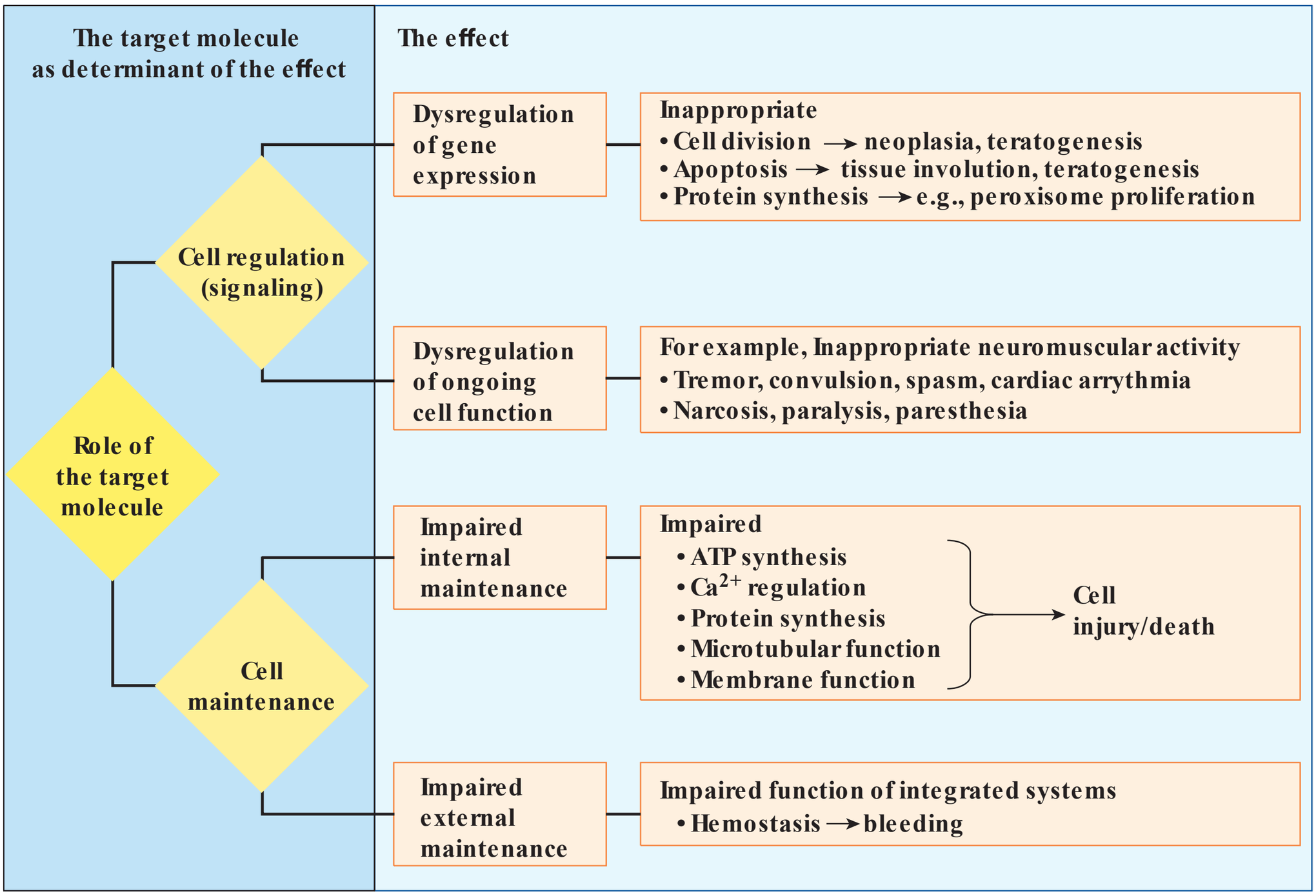

FIGURE 3–6 The third step in the development of toxicity: alteration of the regulatory or maintenance function of the cell.

Toxicant-induced Cellular Dysregulation

Cells are regulated by signaling molecules that activate specific cellular receptors linked to signal-transducing networks that transmit the signals to the regulatory regions of genes and/or functional proteins. Receptor activation may ultimately lead to altered gene expression and/or a chemical modification of specific proteins, typically by phosphorylation. Programs controlling the destiny of cells primarily affect gene expression, whereas those regulating the ongoing activities primarily influence the activity of functional proteins. However, one signal often evokes both responses because of branching and interconnection of signaling networks.

Dysregulation of Gene Expression—Gene expression is the process by which information from a gene is used to synthesize a functional gene product. The central dogma of molecular biology is that information from DNA is transcribed into messenger RNA (mRNA), which is then translated into a protein product. Genes that are transcribed into other types of RNA but not into proteins are called nonprotein-coding genes and they are one source of post-transcriptional control of protein synthesis. Among the alternative RNA types is the recently discovered small silencing RNA, called microRNA (miRNA), which can repress translation of mRNA into proteins. Dysregulation of gene expression may occur at elements that are directly responsible for transcription, at components of the intracellular signal-transduction pathway, and at the synthesis, storage, or release of the extracellular signaling molecules.

Dysregulation of Transcription—Transcription of genetic information from DNA to mRNA is controlled largely by interplay between transcription factors (TFs) and the regulatory or promoter region of genes. By binding to distinctive nucleotide sequences in the promoter or regulatory regions, TFs can facilitate or impede formation of the preinitiation complex, thereby either promoting or repressing transcription of the adjacent gene. Xenobiotics may interact with the promoter region of the gene, the TFs, or other components of the transcription initiation complex. However, altered activation of TFs appears to be the most common modality.

Several endogenous compounds, such as hormones, and vitamins, influence gene expression by binding to and activating TFs or intracellular receptors; xenobiotics may mimic these natural ligands. Either natural or xenobiotic ligands may cause toxicity when present at extreme doses or during critical periods of organism development. In addition to altering the fate of specific cells, compounds that act on ligand-activated TFs can also evoke changes in the metabolism of endogenous and foreign substances by inducing overexpression of relevant

enzymes. The effects of endobiotics and xenobiotics that act on TFs may also be mediated by transcriptional up- or down-regulation of protein-coding genes (i.e., genes transcribed into mRNA) and/or nonprotein-coding genes (i.e., genes transcribed into miRNA). Xenobiotics may also dysregulate transcription by altering the regulatory gene regions and the promoter methylation pattern.

Dysregulation *of* Signal Transduction—Extracellular signaling molecules, such as growth factors, cytokines, hormones, and neurotransmitters, can ultimately activate TFs by utilizing cell surface receptors and intracellular signal-transducing networks. Figure 3–7 depicts such networks and identifies some important signal-activated TFs that control transcriptional activity of genes that influence cell cycle progression and thus determine the fate of cells. An example is the c-Myc protein, which, on dimerizing with Max protein and binding to its cognate nucleotide sequence, transactivates cyclin D and E genes. The cyclins, in turn, accelerate the cell-division cycle by activating cyclin-dependent protein kinases, which are involved in regulating the cell cycle. Mitogenic signaling molecules thus induce cellular proliferation.

The signal from the cell surface receptors to the TFs is relayed by successive protein–protein interactions and protein phosphorylations, that is, a signal molecule phosphorylates another protein like mitogen-activated protein kinase (MAPK), which activates that protein to phosphorylate and activate another. For example, ligands induce growth factor receptors (item 4 in Figure 3–7) on the surface of all cells to self-phosphorylate, and these phosphorylated receptors then bind to adapter proteins through which they activate Ras. The active Ras initiates the MAPK cascade, involving serial phosphorylations of protein kinases, which finally reaches the TFs. These signal transducers are typically, but not always, activated by phosphorylation, which is catalyzed by protein kinases, and are usually inactivated by dephosphorylation, which is carried out by protein phosphatases.

Chemicals most often cause aberrant signal transduction by altering protein phosphorylation, and occasionally by interfering with the GTPase activity or signal termination activity of G proteins (e.g., Ras), disrupting normal protein–protein interactions, establishing abnormal ones, or by altering the synthesis or degradation of signaling proteins. Such interventions may ultimately influence cell cycle progression.

Chemically Altered Signal Transduction with Proliferative Effect: Xenobiotics that facilitate phosphorylation of signal transducers often promote mitosis and tumor formation. For example, the phorbol esters and fumonisin B activate protein kinase C (PKC) by mimicking diacylglycerol (DAG), one of the physiologic activators of PKC (item 6 in Figure 3–7). The other physiologic PKC activator, Ca^{2+}, is mimicked by Pb^{2+}. Activated PKC promotes mitogenic signaling by starting a cascade that activates other kinases and allows certain TFs to bind to DNA. Protein kinases may also be activated by interacting with proteins that have been altered by a xenobiotic.

Aberrant phosphorylation of proteins may result from decreased dephosphorylation by phosphatases or by increased phosphorylation by kinases. Inhibition of phosphatases appears to be the underlying mechanism of the mitogenic effect of various chemicals, oxidative stress, and ultraviolet (UV) irradiation. Soluble protein phosphatase 2A (PP2A) in cells is likely responsible for reversing the growth factor–induced stimulation of MAPK, thereby controlling the extent and duration of MAPK activity. PP2A also removes an activating phosphate from a mitosis-triggering protein kinase. Several natural toxins are extremely potent inhibitors of PP2A, including the blue-green algae poison microcystin-LR and the dinoflagellate-derived okadaic acid.

Apart from phosphatases, there are also other inhibitory binding proteins that can keep signaling under control. For example, IκB binds to NF-κB, subsequently preventing its transfer into the nucleus and its function as a TF (Figure 3–7). Upon phosphorylation, IκB becomes degraded and NF-κB is set free. NF-κB is an important contributor to proliferative and prolife signaling, as well as the acute and chronic inflammatory response. IκB degradation which leads to NF-κB activation can also be induced by oxidative stress.

Chemically Altered Signal Transduction with Antiproliferative Effect: Downturning of increased proliferative signaling after cell injury may compromise replacement of injured cells (follow the path in Figure 3–7: inhibition of Raf → diminished degradation of IκB → diminished binding of NF-κB to DNA → diminished expression of c-Myc mRNA). Down-regulation of a normal mitogenic signal is a step away from survival and toward apoptosis.

Dysregulation *of* Extracellular Signal Production—Hormones of the anterior pituitary exert mitogenic effects on endocrine glands in the periphery by acting on cell surface receptors. Pituitary hormone production is under negative feedback control by hormones of the peripheral glands. Perturbation of this circuit adversely affects pituitary hormone secretion and, in turn, the peripheral glands. Decreased secretion of pituitary hormone produces apoptosis followed by involution of the peripheral target gland.

Dysregulation of Ongoing Cellular Activity—Toxicants can adversely affect ongoing cellular activity in specialized cells by disrupting any step in signal coupling.

Dysregulation *of* Electrically Excitable Cells—Many xenobiotics influence cellular activity in excitable cells, such as neurons, skeletal, cardiac, and smooth muscle cells. Release of neurotransmitters and muscle contraction are controlled by transmitters and modulators synthesized and released by adjacent neurons. Chemicals that interfere with these mechanisms are listed in Table 3–1.

Perturbation of ongoing cellular activity by chemicals may be due to an alteration in (1) the concentration of neurotransmitters, (2) receptor function, (3) intracellular signal transduction, or (4) the signal-terminating processes.

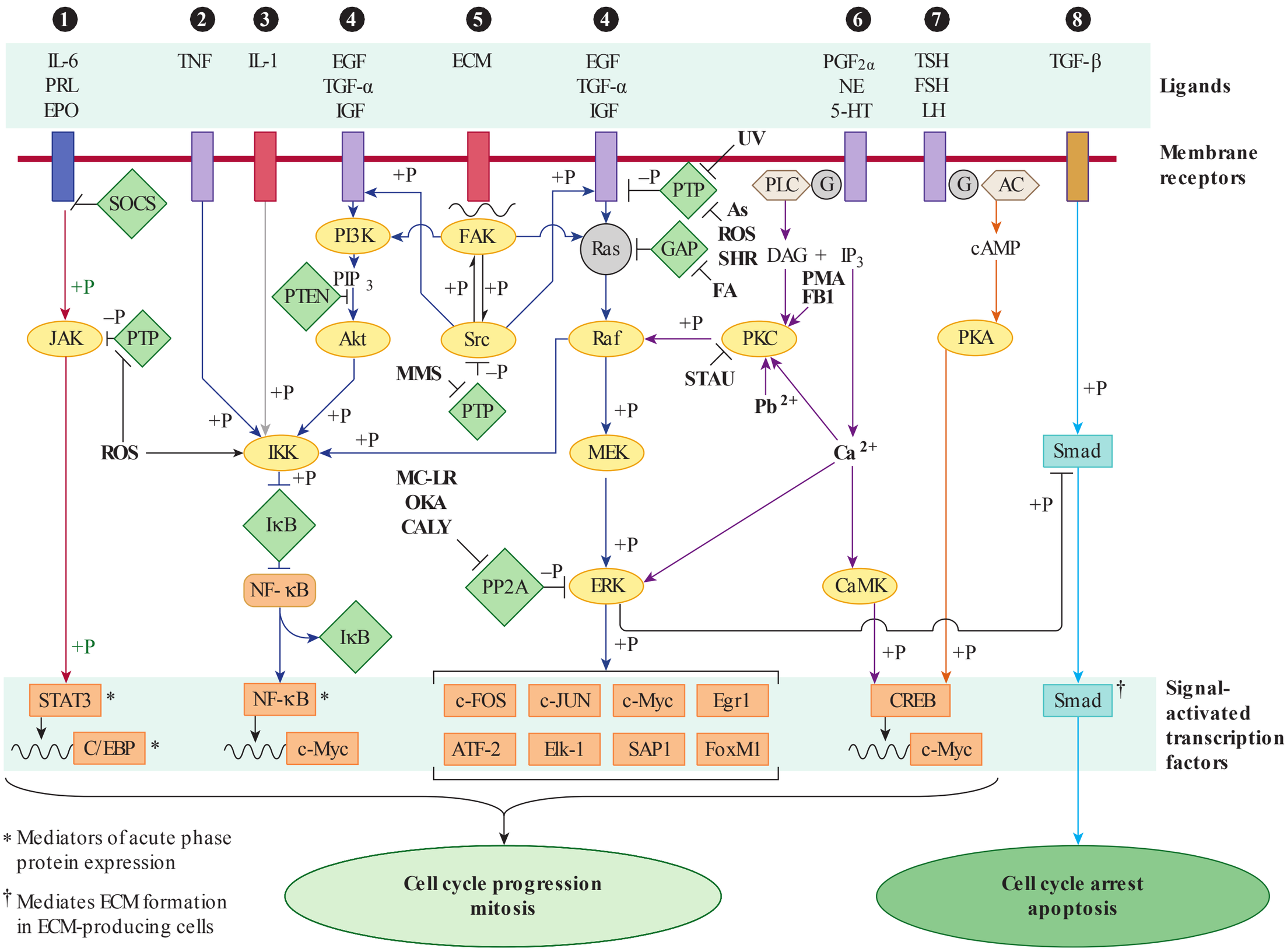

FIGURE 3–7 **Signal-transduction pathways from cell membrane receptors to signal-activated nuclear transcription factors that influence transcription of genes involved in cell-cycle regulation.** The symbols of cell membrane receptors are numbered 1 to 8 and some of their activating ligands are indicated. Circles represent G proteins, oval symbols protein kinases, rectangles transcription factors, wavy lines genes, and diamond symbols inhibitory proteins, such as protein phosphatases (PTP and PP2A) and the lipid phosphatase PTEN, the GTPase-activating protein GAP, and the inhibitory binding protein IκB. Arrowheads indicate stimulation or formation of second messengers (e.g., DAG, IP_3, PIP_3, cAMP, and Ca^{2+}), whereas blunt arrows indicate inhibition. Phosphorylation and dephosphorylation are indicated by + P and –P, respectively. Abbreviations for interfering chemicals are printed in black (As = arsenite; CALY = calyculin A; FA = fatty acids; FB1 = fumonisin B; MC-LR = microcystin-LR; OKA = okadaic acid; MMS = methylmethane sulfonate; PMA = phorbol miristate acetate; ROS = reactive oxygen species; SHR = SH-reactive chemicals, such as iodoacetamide; STAU = staurosporin).

In the center of the depicted networks is the pathway activated by growth factors, such as EGF, that acts on a tyrosine kinase receptor (#6), which uses adaptor proteins (Shc, Grb2, and SOS; not shown) to convert the inactive GDP-bound Ras to active GTP-bound form, which in turn activates the MAP-kinase phosphorylation cascade (Raf, MAPKK, and MAPK). The phosphorylated MAPK moves into the nucleus and phosphorylates transcription factors, thereby enabling them to bind to cognate sequences in the promoter regions of genes to facilitate transcription. There are numerous interconnections between the signal-transduction pathways. Some of these connections permit the use of the growth factor receptor (#6)–MAPK "highway" for other receptors (e.g., 4, 5, and 7) to send mitogenic signals. For example, receptor (#4) joins in via its G protein β/γ subunits and tyrosine kinase Src; the integrin receptor (#5), whose ligands are constituents of the extracellular matrix (ECM), possibly connects via G-protein Rho (not shown) and focal adhesion kinase (FAK); and the G-protein-coupled receptor (#7) via phospholipase C (PLC)-catalyzed formation of second messengers and activation of protein kinase C (PKC). The mitogenic stimulus relayed along the growth factor receptor (#6)–MAPK axis can be amplified by, e.g., the Raf-catalyzed phosphorylation of IκB, which unleashes NF-κB from this inhibitory protein, and by the MAPK-catalyzed inhibitory phosphorylation of Smad that blocks the cell-cycle arrest signal from the TGF-β receptor (#9). Activation of protein kinases (PKC, CaMK, and MAPK) by Ca^{2+} can also trigger mitogenic signaling. Several xenobiotics that are indicated in the figure may dysregulate the signaling network. Some may induce cell proliferation by either activating mitogenic protein kinases (e.g., PKC) or by inhibiting inactivating proteins, such as protein phosphatases (PTP and PP2A), GAP, or IκB. Others, e.g., inhibitors of PKC, oppose mitosis and facilitate apoptosis.

This scheme is oversimplified and tentative in several details. Virtually all components of the signaling network (e.g., G proteins, PKCs, and MAPKs) are present in multiple, functionally different forms whose distribution may be cell specific. The pathways depicted are not equally relevant for all cells. In addition, these pathways regulating gene expression not only determine the fate of cells, but also control certain aspects of the ongoing cellular activity.

TABLE 3–1 Agents acting on signaling systems for neurotransmitters and causing dysregulation of the momentary activity of electrically excitable cells such as neurons and muscle cells.*

Receptor/Channel/Pump		Agonist/Activator		Antagonist/Inhibitor	
Name	**Location**	**Agent**	**Effect**	**Agent**	**Effect**
1. Acetyl-choline nicotinic receptor	Skeletal muscle	Nicotine	Muscle fibrillation, and then paralysis	Tubocurarine, lophotoxin	Muscle paralysis
		Anatoxin-a		α-Bungarotoxin	
		Cytisine		α-Cobrotoxin	
		Ind: ChE inhibitors		α-Conotoxin	
				Erabutoxin b	
				Ind: botulinum toxin	
	Neurons	See above	Neuronal activation	Pb^{2+}, general anesthetics	Neuronal inhibition
2. Glutamate receptor	CNS neurons	N-Methyl-d-aspartate	Neuronal activation → convulsion, neuronal injury ("excitotoxicity")	Phencyclidine	Neuronal inhibition → anesthesia
		Kainate, domoate		Ketamine	Protection against "excitotoxicity"
		Quinolinate		General anesthetics	
		Quisqualate			
		Ind: hypoxia, HCN → glutamate release			
3. $GABA_A$ receptor	CNS neurons	Muscimol, Avermectins, Sedatives (barbiturates, benzodiazepines) General anesthetics (halothane) Alcohols (ethanol)	Neuronal inhibition → sedation, general anesthesia, coma, depression of vital centers	Bicuculline	Neuronal activation → tremor, convulsion
				Picrotoxin	
				Pentylenetetrazole	
				Cyclodiene insecticides	
				Lindane, TCAD	
				Ind: isoniazid	
4. Glycine receptor	CNS neurons, motor neurons	Avermectins (?)	Inhibition of motor neurons → paralysis	Strychnine	Disinhibition of motor neurons → tetanic convulsion
		General anesthetics		Ind: tetanus toxin	
5. Acetylcholine M_2 muscarinic receptor	Cardiac muscle	Ind: ChE inhibitors	Decreased heart rate and contractility	Belladonna alkaloids (e.g., atropine), atropine-like drugs (e.g., TCAD)	Increased heart rate
6. Opioid receptor	CNS neurons, visceral neurons	Morphine and congeners (e.g., heroin, meperidine)	Neuronal inhibition → analgesia, central respiratory depression, constipation, urine retention	Naloxone	Antidotal effects in opiate intoxication
		Ind: clonidine			
7. Voltage-gated Na^+ channel	Neurons, muscle cells, etc.	Aconitine, veratridine	Neuronal activation → convulsion	Tetrodotoxin, saxitoxin	Neuronal inhibition → paralysis, anesthesia
		Grayanotoxin		μ-Conotoxin	Anticonvulsive action
		Batrachotoxin		Local anesthetics	
		Scorpion toxins		Phenytoin	
		Ciguatoxin		Quinidine	
		DDT, pyrethroids			

(Continued)

TABLE 3–1 Agents acting on signaling systems for neurotransmitters and causing dysregulation of the momentary activity of electrically excitable cells such as neurons and muscle cells.* (Continued)

Receptor/Channel/Pump		Agonist/Activator		Antagonist/Inhibitor	
Name	**Location**	**Agent**	**Effect**	**Agent**	**Effect**
8. Voltage-gated Ca^{2+} channel	Neurons, muscle cell, etc.	Maitotoxin (?) Atrotoxin (?) Latrotoxin (?)	Neuronal/muscular activation, cell injury	ω-Conotoxin Pb^{2+}	Neuronal inhibition → paralysis
9. Voltage/Ca^{2+}-activated K^+ channel	Neurons, smooth and skeletal muscle, cardiac muscle	Pb^{2+}	Neuronal/muscular inhibition	Ba^{2+}, apamin (bee venom), dendrotoxin, 20-HETE, hERG inhibitors (e.g., cisapride, terfenadine)	Neuronal/muscular activation → convulsion/spasm vasoconstriction, PMV tachycardia (torsade de pointes)
10. Na^+,K^+-ATPase	Universal			Digitalis glycosides Oleandrin Chlordecone	Increased cardiac contractility, excitability Increased neuronal excitability → tremor
11. Acetylcholine M_3 muscarinic receptor	Smooth muscle, glands	Ind: ChE inhibitors	Smooth muscle spasm Salivation, lacrimation	Belladonna alkaloids (e.g., atropine) Atropine-like drugs (e.g., TCAD)	Smooth muscle relaxation → intestinal paralysis, decreased salivation, decreased perspiration
Acetylcholine M_1 muscarinic receptor	CNS neurons	Oxotremorine Ind: ChE inhibitors	Neuronal activation → convulsion	See above	
12. Adrenergic α_1 receptor	Vascular smooth muscle	(Nor)epinephrine Ind: cocaine, tyramine, amphetamine, TCAD	Vasoconstriction → ischemia, hypertension	Prazosin	Antidotal effects in intoxication with α_1-receptor agonists
13. 5-HT_2 receptor	Smooth muscle	Ergot alkaloids (ergotamine, ergonovine)	Vasoconstriction → ischemia, hypertension	Ketanserine	Antidotal effects in ergot intoxication
14. Adrenergic β_1 receptor	Cardiac muscle	(Nor)epinephrine Ind: cocaine, tyramine, amphetamine, TCAD	Increased cardiac contractility and excitability	Atenolol, metoprolol	Antidotal effects in intoxication with β_1-receptor agonists

*Numbering of the signaling elements in this table corresponds to the numbering of their symbols in Figure 3–7. This tabulation is simplified and incomplete. Virtually all receptors and channels listed occur in multiple forms with different sensitivity to the agents. The reader should consult the pertinent literature for more detailed information.
CNS, central nervous system; ChE, cholinesterase; Ind, indirectly acting (i.e., by altering neurotransmitter level); 20-HETE, 20-hydroxy-5,8,11,14-eicosatetraenoic acid; PMV, polymorphic ventricular; TCAD, tricyclic antidepressant.
The ? indicates there is some uncertainty regarding this action.

Alteration in Neurotransmitter Levels: Chemicals may alter synaptic levels of neurotransmitters by interfering with their synthesis, storage, release, or removal from the vicinity of the receptor. Most pharmaceuticals employ this strategy, including antidepressants, antiseizures, antipsychotics, etc.

Toxicant–Neurotransmitter Receptor Interactions: Some chemicals interact directly with neurotransmitter receptors, including (1) agonists that associate with the ligand-binding site on the receptor and mimic the natural ligand, (2) antagonists that occupy the ligand-binding site but cannot activate the receptor, (3) activators, and (4) inhibitors that bind to a site on the receptor that is not directly involved in ligand binding. In the absence of other actions, agonists and activators mimic, whereas antagonists and inhibitors block, the physiologic responses characteristic of endogenous ligands. Because there are multiple types of receptors for each neurotransmitter, these receptors may be affected differentially by toxicants.

Toxicant–Signal Transducer Interactions: Many chemicals alter neuronal and/or muscle activity by acting on signal-transduction processes. Voltage-gated Na^+ channels, which transduce and amplify excitatory signals generated by ligand-gated cation channels, are activated or inactivated by several toxins (see Table 3–1).

Toxicant–Signal Terminator Interactions: The cellular signal generated by cation influx is terminated by removal of the cations through channels or by transporters. Inhibition of cation export may prolong excitation.

Dysregulation *of* the Activity *of* Other Cells—Whereas many signaling mechanisms operate in nonexcitable cells, such as exocrine secretory cells, Kupffer cells, and pancreatic beta cells, disturbance of these processes is usually less consequential.

Toxic Alteration of Cellular Maintenance

Impairment of Internal Cellular Maintenance: Mechanisms of Toxic Cell Death—For survival, all cells must synthesize endogenous molecules; assemble macromolecular complexes, membranes, and cell organelles; maintain the intracellular environment; and produce energy for operation. Agents that disrupt these functions jeopardize survival and may cause toxic cell death. There are three critical biochemical disorders that chemicals inflicting cell death may initiate, namely, ATP depletion, sustained rise in intracellular Ca^{2+}, and overproduction of ROS and RNS.

Depletion of ATP—ATP plays a central role in cellular maintenance both as a chemical for biosynthesis and as the major source of energy. ATP is utilized in numerous biosynthetic reactions, and is incorporated into cofactors as well as nucleic acids. It is required for muscle contraction and polymerization of the cytoskeleton, fueling cellular motility, cell division, vesicular transport, and the maintenance of cell morphology. ATP drives ion transporters (e.g., Na^+,K^+-ATPase) that maintain conditions essential for various cell functions.

Chemical energy is released by hydrolysis of ATP to ADP or AMP. The ADP is rephosphorylated in the mitochondria by ATP synthase (Figure 3–8) via a process that couples oxidation of hydrogen to water and is termed oxidative phosphorylation.

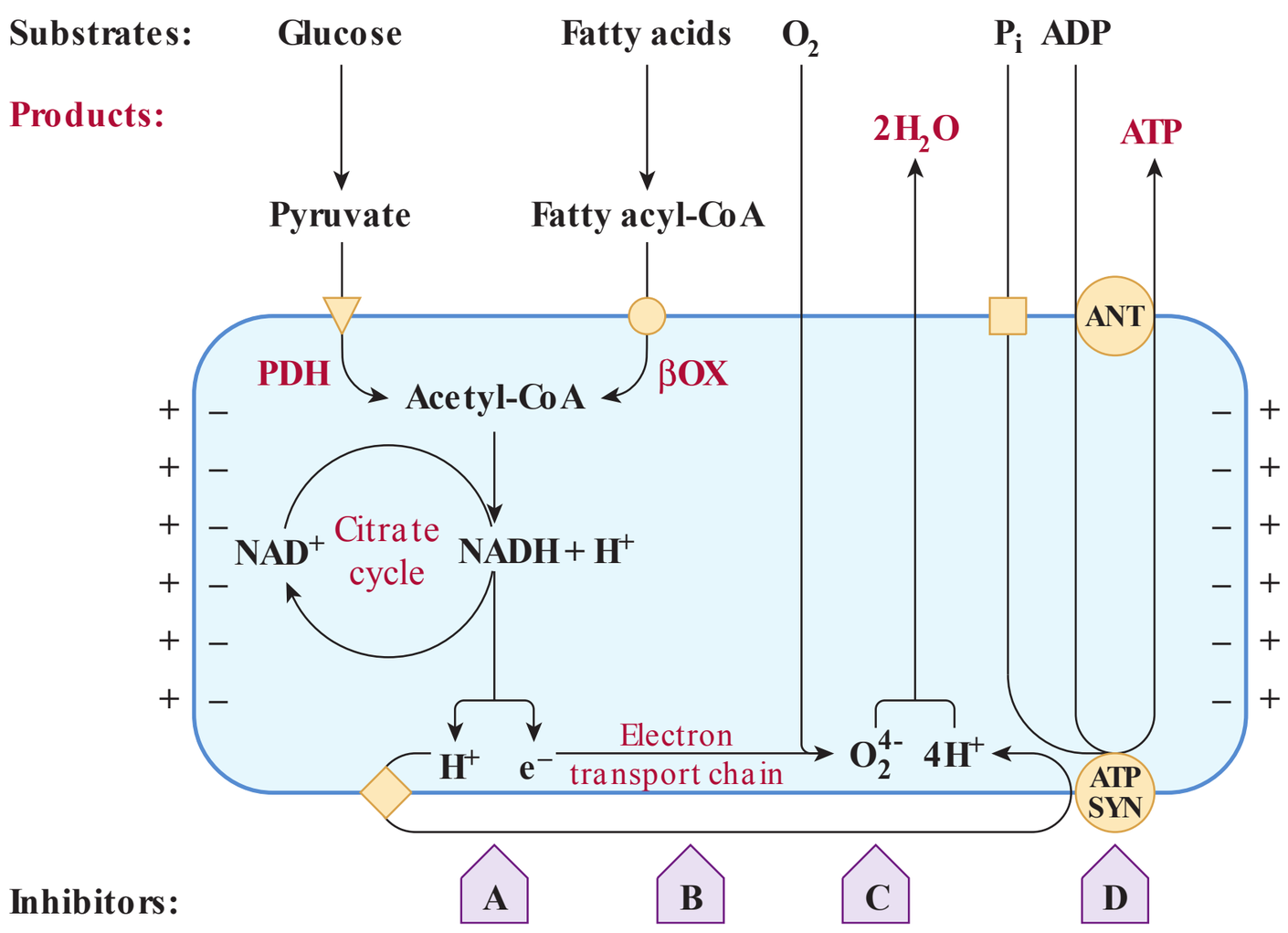

FIGURE 3–8 **ATP synthesis (oxidative phosphorylation) in mitochondria.** Arrows with letters A–D point to the ultimate sites of action of four categories of agents that interfere with oxidative phosphorylation (Table 3–2). For simplicity, this scheme does not indicate the outer mitochondrial membrane and that protons are extruded from the matrix space along the electron transport chain at three sites. βOX= bets-oxidation of fatty acids; e^- = electron; P_i = inorganic phosphate; ANT= adenine nucleotide translocator; ATP SYN= ATP synthase (F_0F_1ATPase).

Oxidative phosphorylation also requires several steps, each of which can be interfered with by toxins, as described in Table 3–2. Impairment of oxidative phosphorylation is detrimental to cells because failure of ADP rephosphorylation results in the accumulation of ADP and its breakdown products, as well as depletion of ATP.

Chemicals that impede oxidative phosphorylation are divided into five groups (Figure 3–8; Table 3–2). Substances in class A interfere with the delivery of hydrogen to the electron transport chain. Class B chemicals inhibit the transfer of electrons along the electron transport chain to oxygen. Class C agents interfere with oxygen delivery to the terminal electron transporter, cytochrome oxidase. Chemicals in class D inhibit oxidative phosphorylation by (1) direct inhibition of ATP synthase, (2) interference with ADP delivery, (3) interference with inorganic phosphate delivery, and (4) deprivation of ATP synthase from its driving force (i.e., the controlled influx of protons into the matrix space). Finally, chemicals causing mitochondrial DNA injury, thereby impairing synthesis of specific proteins encoded by the mitochondrial genome, are listed in group E.

Sustained Rise of Intracellular Ca^{2+}—Intracellular Ca^{2+} levels are highly regulated and maintained by the impermeability of the plasma membrane to Ca^{2+} and by transport

TABLE 3–2 Agents impairing mitochondrial ATP synthesis.*

A. Inhibitors of hydrogen delivery to the electron transport chain acting on/as

1. Glycolysis (critical in neurons): hypoglycemia; iodoacetate, koningic acid, and NO^+ at GAPDH
2. Gluconeogenesis (critical in renal tubular cells): coenzyme A depletors (see below)
3. Fatty acid oxidation (critical in cardiac muscle): hypoglycin, 4-pentenoic acid, 4-ene-valproic acid
4. Pyruvate dehydrogenase: arsenite, DCVC, p-benzoquinone
5. Citrate cycle
   (a) Aconitase: fluoroacetate, $ONOO^-$
   (b) Isocitrate dehydrogenase: DCVC
   (c) Succinate dehydrogenase: malonate, DCVC, PCBD-Cys, 2-bromohydroquinone, 3-nitropropionic acid, cis-crotonalide fungicides
6. Depletors of TPP (inhibit TPP-dependent PDH and α-KGDH): ethanol (when chronically consumed)
7. Depletors of coenzyme A (CoA)
   (a) Thiol-reactive electrophiles: 4-(dimethylamino)phenol, p-benzoquinone
   (b) Drugs enzymatically conjugated with CoA: salicylic acid (the metabolite of aspirin), valproic acid
8. Depletors of NADH
9. Alloxan, t-BHP, NAPBQI, fatty acid hydroperoxides, menadione
10. Activators of poly(ADP-ribose) polymerase: agents causing DNA damage (e.g., MNNG, hydrogen peroxide, $ONOO^-$)

B. Inhibitors of electron transport acting on/as

1. Inhibitors of electron transport complexes
   (a) NADH–coenzyme Q reductase (complex I): rotenone, amytal, MPP^+, paraquat
   (b) Coenzyme Q–cytochrome c reductase (complex III): antimycin-A, myxothiazole
   (c) Cytochrome oxidase (complex IV): cyanide, hydrogen sulfide, azide, formate, $^{\bullet}NO$, phosphine (PH_3)
   (d) Multisite inhibitors: dinitroaniline and diphenylether herbicides, $ONOO^-$
2. Electron acceptors: CCl_4, doxorubicin, menadione, MPP^+

C. Inhibitors of oxygen delivery to the electron transport chain

1. Chemicals causing respiratory paralysis: CNS depressants (e.g., opioids), convulsants
2. Chemicals impairing pulmonary gas exchange: CO_2, "deep pulmonary irritants" (e.g., NO_2, phosgene, perfluoroisobutene)
3. Chemicals inhibiting oxygenation of Hb: carbon monoxide, methemoglobin-forming chemicals
4. Chemicals causing ischemia: ergot alkaloids, cocaine

D. Inhibitors of ADP phosphorylation acting on/as

1. ATP synthase: oligomycin, cyhexatin, DDT, chlordecone
2. Adenine nucleotide translocator: atractyloside, DDT, free fatty acids, lysophospholipids
3. Phosphate transporter: N-ethylmaleimide, mersalyl, p-benzoquinone
4. Chemicals dissipating the mitochondrial membrane potential (uncouplers)
   (a) Cationophores: pentachlorophenol, dinitrophenol-, benzonitrile-, thiadiazole herbicides, salicylate, CCCP, cationic amphiphilic drugs (bupivacaine, perhexiline), valinomycin, gramicidin, calcimycin (A23187)
   (b) Chemicals permeabilizing the mitochondrial inner membrane: PCBD-Cys, chlordecone
5. Multisite inhibitor drugs: phenformin, propofol, salicylic acid (when overdosed)

E. Chemicals causing mitochondrial DNA damage and/or impaired transcription of key mitochondrial proteins

1. Antiviral drugs: zidovudine, zalcitabine, didanosine, fialuridine
2. Antibiotics: chloramphenicol (when overdosed), linezolid
3. Ethanol (when chronically consumed)

*The ultimate sites of action of these agents are indicated in Figure 3–8.
CCCP, carbonyl cyanide m-chlorophenylhydrazone; DCVC, dichlorovinyl-cysteine; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; α-KGDH, α-ketoglutarate dehydrogenase; MNNG, N-methyl-N′-nitro-N-nitrosoguanidine; MPP^+, 1-methyl-4-phenylpyridinium; PCBD-Cys, pentachlorobutadienylcysteine; PDH, pyruvate dehydrogenase; TPP, thyamine pyrophosphate.

TABLE 3–3 Agents causing sustained elevation of cytosolic Ca^{2+}.

A. Chemicals inducing Ca^{2+} influx into the cytoplasm

I. Via ligand-gated channels in neurons
   1. Glutamate receptor agonists ("excitotoxins"): glutamate, kainate, domoate
   2. TRPV1 receptor (capsaicin receptor) agonists: capsaicin, resiniferatoxin
   3. TRPA1 receptor agonists: SH-reactive electrophiles, such as lacrimators (e.g., chlorobenzalmalonitrile), acrolein, methyl isocyanate, phosgene, chloropicrin

II. Via voltage-gated channels: maitotoxin (?), $HO^{\bullet}$

III. Via "newly formed pores": maitotoxin, amphotericin B, chlordecone, methylmercury, alkyltins

IV. Across disrupted cell membrane
   1. Detergents: exogenous detergents, lysophospholipids, free fatty acids
   2. Hydrolytic enzymes: phospholipases in snake venoms, endogenous phospholipase A_2
   3. Lipid peroxidants: carbon tetrachloride
   4. Cytoskeletal toxins (by inducing membrane blebbing): cytochalasins, phalloidin

V. From mitochondria
   1. Oxidants of intramitochondrial NADH: alloxan, t-BHP, NAPBQI, divicine, fatty acid hydroperoxides, menadione, MPP+
   2. Others: phenylarsine oxide, gliotoxin, $^{\bullet}NO$, $ONOO^-$

VI. From the endoplasmic reticulum
   1. IP_3 receptor activators: γ-HCH (lindane), IP_3 formed during "excitotoxicity"
   2. Ryanodine receptor activators: δ-HCH

B. Chemicals inhibiting Ca^{2+} export from the cytoplasm (inhibitors of Ca^{2+}-ATPase in cell membrane and/or endoplasmic reticulum)

I. Covalent binders: acetaminophen, bromobenzene, CCl_4, chloroform, DCE

II. Thiol oxidants: cystamine (mixed disulfide formation), diamide, t-BHP, $O_2^{\bullet -}$, and HOOH generators (e.g., menadione, diquat)

III. Others: vanadate, Cd^{2+}, thapsigargin (specific SERCA inhibitor)

IV. Chemicals impairing mitochondrial ATP synthesis (see Table 3–2)

DCE, 1,1-dichloroethylene; t-BHP, t-butyl hydroperoxide; HCH, hexachlorocyclohexane; MPP^+, 1-methyl-4-phenylpyridinium; NAPBQI, N-acetyl-p-benzoquinoneimine; SERCA, sarco/endoplasmic reticulum calcium ATPase.

mechanisms that remove Ca^{2+} from the cytoplasm. Ca^{2+} is actively pumped from the cytosol across the plasma membrane to the extracellular space, and is also sequestered from the cytosol into the endoplasmic reticulum and mitochondria.

Toxicants induce elevation of cytoplasmic Ca^{2+} levels by promoting Ca^{2+} influx into or inhibiting Ca^{2+} efflux from the cytoplasm (Table 3–3). Opening of the ligand- or voltage-gated Ca^{2+} channels or damage to the plasma membrane causes Ca^{2+} to move down its concentration gradient from extracellular fluid to the cytoplasm. Toxicants also may increase cytosolic Ca^{2+} inducing its leakage from the mitochondria or the endoplasmic reticulum. They also may diminish Ca^{2+} efflux through inhibition of Ca^{2+} transporters or depletion of their driving forces. Sustained elevation of intracellular Ca^{2+} is harmful because it can result in (1) depletion of energy reserves by inhibiting the ATPase used in oxidative phosphorylation, (2) dysfunction of microfilaments, (3) activation of hydrolytic enzymes, and (4) generation of ROS and RNS.

There are at least three mechanisms by which sustained elevations in intracellular Ca^{2+} levels influence the cellular energy balance. First, high cytoplasmic Ca^{2+} levels cause increased mitochondrial Ca^{2+} uptake by the Ca^{2+} "uniporter," which, like ATP synthase, utilizes the inside negative mitochondrial membrane potential as the driving force. Consequently, mitochondrial Ca^{2+} uptake dissipates the membrane potential and inhibits the synthesis of ATP. Moreover, agents that oxidize mitochondrial NADH activate a transporter that extrudes Ca^{2+} from the matrix space. The ensuing continuous Ca^{2+} uptake and export ("Ca^{2+} cycling") by the mitochondria further compromise oxidative phosphorylation.

Second, an uncontrolled rise in cytoplasmic Ca^{2+} causes cell injury by microfilamental dissociation. An increase of cytoplasmic Ca^{2+} causes dissociation of actin filaments from proteins that promote anchoring of the filament to the plasma membrane, predisposing the membrane to rupture.

Third, high Ca^{2+} levels may lead to activation of hydrolytic enzymes that degrade proteins, phospholipids, and nucleic acids. Many integral membrane proteins are targets for Ca^{2+}-activated neutral proteases, or calpains. Indiscriminate activation of phospholipases by Ca^{2+} causes membrane breakdown directly and by the generation of detergents. Activation of a Ca^{2+}–Mg^{2+}–dependent endonuclease causes fragmentation of the chromatin form of DNA.

Overproduction of ROS and RNS—A number of xenobiotics can directly generate ROS and RNS, such as the redox cyclers and transition metals (Figure 3–3). Overproduction of ROS and RNS can be secondary to intracellular hypercalcemia, as Ca^{2+} helps generate ROS and/or RNS by activating dehydrogenases in the citric acid cycle, leading to increased activity in the electron transport chain and increased formation of $O_2^{\bullet -}$ and HOOH, and by activating nitric oxide synthase, which leads to formation of $ONOO^-$.

Interplay between the Primary Metabolic Disorders Spells Cellular Disaster—The primary derailments in cellular biochemistry discussed above may interact and amplify each other in a number of ways:

1. Depletion of cellular ATP reserves deprives the endoplasmic and plasma membrane Ca^{2+} pumps of fuel, causing elevation of Ca^{2+} in the cytoplasm. With the influx of Ca^{2+} into the mitochondria, the mitochondrial membrane potential declines, hindering ATP synthase.
2. Intracellular hypercalcemia facilitates formation of ROS and RNS, which oxidatively inactivates the Ca^{2+} pump and exacerbates the hypercalcemia.
3. ROS and RNS can also drain the ATP reserves. $^{\bullet}NO$ is a reversible inhibitor of cytochrome oxidase, NO^+ (nitrosonium cation, a product of $^{\bullet}NO$) inactivates glyceraldehyde 3-phosphate dehydrogenase and impairs glycolysis, whereas $ONOO^-$ irreversibly inactivates several components of the electron transport chain, inhibiting cellular ATP synthesis.
4. Furthermore, $ONOO^-$ can induce DNA single-strand breaks, which activate poly(ADP-ribose) polymerase

(PARP). As part of the repair strategy, activated PARP transfers multiple ADP-ribose moieties from NAD^+ to nuclear proteins and PARP itself. Because consumption of NAD^+ severely compromises ATP synthesis (see Figure 3–8) and resynthesis of NAD^+ consumes ATP, a cellular energy deficit occurs as a major consequence of DNA damage by $ONOO^-$.

Mitochondrial Permeability Transition (MPT) and the Worst Outcome: Necrosis—Mitochondrial Ca^{2+} uptake, decreased mitochondrial membrane potential, generation of ROS and RNS, depletion of ATP, and consequences of the primary metabolic disorders (e.g., accumulation of inorganic phosphate, free fatty acids, and lysophosphatides) are all considered as causative factors of an abrupt increase in the mitochondrial inner-membrane permeability, termed MPT. This is believed to be caused by the opening of a proteinaceous pore that spans both mitochondrial membranes and is permeable to solutes of 1500 Da. This opening permits free influx into the matrix space of protons, causing rapid and complete dissipation of the membrane potential, cessation of ATP synthesis, and the osmotic influx of water causing mitochondrial swelling. Ca^{2+} accumulated in the matrix space effluxes through the pore, flooding the cytoplasm. Such mitochondria are not only incapable of synthesizing ATP, but also even waste the remaining sources because depolarization of the inner membrane forces the ATP synthase to operate in the reverse mode, as an ATPase, hydrolyzing ATP. Then glycolysis may become compromised by the insufficient ATP supply to the glycolytic enzymes that require ATP (hexokinase and phosphofructokinase). A complete bioenergetic catastrophe ensues in the cell if the metabolic disorders evoked by the toxicant (such as those listed in Tables 3–2 and 3–3) are so extensive that most or all mitochondria undergo MPT, causing depletion of cellular ATP, and culminating in cell lysis or necrosis (see Figure 3–9).

An Alternative Outcome of MPT: Apoptosis—Chemicals that adversely affect cellular energy metabolism, Ca^{2+} homeostasis, and redox state and ultimately cause necrosis may also induce apoptosis. While the necrotic cell swells and lyses, the apoptotic cell shrinks; its nuclear and cytoplasmic materials condense, and then it breaks into membrane-bound fragments (apoptotic bodies) that are phagocytosed.

In contrast to the random sequence of multiple metabolic defects that a cell suffers on its way to necrosis, the routes to apoptosis are ordered, involving cascade-like activation of catabolic processes that finally disassemble the cell. Many details of the apoptotic pathways are presented schematically in Figure 3–10. It appears that most, if not all, chemical-induced cell deaths will involve the mitochondria, and that MPT is a crucial event. Another related event is release into the cytoplasm of cytochrome c (cyt c), a small hemeprotein that normally resides in the mitochondrial intermembrane space attached to the surface of inner membrane.

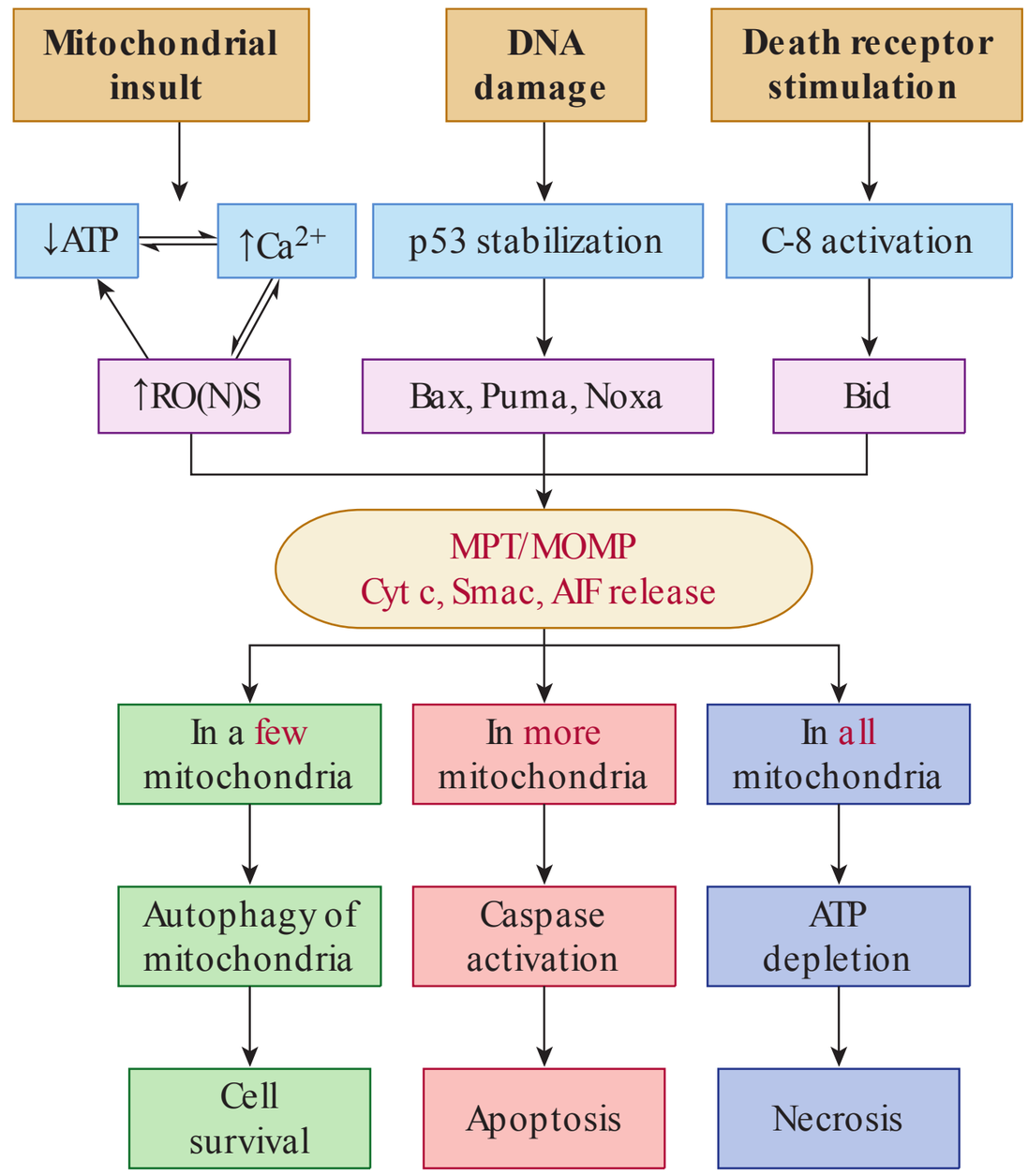

FIGURE 3–9 **"Decision plan" on the fate of injured cell.** See the text for details. MOMP = mitochondrial outer membrane permeabilization; MPT = mitochondrial permeability transition; Puma = p53-upregulated modulator of apoptosis; RO(N)S = reactive oxygen or nitrogen species.

As cyt c is the penultimate link in the mitochondrial electron transport chain, its loss will block ATP synthesis, increase formation of $O_2^{\bullet -}$, and potentially thrust the cell toward necrosis. Simultaneously, the unleashed cyt c represents a signal or an initial link in the chain of events directing the cell to the apoptotic path (Figure 3–10). On binding, together with ATP, to an adapter protein, cyt c can induce proteolytic cleavage of proteins called caspases or cysteine proteases that cleave cytoplasmic proteins into fragments, beginning apoptosis. Some caspases (e.g., 2, 8, and 9) activate procaspases. These signaling caspases carry the activation wave to the so-called effector caspases (e.g., 3, 6, and 7), which activate or inactivate specific cellular proteins. It is the hydrolysis of these specific proteins that accounts (directly or indirectly) for the morphological and biochemical alterations in apoptotic cells.

The decisive mitochondrial events of cell death are controlled by the Bcl-2 family of proteins, which includes members that facilitate (e.g., Bax, Bad, and Bid) and those that inhibit (e.g., Bcl-2 and Bcl-XL) these processes. Death-promoting members can oligomerize and form pores in the mitochondrial outer membrane. By doing so, these may facilitate release of cyt c and other intermembrane proapoptotic proteins. This release of cyt c can be caused by toxic insult of the mitochondria leading to the MPT or the mitochondrial outer membrane permeabilization (MOMP) induced by Bax

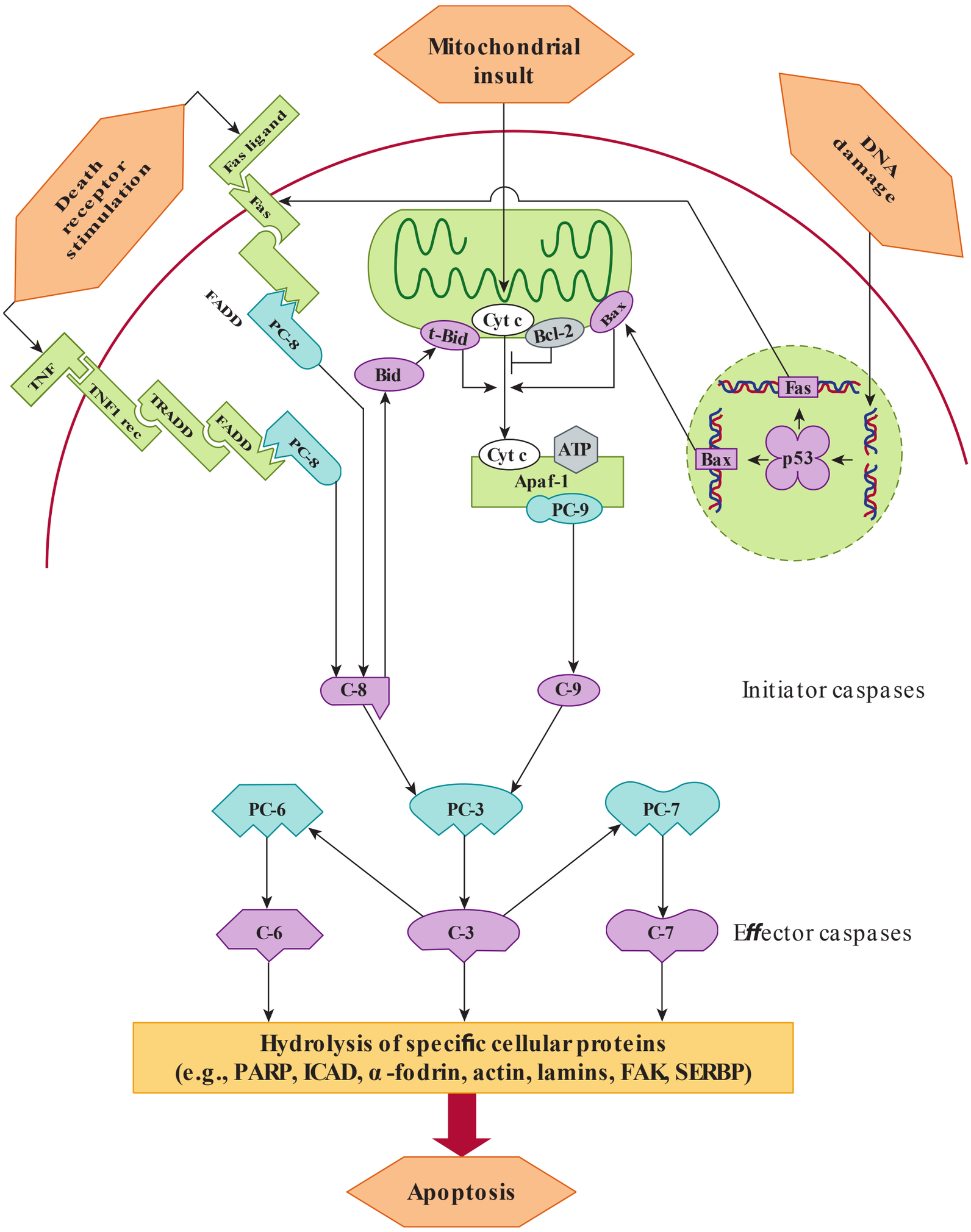

FIGURE 3–10 **Apoptotic pathways initiated by mitochondrial insult, nuclear DNA insult, and Fas or TNF receptor-1 stimulation.** The figure is a simplified scheme of three pathways to apoptosis. (1) Mitochondrial insult ultimately opens the permeability transition pore spanning both mitochondrial membranes and/or causes release of cytochrome c (cyt c) from the mitochondria. Cyt c release is facilitated by Bax or Bid proteins and opposed by Bcl-2 protein. (2) DNA insult, especially double-strand breaks, activates p53 protein, which increases the expression of Bax (that mediates cyt c release) and the membrane receptor protein Fas. (3) Fas ligand or tumor necrosis factor binds to and activates their respective receptor, Fas and TNF1 receptor. These ligand-bound receptors and the released cyt c interact with specific adapter proteins (i.e., FADD, RAIDD, and Apaf-1) through which they proteolytically activate procaspases (PC) to active caspases (C). The latter in turn cleave and activate other proteins (e.g., the precursor of Bid, P-Bid) and PC-3, a main effector procaspase. The active effector caspase-3 activates other effector procaspases (PC-6 and PC-7). Finally, C-3, C-6, and C-7 clip specific cellular proteins, whereby apoptosis occurs. These pathways are not equally relevant in all types of cells and other pathways, such as those employing TGF-β as an extracellular signaling molecule and ceramide as an intracellular signaling molecule. DFF = DNA fragmentation factor; FAK = focal adhesion kinase; PARP = poly(ADP-ribose) polymerase; SREBP = sterol regulatory element binding protein.

and its congeners. The death-suppressing family of proteins can dimerize with the death-inducing counterparts and neutralize them. Thus, the relative amount of these antagonistic proteins functions as a regulatory switch between cell survival and death.

The proapoptotic Bax and Bid proteins also represent links whereby death programs, initiated by DNA damage in the nucleus or by stimulation of death receptors at the cell surface (e.g., TNFR1 and FasR), can trigger the mitochondria into the apoptotic process (Figure 3–10). DNA damage induces

stabilization and activation of p53 protein, which increases expression of the proapoptotic Bcl-2 family of proteins. DNA damage is potentially mutagenic and carcinogenic and apoptosis of cells possessing DNA that is damaged beyond repair is an important self-defense against oncogenesis. Stimulation of death receptors leads to the activation of caspase 8, setting the caspase cascade into motion. Caspase-8 can activate Bid, another member of the Bcl-2 family (Figure 3–10). Thus, apoptosis can be executed via multiple pathways; the preferred route will depend on the initial insult as well as on the type and state of the cell.

What Determines the Form of Cell Death?—The mode of cell death (i.e. necrosis or apoptosis) is not trivial with respect to the fate of surrounding cells, a topic that will be discussed later. Toxicants tend to induce apoptosis at low exposure levels or early after exposure at high levels, whereas they cause necrosis later at high exposure levels. Recent research suggests a larger toxic insult causes necrosis rather than apoptosis because it incapacitates the cell such that it is unable to undergo apoptosis. Three causatively-related cellular events may lead to this incapacitation, namely increasing number of mitochondria undergoing MPT, depletion of ATP, and failed activation of caspases. When few mitochondria undergo MPT, they are removed by selective autophagy and the cell survives. This autophagic mechanism can become overwhelmed and lead to caspase activation as the degree of mitochondrial MPT increases. If all mitochondria undergo MPT, cell lysis occurs. ATP is crucial for executing the apoptotic program and depletion of ATP can prevent caspase activation. Finally, toxicants can directly act on caspases and impede the cell's apoptotic ability.

Induction of Death by Unknown Mechanisms—Mitochondrial integrity and intracellular Ca^{2+} homeostasis are not the only mechanisms by which toxicants induce cell death. Toxicants that affect plasma membranes, lysosomal membranes, cytoskeletal components, protein phosphatase inhibitor, protein synthesis, and cholesterol-lowering drugs (statins) also lead to cell death. The exact mechanism subsequent to target damage remains unknown, however it is likely that cell death is mediated by the modes described above.

Impairment of External Cellular Maintenance—Toxicants may also interfere with cells that are specialized to provide support to other cells, tissues, or the whole organism. Chemicals acting on the liver invoke this type of toxicity.

STEP 4—REPAIR OR DYSREPAIR

The fourth step in the development of toxicity is inappropriate repair (Figure 3–1). Many toxicants alter macromolecules, which, if not repaired, cause damage at higher levels of the biological hierarchy in the organism and influence the progression of toxicity.

Molecular Repair

Damaged molecules may be repaired in different ways. Some chemical alterations, such as oxidation of protein thiols and methylation of DNA, are simply reversed. Hydrolytic removal of the molecule's damaged unit or units and insertion of a newly synthesized unit or units of en occur with chemically altered DNA and peroxidized lipids. In some instances, the damaged molecule is totally degraded and resynthesized.

Repair of Proteins—Thiol groups are essential for the function of numerous proteins. Oxidation of protein thiols can be reversed by enzymatic reduction that is catalyzed by thioredoxin and glutaredoxin. Once oxidized, the catalytic thiol groups in these proteins are recycled by reduction with NADPH.

Soluble intracellular proteins are typically folded into a globular form with their hydrophobic amino acid residues hidden inside, and their hydrophilic residues located externally. Physical or chemical insults may lead to unfolding of the protein (denaturation) or its aggregation. Molecular chaperones, such as the heat-shock proteins, can prevent unfolding by "clamping down" onto the exposed hydrophobic region and utilizing ATP hydrolysis to change that protein's conformation. Proteins denatured beyond repair are ubiquinated multiple times, which targets that protein for degradation in the proteasome. However, oligomerization and aggregation of damaged and unfolded proteins preclude the proteasome from degrading them, and some can even trap the proteasomes and render them nonfunctional.

Repair of Lipids—Peroxidized lipids are repaired by a complex process involving a series of reductants, glutathione peroxidase, and glutathione reductase. NADPH is needed to recycle the reductants that are oxidized in the process.

Repair of DNA—Despite its high reactivity with electrophiles and free radicals, nuclear DNA is remarkably stable, in part because it is packaged in a condensed form, called chromatin, and because several repair mechanisms are available to correct alterations. Mitochondrial DNA, however, is not condensed and lacks efficient repair mechanisms; therefore, mitochondrial DNA is more prone to damage. Different types of damages are corrected by specialized mechanisms, each employing a different set of proteins.

Direct Repair—Certain covalent DNA modifications are directly reversed by enzymes such as DNA photolyase, which cleaves adjacent pyrimidines dimerized by UV light. This chromophore-equipped enzyme functions only in light-exposed cells. Minor adducts, such as methyl groups, that are attached to the O^6 position of guanine may be removed by specialized enzymes, such as O^6-alkylguanine-DNA-alkyltransferase.

Excision Repair—Base excision and nucleotide excision are two mechanisms for removing damaged bases from DNA (Chapters 8 and 9). Lesions that do not cause major distortion

of the helix are removed by base excision, in which the altered base is recognized by a relatively substrate-specific DNA glycosylase that hydrolyzes the N-glycosidic bond, releasing the modified base and creating an apurinic or apyrimidinic (AP) site in the DNA. The AP site is recognized by the AP endonuclease, which hydrolyzes the phosphodiester bond adjacent to the abasic site. After its removal, the abasic sugar is replaced with the correct nucleotide by a DNA polymerase and is sealed in place by a DNA ligase.

Bulky lesions are removed by nucleotide-excision repair. An ATP-dependent nuclease recognizes the distorted double helix and excises a number of intact nucleotides on both sides of the lesion. The excised section of the strand is restored by insertion of nucleotides into the gap by DNA polymerase, using the complementary strand as a template. DNA ligase then forms a continuous strand.

PARP appears to be an important contributor in excision repair. On base damage or single-strand break, PARP binds to the injured DNA and becomes activated. The active PARP cleaves NAD^+ to use the ADP-ribose moiety of this cofactor for attaching long chains of polymeric ADP-ribose to nuclear proteins. This causes the DNA to unwind, giving access to the repair enzymes and allowing the broken DNA to be fixed.

Nonhomologous End Joining—This process repairs DSBs that may be formed when two SSBs occur in close proximity, or when DNA with SSBs undergoes replication. This repair system directly ligates broken strands without the need for a homologous template (as is the case with nucleotide excision repair). Nonhomologous end joining (NHEJ) is more error prone than other types of DNA repair; however, it is unique in that it can operate in any phase of the cell cycle. It is also the mechanism of DSB repair in terminally differentiated cells such as neurons.

Recombinational (or Postreplication) Repair—Recombinational repair is a mechanism that fixes DSBs with higher fidelity than NHEJ because it requires a template from sister chromatids and, therefore, can function only after replication (in S and G2 phases). Recombinational repair can also fix postreplication gaps which may occur for a variety of reasons, such as when excision of a bulky adduct or intrastrand pyrimidine dimer fails to occur before DNA replication begins. This is a complex process in which the two sister chromatids ultimately exchange DNA and represents the "crossing over" that occurs during meiosis. Bifunctional electrophiles (e.g., nitrogen mustard-type drugs, and the cancer drug, cisplatin) produce interstrand cross-links that are fixed by a combination of excision and recombinational repair.

Cellular Repair: A Strategy in Peripheral Neurons

Autophagic removal of damaged cell organelles may be viewed as a universal mechanism of cellular repair, whereas clearance and regeneration of damaged axons is a mechanism specific for neurons.

Autophagy of Damaged Cell Organelles—Cells suffering mild injury may repair themselves by removing and degrading damaged components, such as organelles and protein aggregates, in a process called autophagy. This process is particularly important in terminally differentiated cells, such as neurons, cardiac myocytes, and skeletal myocytes, because renewal by cell replication is not possible.

In autophagy, an "isolation membrane" engulfs the cytoplasmic material and then encapsulates it in a double-membrane vesicle, called an autophagosome. This vesicle moves along microtubules, driven by dynein motors, to the lysosome where it fuses to form an autolysosome. Contents of the autolysosome are degraded into amino acids, lipids, nucleosides, and carbohydrates, which are then transported to the cytosol for further metabolism.

Regeneration of Damaged Axons—Peripheral neurons with axonal damage can regenerate their axons with the assistance of macrophages and Schwann cells. Macrophages remove debris by phagocytosis and produce cytokines and growth factors which activate Schwann cells to proliferate and transdifferentiate from myelinating mode into growth-support mode. Schwann cells play an indispensable role in promoting axonal regeneration by facilitating membrane construction, producing neurotrophic factors, and physically guiding the axon toward its target cell.

In the mammalian central nervous system, axonal regrowth is prevented by growth inhibitory glycoproteins and chondroitin sulfate proteoglycans produced by the oligodendrocytes and by the scar produced by astrocytes. Although damage to central neurons is irreversible, the large number of reserve nerve cells can partly compensate by taking over the functions of lost neurons.

Tissue Repair

In tissues with cells capable of multiplying, damage is repaired by apoptosis or necrosis of the injured cells and regeneration of the tissue by proliferation.

Apoptosis: An Active Deletion of Damaged Cells—Apoptosis initiated by cell injury can be regarded as tissue repair. A cell undergoing apoptosis shrinks as its nuclear and cytoplasmic materials condense, and then it breaks into membrane-bound fragments (apoptotic bodies) that are phagocytosed without inflammation. Also, apoptosis may intercept the process leading to neoplasia by eliminating the cells with potentially mutagenic DNA damage.

Apoptosis of damaged cells may serve as a tissue restoration process only for tissues that are made up of constantly renewing cells (e.g., the bone marrow, the respiratory and GI epithelium, and the epidermis of the skin), or of conditionally dividing cells (e.g., hepatic and renal parenchymal cells), because the apoptotic cells can be replaced. The value of apoptosis as a tissue repair strategy is markedly lessened in organs

containing nonreplicating and nonreplaceable cells, such as the neurons, cardiac muscle cells, and female germ cells.

Proliferation: Regeneration of Tissue—Tissues are composed of various cells and the extracellular matrix. Tissue elements are anchored to each other by transmembrane proteins. Cadherins allow adjacent cells to adhere to one other, whereas connexins connect neighboring cells internally by association of these proteins into gap junctions. Integrins link cells to the extracellular matrix. Therefore, repair of injured tissues involves both regeneration of lost cells and the extracellular matrix and reintegration of the newly formed elements into tissues and organs.

Replacement of Lost Cells by Mitosis—Soon after injury, cells adjacent to the damaged area enter the cell division cycle. Quiescent cells residing in G_0 enter G_1 and progress to mitosis (M).

Sequential changes in gene expression occur in the cells that are destined to divide. Early after injury, intracellular signaling turns on, and expression of numerous genes is increased. Among these so-called immediate-early genes are those that code for TFs that amplify the initial gene-activation process by stimulating other genes directly or through cell surface receptors and their coupled transducing networks. A few hours later, the so-called delayed-early genes are expressed whose products regulate the cell division cycle. Genes for the cell cycle accelerator proteins and also genes whose products decelerate the cell cycle become temporarily overexpressed, suggesting that this duality keeps tissue regeneration precisely regulated. Thus, genetic expression is reprogrammed so that DNA synthesis and mitosis gain priority over specialized cellular activities.

The regenerative process is probably initiated by the release of chemical mediators from damaged cells. Nonparenchymal cells, such as resident macrophages and endothelial cells, are receptive to these chemical signals and produce a host of signaling molecules that promote and propagate the regenerative process. The cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) promote transition of the quiescent cells into cell cycle and makes them receptive to growth factors ("priming"). Growth factors, especially the hepatocyte growth factor (HGF) and transforming growth factor-α (TGF-α), initiate the progression of the "primed" cells in the cycle toward mitosis.

Besides mitosis, cell migration also significantly contributes to restitution of certain tissues. In the mucosa of the GI tract, cells of the residual epithelium rapidly migrate to the site of injury as well as elongate and thin to reestablish the continuity of the surface even before this could be achieved by cell replication. Mucosal repair is dictated by growth factors and cytokines operative in tissue repair elsewhere and also by specific peptides associated with the mucous layer of the GI tract that become overexpressed at sites of mucosal injury.

Replacement of the Extracellular Matrix—The extracellular matrix is composed of proteins, glycosaminoglycans, and the glycoprotein and proteoglycans glycoconjugates. In the liver, these molecules are synthesized by stellate or fat-storing cells located in the space of Disse (between hepatic sinusoids and hepatocytes). Activation of resting stellate cells is mediated chiefly by two growth factors, platelet-derived growth factor (PDGF) and transforming growth factor-β (TGF-β), that may be released from platelets accumulating and degranulating at sites of injury and later from the activated stellate cells themselves. Proliferation of stellate cells is induced by the potent mitogen PDGF, whereas TGF-β acts on the stellate cells to stimulate the synthesis of extracellular matrix components, including collagens, fibronectin, tenascin, and proteoglycans. TGF-β also plays a central role in extracellular matrix formation in other tissues.

Side Reactions to Tissue Injury—In addition to mediators that aid in the replacement of lost cells and the extracellular matrix, resident macrophages and endothelial cells activated by cell injury also produce inflammation, altered production of acute-phase protein, and generalized reactions such as fever.

Inflammation

Cells and Mediators: Alteration of the microcirculation and accumulation of inflammatory cells are largely initiated by resident macrophages secreting cytokines such as TNF-α and IL-1 in response to tissue damage. These cytokines, in turn, stimulate neighboring stromal cells, such as the endothelial cells and fibroblasts, to release mediators that induce dilation of the local microvasculature and cause permeabilization of capillaries. Activated endothelial cells also facilitate the egress of circulating leukocytes into the injured tissue by releasing chemoattractants and expressing cell-adhesion molecules. Subsequently a stronger interaction (adhesion) is established between the endothelial cells and leukocytes with participation of intercellular adhesion molecules (e.g., ICAM-1) and leukocytes are able to enter the tissues by crossing the endothelial layer. This is facilitated by gradients of chemoattractants, including chemotactic cytokines, platelet-activating factor (PAF) and leukotriene B4, that induce expression of leukocyte integrins.

Inflammation Produces Reactive Oxygen and Nitrogen Species: Macrophages as well as leukocytes recruited to a site of injury undergo a respiratory burst, producing free radicals and activated enzymes. Membrane-bound NAD(P)H oxidase, activated in both macrophages and granulocytes, produces $O_2^{\bullet -}$ from molecular oxygen, which can give rise to $HO^{\bullet}$ (Figure 3–3).

Macrophages, but not granulocytes, generate another cytotoxic free radical, $^{\bullet}NO$, from arginine by nitric oxide synthase:

$$\text{l-Arginine} + O_2 \rightarrow \text{l-Citrulline} + {}^{\bullet}NO$$

Subsequently, $O_2^{\bullet -}$ and $^{\bullet}NO$, both of which are products of activated macrophages, can react with each other, yielding peroxynitrite anion; on reaction with carbon dioxide, this decays into two radicals, nitrogen dioxide and carbonate anion radical (Figure 3–3).

Granulocytes, but not macrophages, discharge the lysosomal enzyme myeloperoxidase into engulfed extracellular spaces, the phagocytic vacuoles. Myeloperoxidase catalyzes the formation

of hypochlorous acid (aka bleach, HOCl) from hydrogen peroxide and chloride ion:

$$HOOH + H^+ + Cl^- \rightarrow HOH + HOCl$$

Like HOOH, HOCl can form $HO^\bullet$ as a result of electron transfer from Fe^{2+} or from $O_2^{\bullet-}$ to HOCl:

$$HOCl + O_2^{\bullet-} \rightarrow O_2 + Cl^- + HO^\bullet$$

All the above reactive chemicals, as well as the discharged lysosomal proteases, are destructive products of inflammatory cells. Although these chemicals exert antimicrobial activity at the site of microbial invasion, they can damage adjacent healthy tissues at the site of toxic injury and contribute to propagation of tissue injury. In some cases, the chemical alone is harmless, while the immune response it invokes is the primary cause of injury.

Altered Protein Synthesis: Acute-phase Proteins—Cytokines released from macrophages and endothelial cells of injured tissues, IL-6, IL-1, and TNF, act on cell surface receptors to increase or decrease the transcriptional activity of genes encoding certain proteins, called positive and negative acute-phase proteins.

Positive acute-phase proteins may play roles in minimizing tissue injury and facilitating repair. For example, many of them inhibit lysosomal proteases released from the injured cells and recruited leukocytes.

Because negative acute-phase proteins, including albumin, several biotransformation enzymes, and membrane transporters, play important roles in the toxication and detoxication of xenobiotics, the disposition and toxicity of chemicals may be altered markedly during the acute phase of tissue injury.

Generalized Reactions—Cytokines released from activated macrophages and endothelial cells at the site of injury also may evoke neurohormonal responses. Thus, IL-1, TNF, and IL-6 alter the temperature set point of the hypothalamus, triggering fever. In addition, IL-1 and IL-6 act on the pituitary to induce the release of ACTH, which in turn stimulates the secretion of cortisol from the adrenals. This represents a negative feedback loop because corticosteroids inhibit cytokine gene expression.

Mechanisms of Adaptation

Adaptation may be defined as a harm-induced capability of the organism for increased tolerance to the harm itself. It involves responses acting to preserve or regain the biological homeostasis in the face of increased harm from a noxious stimulus. Adaptation of toxicity may result from biological changes causing (1) diminished delivery of the toxicant to the target, (2) decreased size or susceptibility of the target, (3) increased capacity of the organism to repair itself, and (4) strengthened mechanisms to compensate the toxicant-inflicted dysfunction. Adaptation involves sensing the noxious chemical and/or the initial damage or dysfunction, and a response that typically occurs through altered gene expression. For example, Figure 3–11 illustrates many genes that code for (1) enzymes that detoxify xenobiotics, (2) enzymes that eliminate ROS, (3) proteins that detoxify heme, (4) enzymes involved in glutathione homeostasis, and (5) transporters that pump xenobiotics and their metabolites out of cells.

When Repair and Adaptation Fail

Although operating at molecular, cellular, and tissue levels, repair mechanisms often fail to provide protection against injury. DNA repair mechanisms do not have absolute fidelity, meaning that some lesions may be overlooked or erroneously fixed. Repair fails most typically when the damage overwhelms the repair mechanisms as when necessary enzymes or cofactors are consumed. Sometimes the toxicant-induced injury adversely affects the repair process itself. Finally, some types of toxic injuries cannot be repaired effectively, as occurs when xenobiotics are covalently bound to proteins.

It is also possible that repair contributes to toxicity, as when excessive amounts of NAD^+ are cleaved by PARP when this enzyme assists in repairing broken DNA strands, or when too much NAD(P)H is consumed for the repair of oxidized proteins and endogenous reductants. Either event can compromise oxidative phosphorylation, which is also dependent on the supply of reduced cofactors (see Figure 3–8), thus causing or aggravating ATP depletion that contributes to cell injury. However, repair also may play an active role in toxicity. This is observed after chronic tissue injury, when the repair process goes astray and leads to uncontrolled proliferation instead of tissue remodeling. Such proliferation of cells may yield neoplasia, whereas overproduction of extracellular matrix results in fibrosis.

Adaptation—Although adaptation mechanisms boost the capacity of the organism to withstand toxicant exposure and damage, excessive exposure can overwhelm these protective responses. Toxicants may impair the adaptive process itself, while some adaptive mechanisms may be harmful under extreme conditions. For example, chronic inflammation, tissue injury, or cancer may lead to iron deficiency and anemia because IL-6 reduces iron absorption from the GI tract.

Toxicity Resulting from Inappropriate Repair and Adaptation

Like repair, dysrepair occurs at the molecular, cellular, and tissue levels. Some toxicities involve dysrepair at an isolated level, such as a specific enzyme or process, or at different levels, such as tissue necrosis, fibrosis, and chemical carcinogenesis.

Tissue Necrosis—Several mechanisms that can lead to cell death may involve molecular damage that is potentially reversible by repair mechanisms. Cell injury progresses toward cell necrosis if molecular repair mechanisms are inefficient or the molecular damage is not readily reversible.

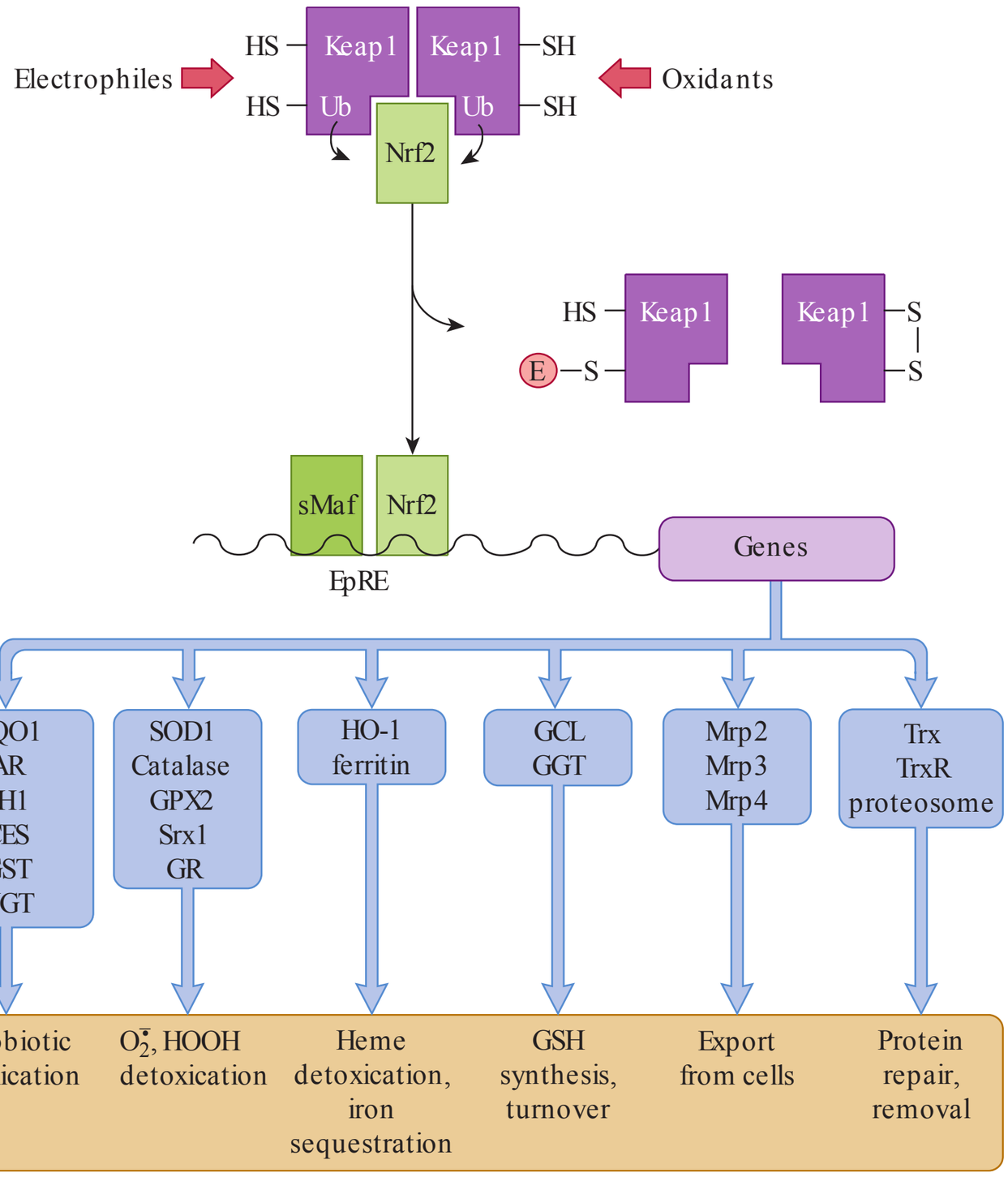

FIGURE 3–11 **Signaling by Keap1/Nrf2 mediates the electrophile stress response.** Normally NF-E2-related factor 2 (Nrf2) is kept inactive and at a low intracellular level by interacting with Keap1 that promotes its proteasomal degradation by ubiquitination. Electrophiles covalently bind to, whereas oxidants oxidize the reactive thiol groups of Keap1, causing Keap1 to release Nrf2. Alternatively, Nrf2 release may follow its phosphorylation by protein kinases. After being released from Keap1, the active Nrf2 accumulates in the cell, translocates into the nucleus, and forms a heterodimer with small Maf proteins to activate genes that contain electrophile response element (EpRE) in their promoter region. These include enzymes, binding proteins, and transporters functioning in detoxication and elimination of xenobiotics, ROS, and endogenous reactive chemicals, as well as some proteins that can repair or eliminate oxidized proteins. Induction of such proteins represents an electrophile stress response that provides protection against a wide range of toxicants. Nrf1, a transcription factor structurally related to Nrf2, also interacts with Keap1 and Maf proteins as well as EpRE and its role is partially overlapping with that of Nrf2. Abbreviations: AR, aldose reductase; CES carboxylesterase; EH1, microsomal epoxide hydrolase; GCL, glutamate–cysteine ligase; GGT, gamma-glutamyl transpeptidase; GPX2, glutathione peroxidase 2; GR, glutathione reductase; GST, glutathione S-transferase; HO-1, heme oxygenase 1; NQO1, NAD(P)H:quinone oxidoreductase; Mrp2, Mrp3, and Mrp4, multidrug resistance protein 2, 3, and 4; SOD1, superoxide dismutase 1; Srx1, sulfiredoxin 1; UGT, UDP-glucuronosyltransferase; Trx, thioredoxin; TrxR, thioredoxin reductase.

Progression of cell injury to tissue necrosis can be intercepted by two repair mechanisms working in concert: apoptosis and cell proliferation. Injured cells can initiate apoptosis, which counteracts the progression of the toxic injury by preventing necrosis of injured cells and the consequent inflammatory response.

Another important repair process that can halt the propagation of toxic injury is proliferation of cells adjacent to the injured cells. Initiated soon after cellular injury, this early cell division is thought to be instrumental in the rapid and complete restoration of the injured tissue and the prevention of necrosis. The sensitivity of a tissue to injury and the capacity of the tissue for repair are apparently two independent variables, both influencing whether tissue restitution ensues with survival or tissue necrosis occurs with death.

The efficiency of repair is also an important determinant of the dose–response relationship for toxicants that cause tissue necrosis. Tissue necrosis is caused by a certain dose of a toxicant not only because that dose ensures sufficient concentration of the ultimate toxicant at the target site to initiate injury, but also because that quantity of toxicant causes a degree of damage sufficient to compromise repair, allowing for progression of the injury. Tissue necrosis occurs because the injury overwhelms and disables the repair mechanisms, including (1) repair of damaged molecules, (2) elimination of damaged cells by apoptosis, and (3) replacement of lost cells by cell division.

Fibrosis—Fibrosis is a pathologic condition characterized by excessive deposition of an extracellular matrix of abnormal composition and is a specific manifestation of dysrepair of the

chronically injured tissue. As discussed above, cellular injury initiates a surge in cellular proliferation and extracellular matrix production, which normally ceases when the injured tissue is remodeled. If increased production of extracellular matrix is not halted, fibrosis develops.

TGF-β appears to be a major mediator of fibrogenesis. T e increased expression of TGF□β is a common response mediat□ ing regeneration of the extracellular matrix af er an acute injury. Normally, TGF□β production ceases when repair is complete. Failure to halt TGF□β overproduction, which leads to fibrosis, could be caused by continuous injury or a defect in the regulation of TGF□β.

T e fibrotic action of TGF□β is due to (1) stimulation of the synthesis of individual matrix components by specific target cells and (2) inhibition of matrix degradation. Interestingly, TGF-β induces transcription of its own gene in target cells, suggesting that the TGF□β produced by these cells can amplify in an autocrine manner the production of the extracellular matrix. T is positive feedback may facilitate fibrogenesis.

Fibrosis involves not only excessive accumulation of the extracellular matrix, but also changes in its composition. Basement membrane components, such as collagens and lam□ inin, increase disproportionately during fibrogenesis.

Carcinogenesis—Chemical carcinogenesis involves inap□ propriate function of various repair mechanisms, including (1) failure of DNA repair, (2) failure of apoptosis, and (3) failure to terminate cell proliferation.

Failure *of* DNA Repair: Mutation, the Initiating Event in Carcinogenesis—Chemical and physical insults may induce neoplastic transformation of cells by genotoxic and nongeno□ toxic mechanisms. Chemicals that react with DNA may cause damage such as adduct formation, oxidative alteration, and strand breakage. If these lesions are not repaired or injured cells are not eliminated, a lesion in the parental DNA strand may induce a heritable alteration, or mutation, in the daughter strand during replication. T e most unfortunate scenario for the organism occurs when the altered genes express mutant proteins that reprogram cells for multiplication. When such cells undergo mitosis, their descendants also have a similar pro□ pensity for proliferation. Moreover, because enhanced cell divi□ sion increases the likelihood of mutations, these cells eventually acquire additional mutations that may further increase their growth advantage over their normal counterparts. T e final outcome of this process is a tumor consisting of transformed, rapidly proliferating cells.

Mutation *of* Proto-oncogenes: Proto□oncogenes are highly con□ served genes encoding proteins that stimulate progression of cells through the cell cycle or oppose apoptosis. T e products of proto□oncogenes that accelerate the cell cycle include (1) growth factors; (2) growth factor receptors; (3) intracellular signal transducers such as G proteins, protein kinases, cyclins, and cyclin□dependent protein kinases; and (4) nuclear TFs. Transient increases in the production or activity of proto□ oncogene proteins are required for regulated growth, as during embryogenesis, tissue regeneration, and stimulation of cells by growth factors or hormones. In contrast, permanent activation and/or overexpression of these proteins favor neoplastic trans□ formation. One mechanism whereby genotoxic carcinogens induce neoplastic cell transformation is by producing an acti□ vating mutation of a proto□oncogene. T e altered gene (called an oncogene) encodes a permanently active protein that forces the cell into the division cycle.

An example of mutational activation of an oncogene protein is that of the Ras proteins. Ras proteins are localized on the inner surface of the plasma membrane and function as crucial mediators in responses initiated by growth factors (see Figure 3–7). Ras serves as a molecular switch, being active in the GTP□bound form and inactive in the GDP□bound form. Some mutations of the Ras gene dramatically lower the GTPase activity of the protein, which, in turn, locks Ras in the perma□ nently active GTP□bound form. Continual, rather than signal□ dependent, activation of Ras can lead eventually to uncontrolled proliferation and transformation.

Mutation *of* Tumor Suppressor Genes: Tumor□suppressor genes ncode proteins that inhibit the progression of cells in the division ycle, or promote DNA repair or apoptosis upon irreparable DNA damage. Some examples include cyclin□dependent protein kinase nhibitors, TFs that transactivate genes encoding cyclin□ dependent protein kinase inhibitors, and proteins that block TFs involved in NA synthesis and cell division (Figure 3–12).

T e p53 tumor□suppressor gene encodes a 53□kDa protein with multiple functions. Acting as a TF, the p53 protein trans□ activates genes whose products arrest the cell cycle, repair damaged DNA, or promote apoptosis. It also activates miRNA□ coding genes whose products repress translation of mitogenic TFs and cell cycle accelerator proteins, and the genes that encode cell cycle accelerators or antiapoptotic proteins. DNA damage activates protein kinases to phosphorylate and stabi□ lize the p53 protein, which causes it to accumulate and either induce cell cycle arrest or apoptosis. In addition to aberrations in critical protein□coding genes, damage in genes coding for miRNA may also contribute to carcinogenesis.

Epigenetic Mechanisms in Carcinogenesis: Inappropriate Activation or Responsiveness *of* the Regulatory Region *of* Critical Genes—Some chemicals cause cancer by reacting with DNA and inducing a mutation, whereas others that do not damage DNA yet still induce cancer af er prolonged exposure are desig□ nated nongenotoxic (or epigenetic) carcinogens. Five examples include (1) xenobiotic mitogens that promote proliferative signaling, (2) endogenous mitogens such as growth factors, (3) toxicants that cause sustained cell injury, (4) xenobiotics that are display differing carcinogenicity between species, and (5) ethi□ onine and diethanolamine, which interfere with formation of the endogenous methyl donor S□adenosyl□methionine (SAM).

Nongenotoxic chemicals eventually influence the expression of proto□oncogenes and/or tumor suppressor genes by

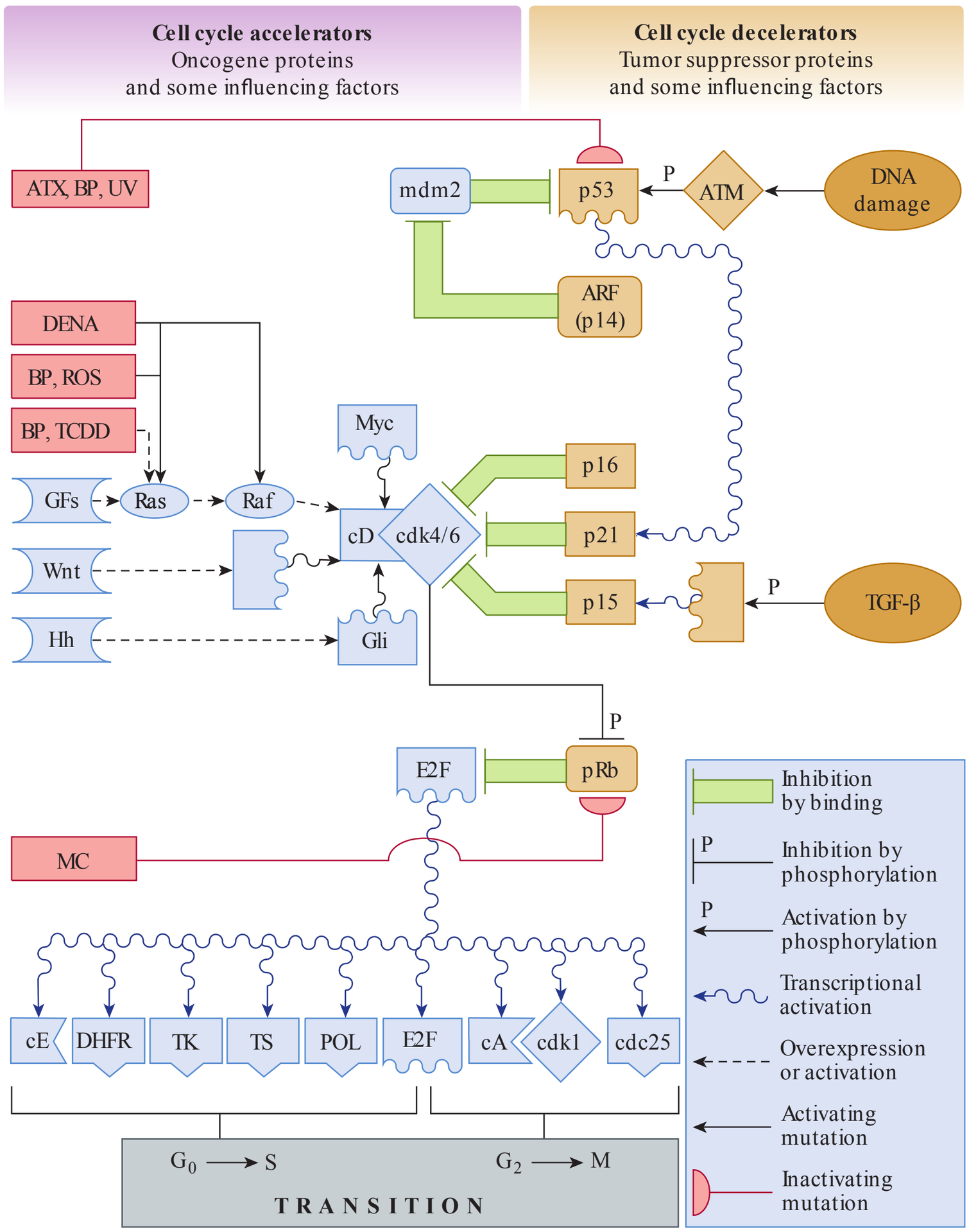

FIGURE 3–12 **Key regulatory proteins controlling the cell-division cycle with some signaling pathways and xenobiotics affecting them.** Proteins on the left, represented by blue symbols, accelerate the cell cycle and are oncogenic if permanently active or expressed at high level. In contrast, proteins on the right, represented by salmon symbols, decelerate or arrest the cell cycle and thus suppress oncogenesis, unless they are inactivated (e.g., by mutation).

Accumulation of cyclin D (cD) is a crucial event in initiating the cell division cycle. cD activates cyclin-dependent protein kinases 4 and 6 (cdk4/6), which in turn phosphorylate the retinoblastoma protein (pRb) causing dissociation of pRb from transcription factor E2F. Then the unleashed E2F is able to bind to and transactivate genes whose products are essential for DNA synthesis, such as dihydrofolate reductase (DHFR), thymidine kinase (TK), thymidylate synthetase (TS), and DNA polymerase (POL), or are regulatory proteins, such as cyclin E (cE), cyclin A (cA), and cyclin-dependent protein kinase 1 (cdk1), which promote further progression of the cell cycle. Expression of cD is increased, e.g., by growth factors signaling through Ras proteins and the MAPK pathway as well as by Wnt and Hedgehog (Hh) ligands that ultimately signal through B-cat and Gli transcription factors, respectively. Some carcinogens, e.g., benzpyrene (BP) and reactive oxygen species (ROS), and diethylnitrosamine (DENA) may cause mutation of the Ras or Raf gene that results in permanently active mutant Ras or Rab protein, but BP as well as TCDD may also induce simple overexpression of normal Ras protein.

Cell cycle progression is counteracted, e.g., by pRb (which inhibits the function of E2F), by cyclin-dependent protein kinase inhibitors (such as p15, p16, and p21), by p53 (which transactivates the p21 gene), and by ARF (also called p14 that binds to mdm2, thereby neutralizing the antagonistic effect of mdm2 on p53). Signals evoked by DNA damage and TGF-β will ultimately result in accumulation of p53 and p15 proteins, respectively, and deceleration of the cell cycle. In contrast, mutations that disable the tumor suppressor proteins facilitate cell cycle progression and neoplastic conversion and are common in human tumors. Aflatoxin B_1 (ATX), BP, and UV light cause such mutations of the p53 gene, whereas pRb mutations occur invariably in methylcholanthrene (MC)–induced transplacental lung tumors in mice.

increasing synthesis of normal proto-oncogene proteins and/or repressing normal tumor suppressor genes. This is in contrast to genotoxic chemicals, which induce the synthesis of permanently active mutant proto-oncogene proteins or permanently inactive mutant tumor suppressor proteins. Secondarily, nongenotoxic carcinogens may also increase mutation of critical genes, which is initiated by genotoxic agents or spontaneous DNA damage. Spontaneous DNA damage commonly occurs in normal human cells at a rate of 1 out of 10^8 to 10^{10} base pairs. Nongenotoxic carcinogens increase the frequency of spontaneous mutations through a mitogenic effect and by inhibiting apoptosis, thereby increasing the number of cells with DNA damage and mutations.

Failure *of* Apoptosis: Promotion *of* Mutation and Clonal Growth: Preneoplastic cells, or cells with mutations, have much higher apoptotic activity than do normal cells. Therefore, apoptosis counteracts clonal expansion of the initiated cells and tumor cells. Facilitation of apoptosis can induce tumor regression, whereas inhibition of apoptosis is detrimental because mutations and clonal expansion of preneoplastic cells are facilitated.

Failure to Terminate Proli*f*eration: Promotion *of* Mutation, Proto-oncogene Expression, and Clonal Growth: Transformation of normal cells with controlled proliferative activity to malignant cells with uncontrolled proliferative activity is driven by three major factors: (1) accumulation of genetic damage in the form of mutant proto-oncogenes and mutant tumor suppressor genes, (2) increased transcription and/or translation of normal proto-oncogenes, and (3) silencing of normal tumor suppressor genes at the transcriptional and/or translational level. Uncontrolled proliferation results from an imbalance between mitosis and apoptosis.

1. Enhanced mitotic activity increases the probability of mutations. With activation of the cell-division cycle, a substantial shortening of the G1 phase occurs, and less time is available for the repair of injured DNA before replication.
2. Enhanced mitotic activity may compromise DNA methylation, which occurs early in the postreplication period. DNA cytosine methyltransferases (DNMTs) copy the methylation pattern of the parental DNA strand to the daughter strand. Limitations of DNMTs by shortened G2 phase or by the presence of other transacting factors might impair methylation and contribute to overexpression of proto-oncogenes.
3. Cell-to-cell communication through gap junctions and intercellular adhesion through cadherins are temporarily disrupted during proliferation, which contributes to the invasiveness of tumor cells.
4. Proliferation also promotes carcinogenesis through clonal expansion of the initial cells to form nodules (foci) and tumors.

Nongenotoxic Carcinogens: Promoters *of* Mitosis and Inhibitors *of* Apoptosis: Many chemicals do not alter DNA or induce mutations yet induce cancer after chronic administration. Designated nongenotoxic or epigenetic carcinogens, these chemicals cause cancer by promoting carcinogenesis initiated by genotoxic agents or spontaneous DNA damage.

According to an emerging theory, cancers may form by genotoxic and/or epigenetic mechanisms in pluripotent stem cell populations. Such cells are characterized by quiescence, self-renewal, and conditional immortality, and thus would potentially supply a lifelong, latent neoplastic population after carcinogenic attack. Finally, further changes in gene expression may occur in these proliferating cells, making them capable of invading other tissues (metastasis).

CONCLUSIONS

Selective or altered toxicity may be due to different or altered (1) exposure; (2) delivery, thus resulting in a different concentration of the ultimate toxicant at the target site; (3) target molecules; (4) biochemical processes triggered by the reaction of the chemical with the target molecules; (5) repair at the molecular, cellular, or tissue level; or (6) mechanisms such as circulatory and thermoregulatory reflexes by which the affected organism can adapt to some of the toxic effects. Although a simplified scheme outlines the development of toxicity (Figure 3–1), the route to toxicity can be considerably more diverse and complicated. An organism has mechanisms that (1) counteract the delivery of toxicants, such as detoxication; (2) reverse the toxic injury, such as repair mechanisms; and (3) offset some dysfunctions, such as adaptive responses. Thus, toxicity is not an inevitable consequence of toxicant exposure because it may be prevented, reversed, or compensated for by such mechanisms. Toxicity develops if the toxicant exhausts or impairs the protective mechanisms and/or overrides the adaptability of biological systems.

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QUESTIONS

1. The severity of a toxin depends, in large part, on the concentration of the toxin at its site of action. Which of the following will decrease the amount of toxin reaching its site of action?
   a. absorption across the skin.
   b. excretion via the kidneys.
   c. toxication.
   d. reabsorption across the intestinal mucosa.
   e. discontinuous endothelial cells of hepatic sinusoids.

2. Toxication (or metabolic activation) is the biotransformation of a toxin to a more toxic and reactive species. Which of the following is not a reactive chemical species commonly formed by toxication?
   a. electrophiles.
   b. nucleophiles.
   c. superoxide anions.
   d. hydroxy radicals.
   e. hydrophilic organic acids.

3. Which of the following is not an important step in detoxication of chemicals?
   a. formation of redox-active reactants.
   b. reduction of hydrogen peroxide by glutathione peroxidase.
   c. formation of hydrogen peroxide by superoxide dismutase.
   d. reduction of glutathione disulfide (GSSG) by glutathione reductase (GR).
   e. conversion of hydrogen peroxide to water and molecular oxygen by catalase.

4. Regarding the interaction of the ultimate toxicant with its target molecule, which of the following is false?
   a. Toxins of en oxidize or reduce their target molecules, resulting in the formation of a harmful by-product.
   b. The covalent binding of a toxin with its target molecule permanently alters the target's function.
   c. The noncovalent binding of a toxin to an ion channel irreversibly inhibits ion flux through the channel.
   d. Abstraction of hydrogen atoms from endogenous compounds by free radicals can result in the formation of DNA adducts.
   e. Several toxins can act enzymatically on their specific target proteins.

5. All of the following are common effects of toxicants on target molecules EXCEPT:
   a. blockage of neurotransmitter receptors.
   b. interference with DNA replication due to adduct formation.
   c. cross-linking of endogenous molecules.
   d. opening of ion channels.
   e. mounting of an immune response.

6. Which of the following proteins functions to prevent the progression of the cell cycle?
   a. NF-κB.
   b. MAPK.
   c. CREB.
   d. c-Myc.
   e. IκB.

7. Which of the following would have the largest negative impact on intracellular ATP levels?
   a. moderately decreased caloric intake.
   b. interference with electron delivery to the electron transport chain.
   c. inability to harvest ATP from glycolysis.
   d. increased synthesis of biomolecules.
   e. active cell division.

8. What happens when a toxin induces elevation of cytoplasmic calcium levels?
   a. Mitochondrial uptake of calcium dissipates the electrochemical gradient needed to synthesize ATP.
   b. Formation of actin filaments increases the strength and integrity of the cytoskeleton.
   c. It decreases the activity of intracellular proteases, nucleases, and phospholipases.
   d. The cell becomes dormant until the calcium is actively pumped from the cell.
   e. The generation of reactive oxygen species slows because of calcium-induced decrease in activity of the TCA cycle.

9. Cytochrome c is an important molecule in initiating apoptosis in cells. All of the following regarding cytochrome c are true EXCEPT:
   a. The release of cytochrome c into the cytoplasm is an important step in apoptosis initiation.
   b. The loss of cytochrome c from the electron transport chain blocks ATP synthesis by oxidative phosphorylation.
   c. Loss of cytochrome c from the inner mitochondrial membrane results in increased formation of reactive oxygen species.
   d. Bax proteins mediate cytochrome c release.
   e. Caspases are proteases that increase cytoplasmic levels of cytochrome c.

10. All of the following regarding DNA repair are true EXCEPT:
    a. In a lesion that does not cause a major distortion of the double helix, the incorrect base is cleaved and the correct base is inserted in its place.
    b. Base excision repair and nucleotide excision repair are both dependent on a DNA polymerase and a DNA ligase.
    c. In nucleotide excision repair, only the adduct is cleaved, and the gap is then filled by DNA polymerase.
    d. Pyrimidine dimers can be cleaved and repaired directly by DNA photolyase.
    e. Recombinational repair requires that a sister strand serve as a template to fill in missing nucleotides.

11. Apoptosis can serve as a tissue repair process in a number of cell types. In which of the following cell types would this be a plausible mechanism of tissue repair?
    a. female germ cells.
    b. gastrointestinal epithelium.
    c. neurons.
    d. retinal ganglion cells.
    e. cardiac muscle cells.

12. Which of the following is NOT associated with carcinogenesis?
    a. mutation.
    b. normal p53 function.
    c. Ras activation.
    d. inhibition of apoptosis.
    e. DNA repair failure.

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CHAPTER

4

Risk Assessment

Elaine M. Faustman and Gilbert S. Omenn

INTRODUCTION AND HISTORICAL CONTEXT

DEFINITIONS

DECISION MAKING

HAZARD IDENTIFICATION

- Assessing Toxicity of Chemicals—Introduction
- Assessing Toxicity of Chemicals—Methods
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- Integrating Qualitative Aspects of Risk Assessment

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EMERGING CONCEPTS

PUBLIC HEALTH RISK MANAGEMENT

SUMMARY

KEY POINTS

- Risk assessment is the systematic scientific characterization of potential adverse health effects resulting from human exposures to hazardous agents or situations.
- Risk is defined as the probability of an adverse outcome under specified conditions.
- Risk management refers to the process by which policy actions are chosen to control hazards.

INTRODUCTION AND HISTORICAL CONTEXT

Toxicologic research and toxicity testing conducted and interpreted by toxicologists constitute the scientific core of an important activity known as risk assessment for chemical exposures. In 1983, the National Research Council detailed the steps of hazard identification, dose–response assessment, exposure analysis, and characterization of risks in Risk Assessment in the Federal Government: Managing the Process (widely known as The Red Book). The scheme shown in Figure 4–1 provides a consistent framework for risk assessment across agencies with bidirectional arrows showing an ideal situation where mechanistic research feeds directly into risk assessments and critical data uncertainty drives research. Often, public policy objectives require extrapolations that go far beyond the observation of

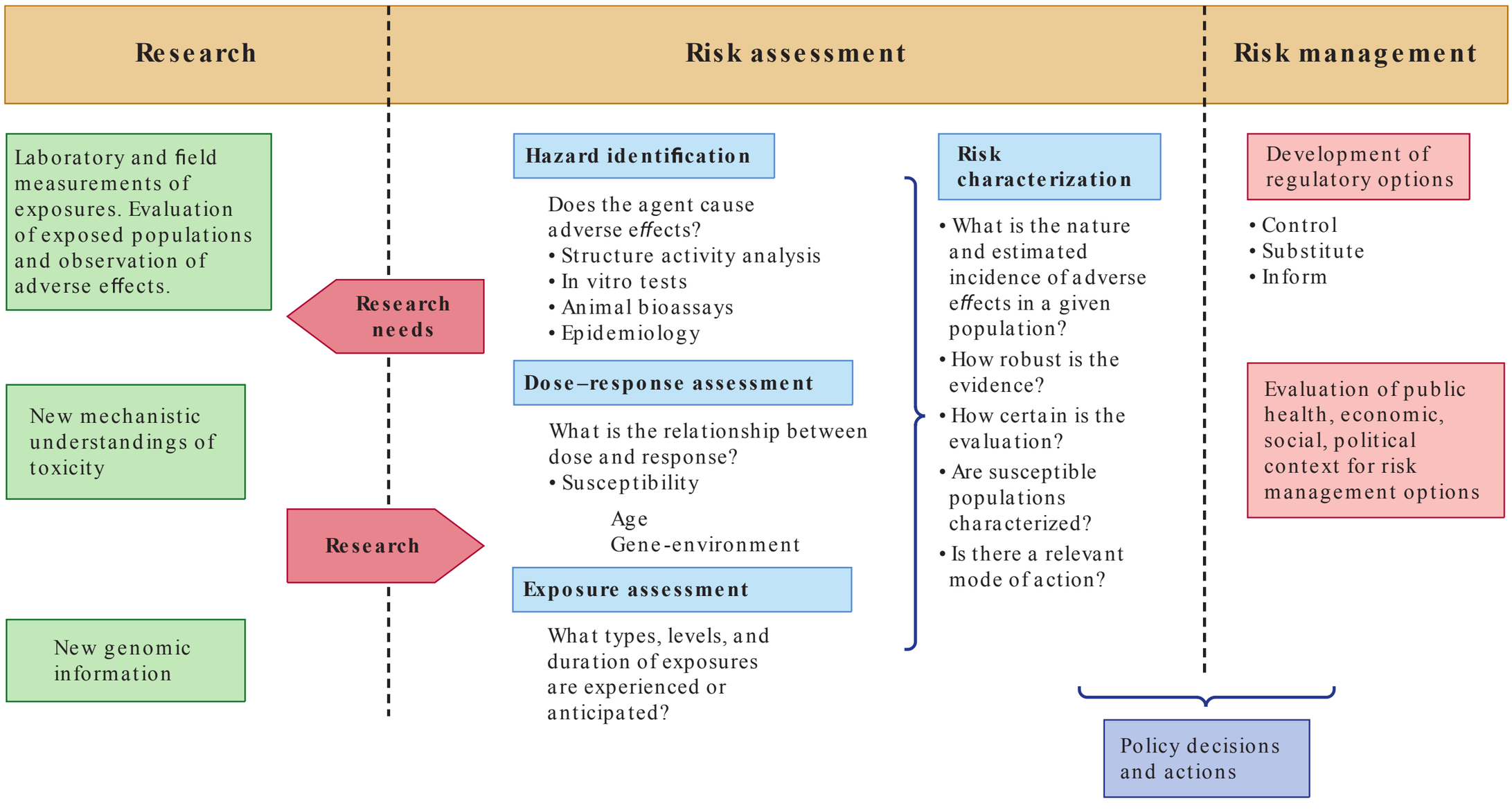

FIGURE 4–1 **Risk assessment/risk management framework.** This framework shows in blue the four key steps of risk assessment: hazard identification, dose–response assessment, exposure assessment, and risk characterization. It shows an interactive, two-way process where research needs from the risk assessment process drive new research, and new research findings modify risk assessment outcomes. (Adapted with permission from Risk Assessment in the Federal Government: Managing the Process, Washington, DC: National Academies Press; 1983.)

actual effects and reflect different tolerances for risks, generating controversy.

A comprehensive framework that applies two crucial concepts: (1) putting each environmental problem or issue into public health and/or ecological context and (2) proactively engaging the relevant stakeholders, affected or potentially affected community groups, from the very beginning of the six-stage process shown in Figure 4–2. Particular exposures and potential health effects must be evaluated across sources and exposure pathways and in light of multiple end points, and not the current general approach of evaluating one chemical in one environmental medium (air, water, soil, food, and products) for one health effect at a time.

DEFINITIONS

Risk assessment is the systematic scientific evaluation of potential adverse health effects resulting from human exposures to hazardous agents or situations. Risk is defined as the probability of an adverse outcome based on the exposure and potency of the hazardous agent(s). The term hazard refers to intrinsic toxic properties, whereas exposure becomes an essential consideration along with hazard for risk determination. Risk assessment requires qualitative information about the strength of the evidence and the nature of the outcomes—as well as quantitative assessment of the exposures, host susceptibility factors, and potential magnitude of the hazard—and then a description of the uncertainties in the estimates and conclusions. The objectives of risk assessment are outlined in Table 4–1.

The phrase characterization *of* risk reflects the combination of qualitative and quantitative analyses. Unfortunately, many users tend to equate risk assessment with quantitative risk assessment, generating a number for an overly precise risk estimate, while ignoring crucial information about the uncertainties of risk assessment, mode of action (MOA), and type of effect across species or context.

Risk management refers to the process by which policy actions are chosen to control hazards identified in the risk assessment/risk characterization stage of the framework (Figure 4–2). Risk managers consider scientific evidence and risk estimates—along with statutory, engineering, economic, social, and political factors—in evaluating alternative options and choosing among those options.

Risk communication is the challenging process of making risk assessment and risk management information comprehensible to community groups, lawyers, local elected officials, judges, business people, labor, environmentalists, etc. A crucial, too-often neglected requirement for communication is listening to the fears, perceptions, priorities, and proposed remedies of these "stakeholders."

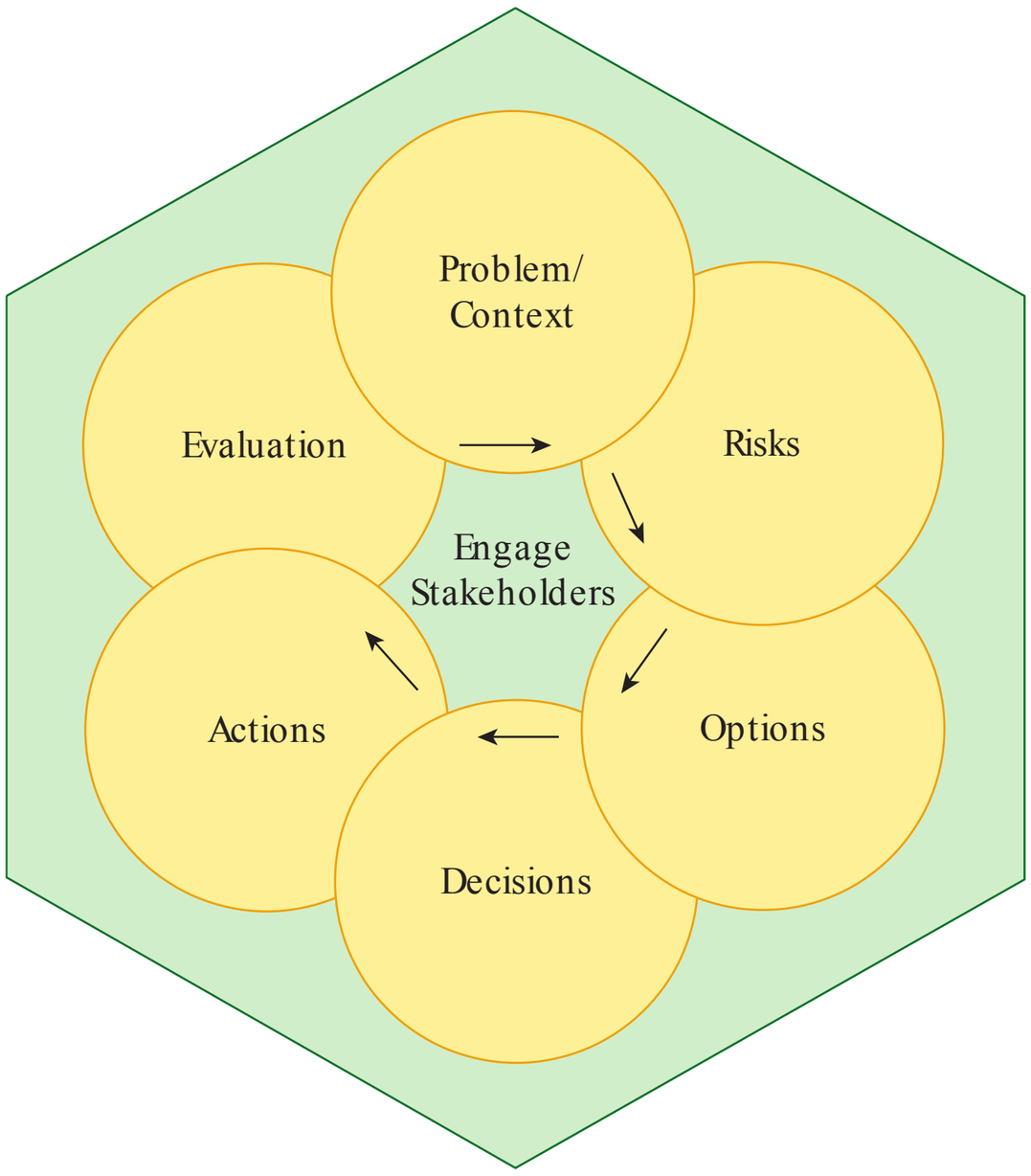

FIGURE 4–2 **Risk management framework for environmental health from the U.S. Commission on Risk Assessment and Risk Management.** The framework comprises six stages: (1) formulating the problem in a broad public health context, (2) analyzing risks, (3) defining options, (4) making risk-reduction decisions, (5) implementing those actions, and (6) evaluating the effectiveness of the taken actions. Interactions with stakeholders are critical and thus have been put at the center of the framework.

DECISION MAKING

Risk management decisions are reached under diverse statutes in the United States and many other countries. Some statutes specify reliance on risk alone, whereas others require a balancing of risks and benefits of the product or activity (Table 4–1). Risk assessments provide a valuable framework for priority setting within regulatory and health agencies, in the chemical development process within companies, and in resource allocation by environmental organizations. Currently, there are significant efforts toward a global harmonization of testing protocols and the assessment of risks and standards.

A major challenge for risk assessment, risk communication, and risk management is to work across disciplines to demonstrate the biological plausibility and clinical significance of the conclusions from studies of chemicals thought to have potential adverse effects. Biomarkers of exposure, effect, or individual susceptibility can link the presence of a chemical in various environmental compartments to specific sites of action in target organs and to host responses. Individual behavioral and social risk factors may be critically important to both the characterization of risk and the reduction of risk. Finally, public and media attitudes toward local polluters, other responsible parties, and relevant government agencies can greatly influence the communication process and the choices for risk management.

TABLE 4–1 Objectives of risk assessment.

1. Protect human and ecological health Toxic substances
2. Balance risks and benefits Drugs Pesticides
3. Set target levels of risk Food contaminants Water pollutants
4. Set priorities for program activities Regulatory agencies Manufacturers Environmental/consumer organizations
5. Estimate residual risks and extent of risk reduction after steps are taken to reduce risks

HAZARD IDENTIFICATION

Assessing Toxicity of Chemicals—Introduction

In order to assess toxicity of chemicals, information from four types of studies is used: structure–activity relationships (SAR), in vitro or short-term studies, in vivo animal bioassays, and information from human epidemiologic studies. In many cases, toxicity information for chemicals is limited; however, recent efforts to mitigate this gap in understanding have been successful.

Assessing Toxicity of Chemicals—Methods

Structure/Activity Relationships (SARs)—Given the cost of $2 to $4 million and the 3 to 5 years required for testing a single chemical in a lifetime rodent carcinogenicity bioassay, initial decisions on whether to continue development of a chemical, submit a premanufacturing notice, or require additional testing may be based largely on SARs and limited short-term assays. A chemical's structure, solubility, stability, pH sensitivity, electrophilicity, volatility, and chemical reactivity can be important information for hazard identification.

SARs have been used for assessment of complex mixtures of structurally related compounds. However, it is difficult to predict activity across chemical classes and especially across multiple toxic end points using a single biological response. Pharmaceutical companies are now using computerized combinatorial chemistry and three-dimensional (3D) molecular modeling approaches to design new drugs (ligands) that can sterically fit into the "receptors of interest." However, computerized SAR methods have given disappointing results because it is rare for environmental pollutants to exhibit selective ligand–receptor binding.

In Vitro and Short-term Tests—The next approach for hazard identification comprises using tests ranging from in vitro bacterial mutation assays to more elaborate short-term tests such as skin-painting studies in mice or altered rat liver foci assays conducted in vivo, as well as other assays that evaluate developmental, reproductive, neuro-, and immunotoxicity.

Short-term assay validation and application is particularly important to risk assessment because such assays can provide information about mechanisms of effects while being faster and less expensive than lifetime bioassays. Validation requires determination of their sensitivity (ability to identify true carcinogens), specificity (ability to recognize noncarcinogens as noncarcinogens), and predictive value for the toxic end point under evaluation. Considerable effort to improve the utility of these tests is continually expended due to their value in providing chemical-specific mechanistic information.

Animal Bioassays—Animal bioassay data are key components of the hazard identification process. A basic premise of risk assessment is that chemicals that cause tumors in animals can cause tumors in humans. All human carcinogens that have been adequately tested in animals produce positive results in at least one animal model. Although this association cannot establish that all agents and mixtures that cause cancer in experimental animals also cause cancer in humans, nevertheless, in the absence of adequate data on humans, it is biologically plausible and prudent to regard agents and mixtures for which there is sufficient evidence of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans—a reflection of the "precautionary principle." In general, the most appropriate rodent bioassays are those that test exposure pathways of most relevance to predicted or known human exposure pathways. Bioassays for reproductive and developmental toxicity and other noncancer end points have a similar rationale.

Consistent features in the design of standard cancer bioassays include testing in two species and both sexes, with 50 animals per dose group and near-lifetime exposure. Important choices include the strains of rats and mice, the number of doses, and dose levels (typically 90%, 50%, and 10% to 25% of the maximally tolerated dose [MTD]), and the details of the required histopathology (number of organs to be examined, choice of interim sacrifice pathology, etc.). Positive evidence of chemical carcinogenicity can include increases in number of tumors at a particular organ site, induction of rare tumors, earlier induction (shorter latency) of commonly observed tumors, and/or increases in the total number of observed tumors.

Critical problems exist in using the hazard identification data from rodent bioassays for quantitative risk assessments. This is because of the limited dose–response data available from these rodent bioassays and nonexistent response information for environmentally relevant exposures. Results thus have traditionally been extrapolated from a dose–response curve in the 10% to 100% biologically observable tumor response range down to 10^{-6} risk estimates (upper confidence limit) or to a benchmark or reference dose-related risk.

Lifetime bioassays have been enhanced with the collection of additional mechanistic data and with the assessment of multiple noncancer end points. It is feasible and desirable to integrate such information together with data from mechanistically oriented short-term tests and biomarker and genetic studies in epidemiology. Such approaches may allow for an extension of biologically observable phenomena to doses lower than those leading to frank tumor development and help to address the issues of extrapolation over multiple orders of magnitude to predict response at environmentally relevant doses.

In an attempt to improve the prediction of cancer risk to humans, transgenic mouse models have been developed as possible alternatives to the standard 2-year cancer bioassay. By using mice that incorporate or eliminate a gene that is linked to human cancer, these transgenic models have the power to improve the characterization of key cellular processes and the mode of action of toxicological responses. It is suggested that these models currently should not replace the 2-year assay, but should be used in conjunction with other types of data to assist in the interpretation of additional toxicological and mechanistic evidence.

Use of Epidemiologic Data in Risk Assessment—The most convincing line of evidence for human risk is a well-conducted epidemiologic study in which a positive association between exposure and disease has been observed. Table 4–2 shows examples of epidemiologic study designs and provides clues on types of outcomes and exposures evaluated. There are important inherent limitations in epidemiologic studies. When the study is exploratory, hypotheses are often weak. Exposure estimates are often crude and retrospective, especially for conditions with long latency before clinical manifestations appear. Generally, there are multiple exposures, especially when a lifetime is considered. There is always a trade-off between detailed information on relatively few persons and very limited information on large numbers of persons. Contributions from lifestyle factors, such as smoking and diet, are a challenge to sort out. Humans are highly outbred, so the method must consider variation in susceptibility among those who are exposed.

Nevertheless, human epidemiology studies provide very useful information for hazard identification and sometimes quantitative information for data characterization. Three major types of epidemiology study designs are available: cross-sectional studies, cohort studies, and case–control studies (Table 4–2). Cross-sectional studies survey groups of humans to identify risk factors (exposure) and disease but are not useful for establishing cause and effect. Cohort studies evaluate individuals selected on the basis of their exposure to an agent under study. These prospective studies monitor over time individuals who initially are disease-free to determine the rates at which they develop disease. In case–control studies, subjects are selected on the basis of disease status: disease cases and matched cases of disease-free individuals. Exposure histories of the two groups are compared to determine key consistent features in their exposure histories. All case–control studies are retrospective studies.

TABLE 4–2 Attributes of three types of epidemiologic study designs.

Methodological Attributes	Type of Study		
	Cohort	Case–Control	Cross-sectional
Initial classification	Exposure–nonexposure	Disease–nondisease	Either one
Time sequence	Prospective	Retrospective	Present time
Sample composition	Nondiseased individuals	Cases and controls	Survivors
Comparison	Proportion of exposed with disease	Proportion of cases with exposure	Either one
Rates	Incidence	Fractional (percent)	Prevalence
Risk index	Relative risk–attributable risk	Relative odds	Prevalence
Advantages	Lack of bias in exposure, yields rates of incidence and risk	Inexpensive, small number of subjects, rapid results, suitable for rare diseases, no attrition	Quick results
Disadvantages	Large number of subjects required, long follow-up, attrition, change in time of criteria and methods, costly, inadequate for rare diseases	Incomplete information, biased recall, problem in selecting control and matching, yields only relative risk—cannot establish causation, population of survivors	Cannot establish causation (antecedent consequence), population of survivors, inadequate for rare diseases

Epidemiologic findings are judged by the following criteria: strength of association, consistency of observations (reproducibility in time and space), specificity (uniqueness in quality or quantity of response), appropriateness of temporal relationship (did the exposure precede responses?), dose–responsiveness, biological plausibility and coherence, verification, and analogy (biological extrapolation). In addition, epidemiologic study designs should be evaluated for their power of detection, appropriateness of outcomes, verification of exposure assessments, completeness of assessing confounding factors, and general applicability of the outcomes to other populations at risk. Power of detection is calculated using study size, variability, accepted detection limits for end points under study, and a specified significance level.

Recent advances from the human genome project, increased sophistication of molecular biomarkers, and improved mechanistic bases for epidemiologic hypotheses have allowed epidemiologists to expand our understanding of biological plausibility and clinical relevance. "Molecular epidemiology" with improved molecular biomarkers of exposure, effect, and susceptibility has allowed investigators to more effectively link molecular events in the causative disease pathway. The range of biomarkers has grown dramatically and includes identification of single nucleotide polymorphisms (SNPs), genomic profiling, transcriptome analysis, and proteomic analysis.

Integrating Qualitative Aspects of Risk Assessment

Qualitative assessment of hazard information should include consideration of the consistency and concordance of findings, including a determination of the consistency of the toxicological findings across species and target organs, an evaluation of consistency across duplicate experimental conditions, and a determination of the adequacy of the experiments to consistently detect the adverse end points of interest. Many agencies use similar evidence classification for both animal and human studies. These classifications include levels of sufficient, limited, inadequate, no evidence, or evidence suggesting lack of carcinogenicity. An overall weight of evidence approach to carcinogenicity uses these evidence classifications, and considers the quality and quantity of data as well as any underlying assumptions.

DOSE–RESPONSE ASSESSMENT

Integrating Quantitative Aspects of Risk Assessment

Quantitative considerations in risk assessment include dose–response assessment, exposure assessment, variation in susceptibility, and characterization of uncertainty.

The fundamental basis of the quantitative relationships between exposure to an agent and the incidence of an adverse response is the dose–response assessment. Analysis of dose–response relationships must start with the determination of the critical effects to be quantitatively evaluated. It is usual practice to choose the data sets with adverse effects occurring at the lowest levels of exposure from studies using the most relevant exposure routes. The "critical" adverse effect is defined as the significant adverse biological effect that occurs at the lowest exposure level.

Threshold Approaches—Threshold dose–response relationship characterization includes identification of "no or lowest observed adverse effect levels" (NOAELs or LOAELs). On the dose–response curve illustrated in Figure 4–3, the threshold, indicated as T, represents the dose below which no additional increase in response is observed. The NOAEL is identified as the highest nonstatistically significant dose tested; in this

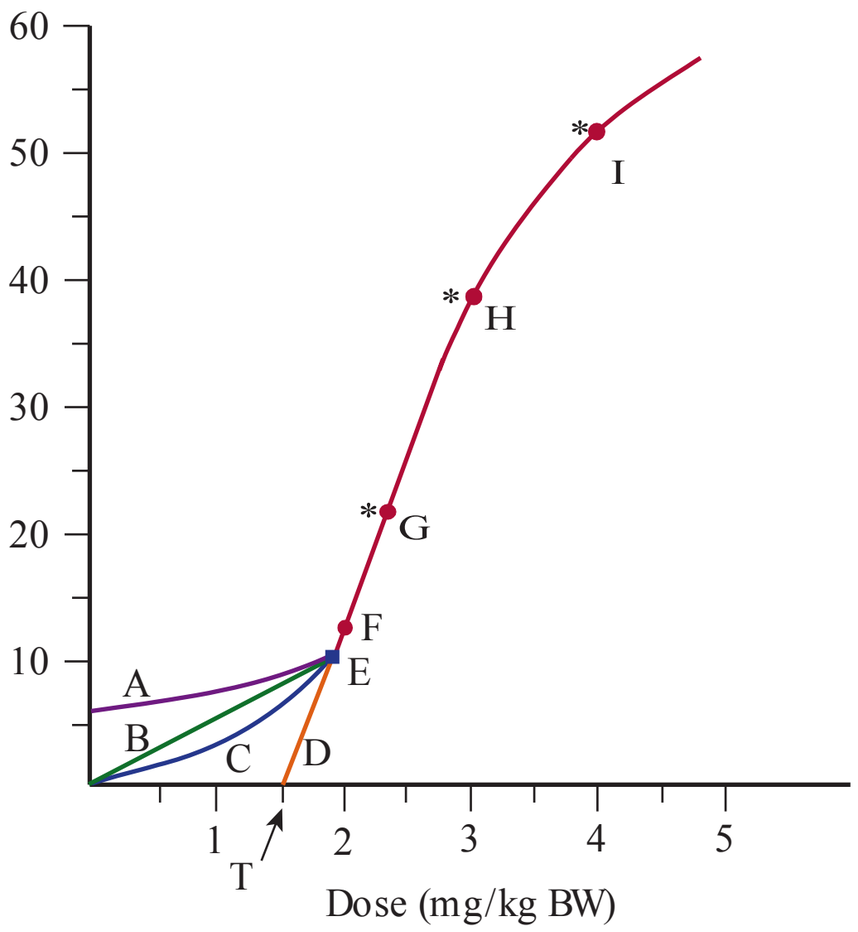

FIGURE 4–3 **Dose–response curve.** This figure is designed to illustrate a typical dose–response curve with points E to I indicating the biologically determined responses. Statistical significance of these responses is indicated with a "*" symbol. The threshold is shown by T, a dose below which no change in biological response occurs. Point E represents the point of departure (POD), the dose near the lower end of the observed dose–response range, below which extrapolation to lower doses is necessary. Point F is the highest nonstatistical significant response point; hence, it is the "no observed adverse effect level" (NOAEL) for this example. Point G is the "lowest observed adverse effect level" (LOAEL) for this example. Curves A to D show some options for extrapolating the dose–response relationship below the range of biologically observed data points and POD.

example it is point F, at 2 mg/kg body weight. Point G is the LOAEL (~2.3 mg/kg body weight), as it is the lowest dose tested with a statistically significant effect. Lines A to D represent possible extrapolations below the point of departure (POD), which is represented on this figure as a square and is labeled as point E. POD is used to specify the estimated dose near the lower end of the observed dose range, below which extrapolation to lower exposures is necessary.

In general, animal bioassays are constructed with sufficient numbers of animals to biological responses at the 10% response range. Significance usually refers to both biological and statistical criteria and is dependent on the number of dose levels tested, the number of animals tested at each dose, and background incidence of the adverse response in the nonexposed control groups. The NOAEL should not be perceived as risk-free.

As described in Chapter 2, approaches for characterizing dose–response relationships include identification of effect levels such as LD_{50} (dose producing 50% lethality), LC_{50} (concentration producing 50% lethality), ED_{10} (dose producing 10% response), as well as NOAELs.

NOAELs have traditionally served as the basis for risk assessment calculations, such as reference doses (RfDs) or acceptable daily intake (ADI) values. RfDs or concentrations (RfCs) are estimates of a daily exposure (oral or inhalation, respectively) to an agent that is assumed to be without adverse health impact in humans. ADI values may be defined as the daily intake of chemical during an entire lifetime, which appears to be without appreciable risk on the basis of all known facts at that time. RfDs and ADI values typically are calculated from NOAEL values by dividing by uncertainty (UF) and/or modifying factors (MF):

$$\text{RfD} = \frac{\text{NOAEL}}{\text{UF} \times \text{MF}}$$

$$\text{ADI} = \frac{\text{NOAEL}}{\text{UF} \times \text{MF}}$$

Tolerable daily intakes (TDIs) can be used to describe intakes for chemicals that are not "acceptable" but are "tolerable" as they are below levels thought to cause adverse health effects. These are calculated in a manner similar to ADI. In principle, dividing by these factors allows for interspecies (animal-to-human) and intraspecies (human-to-human) variability with default values of 10 each. An additional UF can be used to account for experimental inadequacies—e.g., to extrapolate from short-exposure-duration studies to a situation more relevant for chronic study or to account for inadequate numbers of animals or other experimental limitations. If only a LOAEL value is available, then an additional 10-fold factor commonly is used to arrive at a value more comparable to a NOAEL. Traditionally, a safety factor of 100 would be used for RfD calculations to extrapolate from a well-conducted animal bioassay (10-fold factor animal-to-human) and to account for human variability in response (10-fold factor human-to-human variability).

MF can be used to adjust the UF if data on mechanisms, pharmacokinetics, or relevance of the animal response to human risk justify such modification.

Recent efforts have focused on using data-derived and chemical-specific adjustment factors to replace the 10-fold UF traditionally used in calculating RfDs and ADIs. Such efforts have included reviewing the human pharmacologic literature from published clinical trials and developing human variability databases for a large range of exposures and clinical conditions. Intra- and interspecies UF have two components: toxicokinetic and toxicodynamic aspects; Figure 4–4 shows these distinctions. This approach provides a structure for incorporating scientific information on specific aspects of the overall toxicologic process into the RfD calculations; thus, relevant data can replace a portion of the overall "uncertainty" surrounding these extrapolations.

NOAEL values have also been utilized for risk assessment by evaluating a "margin of exposure" (MOE), where the ratio of the NOAEL determined in animals and expressed as mg/kg per day is compared with the level to which a human may be exposed. Low values of MOE indicate that the human levels of exposure are close to levels for the NOAEL in animals. Unlike RfD and RfC, there is usually no factor included in this calculation for differences in human or animal susceptibility or animal-to-human extrapolation. Thus, MOE values of less

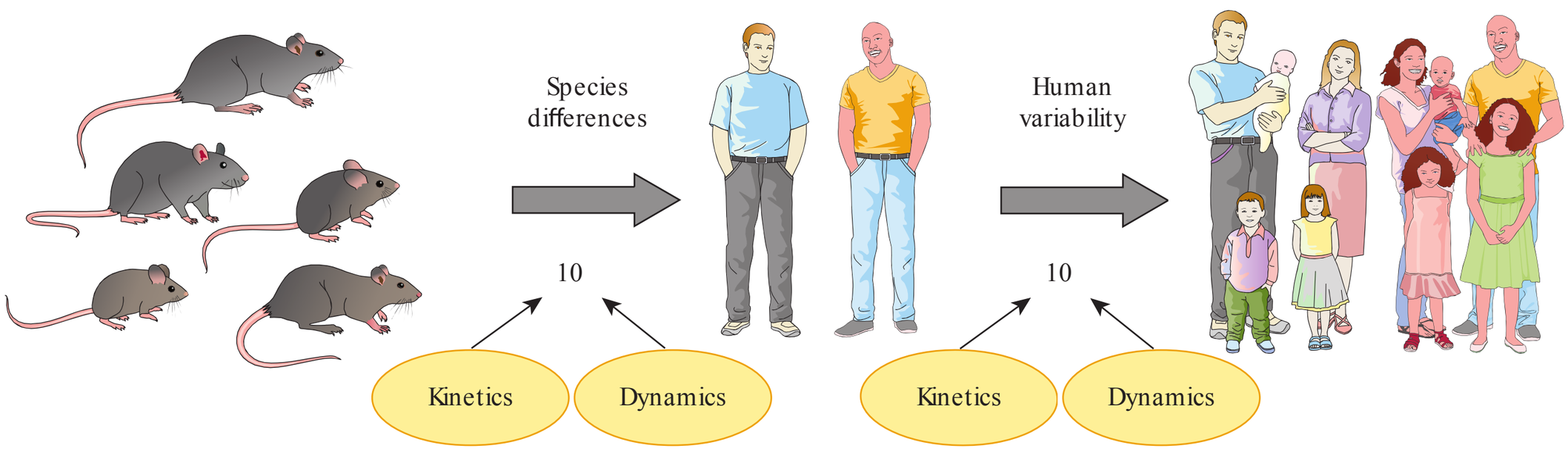

FIGURE 4–4 Toxicokinetic (TK) and toxicodynamic (TD) considerations inherent in interspecies and interindividual extrapolations. Toxicokinetics refers to the processes of absorption, distribution, elimination, and metabolism of a toxicant. Toxicodynamics refers to the actions and interactions of the toxicant within the organism and describes processes at organ, tissue, cellular, and molecular levels. This figure shows how uncertainty in extrapolation both across and within species can be considered as being due to two key factors: a kinetic component and a dynamic component. Refer to the text for detailed explanations.

than 100 have been used by regulatory agencies as flags for requiring further evaluation.

The NOAEL approach has been criticized on several points, including that (1) the NOAEL must, by definition, be one of the experimental doses tested; and (2) once this is identified, the rest of the dose–response curve is ignored. Because of these limitations, an alternative to the NOAEL approach, the benchmark dose (BMD) method, was proposed. In this approach, the dose–response is modeled and the lower confidence bound for a dose at a specified response level (benchmark response [BMR]) is calculated. The BMR is usually specified at 1%, 5%, or 10%. The BMD_x (with x representing the percent BMR) is used as an alternative to the NOAEL value for reference dose calculations. Thus the RfD would be:

$$RfD = \frac{BMD_x}{UF \times MF}$$

The proposed values to be used for the UF and MF for BMDs can range from the same factors as for the NOAEL to lower values due to increased confidence in the response level and increased recognition of experimental variability owing to use of a lower confidence bound on dose.

Advantages of the BMD approach can include (1) the ability to take into account the full dose–response curve; (2) the inclusion of a measure of variability (confidence limit); and (3) the use of a consistent BMR level for RfD calculations across studies. Obviously, limitations in the animal bioassays in regard to minimal test doses for evaluation, shallow dose–responses, and use of study designs with widely spaced test doses will limit the utility of these assays for any type of quantitative assessments, whether NOAEL- or BMD-based approaches.

Nonthreshold Approaches—As Figure 4–3 shows, numerous dose–response curves can be proposed in the low-dose region of the dose–response curve if a threshold assumption is not made. Because the risk assessor generally needs to extrapolate beyond the region of the dose–response curve for which experimentally observed data are available, the choice of models to generate curves in this region has received lots of attention. For nonthreshold responses, methods for dose–response assessments have also utilized models for extrapolation to de minimus (10^{-4} to 10^{-6}) risk levels at very low doses, far below the biologically observed response range and far below the effect levels evaluated for threshold responses.

Statistical or Probability Distribution Models—Two general types of dose–response models exist: statistical (or probability distribution models) and mechanistic models. The distribution models are based on the assumption that each individual has a tolerance level for a test agent and that this response level is a variable following a specific probability distribution function. These responses can be modeled using a cumulative dose–response function. However, extrapolation of the experimental data from 50% response levels to a "safe," "acceptable," or "de minimus" level of exposure—e.g., one in a million risk above background—illustrates the huge gap between scientific observations and highly protective risk limits (sometimes called virtually *safe* doses, or those corresponding to a 95% upper confidence limit on adverse response rates).

Models Derived from Mechanistic Assumptions—This modeling approach designs a mathematical equation to describe dose–response relationships that are consistent with postulated biological mechanisms of response. These models are based on the idea that a response (toxic effect) in a particular biological unit (animal or human) is the result of the random occurrence of one or more biological events (stochastic events).

Radiation research has spawned a series of "hit models" for cancer modeling, where a hit is defined as a critical cellular event that must occur before a toxic effect is produced. The simplest mechanistic model is the one-hit (one-stage) linear model in which only one hit or critical cellular interaction is required for a cell to be altered. As theories of

cancer have grown in complexity, multi-hit models have been developed that can describe hypothesized single-target multi-hit events, as well as multi-target, multi-hit events in carcinogenesis.

Toxicologic Enhancements of the Models—Three exemplary areas of research that have improved the models used in risk extrapolation are time to tumor information, physiologically based toxicokinetic modeling (described in Chapter 7), and biologically based dose–response (BBDR) modeling. The BBDR model aims to make the generalized mechanistic models discussed in the previous section more clearly reflect specific biological processes. Measured rates are incorporated into the mechanistic equations to replace default or computer-generated values.

Development of BBDR models for end points other than cancer is limited; however, several approaches have been explored in developmental toxicity utilizing mode of action information on cell cycle kinetics, enzyme activity, litter effects, and cytotoxicity as critical end points. Approaches have been proposed that link pregnancy-specific toxicokinetic models with temporally sensitive toxicodynamic models for developmental impacts. Unfortunately, the lack of specific, quantitative biological information for most toxicants and for most end points limits study and utilization of these models.

EXPOSURE ASSESSMENT

The primary objectives of exposure assessment are to determine source, type, magnitude, and duration of contact with the agent of interest. Obviously, a critical element of the risk assessment process requires recognition that hazard does not occur in the absence of exposure. However, exposure data are frequently identified as the key area of uncertainty in overall risk determination. The primary goal of using exposure information in quantitative risk assessment is not only to determine the type and amount of total exposure, but also to find out specifically how much may be reaching target tissues. A key step in making an exposure assessment is determining what exposure pathways are relevant for the risk scenario under development. The subsequent steps entail quantitation of each pathway identified as a potentially relevant exposure and then summarizing these pathway-specific exposures for calculation of overall exposure.

Additional considerations for exposure assessments include how time and duration of exposures are evaluated in risk assessments. In general, estimates for cancer risk use averages over a lifetime. In a few cases, short-term exposure limits (STELs) are required and characterization of brief but high levels of exposure is significant. In these cases exposures are not averaged over the lifetime and the effects of high, short-term doses are estimated. With developmental toxicity, a single exposure can be sufficient to produce an adverse developmental effect if exposures occur during a window of developmental susceptibility; thus, daily doses are used, rather than lifetime weighted averages.

RISK CHARACTERIZATION

Variation in Susceptibility

Toxicology has been slow to recognize the marked variation among humans. Generally, assay results and toxicokinetic modeling utilize means and standard deviations to measure variation, or even standard errors of the mean, thereby ignoring variability in response due to differences in age, sex, health status, and genetics.

One key challenge for risk assessment will be interpretation and linking of observations from highly sensitive molecular and genome-based methods with the overall process of toxicity. Biomarkers of early effects, like frank clinical pathology, arise as a function of exposure, response, and time. Early, subtle, and possibly reversible effects can generally be distinguished from irreversible disease states.

The challenge for interpretation of early and highly sensitive response biomarkers is made clear in the analysis of data from gene expression arrays. Because our relatively routine ability to monitor gene responses has grown exponentially in the last decade, the need for toxicologists to interpret such observations for risk assessment and the overall process of toxicity has increased with equal or greater intensity.

Microarray analysis for risk assessment requires sophisticated analyses to arrive at a functional interpretation and linkage to a conventional toxicologic end point. Because of the vast number of measured responses with gene expression arrays, pattern analysis techniques are being used. The extensive databases across chemical classes, pathological conditions, and stages of disease progression that are essential for these analyses are being developed.

INFORMATION RESOURCES

Though numerous information resources are available for risk assessment, a few are listed below in order to provide the reader with examples of risk assessment resources and databases. The Toxicology Data Network (TOXNET) from the National Library of Medicine (http://toxnet.nlm.nih.gov/) provides access to databases on toxicology, hazardous chemicals, and related areas. These information sources vary in the included level of assessment, ranging from just listings of scientific references without comment to extensive peer-reviewed risk assessment information. The World Health Organization (http://who.int/) provides chemical-specific information through the International Programme on Chemical Safety (http://who.int/pcs/IPCS/index.htm) criteria documents and health and safety documents. The International Agency for Research on Cancer (IARC) provides data on specific classes of carcinogens as well as individual agents. The National Institute of Environmental Health Sciences (NIEHS) National Toxicology Program provides technical reports on the compounds tested as a part of this national program (http://ntp.niehs.nih.gov/).

Recently, new toxicogenomic databases that identify and, in some cases, provide characterization of chemicals have become available. The National Center for Biotechnology

Information (NCBI) provides access to an enormous set of biomedical and genomic information which can be valuable for risk assessment, and they have worked to incorporate toxicologically relevant end points. ACToR (http://actor.epa.gov/actor/faces/ACToRHome.jsp), the EPA's online database on chemical toxicity data and potential chemical risks to human health and the environment, is another useful resource for risk assessments. The Comparative Toxicogenomics Database (http://ctd.mdibl.org/) includes data describing cross-species chemical–gene–protein interactions and chemical–gene–disease relationships which illuminate molecular mechanisms underlying variable susceptibility and environmentally induced diseases. Although these databases provide useful hazard identification and mechanistic information, there is little emphasis on exposure data.

RISK PERCEPTION AND COMPARATIVE ANALYSES OF RISK

Individuals respond very differently to information about hazardous situations and products, as do communities and whole societies. Understanding these behavioral responses is critical in stimulating constructive risk communication and evaluating potential risk management options. In a classic study, students, League of Women Voters members, active club members, and scientific experts were asked to rank 30 activities or agents in order of their annual contribution to deaths. Club members ranked pesticides, spray cans, and nuclear power as safer than did other lay persons. Students ranked contraceptives and food preservatives as riskier and mountain climbing as safer than did others. Experts ranked electric power, surgery, swimming, and X-rays as more risky and nuclear power and police work as less risky than did lay persons. There are also group differences in perceptions of risk from chemicals among toxicologists, correlated with their employment in industry, academia, or government.

Psychological factors such as dread, perceived uncontrollability, and involuntary exposure interact with factors that represent the extent to which a hazard is familiar, observable, and "essential" for daily living. Figure 4–5 presents a grid on the parameters controllable/uncontrollable and observable/not observable for a large number of risky activities; for each of the two paired main factors, highly correlated factors are described in the boxes.

Public demand for government regulations often focuses on involuntary exposures (especially in the food supply, drinking

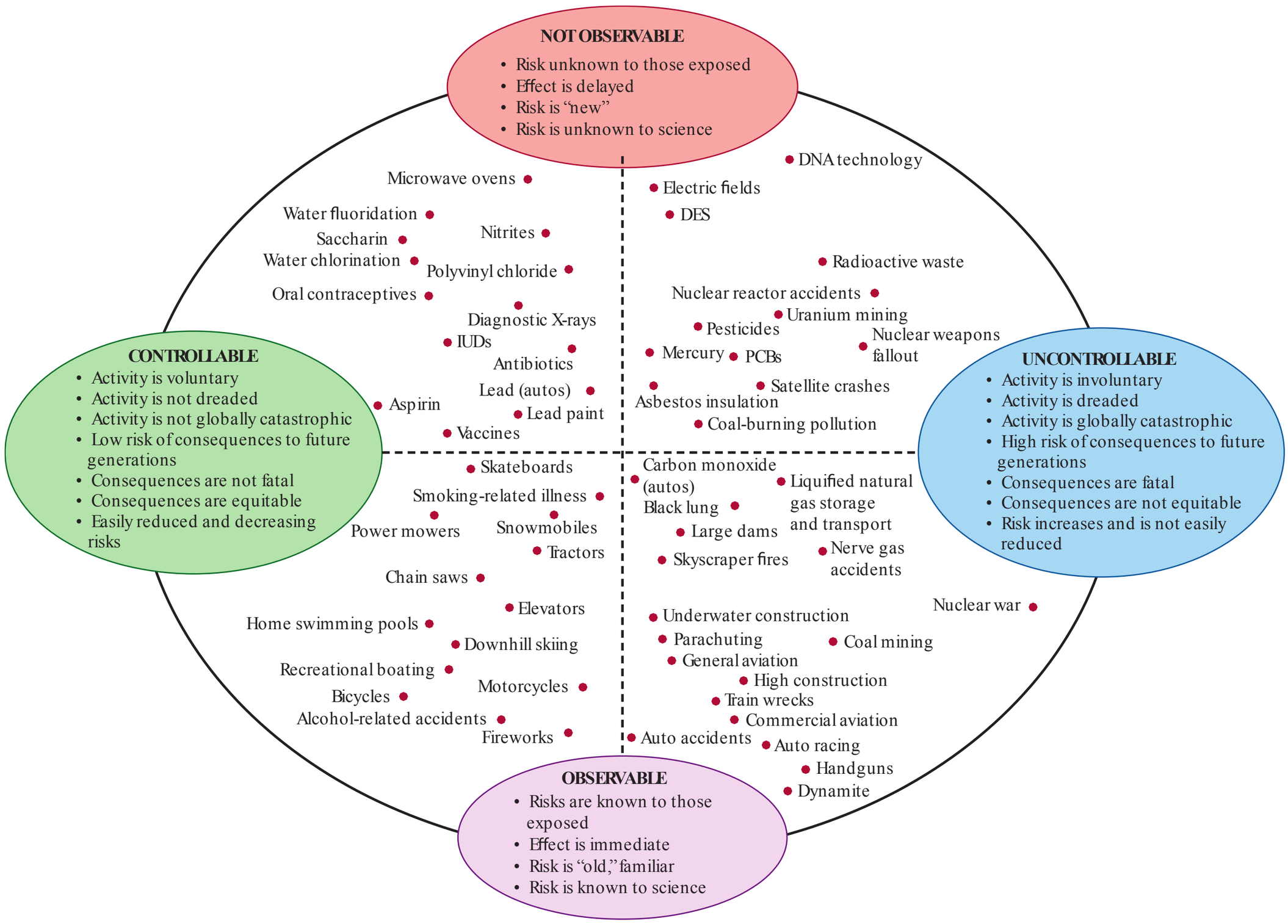

FIGURE 4–5 **Perceptions of risk illustrated using a "risk space" axis diagram.** Risk space has axes that correspond roughly to a hazard's perceived "dreadedness" and to the degree to which it is familiar or observable. Risks in the upper right quadrant of this space are most likely to provoke calls for government regulation.

water, and air) and unfamiliar hazards, such as radioactive waste, electromagnetic fields, asbestos insulation, and genetically modified crops and foods. Many people respond very negatively when they perceive that information about hazards or even about new technologies without reported hazards has been withheld by the manufacturers (genetically modified foods) or by government agencies (HIV-contaminated blood transfusions in the 1980s; extent of hazardous chemical or radioactive wastes).

Most people regularly compare risks of alternative activities—on the job, in recreational pursuits, in interpersonal interactions, and in investments. Determining how best to conduct comparative risk analyses has proved difficult due to the great variety of health and environmental benefits, the gross uncertainties of dollar estimates of benefits and costs, and the different distributions of benefits and costs across the population.

EMERGING CONCEPTS

There is a need to ensure that the risk question(s) is(are) succinctly framed to answer questions in the real world. Environmental health is very dynamic and many divergent emerging environmental challenges such as climate change, energy shortages, and engineered nanoparticles will require an expansion of our context well beyond single-chemical, single-exposure scenarios. In order to accomplish this goal, global and international thinking will be required.

Well-being is increasingly being used to describe human health and the goal of sustainable environmental risk management. Well-being goes beyond "disease-free" existence to freedom from want (including food and water security) and fear (personal safety) and sustainable futures. Recognition that environmental problems are global is essential to how we manage risks and address sustainability. Research and development efforts must examine chemical safety for sustainable and healthy communities with safe and sustainable water, air, and energy resources.

PUBLIC HEALTH RISK MANAGEMENT

Associated with concepts of well-being and sustainability is a public health orientation to use toxicological tests to identify and characterize potential health risks and to prevent the unsafe use of such agents. There are three stages of prevention: primary, whose goal is prevention and risk or hazard avoidance; secondary, whose goal is mitigation or preparedness including risk or vulnerability reduction and risk transfer; and tertiary, where prompt response or recovery is an approach for decreasing residual risk or risk reduction. Figure 4–5 shows an overview of risk assessment and management for public health where concepts of capacity assessment, vulnerability, and impact assessment are included. In this context, vulnerability assessment would include consideration of exposure and susceptibility as part of the vulnerability assessment. Hazard analysis refers to both hazard identification and probability-based frequency of anticipated events. Capacity assessment has been used for identifying strengths and resiliency of a system to impact.

SUMMARY

Risk assessment objectives vary with the issues, risk management needs, and statutory requirements. Hence, setting the context and problem formation for risk evaluation is essential. The frameworks are sufficiently flexible to address various objectives and to accommodate new knowledge while providing guidance for priority setting in industrial, environmental, governmental, and public health agencies. Risk assessment analyzes the science, identifies uncertainty and provides approaches for decisions. Toxicology, epidemiology, exposure assessment, and clinical observations can be linked with biomarkers, cross-species investigations of mechanisms of effects, and systematic approaches to risk assessment, risk communication, and risk management. Advances in toxicology are certain to improve the quality of risk assessments as scientific findings substitute data for assumptions and help to describe and model uncertainty more credibly.

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QUESTIONS

1. Which of the following is NOT important in hazard identification?
   a. structure–activity analysis.
   b. in vitro tests.
   c. animal bioassays.
   d. susceptibility.
   e. epidemiology.

2. The probability of an adverse outcome is defined as:
   a. hazard.
   b. exposure ratio.
   c. risk.
   d. susceptibility.
   e. epidemiology.

3. The systematic scientific characterization of adverse health effects resulting from human exposure to hazardous agents is the definition of:
   a. risk.
   b. hazard control.
   c. risk assessment.
   d. risk communication.
   e. risk estimate.

4. Which of the following is not an objective of risk management?
   a. setting target levels for risk.
   b. balancing risks and benefits.
   c. calculating lethal dosages.
   d. setting priorities for manufacturers.
   e. estimating residual risks.

5. Which of the following is NOT a feature in the design of standard cancer bioassays?
   a. more than one species.
   b. both sexes.
   c. near lifetime exposure.
   d. approximately 50 animals per dose group.
   e. same dose level for all groups.

6. Which of the following types of epidemiologic study is always retrospective?
   a. cohort.
   b. cross-sectional.
   c. case–control.
   d. longitudinal.
   e. exploratory.

7. Which of the following is defined as the highest nonstatistically significant dose tested?
   a. ED_{50}
   b. ED_{100}
   c. NOAEL.
   d. ADI.
   e. COAEL.

8. Which of the following represents the dose below which no additional increase in response is observed?
   a. ED_{10}
   b. LD_{10}
   c. RfC.
   d. threshold.
   e. significance level.

9. Which of the following is NOT needed to calculate the reference dose using the BMD method?
   a. MF.
   b. percent benchmark response.
   c. NOAEL.
   d. UF.
   e. benchmark dose.

10. Virtually safe doses are described at which confidence level?
    a. 90%.
    b. 95%.
    c. 99%.
    d. 99.9%.
    e. 99.99%.

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C H A P T E R

Absorption, Distribution, and Excretion of Toxicants

5

Lois D. Lehman-McKeeman

INTRODUCTION

CELL MEMBRANES

- Passive Transport
  - Simple Diffusion
  - Filtration
- Special Transport
  - Facilitated Diffusion
  - Active Transport
  - Xenobiotic Transporters
  - Additional Transport Processes

ABSORPTION

- Absorption of Toxicants by the Gastrointestinal Tract
- Absorption of Toxicants by the Lungs
  - Gases and Vapors
  - Aerosols and Particles
- Absorption of Toxicants through the Skin
- Absorption of Toxicants after Special Routes of Administration

DISTRIBUTION

- Volume of Distribution
- Storage of Toxicants in Tissues
  - Plasma Proteins as Storage Depot
  - Liver and Kidney as Storage Depots
  - Fat as Storage Depot
  - Bone as Storage Depot
- **Blood–Brain Barrier**
- Passage of Toxicants across the Placenta
- Redistribution of Toxicants

EXCRETION

- Urinary Excretion
- Fecal Excretion
  - Nonabsorbed Ingesta
  - Biliary Excretion
- Exhalation
- Other Routes of Elimination
  - Cerebrospinal Fluid
  - Milk
  - Sweat and Saliva

CONCLUSION

KEY POINTS

- Absorption is the transfer of a chemical from the site of exposure, usually an external or internal body surface, into the systemic circulation.
- Toxicants are removed from the systemic circulation by biotransformation, excretion, and storage at various sites in the body.
- Excretion is the removal of xenobiotics from the blood and their return to the external environment via urine, feces, exhalation, etc.

INTRODUCTION

The disposition of a chemical or xenobiotic is defined as the composite actions of its absorption, distribution, biotransformation, and elimination. The quantitative characterization of xenobiotic disposition is termed pharmacokinetics or toxicokinetics (see Chapter 7).

The toxicity of a substance depends on the dose. The concentration of a chemical at the site of action is usually proportional to the dose, but the same dose of two or more chemicals may lead to vastly different concentrations in a particular target organ of toxicity owing to differences in the disposition of the chemicals. Various factors affecting disposition are depicted in Figure 5–1, such as (1) if the fraction absorbed or the rate of absorption is low, a chemical may never attain a sufficiently high concentration at a potential site of action to cause toxicity; (2) the distribution of a toxicant may be such that it is concentrated in a tissue other than the target organ, thus decreasing toxicity; (3) biotransformation of a chemical may result in the formation of less toxic or more toxic metabolites at a fast or slow rate with obvious consequences for the concentration and, thus, the toxicity at the target site; and (4) the more rapidly a chemical is eliminated from an organism, the lower will be its concentration and hence its toxicity in target tissues. If a chemical is distributed to and stored in fat, its elimination is likely to be slow because very low plasma levels preclude rapid renal clearance or other clearances.

The skin, lungs, and alimentary canal are the main barriers that separate higher organisms from an environment containing a large number of chemicals. Toxicants must cross one or several of these incomplete barriers to exert deleterious effects. Only chemicals that are caustic and corrosive agents (acids, bases, salts, and oxidizers) and act topically at the point of contact are exceptions. A chemical absorbed into the bloodstream through any of these three barriers is distributed throughout the body, including the site where it produces damage, the target organ or target tissue. A chemical may have one or several target organs, and, in turn, several chemicals may have the same target organ(s). Because several factors other than the concentration influence the susceptibility of organs to toxicants, the

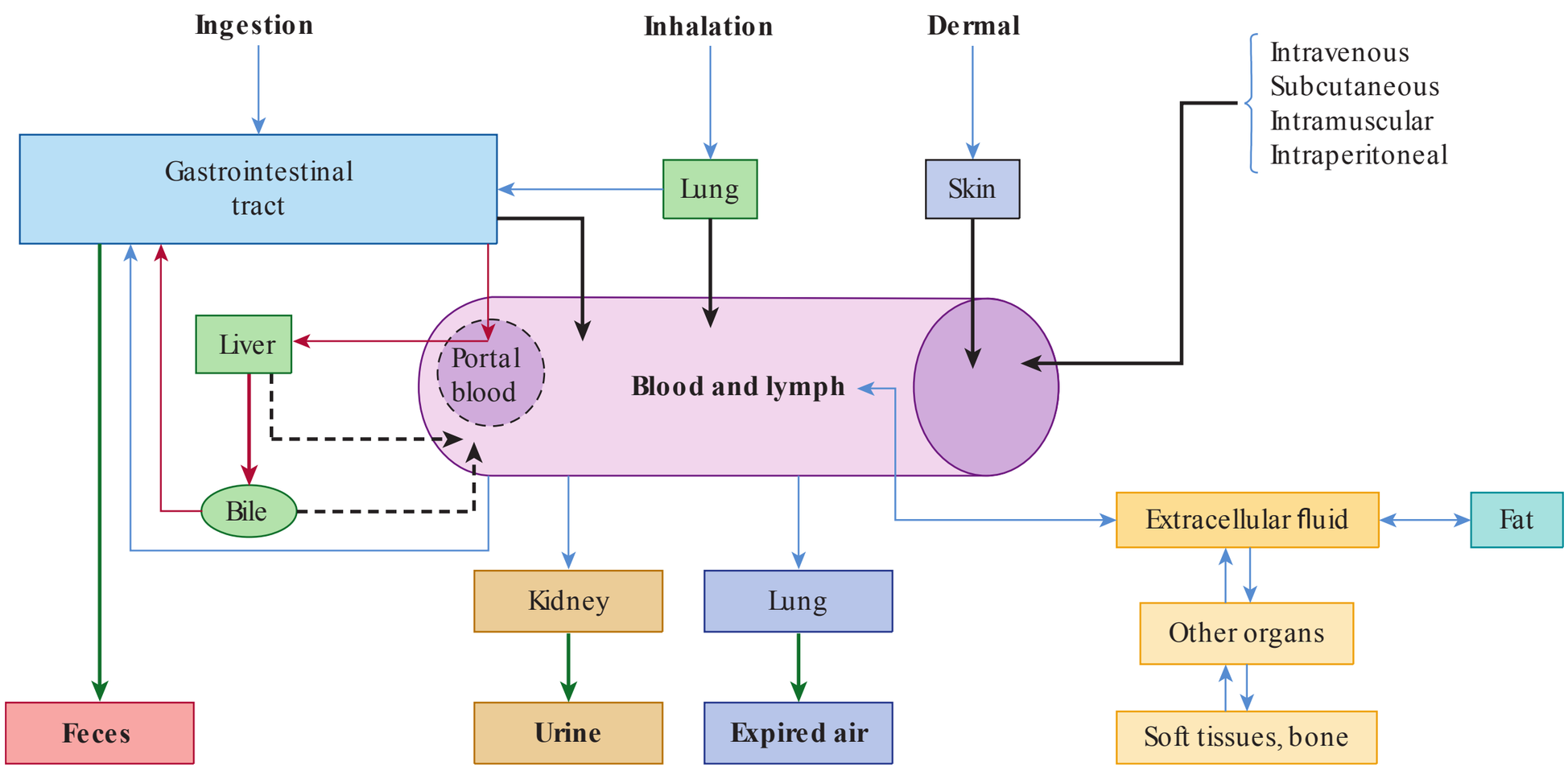

FIGURE 5–1 **Routes of absorption, distribution, and excretion of toxicants in the body.** Black lines represent routes of absorption into the blood stream; blue lines designate distribution; green lines identify pathways of final excretion; red lines show enterohepatic circulation.

organ or tissue with the highest concentration of a toxicant is not necessarily the site of toxicity. It is important to note that the processes comprising xenobiotic disposition are interrelated and influence each other (Figure 5–1).

CELL MEMBRANES

All processes of toxicant distribution involve passage across biological membranes. Toxicants usually pass through the membranes of a number of cells, such as the stratified epithelium of skin, the thin cell layers of lungs or gastrointestinal tract, the capillary endothelium, and the cells of the target organ or tissue; the plasma membranes surrounding all these cells are remarkably similar.

T e basic unit of the cell membrane is a lipid bilayer, composed primarily of phospholipids, glycolipids, and cholesterol. Phospholipids are amphiphilic, consisting of a hydrophilic polar head and a hydrophobic lipid tail. In membranes, the polar head groups are oriented toward the outer and inner surfaces of the membrane, whereas the hydrophobic tails are oriented inward and face each other to form a continuous hydrophobic inner space. Hydrophobic interaction between these fatty acids is the major driving force for the formation of membrane lipid bilayers. Numerous proteins are inserted or embedded in the bilayer, and some transmembrane proteins traverse the entire lipid bilayer, functioning as important biological receptors or allowing the formation of aqueous pores, ion channels, and transporters (Figure 5–2). Fatty acids of the phospholipids and glycolipids do not have a rigid crystalline structure, but are semifluid at physiological temperatures. Many factors influence this fluid character, including degree of unsaturation (lack of double bonds), the presence of cholesterol, and temperature.

A key characteristic of plasma membranes is their ability to be differentially permeable, which regulates what enters into or exits from cells. A toxicant may pass through a membrane by either (1) passive transport, in which the cell expends no energy or (2) specialized transport, in which the cell provides energy to translocate the toxicant across its membrane.

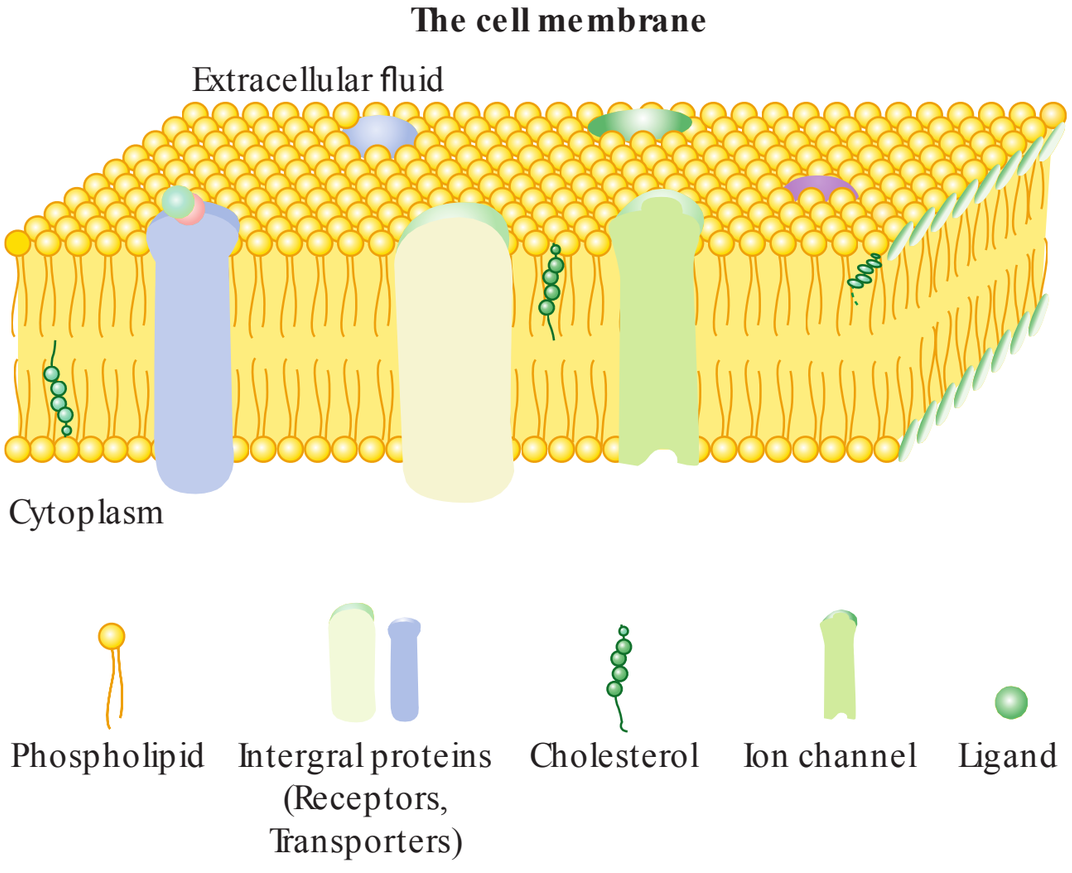

FIGURE 5–2 **Schematic model of a biological membrane.**

Passive Transport

Simp le Dif usion—Most toxicants cross membranes by simple diffusion, following the principles of Fick's law, which establishes that chemicals move from regions of higher concentration to regions of lower concentration without any energy expenditure. Small hydrophilic molecules (up to a molecular weight of about 600 daltons [Da]) permeate membranes through aqueous pores, in a process termed paracellular di*ff*usion. In contrast, hydrophobic molecules diffuse across the lipid domain of membranes, in a process called transcellular diffusion. T e smaller a hydrophilic molecule is, the more readily it traverses membranes by simple diffusion, and, consequently, a small water-soluble compound such as ethanol is rabidly absorbed and distributed.

For larger organic molecules with differing degrees of lipid solubility, the rate of transport across membranes correlates with lipophilicity. T eir rate of transport across membranes correlates with their lipid solubility, which is determined by the octanol/water partition coefficient, P, which is defined as the ratio of the concentration of neutral compound in organic and aqueous phases under equilibrium conditions. T e octanol/water partition coefficient is usually expressed in log form, and is an informative physicochemical parameter relative to assessing potential membrane permeability, with positive values associated with high lipid solubility.

Many chemicals are weak organic acids or bases, which are ionized in solution according to Arrhenius' theory. T e ionized form of weak organic acids or bases usually has low lipid solubility and does not permeate readily through the lipid domain of a membrane. In contrast, the nonionized form is more lipid soluble and diffuses across membranes at a rate that is proportional to its lipid solubility. T e pH at which a weak organic acid or base is 50% ionized is called its pK_a or pK_b. Values for pK_a relay the relative strength or weakness of the acid such that low values indicate a strong acid and high values indicate a weak acid; the opposite is true for bases. Both pK_a and pK_b are defined as the negative logarithm of the ionization constant of a weak organic acid or base. Knowing pK_b, one can calculate pK_a for weak organic bases with the equation $pK_a = 14 - pK_b$. Knowledge of the chemical structure is required to distinguish between organic acids and bases, as the numerical value of pK_a does not indicate this characteristic.

T e degree of ionization of a chemical depends on its pK_a and on the pH of the solution. T e relationship between pK_a and pH is described by the Henderson–Hasselbalch equations:

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

$$\text{For bases } pK_a - pH = \log \frac{[\text{ionized}]}{[\text{nonionized}]}$$

The effect of pH on the degree of ionization of an organic acid (benzoic acid) and an organic base (aniline) is illustrated in Figure 5–3. According to Brönsted–Lowry acid-base theory, an acid is a proton (H^+) donor and a base is a proton acceptor. Thus, the ionized and nonionized forms of an organic acid represent an acid–base pair, with the nonionized moiety being the acid and the ionized moiety being the base.

Filtration—When water flows in bulk across a porous membrane, any solute small enough to pass through the pores flows with it. Passage through these channels is called *filtration*. One of the main differences between various membranes is the size of these channels. In the renal glomeruli, a primary site of blood filtration and subsequent urine formation, these pores are relatively large and allow molecules smaller than albumin (approximately 60 kDa) to pass through. The channels in most cells are much smaller, permitting substantial passage of molecules with molecular weights of no more than a few hundred daltons.

Special Transport

There are numerous compounds whose movement across membranes cannot be explained by simple diffusion or filtration. Some compounds are too large to pass through aqueous pores or too lipid-insoluble to diffuse across the lipid domain of plasma membranes. Nevertheless, these molecules are still transported, often very rapidly, across plasma membranes and even against concentration gradients. Specialized transport systems have been identified to explain these phenomena, and identifying such transporters and their dysfunctions is a developing area of toxicology.

Facilitated Diffusion—Facilitated diffusion is carrier-mediated transport that exhibits the properties of active transport except that the substrate is not moved against an electrochemical or concentration gradient and the transport process does not require the input of energy. Because this process is energy-independent, metabolic poisons do not interfere with this type of transport, as they would with active transport.

Active Transport—Active transport is characterized by (1) movement of chemicals against electrochemical or concentration gradients, (2) saturability at high substrate concentrations, thus exhibiting a transport maximum (T_m), (3) selectivity for certain structural features of chemicals, (4) competitive inhibition by chemical antagonists or compounds that are carried by the same transporter, and (5) requirement for expenditure of energy (often in the form of ATP), so that metabolic inhibitors block the transport process.

Xenobiotic Transporters—Around 5% of all human genes are transporter related, indicating the importance of transport function in normal biological and toxicological outcomes. Transporters mediate the influx (uptake) and efflux of xenobiotics and can be divided into two categories determined by whether they employ active transport or facilitative diffusion (Tables 5–1 and 5–2).

Energy-dependent xenobiotic transporters are part of a large superfamily known as ATP-binding cassette (ABC) transporters, and seven subfamilies (classified A to G) have been now identified. Many of these transporters play key roles in the homeostasis of numerous endogenous substances, including absorption from the GI tract and maintenance of the blood-brain barrier (BBB); mutations can lead to multidrug resistance (MDR). A notable example is from the B subfamily, called MDR1 (ABCB1) that, in cancerous cells, exudes cytotoxic drugs out of the tumor cells thereby protecting the cell from drug-mediated destruction. The C subfamily of ABC transporters is also known as the multidrug resistance–associated protein (MRP) family, and they are also involved in efflux of chemicals from cells.

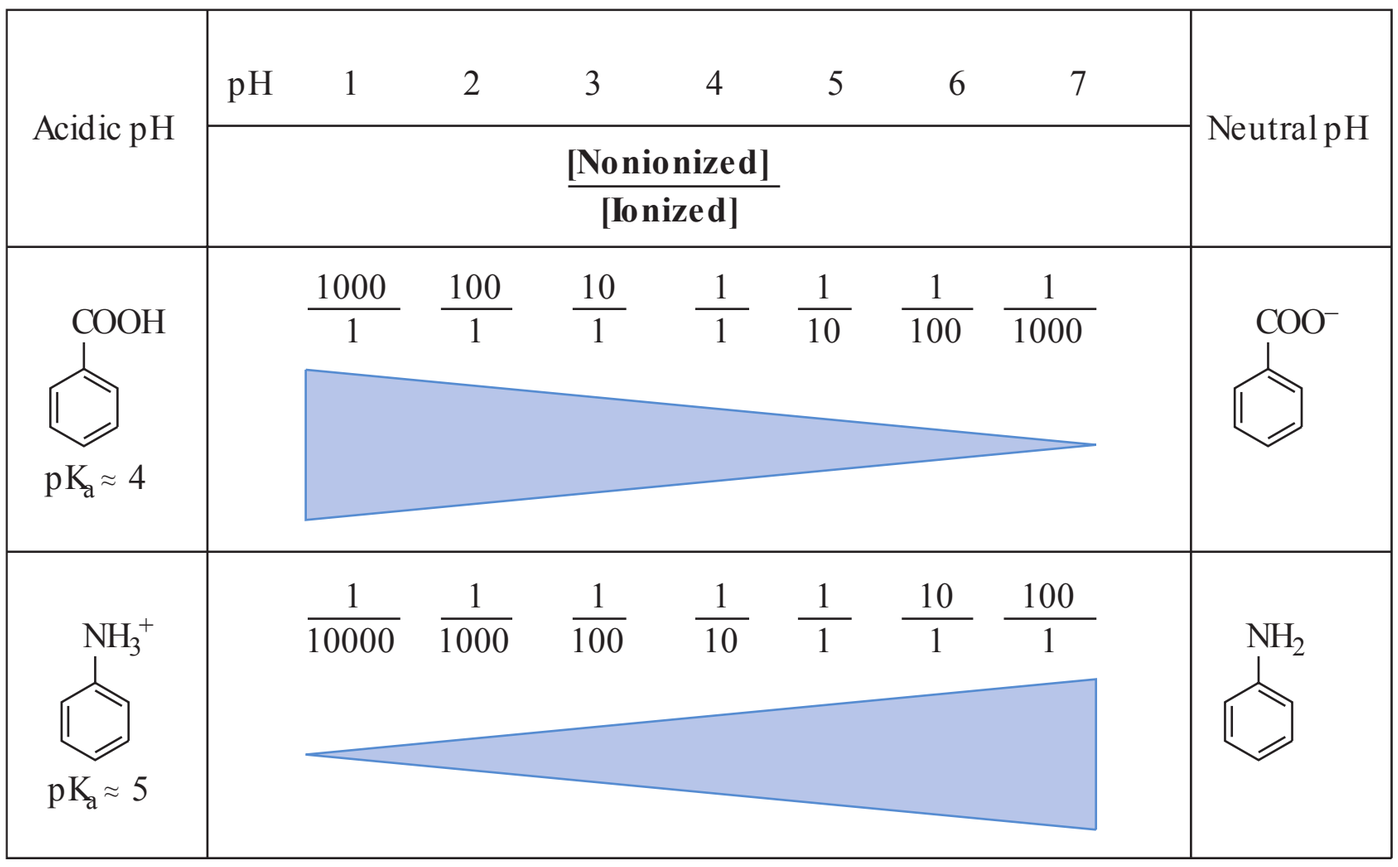

FIGURE 5–3 **Effect of pH on the ionization of benzoic acid ($pK_a = 4$) and aniline ($pK_a = 5$).**

TABLE 5–1 Human ABC transporters: gene family overview and major transporters involved in xenobiotic disposition.

ABC Subfamily	Genes in Family	Gene Symbols
A	12	ABCA1-10, 12, 13
B	**11**	ABCB1-11
C	**13**	ABCC1-13*
D	4	ABCD1-4
E	1	ABCE1
F	3	ABCF1-3
G	5	ABCG1, 2, 4, 5, 8

Bolded subfamily designations are those with a major role in xenobiotic disposition.
*ABCC13 is reported to be a pseudogene.

Gene Symbol	Common Name	General Function
ABCB1	Multidrug resistant protein/P-glycoprotein (MDR)	Ef ux from gut, brain, placenta; biliary excretion
ABCB11	Bile salt export pump (BSEP)	Bile salt transport
ABCC1	Multidrug resistance–associated protein 1 (MRP1)	Multidrug resistance in many tissues; export pump
ABCC2	Multidrug resistance–associated protein 2 (MRP2)	Organic anion ef ux, glucuronide and glutathione conjugates, biliary excretion
ABCC3	Multidrug resistance–associated protein 3 (MRP3)	Organic anion ef ux, glucuronide and glutathione conjugates
ABCC4	Multidrug resistance–associated protein 4 (MRP4)	Nucleoside transport and organic anion ef ux
ABCC5	Multidrug resistance–associated protein 5 (MRP6)	Mainly nucleoside transport
ABCC6	Multidrug resistance–associated protein 6 (MRP6)	Some glutathione conjugates
ABCC10	Multidrug resistance–associated protein 7 (MRP7)*	Organic anions, vinca alkaloids
ABCC11	Multidrug resistance–associated protein 8 (MRP8)*	Cyclic nucleotides and organic anions
ABCC12	Multidrug resistance–associated protein 9 (MRP9)*	Not defined
ABCG2	Breast cancer resistance protein (BCRP)	Organic anion ef ux, many sulfate conjugates, biliary excretion

*There is little functional information available for MRP7, MRP8, and MRP9. A single nucleotide polymorphism in MRP8 is known to determine wet or dry earwax.

T e second major class of xenobiotic transporters is the solute carriers (SLCs), which predominantly function through facilitative diffusion. T ere are 43 SLC gene families identified, and many of the nearly 300 genes comprising the 43 distinct SLC families play important roles in the disposition of endogenous compounds, including glucose, neurotransmitters, nucleotides, essential metals, and peptides. Additionally, there are several families that are vital to xenobiotic disposition, regulating the movement of many diverse organic anions and cations across cell membranes. Organic-anion transporting peptides (OATPs, SLCO family) are one of the major SLCs involved in xenobiotic disposition in humans. OATPs are of particular importance in the liver and kidneys and mediate the sodium-independent transport of a wide range of compounds, including organic bases, acids, and neutral compounds. Peptide transporters (PEPTs) are responsible for the transport of di- and tri-peptides as well as drugs and toxicants such as the β-lactam antibiotics. Finally, the multidrug and toxin extrusion (MATE) transporters are a unique gene family of SLCs expressed predominantly in liver and kidney that function specifically as cation ef ux pumps.

Additional Transport Processes—Other forms of specialized transport (e.g., phagocytosis and pinocytosis used by cell membranes to engulf particles) have been proposed, but their overall importance is not as well established as that of active transport and facilitated diffusion.

ABSORPTION

T e process by which toxicants cross body membranes and enter the bloodstream is referred to as absorption. T ere are no specific systems or pathways for the sole purpose of absorbing toxicants, and xenobiotics penetrate membranes in the same

TABLE 5–2 Major members of the human solute carrier transporter families involved in xenobiotic disposition.

Transporter	Gene Family	Human Proteins	Gene Name	Function
Organi- anion transporting polypeptide (OATP)	SLCO	OATP1A2	SLCO1A2	Transport of organic anions, cations, and neutral compounds
		OATP1B1	SLCO1B1	
		OATP1B3	SLCO1B3	
		OATP1C1	SLCO1C1	
		OATP2A1	SLCO2A1	
		OATP2B1	SLCO2B1	
		OATP3A1	SLCO3A1	
		OATP4A1	SLCO4A1	
		OATP4C1	SLCO4C1	
		OATP5A1	SLCO5A1	
		OATP6A1	SLCO6A1	
Organic-cation transporter (OCT)	SLC22	OCT1	SLC22A1	Transport of organic cations
		OCT2	SLC22A2	
		OCT3	SLC22A3	
Organic-cation/carnitine transporter (OCTN)	SLC22	OCTN1	SLC22A4	Organic cations;
		OCTN2	SLC22A5	OCTN2 specific for carnitine
Organic-anion transporter (OAT)	SLC22	OAT1	SLC22A6	Transport of organic anions
		OAT2	SLC22A7	
		OAT3	SLC22A8	
		OAT4	SLC22A11	
		OAT5	SLC22A10	
Peptide transporter (PEPT)	SLC15	PEPT1	SLC15A1	Transport of di- and tripeptides, some xenobiotics
		PEPT2	SLC15A2	
Multidrug and toxin extrusion transporter (MATE)	SLC47	MATE1	SLC47A1	Ef ux of organic cations;
		MATE2K	SLC47A2	MATE2K localized to kidney

way as biologically essential substances such as oxygen and foodstuffs. T e main sites of absorption are the gastrointestinal (GI) tract, lungs, and skin. Enteral administration includes all routes pertaining to the alimentary canal (sublingual, oral, and rectal), whereas parenteral administration involves all other routes (intravenous, intraperitoneal, intramuscular, subcutaneous, etc.).

Absorption of Toxicants by the Gastrointestinal Tract

Many environmental toxicants enter the food chain and are absorbed together with food from the GI tract. T is site of absorption is also particularly relevant to toxicologists because accidental ingestion is the most common cause of unintentional exposure to a toxicant (especially for children) and intentional overdoses most frequently occur via the oral route.

T e GI tract may be viewed as a tube traversing the body. Although within the body, GI contents can be considered exterior to the body. Unless a noxious agent has caustic or irritating properties, poisons in the GI tract usually do not produce systemic injury to an individual until they are absorbed into the blood stream.

Absorption of toxicants can take place along the entire GI tract, even in the mouth and rectum. If a toxicant is an organic acid or base, it tends to be absorbed by simple diffusion in the

part of the GI tract in which it exists in the most lipid-soluble (nonionized) form. Recall that the degree of ionization is, in part, determined by the pH of its solution according to the Henderson–Hasselbalch equation. Consequently, the lipid solubility of weak organic acids or bases can differ markedly in the GI tract because gastric juice is acidic (pH ~ 2) and the intestinal contents are nearly neutral (pH ~ 7). Other factors that influence the absorption of weak organic acids or bases include the mass action law, intestinal surface area, and blood flow rate. Continuous circulation of blood will help keep blood concentrations low, thereby maintaining a concentration gradient favoring absorption. T e villi and microvilli of the small intestine contribute to an approximate 600-fold increase in surface area, which greatly facilitates absorption.

T e mammalian GI tract has specialized transport systems (carrier mediated) for the absorption of nutrients and electrolytes (Table 5–3). T e number of toxicants actively absorbed by the GI tract is low; most enter the body by simple diffusion. Lipid-soluble substances are absorbed by this process more rapidly and extensively than are water-soluble substances.

Particulate matter can also be absorbed by the GI epithelium. In this case, particle size determines absorption rate, whereas factors such as lipid solubility and ionization characteristics are less important. Particle size is inversely related to absorption rate such that absorption increases with decreasing particle diameter. Large particles enter the GI epithelium by pinocytosis. T ere is increasing interest in particles of very small diameter (less than 100 nm) called nanoparticles or nanomaterials that may be used in a variety of chemical and biological processes (Chapter 28).

Overall, the absorption of a toxicant from the GI tract depends on its physical properties, including lipid solubility and its dissolution rate. An increase in lipid solubility typically increases the absorption of chemicals and the dissolution rate is inversely proportional to particle size.

In addition to the characteristics of the compounds themselves, there are numerous additional factors relating to the GI tract itself that influence the absorption of xenobiotics, including pH, the presence of food, digestive enzymes, bile acids, bacterial microflora, and the motility and permeability of the GI tract.

Chemical resistance or lack of resistance to alteration by the acidic pH of the stomach, enzymes of the stomach or intestine, or the intestinal microflora are extremely important. For example, a toxicant may be hydrolyzed by stomach acid, biotransformed by enzymes in the GI tract, or modified by the resident microflora to new compounds with a toxicity different from that of the parent compound. Simple diffusion is proportional not only to surface area and permeability but also to the residency time within various segments of the GI tract such that longer residencies lead to increased absorption and vice versa.

Experiments have shown that the oral toxicity of some chemicals is increased by diluting the dose. T is phenomenon may be explained by more rapid stomach emptying induced by increased dosage volume, which in turn leads to more rapid absorption in the duodenum because of the larger surface area there.

TABLE 5–3 Site distribution of specialized transport systems in the intestine of man and animals.

	Location of Absorptive Capacity in Small Intestine			
Substrates	**Upper**	**Middle**	**Lower**	**Colon**
Sugar (glucose, galactose, etc.)	++	+++	++	0
Neutral amino acids	++	+++	++	0
Basic amino acids	++	++	++	?
Gamma globulin (newborn animals)	+	++	+++	?
Pyrimidines (thymine and uracil)	+	+	?	?
Triglycerides	++	++	+	?
Fatty acid absorption and conversion to triglyceride	+++	++	+	0
Bile salts	0	+	+++	
Vitamin B_{12}	0	+	+++	0
Na^+	+++	++	+++	+++
H^+ (and/or HCO_3^- secretion)	0	+	++	++
Ca^{2+}	+++	++	+	?
Fe^{2+}	+++	++	+	?
Cl^-	+++	++	+	0

T e amount of a chemical entering the systemic circulation after oral administration depends on the amount absorbed into the GI cells, biotransformation by the GI cells, and extraction by the liver into bile (with or without biotransformation). Transporters can influence this amount by affecting the uptake or ef ux from the cells. T is phenomenon of the removal of chemicals before entrance into the systemic circulation is referred to as presystemic elimination, or *first-pass effect*. Chemicals that have a high first-pass effect will appear to have a lower absorption because they are eliminated as quickly as they are absorbed.

A number of other factors have been shown to alter absorption. For example, heavy metal ions such as lead are not readily absorbed from the GI tract. However, EDTA and other chelators increase the lipid solubility of heavy metals and, thus, absorption of complexed ions. Consumption of grapefruit juice can also influence GI absorption through the actions of naringin, a flavonoid that can increase absorption (though inhibition of MDR1) or decrease absorption (through inhibition of OATPs) of numerous pharmaceutical agents.

Absorption of Toxicants by the Lungs

Toxicants absorbed by the lungs are usually gases, vapors of volatile or volatilizable liquids, and aerosols. Anatomical and physiologic details of the respiratory system are described in Chapter 15.

Gases and Vapors—A vapor is the gas form of a substance that can also exist as a liquid or a solid at atmospheric pressure and normal temperature. Most organic solvents evaporate and produce vapors, and some solids can sublimate directly into a gaseous form. Vapor pressure is that exerted by a vapor above its own liquid in a closed system, such that liquids that have a high vapor pressure have a higher tendency to evaporate. A toxicant with a high vapor pressure at room temperature is considered to be volatile.

T e absorption of inhaled gases takes place mainly in the lungs. However, before a gas reaches the lungs, it passes through the nose. Because the mucosa of the nose is covered by a film of fluid, gas molecules can be retained by the nose and not reach the lungs if they are very water soluble or react with cell surface components. T erefore, the nose acts as a "scrubber" for water-soluble gases and highly reactive gases. Although this may serve to reduce systemic exposure or to protect the lungs, it also increases the risk that the nose could be adversely affected.

When a gas is inhaled into the lungs, gas molecules diffuse from the alveolar space into the blood until equilibrium is reached (i.e., no net movement of inhaled gas between the alveolar space and blood). At equilibrium, the concentration of gas in the alveolar space can be, and often is, different from the concentration of gas in the blood. T is difference is accounted for by Henry's law, which states the concentration in the blood (or any fluid) is directly proportional to the partial vapor pressure of the inhaled gas. While the concentration varies with partial vapor pressure, the ratio of the concentration in the blood to the concentration in the alveolar space is constant. For example, the atmospheric pressure is much lower at the peak of Mt. Everest (~ 252 mm Hg) than it is at sea level (760 mm Hg). T e partial vapor pressure of oxygen is likewise lower atop Mt. Everest (~53 mm Hg) than at sea level (160 mm Hg), but the blood-to-gas partition coefficient would remain the same regardless of elevation. T is solubility ratio is called the blood-to-gas partition coef cient and it is unique for each gas.

Absorption of gasses in the lungs differs from intestinal and percutaneous absorption in that the ionization state and the lipid solubility of molecules are less important factors in pulmonary absorption. T is is because diffusion through cell membranes is not normally rate-limiting in the pulmonary absorption of gases. T e rate of absorption of gases in the lungs is variable and depends on a toxicant's solubility ratio (concentration in blood/concentration in gas phase) at equilibrium. A gas with a high solubility ratio (e.g., > 1) is readily transferred to the blood during each respiratory cycle so that little if any remains in the alveoli just before the next inhalation. Conversely, a gas with a low solubility ratio (e.g., < 1) means the blood is quickly saturated with this gas and a higher concentration of the gas remains in the alveolar space. Gases with high solubility ratios are said to be ventilation-limited because the rate and depth of respiration determine the extent of distribution of the gas. T e rate of blood flow is the primary determinant of distribution for gasses with low solubility ratios and they are, therefore, considered perfusion-limited.

T e blood carries dissolved gas molecules to the rest of the body. In each tissue, gas molecules are transferred from the blood to the tissue until equilibrium is reached. After releasing part of the gas to tissues, blood returns to the lungs to take up more of the gas. T e process continues until a gas reaches equilibrium between blood and each tissue. At this time, no net absorption of gas takes place as long as the exposure concentration remains constant. Of course, if biotransformation and excretion occur, alveolar absorption will continue until a corresponding steady state is established. T e lung can also potentially contribute to the biotransformation or elimination of chemicals before their entrance into the systemic circulation.

Aerosols and Particles—**a erosols are a colloid of solid particles and liquid droplets in air.** T e major characteristics that affect absorption after exposure to aerosols are the aerosol size and water solubility of any chemical present in the aerosol (Chapter 15). T e site of deposition of aerosols depends largely on the size of the particles. In general, the smaller the particle, the further into the respiratory tree the particle will deposit. Particles 5 μm or larger usually are deposited in the nasopharyngeal region (Figure 5–4) and are removed by nose wiping, blowing, or sneezing. T e mucous blanket of the ciliated nasal surface propels insoluble particles by the movement of the cilia. T ese particles and particles inhaled through the mouth are swallowed within minutes. Soluble particles may dissolve in the

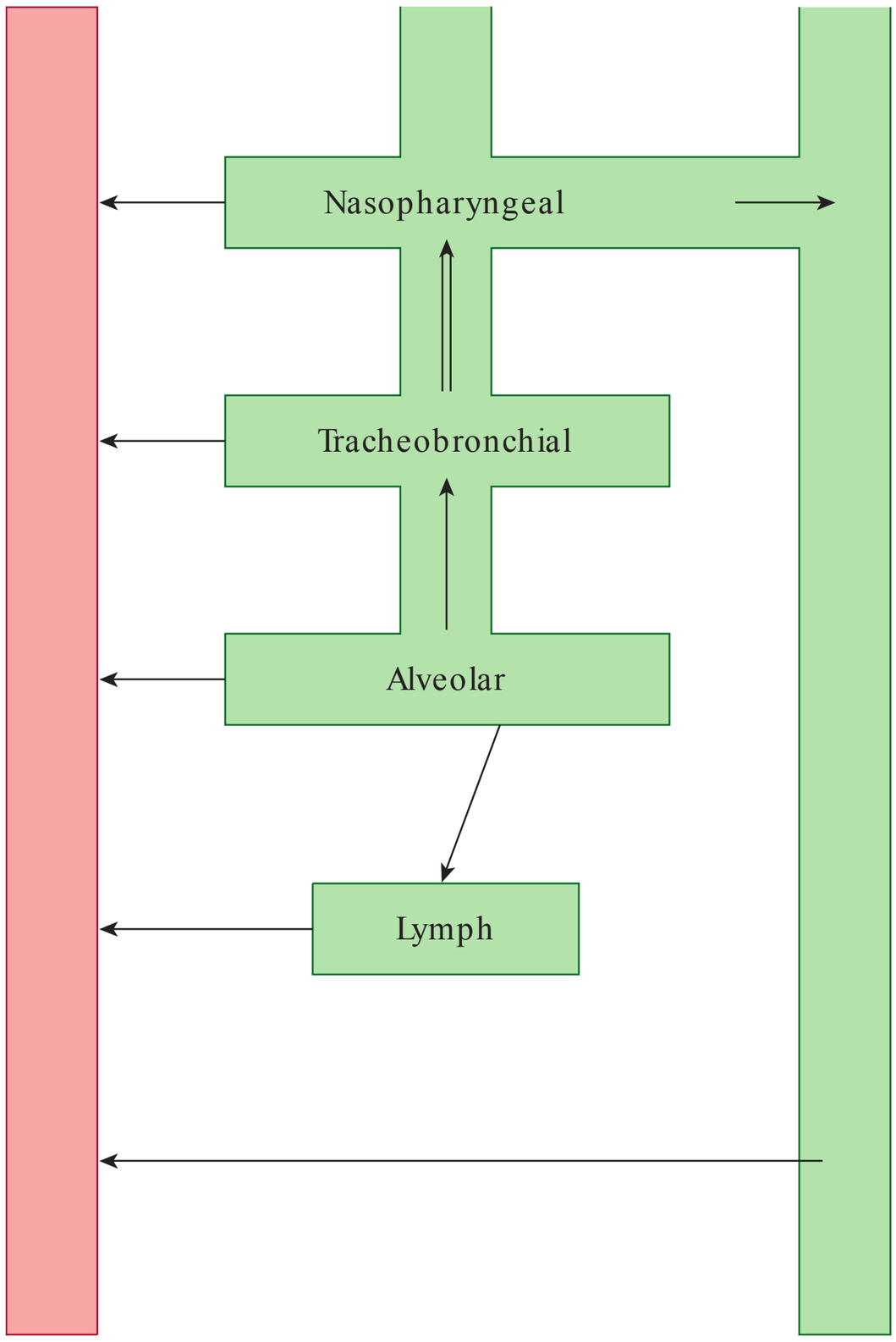

FIGURE 5–4 **Schematic diagram of the absorption and translocation of chemicals by lungs.**

mucus and be carried to the pharynx or may be absorbed through the nasal epithelium into blood.

Particles approximately 2.5 μm are deposited mainly in the tracheobronchiolar regions of the lungs, from which they are cleared by retrograde movement of the mucus layer in the ciliated portions of the respiratory tract. Particles eventually may be swallowed and absorbed from the GI tract. Toxicants or viral infections that damage cilia may impair the efficiency of this process.

Particles 1 μm and smaller penetrate to the alveolar sacs of the lungs. Ultrafine or nanoparticles, particularly those that are approximately 10 to 20 nm in size, have the greatest likelihood of depositing in the alveolar region. T ey may be absorbed into blood or cleared through the lymphatics after being scavenged by alveolar macrophages.

As particle size decreases, the number of potential particles in a unit of space increases along with the total surface area of the particles. T is relationship indicates that nanoparticles have the propensity to deliver a high amount of particulate to the lungs. However, it appears that the surface properties of nanoparticles may be more important determinants of toxic potential than their size or surface area.

Removal or absorption of particulate matter from the alveoli appears to occur by three major mechanisms. First, particles may be removed from the alveoli by a physical process. It is thought that particles deposited on the fluid layer of the alveoli are aspirated onto the mucociliary escalator of the tracheobronchial region. From there, they are transported to the mouth and may be swallowed. Second, particles from the alveoli may be removed via phagocytosis by the alveolar macrophages. T ese cells are found in large numbers in normal lungs and contain many phagocytized particles of both exogenous and endogenous origin. T ey migrate to the distal end of the mucociliary escalator and are cleared and eventually swallowed. T ird, removal may occur via the lymphatics, although particulates may remain in lymphatic tissue for long time periods.

In general, the overall removal of particles from the alveoli is relatively inefficient. T e rate of clearance by the lungs can be predicted by a compound's solubility in lung fluids such that lower solubility means lower clearance rate. T us, removal of particles that enter the alveoli is largely due to dissolution and vascular transport. Some particles may remain in the alveoli indefinitely, as is the case when alveolar macrophages phagocytose indigestible dust particles.

Absorption of Toxicants through the Skin

Skin is the largest body organ and provides a relatively good barrier for separating organisms from their environment. Human skin comes into contact with many toxic chemicals, but exposure is usually limited by its relatively impermeable nature. However, some chemicals can be absorbed by the skin in sufficient quantities to produce systemic effects.

T e skin comprises two major layers, the epidermis and dermis (Figure 5–5). T e epidermis is composed of four or five layers (called strata), depending on location. T e stratum corneum is the outermost layer and is unique in that it represents the single most important barrier to preventing fluid loss from the body while also serving as the major barrier to prevent absorption of xenobiotics into the body. T e dermis is situated beneath the epidermis and consists primarily of fibroblasts, which are cells responsible for synthesizing the collagen and extracellular matrix components of the dermis. A vascular network that provides both the dermis and epidermis with blood supply is also contained within the dermis. Although the stratum corneum is the major barrier to absorption, compounds may also be absorbed through dermal appendages (sweat glands, sebaceous glands, and hair follicles) found within the dermis.

To be absorbed through the skin, a chemical first pass through the stratum corneum and then traverse the other six layers of the skin. All toxicants move across the stratum corneum by passive diffusion. Lipophilic compounds are generally absorbed quickly and in a manner proportional to their lipid solubility, but inversely related to molecular weight. Hydrophilic compounds are absorbed more slowly across the stratum corneum, and they are, therefore, more likely to penetrate through dermal appendages.

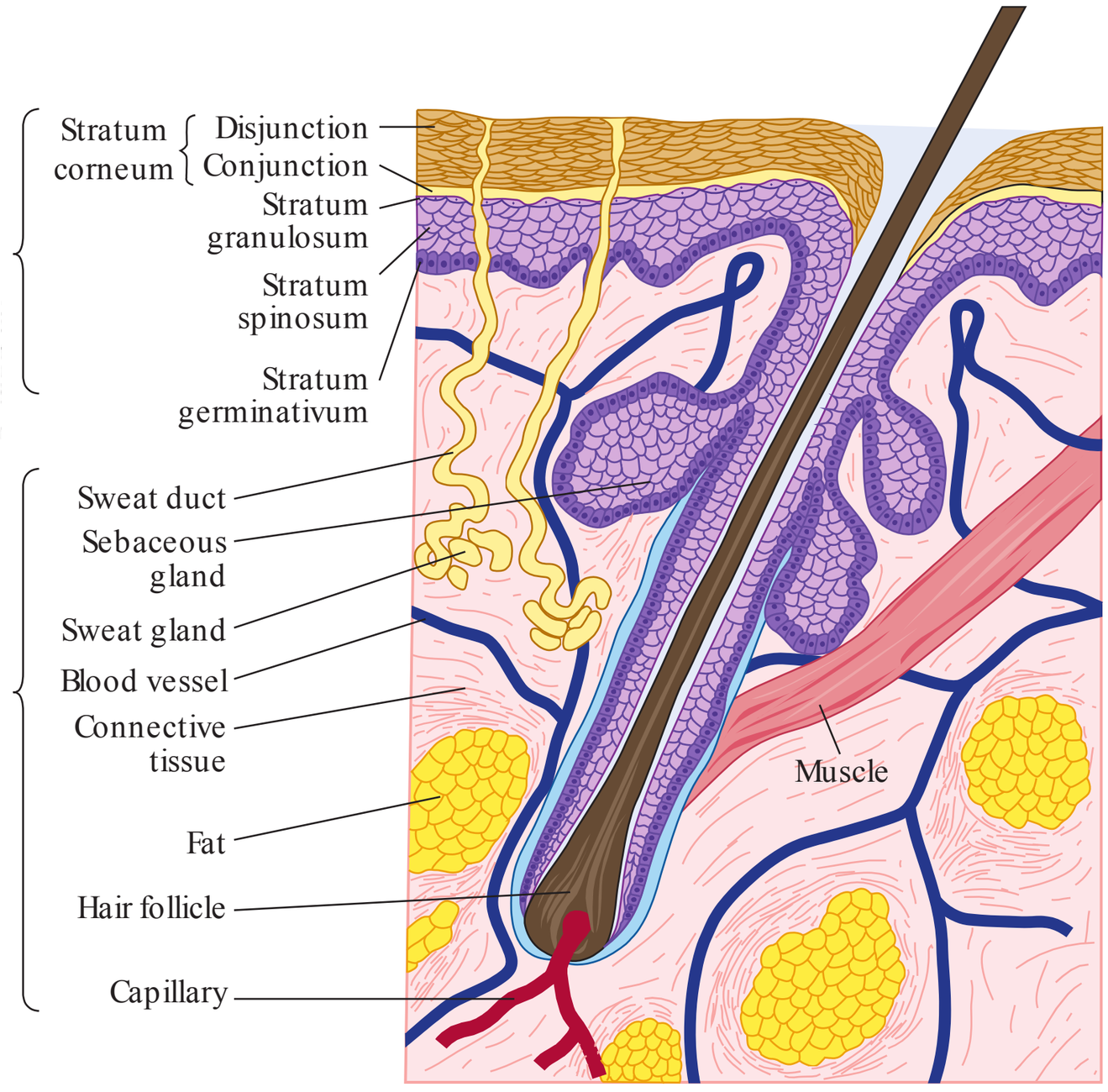

FIGURE 5–5 **Diagram of a cross-section of human skin.**

T e permeability of the skin depends on both the coefficient of diffusion ("diffusivity") and the thickness of the stratum corneum. T e second phase of absorption consists of diffusion through the lower layers of the epidermis and dermis and subsequent entry into systemic circulation through the vasculature of the dermis. T e rate of diffusion here primarily depends on blood flow and interstitial fluid movement.

Several factors that influence the absorption of toxicants through the skin include (1) the integrity of the stratum corneum, (2) the hydration state of the stratum corneum, (3) ambient temperature, (4) solvents as carriers, and (5) molecular size. Absorption is increased by decreasing size, and it is generally recognized that compounds above 400 Da exhibit poor dermal absorption. Paradoxically, the overall absorption of most nanoparticles is relatively low.

Dermal absorption has been studied in most laboratory animals, including rats, mice, rabbits, guinea pigs, primates, and pig, and it has been found to vary widely between species. For instance, dermal absorption across rodent skin is greater than human skin, whereas the cutaneous permeability characteristics of guinea pigs, pigs, and monkeys are more similar to those observed in humans. Inter-species differences in dermal absorption of xenobiotics result from variance in (1) the composition and thickness of the stratum corneum along with the nature of dermal appendages, (2) cutaneous blood flow, (3) biotransformation reactions, and (4) the levels and patterns of xenobiotic transporters.

Absorption of Toxicants after Special Routes of Administration

Besides absorption through the skin, lungs, or GI tract, chemical agents can be administered through other routes, including (1) intravenous, (2) intraperitoneal, (3) subcutaneous, and (4) intramuscular. T e intravenous route introduces the toxicant directly into the bloodstream, eliminating the process of absorption. Intraperitoneal injection results in rapid absorption of xenobiotics because of the rich blood supply and the relatively large surface area of the peritoneal cavity. Intraperitoneally administered compounds are absorbed primarily through the portal circulation and therefore must pass through the liver before reaching other organs by way of systemic circulation. Subcutaneous and intramuscular injections are usually absorbed at slower rates but enter directly into the general circulation.

T e toxicity of a chemical may or may not depend on the route of administration. For example, if a toxicant is injected intraperitoneally, the compound may be completely extracted and biotransformed by the liver with subsequent excretion into the bile without gaining access to the systemic circulation. Any toxicant displaying the first-pass effect with selective toxicity for an organ other than the liver and GI tract is expected to be less toxic when administered intraperitoneally than when injected intravenously, intramuscularly, or subcutaneously because of extraction in the liver.

DISTRIBUTION

After gaining entry into the bloodstream, regardless of route of exposure, a toxicant may distribute to tissues throughout the body. T e rate of distribution to organs or tissues is determined primarily by blood flow and the rate of diffusion out of the capillary bed into the cells of a particular organ or tissue. T e final distribution depends largely on the affinity of a xenobiotic for various tissues.

Volume of Distribution

A key concept in understanding the disposition of a toxicant is its volume *of* distribution (Vd), which is defined as the volume in which the amount of drug would need to be uniformly dissolved in order to produce the observed blood concentration. T e total water in one's body accounts for approximately 60% of body weight and is partitioned into two main compartments: (1) intracellular water and (2) extracellular water. Extracellular water is further divided into interstitial water and plasma water. If a chemical distributes only to the plasma compartment (no tissue distribution), it has a high plasma concentration and a low Vd. In contrast, if a chemical distributes throughout the body (into both compartments), it has a low plasma concentration and a high Vd. T e distribution of toxicants is more complex than this, however, and strongly influenced by factors such as binding to and/or dissolution in fat, liver, and bone.

Some toxicants do not readily cross cell membranes and therefore have restricted distribution, whereas other toxicants rapidly pass through cell membranes and are distributed throughout the body. Some toxicants selectively accumulate in certain parts of the body as a result of protein binding, active transport, or high solubility in fat. T e target organ for toxicity may be the site of accumulation of a toxicant, but this is not always the case. If a toxicant accumulates at a site other than the target organ or tissue, the accumulation may be viewed as a protective process in that plasma levels and consequently the concentration of a toxicant at the site of action are diminished. However, because any chemical in a storage depot is in equilibrium with the free fraction (unbound) of toxicant in plasma, it is released into the circulation as the unbound fraction of toxicant is eliminated.

Storage of Toxicants in Tissues

Since only the free fraction (unbound) of a chemical is in equilibrium throughout the body, binding to or dissolving in certain body constituents greatly alters the distribution of a xenobiotic. Toxicants are often concentrated in a specific tissue, called a storage depot, which may or may not be their site of toxic action. Toxicants in storage depots are always in equilibrium with the free fraction in plasma, so that as a chemical is biotransformed or excreted from the body, more is released from the storage site. As a result, the biological half-life of stored compounds can be very long.

Plasma Proteins as Storage Depot—Several plasma proteins bind xenobiotics as well as some endogenous constituents of the body. As depicted in Figure 5–6, albumin is the major protein in plasma and it binds many different compounds compared to other proteins, such as globulins, lipoproteins, and glycoproteins.

Protein–ligand interactions occur primarily as a result of hydrophobic forces, hydrogen bonding, and van der Waals forces. Because of their high molecular weight, plasma proteins and the toxicants bound to them cannot cross capillary walls. Consequently, the fraction of toxicant bound to plasma proteins is not immediately available for distribution into the extravascular space or filtration by the kidneys. However, the interaction of a chemical with plasma proteins is a reversible process. As unbound chemical diffuses out of capillaries, bound chemical dissociates from the protein until the free fraction reaches equilibrium between the vascular space and the extravascular space. In turn, diffusion in the extravascular space to sites more distant from the capillaries continues, and the resulting concentration gradient causes continued dissociation of the bound fraction in plasma.

T e binding of toxicants to plasma proteins is an important concept in toxicology for two reasons. First, toxicity is typically manifested by the amount of a xenobiotic that is unbound. T erefore, a compound with a high degree of plasma protein binding may not show toxicity when compared to one that is less extensively bound to plasma proteins. Severe toxic reactions can occur if a toxicant is displaced from plasma proteins by another agent, increasing the free fraction of the toxicant in plasma. T is will result in an increased equilibrium concentration of the toxicant in the target organ, with the potential for toxicity. Xenobiotics can also compete with and displace endogenous compounds that are bound to plasma proteins, which can allow the endogenous compound to exert a toxic effect.

Plasma protein binding can also give rise to observed species differences in the disposition of toxicants. Factors that

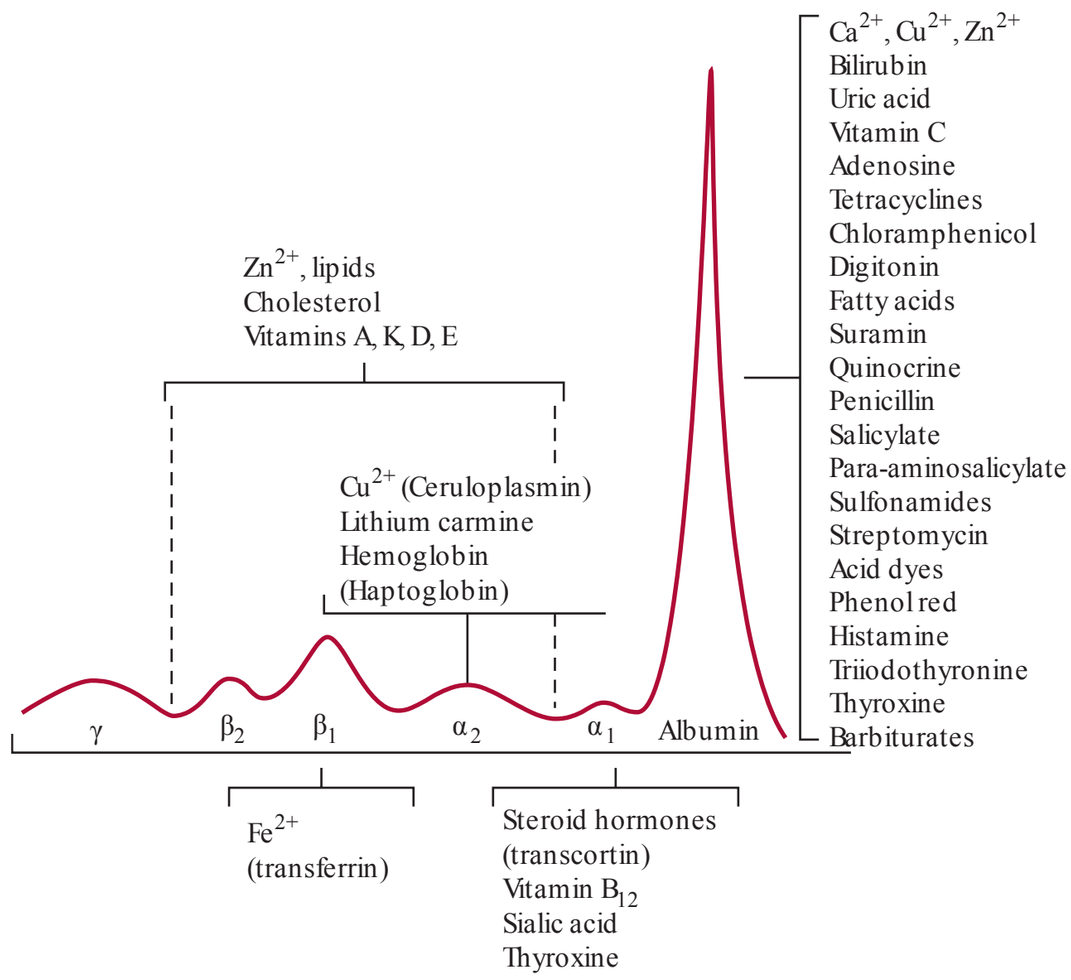

FIGURE 5–6 **Ligand interactions with plasma proteins.**

influence plasma protein binding across species include differences in the concentration of albumin, binding affinity, and/or competitive binding of endogenous substances.

Liver and Kidney as Storage Depots—T e liver and kidney have a high capacity for binding a multitude of chemicals. T ese two organs probably concentrate more toxicants than do all the other organs combined. In most cases, binding to tissue components is likely to be involved.

Fat as Storage Depot—Many highly lipophilic toxicants with a high lipid/water partition coefficient are distributed and concentrated in body fat. Storage lowers the concentration of the toxicant in the target organ; therefore, the toxicity of such a compound can be expected to be less severe in an obese person than in a lean individual. However, the possibility of a sudden increase in the concentration of a chemical in the blood and thus in the target organ of toxicity when rapid mobilization of fat occurs must be considered. Several studies have shown that signs of intoxication can be produced by short-term starvation of experimental animals that were previously exposed to persistent organochlorine insecticides.

Bone as Storage Depot—Skeletal uptake of xenobiotics is essentially a surface chemistry phenomenon, with exchange taking place between the bone surface of hydroxyapatite crystals and the extracellular fluid in contact with it. Deposition and reversible storage of toxicants in bone is dynamic and may or may not be detrimental. For instance, lead is not toxic to bone, but the chronic effects of fluoride deposition (skeletal fluorosis) and radioactive strontium (osteosarcoma and other neoplasms) are well documented.

Blood–Brain Barrier

Access to the brain is restricted by the presence of two barriers: the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB). Although neither represents an absolute barrier to the passage of toxic chemicals into the central nervous system (CNS), many toxicants do not enter the brain in appreciable quantities compared to other body tissues.

T ere are four major anatomical and physiologic reasons why some toxicants do not readily enter the CNS. First, the capillary endothelial cells of the CNS are tightly joined, leaving few or no pores between the cells, which prevents diffusion of polar compounds through paracellular pathways. Second, the capillaries in the CNS are to a large extent surrounded by glial cell processes (astrocytes), which secrete chemical factors that modulate endothelial permeability. T ird, the protein concentration in the interstitial fluid of the CNS is much lower than that in other body fluids, limiting the movement of water-insoluble compounds by paracellular transport, which is possible in a largely aqueous medium only when such compounds are bound to proteins. Fourth, ATP-dependent transporters include members of both ABC and SLC families (Tables 5–1 and 5–2). Ef ux transporters MDR1, BCRP, and MRP1, 2, 4, and 5 are located on the blood side of the capillary endothelium function to move xenobiotics back into the blood and hence limit their distribution into the brain.

T e blood cerebrospinal fluid (CSF) barrier is found between the circulating blood and the circulating CSF in the brain. Certain areas (the choroid plexus, the arachnoid membrane, and the area postrema) are more permeable on the blood side, whereas the epithelium on the CSF side is a barrier. In addition, ef ux transporters contribute to xenobiotic removal from the CSF thereby protecting against toxicant distribution into the CNS.

In general, only the free unbound toxicant equilibrates rapidly with the brain. Lipid solubility and the degree of ionization are important determinants of the rate of entry of a compound into the CNS. Increased lipid solubility enhances the rate of penetration of toxicants into the CNS, whereas ionization greatly diminishes it. A few xenobiotics appear to enter the brain by carrier-mediated processes. Some lipophilic compounds may enter the brain, but are so efficiently removed by these transporters that they never reach appreciable concentrations.

T e BBB is not fully developed at birth, and this is one reason why some chemicals are more toxic to newborns than to adults.

Passage of Toxicants across the Placenta

T e term "placental barrier" has been associated with the concept that the main function of the placenta is to protect the fetus against the passage of noxious substances from the mother. However, the placenta is a multifunctional organ that also provides nutrition, exchanges maternal and fetal blood gases, disposes of fetal excretory material, and maintains pregnancy through complex hormonal regulation. Placental structure and function show more species differences than any other mammalian organ.

Most vital nutrients for fetal development, including vitamins, amino acids, essential sugars, iron, and calcium, are transported by active transport systems from mother to fetus. Many foreign substances can cross the placenta, and the same factors that dictate the passage of xenobiotics across other biological membranes are important determinants of placental transfer. T ese include previously discussed attributes including the degree of ionization, lipophilicity, protein binding, molecular weight, blood flow, and the concentration gradient across the placenta. Among the substances that cross the placenta by passive diffusion, more lipid-soluble substances attain a maternal–fetal equilibrium more rapidly. Under steady-state conditions, the concentrations of a toxic compound in the plasma of the mother and fetus are usually the same. T e concentration in the various tissues of the fetus depends on the ability of fetal tissue to concentrate a toxicant. Differential body composition between mother and fetus may be another reason for an apparent placental barrier. For example, fetuses have very little fat; hence, they do not accumulate highly lipophilic chemicals.

Besides chemicals, viruses (e.g., rubella virus), cellular pathogens (e.g., syphilis spirochetes), and globulin antibodies

can traverse the placenta. In this regard, the placental barrier is not as precise an anatomical unit as the BBB. Anatomically, the placental barrier consists of a number of cell layers—at most six—interposed between the fetal and maternal circulations. Active transport systems and biotransformation enzymes are differentially expressed through the cell layers. T ese help protect the fetus from some xenobiotics while regulating the movement of essential nutrients.

Redistribution of Toxicants

T e most critical factors that affect the distribution of xenobiotics are the organ blood flow and its affinity for a xenobiotic. T e initial phase of distribution is determined primarily by blood flow to the various parts of the body. T erefore, a well-perfused organ such as the liver may attain high initial concentrations of a xenobiotic. However, chemicals may have a high affinity for a binding site (e.g., intracellular protein or bone matrix) or to a cellular constituent (e.g., fat), and, with time, will redistribute to these high-affinity sites.

EXCRETION

Toxicants are eliminated from the body by several routes. Many xenobiotics, though, have to be biotransformed to more water-soluble products before they can be excreted into urine (Chapter 6). All body secretions appear to have the ability to excrete chemicals; toxicants have been found in sweat, saliva, tears, and milk.

Urinary Excretion

Toxic compounds are excreted into urine by the same mechanisms the kidney uses to remove end products of intermediary metabolism from the body: glomerular filtration, tubular excretion by passive diffusion, and active tubular secretion. (See Chapter 14 for greater discussion of renal anatomy and physiology). Compounds up to a molecular weight of about 60 kDa are filtered at the glomeruli. T e degree of plasma protein binding affects the rate of filtration, because protein–xenobiotic complexes are too large to pass through the pores of the glomeruli.

A toxicant filtered at the glomeruli may remain in the tubular lumen and be excreted with urine or may be reabsorbed across the tubular cells of the nephron back into the bloodstream. Toxicants with a high lipid/water partition coefficient are reabsorbed efficiently, whereas polar compounds and ions are excreted with urine. T e pH of urine may vary but is usually slightly acidic (~6.0 to 6.5). Just as the Henderson–Hasselbalch calculations determine the absorption of nonionized compounds from the GI tract, they also determine urinary excretion. In this case, urinary excretion of the ionized moiety is favored, such that bases are excreted to a greater extent at lower pH whereas excretion of acids predominates at higher urinary pH.

Toxic agents can also be excreted from plasma into urine by passive diffusion through the tubule. T is process is probably of minor significance because filtration is much faster than excretion by passive diffusion through the tubules, providing a favorable concentration gradient for reabsorption rather than excretion.

Xenobiotics can also be excreted into urine by active secretion. T is process involves the uptake of toxicants from blood into the cells of the renal proximal tubule, with subsequent ef ux from the cell into the tubular fluid from which urine is formed. Figure 5–7 illustrates the various families of transporters expressed in the human kidney that are directly involved in xenobiotic disposition. T ere are numerous other transporters such as specific glucose transporters or nucleotide transporters

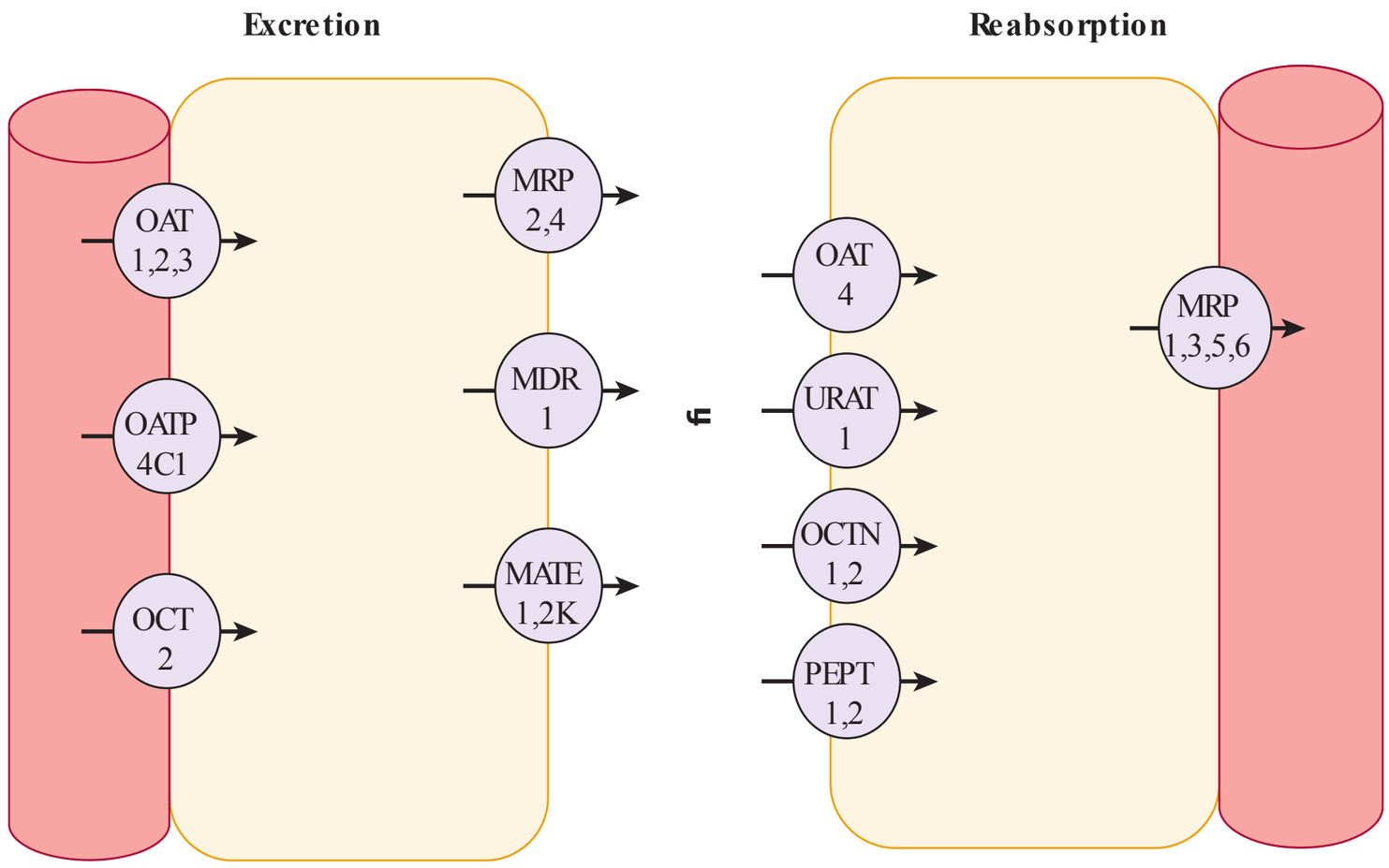

FIGURE 5–7 **Schematic model showing the transport systems in the proximal tubule of the kidney.** The families of transporters are organic-anion transporters (OAT), organic-cation transporters (OCT), multidrug-resistant protein (MDR), multidrug resistance-associated protein (MRP), peptide transporters (PEP), and urate transporter (URAT).

that play a role predominantly in the flux of endogenous substances that are not presented here. Transporters may be expressed on the apical cell membrane where ef ux pumps contribute to tubular secretion and influx pumps are important for reabsorption. Transporters localized to the basolateral membranes serve to transport xenobiotics to and from the systemic circulation or the renal tubular cells and also contribute to reabsorptive and excretory processes. Specific transporters expressed on the basolateral side of renal tubules in humans include OATs, OCTs, OATP4C1, and a subset of MRPs. Brush border transporters include MRPs, MDRs, MATEs, URATs, PEPTs, and OAT4.

Because many functions of the kidney are incompletely developed at birth, some xenobiotics are eliminated more slowly in newborns than in adults and therefore may be more toxic to newborns. For example, the clearance of penicillin by premature infants is only about 20% of that observed in older children. T e renal proximal tubule reabsorbs small plasma proteins that are filtered at the glomerulus. A toxicant binding those small proteins can be carried into the proximal tubule cells and exert toxicity.

Species differences in urinary excretion can be explained by variance in the pH urine, differences in plasma protein binding, and xenobiotic transporter expression, regulation, and function.

Fecal Excretion

Fecal excretion is the second major pathway for the elimination of xenobiotics from the body. Many chemicals in feces directly transfer from blood into the intestinal contents by passive diffusion. In some instances, rapid exfoliation of intestinal cells may contribute to the fecal excretion of some compounds. Intestinal excretion is a relatively slow process that is a major pathway of elimination only for compounds that have low rates of biotransformation and/or low renal or biliary clearance.

Nonabsorbed Ingesta—In addition to indigestible material, varying proportions of nutrients and xenobiotics that are present in food or are ingested voluntarily (drugs) pass through the alimentary canal unabsorbed, contributing to fecal excretion.

Mucosal biotransformation and reexcretion into the intestinal lumen occur with many compounds. It has been estimated that 30% to 42% of fecal dry matter originates from bacteria. Moreover, a considerable proportion of fecally excreted xenobiotic is associated with excreted bacteria. However, chemicals may be profoundly altered by bacteria before excretion with feces. It seems that biotransformation by intestinal flora favors reabsorption rather than excretion. Nevertheless, there is evidence that in many instances xenobiotics found in feces derive from bacterial biotransformation.

Biliary Excretion—T e biliary route of elimination is perhaps the most important contributing source to the fecal excretion of xenobiotics and their metabolites. Hepatic anatomy and physiology and bile formation are discussed in greater detail in Chapter 13. To summarize, nutrients and xenobiotics in portal venous blood from the GI tract are available for uptake by the liver or passage into the systemic circulation. T e liver can extract compounds from blood and prevent their distribution to other parts of the body. Furthermore, the liver is the main site of biotransformation of toxicants, and the metabolites thus formed may be excreted directly into bile or into the hepatic venous blood for systemic distribution. Xenobiotics and/or their metabolites entering the intestine with bile may be excreted with feces or undergo an enterohepatic circulation.

Figure 5–8 illustrates the many transporters localized on hepatic parenchymal cells that move foreign substances from plasma into liver and from liver into bile. Biliary excretion is regulated predominantly by xenobiotic transporters present on the canalicular membrane. Sodium-dependent taurocholate peptide (ntcp) present on the sinusoidal side of the parenchymal cell transports bile acids such as taurocholate into the liver, whereas the bile salt excretory protein (bsep) transports bile acids out of the liver cell into the bile canaliculi. T e sinusoidal membrane of the hepatocyte has a number of transporters including organic-anion transporting polypeptide (oatp) 1 and 2, and oct that move xenobiotics into the liver. Once inside the hepatocyte, the xenobiotic itself can be transported into the blood or bile, or be biotransformed by phase I and II drug-metabolizing enzymes to more water-soluble products that are then transported into the bile or back into the blood. Multidrug-resistant protein one (mdr1) and multidrug resistance–associated protein two (mrp2) are responsible for transporting xenobiotics into bile, whereas mrp3 and mrp6 transport xenobiotics back into the blood.

An important concept relating to biliary excretion is the phenomenon of enterohepatic circulation. Once a compound is excreted into bile and enters the intestine, it can be reabsorbed or eliminated with feces. Many organic compounds are conjugated before excretion into bile. Such polar metabolites are not sufficiently lipid soluble to be reabsorbed. However,

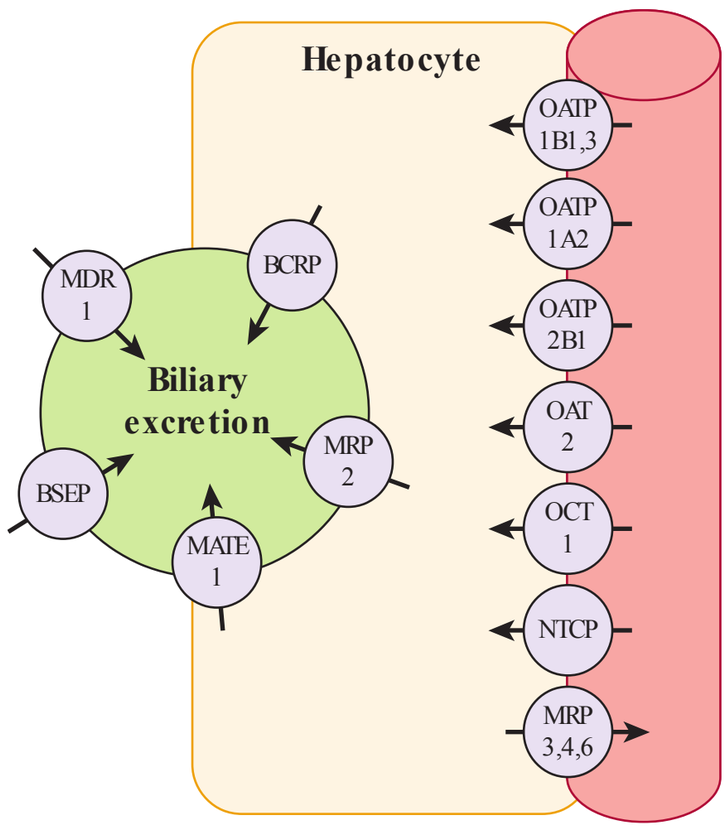

FIGURE 5–8 **Schematic model showing the transport systems in the liver.** OATP = organic-anion transporting polypeptide, OCT = organic-cation transporter, BSEP = bile salt excretory protein, MDR = multidrug-resistant protein, MRP = multidrug resistance-associated protein, BCRP = breast cancer resistance protein, and NTCP = sodium-dependent taurocholate peptide.

intestinal microflora may hydrolyze glucuronide and sulfate conjugates, making them sufficiently lipophilic for reabsorption and enterohepatic cycling. T is principle has been utilized in the treatment of dimethylmercury poisoning; ingestion of a polythiol resin binds the mercury and thus prevents its reabsorption and cycling.

Exhalation

Substances that exist predominantly in the gas phase at body temperature and volatile liquids are eliminated mainly by the lungs. Because volatile liquids are in equilibrium with their gas phase in the alveoli, they may also be excreted via the lungs. T e amount of liquid eliminated via the lungs is proportional to its vapor pressure. A practical application of this principle is seen in the breath analyzer test for determining the amount of ethanol in the body.

No specialized transport systems have been described for the excretion of toxic substances by the lungs. Some xenobiotic transporters, including MRP1 and MDR1, have been identified in the lung, but overall, compounds seem to be eliminated by simple diffusion. Elimination of gases is roughly inversely proportional to the rate of their absorption. T e rate of elimination of a gas with low solubility in blood is perfusion-limited, whereas that of a gas with high solubility in blood is ventilation-limited.

Other Routes of Elimination

Cerebrospinal Fluid—All compounds can leave the CNS with the bulk flow of cerebrospinal fluid (CSF) through arachnoid villi, which allow fluid to flow from CSF to the venous system. In addition, lipid-soluble toxicants also can exit at the site of the BBB. Active transport using the transport systems present in the BCSFB can also remove toxicants.

Milk—T e secretion of toxic compounds into milk is extremely important because (1) a toxicant may be passed with milk from the mother to the nursing offspring and (2) compounds can be passed from cows to humans by way of dairy products. Toxic agents are excreted into milk by simple diffusion. Because milk is more acidic (pH ≈ 6.5) than plasma, basic compounds may be concentrated in milk, whereas acidic compounds may attain lower concentrations in milk than in plasma. About 3% to 4% of milk consists of lipids, and the lipid content of milk after parturition is even higher. Importantly, lipid-soluble xenobiotics diffuse along with fats from plasma into the mammary gland and are excreted with milk during lactation.

Sweat and Saliva—T e excretion of toxic agents in sweat and saliva is quantitatively of minor importance. Again, excretion depends on the diffusion of the nonionized, lipid-soluble form of an agent. Toxic compounds excreted into sweat may produce dermatitis (inflammation of the skin). Substances excreted in saliva enter the mouth, where they are usually swallowed to become available for GI absorption.

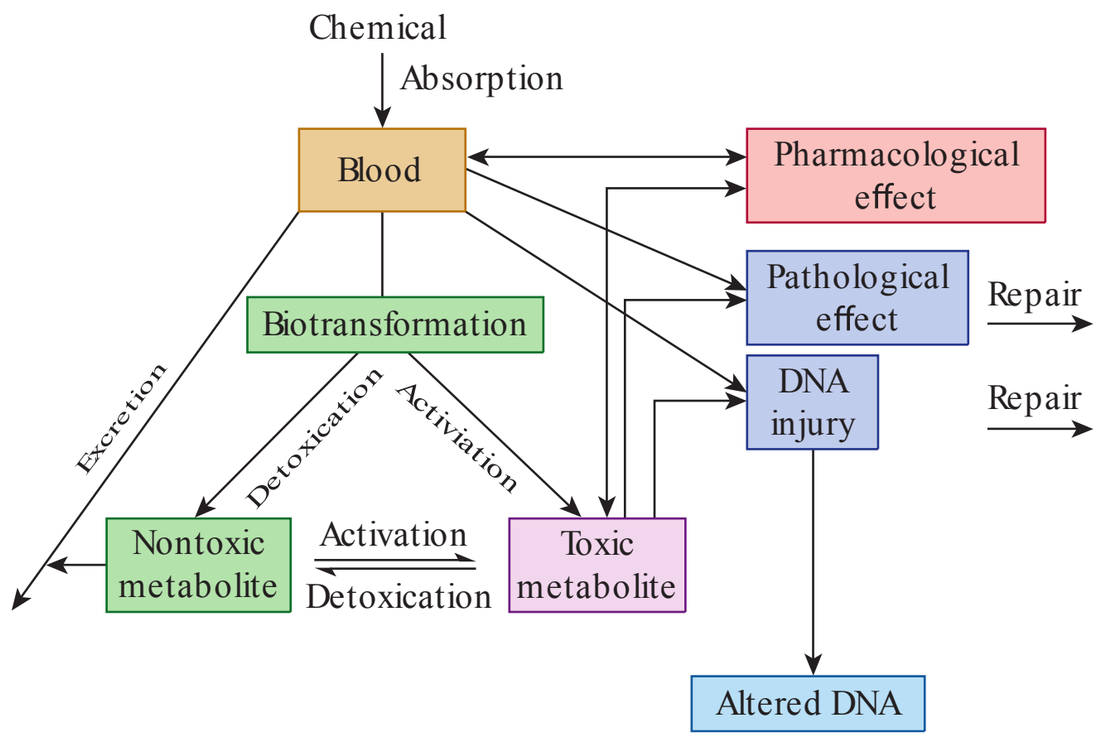

FIGURE 5–9 **Schematic representation of the disposition and toxic ef ects of chemicals.**

CONCLUSION

Humans are in continuous contact with toxic agents. Depending on their physical and chemical properties, toxicants may be absorbed by the GI tract, the lungs, and/or the skin. T e body has the ability to biotransform and excrete these compounds into urine, feces, and air. However, when the rate of absorption exceeds the rate of elimination, toxic compounds may accumulate, reaching a critical concentration at a target site, and toxicity may ensue (Figure 5–9). Whether a chemical elicits toxicity depends not only on its inherent potency and site specificity, but also on how an organism can dispose of that toxicant.

Many chemicals have very low inherent toxicity but have to be activated by biotransformation into toxic metabolites and the toxic response depends on the rate of production of toxic metabolites. Alternatively, a very potent toxicant may be detoxified rapidly by biotransformation. T e fundamental and overarching concept is that adverse toxic effects are related to the unbound concentration of "toxic chemical" at the site of action (in the target organ), whether a chemical is administered or generated by biotransformation in the target tissue or at a distant site. Accordingly, the toxic response exerted by chemicals is critically influenced by the rates of absorption, distribution, biotransformation, and excretion.

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QUESTIONS

1. Biotransformation is vital in removing toxins from the circulation. All of the following statements regarding biotransformation are true EXCEPT:
   - **a.** Many toxins must be biotransformed into a more lipid-soluble form before they can be excreted from the body.
   - **b.** T e liver is the most active organ in the biotransformation of toxins.
   - **c.** Water solubility is required in order for many toxins to be excreted by the kidney.
   - **d.** T e kidney plays a major role in eliminating toxicants from the body.
   - **e.** T e lungs play a minor role in ridding the body of certain types of toxins.

2. Which of the following statements about active transport across cell membranes is FALSE?
   - **a.** Unlike simple or facilitated diffusion, active transport pumps chemicals against an electrochemical or concentration gradient.
   - **b.** Unlike simple diffusion, there is a rate at which active transport becomes saturated and cannot move chemicals any faster.
   - **c.** Active transport requires the expenditure of ATP in order to move chemicals against electrochemical or concentration gradients.
   - **d.** Active transport exhibits a high level of specificity for the compounds that are being moved.
   - **e.** Metabolic inhibitors do not affect the ability to perform active transport.

3. Which of the following might increase the toxicity of a toxin administered orally?
   - **a.** increased activity of the mdr transporter (p-glycoprotein).
   - **b.** increased biotransformation of the toxin by gastrointestinal cells.
   - **c.** increased excretion of the toxin by the liver into bile.
   - **d.** increased dilution of the toxin dose.
   - **e.** increased intestinal motility.

4. Which of the following most correctly describes the first-pass effect?
   - **a.** T e body is most sensitive to a toxin the first time that it passes through the circulation.
   - **b.** Orally administered toxins are partially removed by the GI tract before they reach the systemic circulation.
   - **c.** It only results from increased absorption of toxin by GI cells.
   - **d.** It is often referred to as "postsystemic elimination."
   - **e.** A majority of the toxin is excreted after the first time the blood is filtered by the kidneys.

5. Which of the following is an important mechanism of removing particulate matter from the alveoli?
   - **a.** coughing.
   - **b.** sneezing.
   - **c.** blowing one's nose.
   - **d.** absorption into the bloodstream, followed by excretion via the kidneys.
   - **e.** swallowing.

6. For a toxin to be absorbed through the skin, it must pass through multiple layers in order to reach the systemic circulation. Which of the following layers is the most important in slowing the rate of toxin absorption through the skin?
   - **a.** stratum granulosum.
   - **b.** stratum spinosum.
   - **c.** stratum corneum.
   - **d.** stratum basale.
   - **e.** dermis.

7. A toxin is selectively toxic to the lungs. Which of the following modes of toxin delivery would most likely cause the LEAST damage to the lungs?
   - **a.** intravenous.
   - **b.** intramuscular.
   - **c.** intraperitoneal.
   - **d.** subcutaneous.
   - **e.** inhalation.

8. Which of the following is NOT an important site of toxicant storage in the body?
   a. adipose tissue.
   b. bone.
   c. plasma proteins.
   d. muscle.
   e. liver.

9. Which of the following regarding the blood–brain barrier is TRUE:
   a. T e brains of adults and newborns are equally susceptible to harmful blood-borne chemicals.
   b. T e degree of lipid solubility is a primary determinant in whether or not a substance can cross the blood–brain barrier.
   c. Astrocytes play a role in increasing the permeability of the blood–brain barrier.
   d. Active transport processes increase the concentration of xenobiotics in the brain.
   e. T e capillary endothelial cells of the CNS possess large fenestrations in their basement membranes.

10. Which of the following will result in DECREASED excretion of toxic compounds by the kidneys?
    a. a toxic compound with a molecular weight of 25,000 Da.
    b. increased activity of the multidrug-resistance (mdr) protein.
    c. increased activity of the multiresistant drug protein (mrp).
    d. increased activity of the organic cation transporter.
    e. increased hydrophilicity of the toxic compound.

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CHAPTER

6

Biotransformation of Xenobiotics

Andrew Parkinson, Brian W. Ogilvie, David B. Buckley, Faraz Kazmi, Maciej Czerwinski, and Oliver Parkinson

GENERAL PRINCIPLES

HYDROLYSIS, REDUCTION, AND OXIDATION

- Hydrolysis
  - Carboxylesterases, Cholinesterases, and Paraoxonase
  - Prodrugs and Alkaline Phosphatase
  - Peptidases
  - Epoxide Hydrolase
- Reduction
  - Azo- and Nitro-reduction
  - Carbonyl Reduction
  - Disulfide Reduction
  - Sulfoxide and N-Oxide Reduction
  - Quinone Reduction
  - Dehalogenation
- Oxidation
  - Alcohol Dehydrogenase
  - Aldehyde Dehydrogenase
  - Dihydrodiol Dehydrogenase
  - Molybdenum Hydroxylases
  - Xanthine Oxidoreductase
  - Aldehyde Oxidase
  - Monoamine Oxidase
  - Peroxidase-dependent Cooxidation
  - Flavin Monooxygenases
  - Cytochrome P450
  - Activation of Xenobiotics by Cytochrome P450
  - Inhibition of Cytochrome P450
  - Induction of Cytochrome P450

CONJUGATION

- Glucuronidation
- Sulfonation
- Methylation
- Acetylation
- Amino Acid Conjugation
- Glutathione Conjugation

KEY POINTS

- Biotrans*f*ormation is the metabolic conversion of endogenous and xenobiotic chemicals to more water-soluble compounds.
- Xenobiotic biotransformation is accomplished by a limited number of enzymes with broad substrate specificities.
- Phase I reactions involve hydrolysis, reduction, and oxidation. T ese reactions expose or introduce a functional group (—OH, —NH_2, —SH, or —COOH), and usually result in only a small increase in hydrophilicity.
- Phase II biotransformation reactions include glucuronidation, sulfonation (more commonly called sulfation), acetylation, methylation, and conjugation with glutathione (mercapturic acid synthesis), which usually result in increased hydrophilicity and elimination.

The enzymes that catalyze xenobiotic biotransformation are often called drug-metabolizing enzymes. The acronym ADME stands for absorption, distribution, metabolism, and elimination. This acronym is widely used in the pharmaceutical industry to describe the four main processes governing drug disposition. The acronym is sometimes extended to include drug transport (AMDET) or drug toxicity (ADME-Tox). This chapter describes some fundamental principles of xenobiotic biotransformation, and describes the major enzyme systems involved in the biotransformation (or metabolism) of drugs and other xenobiotics.

GENERAL PRINCIPLES

The following points, which might be considered principles or rules, apply in the majority of cases:

Point 1 Xenobiotic biotransformation or drug metabolism is the process of converting lipophilic (fat-soluble) chemicals, which are readily absorbed from the gastrointestinal tract and other sites, into hydrophilic (water-soluble) chemicals, which are readily excreted in urine or bile. There are exceptions even to this most basic rule. For example, acetylation and methylation are biotransformation reactions that can actually decrease the water solubility of certain xenobiotics.

Point 2 The biotransformation of xenobiotics is catalyzed by various enzyme systems that can be divided into four categories based on the reaction they catalyze: (1) hydrolysis (e.g., carboxylesterase); (2) reduction (e.g., carbonyl reductase); (3) oxidation (e.g., cytochrome P450 [CYP]); and (4) conjugation (e.g., UDP-glucuronosyltransferase [UGT]). The mammalian enzymes involved in the hydrolysis, reduction, oxidation, and conjugation of xenobiotics are listed in Table 6–1, together with their principal subcellular location.

Point 3 In general, individual xenobiotic-biotransforming enzymes are located in a single organelle. In Table 6–1, some enzymes are listed with two or more subcellular locations.

Point 4 In general, xenobiotic biotransformation is accomplished by a limited number of enzymes with broad substrate specificities. In humans, e.g., 2 CYP enzymes—namely, CYP2D6 and CYP3A4—metabolize over half the orally effective drugs in current use. Many of the enzymes involved in xenobiotic biotransformation are arranged in families and subfamilies and named according to nomenclature systems based on the primary amino acid sequence of the individual enzymes. The convention of using italic and regular letters to distinguish between the gene and gene products (mRNA and protein), respectively, and the convention of using lower case letters to designate mouse genes and gene products is not followed in this chapter.

The structure (i.e., amino acid sequence) of a given xenobiotic-biotransforming enzyme may differ among individuals, which can give rise to differences in rates of drug metabolism. The broad substrate specificity of xenobiotic-biotransforming enzymes makes them catalytically versatile but slow compared with most other enzymes (with the exception of hydrolytic reactions). The sequential oxidation, conjugation, and transport of a xenobiotic tend to proceed quicker at each subsequent step, which prevents the accumulation of intracellular metabolites. Were it not for the low catalytic turnover of CYP (one molecule of which may take several seconds or minutes to oxidize a single drug molecule), it would not be possible to achieve the once-a-day dosing characteristic of a large number of drugs.

Point 5 Hydrolysis, reduction, and oxidation expose or introduce a functional group (such as $-OH$, $-NH_2$, $-SH$, or $-COOH$) that can be converted to a water-soluble conjugate. The functional group introduced or exposed by hydrolysis, reduction, or oxidation must be nucleophilic (in the case of glucuronidation, sulfonation, methylation, acetylation, and conjugation with glycine or taurine) or electrophilic (in the case of glutathionylation). The first three reactions (hydrolysis, reduction, and oxidation) are often called Phase 1 reactions, and the conjugation reactions are often called Phase 2 reactions.

Point 6 Oxidation, reduction, hydrolysis, methylation, and acetylation generally cause a modest increase in the water solubility of a xenobiotic, whereas glucuronidation, sulfonation, glutathionylation, and amino acid conjugation generally cause a marked increase in hydrophilicity.

Point 7 Xenobiotics can undergo biotransformation both by enzymes that normally participate in intermediary (endobiotic) metabolism and by enzymes within gut microflora.

Point 8 Just as some xenobiotics are biotransformed by the so-called endobiotic-metabolizing enzymes (Point 7), certain endobiotics are biotransformed by the so-called xenobiotic-metabolizing enzymes. For example, the same CYP enzymes implicated in xenobiotic biotransformation also contribute to the hepatic catabolism of steroid hormones, and the same UGTs that conjugate xenobiotics also glucuronidate bilirubin, thyroid hormones, and steroid hormones. On a case-by-case basis, there is often no clear-cut distinction between endobiotic- and xenobiotic-biotransforming enzymes.

Point 9 Several xenobiotic-biotransforming enzymes are inducible, meaning their expression can be increased (upregulated) usually in response to exposure to high concentrations of xenobiotics. Xenobiotics can act as ligands for certain receptors (so-called xenosensors). Activated xenosensors (i.e., those bound to xenobiotics) interact with DNA-binding proteins which upregulate the transcription of various genes encoding for xenobiotic-biotransforming enzymes. The major xenosensors are aryl hydrocarbon receptor (AhR), which induces CYP1 enzymes, the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR), which induce CYP2B, 2C, and 3A enzymes, and the peroxisome proliferator–activated receptor-α (PPARα), which induces CYP4 enzymes.

Certain xenosensors are activated by endogenous ligands (e.g., bilirubin, bile acids, and fatty acids activate CAR, PXR, and PPARα, respectively). Induction is a reversible, adaptive response to xenobiotic exposure. Induction is also a pleiotropic response: activation of AhR, CAR, PXR, PPARα, and Nrf2 all results in alterations in the expression of numerous genes.

TABLE 6–1 General pathways of xenobiotic biotransformation and their major subcellular location.

Reaction	Enzyme or Specific Reaction	Localization
Hydrolysis	Carboxylesterase	Microsomes, cytosol, lysosomes, blood
	Butyrylcholinesterase	Plasma and most tissues
	Acetylcholinesterase	Erythrocytes and most tissues
	Paraoxonases	Plasma, microsomes, inner mitochondrial membrane
	Alkaline phosphatase	Plasma membrane
	Peptidase	Blood, lysosomes
	β-Glucuronidase	Microsomes, lysosomes, microflora
	Epoxide hydrolase	Microsomes, cytosol
Reduction	Azo- and nitro-reduction	Microflora
	Carbonyl (aldo-keto) reduction	Cytosol, microsomes, blood
	Disulfide reduction	Cytosol
	Sulfoxide reduction	Cytosol
	Quinone reduction	Cytosol, microsomes
	Dihydropyrimidine dehydrogenase	Cytosol
	Reductive dehalogenation	Microsomes
	Dehydroxylation (mARC)*	Mitochondria
	Dehydroxylation (aldehyde oxidase)	Cytosol
Oxidation	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Mitochondria, cytosol
	Aldehyde oxidase	Cytosol
	Xanthine oxidase	Cytosol
	Class I amine oxidases	
	Monoamine oxidase-A and B	Inner mitochondrial membrane, platelets
	Class II amine oxidases (CuAOs)	
	Diamine oxidase	Microsomes, extracellular matrix
	Peroxidase	Microsomes, lysosomes, saliva
	Flavin-monooxygenases	Microsomes
	Cytochrome P450	Microsomes, mitochondria
Conjugation	UDP-glucuronosyltransferase	Microsomes
	Sulfotransferase	Cytosol
	Glutathione transferase	Cytosol, microsomes, mitochondria
	Amino acid transferase	Mitochondria, microsomes
	N-Acetyltransferase	Mitochondria, cytosol
	Methyltransferase	Cytosol, microsomes, blood

*mARC, mitochondrial amidoxime-reducing component.

Suppression (down-regulation) of drug-metabolizing enzymes is often associated with inflammatory diseases (such as arthritis), cancer, infectious diseases (both bacterial and viral), vaccination, and treatment with certain proinflammatory biologics (therapeutic proteins). T ese disease processes activate nuclear factor-kappa B (NF-κB) and other nuclear receptors, which suppress the expression and induction of CYP and other xenobiotic-metabolizing enzymes. T is is because activated NF-κB suppresses all four xenosensors (AhR, CAR, PXR, and PPARα) as well as several other nuclear receptors. By reversing the disease process—such as lessening the inflammation associated with rheumatoid arthritis—some biologics (large drug molecules such as monoclonal antibodies and other types of therapeutic proteins) can reverse the suppression of drug-metabolizing enzymes and restore their activity to normal (pre-disease) levels.

Point 10 Xenobiotic biotransformation can alter the biological properties of a xenobiotic. T e biotransformation of drugs can result in (1) a loss of pharmacological activity, (2) no change in pharmacological activity, or (3) an increase in pharmacological activity.

Point 11 T e toxicity and potential carcinogenicity of electrophilic metabolites produced by CYP and other xenobiotic-biotransforming enzymes is reduced and often altogether eliminated by their conjugation with reduced glutathione (GSH).

Point 12 T e biotransformation of some xenobiotics results in the production of reactive oxygen species (ROS), which can cause cell toxicity (including DNA damage) through oxidative stress and lipid peroxidation. GSH, GSTs, and glutathione peroxidases (GPXs) all limit the toxic effects of ROS just as they limit the toxicity of reactive metabolites formed directly from xenobiotics. Oxidative stress and the formation of electrophilic metabolites reduce GSH levels and thus result in the concurrent oxidation of KEAP-1, which then releases Nrf2, which in turn upregulates the expression of enzymes that detoxify electrophilic metabolites (e.g., epoxides) and those metabolites that generate ROS (e.g., quinones).

Point 13 T e balance between activation and detoxication by xenobiotic-biotransforming enzymes is often a key determinant of chemical toxicity, and is often the basis for organ or species differences in toxicity.

Point 14 Exposure to xenobiotics (especially drugs) is largely through oral ingestion, and the small intestine and liver are highly developed to limit systemic exposure to orally ingested xenobiotics, a process known as f rst-pass elimination (or presystemic elimination). T e enterocytes at the tips of the small intestinal villi express the efflux transporters P-glycoprotein (ABCB1 or MDR1) and BCRP (ABCG2), which serve to limit xenobiotic absorption. Enterocytes and hepatocytes express high levels of certain CYP and UGT enzymes, which biotransform a wide variety of xenobiotics.

Point 15 Although the small intestine and liver contain the highest concentrations, xenobiotic-biotransforming enzymes are nevertheless widely distributed throughout the body.

Point 16 Species differences in xenobiotic-biotransforming enzymes are often the basis for species differences in both the qualitative and quantitative aspects of xenobiotic biotransformation and toxicity.

Point 17 In sexually mature rats and, to a lesser extent, mice there are marked gender differences in the expression of certain xenobiotic-biotransforming enzymes (both oxidative and conjugating enzymes). In other species, including humans, gender differences either do not exist or generally represent less than a twofold difference.

Point 18 Large interindividual differences in pharmacokinetic parameters upon administration or exposure to a chemical can reflect genetically determined differences in the activity of xenobiotic-biotransforming enzymes or transporters (genetic polymorphisms) or environmental factors, such as drug–drug interactions. T e study of the causes, prevalence, and impact of heritable differences in xenobiotic-biotransforming enzymes is known as pharmacogenetics.

Point 19 Stereochemical aspects can play an important role in the interaction between a xenobiotic and its biotransforming enzyme (from both a substrate and an inhibitor perspective), and xenobiotic-biotransforming enzymes can play a key role in converting one stereoisomer to another, a process known as mutarotation or inversion *of* conf guration.

Point 20 Mass spectrometry is widely used to characterize the structure of metabolites. Certain xenobiotic reactions are associated with discrete changes in mass: the loss of 2 atomic mass units (amu) signifies dehydrogenation, whereas the loss of 14 amu usually signifies demethylation ($-CH_2$). Several reactions result in an increase in mass, including reduction ($+2$ amu $= 2H$), methylation ($+14$ amu $= CH_2$), oxidation ($+16$ amu $= O$), hydration ($+18$ amu $= H_2O$), acetylation ($+42$ amu $= C_2H_2O$), sulfonation ($+80$ amu $= SO_3$), glucuronidation ($+176$ amu $= C_6H_8O_6$), carbamoyl glucuronidation ($+220$ amu $= C_7H_8O_8$), and conjugation with GSH ($+305$ amu $= C_{10}H_{15}N_3O_6S$). Conjugation of acidic drugs with CoA (to form acyl-CoA thioesters) increases mass by 749 amu, but these conjugates are not transported out of cells and, hence, are not detected in blood, bile, or urine.

HYDROLYSIS, REDUCTION, AND OXIDATION

Hydrolysis

Carboxylesterases, Cholinesterases, and Paraoxonase—T e hydrolysis of carboxylic acid esters, amides, and thioesters is largely catalyzed by carboxylesterases and by two cholinesterases: true acetylcholinesterase in erythrocyte membranes and pseudocholinesterase, which is also known as butyrylcholinesterase and is located in serum. Phosphoric acid esters are hydrolyzed by paraoxonase, a serum enzyme also known as aryldialkylphosphatase. Phosphoric acid anhydrides are hydrolyzed by a related organophosphatase.

Carboxylesterases in serum and tissues and serum cholinesterase collectively determine the duration and site of action of certain drugs. T e hydrolysis of xenobiotic esters and amides

in humans is largely catalyzed by just two carboxylesterases called hCE1 and hCE2.

Carboxylesterases are glycoproteins that are present in serum and most tissues. Carboxylesterases hydrolyze numerous endogenous lipid compounds and generate pharmacologically active metabolites from several ester or amide prodrugs. In addition, carboxylesterases may convert xenobiotics to toxic and tumorigenic metabolites.

Cholinesterases play an important role in limiting the toxicity of organophosphates, which inhibit acetylcholinesterase and thus the termination of acetylcholine action. Factors that decrease esterase activity potentiate the toxic effects of organophosphates, whereas factors that increase serine esterase activity have a protective effect.

Paraoxonases, calcium-dependent enzymes containing a critical sulfhydryl group, catalyze the hydrolysis of a broad range of organic compounds, including lactones. T us, "lactonase" is a more encompassing name for this group of enzymes.

Prodrugs and Alkaline Phosphatase—Many drugs are designed as prodrugs, meaning biotransformation is required to produce the active species. Hydrolytic enzymes such as carboxylesterases, cholinesterases, and alkaline phosphatase are commonly invoked for this purpose. Potent anticancer agents exist that are highly selective for certain sites and only release active drug in the vicinity of tumor cells.

Peptidases—Numerous human peptides and several recombinant peptide hormones, growth factors, cytokines, soluble receptors, and monoclonal antibodies are used therapeutically. T ese peptides are hydrolyzed in the blood and tissues by a variety of peptidases, which cleave the amide linkage between adjacent amino acids.

Epoxide Hydrolase—Epoxide hydrolase catalyzes the trans-addition of water to alkene epoxides and arene oxides, and is present in virtually all tissues. It plays an important role in detoxifying electrophilic epoxides that might otherwise bind to proteins and nucleic acids and cause cellular toxicity and genetic mutations. T ere are five distinct forms of epoxide hydrolase in mammals: microsomal epoxide hydrolase (mEH), soluble epoxide hydrolase (sEH), cholesterol epoxide hydrolase, LTA_4 hydrolase, and hepoxilin hydrolase. T e latter three enzymes appear to hydrolyze endogenous epoxides exclusively and have virtually no capacity to detoxify xenobiotic oxides.

In contrast to the high degree of substrate specificity displayed by the cholesterol, LTA_4, and hepoxilin epoxide hydrolases, the mEH and sEH hydrolyze many alkene epoxides and arene oxides. Generally, these two forms of epoxide hydrolases and cytochrome P450 enzymes, which are often responsible for producing the toxic epoxides, have a similar cellular localization that presumably ensures the rapid detoxication of alkene epoxides and arene oxides generated during the oxidative biotransformation of xenobiotics.

Epoxide hydrolase is one of the several inducible enzymes in liver microsomes. Induction of epoxide hydrolase is invariably associated with the induction of cytochrome P450.

Reduction

Certain metals and xenobiotics containing an aldehyde, ketone, disulfide, sulfoxide, quinone, N-oxide, alkene, azo, or nitro group are often reduced in vivo. T e reaction may proceed enzymatically or nonenzymatically by interaction with reducing agents, such as the reduced forms of glutathione (GSH), FAD, FMN, and NADP. Likewise, enzymes, such as alcohol dehydrogenase (ADH), aldehyde oxidase, and cytochrome P450, can catalyze both reductive and oxidative reactions depending on the substrate and conditions.

Azo- and Nitro-reduction—Azo- and nitro-reduction are catalyzed by intestinal microflora and under certain conditions (i.e., low oxygen tension), by two liver enzymes: cytochrome P450 and NADPH-quinone oxidoreductase (also known as DT-diaphorase). T e reactions require NADPH and are inhibited by oxygen. T e anaerobic environment of the lower gastrointestinal tract is well suited for azo- and nitro-reduction.

Carbonyl Reduction—T e reduction of certain aldehydes to primary alcohols and of ketones to secondary alcohols is catalyzed by NAD(P)H-dependent reductases belonging to one of the two superfamilies, the aldo-keto reductases (AKRs) and the short-chain dehydrogenases/reductases (SDRs). AKRs are members of a superfamily of cytosolic enzymes that reduce both xenobiotic and endobiotic compounds. SDR carbonyl reductases are monomeric enzymes, present in blood and the cytosolic fraction of various tissues. Hepatic carbonyl reductase activity is present mainly in the cytosolic fraction, with a different carbonyl reductase present in the microsomes.

Disulfide Reduction—Disulfide reduction by glutathione is a three-step process, the last step of which is catalyzed by glutathione reductase. T e first steps can be catalyzed by GST, or they can occur nonenzymatically.

Sulfoxide and N-Oxide Reduction—T ioredoxin-dependent enzymes in liver and kidney cytosol can reduce sulfoxides, which were formed by cytochrome P450. Under reduced oxygen tension, the NADPH-dependent reduction of N-oxides in liver microsomes may be catalyzed by cytochrome P450 or NADPH–cytochrome P450 reductase.

Quinone Reduction—Quinones can be reduced to hydroquinones by two cytosolic flavoproteins, NQO1 and NQO2, without oxygen consumption. NADPH-quinone oxidoreductase-1 (DT-diaphorase) and NADPH-quinone oxidoreductase-2 have different substrate specificities. T e two-electron reduction of quinones also can be catalyzed by carbonyl reductase. T is pathway of quinone reduction is essentially nontoxic and

is not associated with oxidative stress because no oxygen is used.

T e second pathway of quinone reduction catalyzed by microsomal NADPH–cytochrome P450 reductase results in the formation of a semiquinone free radical by a one-electron reduction of the quinone. T e oxidative stress associated with autooxidation of a semiquinone free radical, which produces superoxide anion, hydrogen peroxide, and other active oxygen species, can be extremely cytotoxic.

T e properties of the hydroquinone determine whether, during the metabolism of quinine-containing xenobiotics, NQO functions as a protective antioxidant or a prooxidant activator leading to the formation of reactive oxygen species and reactive semiquinone free radicals.

Dehalogenation—T ere are three major mechanisms for removing halogens (F, Cl, Br, and I) from aliphatic xenobiotics: (1) reductive dehalogenation involves replacement of a halogen with hydrogen, (2) oxidative dehalogenation replaces a halogen and hydrogen on the same carbon atom with oxygen, and (3) double dehalogenation involves the elimination of two halogens on adjacent carbon atoms to form a carbon–carbon double bond. A variation of this third mechanism is dehydrohalogenation, in which a halogen and hydrogen on adjacent carbon atoms are eliminated to form a carbon–carbon double bond.

Oxidation

Alcohol Dehydrogenase—ADH is a cytosolic enzyme present in several tissues including the liver, which has the highest levels, the kidney, the lung, and the gastric mucosa. T ere are five major classes of ADH. T e class I ADH isozymes (α-ADH, β-ADH, and γ-ADH) are responsible for the oxidation of ethanol and other small aliphatic alcohols. Class II ADH (π-ADH) is primarily expressed in liver where it preferentially oxidizes larger aliphatic and aromatic alcohols. Long-chain alcohols (pentanol and larger) and aromatic alcohols are preferred substrates for class III ADH (χ-ADH). Class IV ADH (σ-ADH or μ-ADH), which is not expressed in liver, is the most active of the medium-chain ADHs in oxidizing retinol. Class V ADH has no subunit designation.

Aldehyde Dehydrogenase—Aldehyde dehydrogenase (ALDH) oxidizes aldehydes to carboxylic acids with NAD^+ as the cofactor. T e enzymes also have esterase activity. T e 19 identified ALDHs differ in their primary amino acid sequences and in the quaternary structure. In contrast to ALDH1A1 and ALDH2, which specifically reduce NAD^+, ALDH3A1 reduces both NAD^+ and $NADP^+$.

As shown in Figure 6–1, ALDH2 is a mitochondrial enzyme that, by virtue of its high af nity, is primarily responsible for oxidizing simple aldehydes, such as acetaldehyde. Genetic deficiencies in other ALDHs impair the metabolism of other aldehydes.

Dihydrodiol Dehydrogenase—T e AKR superfamily includes several forms of dihydrodiol dehydrogenases, which are cytosolic, NADPH-requiring oxidoreductases that oxidize various polycyclic aromatic hydrocarbons to potentially toxic metabolites.

Molybdenum Hydroxylases—Two major molybdenum hydroxylases or molybdozymes participate in the biotransformation of xenobiotics: aldehyde oxidase and xanthine oxidoreductase (also known as xanthine oxidase [XO]). Sulfite oxidase, a third molybdozyme, oxidizes sulfite, an irritating air pollutant, to sulfate, which is relatively innocuous. All three molybdozymes are flavoprotein enzymes. During substrate oxidation, aldehyde oxidase and XO are reduced and then reoxidized by molecular oxygen. T e oxygen incorporated into the xenobiotic is derived from water rather than oxygen, which distinguishes the oxidases from oxygenases. Xenobiotics that are good substrates for molybdozymes tend to be poor substrates for cytochrome P450, and vice versa.

Xanthine Oxidoreductase—Xanthine dehydrogenase (XD) and XO are two forms of the same enzyme involved in purine degradation that differ in the electron acceptor used in the final step of catalysis. In the case of XD, the final electron acceptor is NAD^+, whereas in the case of XO the final electron acceptor is oxygen. XD is converted to XO by oxidation of cysteine residues and/or proteolytic cleavage. T e conversion of XD to XO in vivo may be important in ischemia–reperfusion injury, lipopolysaccharide-mediated tissue injury, and alcohol-induced hepatotoxicity. XO contributes to oxidative stress and lipid peroxidation because the oxidase activity of XO involves reduction of molecular oxygen, which can lead to the formation of ROS.

Allopurinol and other xanthine oxidoreductase inhibitors are being evaluated for the treatment of various types of ischemia–reperfusion and vascular injury that appear to be mediated, at least in part, by xanthine oxidoreductase.

Aldehyde Oxidase—T e molybdozyme aldehyde oxidase exists only in the oxidase form. Cytosolic aldehyde oxidase transfers electrons to molecular oxygen, which can generate reactive oxygen species and lead to lipid peroxidation. Aldehyde oxidase plays an important role in the catabolism of biogenic amines and catecholamines.

Monoamine Oxidase—Monoamine oxidases (MAO) are involved in the oxidative deamination of primary, secondary, and tertiary amines, including serotonin and a number of xenobiotics. Oxidative deamination of a primary amine produces ammonia and an aldehyde, whereas oxidative deamination of a secondary amine produces a primary amine and an aldehyde. T e aldehydes formed by MAO are usually oxidized further by other enzymes to the corresponding carboxylic acids. MAO is located throughout the brain and in the outer membrane of mitochondria of the liver, kidney, intestine, and blood platelets.

T e substrate is oxidized by MAO, which itself is reduced using FAD. T e oxygen incorporated into the substrate is

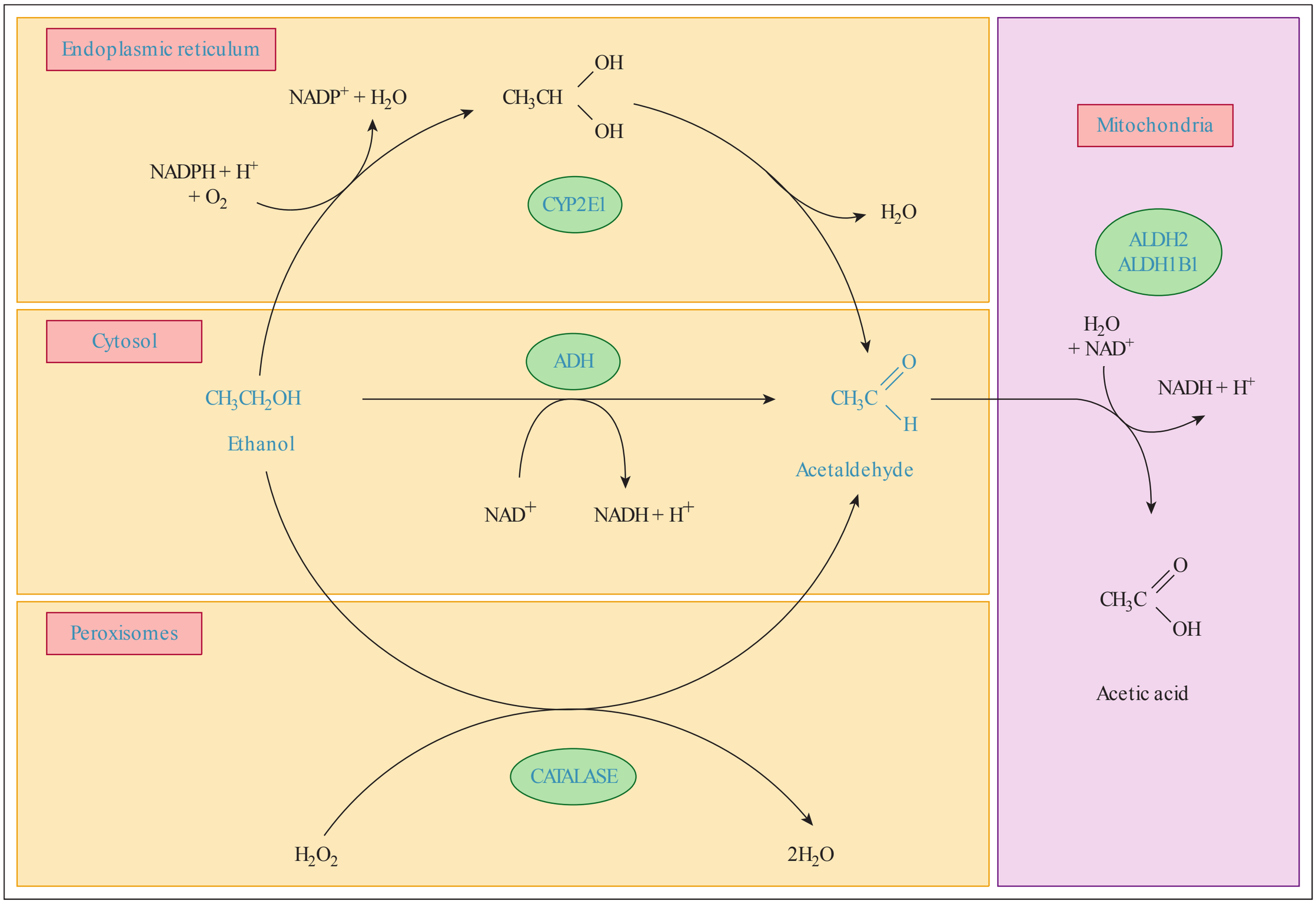


FIGURE 6–1 **Oxidation of ethanol to acetaldehyde by alcohol dehydrogenase (ADH), cytochrome P450 (CYP2E1), and catalase.**
Note: The oxidation of ethanol to acetic acid involves multiple organelles.

derived from water, not molecular oxygen. T e catalytic cycle is completed by reoxidation of the reduced enzyme ($FADH_2 \rightarrow FAD$) by oxygen, which generates hydrogen peroxide.

Semicarbazide-sensitive amine oxidase (SSAO) is a copper-containing enzyme that catalyzes fundamentally the same reaction as MAO. It can be distinguished from MAO by its sensitivity to inhibitors and presence in plasma and various cell surfaces, whereas MAO is found in mitochondria.

Peroxidase-dependent Cooxidation—Oxidative biotransformation of xenobiotics by peroxidases couples the reduction of hydrogen peroxide and lipid hydroperoxides to the oxidation of other substrates via a process known as cooxidation. An important peroxidase is prostaglandin H synthetase (PHS), which possesses two catalytic activities: a cyclooxygenase that converts arachidonic acid to prostaglandins and a peroxidase that converts the hydroperoxide to the corresponding alcohol PGH_2. PHS has two forms (PHS1 and PHS2) that are better known as two forms of cyclooxygenase, namely, COX1 and COX2. PHS peroxidases are important in the activation of xenobiotics to toxic or tumorigenic metabolites, particularly in extrahepatic tissues that contain low levels of cytochrome P450. Oxidation of xenobiotics by peroxidases involves direct transfer of the peroxide oxygen to the xenobiotic, as shown in Figure 6–2 for the conversion of substrate X to the oxidized product XO.

Xenobiotics that serve as electron donors, such as amines and phenols, can also be oxidized to free radicals during the reduction of a hydroperoxide by peroxidases. In this case, the hydroperoxide is still converted to the corresponding alcohol, but the peroxide oxygen is reduced to water instead of being incorporated into the xenobiotic. For each molecule of hydroperoxide reduced (which is a two-electron process), two molecules of xenobiotic can be oxidized (each by a one-electron process). Many of the metabolites produced are reactive electrophiles that can cause tissue damage.

PHS2 may play at least two distinct roles in tumor formation: it may convert certain xenobiotics to DNA-reactive metabolites and initiate tumor formation, and it may promote subsequent tumor growth, perhaps through formation of growth-promoting eicosanoids.

PHS is unique among peroxidases because it can both generate hydroperoxides and catalyze peroxidase-dependent reactions, as shown in Figure 6–2. Xenobiotic biotransformation by PHS is controlled by the availability of arachidonic acid, whereas conversion by other peroxidases is controlled by the availability of hydroperoxide substrates.

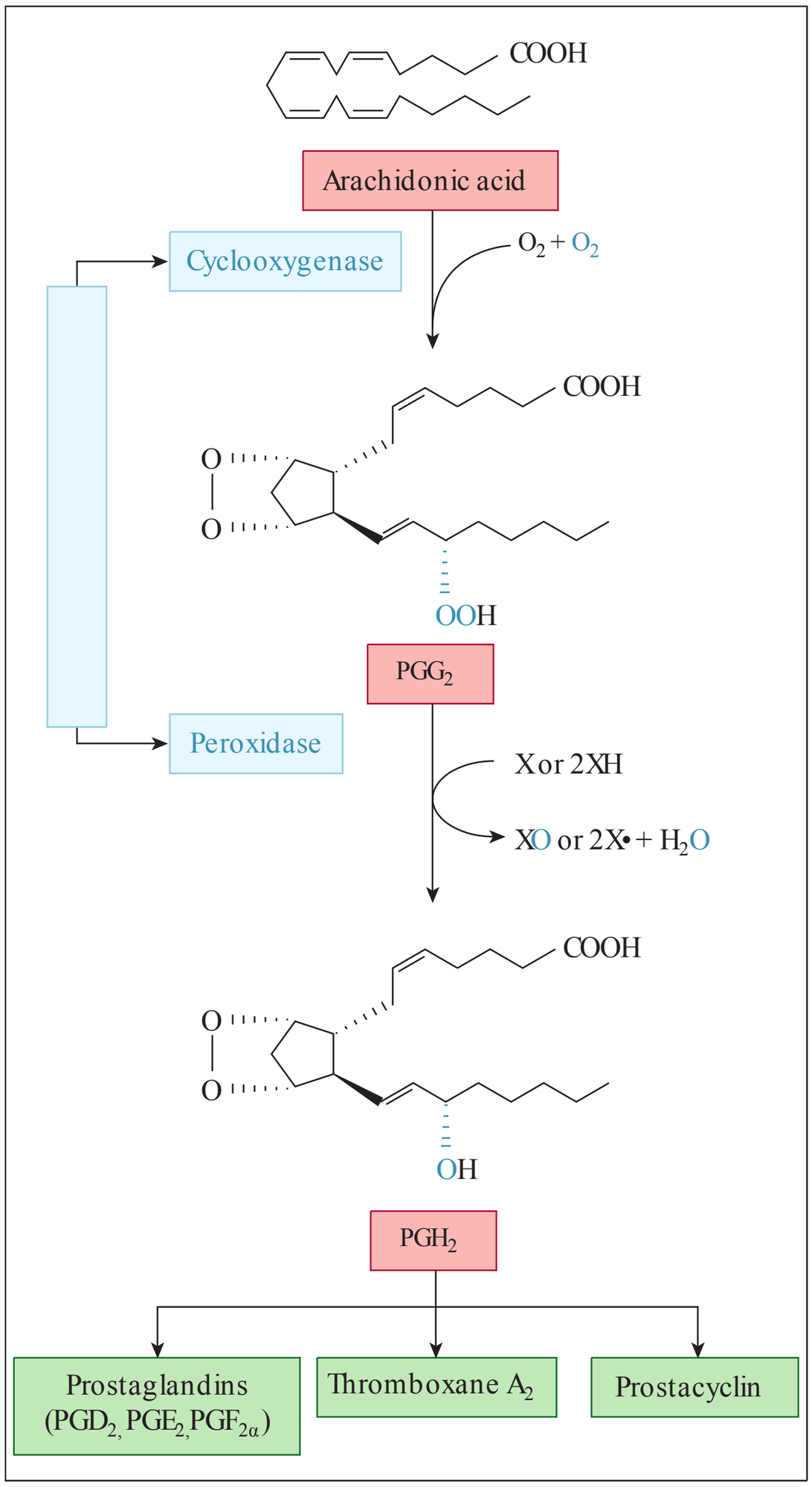


FIGURE 6–2 **Cooxidation of xenobiotics (X) during the conversion of arachidonic acid to PGH_2 by prostaglandin Hsynthase.**

Flavin Monooxygenases—Liver, kidney, intestine, brain, and lung contain one or more FAD-containing monooxygenases (FMO) that oxidize the nucleophilic nitrogen, sulfur, and phosphorus heteroatom of various xenobiotics. T e mammalian FMO gene family comprises five microsomal enzymes that require NADPH and O_2, and many of the reactions catalyzed by FMO can also be catalyzed by cytochrome P450.

T e mechanism of catalysis by FMO is depicted in Figure 6–3. After the FAD moiety is reduced to $FADH_2$ by NADPH, the oxidized cofactor $NADP^+$ remains bound to the enzyme. $FADH_2$ then binds oxygen to produce a relatively stable peroxide. During the oxygenation of xenobiotics, the flavin peroxide oxygen is transferred to the substrate (depicted as X → XO in Figure 6–3). T e final step in the catalytic cycle involves restoration of FAD to its oxidized state and release of

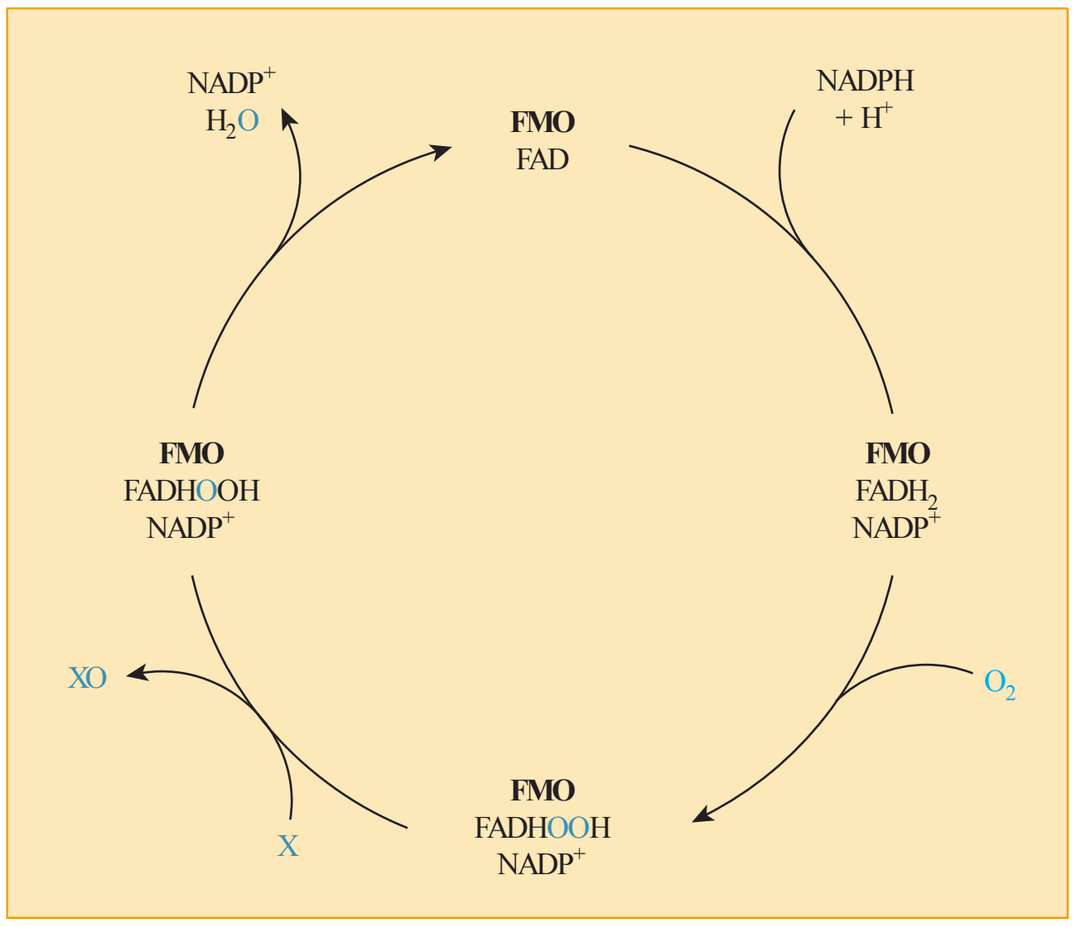


FIGURE 6–3 **Catalytic cycle of f avin monooxygenase (FMO).** Xand XO are the xenobiotic substrate and oxygenated product, respectively. The C(4a)-hydroperoxyflavin and C(4a)-hydroxyflavin of FAD are depicted as FADHOOH and FADHOH, respectively.

$NADP^+$. T is final step is rate-limiting, and it occurs after substrate oxygenation.

Cytochrome P450—T e cytochrome P450 (CYP) system ranks first in terms of catalytic versatility and the sheer number of xenobiotics it detoxifies or activates. T e highest concentration of CYP enzymes involved in xenobiotic biotransformation is found in hepatic endoplasmic reticulum (microsomes), but CYP enzymes are present in virtually all tissues. All CYP enzymes are heme-containing proteins that catalyze the monooxygenation of one atom of oxygen into a substrate, and the other oxygen atom is reduced to water with reducing equivalents derived from NADPH.

During catalysis, CYP does not interact directly with NADPH or NADH. In the endoplasmic reticulum, electrons are relayed from NADPH to cytochrome P450 via a flavoprotein called NADPH–cytochrome P450 reductase. In mitochondria, electrons are transferred from NADPH to CYP via ferredoxin and ferredoxin reductase.

T ere are notable exceptions to the principle that cytochrome P450 requires a second enzyme (i.e., a flavoprotein) for catalytic activity. One exception applies to thromboxane A synthase (CYP5A1) and prostaglandin I_2 synthase (prostacyclin synthase or CYP8A1), which are involved in the conversion of arachidonic acid to eicosanoids. In both cases, cytochrome P450 functions as an isomerase and catalyzes a rearrangement of the oxygen atoms introduced into arachidonic acid by cyclooxygenase. T e second exception involves two CYP enzymes expressed in the bacterium Bacillus megaterium. T ese CYP enzymes are considerably larger than most CYP enzymes because the P450 moiety and oxidoreductase flavoprotein are expressed in a single protein encoded by a single gene.

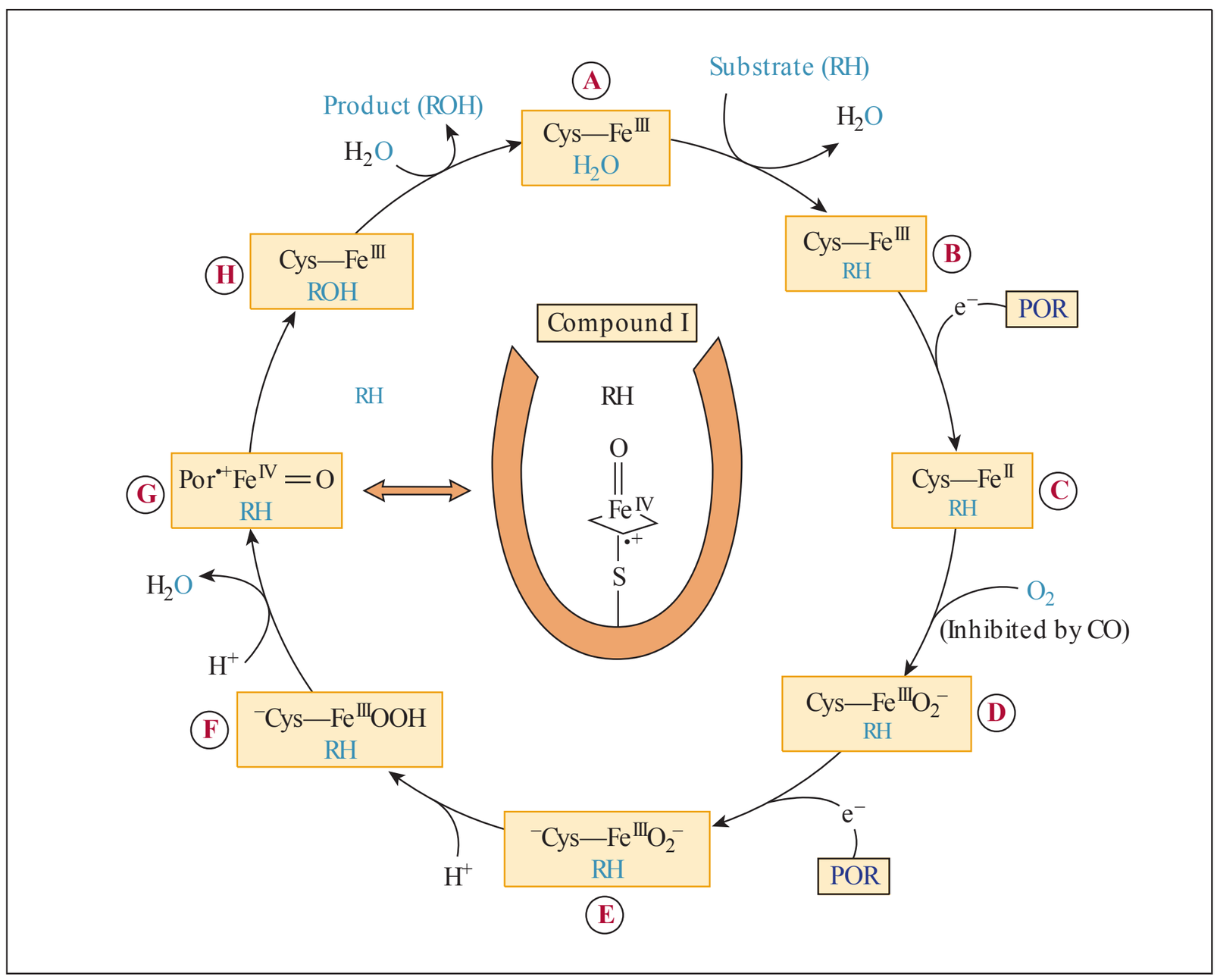
Cytochrome P450 and NADPH–cytochrome P450 reductase are embedded in the phospholipid bilayer of the

endoplasmic reticulum, which facilitates their interaction. As shown in Figure 6–4, the first part of the catalytic cycle involves the activation of oxygen, and the final part involves substrate oxidation, which entails the abstraction of a hydrogen atom or an electron from the substrate followed by oxygen rebound (radical recombination). Following the binding of substrate to the CYP enzyme, the heme iron is reduced from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state by the addition of a single electron from NADPH–cytochrome P450 reductase. Release of the oxidized substrate returns cytochrome P450 to its initial state. If the catalytic cycle is interrupted, oxygen is released as superoxide anion (O_2^-) or hydrogen peroxide (H_2O_2).

Cytochrome P450 catalyzes the following types of oxidation reactions:

1. hydroxylation of an aliphatic or aromatic carbon
2. epoxidation of a double bond
3. heteroatom (S-, N-, and I-) oxygenation and N-hydroxylation
4. heteroatom (O-, S-, N-, and Si-) dealkylation
5. oxidative group transfer
6. cleavage of esters
7. dehydrogenation

Liver microsomes from all mammalian species contain numerous P450 enzymes, each with the potential to catalyze the various reactions shown in Figures 6–5 to 6–12. In general,



Other reactions

One-electron reduction	**C** (Cys—Fe ^{II}RH)	⟶	**A** (Cys—Fe ^{III} + $RH^{-\bullet}$)
Superoxide anion production	**D** (Cys—Fe $^{III}O_2^-$ RH)	⟶	**B** (Cys—Fe ^{III} RH) + $O_2^{-\bullet}$
Hydrogen peroxide production	**E** ($^-$Cys—Fe $^{III}O_2^-$ RH) + $2H^+$	⟶	**B** (Cys—Fe ^{III} RH) + H_2O_2
Hydrogen peroxide shunt	**B** (Cys—Fe ^{III} RH) + H_2O_2	⟶	**F** ($^-$Cys—Fe ^{III}OOH RH) + H^+
Peroxide shunt to form Compound I	**B** (Cys—Fe ^{III} RH) + XOOH	⟶	**G** (Por $^{•+}$Fe ^{IV} = O RH) + XOH

FIGURE 6–4 **Catalytic cycle of cytochrome P450.** Cytochrome P450 is represented as Cys-Fe^{III}, where Cys represents the fifth ligand (a cysteine thiolate) to the ferric heme iron. RH and ROH represent the substrate and product (hydroxylated metabolite), respectively. The intermediates in the catalytic cycle are as follows: A, ferric resting state; B, substrate bound; C, ferrous intermediate; D, ferrisuperoxo anion intermediate; E, ferriperoxo intermediate with an electron delocalized over the Cys thiolate bond; F, ferrihydroperoxy intermediate (with a negative charge on the Cys thiolate bond); G, compound I, an $iron^{IV}$-oxo porphyrin cation, which is responsible for most substrate oxidation reactions; H, enzyme in its resting state prior to the release of product formed by hydrogen abstraction followed by oxygen rebound. Fe^{II}, Fe^{III}, Fe^{IV}, and Fe^{V} refer to iron in the ferrous, ferric, ferryl, and perferryl state, respectively. It should be noted that although it is written as $por^{•+}Fe^{IV}$= O, compound I is in the highly oxidized perferryl (Fe^{V}) state when the oxidation state of the porphyrin ring is also taken into account.

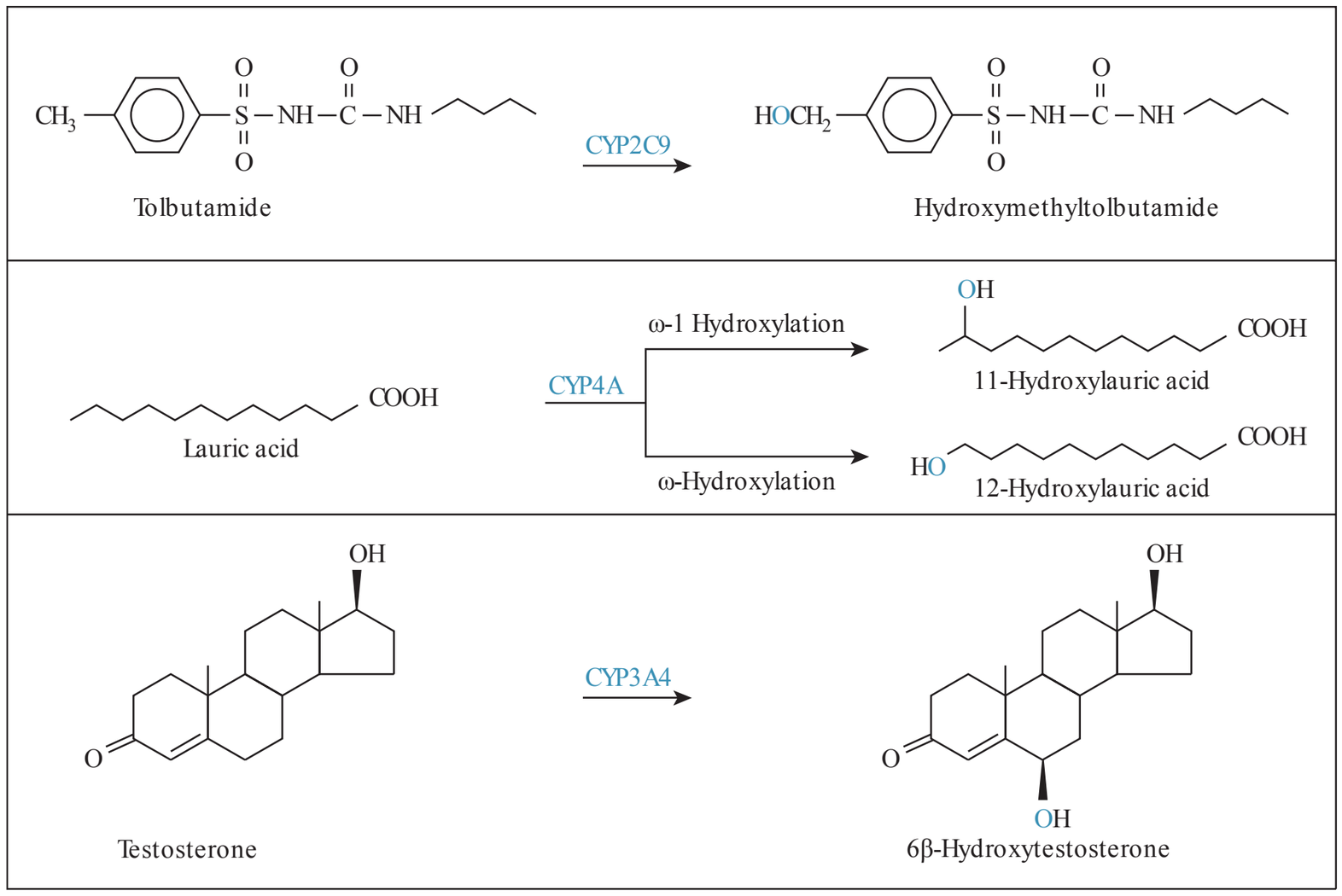


FIGURE 6–5 **Examples of reactions catalyzed by cytochrome P450: hydroxylation of aliphatic carbon.**

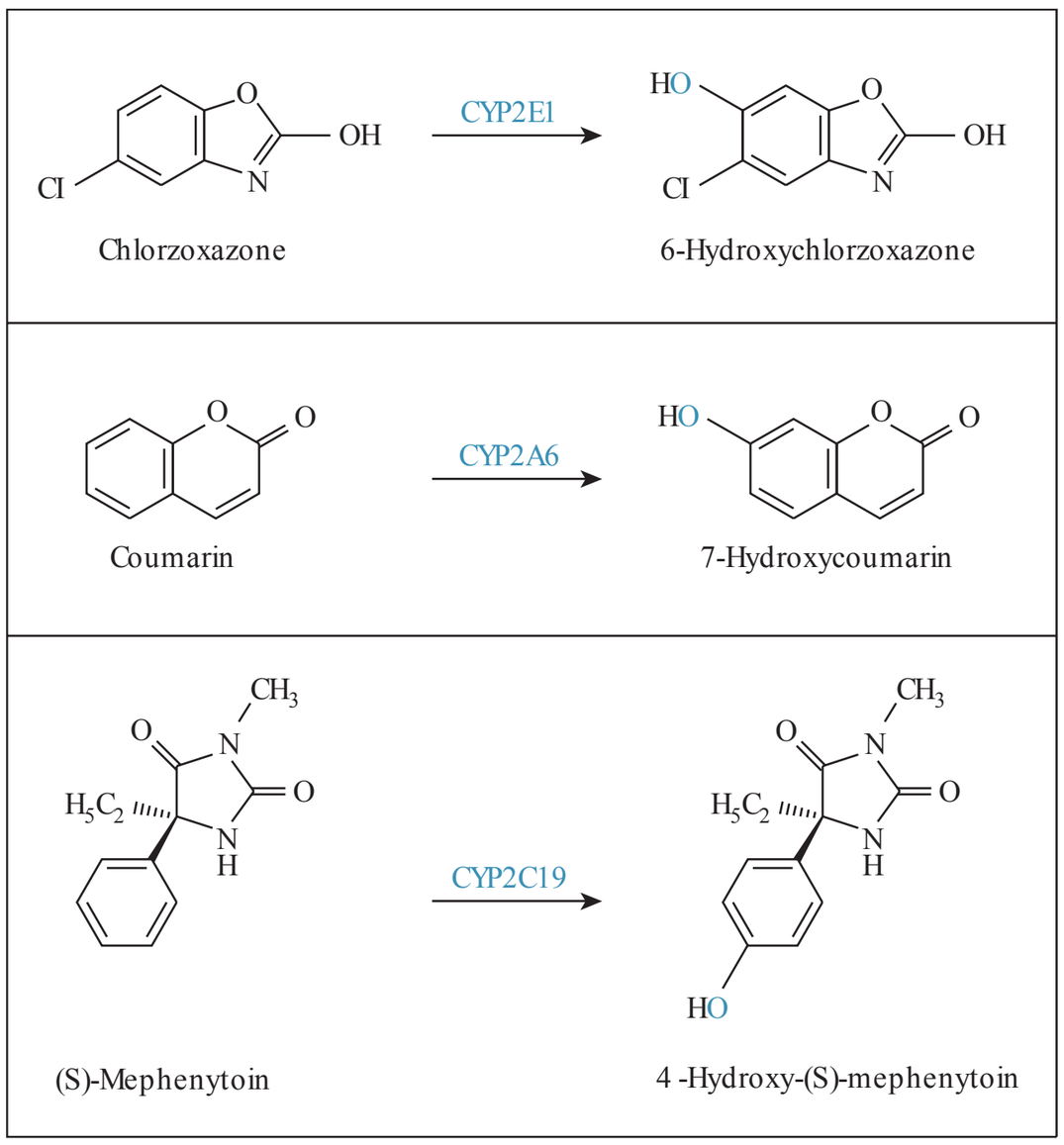


FIGURE 6–6 **Examples of reactions catalyzed by cytochrome P450: hydroxylation of aromatic carbon.**

CYP enzymes are classified into subfamilies based on amino acid sequence identity.

T e function and regulation of CYP1A1, CYP1A2, CYP1B1, CYP2E1, CYP2R1, CYP2S1, CYP2U1, and CYYP2W1 are highly conserved among mammalian species and these proteins have the same names in all mammalian species. In most other cases, the CYP enzymes are named in a species-specific manner.

T e levels and activity of each CYP enzyme vary from one individual to the next, due to environmental and/or genetic factors. Decreased CYP enzyme activity can result from (1) a genetic mutation that either blocks the synthesis of a CYP enzyme or leads to the synthesis of a catalytically compromised, inactive, or unstable enzyme, which gives rise to the poor and intermediate metabolizer genotypes; (2) exposure to an environmental factor (such as an infectious disease or an inflammatory process) that suppresses CYP enzyme expression; or (3) exposure to a xenobiotic that inhibits or inactivates a preexisting CYP enzyme. By inhibiting cytochrome P450, one drug can impair the biotransformation of another, which may lead to an exaggerated pharmacologic or toxicologic response to the second drug. Increased CYP enzyme activity can result from (1) gene duplication leading to overexpression of a CYP enzyme, (2) exposure to drugs and other xenobiotics that induce the synthesis of cytochrome P450, or (3) stimulation of preexisting enzyme by a xenobiotic.

Induction of cytochrome P450 by xenobiotics increases CYP enzyme activity. By inducing cytochrome P450, one drug can stimulate the metabolism of a second drug and thereby decrease or ameliorate its therapeutic effect. Allelic variants, which arise by point mutations in the wild-type gene, are another source of interindividual variation in CYP activity. Environmental factors known to affect CYP levels include medications, foods, social habits (e.g., alcohol consumption and cigarette smoking), and disease status (diabetes, inflammation, viral and bacterial infection, hyperthyroidism, and hypothyroidism). When environmental factors influence CYP enzyme levels, considerable variation may be observed during repeated measures of xenobiotic biotransformation (e.g., drug metabolism) in the same individual. Due to their broad substrate specificity, it is possible that two or more CYP enzymes can contribute to the metabolism of a single compound.

T e pharmacologic or toxic effects of certain drugs are exaggerated in a significant percentage of the population due to a heritable deficiency in a CYP enzyme. Inasmuch as the biotransformation of a xenobiotic in humans is frequently dominated by a single CYP enzyme, the considerable effort in identifying which CYP enzyme or enzymes are involved in eliminating the drug is known as reaction phenotyping or enzyme mapping. Four approaches to reaction phenotyping are as follows:

1. Correlation analysis involves measuring the rate of xenobiotic metabolism by several samples of human liver microsomes and correlating reaction rates with the variation in the level or activity of the individual P450 enzymes in the same microsomal samples.
2. Chemical inhibition evaluates the effects of known CYP enzyme inhibitors on the metabolism of a xenobiotic by human liver microsomes. Inhibitors of cytochrome CYP must be used cautiously because most of them can inhibit more than one CYP enzyme.
3. Antibody inhibition determines the effects of inhibitory antibodies against selected CYP enzymes on the biotransformation of a xenobiotic by human liver microsomes. T is method alone can potentially establish which human CYP enzyme is responsible for biotransforming a xenobiotic.
4. Biotrans*f*ormation by purif ed or recombinant human CYP enzymes establishes whether a particular CYP enzyme can or cannot biotransform a xenobiotic, but it does not address whether that CYP enzyme contributes substantially to reactions catalyzed by human liver microsomes.

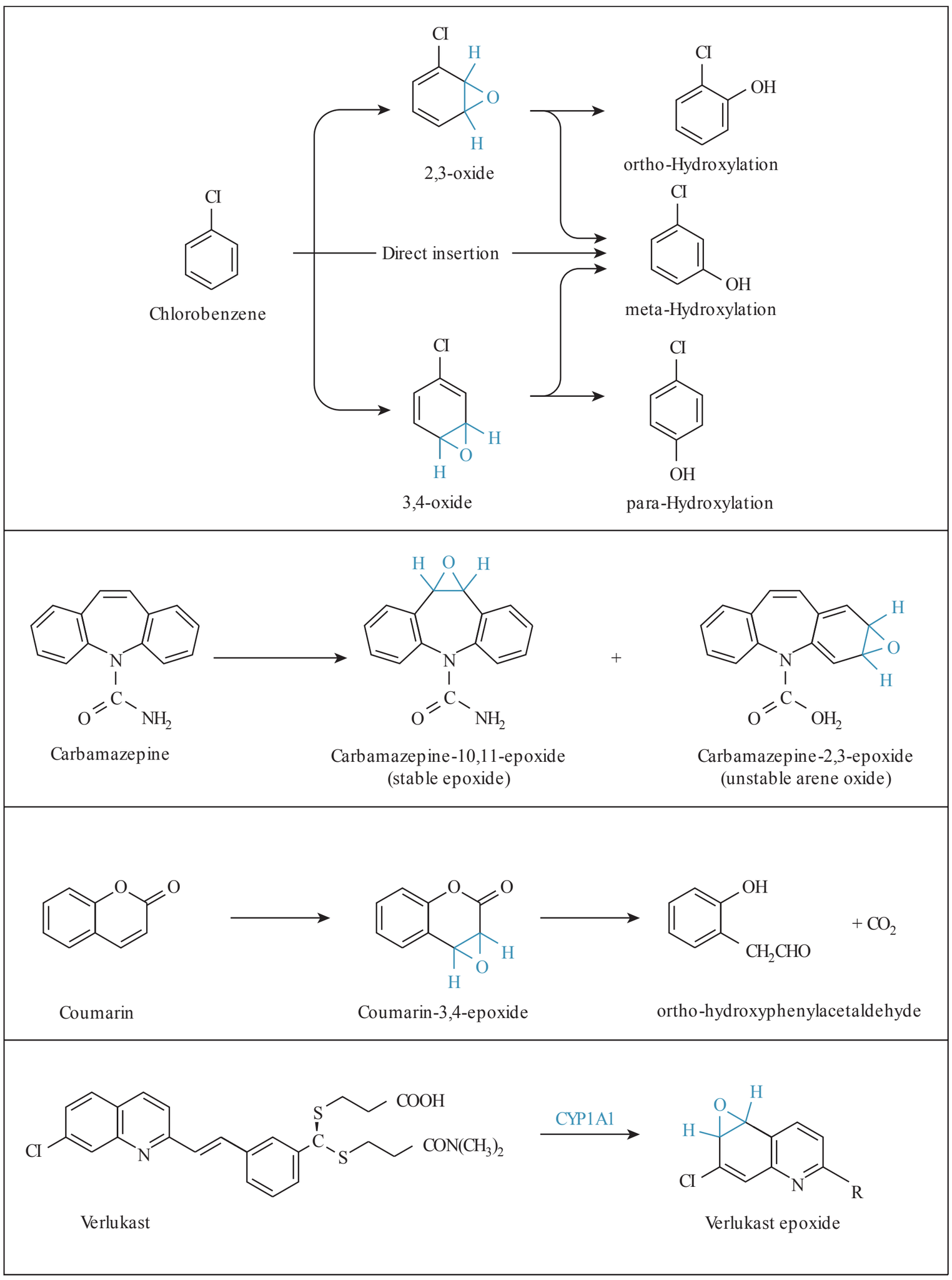


FIGURE 6–7 **Examples of reactions catalyzed by cytochrome P450: epoxidation.**

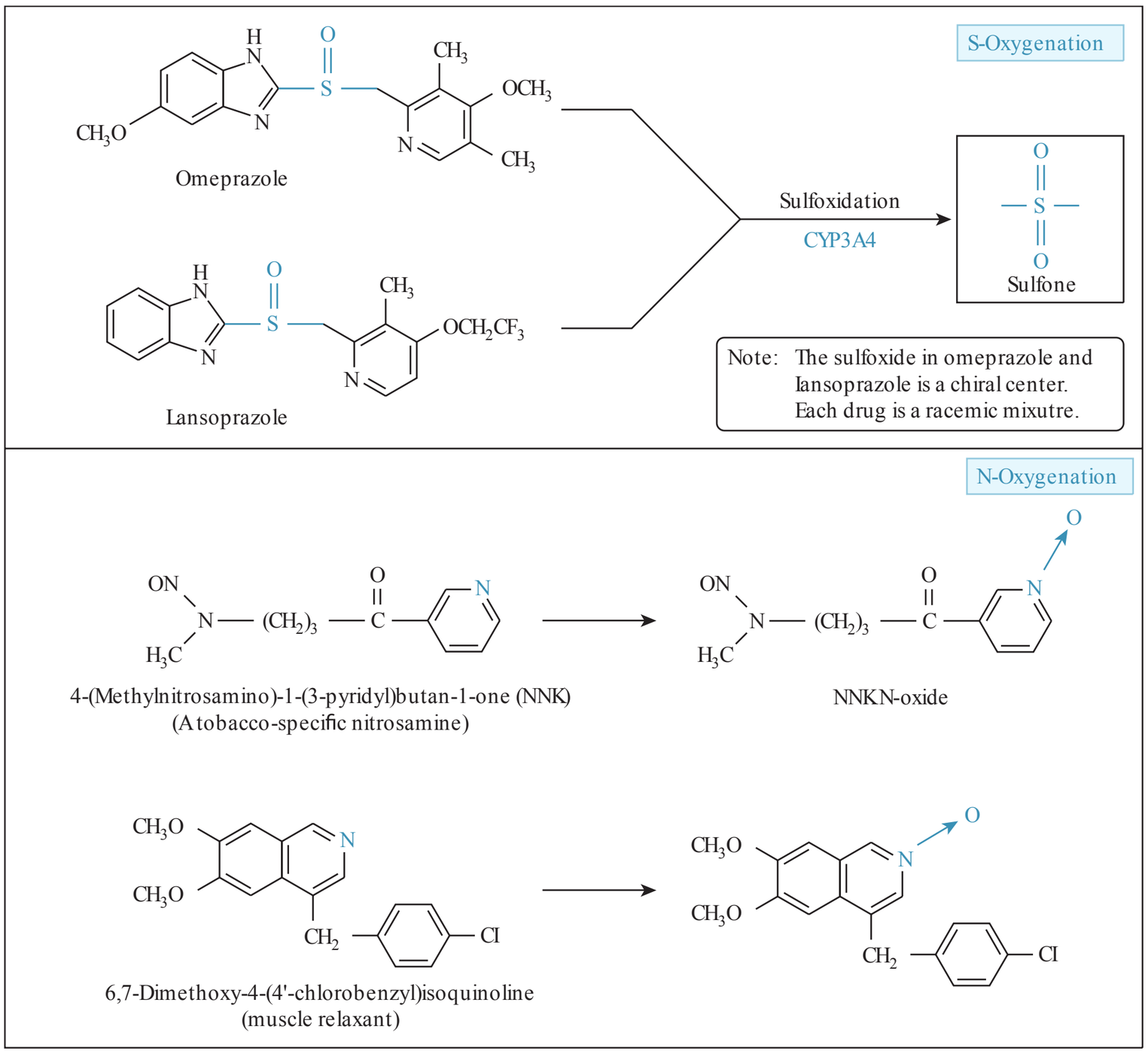


FIGURE 6–8 **Examples of reactions catalyzed by cytochrome P450: heteroatom oxygenation.**

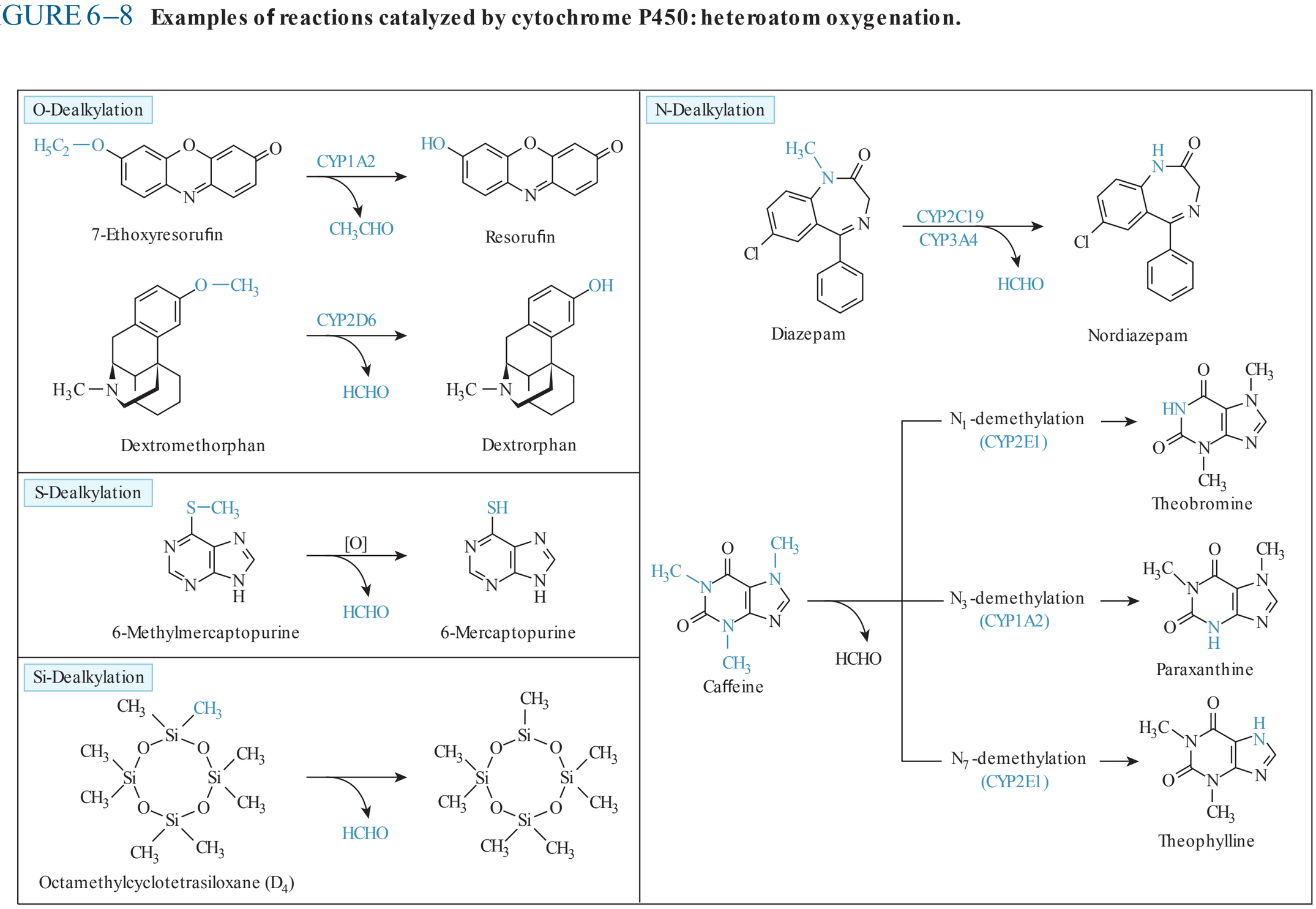


FIGURE 6–9 **Examples of reactions catalyzed by cytochrome P450: heteroatom dealkylation.**

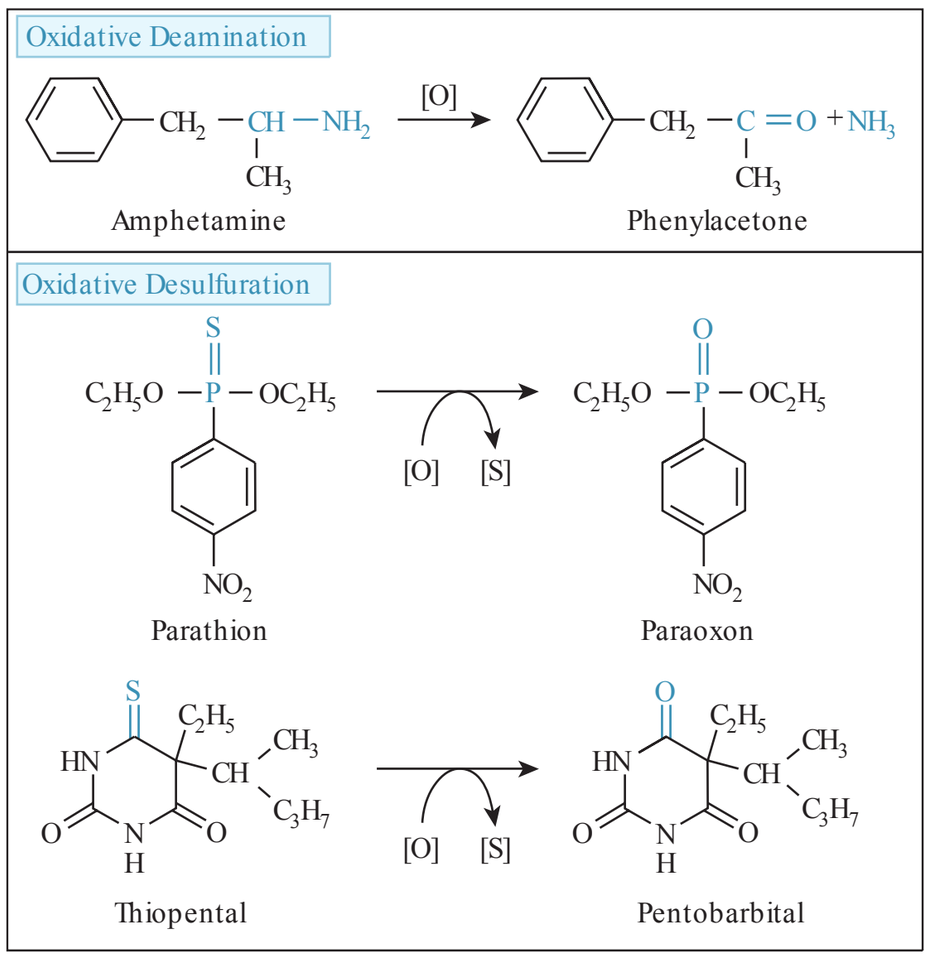


FIGURE 6–10 **Examples of reactions catalyzed by cytochrome P450: oxidative group transfer.**

Examples of substrates, inhibitors, and inducers for each CYP enzyme in human liver microsomes are given in Table 6–2. Because reaction phenotyping in vitro is not always carried out with toxicologically relevant substrate concentrations, the CYP enzyme that appears responsible for biotransforming the drug in vitro may not be the CYP enzyme responsible for biotransforming the drug in vivo.

Activation of Xenobiotics by Cytochrome P450—The role of human CYP enzymes in the activation of procarcinogens and protoxicants and some cytochrome P450–dependent reactions are summarized in Table 6–3. Many of the chemicals listed in Table 6–3 are also detoxified by cytochrome P450 by conversion to less toxic metabolites. In some cases, the same CYP enzyme catalyzes both activation and detoxication reactions. For example, CYP3A4 activates aflatoxin B_1 to the hepatotoxic and tumorigenic 8,9-epoxide, but it also detoxifies aflatoxin B_1 by 3-hydroxylation to aflatoxin Q_1. Complex factors determine the balance between xenobiotic activation and detoxication.

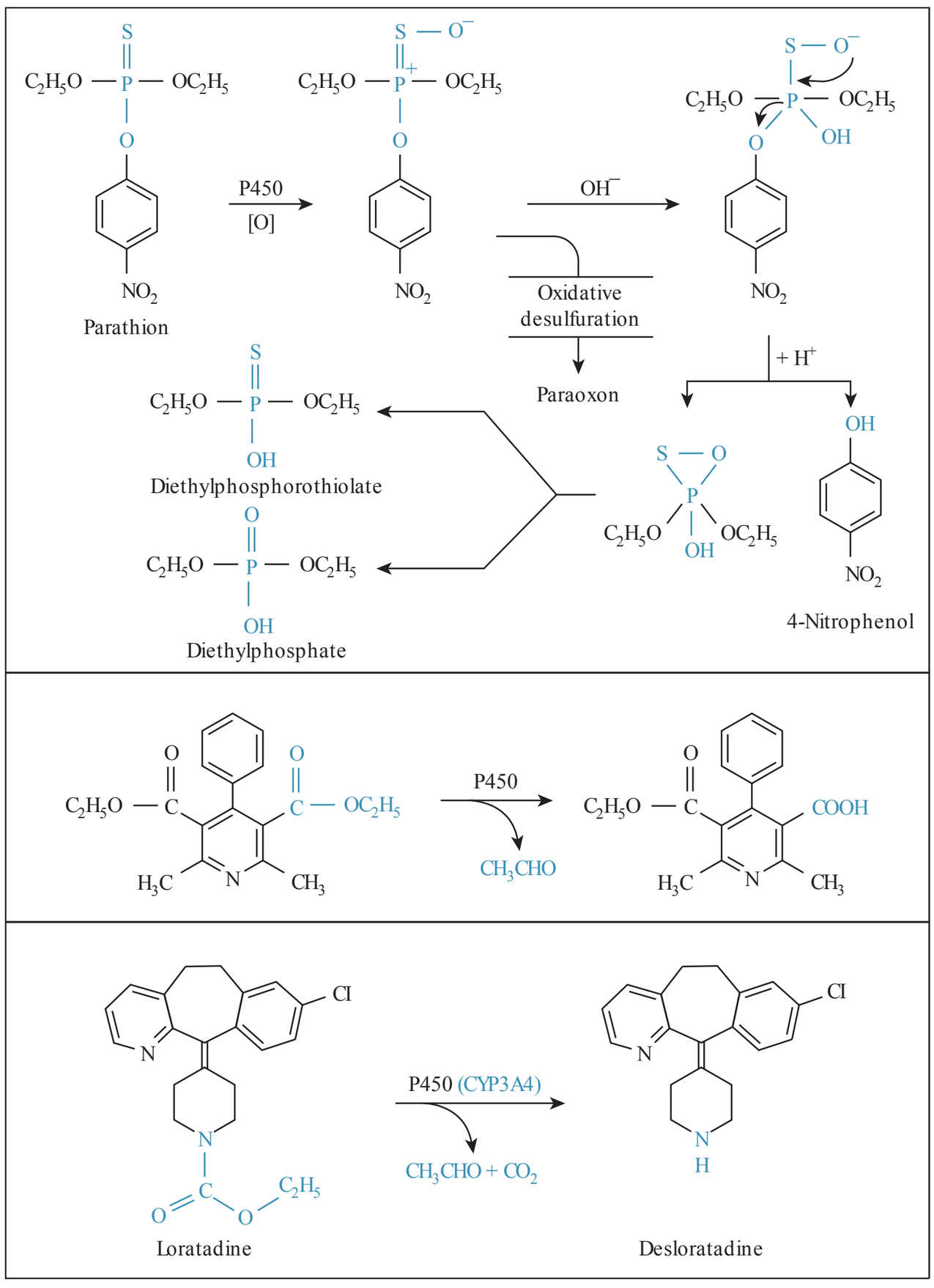


FIGURE 6–11 **Examples of reactions catalyzed by cytochrome P450 that resemble hydrolytic reactions: cleavage of a thiophosphate (parathion), a carboxylic acid ester (2,6-dimethyl-4-phenyl-3,5-pyridinecarboxylic acid diethyl ester), and a carbamate (loratadine).**

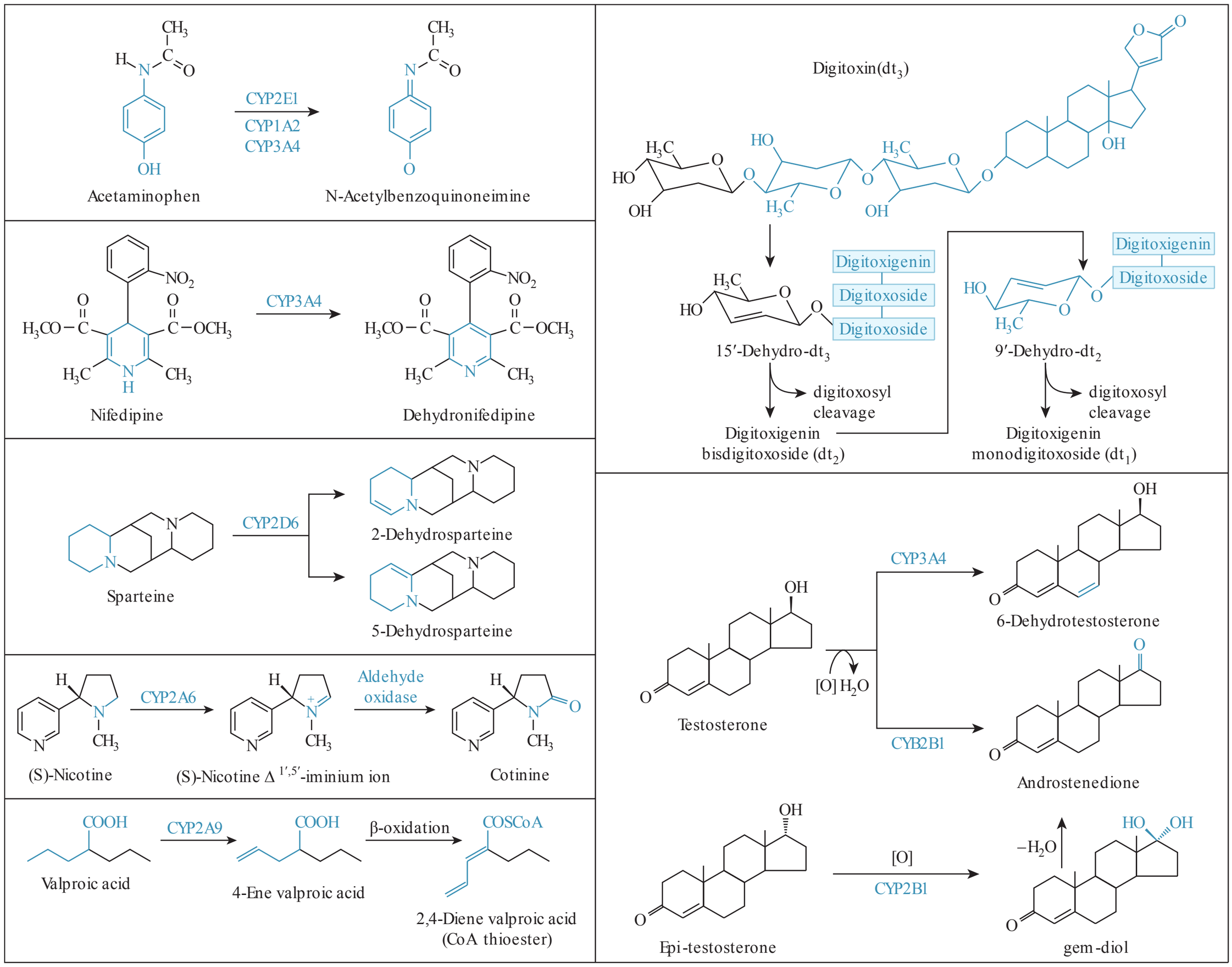


FIGURE 6–12 **Examples of reactions catalyzed by cytochrome P450: dehydrogenation.**

Inhibition of Cytochrome P450—Inhibition of CYP is a major cause of drug–drug interactions and may cause the withdrawal of regulatory approval. The magnitude of the drug–drug interaction depends on the degree of CYP inhibition by the perpetrator drug (those xenobiotics that inhibit or induce the enzyme that is responsible for clearing a victim drug) and the fractional metabolism of the victim drug (xenobiotic whose clearance is largely determined by a single route of elimination, such as a single CYP) by the affected enzyme. Inhibitory drug interactions generally fall into two categories: direct inhibition (which can be competitive, noncompetitive, and uncompetitive) and metabolism-dependent inhibition (which can be irreversible or quasi-irreversible). Direct inhibition can be subdivided into two types. The first involves competition between two drugs that are metabolized by the same CYP enzyme. The second is also competitive in nature, but the inhibitor is not a substrate for the affected CYP enzyme. Metabolism-dependent inhibition occurs when cytochrome P450 converts a xenobiotic to a metabolite that is a more potent inhibitor, either reversible or irreversible, than the parent compound.

Induction of Cytochrome P450—**Xenosensors** The induction (upregulation) of xenobiotic-biotransforming enzymes and transporters is a receptor-mediated, adaptive process that augments xenobiotic elimination during periods of high xenobiotic exposure. It is not a toxicological or pathological response, but enzyme induction is often associated with liver enlargement (due to both hepatocellular hypertrophy and hyperplasia), and it may be associated with toxicological and pharmacological consequences, especially for the safety evaluation of drug candidates in laboratory animals and for clinical practice in humans. In animals and humans, enzyme induction may be associated with pharmacokinetic tolerance, whereby the xenobiotic induces its own elimination.

Inducers of cytochrome P450 increase the rate of xenobiotic biotransformation. Some of the CYP enzymes in human liver microsomes are inducible (Table 6–2). P450 induction typically lowers blood levels, which compromises the therapeutic goal of drug therapy but does not cause an exaggerated response to the drug.

TABLE 6–2 **Examples of clinically relevant substrates, inhibitors, and inducers of the major human liver microsomal P450 enzymes involved in xenobiotic biotransformation.**

	CYP1A2	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2E1
Substrates	Alosetron	Coumarin	Bupropion	Amodiaquine	Diclofenac	Fluoxetine	Aniline
	Caf eine	Nicotine	Efavirenz	Cerivastatin	Fluoxetine	S-Mephenytoin	Chlorzoxazone
	Duloxetine		Propofol	Paclitaxel	Flurbiprofen	Lansoprazole	Lauric acid
	7-Ethoxyresorufin		S-Mephenytoin	Rosiglitazone	Phenytoin	Moclobemide	4-Nitrophenol
	Phenacetin		Cyclophosphamide	Repaglinide	Tolbutamide	Omeprazole	
	Tacrine		Ketamine		S-Warfarin	Pantoprazole	
	Tizanidine		Meperidine				
	Theophylline		Nevirapine				
Inhibitors	Acyclovir	Methoxsalen	Clopidogrel	Gemfibrozil	Amiodarone	Fluvoxamine	Clomethiazole
	Cimetidine	Pilocarpine	3-Isopropenyl-3-methyl diamantane	Montelukast	Capecitabine	Moclobemide	Diallyldisulfide
	Ciprofloxacin	Tranylcypromine	2-Isopropenyl-2-methyladamantane	Quercetin	Fluconazole	Nootkatone	Diethyldithiocarbamate
	Famotidine	Tryptamine	Phencyclidine	Rosiglitazone	Fluoxetine	Omeprazole	Disulfiram
	Fluvoxamine		Sertraline	Rosuvastatin	Fluvoxamine	Ticlopidine	
	Furafylline		Thio-TEPA	Trimethoprim	Oxandrolone		
	Mexilitene		Ticlopidine		Sulfaphenazole		
	α-Naphthoflavone		Phenylethylpiperidine		Sulfinpyrazone		
	Norfloxacin				Tienilic acid		
	Propafenone						
	Verapamil						
	Zileuton						
Inducers	3-Methylcholanthrene	Dexamethasone	Phenobarbital	Phenobarbital	Phenobarbital	Phenobarbital	Ethanol
	β-Naphthoflavone	Pyrazole	Phenytoin	Rifampin	Rifampin	Rifampin	Isoniazid
	Omeprazole		Rifampin				
	Lansoprazole						
	TCDD						
	CYP2AD6		**CYP3A4**				
Substrates	Atomoxetine	(R)-Metoprolol	Alfentanil	Clopidogrel	Fentanyl	Midazolam	Saquinavir
	Amitriptyline	Methylphenidate	Alfuzosin	Cyclosporine	Fluticasone	Mifepristone	Sildenafil
	Aripiprazole	Mexiletine	Alprazolam	Depsipeptide	Gallopamil	Mosapride	Sibutramine
	Brofaromine	Morphine	Amlodipine	Dexamethasone	Gefitinib	Nicardipine	Simvastatin

(Continued)

TABLE 6–2 **Examples of clinically relevant substrates, inhibitors, and inducers of the major human liver microsomal P450 enzymes involved in xenobiotic biotransformation.** (Continued)

	CYP2AD6		CYP3A4				
	(±)-Bufuralol	Nortriptyline	Amprenavir	Dextromethorphan	Gepirone	Nifedipine	Sirolimus
	(S)-Chlorpheniramine	Ondansetron	Aprepitant	Diergotamine	Granisetron	Nimoldipine	Sunitinib
	Chlorpromazine	Paroxetine	Artemether	α-Dihydroergocriptine	Gestodene	Nisoldipine	Tacrolimus
	Clomipramine	Perhexiline	Astemiziole	Disopyramide	Halofantrine	Nitrendipine	Tadalafil
	Codeine	Pimozide	Atazanavir	Docetaxel	Laquinimod	Norethindrone	Telithromycin
	Debrisoquine	Propafenone	Atorvastatin	Domperidone	Imatinib	Oxatomide	Terfenadine
	Desipramine	(+)-Propranolol	Azithromycin	Dutasteride	Indinavir	Oxybutynin	Testosterone
	Dextromethorphan	Sparteine	Barnidipine	Ebastine	Isradipine	Perospirone	Tiagabine
	Dolasetron	Tamoxifen	Bexarotene	Eletriptan	Itraconazole	Pimozide	Tipranavir
	Duloxetine	Thioridazine	Bortezomib	Eplerenone	Karenitecin	Pranidipine	Tirilazad
	Fentanyl	Timolol	Brotizolam	Ergotamine	Ketamine	Praziquantel	Tofisopam
	Haloperidol (reduced)	Tramadol	Budesonide	Erlotinib	Levomethadyl	Quetiapine	Triazolam
	Imipramine	(R)-Venlafaxine	Buspirone	Erythromycin	Lonafarnib	Quinidine	Trimetrexate
	Loperamide		Capravirine	Eplerenone	Lopinavir	Quinine	Vardenafil
			Carbamazepine	Ethosuximide	Loperamide	Reboxetine	Vinblastine
			Cibenzoline	Etoperidone	Lumefantrine	Rifabutin	Vincristine
			Cilastazol	Everolimus	Lovastatin	Ritonavir	Vinorelbine
			Cisapride	Ethinyl estradiol	Medroxyprogesterone	Rosuvastatin	Ziprasidone
			Clarithromycin	Etoricoxib	Methylprednisolone	Ruboxistaurin	Zonisamide
			Clindamycin	Felodipine	Mexazolam	Salmetrol	
Inhibitors	Amiodarone	Fluoxetine	Amiodarone	Cimetidine	Fluvoxamine	Itraconazole	Saquinavir
	Buproprion	Methadone	Amprenavir	Clarithromycin	Fosamprenavir	Mibefradil	St. John's wort
	Chlorpheniramine	Mibefradil	Aprepitant	Diltiazem	Gestodene	Nefazodone	Telithromycin
	Cimetidine	Paroxetine	Atazanavir	Erythromycin	Grapefruit juice	Nelfinavir	Troleandomycin
	Clomipramine	Quinidine	Azamulin	Felbamate	Ketoconazole	Ritonavir	Verapamil
	Duloxetine	Sertraline	Bosentan	Fluconazole	Indinavir	Roxithromycin	
	Haloperidol	Terbinafine	Amprenavir	Efavirenz	Nifedipine	Rifampin	Troglitazone
Inducers	NA		Avasimibe	Etoposide	Omeprazole	Rifapentine	Troleandomycin
			Bosentan	Guggulsterone	Paclitaxel	Ritonavir	Vitamin E
			Carbamazepine	Hyperforin	PCBs	Simvastatin	Vitamin K2
			Clotrimazole	Lovastatin	Phenobarbital	Spironolactone	Yin zhi wuang
			Cyproterone acetate	Mifepristone	Phenytoin	Sulfinpyrazone	
			Dexamethasone	Nelfinavir	Rifabutin	Topotecan	

TABLE 6–3 Examples of xenobiotics activated by human P450.

CYP1A2

Acetaminophen
2-Acetylaminofluorene
4-Aminobiphenyl
2-Aminofluorene
2-Naphthylamine
NNK
Amino acid pyrolysis products (DiMeQx, MeIQ, MeIQx, Glu P-2, IQ, PhIP, Trp P-1, Trp P-2)
Tacrine

CYP2A6 and 2A13

NNK and bulky nitrosamines
N-Nitrosodiethylamine
Aflatoxin B1

CYP2B6

6-Aminochrysene
Cyclophosphamide
Ifosfamide

CYP2C8, 9, 18, 19

Tienilic acid
Phenytoin
Valproic acid

CYP2D6

NNK

CYP2F1

3-Methylindole
Acetaminophen
Valproic acid

CYP1A1 and 1B1

Benzo[a]pyrene and other polycyclic aromatic hydrocarbons

CYP3A4

Acetaminophen
Aflatoxin B1 and G1
6-Aminochrysene
Benzo[a]pyrene 7,8-dihydrodiol
Cyclophosphamide
Ifosfamide
1-Nitropyrene
Sterigmatocystin
Senecionine
Tris(2,3-dibromopropyl) phosphate

CYP2E1

Acetaminophen
Acrylonitrile
Benzene
Carbon tetrachloride
Chloroform
Dichloromethane
1,2-Dichloropropane
Ethylene dibromide
Ethylene dichloride
Ethyl carbamate
Halothane
N-Nitrosodimethylamine
Styrene
Trichloroethylene
Vinyl chloride

CYP4B1

Ipomeanol
3-Methylindole
2-Aminofluorene

NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific nitrosamine.
Data from Guengerich FP, Shimada T: Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol, 1991 Jul-Aug;4(4):391–407.

Although induction of cytochrome P450 may increase the activation of procarcinogens to DNA-reactive metabolites, there is little evidence from either human epidemiologic studies or animal experimentation that P450 induction enhances the incidence or multiplicity of tumors caused by known chemical carcinogens. In fact, most evidence points to a protective role of enzyme induction against chemical-induced neoplasia. Cytochrome P450 induction can cause pharmacokinetic tolerance whereby larger drug doses must be administered to achieve therapeutic blood levels due to increased drug bio-transformation.

CYP induction is mediated by four ligand-activated receptors, namely, AhR, CAR, PXR, and PPARα (Table 6–4). T ese so-called xenosensors resemble other nuclear receptors, such as steroid and thyroid hormone receptors, with cross-talk among xenosensors and cross-talk between xenosensors and other nuclear receptors. Xenosensors have a ligand-binding domain (LBD) and a DNA-binding domain (DBD). In general, CYP induction involves the following steps (with steps 2 and 3 reversed in the case of AhR): (1) binding of ligand (xenobiotic) to the receptor, which triggers conformational changes that promote its dissociation from accessory proteins (such as corepressors, chaperones, and cytoplasm retention proteins) and promote its association with coactivators; (2) dimerization of the ligand-bound receptor with a partner protein to form a DNA-binding heterodimer (which is analogous to the two halves of a clothes peg coming together to form a functional unit); (3) translocation of the functional receptor heterodimer from the cytoplasm to the nucleus; (4) binding of the functional receptor heterodimer to discrete regions of DNA (response elements) that are typically located in the 5′-promoter region of the gene (which is analogous to a clothes peg being fastened to a clothes line); (5) recruitment of other transcription factors and coactivators (such as histone and RNA methyltransferases, histone and chromatin deacetylases, and histone remodeling helicases) and RNA polymerase to form a transcription complex; and (6) gene transcription, which leads to increased levels of CYP mRNA and protein (as well as other xenobiotic-biotransforming enzymes and transporters). As is the case with all nuclear receptors, the details of the process of activating a xenosensor to its transcriptionally active form are complex and multifaceted.

CONJUGATION

Conjugation reactions include glucuronidation, sulfonation (often called sulfation), acetylation, methylation, conjugation with glutathione (mercapturic acid synthesis), and conjugation with amino acids (such as glycine, taurine, and glutamic acid). T e cosubstrates for these reactions, which are shown in Figure 6–13, react with functional groups that are either present

TABLE 6–4 Receptors mediating the induction (or suppression) of cytochrome P450 enzymes and other xenobiotic-biotransforming enzymes and transporters.

Nuclear Receptor	Response Element(s)	Receptor Activators	Regulated Genes*
AhR	XRE	PAHs, TCDD (other PHAHs), β-naphthoflavone, indigoids, tryptophan metabolites, omeprazole, lansoprazole	CYP1A1, 1A2, 1B1, 2S1, UGT1A1, UGT1A6, AKR1A1, AKR1C1-4
CAR	DR-3 DR-4 ER-6	Phenobarbital, phenytoin, carbamazepine, CITCO (human), TCPOBOP (mouse), clotrimazole, (Many PXR agonists are also CAR agonists, and vice versa)	CYP2A6, 2B6, 2C8, 2C9, 2C19, 3A4, UGT1A1, SULT1A1, AKR1D1, ALAS, MRP2, MRP3, MRP4
PXR	DR-3 DR-4 ER-6 ER-8	Amprenavir, avasimibe, bosentan, bile acids, carbamazepine, clindamycin, clotrimazole, cortisol, cyproterone acetate, dicloxacillin, efavirenz, etoposide, dexamethasone, griseofulvin, guggulsterone, hyperforin (SJW), indinavir, lovastatin, mifepristone, nafcillin, nelfinavir, nifedipine, omeprazole, paclitaxel, PCBs, phenobarbital, phthalate monoesters, 5β-pregnane-3,20-dione, rifabutin, rifampin, ritonavir, saquinavir, simvastatin, spironolactone, sulfinpyrazone, TAO, tetracycline, topotecan, transnonachlor, troglitazone, verapamil, vitamin E, vitamin K_2	CYP2A6, 2B6, 2C8, 2C9, 2C19, 3A4, 3A7, 4F12, 7A1↓, CES2, SULT2A1, UGT1A1, 1A3, 1A4, 1A6, GSTA1, AKR1D1, PAPSS2, ALAS, MDR1, MRP2, AhR
PPARα	DR-1	Fibrates, WY-14643, perfluorodecanoic acid	CYP4A, UGT1A9, 2B4
Nrf2	ARE	β-Naphthoflavone, oltipraz, phenolic antioxidants (e.g., BHA and BHT), phenylisothiocyanate, diethyl maleate, phorone	NQO1, mEH, AKR7A, UGTs, GSTA1, γ-GCL, MRP1
GR	GRE	Glucocorticoids (e.g., dexamethasone)	CYP2C9, 2B6, 3A4, 3A5, CAR, PXR
FXR	IR-1	Bile acids, GW4064, AGN29, AGN31	BSEP, I-BABP, MDR3, UGT2B4, SULT2A1, OATP1B3, PPARα, SHP
LXRα	DR-4	GW3965, T0901317, paxiline, F_3methylAA,† acetylpodocarpic dimer (APD)	LRH1, SHP, CYP7A, LXRα, CYP3A4 ↓↓, 2B6 ↓
VDR	DR-3 ER-6 IR-0	1α,25-Dihydroxyvitamin D_3, lithocholate	CYP2B6, 2C9, 3A4, SULT2A1
HNF1α‡			OATP1B1, OATP1B3, CYP7A1, UGT1A6, 1A8, 1A9, 1A10, HNF4α, PXR, kidney-specific expression of OAT1, OAT3, URAT1
HNF4α	DR		CYP2A6, 2B6, 2C9, 2D6, 3A4, DD4, MDR1, PXR, CAR, FXR, PPARα, HNF1α
LRH-1	DR-4		CYP7A, ASBT
SHP	None		Targets of PPARα ↓, AhR↓, PXR↓, CAR↓, LRH-1 ↓, HNF4α ↓, LXRα ↓, GR↓

*A downward arrow indicates downregulation (suppression). All others are upregulated (induced).

†[3-Chloro-4-(3-(7-propyl-3-trifuoromethyl-6-(4,5)-isoxazolyl)propylthio)-phenylacetic acid].

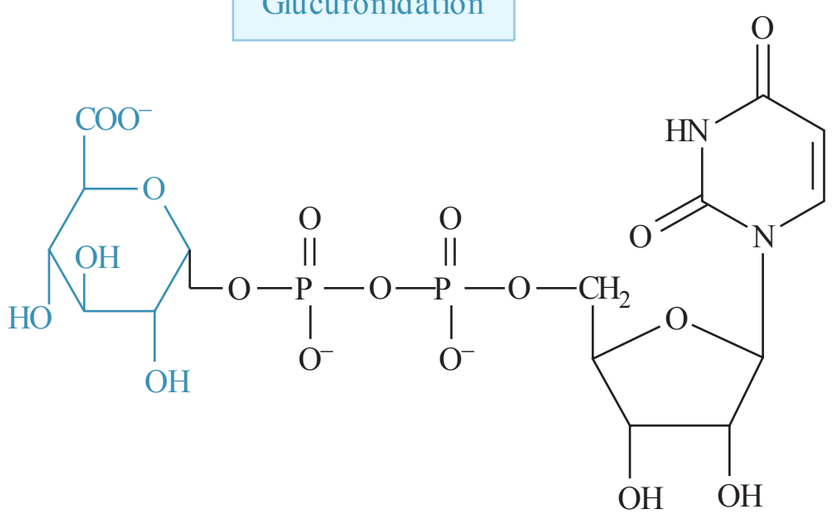
‡The HNF1α consensus sequence is GTTTAATNATTAAC.

on the xenobiotic or are introduced or exposed during oxidation, reduction, or hydrolysis. With the exception of methylation and acetylation, conjugations result in a large increase in xenobiotic hydrophilicity, which greatly facilitates excretion of foreign chemicals. Glucuronidation, sulfation, acetylation, and methylation involve reactions with activated or "high-energy" cosubstrates, whereas conjugation with amino acids or glutathione involves reactions with activated xenobiotics. Except for the glucuronosyltransferases, most conjugation enzymes are mainly located in the cytosol (Table 6–1).

Glucuronidation

Glucuronidation requires the cosubstrate uridine diphosphate-glucuronic acid (UDP-glucuronic acid), and the reaction is

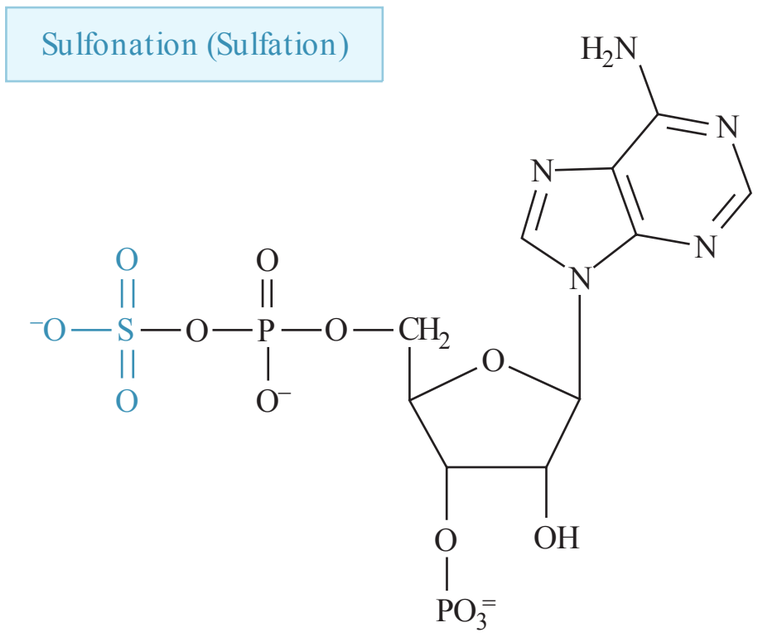
Glucuronidation



D

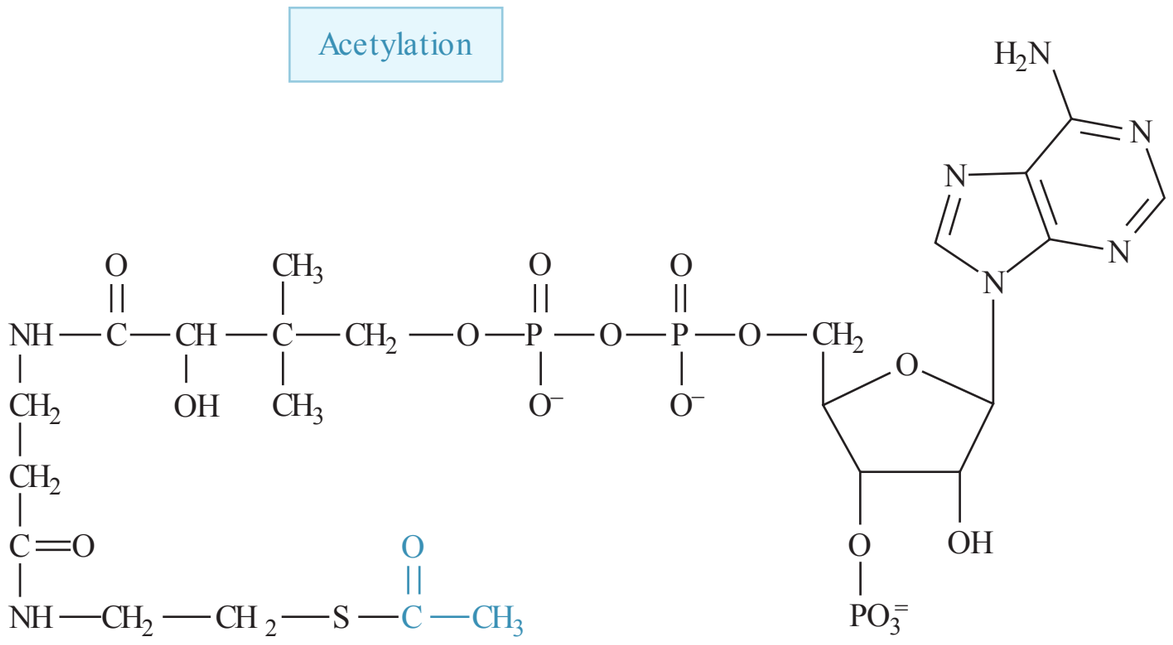
Uridine-5′-diphospho-α-D-glucuronic acid (UDPGA)

Sulfonation (Sulfation)



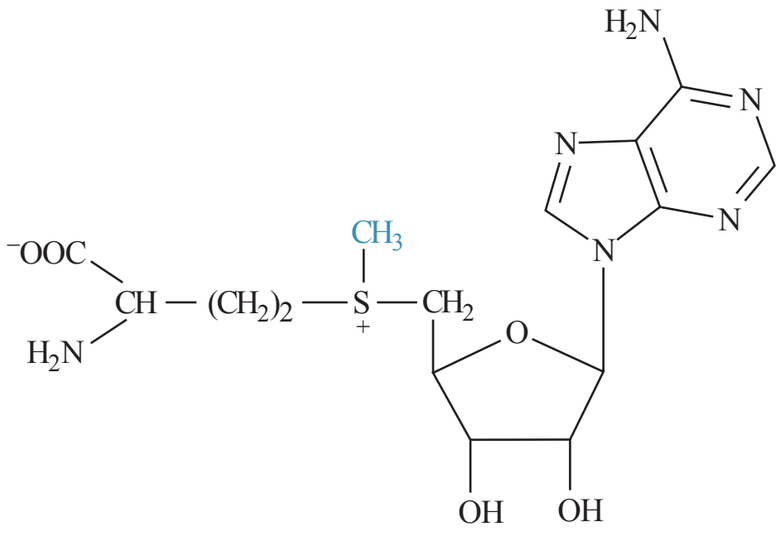
3′-Phosphoadenosine-5′-phosphosulfate (PAPS)

Acetylation



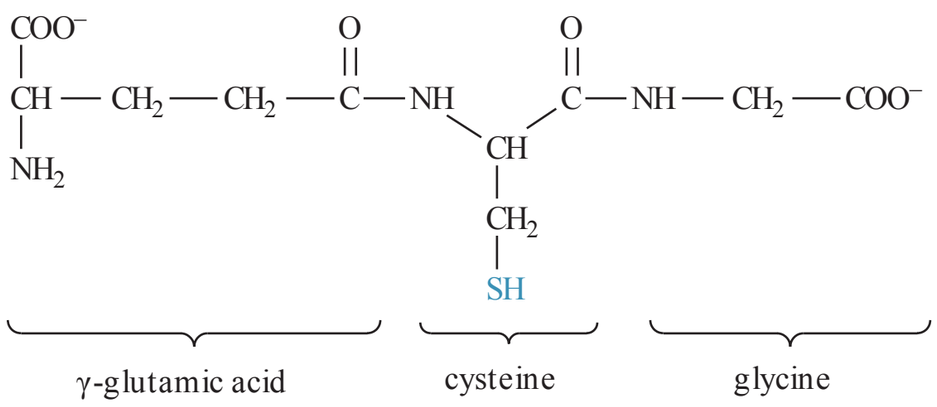
Acetyl-coenzyme A

Methylation



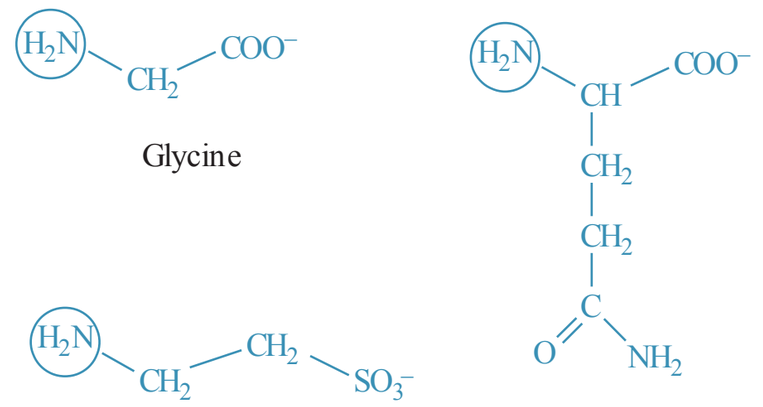
S-Adenosylmethionine (SAM)

Glutathione conjugation



Glutathione

Amino acid conjugation

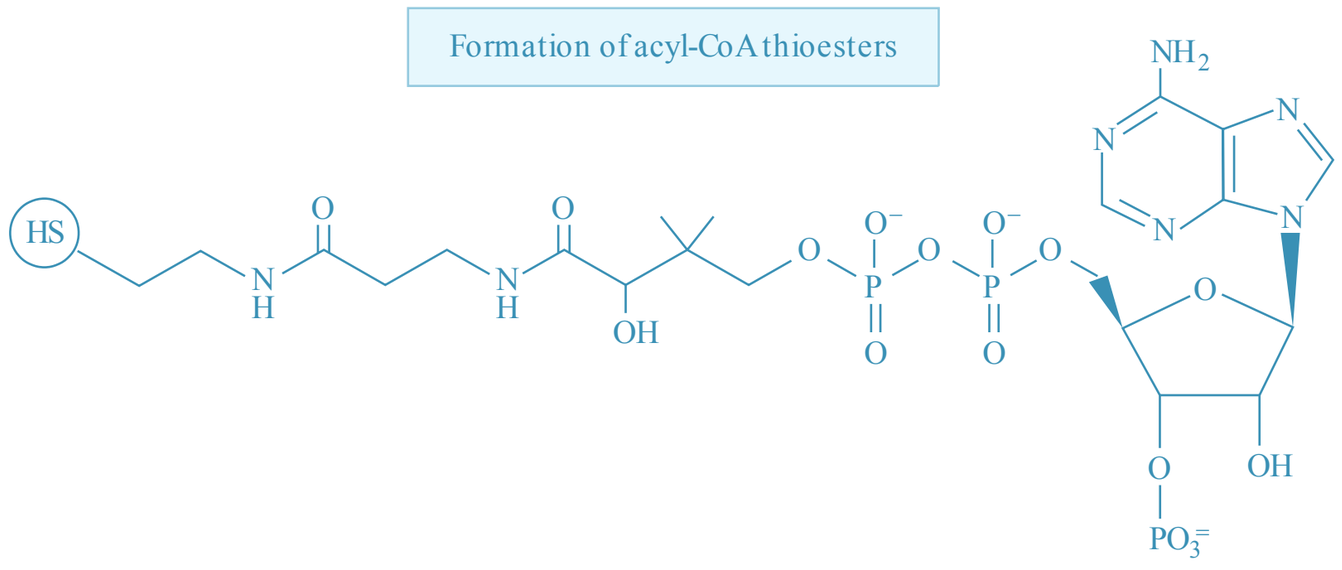


Glycine

Taurine

Glutamine

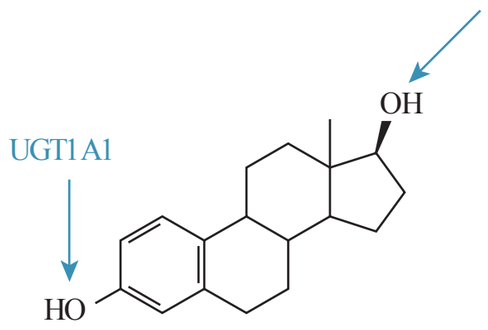
Formation of acyl-CoA thioesters



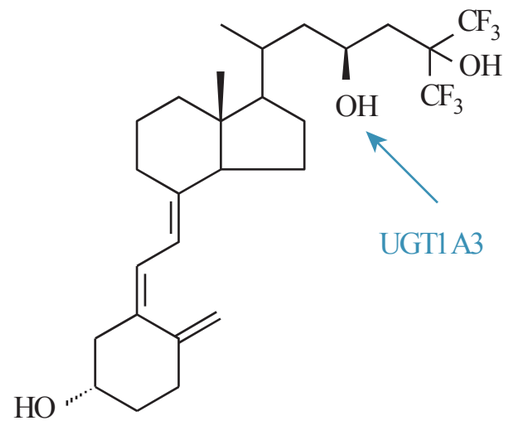
Coenzyme A

FIGURE 6–13 **Structures of cofactors for phase II biotransformation.** The functional group that reacts with or is transferred to the xenobiotic is shown in red.

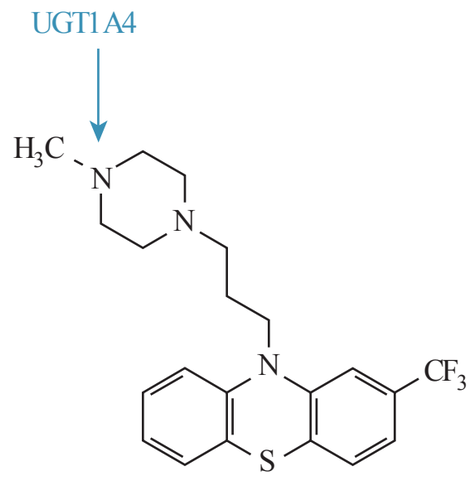
O-Glucuronides (ethers)



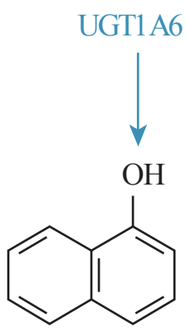
17 β-Estradiol



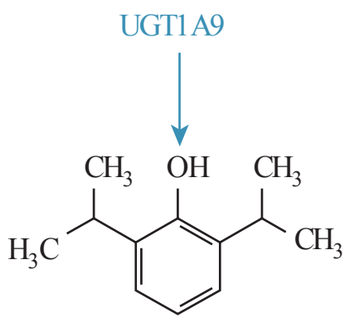
26,26,26,27,27,27,-hexafluoro-1α,23,25-trihydroxyvitamin D3



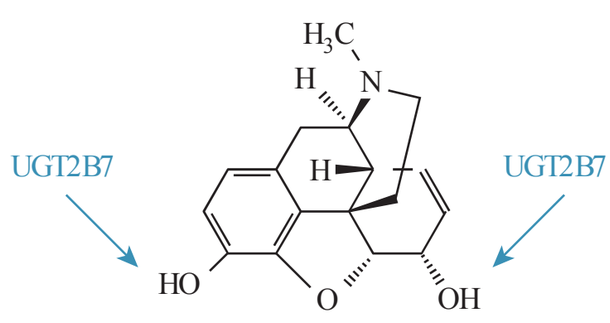
Trifluoperazine



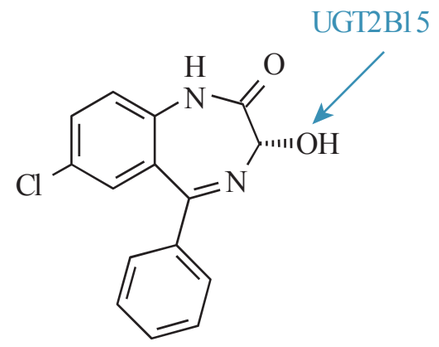
1-Naphthol



Propofol



Morphine

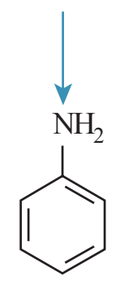


S-Oxazepam

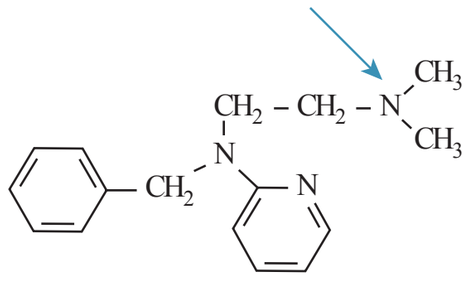
Other Examples

Acetaminophen	Estrone	Naloxone	Progesterone	Testosterone
Codeine	Hexobarbital	4-Nitrophenol	Propranolol	Thyroxine
Dextrophan	Methylphenylcarbinol	Phenolphthalein	Temazepam	Trichloroethanol
Diethystilbestrol				

N-Glucuronides



Aniline

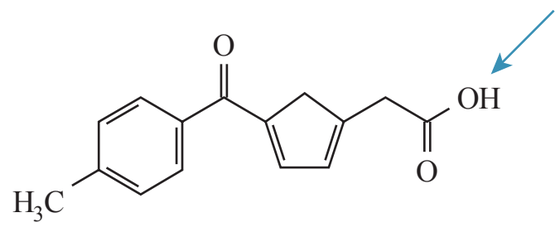


Tripelennamine

Other Examples

Amitryptyline	Cyproheptadine	Meprobamate
Benzidine	N-Hydroxyarylamines	Sulfadimethoxine
Cyclopiroxolamine	Imipramine	Sulfathiazole
Cyclobenzaprine	Lamotrigine	Sulfisoxazole

Acyl-glucuronides
O-Glucuronides (esters)



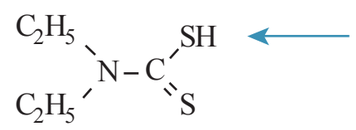
Tolmetin

Other Examples

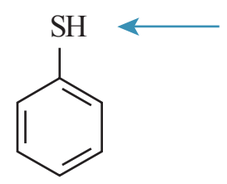
Benoxaprofen

Bilirubin	Etodolac	Naproxen
Diclofenac	Gemfibrozil	Suprofen
Diflunisal	Ketoprofen	Valproic acid
Trifluoperazine	Iopanoic acid	Zomepirac

S-Glucuronides



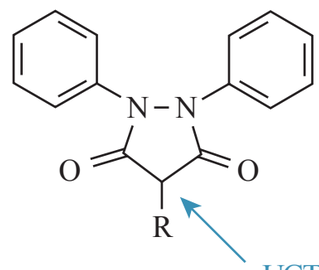
Diethyldithiocarbamate



Thiophenol

Other Examples: Disulfiram Methimazole

C-Glucuronides



Phenylbutazone R = C_4H_9

Sulfinpyrazone R = $(CH_2)_2SOC_6H_5$

Other Examples: Suxibuzone Ethchlorvynol Δ^6-THC

FIGURE 6–14 **Examples of xenobiotics and endogenous substrates that are glucuronidated.** The arrow indicates the site of glucuronidation, with the UGT enzyme if selective.

catalyzed by UDP-glucuronosyltransferases (UGTs). Examples of xenobiotics that are glucuronidated are shown in Figure 6–14. The site of glucuronidation is generally an electron-rich nucleophilic heteroatom (O, N, or S) as found in aliphatic alcohols and phenols, carboxylic acids, primary and secondary aromatic and aliphatic amines, and free sulfhydryl groups. Endogenous substrates for glucuronidation include bilirubin, steroid hormones, and thyroid hormones.

Glucuronide conjugates of xenobiotics and endogenous compounds are polar, water-soluble metabolites. Whether

glucuronides are excreted from the body in bile or urine depends on the size of the aglycone (parent compound or unconjugated metabolite). The carboxylic acid moiety of glucuronic acid, which is ionized at physiologic pH, promotes excretion because (1) it increases the aqueous solubility of the xenobiotic and (2) it is recognized by the biliary and renal organic anion transport systems, which enables glucuronides to be secreted into urine and bile. Glucuronides of xenobiotics are substrates for β-glucuronidase present in the intestinal microflora. The intestinal enzyme can release the aglycone, which undergoes enterohepatic circulation delaying elimination of the xenobiotic.

Cofactor availability can limit the rate of glucuronidation of drugs that are administered in high doses and are conjugated extensively, such as aspirin and acetaminophen.

Sulfonation

Many xenobiotics and endogenous substrates undergo sulfonation. Sulfate conjugation is catalyzed by sulfotransferases, a multigene family of enzymes that generally produces a highly water-soluble sulfuric acid ester. The cosubstrate for the reaction is 3′-phosphoadenosine-5′-phosphosulfate (PAPS; see Figure 6–13).

Sulfate conjugation involves the transfer of sulfonate, not sulfate (i.e., SO_3^-, not SO_4^-) from PAPS to the xenobiotic. (The commonly used terms sul*f*ation and sul*f*ate conjugation are used here, even though sul*f*onation and sul*f*onate conjugation are more appropriate descriptors.) Table 6–4 lists examples of xenobiotics and endogenous compounds that are sulfonated without prior biotransformation by oxidation enzymes. An even greater number of xenobiotics are sulfated after a hydroxyl group is exposed or introduced during oxidative or hydrolytic biotransformation.

Sulfate conjugates of xenobiotics are excreted mainly in urine. Sulfatases present in the endoplasmic reticulum and lysosomes primarily hydrolyze sulfates of endogenous compounds. Some sulfate conjugates are substrates for further biotransformation.

PAPS is synthesized from inorganic sulfate (SO_4^{2-}) and ATP in a two-step reaction. The major source of sulfate required for the synthesis of PAPS appears to be derived from cysteine through a complex oxidation sequence. The low cellular concentration of PAPS (~75 μM versus ~350 μM UDP-glucuronic acid and ~10 mM glutathione) limits the capacity for xenobiotic sulfonation.

Multiple sulfotransferases have been identified in all mammalian species examined. There are two major enzyme classes: membrane-bound enzymes are found in the Golgi apparatus and soluble enzymes are located in the cytoplasm. Sulfotransferases are arranged into gene families (SULT1 to SULT5) that share at least 45% amino acid sequence identity, and are further subdivided into several subfamilies. Each family appears to work on a specific functional group (i.e., phenols, alcohols, and amines) (Table 6–5).

In general, sulfonation is an effective means of decreasing the pharmacologic and toxicologic activity of xenobiotics. However, as shown in Figure 6–15, sulfonation has a role in the activation of aromatic amines, methyl-substituted polycyclic aromatic hydrocarbons, and safrole to tumorigenic metabolites.

Methylation

Methylation, a minor pathway of biotransformation, generally decreases the water solubility of xenobiotics and masks functional groups that might otherwise be conjugated by other enzymes. Methylation can also lead to increased toxicity. The cosubstrate for methylation is S-adenosylmethionine (SAM) (Figure 6–13). The methyl group bound to the sulfonium ion in SAM is transferred to xenobiotics and endogenous substrates by nucleophilic attack from an electron-rich heteroatom (O, N, or S) leaving S-adenosylhomocysteine. Examples of xenobiotics and endogenous substrates that undergo O-, N-, or S-methylation are shown in Figure 6–16.

The O-methylation of phenols and catechols is catalyzed by two different enzymes known as phenol O-methyltransferase (POMT) in microsomes and catechol-O-methyltransferase (COMT) in cytosol and microsomes. In rats and humans, COMT is encoded by a single gene with two different promoters and transcription initiation sites. Transcription at one site produces a cytosolic form of COMT, whereas transcription from the other site produces a membrane-bound form by adding a 50-amino acid segment that targets COMT to the endoplasmic reticulum. Substrates for COMT include several catecholamine neurotransmitters and catechol drugs, such as l-DOPA and methyldopa.

Several N-methyltransferases have been described in humans and other mammals. Phenylethanolamine N-methyltransferase catalyzes the N-methylation of the neurotransmitter norepinephrine to form epinephrine in the adrenal medulla and in certain regions of the brain, and is of minimal significance in xenobiotic biotransformation. However, histamine and nicotine N-methyltransferases expressed in liver, intestine, and/or kidney do methylate xenobiotics.

S-Methylation is an important pathway in the biotransformation of sulfhydryl-containing xenobiotics. In humans, S-methylation is catalyzed by thiopurine methyltransferase in cytosol and thiol methyltransferase in microsomes.

Acetylation

N-Acetylation is a major route of biotransformation for xenobiotics containing an aromatic amine (R—NH_2) or a hydrazine group (R—NH—NH_2), which are converted to aromatic amides (R—NH—$COCH_3$) and hydrazides (R—NH—NH—$COCH_3$), respectively. N-Acetylation masks an amine with a nonionizable group, so that many N-acetylated metabolites are less water soluble than the parent compound. Nevertheless, N-acetylation of certain xenobiotics, such as isoniazid, facilitates their urinary excretion.

Xenobiotic N-acetylation catalyzed by cytosolic N-acetyltransferases requires the cosubstrate acetyl-coenzyme A (acetyl-CoA; Figure 6–13). The two-step reaction involves (1) transfer of the acetyl group from acetyl-CoA to an active site cysteine residue within the enzyme with release of coenzyme A and (2) subsequent transfer of the acetyl group from the acylated enzyme to the amino group of the substrate with regeneration of the enzyme.

NAT1 and NAT2, the two acetyltransferases existing in humans, are 79% to 95% identical in amino acid sequence with

TABLE 6–5 Properties of the human cytosolic sulfotransferases (SULTs).

Human Sult	Polymorphic?	Tissue Distribution	Major Substrates†
SULT1A1	Yes *1–*4	Liver (very high), platelets. placenta, adrenals, endometrium, colon, jejunum, leukocytes, brain (cerebellum, occipital and frontal lobes)	4-Nitrophenol, 4-ethylphenol, 4-cresol, 2-naphthol, other phenols, acetaminophen, minoxidil, N-hydroxy-PhIP, T2, T3, 17β-estradiol (and other phenolic steroids), dopamine, benzylic alcohols, 2-nitropropane, aromatic amines, hydroxylamines, hydroxamic acids, apomorphine, troglitazone, genestein, epinephrine
SULT1A2	Yes *1–*6	Liver, kidney, brain, GI tract, bladder tumors	4-Nitrophenol, N-hydroxy-2-acetylaminofluorene, 2-naphthol, various aromatic hydroxylamines and hydroxamic acids
SULT1A3	Yes *1–*4	Jejunum and colon mucosa (very high), liver (low), platelets, placenta, brain (superior temporal gyrus, hippocampus, and temporal lobe), leukocytes, fetal liver	Dopamine, 4-nitrophenol, 1-hydroxymethylpyrene, norepinephrine, salbutamol, dobutamine, vanillin, albuterol
SULT1A4		Liver, pancreas, colon, brain	Not characterized. Likely similar to SULT1A3
SULT1B1		Colon (highest), liver, leukocytes, small intestine	4-Nitrophenol, T2, T3, r-T3, T4, dopamine, benzylic alcohols
SULT1C2	Yes *1–*5	Fetal lung and kidney, kidney, stomach, thyroid gland	4-Nitrophenol, N-hydroxy-2-AAF, aromatic hydroxylamines, thyroid hormones
SULT1C4		Kidney, ovary, spinal cord, fetal kidney, fetal lung (highest)	4-Nitrophenol, N-hydroxy-2-AAF, 17β-estrone, bisphenol-A, 4-octylphenol, nonylphenol, diethylstilbestrol, 1-hydroxymethylpyrene
SULT1E1		Liver (highest), endometrium, jejunum, adrenals, mammary epithelial cells, fetal liver, fetal lung, fetal kidney	17β-Estradiol, estrone, ethinyl estradiol, 17β-estrone, equilenin, 2-hydroxy-estrone, 2-hydroxy-estradiol, 4-hydroxy-estrone, 4-hydroxy-estradiol, diethylstilbestrol, tamoxifen, thyroid hormones, 4-hydroxylonazolac, pregnenolone, dehydroepiandrosterone, 1-naphthol, naringenin
SULT2A1	Yes *1–*3	Liver (highest), adrenals, ovaries, prostate, jejunum, kidney, brain	Dehydroepiandrosterone (DHEA), 1-hydroxymethylpyrene, 6-hydroxymethylbenzo[a]-pyrene, hycanthone, bile acids, pregnenolone, testosterone, androgens, estrone, 17β-estradiol, other hydroxysteroids, budesonide
SULT2B1a (SULT2B_v1)		Placenta (highest), prostate, trachea, skin	Dehydroepiandrosterone, pregnenolone, oxysterols, other hydroxysteroids
SULT2B1b (SULT2B_v2)		Lung, spleen, thymus, kidney, prostate, ovary, adrenal gland, liver (low), GI tract (low)	Cholesterol, pregnenolone, dehydroepiandrosterone, other hydroxysteroids
SULT4A1a (SULT4A_v1)		Brain: cortex, globus pallidus, islands of Calleja, septum, thalamus, red nucleus, substantia nigra and pituitary	Endogenous: 4 unidentified compounds from mouse brain homogenate Other: T3, T4, estrone, 4-nitrophenol, 2-naphthylamine, 2-naphthol
SULT4A1b (SULT4A_v2)			
SULT6B1	Testis		

†T4 is thyroxine. T2 and T3 are diiodothyronine and triiodothyronine. r-T3 is reverse triiodothyronine.

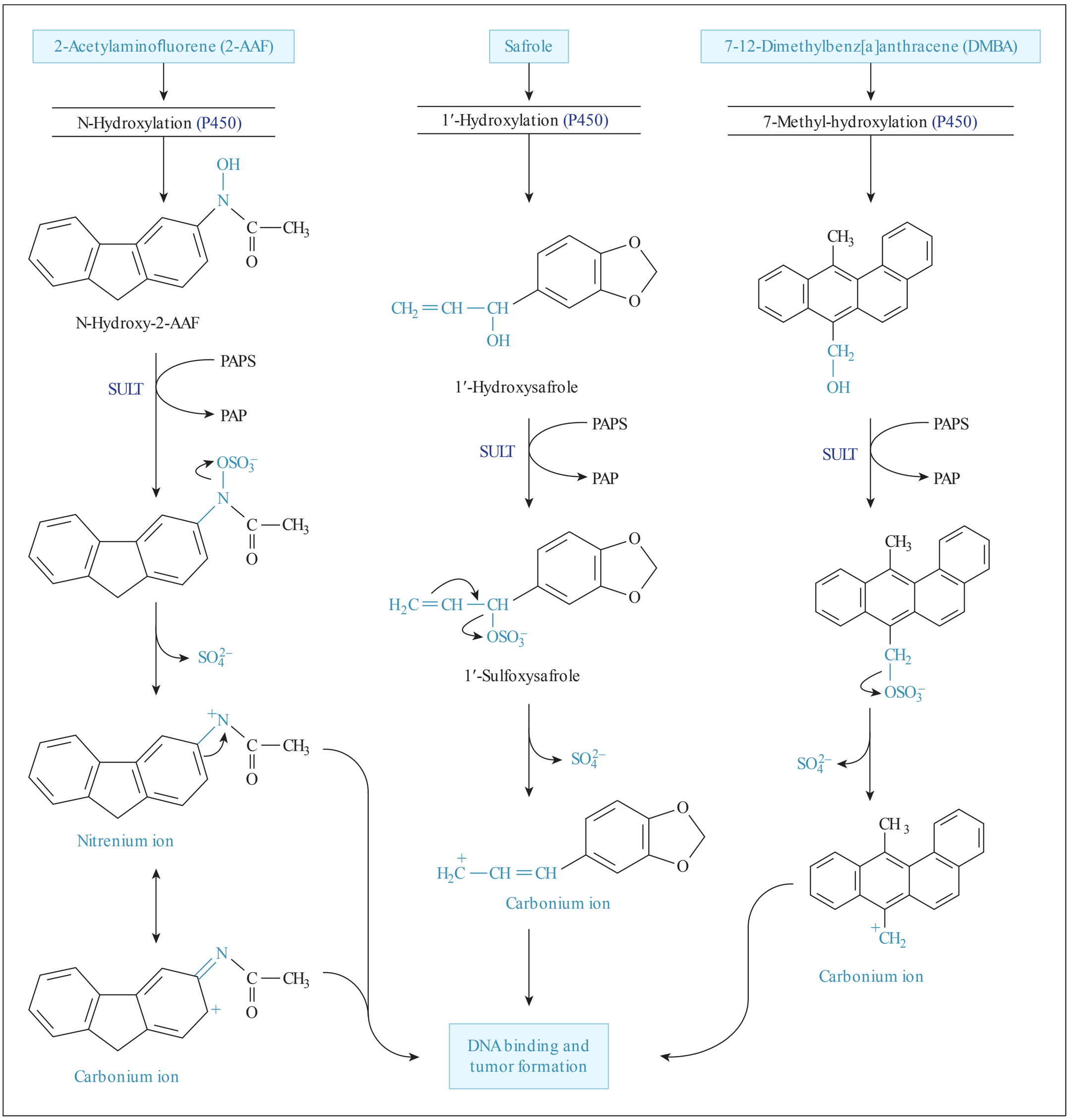


FIGURE 6–15 **Role of sulfonation in the generation of tumorigenic metabolites (nitrenium or carbonium ions) of 2-acetylaminof uorene, safrole, and 7,12-dimethylbenz[a]anthracene (DMBA).**

an active site cysteine residue in the N-terminal region. Although encoded by genes on the same chromosome, NAT1 is expressed in most tissues of the body, whereas NAT2 is mainly expressed only in liver and intestine. Most (but not all) of the tissues that express NAT1 also appear to express low levels of NAT2, at least at the level of mRNA. NAT1 and NAT2 also have different but overlapping substrate specificities. Examples of drugs that are N-acetylated by NAT1 and NAT2 are shown in Figure 6–17.

Genetic polymorphisms for N-acetylation have been documented in humans, hamsters, rabbits, and mice. Polymorphisms in NAT2 have a number of pharmacologic and toxicologic consequences: slow NAT2 acetylators are predisposed to drug toxicities, including excessive hypotension from hydralazine, peripheral neuropathy from isoniazid and dapsone, systemic lupus erythematosus from hydralazine and procainamide, and the toxic effects of coadministration of the anticonvulsant phenytoin with isoniazid.

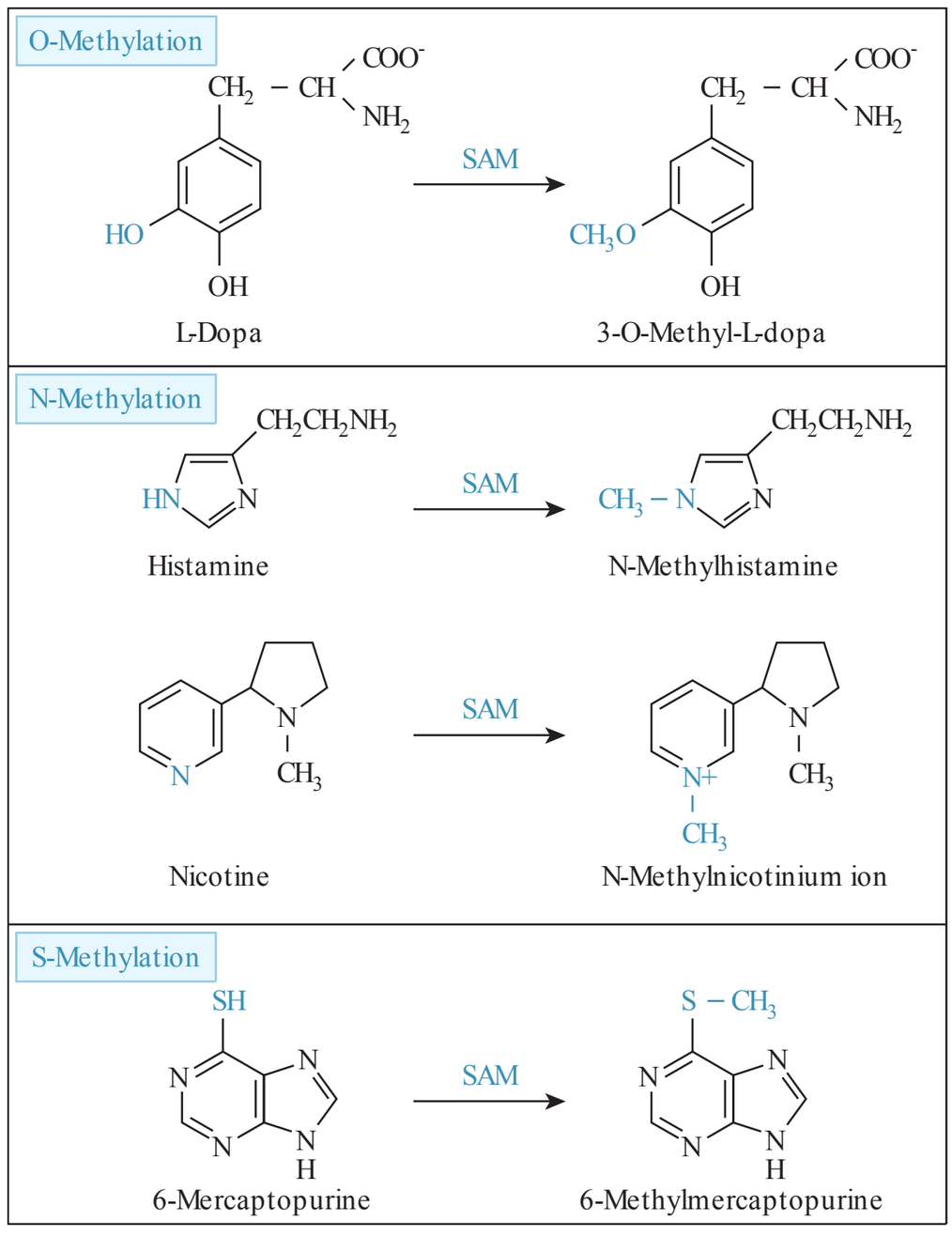


FIGURE 6–16 **Examples of compounds that undergo O-, N-, or S-methylation.**

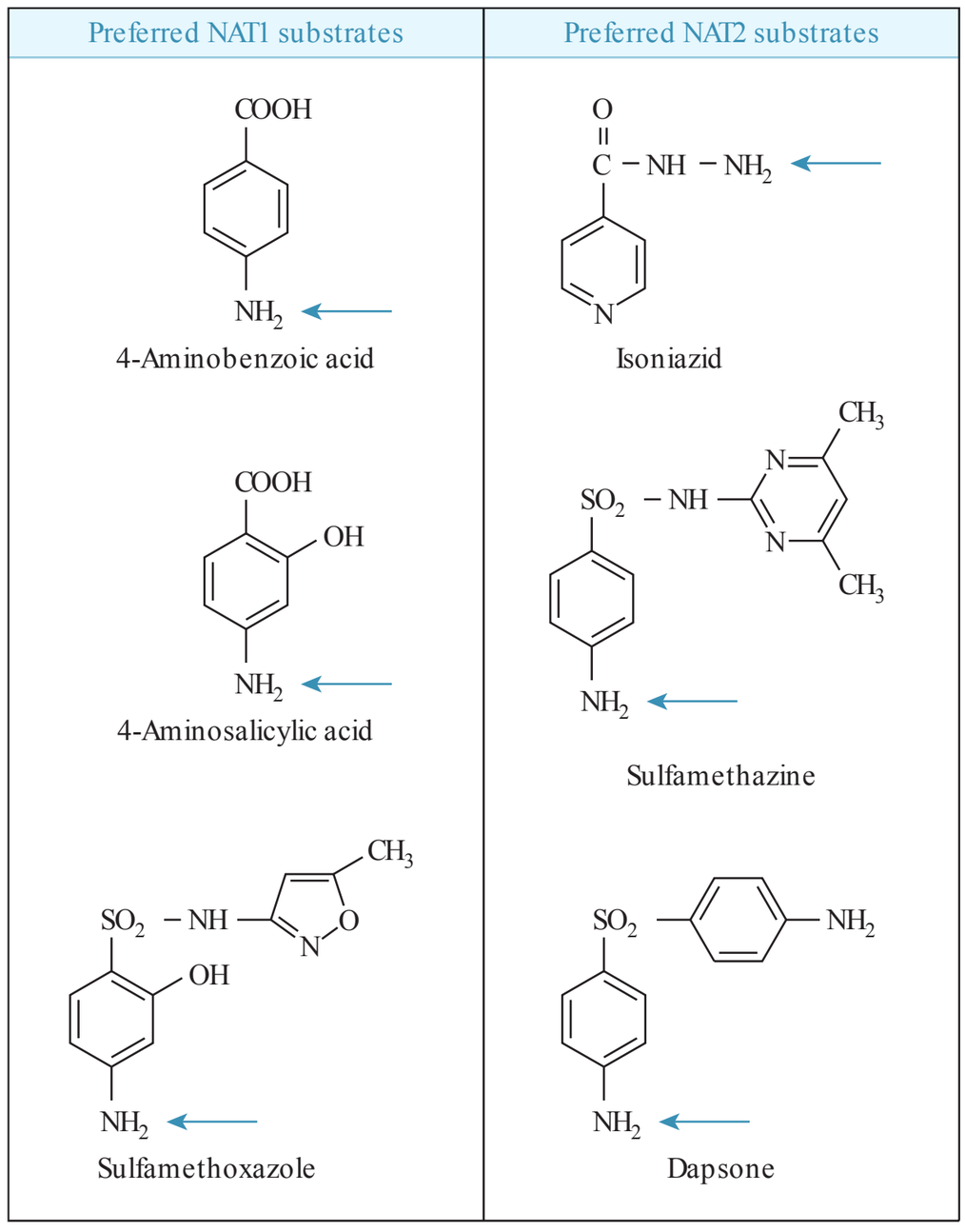


FIGURE 6–17 **Examples of substrates for human N-acetyltransferases, NAT1, and the highly polymorphic NAT2.**

T e N-acetyltransferases detoxify aromatic amines by converting them to the corresponding amides that are less likely to be activated to DNA-reactive metabolites. However, N-acetyltransferases can activate aromatic amines if they are first N-hydroxylated by cytochrome P450. T e acetoxy esters of N-hydroxyaromatic amines, like the corresponding sulfonate esters (Figure 6–15), can break down to form highly reactive nitrenium and carbonium ions that bind to DNA. Whether fast acetylators are protected from or predisposed to the cancer-causing effects of aromatic amines depends on the nature of the aromatic amine and other risk modifiers.

Amino Acid Conjugation

Two principal pathways by which xenobiotics are conjugated with amino acids are illustrated in Figure 6–18. T e first involves conjugation of xenobiotics containing a carboxylic acid group with the amino group of amino acids such as glycine, glutamine, and taurine (see Figure 6–13). After activation of the xenobiotic by conjugation with CoA, the acyl-CoA thioether reacts with the amino group of an amino acid to form an amide linkage. T e second pathway involves conjugation of xenobiotics containing an aromatic hydroxylamine with the carboxylic acid group of such amino acids as serine and proline. T is pathway involves activation of an amino acid by aminoacyl-tRNA synthetase, which reacts with an aromatic hydroxylamine to form a reactive N-ester.

Substrates for amino acid conjugation are restricted to certain aliphatic, aromatic, heteroaromatic, cinnamic, and arylacetic acids. T e ability of xenobiotics to undergo amino acid conjugation depends on steric hindrance around the carboxylic acid group, and by substituents on the aromatic ring or aliphatic side chain. Amino acid conjugates of xenobiotics are eliminated primarily in urine. T e acceptor amino acid used for conjugation is both species- and xenobiotic-dependent.

Amino acid conjugation of N-hydroxy aromatic amines (hydroxylamines) is an activation reaction producing N-esters that can degrade to form electrophilic nitrenium and carbonium ions. Conjugation of hydroxylamines with amino acids is catalyzed by cytosolic aminoacyl-tRNA synthetases and requires ATP (Figure 6–18).

Glutathione Conjugation

Conjugation of xenobiotics with glutathione includes an enormous array of electrophilic xenobiotics, or xenobiotics that can be biotransformed to electrophiles. T e tripeptide glutathione comprises glycine, cysteine, and glutamic acid (Figure 6–13). Glutathione conjugates are thioethers, which form by nucleophilic attack of glutathione thiolate anion (GS^-) with an electrophilic carbon, oxygen, nitrogen, or sulfur atom in the xenobiotic. T is conjugation reaction is catalyzed by a family

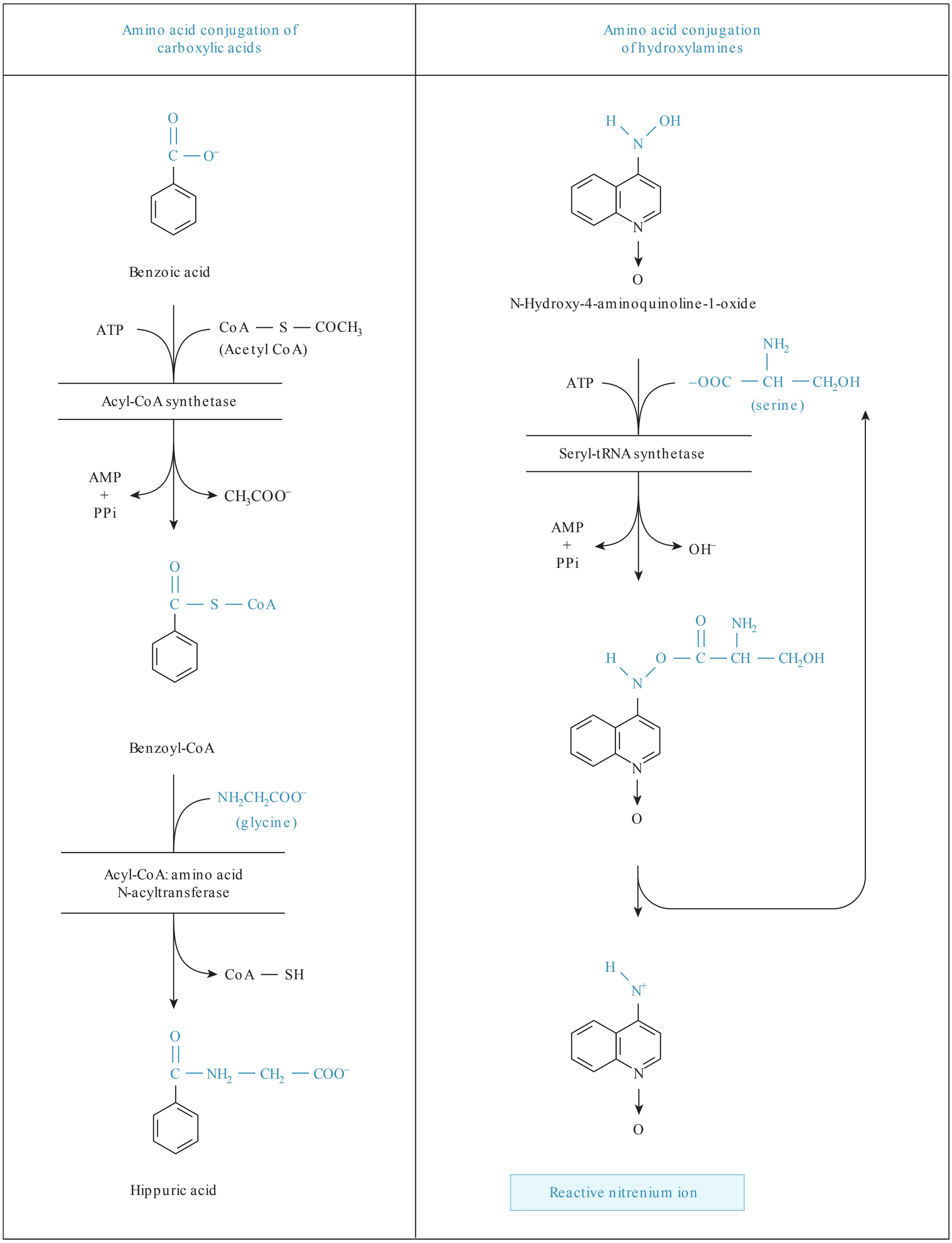


FIGURE 6–18 **Conjugation of xenobiotics with amino acids.**

of glutathione S-transferases that are present in most tissues, where they are localized in the cytoplasm (> 95%) and endoplasmic reticulum (< 5%).

Substrates for glutathione S-transferase are commonly hydrophobic, contain an electrophilic atom, and react nonenzymatically with glutathione at some measurable rate. The mechanism by which glutathione S-transferase increases the rate of glutathione conjugation involves deprotonation of GSH to GS^-. The concentration of glutathione in liver is extremely high (~5 to 10 mM); hence, the nonenzymatic conjugation of certain xenobiotics with glutathione can be significant. However, some xenobiotics are conjugated with glutathione stereoselectively, indicating that the reaction is largely catalyzed by glutathione S-transferase. Like glutathione, the glutathione S-transferases are themselves abundant cellular components, accounting for up to 10% of the total cellular protein. These enzymes bind, store, and/or transport a number of compounds that are not substrates for glutathione conjugation. The cytoplasmic protein formerly known as ligandin, which binds heme, bilirubin, steroids, azo-dyes, polycyclic aromatic hydrocarbons, and thyroid hormones, is an alpha-class GST.

As shown in Figure 6–19, substrates for glutathione conjugation can be divided into two groups: those sufficiently electrophilic to be conjugated directly and those that must first be biotransformed to an electrophilic metabolite prior to conjugation. The conjugation reactions themselves can be divided into two types: displacement reactions, in which glutathione displaces an electron-withdrawing group, and addition reactions, in which glutathione is added to an activated double bond or strained ring system.

The displacement of an electron-withdrawing group by glutathione typically occurs when the substrate contains halide, sulfate, sulfonate, phosphate, or a nitro group (i.e., good leaving groups) attached to an allylic or benzylic carbon atom.

The addition of glutathione to a carbon–carbon double bond is also facilitated by the presence of a nearby electron-withdrawing group; hence, substrates for this reaction typically contain a double bond attached to —CN, —CHO, —COOR, or —COR.

Glutathione can also conjugate xenobiotics with an electrophilic heteroatom (O, N, and S). In each of the examples shown in Figure 6–20, the initial conjugate formed between

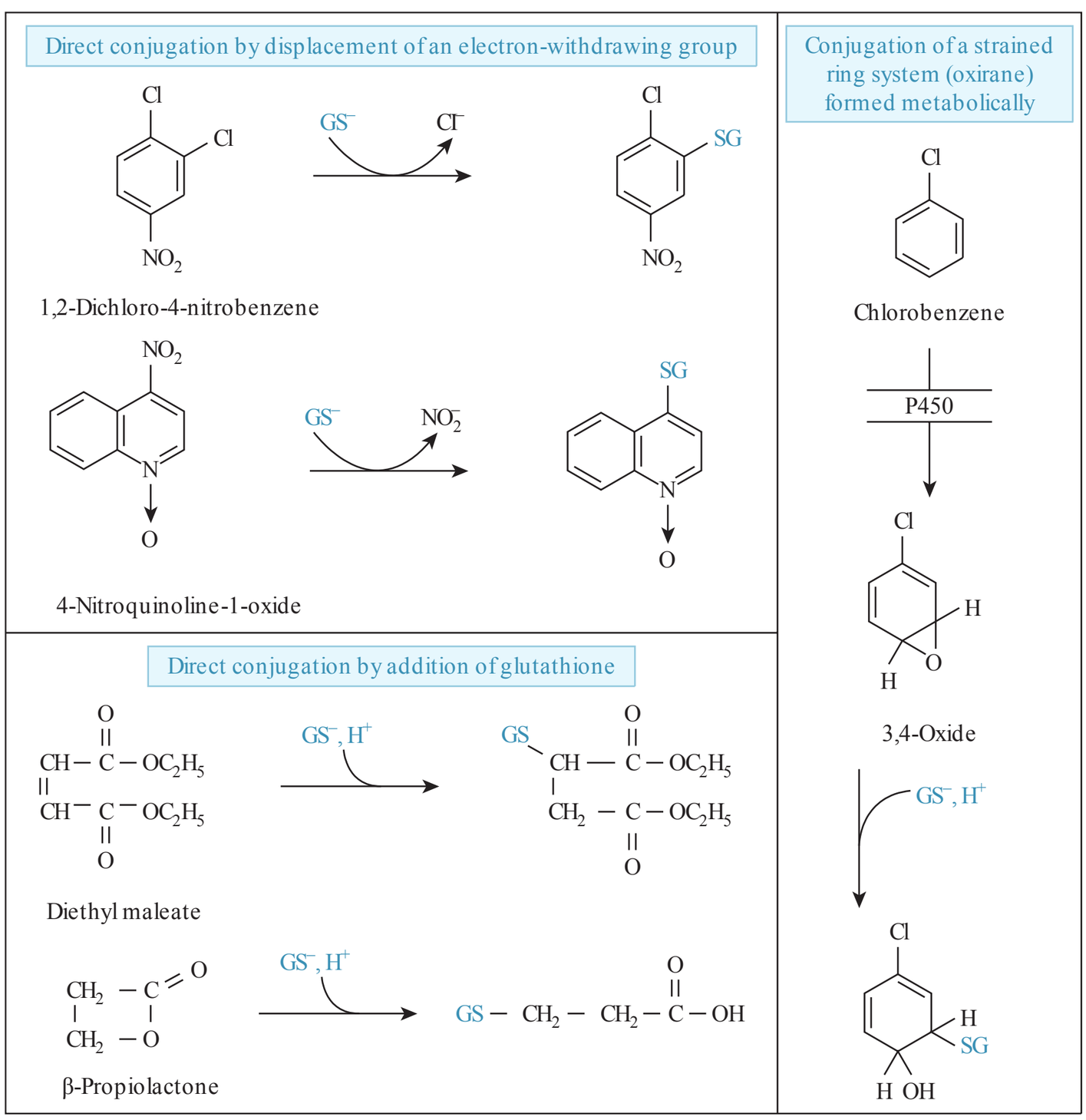


FIGURE 6–19 **Examples of glutathione conjugation of xenobiotics with an electrophilic carbon.** GS^- represents the anionic form of glutathione.

glutathione and the heteroatom is cleaved by a second molecule of glutathione to form oxidized glutathione (GSSG). T e initial reactions are catalyzed by glutathione S-transferase, whereas the second reaction (which leads to GSSG formation) generally occurs nonenzymatically.

Glutathione conjugates formed in the liver can be effluxed into bile and blood, and they can be converted to mercapturic acids in the kidney and excreted in urine. As shown in Figure 6–21, the conversion of glutathione conjugates to mercapturic acids involves the sequential cleavage of glutamic acid and glycine from the glutathione moiety, followed by N-acetylation of the resulting cysteine conjugate.

Glutathione S-transferases are dimers composed of identical subunits, although some forms are heterodimers. Each subunit contains 199 to 244 amino acids and one catalytic site. Numerous subunits have been cloned and sequenced and differ in substrate specificity, tissue location, and cellular location. Conjugation with glutathione represents an important detoxication reaction because electrophiles are potentially toxic species that can bind to critical nucleophiles, such as proteins and nucleic acids, causing cellular damage and genetic mutations (see Chapter 8 for more information). Glutathione is also a cofactor for glutathione peroxidase, which is important in protecting cells against lipid and hemoglobin peroxidation.

In some cases, conjugation with glutathione enhances the toxicity of a xenobiotic. Glutathione conjugates of various compounds can activate xenobiotics to become toxic by releasing a toxic metabolite, being inherently toxic itself, or being degraded to a toxic metabolite.

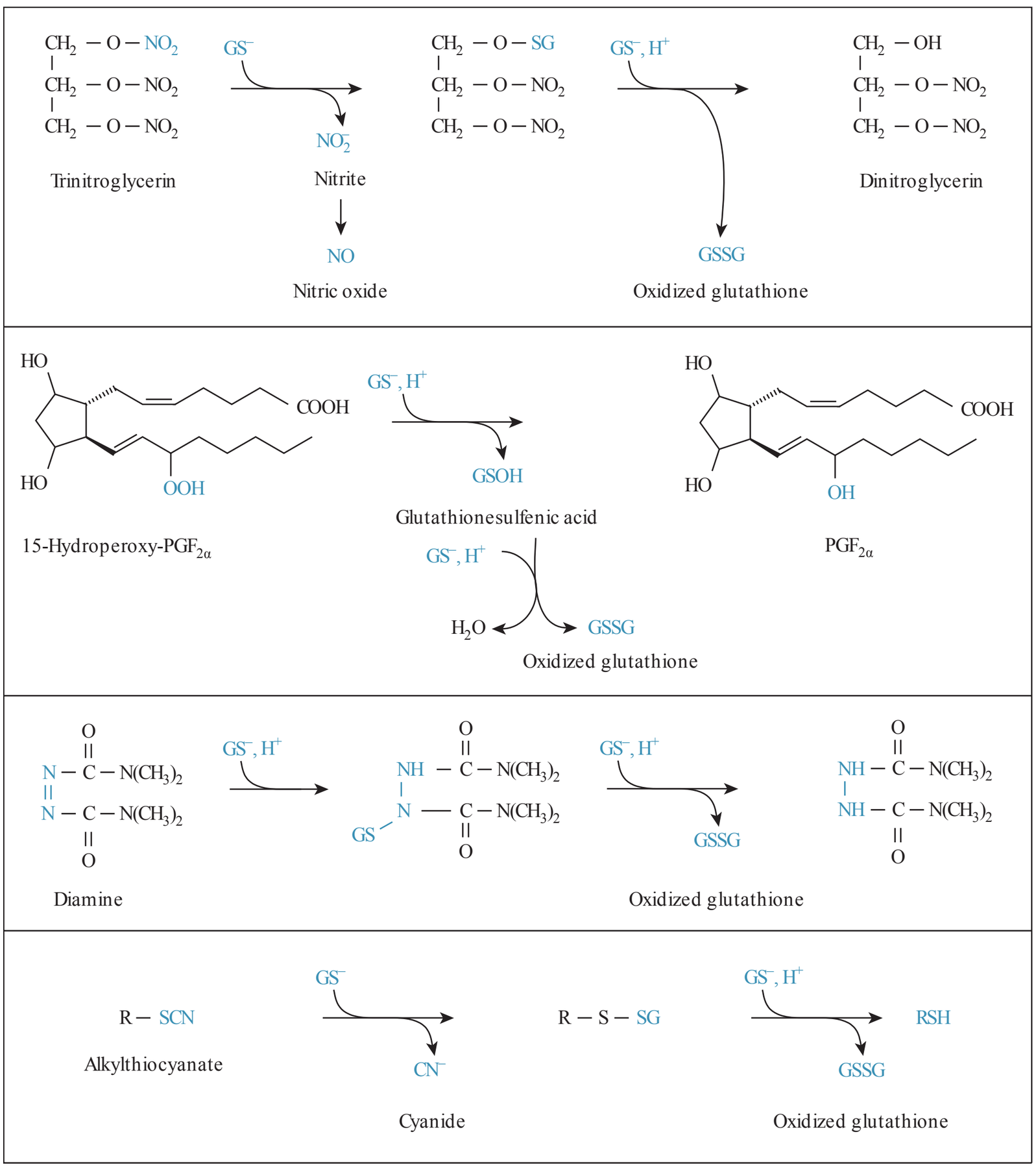


FIGURE 6–20 **Examples of glutathione conjugation of electrophilic heteroatoms.**

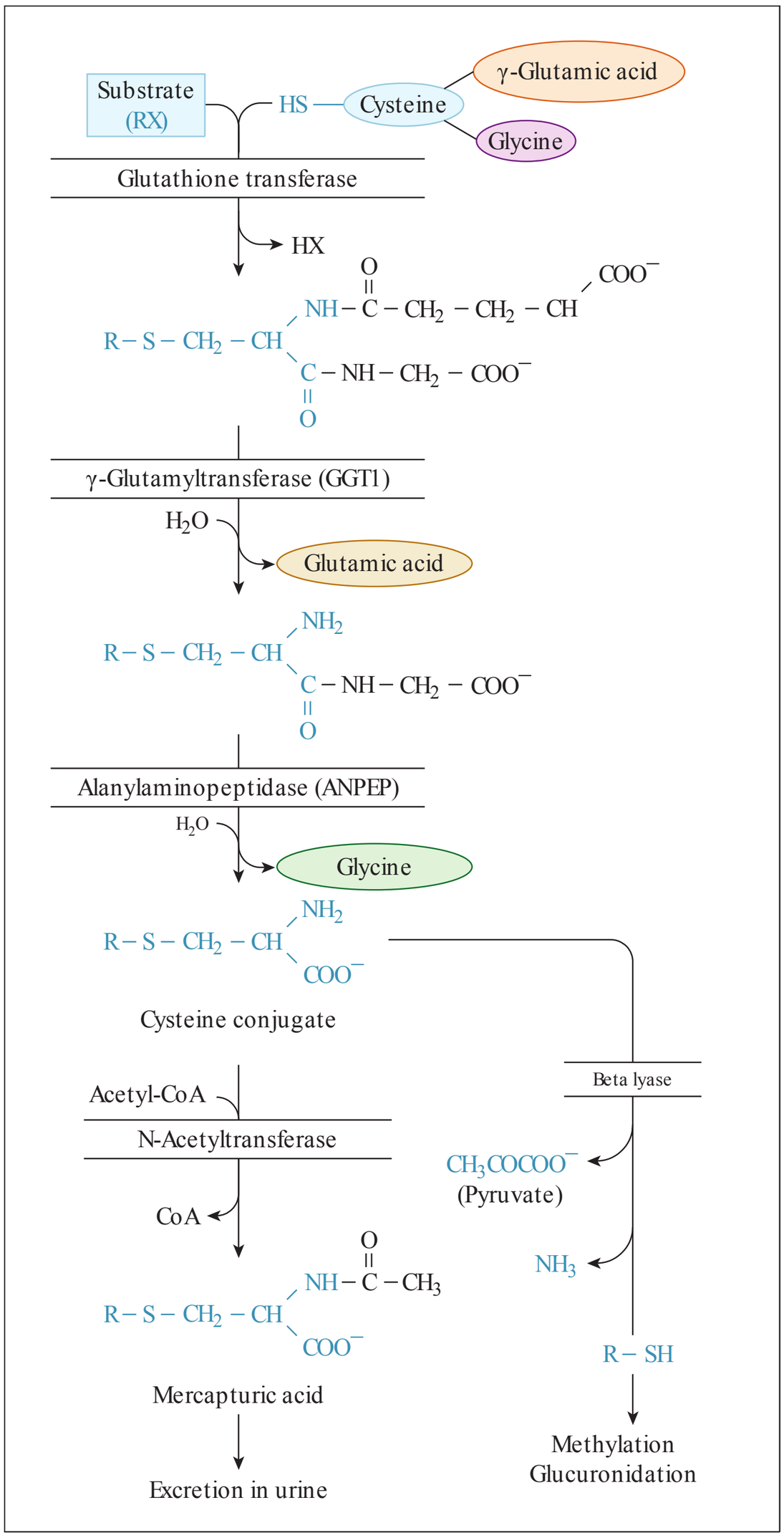


FIGURE 6–21 **Glutathione conjugation and mercapturic acid biosynthesis.**

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QUESTIONS

1. Xenobiotic biotransformation is performed by multiple enzymes in multiple subcellular locations. Where would one of these enzymes most likely NOT be located?
   a. cytosol.
   b. Golgi apparatus.
   c. lysosome.
   d. mitochondria.
   e. microsome.

2. All of the following statements regarding hydrolysis, reduction, and oxidation biotransformations are true EXCEPT:
   a. T e xenobiotic can be hydrolyzed.
   b. T e xenobiotic can be reduced.
   c. T ere is a large increase in hydrophilicity.
   d. T e reactions introduce a functional group to the molecule.
   e. T e xenobiotic can be oxidized.

3. Which of the following is often conjugated to xenobiotics during phase II biotransformations?
   a. alcohol group.
   b. sulfhydryl group.
   c. sulfate group.
   d. aldehyde group.
   e. carbonyl group.

4. Which of the following is a true statement about the biotransformation of ethanol?
   a. Alcohol dehydrogenase is only present in the liver.
   b. Ethanol is reduced to acetaldehyde by alcohol dehydrogenase.
   c. Ethanol and hydrogen peroxide combine to form acetaldehyde with the aid of catalase.
   d. In spite of its catalytic versatility, cytochrome P450 does not aid in ethanol oxidation.
   e. Acetaldehyde is oxidized to acetic acid in the mitochondria by aldehyde dehydrogenase.

5. Which of the following enzymes is responsible for the biotransformation and elimination of serotonin?
   a. cytochrome P450.
   b. monoamine oxidase.
   c. flavin monooxygenase.
   d. xanthine oxidase.
   e. paraoxonase.

6. Which of the following reactions would likely NOT be catalyzed by cytochrome P450?
   a. dehydrogenation.
   b. oxidative group transfer.
   c. epoxidation.
   d. reductive dehalogenation.
   e. ester cleavage.

7. All of the following statements regarding cytochrome P450 are true EXCEPT:
   a. Poor metabolism or biotransformation of xenobiotics is often due to a genetic deficiency in cytochrome P450.
   b. Cytochrome P450 can be inhibited by both competitive and noncompetitive inhibitors.
   c. Certain cytochrome P450 enzymes can be induced by one's diet.
   d. Increased activity of cytochrome P450 always slows the rate of xenobiotic activation.
   e. Induction of cytochrome P450 can lead to increased drug tolerance.

8. Which of the following statements regarding phase II biotransformation (conjugation) reactions is true?
   a. Phase II reactions greatly increase the hydrophilicity of the xenobiotic.
   b. Phase II reactions are usually the rate-determining step in the biotransformation and excretion of xenobiotics.
   c. Carboxyl groups are very common additions of phase II reactions.
   d. Most phase II reactions occur spontaneously.
   e. Increased phase II reactions result in increased xenobiotic storage in adipocytes.

9. Where do most phase II biotransformations take place?
   a. mitochondria.
   b. ER.
   c. blood.
   d. nucleus.
   e. cytoplasm.

10. Which of the following is not an important cosubstrate for phase II biotransformation reactions?
    a. UDP-glucuronic acid.
    b. 3′-phosphoadenosine-5′-phosphosulfate (PAPS).
    c. S-adenosylmethionine (SAM).
    d. N-nitrosodiethylamine.
    e. acetyl CoA.

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CHAPTER 7

Toxicokinetics

Danny D. Shen

INTRODUCTION

CLASSIC TOXICOKINETICS

- One-compartment Model
- Two-compartment Model
- Elimination
- Apparent Volume of Distribution
- Clearance
- Relationship of Elimination Half-life to Clearance and Volume
- Absorption, Bioavailability, and Metabolite Kinetics
- Saturation Toxicokinetics
- Accumulation during Continuous or Intermittent Exposure
- Conclusion

PHYSIOLOGIC TOXICOKINETICS

- Basic Model Structure
- Compartments
- Parameters
  - Anatomical
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- Perfusion-limited Compartments
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  - Liver
  - Blood

CONCLUSION

KEY POINTS

- Toxicokinetics is the study of the modeling and mathematical description of the time course of disposition (absorption, distribution, biotransformation, and excretion) of xenobiotics in the whole organism.
- The apparent volume of distribution (V_d) is the space into which an amount of chemical is distributed in the body to result in a given plasma concentration.
- Clearance describes the rate of chemical elimination from the body in terms of volume of fluid containing chemical that is cleared per unit of time.
- The half-life of elimination ($T_{1/2}$) is the time required for the blood or plasma chemical concentration to decrease by one-half.

INTRODUCTION

Toxicokinetics is the study of the modeling and mathematical description of the time course of disposition (absorption, distribution, biotransformation, and excretion) of xenobiotics in the whole organism. In the classic model, chemicals are said to move throughout the body as if there were one or more compartments that may have no apparent physiologic or anatomical reality. An alternate and newer approach, physiologically based toxicokinetic modeling, attempts to portray the body as an elaborate system of discrete tissue or organ compartments that are interconnected via the circulatory system. There is no inherent contradiction between the classic and physiologically based approaches, yet certain

assumptions differ between the two models. Ideally, physiologic models can predict tissue concentrations, whereas classic models cannot.

CLASSIC TOXICOKINETICS

The least invasive and simplest method to gather information on absorption, distribution, metabolism, and elimination of a compound is by sampling blood or plasma over time. Assuming that the concentration of a compound in blood or plasma is in equilibrium with concentrations in tissues, then changes in plasma toxicant concentrations should reflect changes in tissue toxicant concentrations. Compartmental pharmacokinetic models consist of a central compartment representing plasma and tissues that rapidly equilibrate with chemical, connected to one or more peripheral compartments that represent tissues that more slowly equilibrate with the chemical (Figure 7–1). Chemical is administered into the central compartment and distributes between central and peripheral compartments. Chemical elimination occurs from the central compartment, which is assumed to contain rapidly perfused tissues capable of eliminating the chemical (e.g., kidneys, lungs, and liver). Compartmental pharmacokinetic models require no information on tissue physiology or anatomical structure, and they are valuable in predicting the plasma chemical concentrations at different doses, establishing the time course of chemical in plasma and tissues and the extent of chemical accumulation with multiple doses, and determining effective dose and dose regimens in toxicity studies.

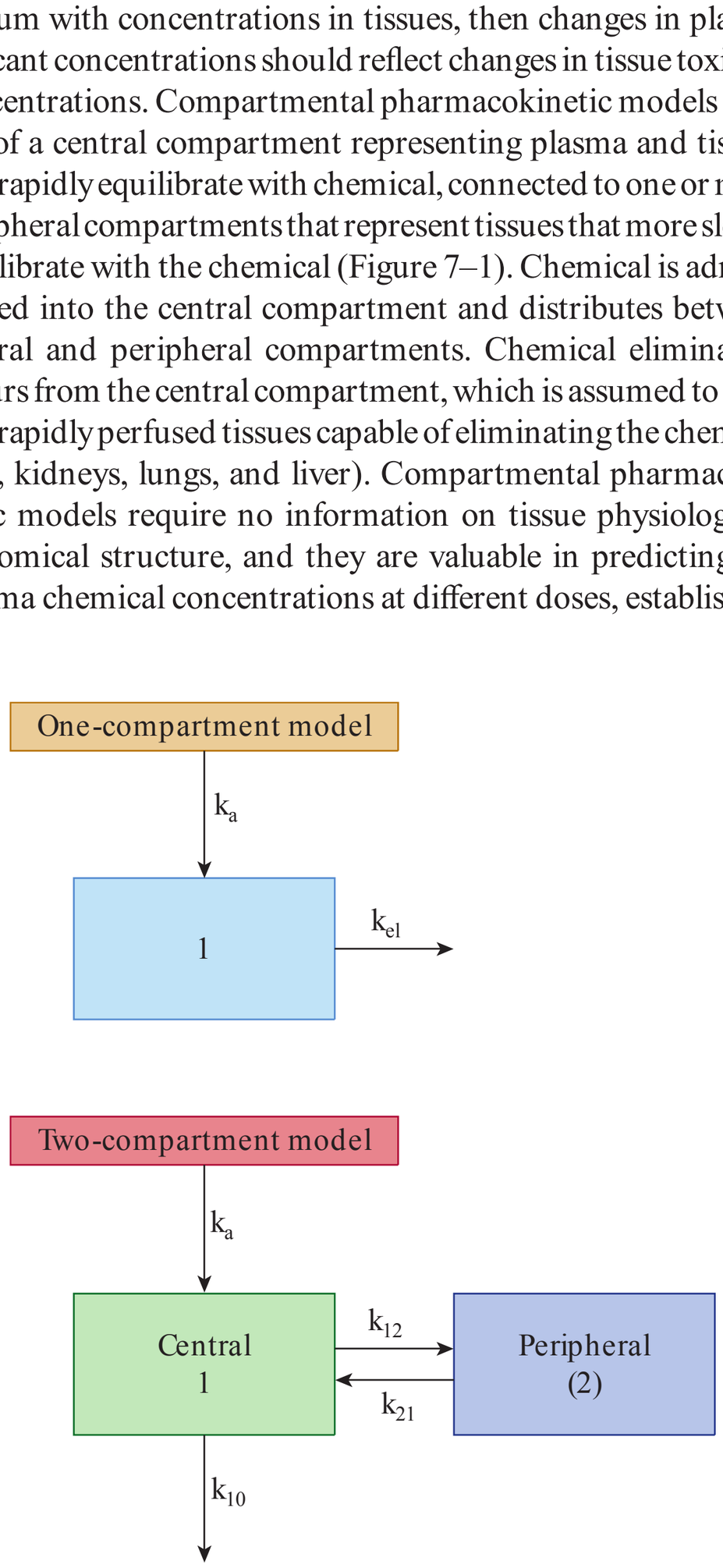

FIGURE 7–1 **Compartmental pharmacokinetic models.** k_a is the first-order extravascular absorption rate constant into the central compartment (1), k_{el} is the first-order elimination rate constant from the central compartment (1), and k_{12} and k_{21} are the first-order rate constants for distribution of chemical into and out of the peripheral compartment (2) in a two-compartment model, whereas k_{10} is the first-order elimination rate constant from the central compartment in a two compartment model.

One-compartment Model

The simplest toxicokinetic analysis entails measurement of the plasma concentrations of a xenobiotic at several time points after the administration of a bolus intravenous injection. If the data obtained yield a straight line when they are plotted as the logarithm of plasma concentrations versus time, the kinetics of the xenobiotic can be described with a one-compartment model (Figure 7–2). Compounds whose toxicokinetics can be described with a one-compartment model rapidly equilibrate, or mix uniformly, between blood and the various tissues relative to the rate of elimination. The one-compartment model depicts the body as a homogeneous unit. This does not mean that the concentration of a compound is the same throughout the body, but it does assume that the changes that occur in the plasma concentration reflect proportional changes in tissue chemical concentrations.

In the simplest case, a curve of this type can be described by the following expression:

$$C = C_0 e^{-k_{el}t}$$

where C is the blood or plasma chemical concentration over time t, C_0 the initial blood concentration at time $t = 0$, and k_{el} the first-order elimination rate constant with dimensions of reciprocal time (e.g., t^{-1}). k_{el} represents the overall elimination of the chemical, which includes biotransformation, exhalation, and/or excretion pathways.

Two-compartment Model

After the rapid intravenous administration of some chemicals, the semilogarithmic plot of plasma concentration versus time yields a curve rather than a straight line, which implies that there is more than one dispositional phase. In these instances, the chemical requires a longer time for tissue concentrations to reach equilibrium with the concentration in plasma, and a multicompartmental analysis of the results is necessary (Figure 7–2). A multiexponential mathematical equation then best characterizes the elimination of the xenobiotic from the plasma.

Generally, a curve of this type can be resolved into two monoexponential terms (a two-compartment model) and is described by:

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$

where A and B are proportionality constants and α and β the first-order distribution and elimination rate constants, respectively (Figure 7–2). During the distribution (α) phase, concentrations of the chemical in the plasma decrease more rapidly than they do in the postdistributional elimination (β) phase. The distribution phase may last for only a few minutes or for

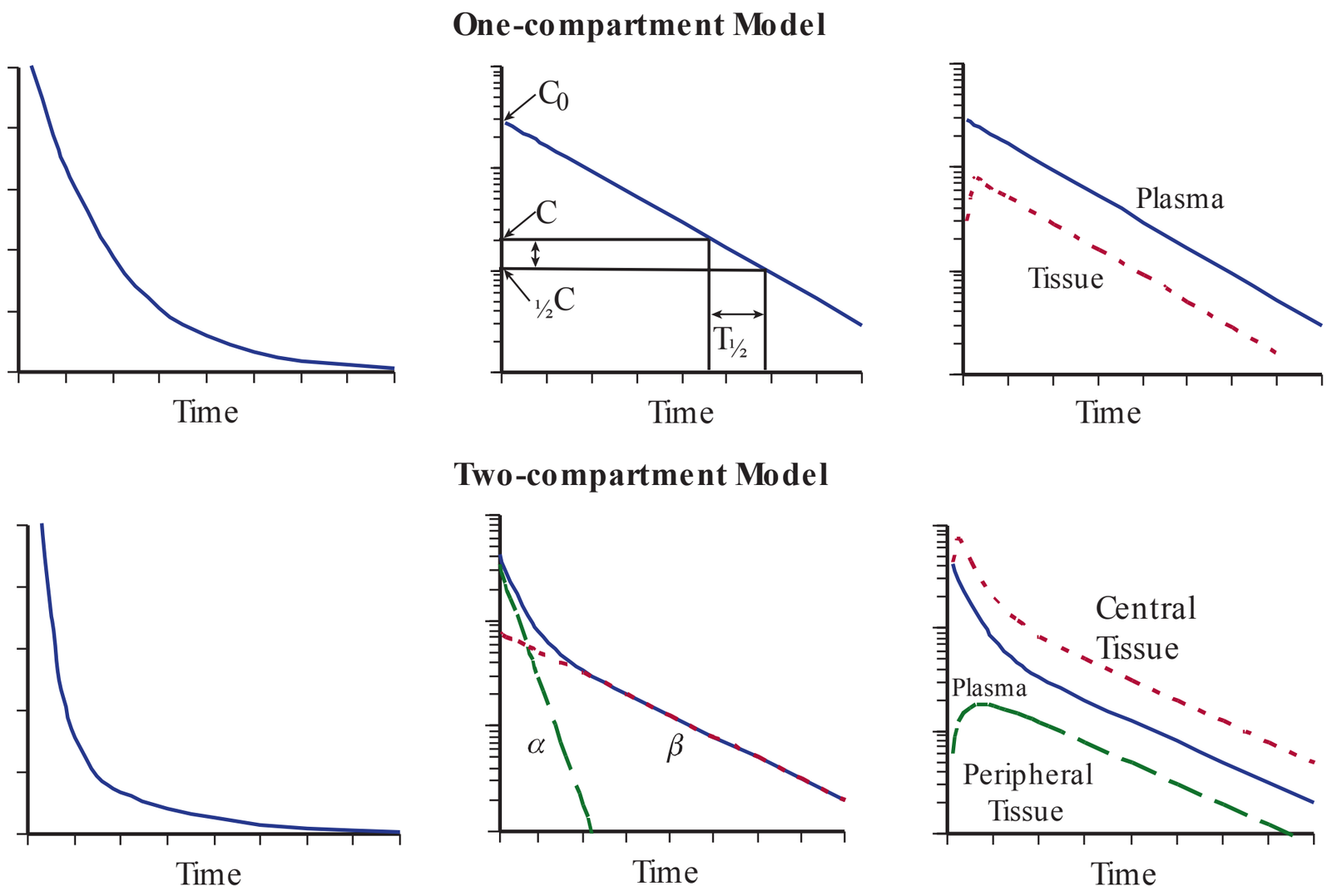

FIGURE 7–2 **Plasma concentration versus time curves of toxicants exhibiting kinetic behavior conforming to a one-compartment model (top row) and a two-compartment model (bottom row) following intravenous bolus injection.** Left and middle panels show the plots on a rectilinear and semilogarithmic scale, respectively. Right panels illustrate the relationship between tissue (dashed lines) and plasma (solid line) concentration over time. The right panel for the one-compartment model shows a typical tissue with a higher concentration than plasma. Note that tissue concentration can be higher, nearly the same, or lower than plasma concentration. Tissue concentration peaks almost immediately, and thereafter declines in parallel with plasma concentration. The right panel for the two-compartment model shows typical tissues associated with the central (1) and peripheral (2) compartments as represented by short-and-long dash lines, respectively. For tissues associated with the central compartment, their concentrations decline in parallel with plasma. For tissues associated with peripheral compartment, toxicant concentration rises, while plasma concentration declines rapidly during the initial phase; it then reaches a peak and eventually declines in parallel with plasma in the terminal phase. Elimination rate constant k_{el} for one-compartment model and the terminal exponential rate constant β are determined from the slope of the log-linear concentration versus time curve. Half-life ($T_{1/2}$) is the time required for plasma toxicant concentration to decrease by one-half. C_0 is the concentration of a toxicant for a one-compartment model at $t = 0$ determined by extrapolating the log-linear concentration time curve to the Y-axis.

hours or days. The equivalent of k_{el} in a one-compartment model is β in a two-compartment model.

Elimination

Elimination includes biotransformation, exhalation, and excretion. The elimination of a chemical from the body whose disposition is described by a one-compartment model usually occurs through a first-order process; that is, the rate of elimination at any time is proportional to the amount of the chemical in the body at that time. First-order reactions occur at chemical concentrations that are not sufficiently high to saturate elimination processes.

The equation for a monoexponential model, $C = C_0e^{-k_{el}t}$, can be transformed to a logarithmic equation that has the general form of a straight line, $y = mx + b$:

$$\log C = \left(\frac{k_{el}}{2.303}\right)t + \log C_0$$

where $\log C_0$ represents the y-intercept or initial concentration and $(k_{el}/2.303)$ the slope of the line. The first-order elimination rate constant (k_{el}) can be determined from the slope of the log C versus time plot (i.e., $k_{el} = 2.303 \times$ slope). The first-order elimination rate constants, k_{el} and β, have units of reciprocal time (e.g., min^{-1} and h^{-1}) and are independent of dose.

Mathematically, the fraction of dose remaining in the body over time (C/C_0) is calculated using the elimination rate constant by rearranging the equation for the monoexponential function and taking the antilog to yield:

$$\frac{C}{C_0} = \text{Antilog}\left[\left(\frac{-k_{el}}{2.303}\right)t\right]$$

Apparent Volume of Distribution

In a one-compartment model, all chemical is assumed to distribute and equilibrate into plasma and tissues instantaneously. The apparent volume of distribution (V_d) is a proportionality constant that relates the total amount of chemical in the body to its concentration in plasma, and typically has units of liters or liters per kilogram of body weight. V_d is the apparent space into

which an amount of chemical is distributed in the body to result in a given plasma concentration. The apparent volume of distribution of a chemical in the body is determined after intravenous bolus administration, and is mathematically defined as the quotient of the amount of chemical in the body and its plasma concentration. V_d is calculated as follows:

$$V_d = \frac{Dose_{iv}}{\beta \times AUC_0^\infty}$$

where $Dose_{iv}$ is the intravenous dose or known amount of chemical in body at time zero, β the elimination rate constant, and AUC_0^∞ the area under the chemical concentration versus time curve from time zero to infinity. The product, $\beta \times AUC_0^\infty$, is the concentration of xenobiotic in plasma.

For a one-compartment model, V_d can be simplified by the following equation:

$$V_d = \frac{Dose_{iv}}{C_0}$$

where C_0 is the concentration of chemical in plasma at time zero. C_0 is determined by extrapolating the plasma disappearance curve after intravenous injection to the zero time point (Figure 7–2). V_d is called the apparent volume *of* distribution. The magnitude of the V_d term is chemical-specific and represents the extent of distribution of chemical out of plasma and into other body tissues. Thus, a chemical with high affinity for tissues will also have a large volume of distribution. Alternatively, a chemical that predominantly remains in the plasma will have a low V_d that approximates the volume of plasma. Once the V_d for a chemical is known, it can be used to estimate the amount of chemical remaining in the body at any time if the plasma concentration at that time is also known by the relationship $X_c = V_d C_p$, where X_c is the amount of chemical in the body and C_p the plasma chemical concentration.

Clearance

Clearance describes the rate of chemical elimination from the body in terms of volume of fluid containing chemical that is cleared per unit of time. Thus, clearance has the units of flow (mL/min). A clearance of 100 mL/min means that 100 mL of blood or plasma containing xenobiotic is completely cleared of the substance each minute.

The overall efficiency of the removal of a chemical from the body can be characterized by clearance. High values of clearance indicate efficient and generally rapid removal, whereas low clearance values indicate slow and less efficient removal of a xenobiotic from the body. Total body clearance is defined as the sum of clearances by individual eliminating organs:

$$Cl = Cl_{renal} + Cl_{hepatic} + Cl_{intestinal} + \square$$

Each organ clearance is determined by blood perfusion flow through the organ and the fraction of toxicant in the arterial inflow that is irreversibly removed. After bolus intravenous administration, total body clearance is defined as:

$$Cl = \frac{Dose_{iv}}{AUC_0^\infty}$$

Clearance can also be calculated if the volume of distribution and elimination rate constants are known, and can be defined as $Cl = V_d k_{el}$ for a one-compartment model and $Cl = V_d \beta$ for a two-compartment model.

Relationship of Elimination Half-life to Clearance and Volume

The half-life of elimination ($T_{1/2}$) is the time required for the blood or plasma chemical concentration to decrease by one-half, and is dependent on both volume of distribution and clearance. $T_{1/2}$ can be calculated from V_d and Cl:

$$T_{1/2} = \frac{0.693\, V_d}{Cl}$$

Because of the relationship $T_{1/2} = 0.693 k_{el}$, the half-life of a compound can be calculated after k_{el} (or β) has been determined from the slope of the line that designates the elimination phase on the log C versus time plot. The $T_{1/2}$ can also be determined by means of visual inspection of the log C versus time plot, as shown in Figure 7–2. For compounds eliminated by first-order kinetics, the time required for the plasma concentration to decrease by one-half is constant. After seven half-lives, 99.2% of a chemical is eliminated, which can be practically viewed as complete elimination. The half-life of a chemical obeying first-order elimination kinetics is independent of the dose, and does not change with increasing dose.

Absorption, Bioavailability, and Metabolite Kinetics

For most chemicals in toxicology, exposure occurs by extravascular routes (inhalation, dermal, or oral), and absorption is often incomplete. The extent of absorption of a xenobiotic can be experimentally determined by comparing the plasma AUC_0^∞ after intravenous and extravascular dosing. The resulting index quantifies the fraction of dose absorbed systemically and is called bioavailability (F). Bioavailability can be determined by using different doses, provided that the compound does not display dose-dependent or saturable kinetics. Pharmacokinetic data following intravenous administration are used as the reference from which to compare extravascular absorption because all of the chemical is delivered to the systemic circulation (100% bioavailable). For example, bioavailability following an oral exposure can be determined as follows:

$$F = \frac{AUC_{po} / Dose_{po}}{Dose_{iv} / AUC_{iv}}$$

where AUC_{po}, AUC_{iv}, $Dose_{po}$, and $Dose_{iv}$ are the respective area under the plasma concentration versus time curves and doses for oral and intravenous administration. Bioavailabilities for various chemicals range in values between 0 and 1. Complete availability of chemical to systemic circulation is demonstrated by F = 1. When F < 1, less than 100% of the dose reaches systemic circulation. The fraction of a chemical that reaches the systemic circulation is of critical importance in determining toxicity. Several factors can greatly alter this systemic availability, including (1) limited absorption after oral dosing, (2) intestinal first-pass effect, (3) hepatic first-pass effect, and (4) mode of formulation, which affects, e.g., dissolution rate or incorporation into micelles (for lipid-soluble compounds).

The toxicity of a chemical is in some cases attributed to its biotransformation product(s). Hence, the formation and disposition kinetics of a toxic metabolite is of considerable interest. As expected, the plasma concentration of a metabolite rises as the parent drug is transformed into the metabolite. A biologically active metabolite assumes toxicologic significance when it is the major metabolic product and is cleared much less efficiently than the parent compound.

Saturation Toxicokinetics

As the dose of a compound increases, its volume of distribution or its rate of elimination may change, owing to saturation kinetics. Biotransformation, active transport processes, and protein binding have finite capacities and can be saturated. When the concentration of a chemical in the body is higher than the K_m (chemical concentration at one-half V_{max}, the maximum metabolic capacity), the rate of elimination is no longer proportional to the dose. The transition from first-order to saturation kinetics is important in toxicology because it can lead to prolonged residency time of a compound in the body or increased concentration at the target site of action, which may result in increased toxicity.

Nonlinear toxicokinetics are indicated by the following: (1) the decline in the levels of the chemical in the body is not exponential, (2) AUC_0^∞ is not proportional to the dose, (3) V_d, Cl, k_{el} (or β), or $T_{1/2}$ changes with increasing dose, (4) the composition of excretory products changes quantitatively or qualitatively with the dose, and (5) dose–response curves show a nonproportional change in response to an increasing dose, starting at the dose level at which saturation effects become evident.

Important characteristics of zero-order processes are as follows: (1) an arithmetic plot of plasma concentration versus time yields a straight line, (2) the rate or amount of chemical eliminated at any time is constant and is independent of the amount of chemical in the body, and (3) a true $T_{1/2}$ or k_{el} does not exist, but differs depending on dose.

By comparison, the important characteristics of first-order elimination are (1) the rate at which a chemical is eliminated at any time is directly proportional to the amount of that chemical in the body at that time; (2) a semilogarithmic plot of plasma concentration versus time yields a single straight line; (3) the elimination rate constant (k_{el} or β), apparent volume of distribution (V_d), clearance (Cl), and half-life ($T_{1/2}$) are independent of dose; and (4) the concentration of the chemical in plasma and other tissues decreases similarly by some constant fraction per unit of time, the elimination rate constant (k_{el} or β).

Accumulation during Continuous or Intermittent Exposure

Chronic exposure to a chemical leads to its cumulative intake and accumulation in the body. At a fixed level of continuous exposure, accumulation of a toxicant in the body eventually reaches a point when the intake rate of the toxicant equals its elimination rate, the steady state.

Accumulation can also occur with intermittent exposure. For a chemical with a relatively short half-life compared with the interval between episodes of exposure, little accumulation is expected. In contrast, for a chemical with an elimination half-life approaching or exceeding the between-exposure interval, progressive accumulation is expected over the intervals.

Conclusion

For many chemicals, blood or plasma chemical concentration versus time data can be adequately described by a one- or two-compartment, classic pharmacokinetic model when basic assumptions are made (e.g., instantaneous mixing within compartments and first-order kinetics). In some instances, more sophisticated models with increased numbers of compartments will be needed to describe blood or plasma toxicokinetic data. Knowledge of toxicokinetic data and compartmental modeling are useful in deciding what dose or dosing regimen of chemical to use in the planning of toxicology studies (e.g., targeting a toxic level of exposure), in choosing appropriate sampling times for biological monitoring, and in seeking an understanding of the dynamics of a toxic event (e.g., what blood or plasma concentrations are achieved to produce a specific response, how accumulation of a chemical controls the onset and degree of toxicity, and the persistence of toxic effects following termination of exposure).

PHYSIOLOGIC TOXICOKINETICS

In classic kinetics, the rate constants are defined by the data and these models are often referred to as data-based. In physiologically based models, the rate constants represent known or hypothesized biological processes. The advantages of physiologically based models are that (1) these models can provide the time course of distribution of xenobiotics to any organ or tissue, (2) they allow estimation of the effects of changing physiologic parameters on tissue concentrations, (3) the same model can predict the toxicokinetics of chemicals across species by allometric scaling, and (4) complex dosing regimens and saturable processes such as metabolism and binding are easily accommodated.

The disadvantages are that (1) much more information is needed to implement these models compared with classic

models, (2) the mathematics can be difficult for many toxicologists to handle, and (3) values for parameters are often poorly defined in various species and pathophysiologic states. Nevertheless, physiologically based toxicokinetic models are conceptually sound and are potentially useful tools for gaining rich insight into the kinetics of toxicants beyond what classic toxicokinetic models can provide.

Basic Model Structure

Physiologic models often look like a number of classic one-compartment models that are linked together by the circulatory system. The actual model structure, or how the compartments are linked together, depends on both the chemical and the organism being studied. It is important to realize that there is no generic physiologic model. Models are simplifications of reality and ideally should contain elements believed to be important in describing a chemical's disposition.

Physiologic modeling has enormous potential predictive power compared with classic compartmental modeling. Because the kinetic constants in physiologic models represent measurable biological or chemical processes, the resultant physiologic models have the potential for extrapolation from observed data to predicted situations.

One of the best illustrations of the predictive power of physiologic models is their ability to extrapolate kinetic behavior from laboratory animals to humans. Simulations are the outcomes or results (such as a chemical's concentration in blood or tissue) of numerically integrating model equations over a simulated time period, using a set of initial conditions (such as intravenous dose) and parameter values (such as organ weights and blood flow). Whereas the model structures for the kinetics of chemicals in rodents and humans may be identical, the parameter values, such as organ weight, heart beat rate, and respiration rate, for rodents and humans are different. Other parameters, such as solubility in tissues, are similar in the rodent and human models because the composition of tissues in different species is similar. Because the parameters underlying the model structure represent measurable biological and chemical determinants, the appropriate values for those parameters can be chosen for each species, forming the basis for successful interspecies extrapolation. Because physiologic models represent real, measurable values, such as blood flows and ventilation rates, the same model structure can resolve such disparate kinetic behaviors among species.

Compartments

The basic unit of the physiologic model is the lumped compartment (Figure 7–3), which is a single region of the body with a uniform xenobiotic concentration. A compartment may be a particular functional or anatomical portion of an organ, a single blood vessel with surrounding tissue, an entire discrete organ such as the liver or kidney, or a widely distributed tissue type such as fat or skin. Compartments consist of three individual well-mixed phases, or subcompartments. These subcompartments are

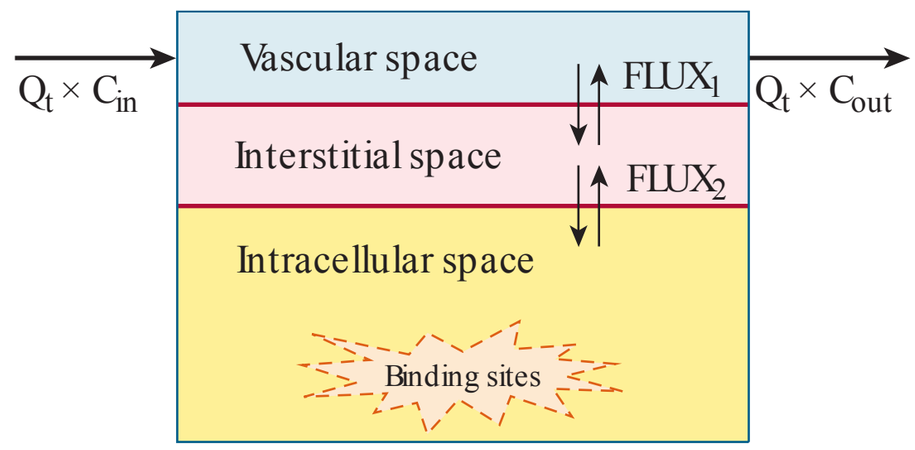

FIGURE 7–3 **Schematic representation of a lumped compartment in a physiologic model.** The blood capillary and cell membranes separating the vascular, interstitial, and intracellular subcompartments are depicted in black. The vascular and interstitial subcompartments are often combined into a single extracellular subcompartment. Q_t is blood flow, C_{in} is chemical concentration into the compartment, and C_{out} is chemical concentration out of the compartment.

(1) the vascular space through which the compartment is perfused with blood, (2) the interstitial space that surrounds the cells, and (3) the intracellular space consisting of the cells in the tissue.

As shown in Figure 7–3, the toxicant enters the vascular subcompartment at a certain rate in mass per unit of time (e.g., mg/h). The rate of entry is a product of the blood flow rate to the tissue (Q_t, L/h) and the concentration of the toxicant in the blood entering the tissue (C_{in}, mg/L). Within the compartment, the toxicant moves from the vascular space to the interstitial space at a certain net rate ($Flux_1$) and from the interstitial space to the intracellular space at a different net rate ($Flux_2$). Some toxicants can bind to cell components; thus, within a compartment there may be both free and bound toxicants. The toxicant leaves the vascular space at a certain venous concentration (C_{out}). C_{out} is equal to the concentration of the toxicant in the vascular space.

Parameters

The most common types of parameters, or information required, in physiologic models are anatomical, physiologic, thermodynamic, and transport.

Anatomical—Anatomical parameters are used to physically describe the various compartments. The size of each of the compartments in the physiologic model must be known. The size is generally specified as a volume (milliliters or liters) because a unit density is assumed even though weights are most frequently obtained experimentally. If a compartment contains subcompartments such as those in Figure 7–3, those volumes also must be known. Volumes of compartments often can be obtained from the literature or from specific toxicokinetic experiments.

Physiologic—Physiologic parameters encompass various processes including blood flow, ventilation, and elimination. The blood flow rate (Q_t, in volume per unit time, such as mL/min or L/h) to individual compartments must be known.

Additionally, information on the total blood flow rate or cardiac output (Q_c) is necessary. If inhalation is the route for exposure to the xenobiotic or is a route of elimination, the alveolar ventilation rate (Q_p) also must be known. Blood flow rates and ventilation rates can be taken from the literature or obtained experimentally. Parameters for renal excretion and hepatic metabolism are another subset of physiologic parameters that are required if these processes are important in describing the elimination of a xenobiotic.

Thermodynamic—Thermodynamic parameters relate the total concentration of a xenobiotic in a tissue (C_t) to the concentration of *free* xenobiotic in that tissue (C_f). Two important assumptions are that (1) total and free concentrations are in equilibrium with each other and (2) only free xenobiotic can be exchanged between the tissue subcompartments. Whereas total concentration is measured experimentally, it is the free concentration that is available for binding, metabolism, or removal from the tissue by blood. The extent to which a xenobiotic partitions into a tissue is directly dependent on the composition of the tissue and independent of the concentration of the xenobiotic. Thus, the relationship between free and total concentration becomes one of proportionality: total = free × partition coefficient, or $C_t = C_f P_t$. Knowledge of the value of P_t, a partition or distribution coefficient, permits an indirect calculation of the free concentration of xenobiotic or $C_f = C_t/P_t$.

Table 7–1 compares the partition coefficients for a number of toxic volatile organic chemicals. The larger values for the fat/blood partition coefficients compared with those for other tissues suggest that these chemicals distribute into fat to a greater extent than they distribute into other tissues.

A more complex relationship between the free concentration and the total concentration of a chemical in tissues occurs when the chemical may bind to saturable binding sites on tissue components. In these cases, nonlinear functions relating the free concentration in the tissue to the total concentration are necessary.

Transport—The passage of a chemical across a biological membrane is complex and may occur by passive diffusion, carrier-mediated transport, facilitated transport, or a combination of processes. The simplest of these processes—passive diffusion—is a first-order process described by Fick's law. Diffusion of xenobiotics can occur across the blood capillary endothelium ($Flux_1$ in Figure 7–3) or across the cell membrane ($Flux_2$ in Figure 7–3). Flux refers to the rate of transfer of a chemical across a boundary. For simple diffusion, the net flux (mg/h) from one side of a membrane to the other is described as Flux = permeability coefficient × driving force, or:

$$\text{Flux} = [PA](C_1 - C_2) = [PA]C_1 - [PA]C_2$$

The term PA is often called the permeability–area product for the membrane or cellular barrier in flow units (e.g., L/h), and is a product of the barrier permeability coefficient (P in velocity units, e.g., μm/h) for the toxicant and the total barrier surface area (A, in μm^2). The permeability constant takes into account the rate of diffusion of the specific xenobiotic and the thickness of the cell membrane. C_1 and C_2 are the *free* concentrations of xenobiotic on each side of the membrane.

For any given xenobiotic, thin membranes, large surface areas, and large concentration differences enhance diffusion. Membrane transporters offer an additional route of entry into cells, and allow more effective tissue penetration for toxicants that have limited passive permeability. Alternately, the presence of efflux transporters at epithelial or endothelial barriers can limit toxicant penetration into critical organs, even for highly permeable toxicants. There are two limiting conditions for the uptake of a toxicant into tissues: perfusion-limited and diffusion-limited.

Perfusion-limited Compartments

A perfusion-limited compartment is also referred to as blood flow–limited, or simply flow-limited. A flow-limited compartment can be developed if the cell membrane permeability coefficient [PA] for a particular xenobiotic is much greater than the blood flow rate to the tissue (Q_t). In this case, uptake of xenobiotic by tissue subcompartments is limited by the rate at which the blood containing a xenobiotic arrives at the tissue and not by the rate at which the xenobiotic crosses the cell membranes. In most tissues, transport across vascular cell membranes is perfusion-limited. In the generalized tissue compartment in Figure 7–3, this means that transport of the xenobiotic through the loosely knit blood capillary walls of most tissues is rapid compared with delivery of the xenobiotic to the tissue by the blood. As a result, the vascular blood is in equilibrium with the interstitial subcompartment and the two subcompartments are usually lumped together as a single compartment that is often called the extracellular space.

As indicated in Figure 7–3, the cell membrane separates the extracellular compartment from the intracellular compartment. The cell membrane is the most important diffusional barrier in a tissue. Nonetheless, for molecules that are very small (molecular weight < 100) or lipophilic, cellular permeability generally does not limit the rate at which a molecule moves across cell membranes. For these molecules, flux across the cell membrane is fast compared with the tissue perfusion rate (PA_2 □ Q_t), and the molecules rapidly distribute throughout the subcompartments. In this case, free toxicant in the

TABLE 7–1 Partition coefficients for four volatile organic chemicals in several tissues.

Chemical	Blood/Air	Muscle/Blood	Fat/Blood
Isoprene	3	0.67	24
Benzene	18	0.61	28
Styrene	40	1	50
Methanol	1,350	3	11

intracellular compartment is always in equilibrium with the extracellular compartment, and these tissue subcompartments can be lumped as a single compartment. T is flow-limited tissue compartment is shown in Figure 7–4. Movement into and out of the entire tissue compartment can be described by a single equation:

$$V_t \frac{dC_t}{dt} = Q_t(C_{in} - C_{out})$$

where V_t is the volume of the tissue compartment, C_t the concentration of free xenobiotic in the compartment (V_tC_t equals the amount of xenobiotic in the compartment), $V_t(dC_t/dt)$ the change in the amount of xenobiotic in the compartment with time expressed as mass per unit of time, Q_t the blood flow to the tissue, C_{in} the xenobiotic concentration entering the compartment, and C_{out} the xenobiotic concentration leaving the compartment. Equations of this type are called mass-balance differential equations. Differential refers to the term dC_t/dt. Mass balance refers to the requirement that the rate of change in the amount of toxicant in a compartment equals the difference in the rate of entry via arterial inflow and the rate of departure via venous outflow.

In the perfusion-limited case, the concentration of toxicant in the venous drainage from the tissue is equal to the concentration of toxicant in the tissue when the toxicant is not bound to blood constituents (i.e., $C_{out} = C_t = C_f$). As was noted previously, when there is binding of toxicant to tissue constituents, C_f (or C_{out}) can be related to the total concentration of toxicant in the tissue through a simple linear partition coefficient, $C_{out} = C_f = C_t/P_t$. In this case, the differential equation describing the rate of change in the amount of a toxicant in a tissue becomes:

$$V_t \frac{dC_t}{dt} = Q_t\left(C_{in} - \frac{C_t}{P_t}\right)$$

In a flow-limited compartment, the assumption is that the concentrations of a xenobiotic in all parts of the tissue are in equilibrium. Additionally, estimates of flux are not required to develop the mass balance differential equation for the compartment. Given the challenges in measuring flux across the vascular endothelium and cell membrane, this is a simplifying assumption that significantly reduces the number of parameters required in the physiologic model.

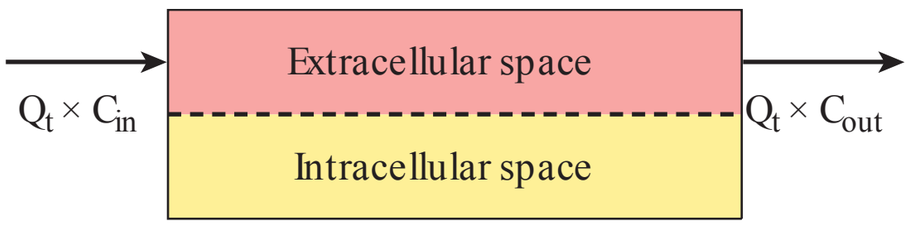

FIGURE 7–4 **Schematic representation of a compartment that is blood f ow–limited.** Rapid exchange between the extracellular space (salmon) and intracellular space (bisque) maintains the equilibrium between them as symbolized by the dashed line. Q_t is blood flow, C_{in} is chemical concentration into the compartment, and C_{out} is chemical concentration out of the compartment.

Diffusion-limited Compartments

When uptake of a toxicant into a compartment is governed by its diffusion or transport across cell membrane barriers, the model is said to be diffusion-limited or barrier-limited. Diffusion-limited uptake or release occurs when the flux, or the transport of a toxicant across cell barriers, is slow compared with blood flow to the tissue. In this case, the permeability–area product is small compared with blood flow, that is, PA □ Q_t. Figure 7–5 shows the structure of such a compartment. T e toxicant concentrations in the vascular and interstitial spaces are in equilibrium and make up the extracellular subcompartment, where uptake from the incoming blood is flow-limited. T e rate of toxicant uptake across the cell membrane from the extracellular space into the intracellular space is limited by membrane permeability. Two mass-balance differential equations are necessary to describe the events in these two subcompartments:

1. Extracellular space:

$$V_{t1} \frac{dC_{t1}}{dt} = Q_t(C_{in} - C_{out}) - PA_t\left(\frac{C_{t1}}{P_{t1}}\right) + PA_t\left(\frac{C_{t2}}{P_{t2}}\right)$$

2. Intracellular space:

$$V_{t2} \frac{dC_{t2}}{dt} = PA_t\left(\frac{C_{t1}}{P_{t1}}\right) - PA_t\left(\frac{C_{t2}}{P_{t2}}\right)$$

Here, Q_t is blood flow and C the *free* xenobiotic concentration in entering blood (in), exiting blood (out), extracellular space (1), or intracellular space (2). Both equations contain terms for flux, or transfer across the cell membrane [PA]($C_1 - C_2$).

Specialized Compartments

Lung—T e inclusion of a lung compartment in a physiologic model is an important consideration because inhalation is a common route of exposure to many toxic chemicals. T e

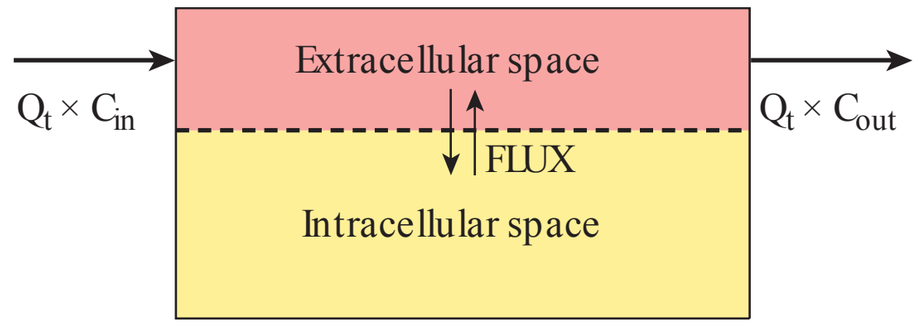

FIGURE 7–5 **Schematic representation of a compartment that is membrane-limited.** Perfusion of blood into and out of the extracellular compartment is depicted by thick arrows. Transmembrane transport (flux) from the extracellular to the intracellular subcompartment is depicted by thin double arrows. Q_t is blood flow, C_{in} is chemical concentration into the compartment, and C_{out} is chemical concentration out of the compartment.

assumptions inherent in this compartment description are as follows: (1) ventilation is continuous, not cyclic; (2) conducting airways (nasal passages, larynx, trachea, bronchi, and bronchioles) function as inert tubes, carrying the vapor to the alveoli where gas exchange occurs; (3) diffusion of vapor across the alveolar epithelium and capillary walls is rapid compared with blood flow through the alveolar region; (4) all chemicals disappearing from the inspired air appears in the arterial blood (i.e., there is no hold-up of chemical in the lung tissue and insignificant lung mass); and (5) vapor in the alveolar air and arterial blood within the lung compartment are in rapid equilibrium.

In the lung compartment depicted in Figure 7–6, the rate of inhalation of xenobiotic is controlled by the ventilation rate (Q_p) and the inhaled concentration (C_{inh}). The rate of exhalation of a xenobiotic is a product of the ventilation rate and the xenobiotic concentration in the alveoli (C_{alv}). Xenobiotic also can enter the lung compartment via venous blood returning from the heart, represented by the product of cardiac output (Q_c) and the concentration of xenobiotic in venous blood (C_{ven}). Xenobiotic leaving the lungs via the blood is a function of both cardiac output and the concentration of xenobiotic in arterial blood (C_{art}). Putting these four processes together, a mass balance differential equation can be written for the rate of change in the amount of xenobiotic in the lung compartment (L):

$$\frac{dL}{dt} = Q_p(C_{inh} - C_{alv}) + Q_c(C_{ven} - C_{art})$$

During continuous exposure at steady state, the rate of change in the amount of xenobiotic in the lung compartment becomes equal to zero ($dL/dt = 0$). C_{alv} can be replaced by $C_{art}/P_{b/a}$, and the differential equation can be solved for the arterial blood concentration:

$$C_{art} = \frac{Q_p C_{inh} + Q_c C_{ven}}{Q_c + (Q_p / P_{b/a})}$$

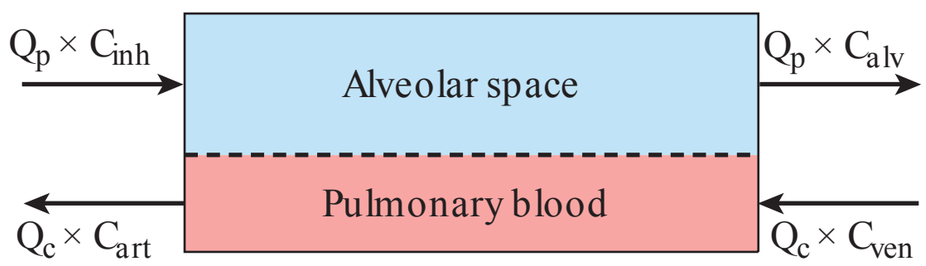

FIGURE 7–6 **Simple model of gas exchange in the alveolar region of the respiratory tract.** Rapid exchange in the lumped lung compartment between the alveolar gas (blue) and the pulmonary blood (salmon) maintains the equilibrium between them as symbolized by the dashed line. Q_p is alveolar ventilation (L/h); Q_c is cardiac output (L/h); C_{inh} is inhaled vapor concentration (mg/L); C_{art} is concentration of vapor in the arterial blood; C_{ven} is concentration of vapor in the mixed venous blood. The equilibrium relationship between the chemical in the alveolar air (C_{alv}) and the chemical in the arterial blood (C_{art}) is determined by the blood/air partition coefficient P_b, e.g., $C_{alv} = C_{art}/P_{b/a}$.

The lung is viewed here as a portal of entry and not as a target organ, and the concentration of a xenobiotic delivered to other organs by the blood, or the arterial concentration of that xenobiotic, is of primary interest. The assumptions of continuous ventilation, rapid equilibration with arterial blood, and no hold-up in lung tissues have proved applicable with many volatile organics. The use of these assumptions simplifies and speeds up model calculations and may be entirely adequate for describing the toxicokinetic behavior of relatively inert vapors with low water solubility.

Liver—The liver is almost always featured as a distinct compartment in physiologic models because biotransformation is an important route of elimination for many toxicants and the liver is considered the major organ for biotransformation of xenobiotics. A simple compartmental structure for the liver is one where uptake into the liver compartment is assumed to be flow-limited. This liver compartment is similar to the general tissue compartment in Figure 7–4, except that the liver compartment contains an additional process for metabolic elimination. Under first-order elimination, the rate of hepatic metabolism (R) by the liver can be presented as:

$$R = Cl_l \cdot C_f$$

where C_f is the free concentration of toxicant in the liver (mg/L), and Cl_l is the clearance of free toxicant within the liver (L/h).

In the case of a single enzyme mediating the biotransformation and Michaelis–Menten kinetics are obeyed, Cl_l is related to the maximum rate of metabolism V_{max} (in mg/h) and the Michaelis constant K_M (in mg/L). As a result, the rate of hepatic metabolism can be expressed in terms of the Michaelis parameters:

$$R = \left[\frac{V_{max}}{K_M + C_f}\right] C_f$$

Under nonsaturating or first-order condition (i.e., $C_f \ll K_M$), Cl_l becomes equal to the ratio of V_{max}/K_M. Because many toxicants at high exposure levels display saturable metabolism, the above equation is often invoked for simulation of toxicant disposition across a wide range of doses.

Other, more complex expressions for metabolism also can be incorporated into physiologic models. Bi-substrate second-order reactions, reactions involving the destruction of enzymes, inhibition of enzymes, or the depletion of cofactors, have been simulated using physiologic models. Metabolism can be also included in other compartments in much the same way as described for the liver.

Blood—In a physiologic model, the tissue compartments are linked together by the circulatory network. The decision to represent blood as an explicit physiologic compartment depends on the role the blood plays in disposition and the type of

application. If the toxicokinetics after intravenous injection is to be simulated or if binding to or metabolism by blood components is suspected, a separate compartment for the blood that incorporates these additional processes is required. A blood compartment is obviously needed if the model were developed to explain a set of blood concentration–time data for a toxicant. However, if blood is simply a conduit to the other compartments, as in the case for inhaled volatile organics, an algebraic solution is acceptable.

CONCLUSION

Biological monitoring or biomonitoring is defined as the systematic sampling of body fluids, and at times body tissue, for the purpose of estimating an individual's internal dose from exposure to chemicals in the workplace or assessing the range of internal exposure within a select population to environmental pollutants. T e advantages of biomonitoring over traditional environmental monitoring, such as ambient or personal air sampling or dermal dosimetry, include the accounting of other unanticipated routes of exposure, individual differences in toxicant absorption and disposition, and critical personal or lifestyle variables, such as body size and composition, workload that affects pulmonary ventilation, or cigarette smoking that could affect the metabolic status of an individual. Linking environmental exposure or dose to measurements of concentration of the parent chemical or its metabolite(s) in a biological sample is essentially an exercise in toxicokinetics.

Although simpler elements of physiologic models and the important assumptions that underlie model structures are presented, toxicologists are developing increasingly more sophisticated applications. T ree-dimensional visualizations of xenobiotic transport, physiologic models of a parent chemical linked in series with one or more active metabolites, models describing biochemical interactions among xenobiotics, and more biologically realistic descriptions of tissues previously viewed as simple lumped compartments are just a few of the more sophisticated applications. Finally, physiologically based toxicokinetic models are now being linked to biologically based toxicodynamic models to simulate the entire exposure → dose → response paradigm that is basic to the science of toxicology.

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QUESTIONS

1. Regarding the two-compartment model of classic toxicokinetics, which of the following is true?
   a. There is rapid equilibration of chemical between central and peripheral compartments.
   b. The logarithm of plasma concentration versus time data yields a linear relationship.
   c. There is more than one dispositional phase.
   d. It is assumed that the concentration of a chemical is the same throughout the body.
   e. It is ineffective in determining effective doses in toxicity studies.

2. When calculating the fraction of a dose remaining in the body over time, which of the following factors need not be taken into consideration?
   a. half-life.
   b. initial concentration.
   c. time.
   d. present concentration.
   e. elimination rate constant.

3. All of the following statements regarding apparent volume of distribution (V_d) are true EXCEPT:
   a. V_d relates the total amount of chemical in the body to the concentration of chemical in the plasma.
   b. V_d is the apparent space into which an amount of chemical is distributed in the body to result in a given plasma concentration.
   c. A chemical that usually remains in the plasma has a low V_d.
   d. V_d will be low for a chemical with high affinity for tissues.
   e. V_d can be used to estimate the amount of chemical in the body if the plasma concentration is known.

4. Chemical clearance:
   a. is independent of V_d.
   b. is unaffected by kidney failure.
   c. is indirectly proportional to V_d.
   d. is performed by multiple organs.
   e. is not appreciable in the GI tract.

5. A chemical with which of the following half-lives ($T_{1/2}$) will remain in the body for the longest period of time when given equal dosage of each?
   a. $T_{1/2}$ = 30 min.
   b. $T_{1/2}$ = 1 day.
   c. $T_{1/2}$ = 7 h.
   d. $T_{1/2}$ = 120 s.
   e. $T_{1/2}$ = 1 month.

6. With respect to first-order elimination, which of the following statements is FALSE?
   a. The rate of elimination is directly proportional to the amount of the chemical in the body.
   b. A semilogarithmic plot of plasma concentration versus time shows a linear relationship.
   c. Half-life ($T_{1/2}$) differs depending on the dose.
   d. Clearance is dosage-independent.
   e. The plasma concentration and tissue concentration decrease similarly with respect to the elimination rate constant.

7. The toxicity of a chemical is dependent on the amount of chemical reaching the systemic circulation. Which of the following does NOT greatly influence systemic availability?
   a. absorption after oral dosing.
   b. intestinal motility.
   c. hepatic first-pass effect.
   d. intestinal first-pass effect.
   e. incorporation into micelles.

8. Which of the following is NOT an advantage of a physiologically based toxicokinetic model?
   a. Complex dosing regimens are easily accommodated.
   b. The time course of distribution of chemicals to any organ is obtainable.
   c. The effects of changing physiologic parameters on tissue concentrations can be estimated.
   d. The rate constants are obtained from gathered data.
   e. The same model can predict toxicokinetics of chemicals across species.

9. Which of the following will not help to increase the flux of a xenobiotic across a biological membrane?
   a. decreased size.
   b. decreased oil:water partition coefficient.
   c. increased concentration gradient.
   d. increased surface area.
   e. decreased membrane thickness.

10. Which of the following statements is true regarding diffusion-limited compartments?
    a. Xenobiotic transport across the cell membrane is limited by the rate at which blood arrives at the tissue.
    b. Diffusion-limited compartments are also referred to as flow-limited compartments.
    c. Increased membrane thickness can cause diffusion-limited xenobiotic uptake.
    d. Equilibrium between the extracellular and intracellular space is maintained by rapid exchange between the two compartments.
    e. Diffusion of gases across the alveolar septa of a healthy lung is diffusion-limited.

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CHAPTER

Chemical Carcinogenesis

8

James E. Klaunig

OVERVIEW
- Definitions

MULTISTAGE CARCINOGENESIS
- Initiation
- Promotion
- Progression

MECHANISMS OF ACTION OF CHEMICAL CARCINOGENS
- Genotoxic/DNA-Reactive Carcinogens
  - Direct-acting (Activation-independent) Carcinogens
  - Indirect-acting Genotoxic Carcinogens
- Mutagenesis
- Damage by Alkylating Electrophiles
- DNA Repair
- DNA Repair Mechanisms
  - Mismatch Repair of Single-base Mispairs
  - Excision Repair
  - End-joining Repair of Nonhomologous DNA
- Classes of Genotoxic Carcinogens
  - Polyaromatic Hydrocarbons
  - Alkylating Agents
  - Aromatic Amines and Amides
- Inorganic Carcinogens
- Nongenotoxic (Epigenetic) Carcinogens
  - Cytotoxicity
  - Receptor Mediated
  - Hormonal Mode of Action
  - DNA Methylation and Carcinogenesis
  - Oxidative Stress and Chemical Carcinogenesis
- Gap Junctional Intercellular Communication and Carcinogenesis
- Modifiers of Chemical Carcinogenic Effects
- Polymorphisms in Carcinogen Metabolism and DNA Repair
- Proto-oncogenes and Tumor-suppressor Genes
  - Retroviruses
  - DNA Viruses
  - Proto-oncogenes
  - Tumor-suppressor Genes
- Hormesis, Dose Response, and Carcinogenesis
- Chemoprevention

TEST SYSTEMS FOR CARCINOGENICITY ASSESSMENT
- Short-term Tests for Mutagenicity
  - In Vitro Gene Mutation Assays
  - In Vivo Gene Mutation Assays
  - Chromosomal Alterations
  - DNA Damage
  - Transformation Assays
- Chronic Testing for Carcinogenicity
  - Chronic (2-Year) Bioassay
  - Organ-specific Bioassays and Multistage Animal Models
- Transgenic Animals in Carcinogenicity Assessment

CHEMICAL CARCINOGENESIS IN HUMANS
- Classification Evaluation of Carcinogenicity in Humans

KEY POINTS

- T e term cancer describes a subset of neoplastic lesions.
- A neoplasm is defined as a heritably altered, relatively autonomous growth of tissue with abnormal regulation of gene expression.
- Metastases are secondary growths of cells from the primary neoplasm.
- A carcinogen is an agent whose administration to previously untreated animals leads to a statistically significant increased incidence of neoplasms of one or more histogenetic types as compared with the incidence in appropriate untreated animals.
- Initiation requires one or more rounds of cell division for the "fixation" of the DNA damage.
- Promotion results from the selective functional enhancement of the initiated cell and its progeny by the continuous exposure to the promoting agent.
- Progression is the transition from early progeny of initiated cells to the biologically malignant cell population of the neoplasm.

OVERVIEW

Cancer is a disease of cellular mutation, proliferation, and aberrant cell growth. It ranks as one of the leading causes of death in the world. Multiple causes of cancer have been either firmly established or suggested, including infectious agents, radiation, and chemicals. Estimates suggest that 70% to 90% of all human cancers have a linkage to environmental, dietary, and behavioral factors.

Definitions

Table 8–1 lists definitions of terms commonly used in discussing chemical carcinogenesis. For benign neoplasms, the tissue of origin is frequently followed by the suffix "oma"; e.g., a benign fibrous neoplasm would be termed f broma, and a benign glandular epithelium termed an adenoma. Malignant neoplasms from epithelial origin are called carcinomas, whereas those derived from mesenchymal origin are referred to as sarcoma. T us, a malignant neoplasm of fibrous tissue would be a f brosarcoma, whereas that derived from bone would be an osteosarcoma.

Carcinogens may be genotoxic, meaning that they interact physically with DNA to damage or change its structure. Other carcinogens may change how DNA expresses information without modifying or directly damaging its structure, or may create a situation in a cell or tissue that makes it more susceptible to DNA damage from other sources. Chemicals belonging to this latter category are referred to as nongenotoxic carcinogens. Common features of genotoxic and nongenotoxic carcinogens are shown in Table 8–2.

MULTISTAGE CARCINOGENESIS

T e carcinogenesis process involves a series of definable and reproducible stages. Operationally, these stages have been defined as initiation, promotion, and progression (Figure 8–1).

TABLE 8–1 Terminology.

Neoplasia	New growth or autonomous growth of tissue
Neoplasm	The lesion resulting from the neoplasia
Benign	Lesions characterized by expansive growth, frequently exhibiting slow rates of proliferation that do not invade surrounding tissues
Malignant	Lesions demonstrating invasive growth, capable of metastases to other tissues and organs
Metastases	Secondary growths derived from a primary malignant neoplasm
Tumor	Lesion characterized by swelling or increase in size, may or may not be neoplastic
Cancer	Malignant neoplasm
Carcinogen	A physical or chemical agent that causes or induces neoplasia
Genotoxic	Carcinogens that interact with DNA resulting in mutation
Nongenotoxic	Carcinogens that modify gene expression but do not damage DNA

TABLE 8–2 Features of genotoxic and nongenotoxic carcinogens.

Genotoxic carcinogens
• Mutagenic
• Can be complete carcinogens
• Tumorigenicity is dose responsive
• No theoretical threshold
Nongenotoxic carcinogens
• Nonmutagenic
• Threshold, reversible
• Tumorigenicity is dose responsive
• May function at tumor promotion stage
• No direct DNA damage
• Species, strain, tissue specificity

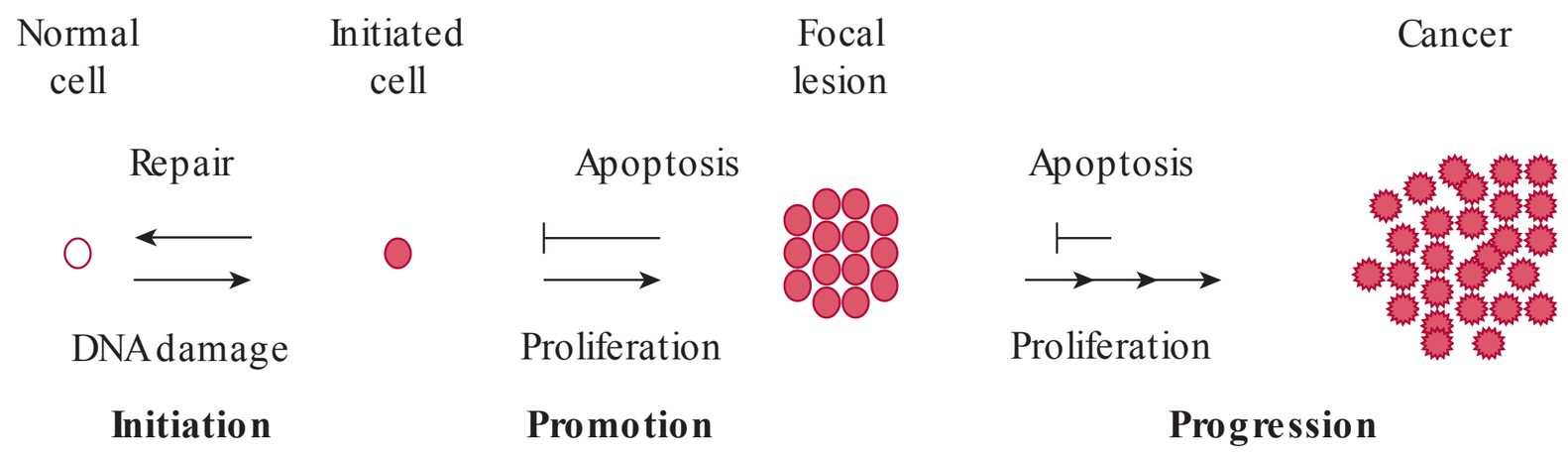

FIGURE 8–1 **Multistage model of carcinogenesis.**

The defining characteristics of each of these stages are outlined in Table 8–3.

Initiation

The first stage of the cancer process involves initiation, a process that is defined as a stable, heritable change. This stage is a rapid, irreversible process that results in a carcinogen-induced mutational event. Chemical and physical agents that interact with cellular components at this stage are referred to as initiators or initiating agents. Initiating agents lead to genetic changes including mutations and deletions. Chemical carcinogens that covalently bind to DNA and form adducts that result in mutations are initiating agents. The initiating event becomes "fixed" when the DNA damage is not correctly or completely repaired prior to DNA synthesis and cell division.

TABLE 8–3 Characteristics of the stages of carcinogenesis process.

Initiation
DNA modification
Mutation
Genotoxic
One cell division necessary to lock-in mutation
Modification is not enough to produce cancer
Nonreversible
Single treatment can induce mutation
Promotion
No direct DNA modification
Nongenotoxic
No direct mutation
Multiple cell divisions necessary
Clonal expansion of the initiated cell population
Increase in cell proliferation or decrease in cell death (apoptosis)
Reversible
Multiple treatments (prolonged treatment) necessary
Threshold
Progression
DNA modification
Genotoxic event
Mutation, chromosome disarrangement
Changes from preneoplasia to neoplasia benign/malignant
Irreversible
Number of treatments needed with compound unknown (may require only single treatment)

Once initiated cells are formed, their fate has multiple potential outcomes: (1) the initiated cell can remain in a static nondividing state; (2) the initiated cell may possess mutations incompatible with viability or normal function and be deleted through apoptotic mechanisms; or (3) the cell may undergo cell division resulting in the proliferation of the initiated cell. Besides the production of an initiated cell through carcinogen binding and misrepair, additional evidence has come forth showing that induction of continual stress, resulting in continual cell proliferation, can also produce new mutated, initiated cells.

Promotion

The second stage of the carcinogenesis process involves the selective clonal expansion of initiated cells to produce a preneoplastic lesion. This is referred to as the promotion stage of the carcinogenesis process. Both exogenous and endogenous agents that operate at this stage are referred to as tumor promoters. Tumor promoters are not mutagenic and generally are not able to induce tumors by themselves; rather they act through several mechanisms involving gene expression changes that result in sustained cell proliferation through increases in cell proliferation and/or the inhibition of apoptosis. Promotion is reversible upon removal of the promoting agent, and the focal cells may return to single initiated cell thresholds. In addition, these agents demonstrate a well-documented threshold for their effects—below a certain dose or frequency of application, tumor promoters are unable to induce cell proliferation. Tumor promoters generally show organ-specific effects, e.g., a tumor promoter of the liver, such as phenobarbital, will not function as a tumor promoter in the skin or other tissues.

Progression

Progression involves the conversion of benign preneoplastic lesions into neoplastic cancer. In this stage, due to an increase in DNA synthesis and cell proliferation in the preneoplastic lesions, additional genotoxic events may occur, resulting in further DNA damage including chromosomal aberrations and translocations. The progression stage is irreversible in that neoplasm formation, whether benign or malignant, occurs. With the formation of neoplasia, an autonomous growth and/or lack of growth control is achieved. Spontaneous progression can occur from spontaneous karyotypic changes that occur in

mitotically active initiated cells during promotion. An accumulation of nonrandom chromosomal aberrations and karyotypic instability are hallmarks of progression.

MECHANISMS OF ACTION OF CHEMICAL CARCINOGENS

T e formation of a neoplasm is a multistage, multistep process that involves the ultimate release of the neoplastic cells from normal growth control processes and creating a tumor microenvironment. T e eight properties of carcinogenesis are listed in Table 8–4. An important concept is that tumors are not just a collection of clonal neoplastic cells but a complex tissue with multiple cell populations that interact with one another and function as a unique tissue. T is tumor microenvironment involves the recruitment of normal stromal and inflammatory cells that contribute to the growth the development of the neoplasm.

Genotoxic/DNA-Reactive Carcinogens

Genotoxic compounds directly interact with the nuclear DNA of a target cell. If this damage is unrepairable, DNA damage is inherited in subsequent daughter cells. DNA reactive carcinogens can be further subdivided according to whether they are active in their parent form (i.e., direct-acting carcinogens—agents that can directly bind to DNA without being metabolized) and those that require metabolic activation (i.e., indirect-acting carcinogens—compounds that require metabolism in order to react with DNA).

TABLE 8–4 Proposed modes of action for selected nongenotoxic chemical carcinogens.

Mode of Action	Example
Cytotoxicity	Chloroform Melamine
α_{2u}-Globulin-binding	D-limonene, 1,4-dichlorobenzene
Receptor-mediated CAR	Phenobarbital
PPARα	Trichloroethylene Perchloroethylene Diethylhexylphthalate Fibrates (e.g., clofibrate)
AhR	TCDD Polychlorinated biphenyls (PCBs) Polybrominated biphenyls (PBBs)
Hormonal	Biogenic amines Steroid and peptide hormones DES Phytoestrogens (bisphenol-A) Tamoxifen Phenobarbital
Altered methylation	Phenobarbital Choline deficiency Diethanolamine
Oxidative stress inducers	Ethanol TCDD Lindane Dieldrin Acrylonitrile

Direct-acting (Activation-independent) Carcinogens—Direct-acting carcinogens are highly reactive electrophilic molecules that can interact with and bind to nucleophiles, such as cellular macromolecules, including DNA without needing to be biotransformed into a reactive toxicant. Generally, these highly reactive chemicals frequently result in tumor formation at the site of chemical exposure.

T e relative carcinogenic strength of direct-acting carcinogens depends in part on the relative rates of interaction between the chemical and genomic DNA, as well as competing reactions with the chemical and other cellular nucleophiles. Chemical stability, transport, and membrane permeability determine the carcinogenic activity of the chemical. Direct-acting carcinogens are typically carcinogenic at multiple sites and in all species examined.

Indirect-acting Genotoxic Carcinogens—T e majority of DNA reactive carcinogens are found as parent compounds, or procarcinogens, chemicals that require subsequent metabolism to be carcinogenic. Terms have been coined to define the parent compound (procarcinogen) and its metabolite form, either intermediate (proximate carcinogen) or final (ultimate carcinogen), that reacts with DNA. T e ultimate form of the carcinogen may not be known or may be several forms depending on metabolic pathway, but it is most likely the chemical species that results in mutation and neoplastic transformation. It is important to note that besides activation of the procarcinogen to a DNA reactive form, detoxification pathways may also occur resulting in inactivation of the carcinogen.

Indirect-acting genotoxic carcinogens usually produce their neoplastic effects at the target tissue where the metabolic activation of the chemical occurs and not at the site of exposure (as seen with direct-acting genotoxic carcinogens).

Mutagenesis

Effects of mutations depend on when in the cell cycle the adducts are formed, where the adducts are formed, and the type of repair process used in response to the damage. Mutagenesis may result from misread DNA (through transitions or transversions), frame-shif ing, or broken DNA strands.

Damage by Alkylating Electrophiles

As noted above, most chemical carcinogens require metabolic activation to exert a carcinogenic effect. T e ultimate carcinogenic forms of these chemicals are frequently strong electrophiles (Figure 8–2) that can readily form covalent adducts with nucleophilic targets. In general, the stronger electrophiles

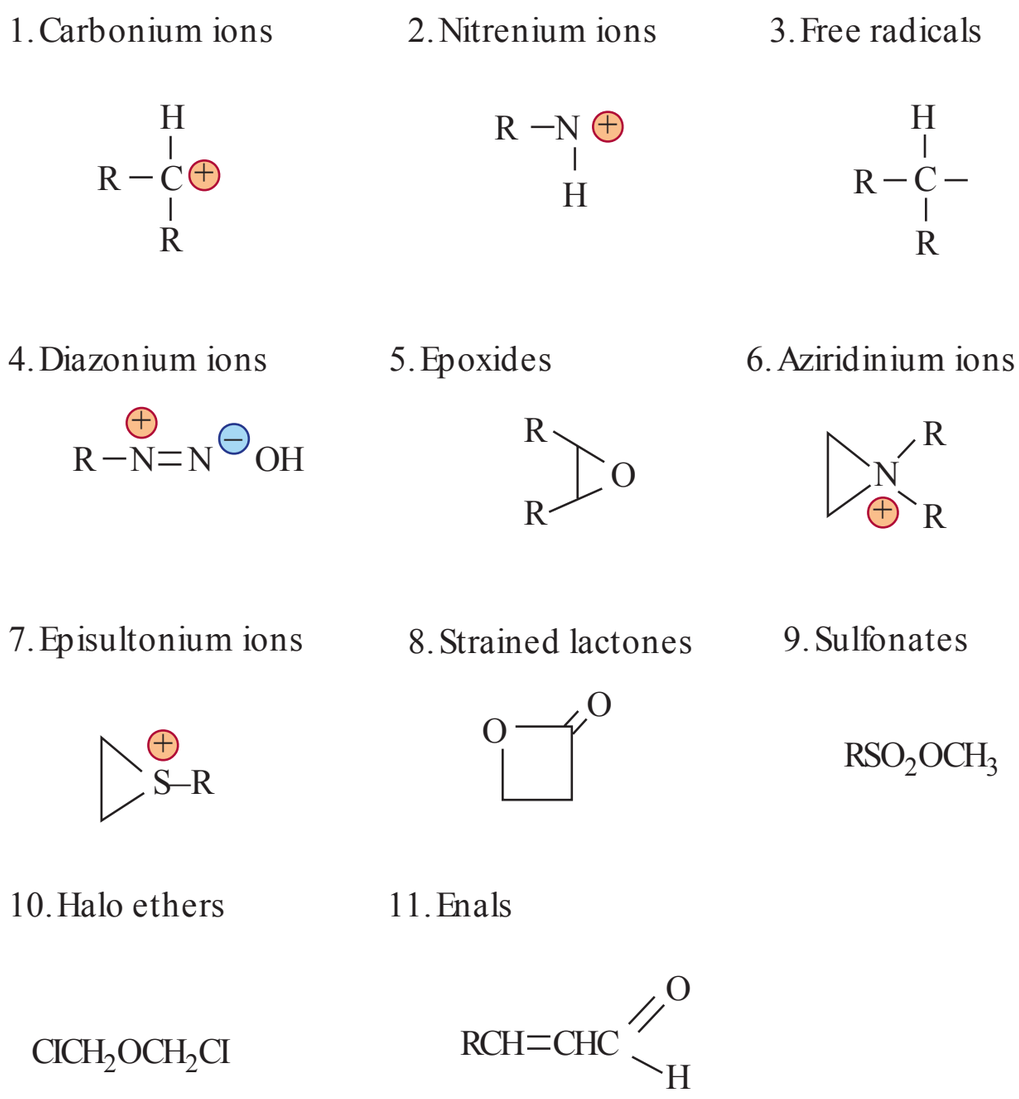

FIGURE 8–2 **Structures of reactive electrophiles.**

display a greater range of nucleophilic targets, whereas weak electrophiles are only capable of alkylating strong nucleophiles.

An important and abundant source of nucleophiles is contained not only in the DNA bases, but also in the phosphodiester backbone. Different electrophilic carcinogens will often display different preferences for nucleophilic sites in DNA and, thus, a different spectra of damage.

Another common modification to DNA is the hydroxylation of DNA bases. Oxidative DNA adducts have been identified in all four DNA bases. The source of oxidative DNA damage is typically formed from free radical reactions that occur endogenously in the cell or from exogenous sources.

Methylation of DNA results in heritable expression or repression of genes, with hypomethylation associated with active transcription of genes, whereas hypermethylated genes tend to be rarely transcribed. Chemical carcinogens may inhibit DNA methylation by forming covalent adducts, single-strand breaks in the DNA, alteration of methionine pools, and inactivation of the DNA methyltransferase responsible for methylation. Whether a particular DNA adduct will result in mutation depends in part on the process of DNA replication and in part on DNA repair.

DNA Repair

Following the formation of a carcinogen-DNA adduct, the persistence of the adduct is a major determinant of the outcome. This persistence depends on the ability of the cell to repair the altered DNA. However, the presence of a DNA adduct is not sufficient for the carcinogenesis process to proceed. The relative rates or persistence of particular DNA adducts may be an important determinant of carcinogenicity. Differences in susceptibility to carcinogenesis are likely the result of a number of factors, including DNA replication within a tissue and repair of a DNA adduct. The development of cancer following exposure to chemical carcinogens is a relatively rare event because of a cell's ability to recognize and repair damaged DNA. The DNA region containing the adduct is removed and a new patch of DNA is synthesized, using the opposite intact strand as a template. The new DNA segment is then spliced into the DNA molecule in place of the defective one. To be effective in restoring a cell to normal, repair of DNA must occur prior to cell division.

DNA Repair Mechanisms

Although cells possess mechanisms to repair many types of DNA damage, these are not always completely effective, and residual DNA damage can lead to the synthesis of altered protein. Mutations in an oncogene, tumor-suppressor gene, or gene that controls the cell cycle can result in a clonal cell population with a survival advantage. The development of a tumor requires many such events, occurring over a long period of time, and for this reason human cancer induction often takes place within the context of chronic exposure to chemical carcinogens.

Cells have several mechanisms for repairing DNA damage. Repair of DNA damage does not always occur prior to cell replication, and repair of DNA damage by some chemicals is relatively inefficient.

Mismatch Repair of Single-base Mispairs—Many spontaneous mutations are point mutations, a change in a single-base pair in the DNA sequence. Depurination is a fairly common occurrence and a spontaneous event in mammals, and results in the formation of apurinic sites. All mammalian cells possess apurinic endonucleases that function to cut DNA near apurinic sites. The cut is then extended by exonucleases, and the resulting gap repaired by DNA polymerases and ligases.

Excision Repair—DNA regions containing chemically modified bases, or DNA chemical adducts, are typically repaired by excision repair processes. Proteins that slide along the surface of a double-stranded DNA molecule recognize irregularities in the shape of the double helix, and induce repair of the lesion.

End-joining Repair of Nonhomologous DNA—A cell that has double-strand breaks can be repaired by joining the free DNA ends. The joining of broken ends from different chromosomes, however, will lead to the translocation of DNA pieces from one chromosome to another, translocations that have the potential to enable abnormal cell growth. Homologous recombination is one of two mechanisms responsible for the repair of double-strand breaks. In this process, the double-strand break on one chromosome is repaired using the information on the homologous, intact chromosome.

The predominant mechanism for double-stranded DNA repair in multicellular organisms is nonhomologous repair,

which involves the rejoining of the ends of the two DNA molecules. Although this process yields a continuous double-stranded molecule, several base pairs are lost at the joining point. T is type of deletion may produce a potentially mutagenic coding change.

Classes of Genotoxic Carcinogens

Polyaromatic Hydrocarbons—Polyaromatic hydrocarbons such as benzo(a)pyrene are found at high levels in charcoal broiled foods, cigarette smoke, and in diesel exhaust.

Alkylating Agents—Whereas some alkylating chemicals are direct-acting genotoxic agents, many require metabolic activation to produce electrophilic metabolites that can react with DNA. Alkylating agents readily react with DNA at more than 12 sites. T e N^7 position of guanine and the N^3 position of adenine are the most reactive sites in DNA for alkylating chemicals.

Aromatic Amines and Amides—Aromatic amines and amides encompass a class of chemicals with varied structures. Classically, exposure to these chemicals was through the dye industry, although exposure still occurs through cigarette smoke and other environmental sources. T e aromatic amines undergo phase-I (hydrolysis, reduction, and oxidation) and phase-II (conjugation) metabolism. Phase-I reactions occur mainly by cytochrome P450–mediated reactions, yielding hydroxylated metabolites that are of en associated with adduct formation in proteins and DNA, and produce liver and bladder carcinogenicity.

Inorganic Carcinogens

Several metals exhibit carcinogenicity in experimental animals and/or humans, including arsenic, beryllium, cadmium, chromium, nickel, and lead. T e carcinogenic manifestations are varied as well and include increased risk for skin, lung, and liver tumors. Additional discussion of selected metals is in Chapter 23.

Nongenotoxic (Epigenetic) Carcinogens

T e targets induced by nongenotoxic carcinogens are of en in tissues where a significant incidence of background, spontaneous tumors is seen in the animal model. Prolonged exposure to relatively high levels of chemicals is usually necessary for the production of tumors. Examples of the diverse modes of action for non-DNA-reactive carcinogens are listed in Table 8–4.

Cytotoxicity—Chemicals that function through this mechanism produce sustained cell death that is accompanied by persistent regenerative growth. T is results in the potential for the acquisition of "spontaneous" DNA mutations and allowing mutated cells to accumulate and proliferate. T is process then gives rise to preneoplastic focal lesions that, upon expansion, can lead to tumor formation. T e induction of cytotoxicity may be observed with many carcinogens both genotoxic and nongenotoxic when high toxic exposures occur. T us, the induction of cytotoxicity with compensatory hyperplasia may contribute to the observed tumorigenicity of many carcinogenic chemicals at high doses.

Receptor Mediated

P450 Inducers: Phenobarbital-like Carcinogens—Phenobarbital is a commonly studied non-DNA reactive compound that is known to cause tumors by a nongenotoxic mechanism involving liver hyperplasia. T e induction of CYP2B by phenobarbital is mediated by activation of the constitutive androstane receptor (CAR), a member of the nuclear receptor family. Other CAR-dependent phenobarbital responses that are critical for tumor formation include increased cell proliferation, inhibition of apoptosis, inhibition of gap junctional communication, hypertrophy, and development of preneoplastic focal lesions in the liver.

Peroxisome Proliferator–activated Receptor-α (PPARα)—Various chemicals are capable of increasing the number and volume of peroxisomes in the cytoplasm of cells. These so-called peroxisome proliferators include chemicals such as herbicides, chlorinated solvents (e.g., trichloroethylene and perchloroethylene), plasticizers (e.g., diethylhexylphthalate and other phthalates), lipid-lowering fibrate drugs (e.g., ciprofibrate and clofibrate), and natural products. T e currently accepted mode of action for this class of chemicals involves agonist binding to the nuclear hormone receptor, PPARα. PPARα is highly expressed in cells that have active fatty acid oxidation capacity. PPARα plays a central role in lipid metabolism and acts as a transcription factor to modulate gene expression following ligand activation.

Hormonal Mode of Action—Hormonally active chemicals include biogenic amines, steroids, and peptide hormones that cause tissue-specific changes through interaction with a receptor. A number of non-DNA-reactive chemicals induce neoplasia through receptor-mediated mechanisms, and/or perturbation of hormonal balance. Trophic hormones are known to induce cell proliferation at their target organs. T is action may lead to the development of tumors when the mechanisms of hormonal control are disrupted and some hormone shows persistently increased levels.

Estrogenic agents can induce tumors in estrogen-dependent tissue. Individuals with higher circulating estrogen levels and those with exposure to the potent estrogenic agent diethylstilbestrol (DES) are at increased risk of cancer development. DES has been causally linked to the higher incidence of adenocarcinomas of the vagina and cervix in daughters of women treated with the hormone during pregnancy. T e effects of steroidal chemicals on the cell cycle and on microtubule assembly may be important in the aneuploidy inducing effects of some hormonal agents.

A number of chemicals that reduce thyroid hormone concentrations (T4 and/or T3) and increase thyroid-stimulating hormone (TSH) have been shown to induce neoplasia in the rodent thyroid. TSH demonstrates proliferative activity in the

thyroid, with chronic drug-induced TSH increases leading to progression of follicular cell hypertrophy, hyperplasia, and eventually neoplasia.

DNA Methylation and Carcinogenesis—Post-DNA synthetic methylation of the five position on cytosine is a naturally occurring modification to DNA in higher eukaryotes that influences gene expression. Under normal conditions, DNA is methylated symmetrically on both strands. Immediately following DNA replication, the newly synthesized double-stranded DNA contains hemimethylated sites that signal for DNA maintenance methylases to transfer methyl groups from S-adenosylmethionine to cytosine residues on the new DNA strand. T e degree of methylation within a gene inversely correlates with the expression of that gene. Several chemical carcinogens are known to modify DNA methylation, methyltransferase activity, and chromosomal structure. During carcinogenesis, both hypomethylation and hypermethylation of the genome have been observed. Tumor-suppressor genes have been reported to be hypermethylated in tumors. Hypomethylation has been associated with increased mutation rates because many oncogenes are hypomethylated and their expression is amplified.

Reactive oxygen species have also been shown to modify DNA methylation by interfering with the ability of methyltransferases to interact with DNA; the resulting hypomethylation allows for the expression of normally quiescent genes. Also, the abnormal methylation pattern observed in cells transformed by chemical oxidants may contribute to an overall aberrant gene expression and promote tumorigenesis.

Oxidative Stress and Chemical Carcinogenesis—Oxygen radicals can be produced by both endogenous and exogenous sources and are typically counterbalanced by antioxidants. Antioxidant defenses are both enzymatic (e.g., superoxide dismutase, glutathione peroxidase, and catalase) and nonenzymatic (e.g., vitamin E, vitamin C, β-carotene, and glutathione). Endogenous sources of reactive oxygen species include oxidative phosphorylation, P450 metabolism, peroxisomes, and inflammatory cell activation. T rough these or other currently unknown mechanisms, a number of chemicals that induce cancer (e.g., chlorinated compounds, radiation, metal ions, barbiturates, and some PPARα agonists) induce reactive oxygen species formation and/or oxidative stress.

Oxidative DNA Damage and Carcinogenesis—Reactive oxygen species lef unbalanced by antioxidants can result in damage to cellular macromolecules. In DNA, reactive oxygen species can produce single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA crosslinks. Although many pathways exist that enable the formation of oxidative DNA damage, mammalian cells also possess specific repair pathways for the remediation of oxidative DNA damage.

Mutations and oxidative damage to mitochondrial DNA have been identified in a number of cancers. Compared to nuclear DNA, the mitochondrial genome is relatively susceptible to oxidative base damage due to (1) close proximity to the electron transport system, a major source of reactive oxygen species; (2) mitochondrial DNA is not protected by histones; and (3) DNA repair capacity is limited in the mitochondria.

Aside from oxidized nucleic acids, oxygen radicals can damage cellular biomembranes resulting in lipid peroxidation. Peroxidation of biomembranes generates a variety of products including reactive electrophiles such as epoxides and aldehydes, including malondialdehyde.

Oxidative Stress and Cell Growth Regulation—Reactive oxygen species production and oxidative stress can affect both cell proliferation and apoptosis. It has been demonstrated that low levels of reactive oxygen species influence signal transduction pathways and alter gene expression. Many xenobiotics, by increasing cellular levels of oxidants, alter gene expression through activation of signaling pathways including cAMP-mediated cascades, calcium-calmodulin pathways, transcription factors such as AP-1 and NF-κB, as well as signaling through mitogen-activated protein (MAP) kinases. Activation of these signaling cascades ultimately leads to altered gene expression for a number of genes including those affecting proliferation, differentiation, and apoptosis.

Gap Junctional Intercellular Communication and Carcinogenesis

Cells within an organism communicate in a variety of ways including through gap junctions, which are aggregates of connexin proteins that form a conduit between two adjacent cells. Gap junctional intercellular communication appears to play an important role in the regulation of cell growth and cell death, in part through the ability to exchange small molecules (< 1 kDa) between cells. If cell communication is blocked between tumor and normal cells, the exchange of growth inhibitory signals from normal cells to initiated cells is prevented, thus allowing the potential for unregulated growth and clonal expansion of initiated cell populations.

Modifiers of Chemical Carcinogenic Ef ects

Genetic and environmental factors have a significant impact on the way in which individuals and/or organisms respond to carcinogen exposure. As with most genes, enzymes that metabolize carcinogens are expressed in a tissue-specific manner. Within tissues, the enzymatic profile can vary with cell type or display differential localization within cells. Further, carcinogen metabolizing enzymes are differentially expressed among species. T ese differences may represent an underlying factor explaining the differential responses to chemical carcinogens across species.

Polymorphisms in Carcinogen Metabolism and DNA Repair

Genetic polymorphisms arise from human genetic variability. A genetic polymorphism is when a gene has more than one allele. In assessing variability in the human genome project it was found that base variations occurred at approximately once in every 1 000 base pairs. T erefore, there may be over one million genetic variations between any two individuals. A single nucleotide polymorphism (SNP) is a variant in DNA sequence found in greater than 1% of the population. T us, by definition, changes in DNA sequence go from mutation to polymorphism when a unique genotype is seen in over 1% of the population. Over three million candidate SNPs have been identified to date with up to 10 million being estimated to be present within the human genome. In carcinogenesis, genetic polymorphisms may account for the susceptibility of some individuals to certain cancers. In carcinogenesis, genetic polymorphisms may account for the susceptibility of some individuals to certain cancers. A number of polymorphisms have been described in carcinogen-metabolizing enzymes, with certain alleles linked to altered risk of selective cancers. Glutathione S-transferases (GSTs) are highly polymorphic in humans. T e GSTM1 isoform is particularly important in carcinogenesis, because of its high reactivity toward epoxides.

Carcinogenic risk depends on both exposure (dose and duration) as well as genetic susceptibility. For example, if the genetic susceptibility is high, then exposure to a chemical carcinogen will result in a higher risk for cancer development.

Proto-oncogenes and Tumor-suppressor Genes

Proto-oncogenes and tumor-suppressor genes encode a wide array of proteins that function to control cell growth and proliferation. Common characteristics of oncogenes and tumor-suppressor genes are shown in Table 8–5. Mutations in both oncogenes and tumor-suppressor genes contribute to the progressive development of human cancers. Accumulated damage to multiple oncogenes and/or tumor-suppressor genes can result in altered cell proliferation, differentiation, and/or survival of cancer cells.

Retroviruses—T e Rous sarcoma virus (RSV) is capable of transforming a normal cell and producing sarcomas. T e genome of RSV and other retroviruses consists of two identical copies of mRNA, which is then reverse transcribed into DNA and incorporated into the host-cell genome. Oncogenic transforming viruses like RSV contain the v-src gene, a gene required for cancer induction. Normal cells contain a gene closely related to v-src in RSV. T is discovery showed that cancer may be induced by the action of normal, or nearly normal, genes.

DNA Viruses—Unlike retroviral oncogenes, which are derived from normal cellular genes and have no function for the virus, the known oncogenes of DNA viruses are integral parts of the viral genome required for viral replication. Infection by small DNA viruses is lethal to most non-host animal cells; however, a small proportion integrates the viral DNA into the host-cell genome. T e cells that survive infection become permanently transformed due to the presence of one or more oncogenes in the viral DNA. Papilloma viruses can infect and cause tumors in humans. Some examples of oncogenic DNA viruses include human papilloma viruses, Epstein–Barr virus, hepatitis B virus, and herpes viruses.

Proto-oncogenes—An oncogene encodes a protein that is capable of transforming cells in culture or inducing cancer in animals. Of the known oncogenes, the majority appear to have been derived from normal genes (i.e., proto-oncogenes), and are involved in cell signaling cascades. Because most proto-oncogenes are essential for maintaining viability, they are highly conserved. Activation of proto-oncogenes to oncogenes arises through mutational events occurring within proto-oncogenes. It has been recognized that a number of chemical carcinogens are capable of inducing mutations in proto-oncogenes. Oncogene products can operate at multiple levels of signaling cascades, including ligand, receptor, second messengers, and transcription factor stages of transduction.

TABLE 8–5 Characteristics of proto-oncogenes, cellular oncogenes, and tumor-suppressor genes.

Proto-oncogenes	Oncogenes	Tumor-suppressor Genes
Dominant	Dominant	Recessive
Broad tissue specificity for cancer development	Broad tissue specificity for cancer development	Considerable tissue specificity for cancer development
Germ line inheritance rarely involved in cancer development	Germ line inheritance frequently involved in cancer development	Germ line inheritance frequently involved in cancer development
Analogous to certain viral oncogenes	No known analogs in oncogenic viruses	No known analogs in oncogenic viruses
Somatic mutations activated during all stages of carcinogenesis	Somatic mutations activated during all stages of carcinogenesis	Germ line mutations may initiate, but mutation to neoplasia occurs only during progression stage

Tumor-suppressor Genes—In contrast to oncogenes, the proteins encoded by most tumor-suppressor genes act as inhibitors of cell proliferation or cell survival (Table 8–6). T e prototype tumor-suppressor gene, Rb, was identified by studies of inheritance of retinoblastoma.

Retinoblastoma (Rb) Gene—Loss or mutational inactivation of Rb contributes to the development of a wide variety of human cancers. In its unphosphorylated form, Rb binds to the E2F transcription factors preventing E2F-mediated transcriptional activation of a number of genes whose products are required for DNA synthesis. Rb becomes phosphorylated during the late G1, causing dissociation from E2F—a process that allows E2F to induce synthesis of DNA replication enzymes, resulting in a commitment to the cell cycle.

p53 Gene—T e p53 protein is a tumor-suppressor gene that is essential for checkpoint control and arrests the cell cycle in cells with damaged DNA in G1. Cells with functional p53 arrest in G1 when exposed to DNA damaging agents, whereas cells lacking functional p53 are unable to block the cell cycle. p53 is activated by a wide array of stressors including ultraviolet light, γ irradiation, heat, and several carcinogens.

In most cells, accumulation of p53 also leads to induction of proteins that promote apoptosis, and therefore would prevent proliferation of cells that are likely to accumulate multiple mutations. When the p53 checkpoint control does not operate properly, damaged DNA can replicate, producing mutations and DNA rearrangements that contribute to the development of transformed cells.

BRCA1 Gene—Genetic analysis of breast tumors has revealed a hereditary predisposition for breast cancer linked to BRCA1, a tumor-suppressor gene. Mutation of a single BRCA1 allele results in a 60% probability of developing breast cancer by age 50. A number of investigators have shown that germ line mutations lead to loss of function of the BRCA1 gene. However, no mutations have been observed in sporadic breast cancer, suggesting that BRCA1 gene silencing may occur through nonmutational mechanisms.

TABLE 8–6 Examples of tumor-suppressor genes and cancer association.

Tumor Suppressor	Disorder	Neoplasm
Rb1	Retinoblastoma	Small-cell lung carcinoma
p53	Li-Fraumeni syndrome	Breast, colon, lung cancers
BRCA1	Unknown	Breast carcinoma
WT-1	Wilms'tumor	Lung cancer
p16	Unknown	Melanoma

Wilms' Tumor Gene (WT1)—Wilms' tumor occurs in the developing kidney at a rate of approximately one per 10 000 children. T e W*T*1 gene is believed to be responsible for tumor development and is thought to function as a transcription factor.

p16 Gene—T e group of proteins that function as cyclin-kinase inhibitors play an important role in cell cycle regulation. Mutations, especially deletions of the p16 gene that inactivate the ability of p16 to inhibit cyclin D–dependent kinase activity, are common in several human cancers including a high percentage of melanomas. Loss of p16 would mimic cyclin D1 overexpression, leading to Rb overactivation and release of active E2F transcription factor. T us, p16 normally acts as a tumor suppressor. As with the BRCA1 gene, relatively few mutations have been found in this gene, and some researchers have speculated that epigenetic mechanisms such as gene silencing by DNA methylation may occur during tumorigenesis.

Hormesis, Dose-Response, and Carcinogenesis

Hormesis is defined as a dose–response curve in which a U-, J-, or inverted U-shaped dose–response is observed, with low-dose exposures of en resulting in beneficial rather than harmful effects. Adaptive responses have been proposed to explain the hormetic effects observed by chemical carcinogens. T ese responses usually involve actions of the chemical on cellular signaling pathways that lead to changes in gene expression, resulting in enhanced detoxification and excretion of the chemical, as well as preserving the cell cycle and programmed cell death. It has been proposed that following very low doses of chemicals, the upregulation of these mechanisms overcompensates for cell injury such that a reduction in tumor promotion and/or tumor development is seen, and would explain the U- or J-shaped response curves obtained following carcinogen exposure. A common feature of chemical carcinogens for which hormetic effects have been proposed is the formation of reactive oxygen species and the induction of cytochrome P450 isoenzymes.

Chemoprevention

T e study of chemicals that prevent, inhibit, or slow down the process of cancer is referred to as chemoprevention. A number of chemicals, including drugs, antioxidants, foodstuffs, and vitamins, have been found to inhibit or retard the components of the cancer process in both in vitro and in vivo models. A basic assumption in chemoprevention is that treating early stages of malignant process will halt or delay the progression to neoplasia. Chemopreventive agents may function as inhibitors of carcinogen formation, blocking agents, and/or suppressing agents. Blocking agents serve to prevent the metabolic activation of genotoxic or nongenotoxic carcinogens by either inhibiting its metabolism or by enhancing the detoxification mechanisms. Suppressing agents induce tissue differentiation, may counteract oncogenes, enhance tumor-suppressor gene activities,

inhibit proliferation of premalignant cells, or modify the effect of the carcinogen on the target tissue.

TEST SYSTEMS FOR CARCINOGENICITY ASSESSMENT

A number of in vivo and in vitro experimental systems are available to assess the potential carcinogenicity of chemicals. T e types of tests available to identify chemicals with carcinogenic potential can be classified into general categories, based on the duration required to conduct the test. Short-term tests are typically of the duration of days to a few weeks, intermediate-term tests last from weeks up to a year, whereas chronic long-term tests usually encompass six months to two years exposure to a chemical. T ese bioassays use bacterial and mammalian targets.

Short-term Tests for Mutagenicity

Short-term tests for mutagenicity were developed to identify potentially carcinogenic chemicals based on their ability to induce mutations in DNA either in vivo or in vitro. T e majority of these tests measure the mutagenicity of chemicals as a surrogate for carcinogenicity. Although usually very predictive of indirect action and direct action (if a metabolic source is provided), these tests routinely fail to detect nongenotoxic carcinogens.

In Vitro Gene Mutation Assays—T e most widely used short-term test is the Ames assay. Salmonella typhimurium strains deficient in DNA repair and unable to synthesize histidine are treated with several dose levels of the test compound, af er which reversion to the histidine-positive phenotype is ascertained.

T e mouse lymphoma assay is a mutagenicity assay used to determine whether a chemical is capable of inducing mutation in eukaryotic cells. T e ability of the cell cultures to acquire resistance to trifluorothymidine (the result of forward mutation at the thymidine kinase locus) is quantified. Another mammalian cell mutation assay, the Chinese hamster ovary (CHO) test, is also commonly used to assess the potential mutagenicity of chemicals. T is assay uses the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene as the end point.

In Vivo Gene Mutation Assays—T e in vivo tests have advantages over the in vitro test systems in that they take into account the whole animal processes such as absorption, tissue distribution, metabolism, and excretion of chemicals and their metabolites. T e commonly used in vivo models include transgenic rodent mutation assay systems based on the genes of the lac operon, Muta™Mouse, and Big Blue®

To detect mutations following exposure of mice to test chemicals, mutations are analyzed in high molecular weight DNA isolated from the tissue under investigation. T e ratio of mutants to the total population will provide a mutation frequency for each chemical and each organ tested. In vivo genotoxicity test systems will fail to identify nongenotoxic/non-DNA reactive compounds.

Chromosomal Alterations—Chromosomal alterations are quite common in malignant neoplasms. Both in vivo and in vitro assays are available to assess chromosomal alterations. To assess induction of chromosomal alterations, cells are harvested in their first mitotic division af er the initiation of chemical exposure. Cells are stained with Giemsa and scored for completeness of karyotype (21 ± 2 chromosomes). T e classes of aberrations recorded include breaks and terminal deletions, rearrangements and translocations, as well as despiralized chromosomes, and cells containing 10 or more aberrations.

Sister chromatid exchanges (SCEs) are a measure of DNA damage events that are associated with mutation induction and cancer. SCEs are a reflection of an interchange of DNA between different chromatids at homologous loci within a replicating chromosome. Second-division metaphase cells are scored to determine the frequency of SCE/cell for each dose level. Disruption of the DNA replication process or damage to chromosomes by chemicals can alter the genetic material distributed to either of the two daughter nuclei. When this occurs, the genetic material that is not incorporated into a new nucleus may form its own "micronucleus," which is clearly visible with a microscope. For this assay, animals are exposed to chemicals and the frequency of micronucleated cells is determined at some specified time af er treatment.

DNA Damage—Primary DNA damage represents possible pre-mutational events that can be detected using mammalian cells in culture or using rodent tissue. Unscheduled DNA synthesis (UDS) is a commonly used assay that measures the ability of a chemical to induce DNA lesions by measuring the increase in DNA repair. Among the available techniques is the measurement of DNA strand breaks both in vivo and in vitro.

Transformation Assays—Various in vitro test systems have been developed to assess the carcinogenic potential of chemicals. T e C3H/10T$^{1/2}$ cell line has been widely used in the transformation assays. It was originally derived from fibroblasts taken from the prostate of a C3H mouse embryo. T e cells are approximately tetraploid but the chromosome number in the cells varies widely. As such, these cells are chromosomally abnormal and have already passed through some of the stages that might be involved in the production of a cancerous cell. On plating these cells, they will stop growing when their density is sufficiently high (contact growth inhibition). However, the contact inhibition can fail, resulting in cell piling forming a transformed colony. T erefore, following exposure to xenobiotics, this assay assesses carcinogenic potential based on the percentage of colonies that are transformed.

T e most frequently used end point for cell transformation is morphological transformation of mammalian cell fibroblasts in culture. Transformation assays using Syrian hamster embryo (SHE) cells are available for the assessment of the carcinogenic potential of chemicals. T e SHE cell assay measures

carcinogenic potential of xenobiotics by assessing transformed colonies based on morphological criteria.

Chronic Testing for Carcinogenicity

Chronic (2-Year) Bioassay—Two-year studies over the lifespan of rodents remain the primary method by which chemicals or physical agents are identified as having the potential to be hazardous to humans. In the chronic bioassay, two or three dose levels (up to the maximum tolerated dose, MTD) of a test chemical and a vehicle control are administered to 50 males and 50 females (mice and rats), beginning at 8 weeks of age, continuing throughout their lifespan. During the study, food consumption and bodyweight gain are monitored, and the animals are observed clinically on a regular basis, and at necropsy the tumor number, location, and pathological diagnosis for each animal are thoroughly assessed.

Organ-specific Bioassays and Multistage Animal Models—Many tissue-specific bioassays have been developed with the underlying goal being to produce a sensitive and reliable assay that could be conducted in a time frame shorter in duration than the 2-year chronic bioassay. These assays are commonly used to detect carcinogenic activity of chemicals in various target organs.

Carcinogenicity Testing in the Liver—The liver represents a major target organ for chemical carcinogens. It has been estimated that nearly half of the chemicals tested in the 2-year chronic bioassay by the National Toxicology Program showed an increased incidence of liver cancer. Liver carcinogenesis assays have been developed to study and distinguish chemicals that affect the initiation or promotion stage of hepatocarcinogenesis. The ability of the test chemical to promote the growth of preneoplastic lesions can be assessed.

Carcinogenicity Testing in the Skin—The mouse skin model has been used to dissect mechanisms of carcinogenesis and also is purported to be a useful intermediate-term cancer bioassay. This model exploits many of the unique properties of the mouse skin, one major advantage being that the development of neoplasia can be followed visually. In addition, the number and relative size of papillomas and carcinomas can be quantified as the tumors progress. Both initiating and promoting activities of chemical carcinogens can be assessed using this model. Grossly, initiated cells of the skin appear identical to normal skin. Because the terminally differentiated cells in the skin are no longer capable of undergoing cell division, only initiated cells retain their proliferative capacity and thus represent the cell populations that give rise to tumors. On repeated application of tumor promoters, selective clonal expansion of initiated keratinocytes occurs, resulting in skin papillomas, which over time can progress to carcinomas.

Carcinogenicity Testing in Other Organs—Test systems to examine the ability of a chemical to promote neoplastic development at organ sites other than liver and skin have also been developed. The available systems include animal models directed at examining carcinogenicity in the lung, kidney, bladder, pancreas, stomach, colon, small intestine, and oral cavity. These models vary in the initiating carcinogen used, and frequency, duration, and site of application, as well as the duration of promoting chemical exposure.

Transgenic Animals in Carcinogenicity Assessment

Animal models with genetic alterations that invoke a susceptibility to carcinogenesis by chemical agents include Tg.AC and rasH2 transgenic mice, and $p53^{+/-}$ and $XPA^{-/-}$ knockout mice. Recently, the feasibility of the use of these animal models as alternative assays for the 2-year chronic bioassay was assessed by the Health and Environmental Sciences Institute branch of the International Life Sciences Institute. The conclusions drawn from the scientific review suggested that these models appear to have usefulness as screening models for assessment of chemical carcinogenicity; however, they do not provide definitive proof of potential human carcinogenicity. Further, the scientific panel suggested that these models could be used in place of the mouse 2-year bioassay. Coupled with information on genotoxicity, particularly DNA reactivity, structure–activity relationships, results from other bioassays, and the results of other mechanistic investigations including toxicokinetics, metabolism, and mechanistic information, these alternate mouse models for carcinogenicity appear to be useful models for assessing the carcinogenicity of chemical agents.

CHEMICAL CARCINOGENESIS IN HUMANS

Many factors have been implicated in the induction of cancer in humans. Infectious agents, lifestyle, medical treatments, and environmental and occupational exposure account either directly or indirectly for the majority of cancers seen in humans. Of these, the component that contributes the most to human cancer induction and progression is lifestyle: tobacco use, alcohol use, and poor diet (Table 8–7). Tobacco usage either through

TABLE 8–7 Carcinogenic factors associated with lifestyle.

Chemical(s)	Neoplasm(s)
Alcohol beverage	Esophagus, liver, oropharynx, and larynx
Aflatoxins	Liver
Betel chewing	Mouth
Dietary intake (fat, protein, calories)	Breast, colon, endometrium, gallbladder
Tobacco smoking	Mouth, pharynx, larynx, lung, esophagus, bladder

TABLE 8–8 Occupational human carcinogens.

Agent	Industrial Process	Neoplasms
Asbestos	Construction, asbestos mining	Peritoneum, bronchus
Arsenic	Mining and smelting	Skin, bronchus, liver
Alkylating agents (mechloroethamine hydrochloride and bis[chloromethyl]ether)	Chemical manufacturing	Bronchus
Benzene	Chemical manufacturing	Bone marrow
Benzidine, beta-naphthylamine	Dye and textile	Urinary bladder
Chromium and chromates	Tanning, pigment making	Nasal sinus, bronchus
Nickel	Nickel refining	Nasal sinus, bronchus
Polynuclear aromatic hydrocarbons	Steel making, roofing, chimney cleaning	Skin, scrotum, bronchus
Vinyl chloride monomer	Chemical manufacturing	Liver
Wood dust	Cabinet making	Nasal sinus
Beryllium	Aircraft manufacturing, electronics	Bronchus
Cadmium	Smelting	Bronchus
Ethylene oxide	Production of hospital supplies	Bone marrow
Formaldehyde	Plastic, textile, and chemical	Nasal sinus, bronchus
Polychlorinated biphenyls	Electrical-equipment production and maintenance	Liver

smoking tobacco, chewing tobacco, or tobacco snuff-type products is estimated to be responsible for 25% to 40% of all human cancers. In particular, a strong correlation between tobacco usage and mouth, larynx, lung, esophageal, and bladder cancer exists. It has been estimated that 85% to 90% of all lung cancer cases in the United States are a direct result of tobacco use. The induction of pancreatic cancer also appears to have a linkage to tobacco use. Alcohol consumption contributes anywhere from 2% to 4% of cancers of the esophagus, liver, and larynx.

Poor diets, occupational exposures, and chemotherapeutic therapy account for many human cancers. High-fat and high-calorie diets have been linked to breast, colon, and gallbladder cancer in humans (see Chapter 27). Diets poor in antioxidants and/or vitamins such as vitamin A and vitamin E probably also contribute to the onset of cancer. The method of cooking may also influence the production of carcinogens produced in the cooking process. Carcinogenic heterocyclic amines and polycyclic aromatic hydrocarbons are formed during broiling and grilling of meat. Acrylamide, a suspected human carcinogen, has been found in fried foods at low concentrations. A number of occupations associated with the development of specific cancers are listed in Table 8–8. A number of medical therapeutic and diagnostic tools have also been linked to the induction of human cancer (Table 8–9). Therapeutic immunosuppression given to transplant patients or arising secondary to selective diseases such as acquired immune deficiency syndrome (AIDS) result in an increase in a variety of different neoplasms. These results further support the role of the immune system in identifying and removing early preneoplastic cells from the body.

TABLE 8–9 Human carcinogenic chemicals associated with medical therapy and diagnosis.

Chemical or Drug	Associated Neoplasms
Alkylating agents (cyclophosphamide, melphalan)	Bladder, leukemia
Azathioprine	Lymphoma, reticulum cell sarcoma, skin, Kaposi's sarcoma (?)
Chloramphenicol	Leukemia
Diethylstilbestrol	Vagina (clear cell carcinoma)
Estrogens	Liver cell adenoma, endometrium, skin
Phenacetin	Renal pelvis (carcinoma)
Phenytoin	Lymphoma, neuroblastoma
Thorotrast	Liver (angiosarcoma)

TABLE 8–10 IARC classification of the evaluation of carcinogenicity for human beings.

Group	Evidence
1. Agent is carcinogenic to humans	Human data strong Animal data strong
2A. Agent is probably carcinogenic to humans	Human epidemiology data suggestive Animal data positive
2B. Agent is possibly carcinogenic to humans	Human epidemiology data weak Animal data positive
3. Agent is not classifiable as to carcinogenicity to humans	Human and animal data inadequate
4. Agent is probably not carcinogenic to humans	Human and animal data negative

Classification Evaluation of Carcinogenicity in Humans

The assessment and designation of a chemical or a mixture of chemicals as carcinogenic in humans is evaluated by various agencies worldwide. The evaluation usually encompasses both epidemiological and experimental animal and in vitro data utilizing assays as described earlier in this chapter. One of the first devised schemes for the classification of an agent's carcinogenicity was devised by the International Agency for Research on Cancer (IARC) (Table 8–10). The IARC approach assigns the chemical or mixture to one of five groupings based on strength of evidence for the agent's possible, probable, or definite carcinogenicity to humans. Similar classifications exist for the U.S. EPA, the U.S. Food and Drug Administration, and the European Community (EC). The classification of agents with regard to human carcinogenicity can be very difficult, in particular when animal data and/or epidemiological data in humans are inconclusive or confounded.

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QUESTIONS

1. T ere is evidence that certain dietary components are carcinogenic. Which of the following is NOT tabbed as a dietary carcinogen?
   a. excessive caloric intake.
   b. excessive alcohol consumption.
   c. aflatoxin B1 (a food contaminant).
   d. insufficient caloric intake.
   e. nitrites (found in some lunchmeats).

2. Which of the following statements regarding mechanisms of chemical carcinogenesis is FALSE?
   a. Procarcinogens require metabolism in order to exert their carcinogenic effect.
   b. Free radicals are highly reactive molecules that have a single, unpaired electron.
   c. DNA adducts interfere with the DNA replication machinery.
   d. Mutations in the DNA and failure to repair those mutations can be highly carcinogenic.
   e. Biological reduction of molecular oxygen is the only way free radicals can be formed.

3. In addition to being necessary for transcription to occur, which of the following transcription factors also plays a crucial role in nucleotide excision repair?
   a. TFIIA.
   b. TFIIB.
   c. TFIID.
   d. TFIIF.
   e. TFIIH.

4. Which of the following statements regarding DNA repair is true?
   a. Base excision repair requires the removal of a longer piece of DNA in comparison with nucleotide excision repair.
   b. T e repair of double-stranded DNA breaks is more prone to error than is base excision repair.
   c. Dimerization of pyrimidines is repaired via base excision repair.
   d. Mismatch repair can only recognize normal nucleotides that are paired with a noncomplementary nucleotide.
   e. Nucleotide excision and base excision are tolerance mechanisms used to respond to DNA damage.

5. Which of the following statements is a characteristic of the initiation stage of carcinogenesis?
   a. Initiation is reversible in viable cells.
   b. T e dose–response exhibits an easily measurable threshold.
   c. Cell division is required for the fixation of the process.
   d. All initiated cells survive over the lifespan of the organism.
   e. Spontaneous initiation of cells is a rare occurrence.

6. Tumor suppressor genes are mutated in a majority of cancers. Which of the following is NOT a characteristic of a tumor suppressor gene?
   a. A mutation in a tumor suppressor gene is dominant.
   b. Germ line inheritance of a mutated tumor suppressor gene is of en involved with cancer development.
   c. T ere is considerable tissue specificity for cancer development.
   d. T e p53 gene is a tumor suppressor gene that also acts as a transcription factor.
   e. Mutations in tumor suppressor genes can result in loss of cell cycle control.

7. Which of the following molecules does NOT play an important role in cell cycle regulation?
   a. p53.
   b. cyclin-D.
   c. MAPK.
   d. MHC.
   e. E2F.

8. Which of the following environmental factors is proportionally responsible for the LEAST amount of cancer deaths?
   a. tobacco.
   b. infection.
   c. diet.
   d. sexual behavior.
   e. alcohol.

9. The evidence of the carcinogenicity of dietary intake is sufficient to include one's diet as associated with neoplasms of all of the following EXCEPT:
   a. colon.
   b. breast.
   c. pancreas.
   d. endometrium.
   e. gallbladder.

10. Which of the following is the correct definition of a complete carcinogen?
    a. a chemical capable only of initiating cells.
    b. a chemical possessing the ability of inducing cancer from normal cells, usually possessing properties of initiating, promoting, and progression agents.
    c. a chemical capable of converting an initiated cell or a cell in the stage of promotion to a potentially malignant cell.
    d. a chemical capable of causing the expansion of initiated cell clones.
    e. a chemical that will cause cancer 100% of the time that it is administered.

CHAPTER

9

Genetic Toxicology

R. Julian Preston and George R. Hof mann

HEALTH IMPACT OF GENETIC ALTERATIONS

Somatic Cells
Germ Cells

CANCER AND GENETIC RISK ASSESSMENTS

Cancer Risk Assessment
Genetic Risk Assessment

MECHANISMS OF INDUCTION OF GENETIC ALTERATIONS

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Ionizing Radiations
Ultraviolet Light
Chemicals
Endogenous Agents
DNA Repair
Base Excision Repair
Nucleotide Excision Repair
Double-strand Break Repair
Mismatch Repair
O^6-Methylguanine-DNA Methyltransferase Repair
Formation of Gene Mutations
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Formation of Chromosomal Alterations
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ASSAYS FOR DETECTING GENETIC ALTERATIONS

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Micronuclei
Sister Chromatid Exchange
Aneuploidy
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HUMAN POPULATION MONITORING

NEW APPROACHES FOR GENETIC TOXICOLOGY

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CONCLUSION

KEY POINTS

- Genetic toxicology assesses the effects of chemical and physical agents on the hereditary material (DNA) and on the genetic processes of living cells.
- Oncogenes are genes that stimulate the transformation of normal cells into cancer cells.
- Genetic toxicology assays serve to identify mutagens for purposes of hazard identification, and to characterize dose–response relationships and mutagenic mechanisms.
- A broad range of short-term assays for genetic toxicology serve to identify many mutagens and address the relationship between mutagens and cancer-causing agents.

Genetic toxicology assesses the effects of chemical and physical agents on both DNA and on the genetic processes of living cells. T is chapter addresses the assays for qualitative and quantitative assessment of cellular changes induced by chemical and physical agents, the underlying molecular mechanisms for these changes, and how such information can be incorporated in risk assessments.

HEALTH IMPACT OF GENETIC ALTERATIONS

Somatic Cells

Mutational alteration of proto-oncogenes can lead to overexpression of their growth-stimulating activity, whereas mutations that inactivate tumor-suppressor genes, which normally restrain cellular proliferation, free cells from their inhibitory influence. The action of oncogenes is genetically dominant in that a single active oncogene is expressed even though its normal allele is present in the same cell. Among chromosomal alterations that activate proto-oncogenes, translocations are especially prevalent. A translocation can activate a proto-oncogene by moving it to a new chromosomal location with a more active promoter, where its expression is enhanced. Unlike oncogenes, the cancer-causing alleles that arise from tumor-suppressor genes are typically recessive in that they are not expressed when they are heterozygous.

Six acquired characteristics are essential for the formation of all tumors irrespective of tumor type and species. T ese include (1) self-sufficiency in growth signals, (2) insensitivity to antigrowth signals, (3) evasion of apoptosis, (4) limitless replicative potential, (5) sustained angiogenesis, and (6) tissue invasion and metastasis. It seems probable that there is no specific order for obtaining these characteristics.

Gene mutations, chromosome aberrations (morphologic abnormality), and aneuploidy (abnormal number of chromosomes) are all implicated in the development of cancer. Many mutagens and clastogens (chromosome-breaking agents) contribute to carcinogenesis as initiators; however, mutagens, clastogens, and aneugens also may contribute to multiple genetic alterations.

Germ Cells

T e relevance of gene mutations to health is evident from the many disorders often caused by base-pair substitutions or small deletions that are inherited as simple Mendelian characteristics. Many genetic disorders (e.g., cystic fibrosis, phenylketonuria, and Tay–Sachs disease) are caused by the expression of recessive mutations. T ese mutations are mainly inherited from previous generations and are expressed when an individual inherits the mutant gene from both parents.

Besides causing diseases that exhibit Mendelian inheritance, gene mutations undoubtedly contribute to human disease through the genetic component of disorders with a complex etiology such as heart disease, hypertension, and diabetes. Refined cytogenetic methods have led to the discovery of minor variations in chromosome structure that have no apparent effect. Nevertheless, other chromosome aberrations cause fetal death or serious abnormalities. Aneuploidy also contributes to fetal deaths and causes disorders such as Down syndrome. Much of the effect of chromosomal abnormalities occurs prenatally. Among the abnormalities, aneuploidy is the most common, followed by polyploidy. Structural aberrations constitute about 5% of the total. Most chromosomal anomalies detected in newborns arise de novo in the germ cells of the parents.

CANCER AND GENETIC RISK ASSESSMENTS

Cancer Risk Assessment

Cancer risk assessment involves investigation of sensitivity of different species and subpopulations to tumor induction by a chemical and development of a dose–response curve of mutations to a chemical.

Genetic Risk Assessment

To investigate genetic risk, the frequency of genetic alteration in human germ cells is estimated by extrapolation from data from rodent germ cells and somatic cells. For a complete estimate of genetic risk, it is necessary to obtain an estimate of the frequency of genetic alterations transmitted to the offspring (Figure 9–1).

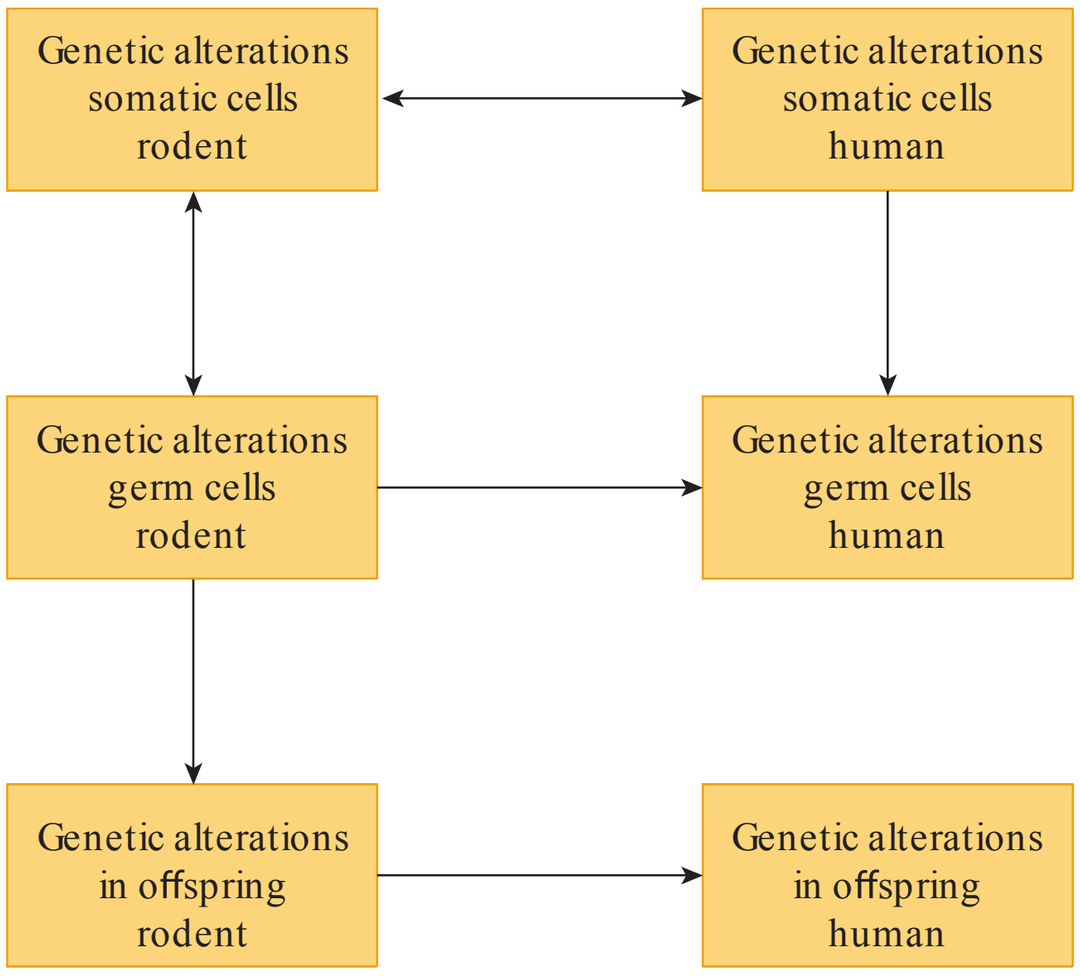

FIGURE 9–1 **Parallelogram approach for genetic risk assessment.** Data obtained for genetic alterations in rodent somatic and germ cells and human somatic cells are used to estimate the frequency of the same genetic alterations in human germ cells. The final step is to estimate the frequency of these genetic alterations that are transmitted to offspring.

MECHANISMS OF INDUCTION OF GENETIC ALTERATIONS

DNA Damage

The types of DNA damage range from single- and double-strand breaks in the DNA backbone to cross-links between DNA bases and between DNA bases and proteins and chemical addition to the DNA bases (adducts) (Figure 9–2).

Ionizing Radiations—Ionizing radiations such as x-rays, γ-rays, and α particles produce DNA single- and double-strand breaks and a broad range of base damages from oxidative processes. Multiple damaged sites or cluster lesions appear to be more difficult to repair. These multiple lesions can be formed in DNA from the same radiation energy deposition event. The relative proportions of these different classes of DNA damage vary with type of radiation.

Ultraviolet Light—Ultraviolet light (a nonionizing radiation) induces two predominant lesions, cyclobutane pyrimidine dimers and 6,4-photoproducts. These lesions can be quantitated by chemical and immunologic methods.

Chemicals—Chemicals can produce DNA alterations either directly (DNA-reactive) as adducts or indirectly by intercalation of a chemical between the base pairs. Many electrophilic chemicals react with DNA, forming covalent addition products (adducts). Alkylated bases can also lead to base loss from DNA, which leaves an apurinic or apyrimidinic site, commonly called an AP site. The insertion of incorrect bases into AP sites causes mutations. Bulky DNA adducts are recognized by the cell in a similar way to UV damages and are also repaired similarly. Such adducts can also hinder polymerases and cause mutation as a consequence of errors that they trigger in replication.

Endogenous Agents—Endogenous agents are responsible for several hundred DNA damages per cell per day. The majority of these damages are altered DNA bases (e.g., 8-oxoguanine and thymine glycol) and AP sites. The cellular processes that can lead to DNA damage are the formation of reactive active oxygen species and deamination of cytosines and S-methylcytosines leading to uracils and thymines, respectively. The process of DNA replication itself is error-prone, and an incorrect base can be added by the polymerase.

DNA Repair

Two processes enable the cell to cope with the DNA damage that it sustains. With extensive damage, the cell can undergo apoptosis. If the damage is less severe, cells have developed a range of repair processes that return the DNA to its undamaged state (error-free repair) or to an improved but still altered state (error-prone repair). The basic principles underlying most repair processes (but not translesion synthesis) are damage recognition, followed by either direct reversal of the damage (e.g., sealing of strand breaks or cleavage of pyrimidine dimers) or removal of the damage, repair DNA synthesis, and ligation.

Base Excision Repair—The major pathways by which DNA base damages are repaired involve removal of the damaged base. The resulting gap can be filled by a DNA polymerase, followed by ligation to the parental DNA. Sites of oxidative damage, either background or induced, are important substrates for base excision repair.

Nucleotide Excision Repair—The nucleotide excision repair (NER) system provides the cell's ability to remove bulky lesions from DNA. NER removes a damage-containing oligonucleotide from DNA by damage recognition, incision, excision, repair synthesis, and ligation. The DNA damage in actively transcribing genes, and specifically the transcribed strand, is preferentially and more rapidly repaired than the DNA damage in the rest of the genome. Thus, the cell protects the integrity of the transcription process.

Double-strand Break Repair—Cell survival is seriously compromised by the presence of broken chromosomes. Unrepaired double-strand breaks trigger one or more DNA damage response systems to either check cell-cycle progression or induce apoptosis. There are two general pathways for repair of DNA double-strand breaks: homologous recombination and nonhomologous end-joining (NHEJ).

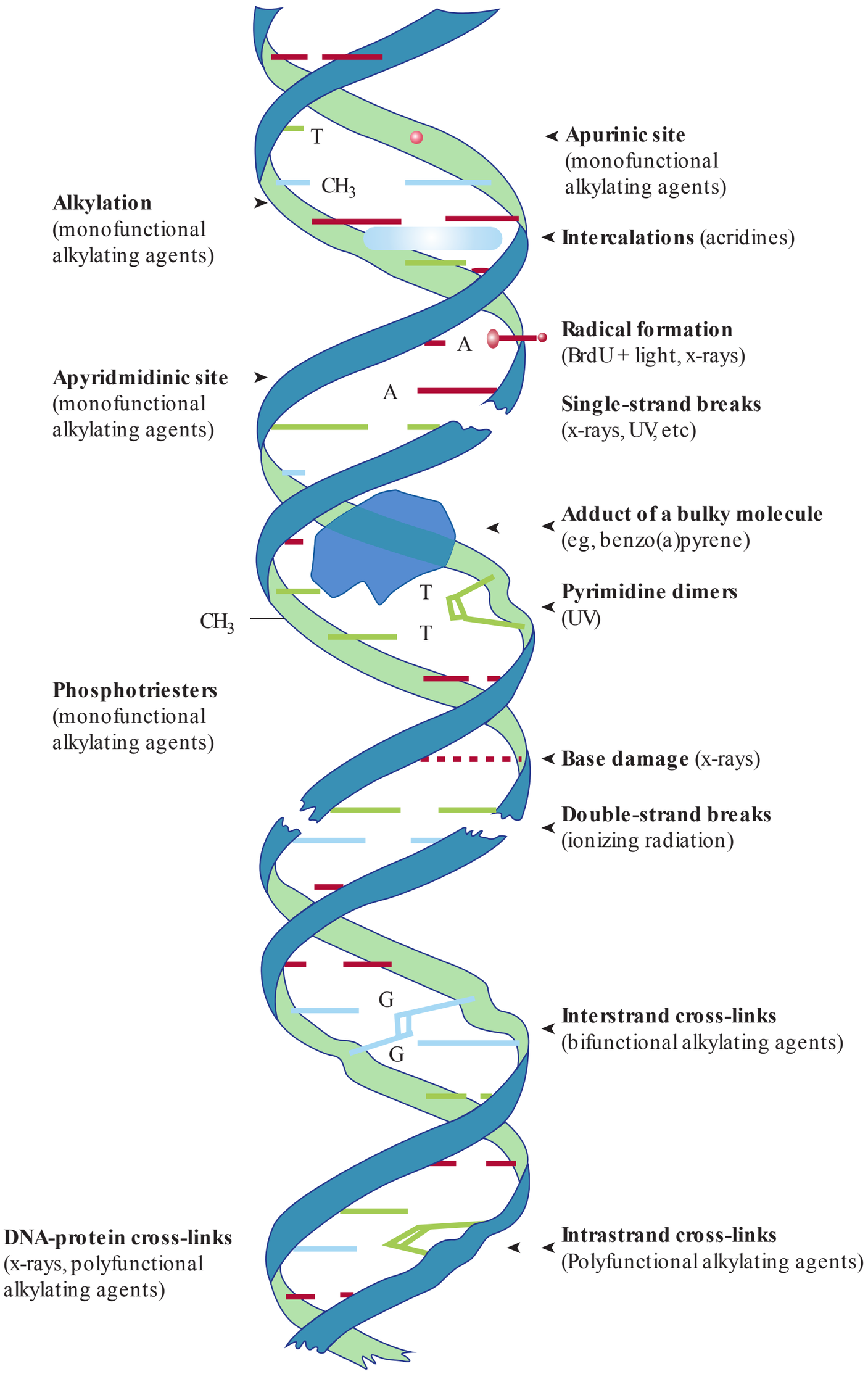

FIGURE 9–2 **Spectrum of DNA damage induced by physical and chemical agents.**

Homologous Recombination—The repair of double-strand breaks (and single-strand gaps) uses the following basic steps. The initial step is the production of a 3′-ended single-stranded tail by exonucleases or helicase activity. Through strand invasion, whereby the single-stranded tail invades an undamaged homologous DNA molecule, together with DNA synthesis, a so-called Holliday junction DNA complex is formed. By cleavage of this junction, two DNA molecules are produced (with or without a structural crossover), neither of which now contain a strand break.

Nonhomologous End-joining—This type of recombination requires the production of double-strand breaks, recombination of DNA pieces, and subsequent religation. A major component of the NHEJ repair complex is a DNA-dependent protein kinase (DNA-PK). This protein probably functions to align the broken DNA ends to facilitate their ligation or to serve as a signal molecule for recruiting other repair proteins.

Mismatch Repair—The principal steps of DNA mismatch repair are damage recognition by a specific protein that binds to

the mismatch, stabilization of the binding by the addition of one or more proteins, cutting the DNA at a distance from the mismatch, excision past the mismatch, resynthesis, and ligation.

O^6-Methylguanine-DNA Methyltransferase Repair—The enzyme O^6-methylguanine-DNA methyltransferase (MGMT) protects cells against the toxic effects of simple alkylating agents by transferring the methyl group from O^6-methylguanine in DNA to a cysteine residue in MGMT. The adducted base is reverted to a normal one by the enzyme, which is itself inactivated by the reaction.

Formation of Gene Mutations

Somatic Cells—Gene mutations, considered to be small DNA-sequence changes confined to a single gene, are substitutions, small additions, and small deletions. Base substitutions are the replacement of the correct nucleotide by an incorrect one; they can be further subdivided as transitions, where the change is purine for purine or pyrimidine for pyrimidine, and transversions where the change is purine for pyrimidine or vice versa. Frameshift mutations are the addition or deletion of one or a few base pairs (not in multiples of 3) in protein-coding regions.

The great majority of so-called spontaneous (background) mutations arise from replication of an altered template. These DNA alterations are either the result of oxidative damage or produced from the deamination of 5-methyl cytosine to thymine at CpG sites resulting in G:C → A:T transitions. Mutations induced by ionizing radiations tend to be deletions ranging in size from a few bases to multilocus events.

Gene mutations produced by a majority of chemicals and nonionizing radiations are base substitutions, frameshifts, and small deletions. Of these mutations, most are produced by errors of DNA replication on a damaged template. The relative mutation frequency will be the outcome of the race between repair and replication, that is, the more repair that takes place prior to replication, the lower the mutation frequency for a given amount of induced DNA damage. Significant regulators of the race are cell-cycle checkpoint genes (e.g., P53) because if the cell is checked from entering the S phase at a G_1/S checkpoint, then more repair can take place prior to the cell starting to replicate its DNA.

Germ Cells—The mechanism of production of gene mutations in germ cells is basically the same as in somatic cells. Ionizing radiations produce mainly deletions via errors of DNA repair; the majority of chemicals induce base substitutions, frameshifts, and small deletions by errors of DNA replication.

An important consideration for assessing gene mutations induced by chemicals in germ cells is the relationship between exposure and the timing of DNA replication (i.e., if there is damage, is it able to be repaired before replication?). The spermatogonial stem cell is the major contributor to genetic risk assessment because it is present generally throughout the reproductive lifetime of an individual. Each time a spermatogonial stem cell divides, it produces a differentiating spermatogonium and a stem cell. This stem cell can accumulate genetic damage from chronic exposures.

In oogenesis, the primary oocyte arrests prior to birth, and there is no further S phase until the zygote. For this reason, the oocyte is resistant to the induction of gene mutations by most chemicals.

Formation of Chromosomal Alterations

Somatic Cells

Structural Chromosome Aberrations—There are common components between the formation of chromosome aberrations, sister chromatid exchanges (SCEs; the apparently reciprocal exchange between the sister chromatids of a single chromosome), and gene mutations. In particular, damaged DNA serves as the substrate leading to chromosomal aberrations. However, chromosome aberrations induced by ionizing radiations are generally formed by errors of DNA repair, whereas those produced by nonradiomimetic chemicals are generally formed by errors of DNA replication on a damaged DNA template.

The DNA repair errors that lead to the formation of chromosome aberrations following ionizing radiation exposure arise from misligation of double-strand breaks or interaction of coincidentally repairing regions during nucleotide excision repair of damaged bases. Incorrect rejoining of chromosomal pieces during repair leads to chromosomal exchanges within and between chromosomes. Failure to rejoin double-strand breaks or to complete repair of other types of DNA damage leads to terminal deletions.

The failure to incorporate an acentric fragment into a daughter nucleus at anaphase/telophase, or the failure of a whole chromosome to segregate to the cellular poles at anaphase, can result in the formation of a micronucleus that resides in the cytoplasm. Errors of DNA replication on a damaged template can lead to a variety of chromosomal alterations. The majority of these involve deletion or exchanges of individual chromatids but some can involve both chromatids.

Numerical Chromosome Changes—Numerical changes (e.g., monosomies, trisomies, and ploidy changes) can arise from errors in chromosomal segregation due to any of the numerous possible impairments of mitotic control processes. Alteration of various cellular components can result in failure to segregate the sister chromatids to separate daughter cells or in failure to segregate a chromosome to either pole.

Sister Chromatid Exchange—SCEs are produced during S phase and are presumed to be a consequence of errors in the replication process.

Germ Cells—The formation of chromosomal alterations in germ cells is basically the same as that for somatic cells, namely, via misrepair for ionizing radiations and radiomimetic chemicals for treatments in G_1 and G_2, and by errors of replication for all radiations and chemicals for DNA damage present during the S phase.

The types of aberrations formed in germ cells are the same as those formed in somatic cells. The specific segregation of chromosomes during meiosis influences the probability of recovery of an aberration, particularly a reciprocal translocation, in the offspring of a treated parent.

ASSAYS FOR DETECTING GENETIC ALTERATIONS

Introduction to Assay Design

Genetic toxicology assays serve two interrelated but distinct purposes in the toxicologic evaluation of chemicals: (1) identifying mutagens for purposes of hazard identification and (2) characterizing dose–response relationships and mutagenic mechanisms.

Table 9–1 lists many of the assays employed in genetic toxicology. Some assays for gene mutations detect forward mutations, whereas others detect reversion. Forward mutations are genetic alterations in a wild-type gene and are detected by a change in phenotype caused by the alteration or loss of gene function. In contrast, a back mutation or reversion is a mutation that restores gene function in a mutant and thereby brings about a return to the wild-type phenotype. The simplest gene mutation assays rely on selection techniques to detect mutations. By imposing experimental conditions under which only cells or organisms that have undergone mutation can grow, selection techniques greatly facilitate the identification of rare cells that have experienced mutation among the many cells that have not.

Studying mutagenesis in intact animals requires more complex assays, which range from inexpensive short-term tests that can be performed in a few days to complicated assays for mutations in mammalian germ cells. Typically, there remains a gradation in which an increase in relevance for human risk entails more elaborate and costly tests.

Many compounds that are not themselves mutagenic or carcinogenic can be activated into mutagens and carcinogens by mammalian metabolism. Such compounds are called promutagens and procarcinogens. The most widely used metabolic activation system in microbial and cell culture assays is a postmitochondrial supernatant from a rat liver homogenate, along with appropriate buffers and cofactors. Most of the short-term assays in Table 9–1 require exogenous metabolic activation to detect promutagens. Exceptions are those in intact mammals.

Despite their usefulness, in vitro metabolic activation systems cannot mimic mammalian metabolism perfectly. There are differences among tissues in reactions that activate or inactivate foreign compounds, and organisms of the normal flora of the gut can contribute to metabolism in intact mammals. Agents that induce enzyme systems or otherwise alter the physiological state can also modify the metabolism of toxicants, and the balance between activation and detoxication reactions in vitro may differ from that in vivo.

Structural Alerts and In Silico Assays

The first indication that a chemical is a mutagen often lies in chemical structure. Potential electrophilic sites in a molecule serve as an alert to possible mutagenicity and carcinogenicity because such sites confer reactivity with nucleophilic sites in DNA. Developmental work to formalize the structural prediction through automated computer programs has not yet led to an ability to predict mutagenicity and carcinogenicity of new chemicals with great accuracy. These computer-based systems for predicting genotoxicity based on chemical properties are sometimes called in silico assays. These assays include computational and structural programs and the modeling of quantitative structure–activity relationships. Although there is much skepticism that such approaches can replace biological testing, they hold promise of improving the efficiency of testing strategies and reducing current levels of animal use.

DNA Damage and Repair Assays

Some assays measure DNA damage itself rather than mutational consequences of DNA damage. They may do so directly, through such indicators as chemical adducts or strand breaks in DNA, or indirectly, through measurement of biological repair processes. Adducts in DNA can be detected by ^{32}P-postlabeling, high-performance liquid chromatography (HPLC), fluorescence-based methods, mass spectrometry, immunological methods using antibodies against specific adducts, isotope-labeled DNA binding, and electrochemical detection.

A rapid method of measuring DNA damage is the comet assay. In this assay, cells are incorporated into agarose on slides, lysed so as to liberate their DNA, and subjected to electrophoresis. The DNA is stained with a fluorescent dye for observation and image analysis. Because broken DNA fragments migrate more quickly than larger pieces of DNA, a blur of fragments (a "comet") is observed when the DNA is extensively damaged. The extent of DNA damage can be estimated from the length and other attributes of the comet tail. The comet assay appears to be a sensitive indicator of DNA damage with broad applicability among diverse species, including plants, worms, mollusks, fish, and amphibians.

The occurrence of DNA repair can serve as a readily measured indicator of DNA damage. A common excision repair assay in mammalian cells measures unscheduled DNA synthesis (UDS). The occurrence of UDS indicates that the DNA had been damaged. The absence of UDS, however, does not provide evidence that DNA has not been damaged because some classes of damage are not readily excised, and some excisable damage may not be detected as a consequence of assay insensitivity.

Gene Mutations in Prokaryotes

The most common means of detecting mutations in microorganisms is selecting for reversion in strains that have a specific nutritional requirement differing from wild-type members of

TABLE 9–1 Overview of genetic toxicology assays.

Assays

I. Prediction of genotoxicity
  A. Interpretation of chemical structure
    Structural alerts to genotoxicity
  B. In silico predictive models
    Computational and structural programs: MCASE, TOPKAT, DEREK
    Quantitative structure–activity relationship (QSAR) modeling

II. DNA damage and repair assays
  A. Direct detection of DNA damage
    Alkaline elution assays for DNA strand breakage in hepatocytes
    Comet assay (single-cell gel electrophoresis) for DNA strand breakage
    Comet-FISH assay for region-specific DNA damage and repair
    Nonmammalian comets in ecotoxicology
    Assays for chemical adducts in DNA
  B. DNA repair, recombination, and genotoxic stress responses as indicators of damage
    Differential killing of repair-deficient and wild-type bacteria
    Induction of the bacterial SOS system
    "Green Screen" for GADD45a gene induction in TK6 human cells
    Unscheduled DNA synthesis (UDS) in isolated rat hepatocytes or rodents in vivo
    Induction of mitotic recombination

III. Prokaryote gene mutation assays
  A. Bacterial reverse mutation assays
    Salmonella/mammalian microsome assay (Ames test)
    E. coli WP2 tryptophan reversion assay
    Salmonella-specific base-pair substitution assay (Ames II assay)
    E. coli lacZ-specific reversion assay
  B. Bacterial forward mutation assays
    E. coli lacI assay
    Resistance to toxic metabolites or analogs in Salmonella

IV. Assays in nonmammalian eukaryotes
  A. Fungal assays
    Forward mutations, reversion, and small deletions
    Mitotic crossing over, gene conversion, and homology-mediated deletions in yeast
    Genetic detection of mitotic and meiotic aneuploidy in yeast
  B. Plant assays
    Gene mutations affecting chlorophyll in seedlings, the waxy locus in pollen, or Tradescantia stamen hair color
    Chromosome aberrations and micronuclei in mitotic and meiotic cells of corn, Tradescantia, and other plants
  C. Drosophila assays
    Sex-linked recessive lethal test in germ cells
    Heritable translocation assays
    Mitotic recombination and LOH in eyes or wings

V. Mammalian gene mutation assays
  A. In vitro assays for forward mutations
    tk mutations in mouse lymphoma or human cells
    hprt or xprt mutations in Chinese hamster or human cells
    CD59 mutations in CHO-human hybrid AL cells
  B. In vivo assays for gene mutations in somatic cells
    Mouse spot test (somatic cell specific-locus test)
    hprt mutations (6-thioguanine-resistance) in rodent lymphocytes
    Pig-a mutations (immunological detection of mutations blocking glycosylphosphatidylinositol synthesis)
  C. Transgenic assays
    Mutations in the bacterial lacI gene in "Big Blue" mice and rats
    Mutations in the bacterial lacZ gene in the "Muta Mouse"
    Mutations in the phage cII gene in lacI or lacZ transgenic mice
    Point mutations and deletions in the lacZ plasmid mouse
    Point mutations and deletions in delta gpt mice and rats
    Forward mutations and reversions in ΦX174 transgenic mice
    Inversions and deletions arising in pKZ1 mice by intrachromosomal recombination

VI. Mammalian cytogenetic assays
  A. Chromosome aberrations
    Metaphase analysis in cultured Chinese hamster or human cells
    Metaphase analysis of rodent bone marrow or lymphocytes in vivo
    Chromosome painting and other FISH applications in vitro and in vivo
  B. Micronuclei
    Cytokinesis-block micronucleus assay in human lymphocytes
    Micronucleus assay in mammalian cell lines
    In vivo micronucleus assay in rodent bone marrow or blood
    In vivo micronucleus assay in tissues other than marrow or blood
  C. Sister chromatid exchange
    SCE in human cells or Chinese hamster cells
    SCE in rodent tissues, especially bone marrow
  D. Aneuploidy in mitotic cells
    Hyperploidy detected by chromosome counting or FISH in cell cultures or bone marrow
    Micronucleus assay with centromere/kinetochore labeling in cell cultures
    Altered parameters in flow-cytometric detection of micronuclei in CHO cells
    Mouse bone marrow micronucleus assay with centromere labeling

VII. Germ cell mutagenesis
  A. Measurement of DNA damage
    Molecular dosimetry based on mutagen adducts in reproductive cells
    UDS in rodent germ cells
    Alkaline elution assays for DNA strand breaks in rodent testes
    Comet assay in sperm and gonadal tissue
  B. Gene mutations
    Mouse specific-locus test for gene mutations and deletions
    Mouse electrophoretic specific-locus test
    Dominant mutations causing mouse skeletal defects or cataracts
    ESTR assay in mice
    Germ cell mutations in transgenic assays
  C. Chromosomal aberrations
    Cytogenetic analysis of oocytes, spermatogonia, spermatocytes, or zygotes
    Direct detection in sperm by FISH
    Micronuclei in mouse spermatids
    Mouse heritable translocation test
  D. Dominant lethal mutations
    Mouse or rat dominant lethal assay
  E. Aneuploidy
    Cytogenetic analysis for aneuploidy arising by nondisjunction
    Sex chromosome loss test for nondisjunction or breakage
    Micronucleus assay in spermatids with centromere labeling
    FISH with probes for specific chromosomes in sperm

the species; such strains are called auxotrophs. In the Ames assay, one measures the frequency of histidine-independent bacteria that arise in a histidine-requiring strain in the presence or absence of the chemical being tested. Auxotrophic (nutrient-deficient) bacteria are treated with the chemical of interest and plated on medium that is deficient in histidine; if the colony survives, it must have a reversion mutation that allows it to survive without exogenous histidine.

The development of specific reversion assays of histidine mutations in Salmonella strains and of lacZ mutations in Escherichia coli has made the identification of specific base-pair substitutions more straightforward. Bacterial forward mutation assays, such as selections for resistance to arabinose or to purine or pyrimidine analogs in Salmonella, are also used in research and testing, although less extensively than reversion assays.

Genetic Alterations in Nonmammalian Eukaryotes

Gene Mutations and Chromosome Aberrations—The fruit fly, Drosophila, has long occupied a prominent place in genetic research because of the sex-linked recessive lethal (SLRL) test. The SLRL test permits the detection of recessive lethal mutations at 600 to 800 different loci on the X chromosome by screening for the presence or absence of wild-type males in the offspring of specifically designed crosses. A significant increase over the frequency of spontaneous SLRLs in the lineages derived from treated males indicates mutagenesis. The SLRL test yields information about mutagenesis in germ cells, which is lacking in microbial and cell culture systems.

Genetic and cytogenetic assays in plants continue to find use in special applications, such as in situ monitoring for mutagens and exploration of the metabolism of promutagens by agricultural plants. In situ monitoring entails looking for evidence of mutagenesis in organisms that are grown in the environment of interest.

Mitotic Recombination—Assays in nonmammalian eukaryotes are important for the study of induced recombination. Recombinogenic effects in yeast have long been used as a general indicator of genetic damage. The best characterized assays for recombinogens are those that detect mitotic crossing over and mitotic gene conversion in the yeast Saccharomyces cerevisiae.

Gene Mutations in Mammals

Gene Mutations In Vitro—Mutagenicity assays in cultured mammalian cells have some of the same advantages as microbial assays with respect to speed and cost, and they follow quite similar approaches. The most widely used assays for gene mutations in mammalian cells detect forward mutations that confer resistance to a toxic chemical.

Gene Mutations In Vivo—In vivo assays involve treating intact animals and analyzing genetic effects in appropriate tissues. Mutations may be detected either in somatic cells or in germ cells.

The mouse spot test is a traditional genetic assay for gene mutations in somatic cells. Visible spots of altered phenotype in mice heterozygous for coat color genes indicate mutations in the progenitor cells of the altered regions.

Besides determining whether agents are mutagenic, mutation assays also provide information on mechanisms of mutagenesis. Base-pair substitutions and large deletions can be differentiated through the use of probes for the target gene and Southern blotting, in that base substitutions are too subtle to be detectable on the blots. Gene mutations have been characterized at the molecular level by DNA-sequence analysis both in transgenic rodents and in endogenous mammalian genes.

Transgenic Assays—Transgenic animals are products of DNA technology in which the animal contains foreign DNA sequences that have been added to the genome and are transmitted through the germ line. The foreign DNA is therefore represented in all the somatic cells of the animal.

Mice that carry lac genes from E. coli use either lacI or lacZ as a target for mutagenesis. After mutagenic treatment of the transgenic animals, the lac genes are recovered from the animal, packaged into phage λ, and transferred to E. coli for mutational analysis. Mutant plaques are identified on the basis of phenotype, and mutant frequencies can be calculated for different tissues of the treated animals.

Mammalian Cytogenetic Assays

Chromosome Aberrations—Genetic assays without DNA sequencing are indirect, in that one observes a phenotype and reaches conclusions about genes. In contrast, cytogenetic assays use microscopy for direct observation of the effect of interest. In conventional cytogenetics, metaphase analysis is used to detect chromosomal anomalies. Cells should be treated during a sensitive period of the cell cycle (typically S), and aberrations should be analyzed at the first mitotic division after treatment. Examples of chromosome aberrations are shown in Figure 9–3.

It is essential that sufficient cells be analyzed because a negative result in a small sample is equivocal and inconclusive. Results should be recorded for specific classes of aberrations, not just as an overall index of aberrations per cell.

In interpreting results on the induction of chromosome aberrations in cell cultures, questionable positive results have been found at highly cytotoxic doses, high osmolality, and pH extremes. Although excessively high doses may lead to artifactual positive responses, the failure to test sufficiently high doses also undermines the utility of a test; therefore, testing should be conducted at an intermediate dose and extended to a dose at which some cytotoxicity is observed.

In vivo assays for chromosome aberrations involve treating intact animals and later collecting cells for cytogenetic analysis. The main advantage of in vivo assays is that they

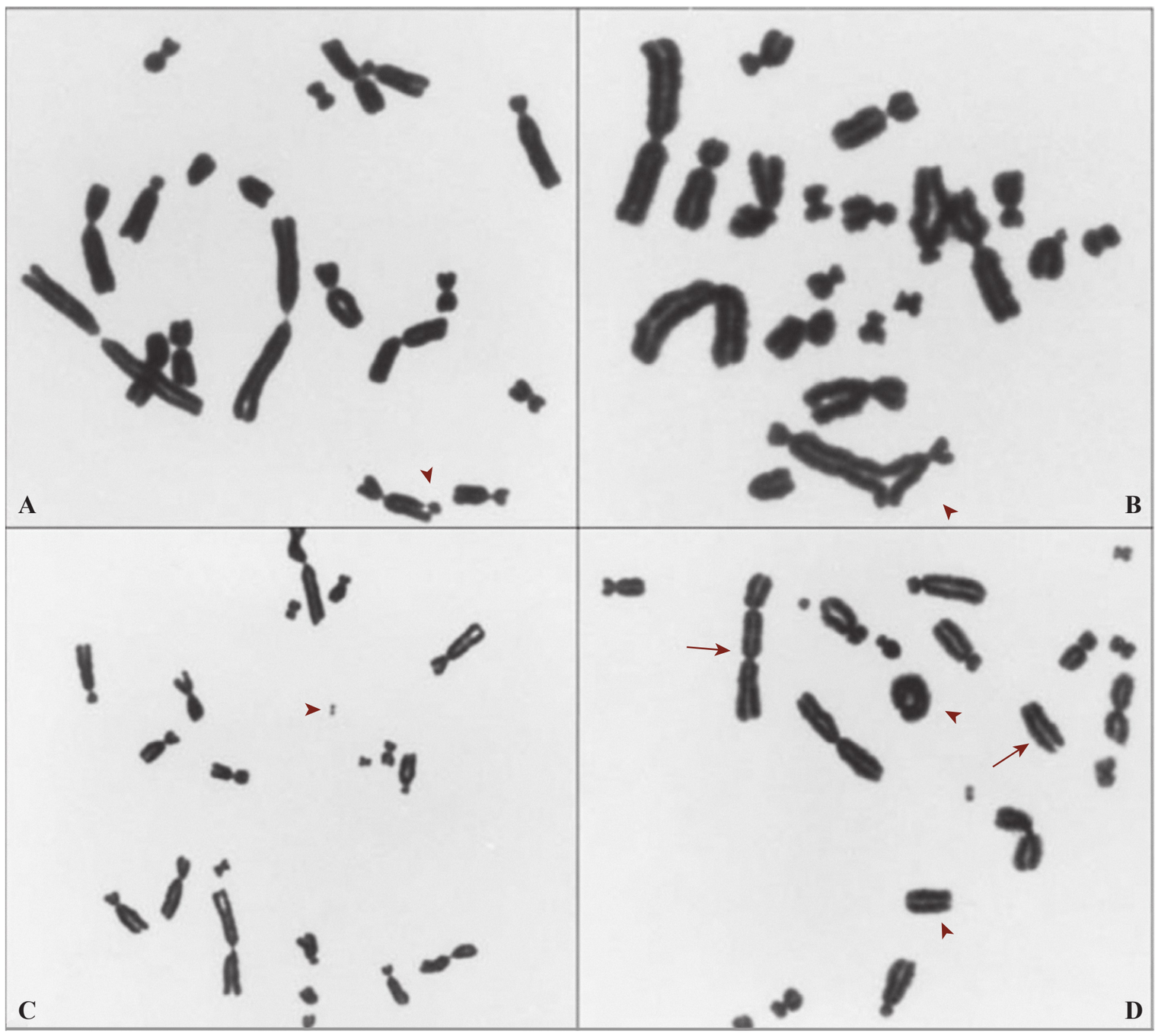

FIGURE 9–3 **Chromosome aberrations induced by x-rays in Chinese hamster ovary (CHO) cells.** A. A chromatid deletion (▶). B. A chromatid exchange called a triradial (▶). C. A small interstitial deletion (▶) that resulted from chromosome breakage. D. A metaphase with more than one aberration: a centric ring plus an acentric fragment (▶) and a dicentric chromosome plus an acentric fragment (→).

include mammalian metabolism, DNA repair, and pharmacodynamics. The target is a tissue from which large numbers of dividing cells are easily prepared for analysis such as bone marrow.

In interphase cell analysis by fluorescence in situ hybridization (FISH; Figure 9–4), a nucleic acid probe is hybridized to complementary sequences in chromosomal DNA. The probe is labeled with a fluorescent dye so that the chromosomal location to which it binds is visible by fluorescence microscopy; often, probes are used that cover the whole chromosome, called "chromosome painting."

Chromosome painting facilitates cytogenetic analysis, because aberrations are easily detected by the number of fluorescent regions in a painted metaphase. FISH permits the scoring of stable aberrations, such as translocations and insertions, which are not readily detected in traditional metaphase analysis of unbanded chromosomes.

Micronuclei—Micronuclei are membrane-bounded structures that contain chromosomal fragments, or sometimes whole chromosomes, that were not incorporated into a daughter nucleus at mitosis. Micronuclei usually represent acentric chromosomal fragments, and they are commonly used as simple indicators of chromosomal damage. Micronuclei in a binucleate human lymphocyte are shown in Figure 9–5.

FIGURE 9–4 **Chromosome aberrations identified by FISH.** Human breast cancer cell with aneuploidy for some chromosomes and with reciprocal translocations identified by color switches along a chromosome.

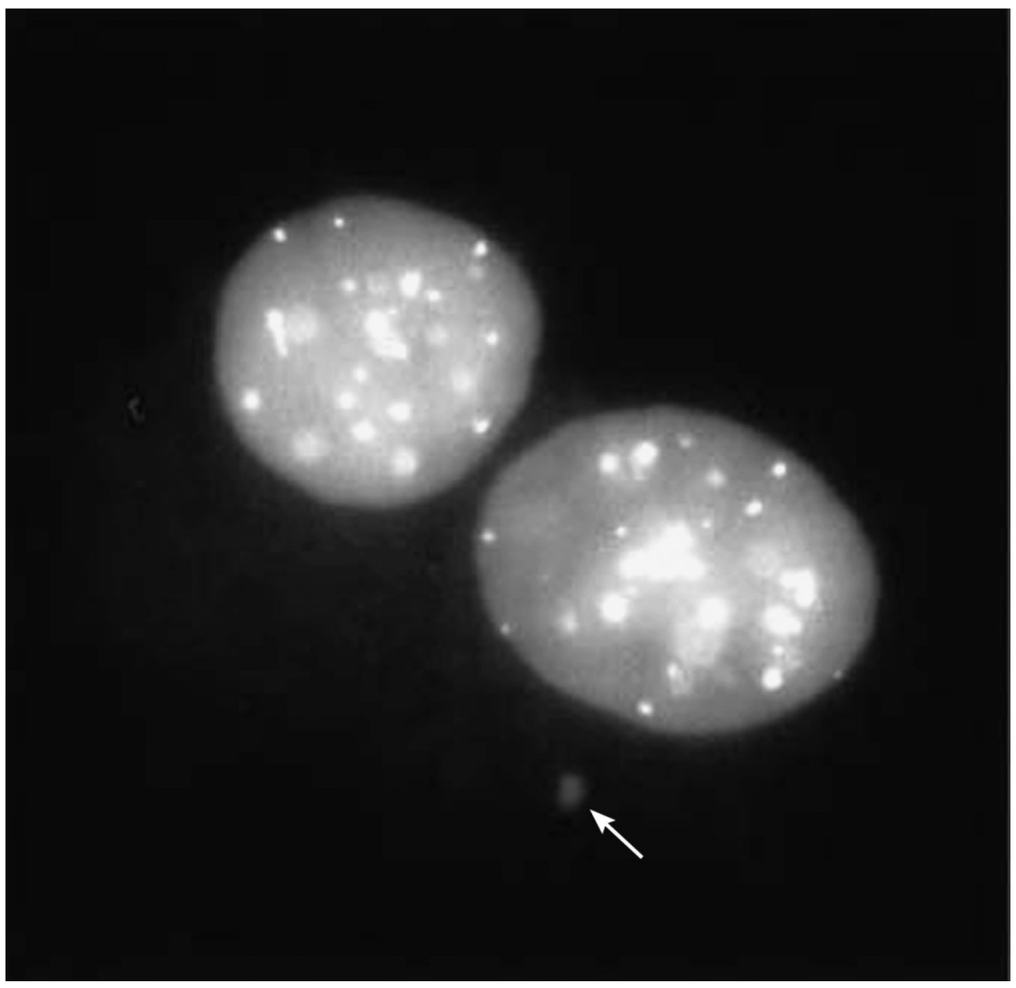

FIGURE 9–5 **Micronucleus in a human lymphocyte.** The cytochalasin B method was used to inhibit cytokinesis that resulted in a binucleate nucleus. The micronucleus (arrow) resulted from failure of an acentric chromosome fragment or a whole chromosome being included in a daughter nucleus following cell division. (Used with permission of James Allen, Jill Barnes, and Barbara Collins.)

Sister Chromatid Exchange—SCE, in which apparently reciprocal segments have been exchanged between the two chromatids of a chromosome, is visible cytologically through differential staining of chromatids (Figure 9–6). SCE assays are general indicators of mutagen exposure, rather than measures of a mutagenic effect.

Aneuploidy—Assays for aneuploidy include chromosome counting, the detection of micronuclei that contain kinetochores, and the observation of abnormal spindles or spindle–chromosome associations in cells in which spindles and chromosomes have been differentially stained.

The presence of the spindle attachment region of a chromosome (kinetochore) in a micronucleus can indicate that it contains a whole chromosome. Aneuploidy may therefore be detected by means of antikinetochore antibodies with a fluorescent label or FISH with a probe for centromere-specific DNA. Frequencies of micronuclei ascribable to aneuploidy and to clastogenic effects may therefore be determined concurrently by tabulating micronuclei with and without kinetochores.

Germ Cell Mutagenesis

Gene Mutations—Mammalian germ cell assays provide the best basis for assessing risks to human germ cells. Mammalian assays permit the measurement of mutagenesis at different germ cell stages. Late stages of spermatogenesis are often found to be sensitive to mutagenesis, but spermatocytes, spermatids, and spermatozoa are transitory. Mutagenesis in stem cell spermatogonia and resting oocytes is of special interest in genetic risk assessment because of the persistence of these stages throughout reproductive life.

Chromosomal Alterations—Knowledge of the induction of chromosome aberrations in germ cells is important for assessing risks to future generations. A germ cell micronucleus assay has been developed, in which chromosomal damage induced in meiosis is measured by observation of rodent spermatids. Aneuploidy originating in mammalian germ cells may be detected cytologically through chromosome counting for hyperploidy or genetically in the mouse sex-chromosome loss test.

Besides cytological observation, indirect evidence for chromosome aberrations is obtained in the mouse heritable translocation assay, which measures reduced fertility in the offspring of

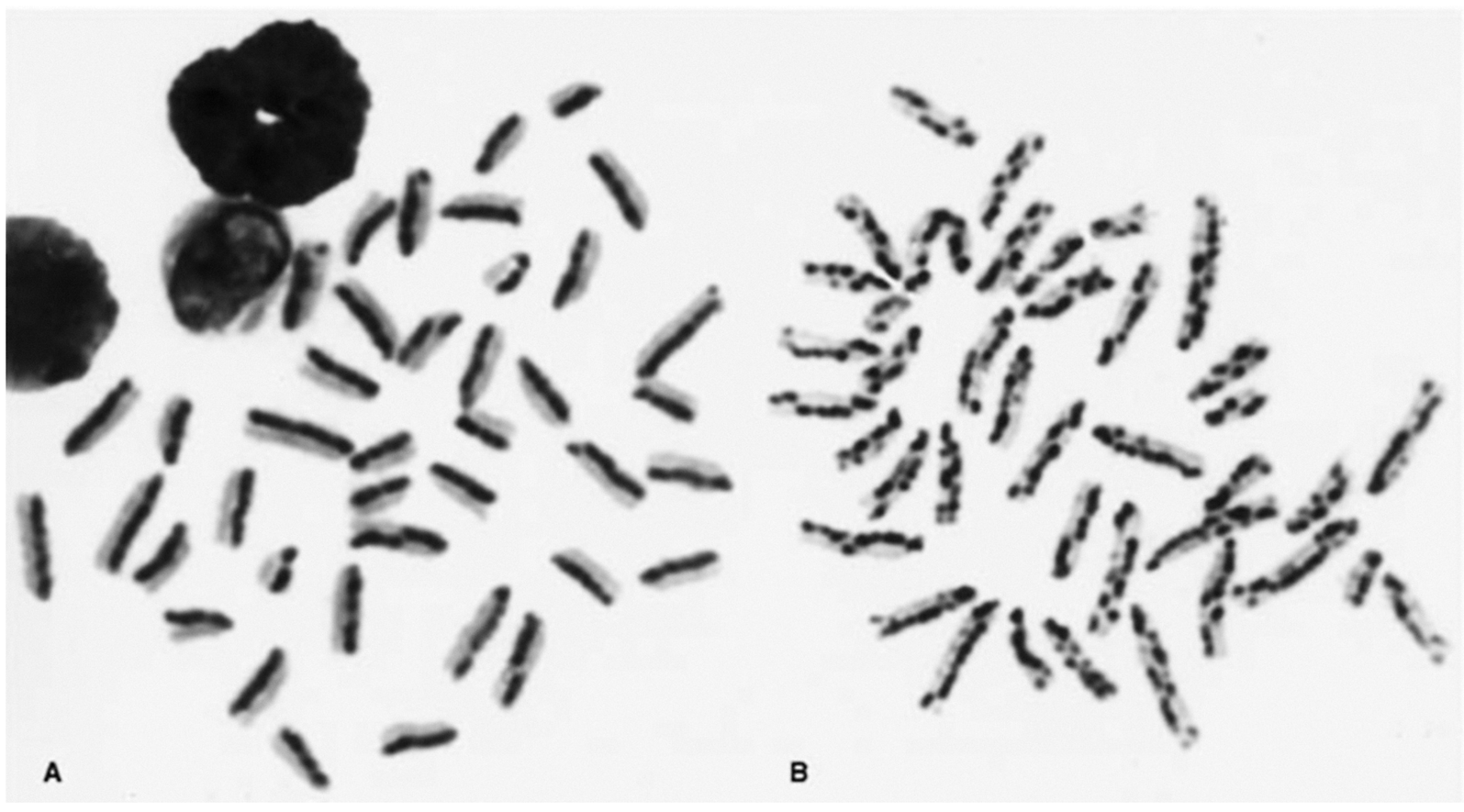

FIGURE 9–6 **Sister chromatid exchanges (SCEs) in human lymphocytes.** A. SCEs in untreated cell. B. SCEs in cell exposed to ethyl carbamate. The treatment results in a very large increase in the number of SCEs. (Used with permission of James Allen and Barbara Collins.)

treated males. T is presumptive evidence of chromosomal rearrangements can be confirmed through cytogenetic analysis.

Dominant Lethal Mutations—T e mouse or rat dominant lethal assay offers an extensive database on the induction of genetic damage in mammalian germ cells. Commonly, males are treated on an acute or subchronic basis with the agent of interest and then mated with virgin females. T e females are killed and necropsied during pregnancy so that embryonic mortality, assumed to be due to chromosomal anomalies, may be characterized and quantified.

Development of Testing Strategies

Concern about adverse effects of mutation on human health, principally carcinogenesis and the induction of transmissible damage in germ cells, has provided the impetus to identify environmental mutagens. Genetic toxicology assays may be used to screen chemicals to detect mutagens and to obtain information on mutagenic mechanisms and dose–responses that contribute to an evaluation of hazards. Besides testing pure chemicals, environmental samples are tested because many mutagens exist in complex mixtures. T e analysis of complex mixtures often requires a combination of mutagenicity assays and refined analytical methods.

Assessment of a chemical's genotoxicity requires data from well-characterized genetic assays. Sensitivity refers to the proportion of carcinogens that are positive in the assay, whereas specificity is the proportion of noncarcinogens that are negative. Sensitivity and specificity both contribute to the predictive reliability of an assay. Assays are said to be validated when they have been shown to perform reproducibly and reliably with many compounds from diverse chemical classes in several laboratories.

Rather than trying to assemble batteries of complementary assays, it is prudent to emphasize mechanistic considerations in choosing assays. Such an approach makes a sensitive assay for gene mutations (e.g., the Ames assay) and an assay for clastogenic effects in mammals pivotal in the evaluation of genotoxicity. Beyond gene mutations, one should evaluate damage at the chromosomal level with a mammalian in vitro or in vivo cytogenetic assay. Other assays offer an extensive database on chemical mutagenesis (Drosophila SLRL), a unique genetic end point (i.e., aneuploidy; mitotic recombination), applicability to diverse organisms and tissues (i.e., DNA damage assays, such as the comet assay), or special importance in the assessment of genetic risk (i.e., germ cell assays).

HUMAN POPULATION MONITORING

For cancer risk assessment considerations, the human data utilized most frequently, in the absence of epidemiologic data, are those collected from genotoxicity/mutagenicity assessments in human populations. T e studies conducted most frequently are for chromosome aberrations, micronuclei, and SCEs in peripheral lymphocytes.

T e size of each study group should be sufficiently large to avoid any confounder having undue inf uence. Certain characteristics should be matched among exposed and unexposed groups. T ese include age, sex, smoking status, and general dietary features. Study groups of 20 or more individuals can be used as a reasonable substitute for exact matching because confounders will be less inf uential on chromosome alteration or mutation frequency in larger groups. In some instances, it might be informative to compare exposed groups with a historical control, as well as to a concurrent control.

Reciprocal translocations are transmissible from cell generation to generation, and frequency can be representative of an accumulation over time of exposure. T e importance of this is that stable chromosome aberrations observed in peripheral lymphocytes exposed in vivo, but assessed following in vitro culture, are produced in vivo in hematopoietic stem cells or other precursor cells of the peripheral lymphocyte pool.

NEW APPROACHES FOR GENETIC TOXICOLOGY

T e ability to manipulate and characterize DNA, RNA, and proteins has been at the root of the advance in our understanding of basic cellular processes and how they can be perturbed. However, the development of sophisticated molecular biology does not in itself imply a corresponding advance in the utility of genetic toxicology and its application to risk assessment. Knowing the types of studies to conduct and knowing how to interpret the data remain as fundamental as always. T ere is a need for genetic toxicology to avoid the temptation to use more and more sophisticated techniques to address the same questions and in the end make the same mistakes as have been made previously.

Advances in Cytogenetics

Conventional chromosome staining with DNA stains such as Giemsa or the process of chromosome banding requires considerable expenditure of time and a rather high level of expertise. Chromosome banding does allow for the assessment of transmissible aberrations such as reciprocal translocations and inversions with a fairly high degree of accuracy. Stable aberrations are transmissible from parent to daughter cell, and they represent effects of chronic exposures. T e more readily analyzed but cell-lethal, nontransmissible aberrations such as dicentrics and deletions ref ect only recent exposures and then only when analyzed at the first division after exposure.

Specific chromosomes, specific genes, and chromosome alterations can be detected readily since the development of FISH. In principle, the technique relies on amplification of DNA from particular genomic regions such as whole chromosomes or gene regions and the hybridization of these amplified DNAs to metaphase chromosome preparations or interphase nuclei. Regions of hybridization can be determined by the use of f uorescent antibodies that detect modified DNA bases incorporated during amplification or by incorporating f uorescent bases during amplification. T e f uorescently labeled,

hybridized regions are detected by fluorescence microscopy. Alterations in tumors can also be detected on a whole-genome basis. Comparative genomic hybridization (CGH) has allowed an accurate and sensitive assessment of chromosomal alterations present in tumors. CGH is adapted for automated screening approaches using biochips.

The types of data collected will affect our understanding of how tumors develop. Data on the dose–response characteristics for a specific chromosomal alteration as a proximate marker of cancer can enhance the cancer risk assessment process by describing effects of low exposures that are below those for which tumor incidence can be reliably assessed. Cytogenetic data can also improve extrapolation of data generated with laboratory animals to humans.

Molecular Analysis of Mutations and Gene Expression

With technological advances, the exact basis of a mutation at the level of the DNA sequence can be established. With hybridization of test DNAs to oligonucleotide arrays, specific genetic alterations or their cellular consequences can be determined rapidly and automatically. cDNA microarray technologies allow the measurement of changes in expression of hundreds or even thousands of genes at one time. The level of expression at the mRNA level is measured by amount of hybridization of isolated cDNAs to oligonucleotide fragments from known genes or expressed sequence tags (EST) on a specifically laid out grid. This technique holds great promise for establishing a cell's response to exposure to chemical or physical agents in the context of normal cellular patterns of gene expression.

These microarray-based techniques are now being replaced by massively parallel sequencing or ultrahigh throughput sequencing approaches that can quantitatively assess gene expression changes in response to exposures. Such sequencing-based techniques have the great advantage that they are based on molecule counting approaches rather than on hybridization, thereby making them more quantitative and able to detect very low level transcripts. There are parallel efforts in the area of proteomics and metabolomics whereby changes in a broad range of cellular proteins can be assessed in response to endogenous or exogenous factors, potentially leading to the development of biomarkers of effect.

The move in the field of genetic toxicology is away from the "yes/no" approach to hazard identification and much more toward a mechanistic understanding of how a chemical or physical agent can produce adverse cellular and tissue responses. In turn such knowledge can be used for the development of informative bioindicators representing the key events along the pathway from initial interactions with cells to adverse outcome. The move is clearly toward analysis at the whole genome level and away from single gene responses.

CONCLUSION

Genetic toxicology demonstrated that ionizing radiations and chemicals could induce mutations and chromosome alterations in plant, insect, and mammalian cells. Various short-term assays for genetic toxicology identified many mutagens and address the relationship between mutagens and carcinogens. Failure of the assays to be completely predictive resulted in the identification of nongenotoxic carcinogens. Key cellular processes related to mutagenesis have been identified, including multiple pathways of DNA repair, cell-cycle controls, and the role of checkpoints in ensuring that the cell cycle does not proceed until the DNA and specific cellular structures are checked for fidelity. Recent developments in genetic toxicology have improved our understanding of basic cellular processes and alterations that can affect the integrity of the genetic material and its functions. The ability to detect and analyze mutations in mammalian germ cells continues to improve and contribute to a better appreciation for the long-term consequences of mutagenesis in human populations.

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QUESTIONS

1. Oncogenes:
   a. maintain normal cellular growth and development.
   b. exert their action in a genetically recessive fashion.
   c. are often formed via translocation to a location with a more active promoter.
   d. can be mutated to form proto-oncogenes.
   e. include growth factors and GTPases, but not transcription factors.

2. Which of the following is NOT one of the more common sources of DNA damage?
   a. ionizing radiation.
   b. UV light.
   c. electrophilic chemicals.
   d. DNA polymerase error.
   e. x-rays.

3. Which of the following pairs of DNA repair mechanisms is most likely to introduce mutations into the genetic composition of an organism?
   a. nonhomologous end-joining (NHEJ) and base excision repair.
   b. nonhomologous end-joining and homologous recombination.
   c. homologous recombination and nucleotide excision repair.
   d. nucleotide excision repair and base excision repair.
   e. homologous recombination and mismatch repair.

4. Which of the following DNA mutations would NOT be considered a frameshift mutation?
   a. insertion of 5 nucleotides.
   b. insertion of 7 nucleotides.
   c. deletion of 18 nucleotides.
   d. deletion of 13 nucleotides.
   e. deletion of 1 nucleotide.

5. Which of the following base-pair mutations is properly characterized as a transversion mutation?
   a. T → C.
   b. A → G.
   c. G → A.
   d. T → U.
   e. A → C.

6. All of the following statements regarding nondisjunction during meiosis are true EXCEPT:
   a. Nondisjunction events can happen during meiosis I or meiosis II.
   b. All gametes from nondisjunction events have an abnormal chromosome number.
   c. Trisomy 21 (Down syndrome) is a common example of nondisjunction.
   d. In a nondisjunction event in meiosis I, homologous chromosomes fail to separate.
   e. T e incorrect formation of spindle fibers is a common cause of nondisjunction during meiosis.

7. Which of the following diseases does NOT have a recessive inheritance pattern?
   a. phenylketonuria.
   b. cystic fibrosis.
   c. Tay–Sachs disease.
   d. sickle cell anemia.
   e. Huntington's disease.

8. What is the purpose of the Ames assay?
   a. to determine the threshold of UV light that bacteria can receive before having mutations in their DNA.
   b. to measure the frequency of aneuploidy in bacterial colonies treated with various chemicals.
   c. to determine the frequency of a reversion mutation that allows bacterial colonies to grow in the absence of vital nutrients.
   d. to measure rate of induced recombination in mutagen-treated fungi.
   e. to measure induction of phenotypic changes in Drosophila.

9. In mammalian cytogenic assays, chromosomal aberrations are measured after treatment of the cells at which sensitive phase of the cell cycle?
   a. interphase.
   b. M phase.
   c. S phase.
   d. G1.
   e. G2.

10. Which of the following molecules is used to gauge the amount of a specific gene being transcribed to mRNA?
    a. protein.
    b. mRNA.
    c. DNA.
    d. cDNA.
    e. CGH.

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CHAPTER

10

Developmental Toxicology

John M. Rogers

SCOPE OF PROBLEM—THE HUMAN EXPERIENCE

- Thalidomide
- Diethylstilbestrol
- Ethanol
- Tobacco Smoke
- Cocaine
- Retinoids
- Antiepileptic Drugs
- Angiotensin Converting Enzyme (ACE) Inhibitors and Angiotensin Receptor Antagonists

PRINCIPLES OF DEVELOPMENTAL TOXICOLOGY

- Critical Periods of Susceptibility and End Points of Toxicity
- Dose–Response Patterns and the Threshold Concept

MECHANISMS AND PATHOGENESIS OF DEVELOPMENTAL TOXICITY

- Advances in the Molecular Basis of Dysmorphogenesis

PHARMACOKINETICS AND METABOLISM IN PREGNANCY

RELATIONSHIPS BETWEEN MATERNAL AND DEVELOPMENTAL TOXICITY

- Maternal Factors Affecting Development
  - Genetics
  - Disease
  - Nutrition
  - Stress
  - Placental Toxicity
  - Maternal Toxicity

DEVELOPMENTAL TOXICITY OF ENDOCRINE-DISRUPTING CHEMICALS

- Laboratory Animal Evidence
- Human Evidence
- Impact on Screening and Testing Programs

MODERN SAFETY ASSESSMENT

- Regulatory Guidelines for In Vivo Testing
- Multigeneration Tests
- Children's Health
- Alternative Testing Strategies
- Epidemiology
- Concordance of Data
- Elements of Risk Assessment

PATHWAYS TO THE FUTURE

KEY POINTS

- Developmental toxicology encompasses the study of pharmacokinetics, mechanisms, pathogenesis, and outcomes following exposure to agents or conditions leading to abnormal development.
- Developmental toxicology includes teratology, or the study of structural birth defects.
- Gametogenesis is the process of forming the haploid germ cells: the egg and the sperm.
- Organogenesis is the period during which most bodily structures are established. T is period of heightened susceptibility to malformations extends from the third to the eighth week of gestation in humans.

SCOPE OF PROBLEM—THE HUMAN EXPERIENCE

Successful pregnancy outcome in the general population occurs at a surprisingly low frequency. Estimates of adverse outcomes include postimplantation pregnancy loss, 31%; major birth defects, 2% to 3% at birth and increasing to 6% to 7% at 1 year as more manifestations are diagnosed; minor birth defects, 14%; low birth weight, 7%; infant mortality (prior to 1 year of age), 1.4%; and abnormal neurologic function, 16% to 17%. Thus, less than half of all human conceptions result in the birth of a completely normal, healthy infant. Many hundreds of chemicals are teratogens; most of them produce birth defects by an unknown mechanism. However, Table 10–1 lists chemicals, chemical classes, or conditions known to alter prenatal development in humans.

Thalidomide

In 1960, a large increase in newborns with rare limb malformations of amelia (absence of the limbs) or various degrees of phocomelia (reduction of the long bones of the limbs) was recorded in West Germany. Congenital heart disease; ocular, intestinal, and renal anomalies; and malformations of the external and inner ears were also involved. Thalidomide, identified as the causative agent, was used throughout much of the world as a sleep aid and to ameliorate nausea and vomiting in pregnancy. It had no apparent toxicity or addictive properties in adult humans or rodents at therapeutic exposure levels.

As a result of this catastrophe, regulatory agencies developed requirements for evaluating the effects of drugs on pregnancy outcomes.

Diethylstilbestrol

Diethylstilbestrol (DES) is a synthetic nonsteroidal estrogen widely used from the 1940s to the 1970s in the United States to prevent threatened miscarriage. It was soon linked to clear cell adenocarcinoma of the vagina. Maternal use of DES prior to the 18th week of gestation appeared to be necessary for induction of the genital tract anomalies in offspring; the overall incidence of noncancerous alterations in the vagina and cervix was estimated to be as high as 75%. In male offspring of exposed pregnancies, a high incidence of reproductive tract anomalies along with low ejaculated semen volume and poor semen quality were observed. The realization of the latent and devastating manifestations of prenatal DES exposure has broadened the magnitude and scope of potential adverse outcomes of intrauterine exposures. A recent study in mice suggests that the increased susceptibility to abnormalities conferred by DES exposure may be passed on to future generations of exposed mothers.

Ethanol

Although the developmental toxicity of ethanol can be traced to biblical times (e.g., Judges 13:3–4), only since the description of the Fetal Alcohol Syndrome (FAS) in 1971 has a clear acceptance

TABLE 10–1 Human developmental toxicants.

Radiation
- Atomic fallout
- Radioiodine
- Therapeutic

Infections
- Cytomegalovirus
- Herpes simplex virus 1 and 2
- Parvovirus B-19 (erythema infectiosum)
- Rubella virus
- Syphilis
- Toxoplasmosis
- Varicella virus
- Venezuelan equine encephalitis virus

Maternal trauma and metabolic imbalances
- Alcoholism
- Amniocentesis, early
- Chorionic villus sampling (before day 60)
- Cretinism
- Diabetes
- Folic acid deficiency
- Hyperthermia
- Phenylketonuria
- Rheumatic disease and congenital heart block
- Sjögren's syndrome
- Virilizing tumors

Drugs and chemicals
- Aminoglycosides
- Androgenic hormones
- Angiotensin converting enzyme inhibitors: captopril, enalapril
- Angiotensin receptor antagonists: sartans
- Anticonvulsants: diphenylhydantoin, trimethadione, valproic acid, carbamazepine
- Busulfan
- Carbon monoxide
- Chlorambucil
- Cocaine
- Coumarins
- Cyclophosphamide
- Cytarabine
- Diethylstilbestrol
- Danazol
- Ergotamine
- Ethanol
- Ethylene oxide
- Fluconazole
- Folate antagonists: aminopterin, methotrexate
- Iodides
- Lead
- Lithium
- Mercury, organic
- Methimazole
- Methylene blue
- Misoprostal
- Penicillamine
- Polychlorobiphenyls
- Quinine (high dose)
- Retinoids: accutane, isotretinoin, etretinate, acitretin
- Tetracyclines
- Thalidomide
- Tobacco smoke
- Toluene
- Vitamin A (high dose)

of alcohol's developmental toxicity occurred. FAS comprises craniofacial dysmorphism, intrauterine and postnatal growth retardation, retarded psychomotor and intellectual development, and other nonspecific major and minor abnormalities.

In utero exposure to lower levels of ethanol than those that produce full-blown FAS has been associated with a wide range of effects, including isolated components of FAS and milder forms of neurologic and behavioral disorders that have been termed fetal alcohol spectrum disorder (FASD). Alcohol consumption can affect birth weight in a dose-related fashion.

Tobacco Smoke

Prenatal and early postnatal exposure to tobacco smoke or its constituents may well represent the leading cause of environmentally induced developmental disease and morbidity today. Approximately 25% of women in the United States continue to smoke during pregnancy, despite public health programs aimed at curbing this behavior. T e consequences of developmental tobacco smoke exposure include spontaneous abortions, perinatal deaths, increased risk of sudden infant death syndrome (SIDS), increased risk of learning, behavioral, and attention disorders, and lower birth weight. One component of tobacco smoke, nicotine, is a known neuroteratogen in experimental animals and can by itself produce many of the adverse developmental outcomes associated with tobacco smoke. Perinatal exposure to tobacco smoke can also affect branching morphogenesis and maturation of the lung, leading to altered physiologic function. Environmental (second-hand) tobacco smoke also represents a significant risk to the pregnant nonsmoker and her baby, and exposure to second-hand smoke has been associated with many of the effects caused by active maternal smoking.

Cocaine

Cocaine is a local anesthetic with vasoconstrictor properties. Effects on the fetus are complicated and controversial and demonstrate the difficulty of monitoring the human population for adverse reproductive outcomes. Accurate exposure ascertainment is difficult, as many confounding factors including socioeconomic status and concurrent use of cigarettes, alcohol, and other drugs of abuse may be involved. In addition, reported effects on the fetus and infant (neurologic and behavioral changes) are difficult to identify and quantify. Nevertheless, adverse effects reliably associated with cocaine exposure in humans include abruptio placentae, premature labor and delivery, microcephaly, altered prosencephalic development, decreased birth weight, SIDS, and a neonatal neurologic syndrome of abnormal sleep, tremor, poor feeding, irritability, and occasional seizures.

Retinoids

Vitamin A (retinol) exposure can cause malformations of the face, limbs, heart, central nervous system, and skeleton. Spontaneous abortion, live-born infants having at least one major malformation, and numerous exposed children having full-scale IQ scores below 85 at age 5 years have been documented.

Antiepileptic Drugs

Clinical management of women of childbearing age who have epilepsy is difficult. Although control of seizures during pregnancy is crucial, most current antiepileptic drugs (AEDs) have been shown to carry risk of developmental toxicity including birth defects, cognitive impairment, and fetal death. As a class, including phenytoin, carbamazepine, and valproic acid, AEDs are considered human teratogens. Studies to date suggest that newer AEDs such as gabapentin, lamotrigine, oxcarbazone, topiramate, and zonizamide may be safer than the older AEDs.

Angiotensin Converting Enzyme (ACE) Inhibitors and Angiotensin Receptor Antagonists

T e renin–angiotensin system is a key controller of blood pressure. T e active signaling messenger of this system is angiotensin II, which binds to angiotensin II (AT1) receptors to cause vasoconstriction and fluid retention, resulting in elevation of blood pressure. ACE inhibitors and angiotensin receptor blockers are widely prescribed and, when used in the second half of pregnancy, are known to cause oligohydramnios (low amniotic fluid volume), fetal growth retardation, pulmonary hypoplasia, joint contractures, hypocalvaria, neonatal renal failure, hypotension, and death. Some studies suggest that exposure in the first trimester should be avoided.

PRINCIPLES OF DEVELOPMENTAL TOXICOLOGY

Some basic principles of teratology put forth by Jim Wilson in 1959 are listed in Table 10–2; they are still valid today.

TABLE 10–2 Wilson's general principles of teratology.

I. Susceptibility to teratogenesis depends on the genotype of the conceptus and the manner in which this interacts with adverse environmental factors
II. Susceptibility to teratogenesis varies with the developmental stage at the time of exposure to an adverse influence
III. Teratogenic agents act in specific ways (mechanisms) on developing cells and tissues to initiate sequences of abnormal developmental events (pathogenesis)
IV. The access of adverse influences to developing tissues depends on the nature of the influence (agent)
V. The four manifestations of deviant development are death, malformation, growth retardation, and functional deficit
VI. Manifestations of deviant development increase in frequency and degree as dosage increases, from the no ef ect to the totally lethal level

Data from Wilson JG: Environment and Birth Defects. New York, NY: Academic Press/Elsevier; 1973.

Critical Periods of Susceptibility and End Points of Toxicity

Development is characterized by various changes that are orchestrated by a cascade of factors regulating gene transcription throughout development. Intercellular and intracellular signaling pathways essential for normal development rely on transcriptional, translational, and posttranslational controls. The rapid changes occurring during development alter the nature of the embryo/fetus as a target for toxicity. Timing of some key developmental events in humans and experimental animal species is presented in Table 10–3.

Gametogenesis is the process of forming the haploid germ cells: the egg and the sperm. These gametes fuse in the process of fertilization to form the diploid zygote, or one-celled embryo. Gametogenesis and fertilization are vulnerable to toxicants. It is now known that the maternal and paternal genomes are not equivalent in their contributions to the zygotic genome. The process of imprinting (which involves cytosine methylation and changes in chromatin conformation) occurs during gametogenesis, conferring to certain allelic genes a differential expressivity depending on whether they are of maternal or paternal origin.

Epigenetics refers to the biochemical changes in chromatin that lead to changes in conformation and gene expression. Epigenetic changes include DNA methylation, histone modifications, and expression of microRNAs. There are specific stages of the life cycle during which epigenetic marks may be erased and reestablished, including two periods of development during which large-scale demethylations of the genome are known to occur. One is during migration and proliferation of the primordial gem cells in which imprinted genes are demethylated, with remethylation occurring in a gender-specific manner during gametogenesis in the offspring. The other period of widespread epigenetic reprogramming occurs shortly after formation of the zygote and in the early embryo, with total genomic methylation reaching a nadir at the blastocyst stage.

Following fertilization, the embryo moves down the fallopian tube (oviduct) and implants in the wall of the uterus. The preimplantation period comprises mainly an increase in cell number through a rapid series of cell divisions with little growth in size (cleavage of the zygote) and cavitation of the embryo to form a fluid-filled blastocoele. This stage, termed the blastocyst, contains cells destined to give rise to the embryo proper and other cells that give rise to extraembryonic membranes and support structures.

Toxicity during preimplantation is generally thought to result in no or slight effect on growth (because of regulative growth) or in death (through overwhelming damage or failure

TABLE 10–3 Timing of key developmental events in some mammalian species.*

	Rat	Rabbit	Monkey	Human
Blastocyst formation	3–5	2.6–6	4–9	4–6
Implantation	5–6	6	9	6–7
Organogenesis	6–17	6–18	20–45	21–56
Primitive streak	9	6.5	18–20	16–18
Neural plate	9.5	—	9–21	18–20
First somite	10	—	—	20–21
First branchial arch	10	—	—	20
First heartbeat	10.2	—	—	22
10 somites	10–11	9	23–24	25–26
Upper limb buds	10.5	10.5	25–26	29–30
Lower limb buds	11.2	11	26–27	31–32
Testes differentiation	14.5	20	—	43
Heart septation	15.5	—	—	46–47
Palate closure	16–17	19–20	45–47	56–58
Urethral groove closed in male	—	—	—	90
Length of gestation	21–22	31–34	166	267

*Developmental ages are days of gestation.
Data from Shepard TH: Catalog of Teratogenic Agents, 9th ed. Baltimore, MD: The Johns Hopkins University Press; 1998.

to implant). Because of the rapid mitoses occurring during the preimplantation period, chemicals affecting DNA synthesis/integrity or those affecting microtubule assembly would be expected to be particularly toxic if given access to the embryo.

Following implantation the embryo undergoes gastrulation, the process of formation of the three primary germ layers—the ectoderm, mesoderm, and endoderm. During gastrulation, cells migrate through a midline structure called the primitive streak, and their movements set up basic morphogenetic fields in the embryo. As a prelude to organogenesis, the period of gastrulation is quite susceptible to teratogens. A number of toxicants administered during gastrulation produce malformations of the eye, brain, and face. T ese malformations are indicative of damage to the anterior neural plate, one of the regions defined by the cellular movements of gastrulation.

T e formation of the neural plate in the ectoderm marks the onset of organogenesis, during which the rudiments of most bodily structures are established. T is period of heightened susceptibility to malformations extends from approximately the third to the eighth week of gestation in humans. T e rapid changes of organogenesis require cell proliferation, cell migration, cell–cell interactions, and morphogenetic tissue remodeling. Within organogenesis, there are periods of peak susceptibility for each forming structure. T e peak incidence of each malformation coincides with the timing of key developmental events in the affected structure.

T e end of organogenesis marks the beginning of the fetal period, which is characterized primarily by tissue differentiation, growth, and physiologic maturation. All organs are present and grossly recognizable, although not yet completely developed.

Exposure during the fetal period is most likely to result in effects on growth and functional maturation. Functional anomalies of the central nervous system and reproductive organs—including behavioral, mental, and motor deficits as well as decreases in fertility—are among the possible adverse outcomes.

Over the past two decades, the concept of "developmental programming" has emerged, in which the developmental environment is thought to influence the metabolic parameters of the offspring that will persist throughout life and may affect lifelong risk of disease. Much of the work on fetal programming has focused on the role of maternal nutrition, and there is a paucity of data concerning the long-term effects of chemical exposure during the fetal and early postnatal periods. Some effects could require years to become apparent (such as those noted above for DES), and others may even result in the premature onset of senescence and/or organ failure late in life.

Dose–Response Patterns and the Threshold Concept

T e major effects of prenatal exposure, observed at the time of birth in developmental toxicity studies, are embryo lethality, malformations, and growth retardation. For some agents, these end points may represent a continuum of increasing toxicity, with low dosages producing growth retardation and increasing dosages producing malformations and then lethality.

Another key element of the dose–response relationship is the shape of the dose–response curve at low exposure levels. Because of the high restorative growth potential of the mammalian embryo, cellular homeostatic mechanisms, and maternal metabolic defenses, mammalian developmental toxicity has generally been considered a threshold phenomenon. Assumption of a threshold means that there is a maternal dosage below which an adverse response is not elicited because some repair or defense system is able to combat the exposure.

MECHANISMS AND PATHOGENESIS OF DEVELOPMENTAL TOXICITY

T e term mechanisms refers to cellular-level events that initiate the process leading to abnormal development. Pathogenesis comprises the cell-, tissue-, and organ-level sequelae that ultimately manifest in abnormality. Mechanisms of teratogenesis include mutations, chromosomal breaks, altered mitosis, altered nucleic acid integrity or function, diminished supplies of precursors or substrates, decreased energy supplies, altered membrane characteristics, osmolar imbalance, and enzyme inhibition. Although these cellular insults are not unique to development, they may trigger unique pathogenetic responses in the embryo, such as reduced cell proliferation, cell death, altered cell–cell interactions, reduced biosynthesis, inhibition of morphogenetic movements, or mechanical disruption of developing structures.

Cell death plays a critical role in normal morphogenesis. T e term programmed cell death refers specifically to apoptosis, which is under genetic control in the embryo. Apoptosis is necessary for sculpting the digits from the hand plate and for assuring appropriate functional connectivity between the central nervous system and distal structures. Cell proliferation rates change both spatially and temporally during ontogenesis. T ere is a delicate balance between cell proliferation, cell differentiation, and apoptosis in the embryo. DNA damage might lead to cell cycle perturbations and cell death.

As discussed in Chapter 9, DNA damage can inhibit cell cycle progression at the G_1–S transition, through the S phase, and at the G_2–M transition. If DNA damage is repaired, the cell cycle can return to normal, but if damage is too extensive or cell cycle arrest too long, apoptosis may be triggered. T e relationship between DNA damage and repair, cell cycle progression, and apoptosis is depicted in Figure 10–1. From the multiple checkpoints and factors present to regulate the cell cycle and apoptosis, it is clear that different cell populations may respond differently to a similar stimulus, in part because cellular predisposition to apoptosis can vary.

Besides affecting proliferation and cell viability, molecular and cellular insults can alter cell migration, cell–cell interactions, differentiation, morphogenesis, and energy metabolism. Although the embryo has compensatory mechanisms to offset such effects, production of a normal or malformed offspring will depend on the balance between damage and repair at each step in the pathogenetic pathway.

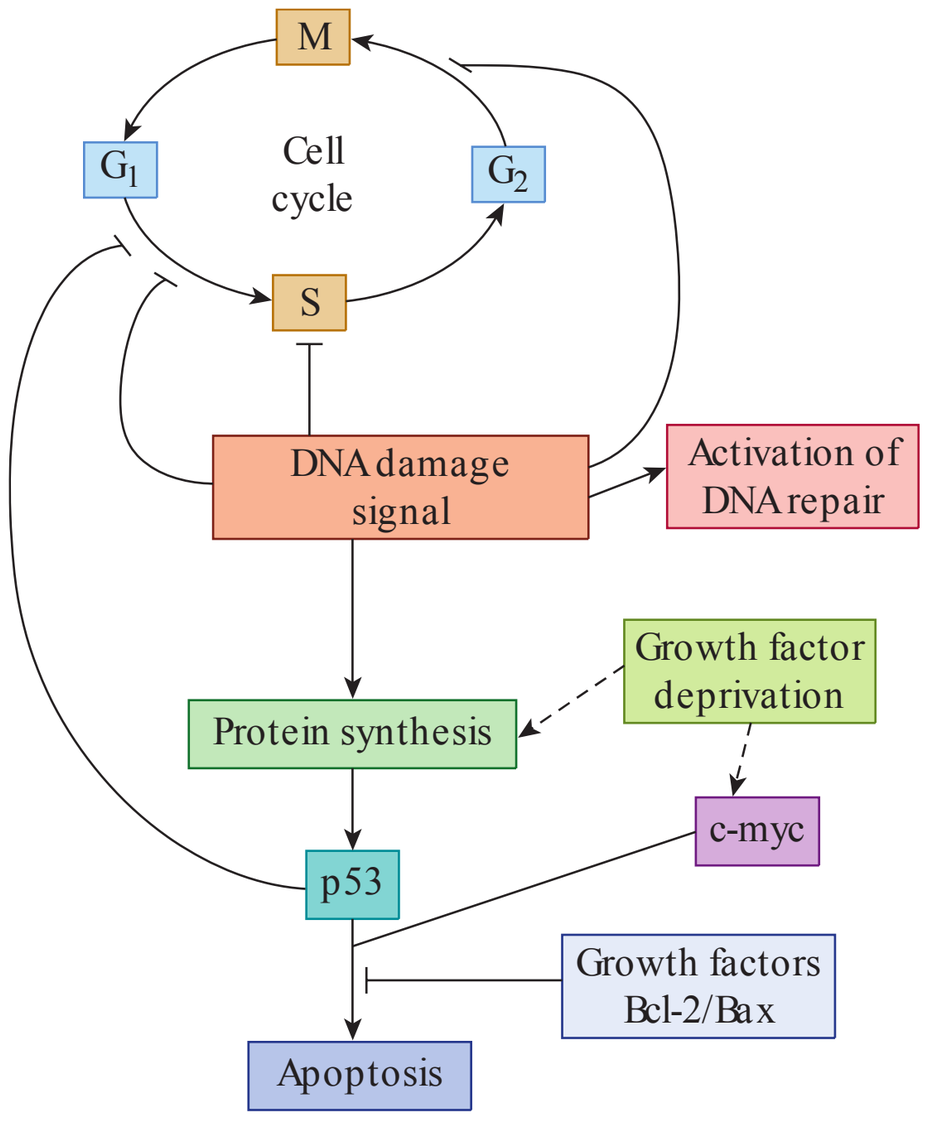

FIGURE 10–1 **Relationships between DNA damage and the induction of cell cycle arrest or apoptosis.** DNA damage can signal inhibition of the cell cycle between G_1 and S, in S phase, or between G_2 and mitosis. The signal(s) can also activate DNA repair mechanisms and synthesis of proteins, including p53, that can initiate apoptosis. Growth factors and products of the proto-oncogene c-myc and the Bcl-2/Bax gene family, as well as dif erentiation state and cell cycle phase, are important determinants of the ultimate outcome of embryonal DNA damage.

Advances in the Molecular Basis of Dysmorphogenesis

Advances in gene targeting and transgenic strategies now allow modification of gene expression at specific points in development and in specific cell types. Conditional knockouts (cKO) or knockins (cKI), inducible gene expression, and other techniques are being used to study the effects of specific gene products on development in great detail. T e use of synthetic antisense oligonucleotides allows temporal and spatial restriction of gene ablation by hybridizing to mRNA in the cell, thereby inactivating it. In this way, gene function can be turned off at specific times. RNA interference is a more recent gene knockdown technique, exploiting the discovery of the RNA interference pathway. Small interference (si)RNA, plasmid-, and virus-encoded small RNAs can be used to down-regulate the expression of specific genes posttranscriptionally.

Gain of gene function can also be studied by engineering genetic constructs with an inducible promoter attached to the gene of interest. Ectopic gene expression can be made ubiquitous or site-specific depending on the choice of promoter to drive expression. Transient overexpression of specific genes can be accomplished by adding extra copies using adenoviral transduction.

PHARMACOKINETICS AND METABOLISM IN PREGNANCY

T e extent and the form in which chemicals reach the conceptus are important determinants of whether the agent can impact development. T e maternal, placental, and embryonic compartments comprise independent, yet interacting, systems that undergo profound changes throughout the course of pregnancy. Alterations in placental physiology can have significant impact on the uptake, distribution, metabolism, and elimination of xenobiotics. For example, decreases in intestinal motility and increases in gastric emptying time result in longer retention of ingested chemicals in the upper gastrointestinal tract in the mother. Cardiac output increases by 50% during the first trimester in humans and remains elevated throughout pregnancy, whereas blood volume increases and plasma proteins and peripheral vascular resistance decrease. T e relative increase in blood volume over red cell volume leads to borderline anemia and a generalized edema with a 70% elevation of extracellular space. T us, the volume of distribution of a chemical and the amount bound by plasma proteins may change considerably during pregnancy. Other changes occur in the renal, hepatic, and pulmonary systems as well. Clearly, maternal handling of a chemical influences the extent of embryotoxicity.

T e placenta also influences embryonic exposure by helping to regulate blood flow, offering a transport barrier, and metabolizing chemicals. T e placenta acts as a lipid membrane that permits bidirectional transfer of substances between maternal and fetal compartments. It is important to note that virtually any substance present in the maternal plasma will be transported to some extent by the placenta. T e passage of most drugs across the placenta seems to occur by simple passive diffusion. Important modifying factors to the rate and extent of transfer include lipid solubility, molecular weight, protein binding, the type of transfer (passive diffusion, and facilitated or active transport), the degree of ionization, and placental metabolism. Blood flow probably constitutes the major rate-limiting step for more lipid-soluble compounds.

Maternal metabolism of xenobiotics is an important and variable determinant of developmental toxicity. As for other health end points, the field of pharmacogenomics offers hope for increasing our ability to predict susceptible subpopulations based on empirical relationships between maternal genotype and fetal phenotype.

RELATIONSHIPS BETWEEN MATERNAL AND DEVELOPMENTAL TOXICITY

Although all developmental toxicity must ultimately result from an insult to the conceptus at the cellular level, the insult may occur through a direct effect on the embryo/fetus, indirectly through toxicity of the agent to the mother and/or the placenta, or a combination of direct and indirect effects. Maternal factors known to affect fetal development include

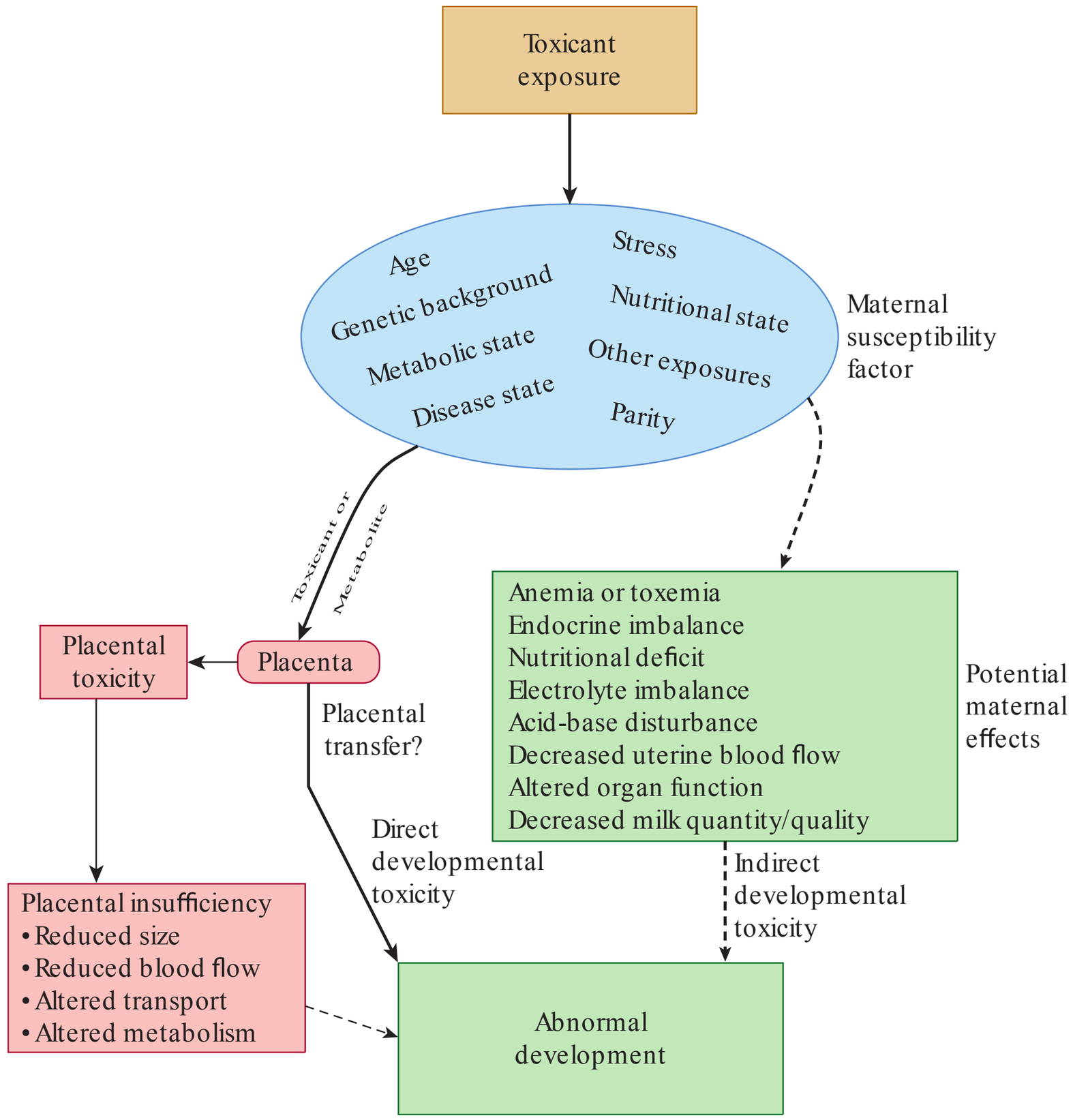

FIGURE 10–2 **Interrelationships between maternal susceptibility factors, metabolism, induction of maternal physiologic or functional alterations, placental transfer and toxicity, and developmental toxicity.** A developmental toxicant can cause abnormal development through any one or a combination of these pathways. Maternal susceptibility factors determine the predisposition of the mother to respond to a toxic insult, and the maternal ef ects listed can adversely af ect the developing conceptus. Most chemicals traverse the placenta in some form, and the placenta can also be a target for toxicity. In most cases, developmental toxicity is probably mediated through a combination of these pathways.

genetics, disease, nutrition, stress, placental toxicity, and maternal toxicity. Some conditions that may adversely affect the fetus are depicted in Figure 10–2.

T e distinction between direct and indirect developmental toxicity is important for interpreting safety assessment results in pregnant animals, as the highest dosage level in these experiments is chosen based on its ability to produce some maternal toxicity (e.g., decreased food or water intake, weight loss, and clinical signs). However, maternal toxicity defined only by such crude manifestations gives little insight to the toxic actions of a xenobiotic. When developmental toxicity is observed only in the presence of maternal toxicity, the developmental effects may be indirect (i.e., caused by an inappropriate growing condition because of an altered maternal environment rather than by a direct interaction of the fetus with the toxin). Greater understanding of the physiologic changes underlying the observed maternal toxicity and elucidation of the association with developmental effects is needed before one can begin to address the relevance of the observations to human safety assessment.

Maternal Factors Af ecting Development

Genetics—T e genetic makeup of the pregnant female has been well documented as a determinant of developmental outcome in both humans and animals. T e incidence of clef lip and/or palate [CL(P)], which occurs more frequently in whites than in blacks, has been investigated in offspring of interracial couples in the United States. Offspring of white mothers had a higher incidence of CL(P) than offspring of black mothers af er correcting for paternal race, whereas offspring of white fathers did not have a higher incidence of CL(P) than offspring of black fathers af er correcting for maternal race.

Disease—Chronic hypertension in the mother, uncontrolled maternal diabetes mellitus, and certain infections in the mother (i.e., cytomegalovirus and Toxoplasma gondii) are leading causes of several types of defects in the fetus. Exposure to hyperthermia (such as febrile illness in the mother) is also implicated in neural defects in the fetus.

Nutrition—A wide spectrum of dietary insufficiencies ranging from protein-calorie malnutrition to deficiencies of vitamins, trace elements, and/or enzyme cofactors is known to adversely affect pregnancy. In fact, folate supplementation by pregnant women can reduce neural tube defect recurrence by over 70%.

Stress—Diverse forms of maternal toxicity may have in common the induction of a physiologic stress response. Various forms of physical stress have been applied to pregnant animals in attempts to isolate the developmental effects of stress. Noise stress of pregnant rats or mice throughout gestation can produce developmental toxicity. Restraint stress produces increased fetal death in rats, and malformations of clef palate, fused and supernumerary ribs, and encephaloceles in mice. T ere is a positive correlation in humans between stress and adverse developmental effects, including low birth weight and congenital malformations.

Placental Toxicity—T e placenta is the interface between the mother and the conceptus, providing attachment, nutrition, gas exchange, and waste removal. T e placenta also produces hormones critical to the maintenance of pregnancy, and it can metabolize and/or store xenobiotics. Placental toxicity may compromise these functions. Known placental toxicants include cadmium, arsenic or mercury, cigarette smoke, ethanol, cocaine, endotoxin, and sodium salicylate.

Maternal Toxicity—A retrospective analysis of relationships between maternal toxicity and specific types of prenatal effects found species-specific associations between maternal toxicity and specific adverse developmental effects. Various adverse developmental outcomes include increased intrauterine death, decreased fetal weight, supernumerary ribs, and enlarged renal pelvises.

A number of studies directly relate specific forms of maternal toxicity to developmental toxicity, including those in which the test chemical causes maternal effects that exacerbate the agent's developmental toxicity. However, clear delineation of the relative role(s) of indirect maternal and direct embryo/fetal toxicity is difficult.

Diflunisal, an analgesic and anti-inflammatory drug, causes axial skeletal defects in rabbits. Developmentally toxic dosages resulted in severe maternal anemia and depletion of erythrocyte ATP levels. Teratogenicity, anemia, and ATP depletion were unique to the rabbit. T e teratogenicity of diflunisal in the rabbit was probably due to hypoxia resulting from maternal anemia.

Phenytoin, an anticonvulsant, can affect maternal folate metabolism in experimental animals, and these alterations may play a role in the teratogenicity of this drug. A mechanism of teratogenesis was proposed relating depressed maternal heart rate and embryonic hypoxia. Supporting studies have demonstrated that hyperoxia reduces the teratogenicity of phenytoin in mice.

DEVELOPMENTAL TOXICITY OF ENDOCRINE-DISRUPTING CHEMICALS

T ere is the growing concern that exposure to chemicals that can interact with the endocrine system may pose a serious health hazard. An "endocrine disruptor" has been broadly defined as an exogenous agent that interferes with the production, release, transport, metabolism, binding, action, or elimination of natural hormones responsible for the maintenance of homeostasis and the regulation of developmental processes. Due to the critical role of hormones in directing differentiation in many tissues, the developing organism is particularly vulnerable to fluctuations in the timing or intensity of exposure to chemicals with hormonal or antihormonal activity. Various chemical classes induce developmental toxicity via at least three modes of action involving the endocrine system: (1) by serving as ligands of steroid receptors, (2) by modifying steroid hormone metabolizing enzymes, and (3) by perturbing hypothalamic-pituitary release of trophic hormones. Interactions with the functions of estrogens, androgens, and thyroid hormones have been the most studied, but the underlying principles apply to other hormones also.

Laboratory Animal Evidence

Estrogenic or antiestrogenic developmental toxicants include DES, estradiol, antiestrogenic drugs such as tamoxifen and clomiphene citrate, and some pesticides and industrial chemicals. T e pattern of outcomes is generally similar across different estrogens. Female offspring are generally more sensitive to these toxicants than males, and altered pubertal development, reduced fertility, and reproductive tract anomalies are common findings.

Antiandrogens represent another major class of endocrine-disrupting chemicals. Principal manifestations of developmental exposure to an antiandrogen are generally restricted to males, and include hypospadias, retained nipples, reduced testes and accessory sex gland weights, and decreased sperm production.

Hypothyroidism during pregnancy and early postnatal development causes growth retardation, cognitive defects, delayed eye-opening, hyperactivity, and auditory defects. Polychlorinated biphenyls (PCBs) may act at several sites to lower thyroid hormone levels during development, and cause these developmental abnormalities.

Human Evidence

Whether human health is being adversely impacted from exposures to endocrine disruptors present in the environment is equivocal. Reports in humans are of two types:

1. Observations of adverse effects on reproductive system development and function following exposure to chemicals with known endocrine activities that are present in medicines, contaminated food, or the workplace. T ese have tended to involve relatively higher exposure to chemicals with known endocrine effects.
2. Epidemiologic evidence of increasing trends in reproductive and developmental adverse outcomes that have an endocrine basis. For example, secular trends have been reported for cryptorchidism, hypospadias, semen quality, and testicular cancer, but due to the lack of exposure assessment, such studies provide limited evidence of a cause and effect relationship.

Impact on Screening and Testing Programs

T e findings of altered reproductive development following early life-stage exposures to endocrine-disrupting chemicals helped prompt revision of traditional safety evaluation tests. T ese include assessments of female estrous cyclicity, sperm motility, and sperm morphology in both parental and F1 generations, the age at puberty in the F1s, histopathology of target organs, anogenital distance in the F2s, and primordial follicular counts in the parental and F1 generations. For the new prenatal developmental toxicity test guidelines, one important modification aimed at improved detection of endocrine disruptors was the expansion of the period of dosing from the end of organogenesis (i.e., palatal closure) to the end of pregnancy in order to include the developmental period of urogenital differentiation.

MODERN SAFETY ASSESSMENT

Experience with chemicals that have the potential to induce developmental toxicity indicates that both laboratory animal testing and surveillance of the human population (i.e., epidemiologic studies) as well as alert clinical evaluation af er potential exposure are all necessary to provide adequate public health protection. Laboratory animal investigations are guided by both regulatory requirements for drug or chemical marketing and the need to understand mechanisms of toxicity.

Regulatory Guidelines for In Vivo Testing

New and internationally accepted testing protocols rely on the investigator to meet the primary goal of detecting and bringing to light any indication of toxicity to reproduction. Key elements of various tests are provided in Table 10–4. T e general goal of these studies is to identify the NOAEL, which is the highest dosage level that does not produce a significant increase in adverse effects in the offspring or juvenile animals. T ese NOAELs are then used in the risk assessment process to assess the likelihood of effects in humans given certain exposure conditions.

Multigeneration Tests

Information pertaining to developmental toxicity can also be obtained from studies in which animals are exposed to the test substance continuously over one or more generations. For additional information on this approach, see Chapter 20.

Children's Health

Infants and children differ both qualitatively and quantitatively from adults in their exposure to pesticide residues in food because of different dietary composition, intake patterns, and different activities, such as crawling on the floor or ground, putting their hands and foreign objects in their mouths, and raising dust and dirt during play. Even the level of their activity (i.e., closer to the ground) can affect their exposure to some toxicants. In addition to exposure differences, children are growing and developing, which makes them more susceptible to some types of insults. Effects of early childhood exposure, including neurobehavioral effects and cancer, may not be apparent until later in life. Debate continues over the approach to be used in risk assessment in consideration of infants and children.

Alternative Testing Strategies

Various alternative test systems have been proposed to refine, reduce, or replace the standard regulatory mammalian tests for assessing prenatal toxicity (Table 10–5). T ese can be grouped into assays based on cell cultures, cultures of embryos in vitro (including submammalian species), and short-term in vivo tests. It was initially hoped that the alternative approaches would become generally applicable to all chemicals, and help prioritize full-scale testing; this has not yet been accomplished. Indeed, given the complexity of embryogenesis and the multiple mechanisms and target site of potential teratogens, it was perhaps unrealistic to have expected a single test, or even a small battery, to accurately prescreen the activity of chemicals in general.

An exception to the poor acceptance of alternate tests for prescreening for developmental toxicity is the Chernoff/Kavlock in vivo test. In this test, pregnant females are exposed during the period of major organogenesis to a limited number of dosage levels near those inducing maternal toxicity, and offspring are evaluated over a brief neonatal period for external malformations, growth, and viability. It has proven reliable over a large number of chemical agents and classes.

Epidemiology

Reproductive epidemiology studies associations between specific exposures of the father or pregnant woman and her conceptus and the outcome of pregnancy. T e likelihood of

TABLE 10–4 Summary of in vivo regulatory protocol guidelines for evaluation of developmental toxicity.

Study	Exposure	End Points Covered	Comments
Segment I: fertility and general reproduction study	Males: 10 weeks prior to mating Females: 2 weeks prior to mating	Gamete development, fertility, pre and postimplantation viability, parturition, lactation	Assesses reproductive capabilities of male and female following exposure over one complete spermatogenic cycle or several estrous cycles
Segment II: teratogenicity test	Implantation (or mating) through end of organogenesis (or term)	Viability, weight, and morphology (external, visceral, and skeletal) of conceptuses just prior to birth	Shorter exposure to prevent maternal metabolic adaptation and to provide high exposure to the embryo during gastrulation and organogenesis. Earlier dosing option for bioaccumulative agents or those impacting maternal nutrition. Later dosing option covers male reproductive tract development and fetal growth and maturation
Segment III: perinatal study	Last trimester of pregnancy through lactation	Postnatal survival, growth, and external morphology	Intended to observe effects on development of major organ functional competence during the perinatal period, and thus may be relatively more sensitive to adverse effects at this time
ICH 4.1.1: fertility protocol	Males: 4 weeks prior to mating Females: 2 weeks prior to mating	Males: Reproductive organ weights and histology, sperm counts, and motility Females: viability of conceptuses at midpregnancy or later	Improved assessment of male reproductive end points; shorter treatment duration than Segment I
ICH 4.1.2: effects on prenatal and postnatal development, including maternal function	Implantation through end of lactation	Relative toxicity to pregnant versus nonpregnant female; postnatal viability, growth, development, and functional deficits (including behavior, maturation, and reproduction)	
ICH 4.1.3: effects on embryo/fetal development	Implantation through end of organogenesis	Viability and morphology (external, visceral, and skeletal) of fetuses just prior to birth	Similar to Segment II study. Usually conducted in two species (rodent and nonrodent)
OECD 414: prenatal developmental	Implantation (or mating) through day prior to cesarean section	Viability and morphology (external, visceral, and skeletal) of fetuses just prior to birth	Similar to Segment II study. Usually conducted in two species (rodent and nonrodent)

linking a particular exposure with a series of case reports increases with the rarity of the defect, the rarity of the exposure in the population, a small source population, a short time span for study, and biological plausibility for the association. In other situations, such as occurred with ethanol and valproic acid, associations are sought through either a case–control or a cohort approach. Both approaches require accurate ascertainment of abnormal outcomes and exposures, and a large enough effect and study population to detect an elevated risk. Another challenge to epidemiologists is the high percentage of human pregnancy failures related to a particular exposure that may go undetected in the general population. With the availability of prenatal diagnostic procedures, additional pregnancies of malformed embryos (particularly neural tube defects) are electively aborted. Thus, the incidence of abnormal outcomes at birth may not reflect the true rate of abnormalities, and the term prevalence, rather than incidence, is preferred when the denominator is the number of live births rather than total pregnancies.

Other issues particularly relevant to reproductive epidemiology include homogeneity, recording proficiency, and confounding. Homogeneity refers to the fact that a particular outcome may be described differently by various recording units and that there can be multiple pathogenetic origins for a given specific outcome. Recording difficulties relate to inconsistencies of definitions and nomenclature, and to difficulties in ascertaining or recalling outcomes as well as exposures. For example, birth weights are usually accurately determined and recalled, but spontaneous abortions and certain malformations may not be. Last, confounding by factors such as maternal age and parity, dietary factors, diseases and drug usage, and social characteristics must be considered in order to control for variables that affect both exposure and outcome.

TABLE 10–5 Brief survey of alternative test methodologies for developmental toxicity.

Assay	Brief Description and End Points Evaluated
Mouse ovarian tumor	Labeled mouse ovarian tumor cells added to culture dishes with concanavalin A-coated disks for 20 min. End Point is inhibition of attachment of cells to disks
Human embryonic palatal mesenchyme	Human embryonic palatal mesenchyme cell line grown in attached culture. Cell number assessed after 3 days
Micromass culture	Midbrain or limb bud cells dissociated from rat embryos and grown in micromass culture for 5 days. Cell proliferation and biochemical markers of dif erentiation assessed
Mouse embryonic stem cell test (EST)	Mouse embryonic stem cells and 3T3 cells in 96-well plates assessed for viability after 3 and 5 days. Embryonic stem cells grown for 3 days in hanging drops form embryoid bodies which are plated and examined after 10 days for dif erentiation into cardiocytes
Chick embryo neural retina cell culture	Neural retinas of day 6.5 chick embryos dissociated and grown in rotating suspension culture for 7 days. End points include cellular aggregation, growth, dif erentiation, and biochemical markers
Drosophila	Fly larvae grown from egg disposition through hatching of adults. Adult flies examined for specific structural defects (bent bristles and notched wing)
Hydra	Hydra attenuata cells are aggregated to form an "artificial embryo" and allowed to regenerate. Dose response compared to that for adult Hydra toxicity
FETAX	Midblastula stage Xenopus embryos exposed for 96 h and evaluated for viability, growth, and morphology
Rodent whole embryo culture	Postimplantation rodent embryos grown in vitro for up to 2 days and evaluated for growth and development
Zebrafish	Zebrafish eggs or blastulae exposed to chemical in water (can be in multiwell plates) for up to 4 days and evaluated for growth, development, and (in some cases) gene expression
Chernof /Kavlock assay	Pregnant mice or rats exposed during organogenesis and allowed to deliver. Postnatal growth, viability, and gross morphology of litters assessed

Epidemiologic studies of abnormal reproductive outcomes are usually undertaken with three objectives in mind: the first is scientific research into the causes of abnormal birth outcomes and usually involves analysis of case reports or clusters; the second objective is prevention and is targeted at broader surveillance of trends by birth defect registries around the world; and the last objective is informing the public and providing assurance. Cohort studies, with their prospective exposure assessment and ability to monitor both adverse and beneficial outcomes, may be the most methodologically robust approach to identifying human developmental toxicants.

Information on differential genetic susceptibility to birth defects continues to accrue. T is new knowledge promises to elucidate links between genetics and disease susceptibility. Understanding the genetic basis of vulnerability to environmentally induced birth defects will allow more inclusive risk assessments and a better appreciation of the mechanisms of action of developmental toxicants.

Concordance of Data

Studies of the similarity of responses of laboratory animals and humans for developmental toxicants support the assumption that results from laboratory tests are predictive of potential human effects. Concordance is strongest when there are positive data from more than one test species. Humans tend to be more sensitive to developmental toxicants than is the most sensitive test species.

Elements of Risk Assessment

Extrapolation of animal test data for developmental toxicity follows two basic directions, one for drugs where exposure is voluntary and usually to high dosages and the other for environmental agents where exposure is generally involuntary and to low levels. For drugs, a use-in-pregnancy rating is utilized, wherein the letters A, B, C, D, and X are used to classify the evidence that a chemical poses a risk to the human conceptus. For example, drugs are placed in category A if

adequate, well-controlled studies in pregnant humans have failed to demonstrate a risk, and in category X (contraindicated for pregnancy) if studies in animals or humans, or investigational or postmarketing reports, have shown fetal risk that clearly outweighs any possible benefit to the patient. The default category C (risks cannot be ruled out) is assigned when there is a lack of human studies and animal studies are either lacking or are positive for fetal risk, but the benefits may justify the potential risk. Categories B and D represent areas of relatively lesser or greater concern for risk, respectively.

For environmental agents, the purpose of the risk assessment process for developmental toxicity is generally to define the dose, route, timing, and duration of exposure that induces effects at the lowest level in the most relevant laboratory animal model. The exposure associated with this "critical effect" is then subjected to a variety of safety or uncertainty factors in order to derive an exposure level for humans that is presumed to be relatively safe. In the absence of definitive animal test data, certain default assumptions are generally made:

1. An agent that produces an adverse developmental effect in experimental animals will potentially pose a hazard to humans following sufficient exposure during development.
2. All four manifestations of developmental toxicity (death, structural abnormalities, growth alterations, and functional deficits) are of concern.
3. The specific types of developmental effects seen in animal studies are not necessarily the same as those that may be produced in humans.
4. The most appropriate species is used to estimate human risk when data are available (in the absence of such data, the most sensitive species is appropriate).
5. In general, a threshold is assumed for the dose–response curve for agents that produce developmental toxicity.

Two approaches to aid defining developmental risk include the benchmark-dose approach and biologically based dose–response modeling. The use of uncertainty factors applied to an experimentally derived NOAEL to arrive at a presumed safe level of human exposure assumes that a threshold for developmental toxicity exists. The available USEPA's Benchmark Dose Software is helping to make this approach a method of choice for many risk assessment organizations. The biologically based dose–response model integrates pharmacokinetic data on tissue dosimetry with molecular, cellular and tissue response, and developmental toxicity.

TABLE 10–6 Seventeen intercellular signaling pathways used in development by most metazoans.

Period during Development	Signaling Pathway
Before organogenesis; later for growth and tissue renewal	1. Wingless–Int pathway 2. Transforming growth factor β pathway 3. Hedgehog pathway 4. Receptor tyrosine kinase pathway 5. Notch–Delta pathway 6. Cytokine pathway (STAT pathway)
Organogenesis and cytodifferentiation; later for growth and tissue renewal	7. Interleukin-1-toll nuclear factor-kappa B pathway 8. Nuclear hormone receptor pathway 9. Apoptosis pathway 10. Receptor phosphotyrosine phosphatase pathway
Larval and adult physiology	11. Receptor guanylate cyclase pathway 12. Nitric oxide receptor pathway 13. G-protein-coupled receptor (large G proteins) pathway 14. Integrin pathway 15. Cadherin pathway 16. Gap junction pathway 17. Ligand-gated cation channel pathway

PATHWAYS TO THE FUTURE

There are several mechanisms of normal development that are conserved in diverse animals, including the fruit fly, roundworm, zebrafish, frog, chick, and mouse. Seventeen conserved intercellular signaling pathways are described that are used repeatedly at different times and locations during development of these and other animal species, as well as in humans (Table 10–6). The conserved nature of these key pathways provides a strong scientific rationale for using these animal models to advantage for developmental toxicology. These organisms have well-known genetics, embryology, and rapid generation times, and they are also amenable to genetic manipulation to enhance the sensitivity of specific developmental pathways or to incorporate human genes to answer questions of interspecies extrapolation.

Increased understanding of human genetic polymorphisms and their contribution to susceptibility to birth defects, use of sensitized animal models for high- to low-dose extrapolation, use of stress/checkpoint pathways as indicators of developmental toxicity, implementation of bioinformatic systems to improve data archival and retrieval, and increased multidisciplinary education and research on the causes of birth defects will aid assessment of the developmental risk of toxicants.

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QUESTIONS

1. Diethylstilbestrol (DES):
   a. was used to treat morning sickness from the 1940s to the 1970s.
   b. was found to affect only female offspring in exposed pregnancies.
   c. greatly affects the development of the fetal brain.
   d. exposure increases the risk of clear cell adenocarcinoma of the vagina.
   e. is now used to treat leprosy patients.

2. Early (prenatal) exposure to which of the following teratogens is most often characterized by craniofacial dysmorphism?
   a. thalidomide.
   b. retinol.
   c. ethanol.
   d. tobacco smoke.
   e. diethylstilbestrol (DES).

3. The nervous system is derived from which of the following germ layers?
   a. ectoderm.
   b. mesoderm.
   c. epidermal placodes.
   d. paraxial mesoderm.
   e. endoderm.

4. Toxin exposure during which of the following periods is likely to have the LEAST toxic effect on the developing fetus?
   a. gastrulation.
   b. organogenesis.
   c. preimplantation.
   d. third trimester.
   e. first trimester.

5. Regarding prenatal teratogen exposure, which of the following statements is FALSE?
   a. Major effects include growth retardation and malformations.
   b. Exposure to teratogens during critical developmental periods will have more severe effects on the fetus.
   c. There is considered to be a toxin level threshold below which the fetus is capable of repairing itself.
   d. The immune system of the fetus is primitive, so the fetus has little to no ability to fight off chemicals and repair itself.
   e. Embryo lethality becomes more likely as the toxic dose is increased.

6. Which of the following stages of the cell cycle are important in monitoring DNA damage and inhibiting progression of the cell cycle?
   a. G_1–S, anaphase, M–G_1.
   b. G_1–S, S, G_2–M.
   c. S, prophase, G_1.
   d. G_2–M, prophase.
   e. M–G_1, anaphase.

7. Which of the following molecules is NOT important in determining the ultimate outcome of embryonal DNA damage?
   a. p53.
   b. Bax.
   c. Bcl-2.
   d. c-Myc.
   e. NF-κB.

8. Which of the following is NOT a physiologic response to pregnancy?
   a. increased cardiac output.
   b. increased blood volume.
   c. increased peripheral vascular resistance.
   d. decreased plasma proteins.
   e. increased extracellular space.

9. All of the following statements are true EXCEPT:
   a. Offspring of white mothers have a higher incidence of cleft lip or palate than do black mothers, after adjusting for paternal race.
   b. Cytomegalovirus (CMV) is a common viral cause of birth defects.
   c. Folate supplementation during pregnancy decreases the risk of neural tube defects.
   d. Cigarette smoke and ethanol are both toxic to the placenta.
   e. In humans, there is a negative correlation between stress and low birth weight.

10. Which of the following is NOT a mechanism involving the endocrine system by which chemicals induce developmental toxicity?
    a. acting as steroid hormone receptor ligands.
    b. disrupting normal function of steroid hormone metabolizing enzymes.
    c. disturbing the release of hormones from the hypothalamus.
    d. disturbing the release of hormones from the pituitary gland.
    e. elimination of natural hormones.

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CHAPTER 11

Toxic Responses of the Blood

John C. Bloom, Andrew E. Schade, and John T. Brandt

BLOOD AS A TARGET ORGAN

HEMATOPOIESIS

TOXICOLOGY OF THE ERYTHRON

The Erythrocyte
Alterations in Red Cell Production
Alterations in the Respiratory Function of Hemoglobin
Homotropic Effects
Heterotropic Effects
Alterations in Erythrocyte Survival
Nonimmune Hemolytic Anemia
Immune Hemolytic Anemia

TOXICOLOGY OF THE LEUKON

Components of Blood Leukocytes
Evaluation of Granulocytes
Toxic Effects on Granulocytes
Effects on Proliferation
Effects on Function
Idiosyncratic Toxic Neutropenia
Mechanisms of Toxic Neutropenia

LEUKEMOGENESIS AS A TOXIC RESPONSE

Human Leukemias
Mechanisms of Toxic Leukemogenesis
Leukemogenic Agents

TOXICOLOGY OF PLATELETS AND HEMOSTASIS

Toxic Effects on Platelets
The Thrombocyte
Thrombocytopenia
Toxic Effects on Platelet Function
Toxic Effects on Fibrin Clot Formation
Coagulation
Decreased Synthesis of Coagulation Proteins
Increased Clearance of Coagulation Factors
Toxicology of Agents Used to Modulate Hemostasis
Oral Anticoagulants
Heparin
Fibrinolytic Agents
Inhibitors of Fibrinolysis

RISK ASSESSMENT

KEY POINTS

- Hematotoxicology is the study of adverse effects of exogenous chemicals on blood and blood-forming tissues.
- Direct or indirect damage to blood cells and their precursors includes tissue hypoxia, hemorrhage, and infection.
- Xenobiotic-induced aplastic anemia is a life-threatening disorder characterized by peripheral blood pancytopenia, reticulocytopenia, and bone marrow hypoplasia.
- Idiosyncratic xenobiotic-induced agranulocytosis may involve a sudden depletion of circulating neutrophils concomitant with exposure that persists as long as the agent or its metabolites are in the circulation.
- Leukemias are proliferative disorders of hematopoietic tissue that originate from individual bone marrow cells.
- Xenobiotic-induced thrombocytopenia may result from increased platelet destruction or decreased platelet production, which lead to decreased platelet aggregation and bleeding disorders.
- Blood coagulation is a complex process involving a number of proteins whose synthesis and function can be altered by many xenobiotics.

BLOOD AS A TARGET ORGAN

Hematotoxicology is the study of adverse effects of exogenous chemicals on blood and blood-forming tissues. T e delivery of oxygen to tissues throughout the body, maintaining vascular integrity and providing the many affector and effector immune functions necessary for host defense, requires a prodigious proliferative and regenerative capacity. Each of the various blood cells (erythrocytes, granulocytes, and platelets) is produced at a rate of approximately 1–3 million/s in a healthy adult; this characteristic makes hematopoietic tissue a particularly sensitive target for cytoreductive or antimitotic agents, such as those used to treat cancer, infection, and immune-mediated disorders. T is tissue is also susceptible to secondary effects of toxic agents that affect the supply of nutrients, such as iron; the clearance of toxins and metabolites, such as urea; or the production of vital growth factors, such as erythropoietin (EPO) and granulocyte colony-stimulating factor (G-CSF). T e consequences of direct or indirect damage to blood cells and their precursors are predictable and potentially life-threatening. T ey include hypoxia, hemorrhage, and infection.

Hematotoxicity may be regarded as primary toxicity, where one or more blood components are directly affected, or secondary, where the toxic effect is a consequence of other tissue injury or systemic disturbances. Primary toxicity is regarded as among the serious effects of xenobiotics, particularly drugs. Secondary toxicity is exceedingly common, due to the propensity of blood cells to reflect various local and systemic effects of toxicants on other tissues.

HEMATOPOIESIS

T e production of blood cells, or hematopoiesis, is a highly regulated sequence of events by which blood cell precursors proliferate and differentiate. T e location of hematopoiesis changes throughout one's life. For instance, fetal hematopoiesis is located in the liver, spleen, bone marrow, thymus, and lymph nodes, while the primary location in adults is the bone marrow of the axial skeleton and proximal limbs. Two types of bone marrow exist: (1) red marrow, which is active in hematopoiesis, and (2) yellow marrow, which is called so because it turns fatty as it ceases participation in hematopoiesis.

Whereas the central function of bone marrow is hematopoiesis and lymphopoiesis (production of a subset of white blood cells), bone marrow is also one of the sites of the mononuclear phagocyte system (MPS), contributing monocytes that differentiate into phagocytic cells in other tissues. A complex interplay of developing cells with stromal (connective tissue) cells, extracellular matrix components, and cytokines makes up the hematopoietic inductive microenvironment. Each lineage is supported within a specific niche, and an array of cytokines and chemokines directs a particular progenitor cell to the appropriate niche.

TOXICOLOGY OF THE ERYTHRON

The Erythrocyte

Erythrocytes (red blood cells [RBCs]) comprise 40% to 45% of the circulating blood volume and serve as the principal vehicle for transportation of oxygen from the lungs to peripheral tissues and of carbon dioxide from tissues to the lung. Erythrocytes are also involved as a carrier and/or reservoir for drugs and toxins. Xenobiotics may affect the production, function, and survival of erythrocytes. T ese effects most frequently manifest as a change in the circulating red cell mass, usually resulting in a decrease (anemia). Occasionally, agents that affect the oxygen affinity of hemoglobin lead to an increase in the red cell mass (erythrocytosis), but this is distinctly less common. Shif s in plasma volume can alter the relative concentration of erythrocytes (and, therefore, hemoglobin concentration) and can be confused with true anemia or erythrocytosis.

Two general mechanisms that lead to true anemia are either decreased production or increased destruction of erythrocytes.

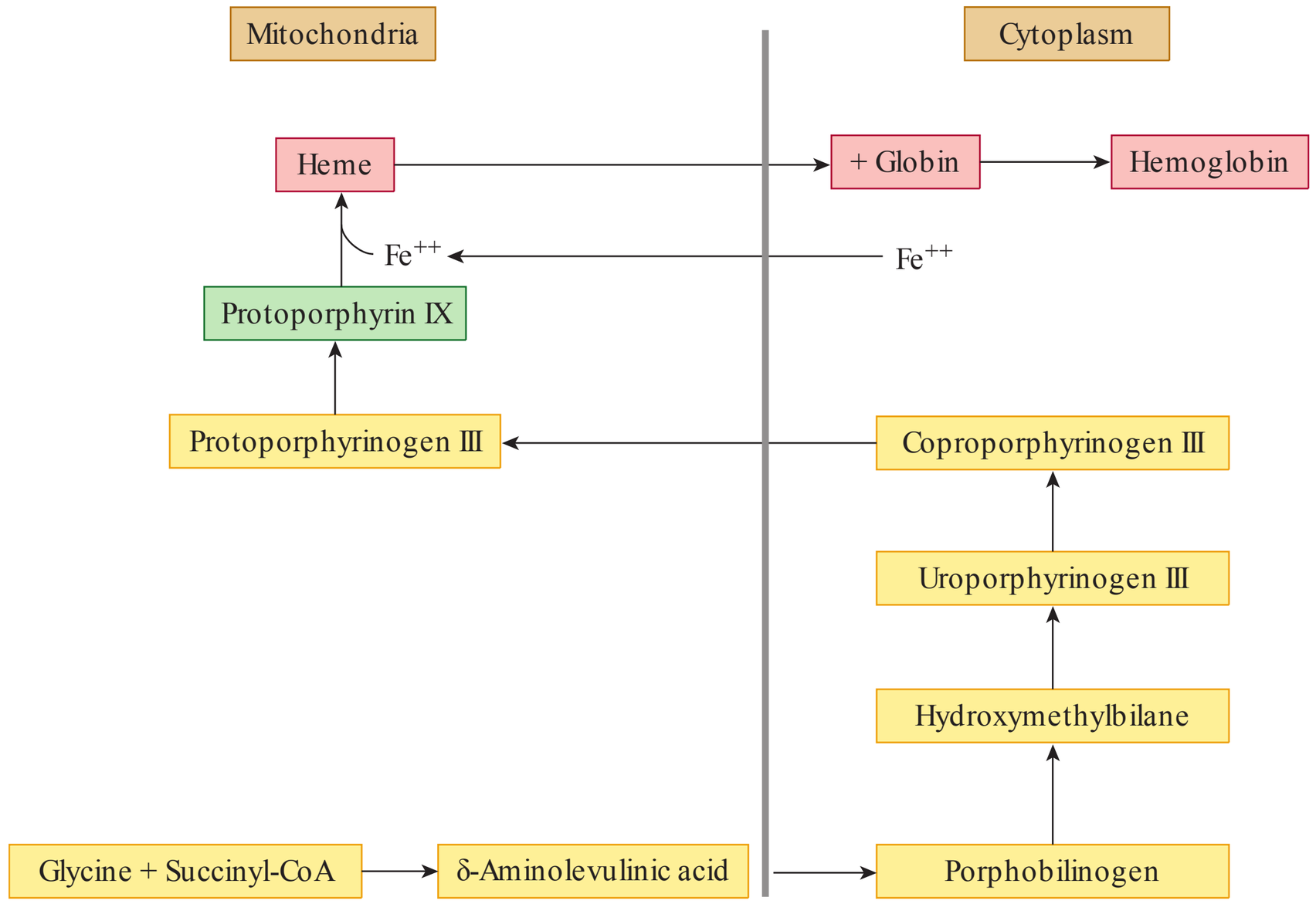

FIGURE 11–1 **Heme and hemoglobin synthesis.** The initial step in heme synthesis is the mitochondria synthesis of δ-aminolevulinic acid, a step that is commonly affected by xenobiotics, including lead. Ferrochelatase catalyzes the incorporation of ferrous iron into the tetrapyrrole protoporphyrin IX. Inhibition of the synthetic pathway leading to protoporphyrin IX, as occurs in the sideroblastic anemias, can cause an imbalance between iron concentration and ferrochelatase activity, resulting in iron deposition within mitochondria. Mitochondrial accumulation of iron is the hallmark lesion of the sideroblastic anemias.

T e usual parameters of a complete blood count (CBC), including RBC count, hemoglobin concentration, and hematocrit (also referred to as packed cell volume [PCV]) can establish the presence of anemia. Two additional parameters that are helpful in classifying an anemia are the mean corpuscular volume (MCV) and the reticulocyte count. Increased destruction is usually accompanied by an increase in reticulocytes (young erythrocytes containing residual RNA). Two related processes contribute to the increased number of reticulocytes in humans. First, increased destruction is accompanied by a compensatory increase in bone marrow production, with an increase in the number of cells being released from the marrow into the circulation. Second, during compensatory erythroid hyperplasia, the marrow releases reticulocytes earlier in their life span and thus the reticulocytes persist for a longer period in the peripheral blood.

Alterations in Red Cell Production

Erythrocyte production is a continuous process that is dependent on frequent cell division and a high rate of hemoglobin synthesis. Adult hemoglobin (hemoglobin A) is a tetramer composed of two α-globin chains and two β-globin chains, each with a heme residue.

Abnormalities that lead to decreased hemoglobin synthesis are relatively common (e.g., iron deficiency). An imbalance between α- and β-chain production is the basis of congenital thalassemia syndromes and results in decreased hemoglobin production and microcytosis. Xenobiotics can affect globin chain synthesis and alter the composition of hemoglobin within erythrocytes.

Synthesis of heme requires incorporation of iron into a porphyrin ring (Figure 11–1). Iron deficiency is usually the result of dietary deficiency or increased blood loss. Any drug that contributes to blood loss may potentiate the risk of developing iron def ciency anemia. Defects in the synthesis of porphyrin ring of heme can lead to sideroblastic anemia, with its characteristic accumulation of iron in bone marrow erythroblasts. T e accumulated iron precipitates within mitochondria causing injury. A number of xenobiotics (Table 11–1) interfere with

TABLE 11–1 Xenobiotics associated with sideroblastic anemia.

Chloramphenicol	Isoniazid
Copper chelation/deficiency	Lead intoxication
Cycloserine	Pyrazinamide
Ethanol	Zinc intoxication

one or more steps in erythroblast heme synthesis and result in sideroblastic anemia.

Hematopoiesis requires active DNA synthesis and frequent mitoses. Folate and vitamin B_{12} are necessary to maintain synthesis of thymidine for incorporation into DNA. Deficiency of folate and/or vitamin B_{12} results in megaloblastic anemia, a result of improper cell division. Xenobiotics that may contribute to a deficiency of vitamin B_{12} and/or folate are listed in Table 11–2.

Many antiproliferative agents used in the treatment of malignancy predictably inhibit hematopoiesis, including erythropoiesis. T e resulting bone marrow toxicity may be dose-limiting. Drugs, such as amifostine, have been developed that may help protect against the marrow toxicity of these agents.

Drug-induced aplastic anemia may represent either a predictable or idiosyncratic reaction to a xenobiotic. T is life-threatening disorder is characterized by peripheral blood pancytopenia, reticulocytopenia, and bone marrow hypoplasia. Agents associated with the development of aplastic anemia are listed in Table 11–3. Pure red cell aplasia is a syndrome that may be due to genetic defects, infection, immune-mediated injury, myelodysplasia, drugs, or other toxicants, in which the decrease in marrow production is limited to the erythroid lineage.

TABLE 11–2 Xenobiotics associated with megaloblastic anemia.

B_{12} Deficiency	Folate Deficiency
Antimetabolites	Neomycin
p-Aminosalicylic acid	Omeprazole
Carbamazepine	Phenobarbital
Cholestyramine	Phenytoin
Colchicine	Primidone
Ethanol	Sulfasalazine
Fish tapeworm	Triamterine
Hemodialysis	Zidovudine
Malabsorption syndromes	

Alterations in the Respiratory Function of Hemoglobin

Hemoglobin transports oxygen and carbon dioxide between the lungs and tissues. T e individual globin units show cooperativity in the binding of oxygen, resulting in the

TABLE 11–3 Drugs and chemicals associated with the development of aplastic anemia.

Allopurinol	Diclofenac	Penicillin
Amphotericin B	Dinitrophenol	Phenylbutazone
Azidothymidine	Ethosuximide	Potassium perchlorate
Benzene	Felbamate	Propylthiouracil
Bismuth	Gold	Pyrimethamine
Carbamazepine	Indomethacin	Quinacrine
Carbimazole	Isoniazid	Streptomycin
Carbon tetrachloride	Mefloquine	Sulfamethoxypyridazine
Carbutamide	Mepazine	Sulfisoxazole
Chloramphenicol	Meprobamate	Sulfonamides
Chlordane	Mercury	Tetracycline
Chlordiazepoxide	Methazolamide	Thiocyanate
Chlorphenothane	Methicillin	Ticlopidine
Chlorpropamide	Methylphenylethylhydantoin	Tolbutamide
Chlorpromazine	Methylmercaptoimidazole	Trifluoroperazine
Chlortetracycline	Metolazone	Trimethadione
Cimetidine	Organic arsenicals	Tripelennamine
D-Penicillamine	Parathion	

familiar sigmoid shape to the oxygen dissociation curve (Figure 11–2).

Homotropic E**ff**ects—One of the most important homotropic (intrinsic) properties of oxyhemoglobin is the slow but consistent oxidation of heme iron to the ferric state to form methemoglobin, which is not capable of binding and transporting oxygen. The presence of methemoglobin in a hemoglobin tetramer results in a leftward shift of the oxygen dissociation curve (Figure 11–2). The combination of decreased oxygen content and increased affinity may significantly impair delivery of oxygen to tissues, as the oxygen will not be readily released from hemoglobin in the periphery.

T e normal erythrocyte has metabolic mechanisms for reducing heme iron back to the ferrous state. Failure of these control mechanisms leads to increased levels of methemoglobin, or methemoglobinemia. Various chemicals that cause methemoglobinemia are shown in Table 11–4. Most patients tolerate low levels (< 10%) of methemoglobin without clinical symptoms. Higher levels lead to tissue hypoxemia that is eventually fatal.

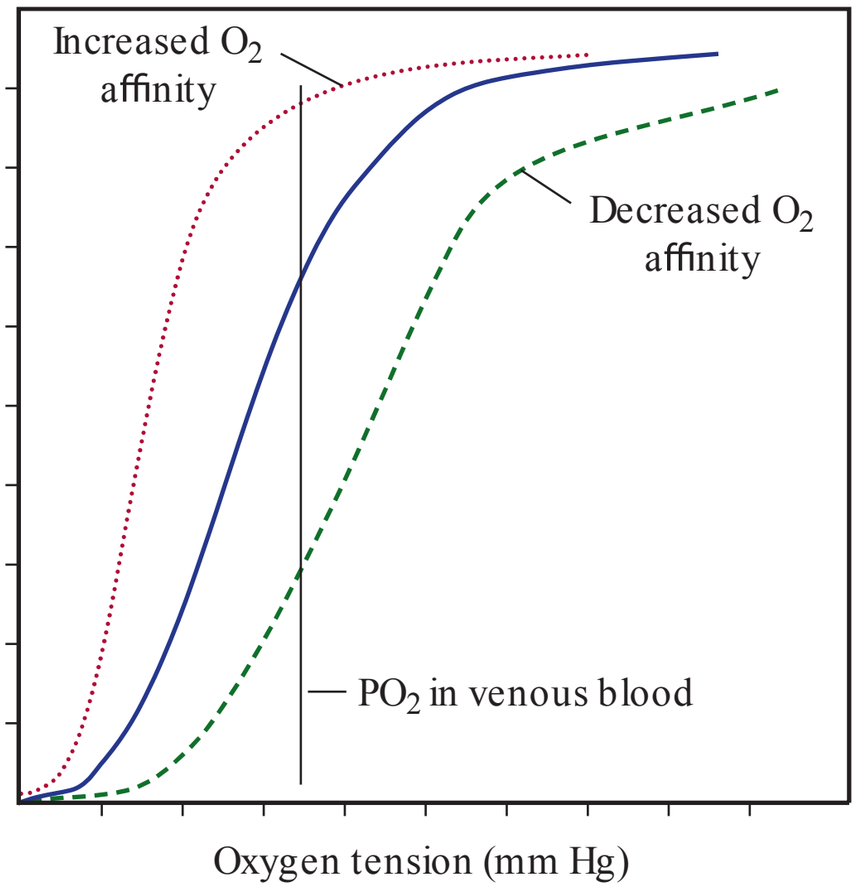

FIGURE 11–2 **Hemoglobin-oxygen dissociation curves.** The normal oxygen dissociation curve (solid line) has a sigmoid shape due to the cooperative interaction between the four globin chains in the hemoglobin molecule. Fully deoxygenated hemoglobin has a relatively low af nity for oxygen. Interaction of oxygen with one heme–iron moiety induces a conformational change in that globin chain. Through surface interactions, that conformational change affects the other globin chains, causing a conformational change in all of the globin chains that increases their af nity for oxygen. Homotropic and heterotropic parameters also affect the af nity of hemoglobin for oxygen. An increase in oxygen af nity results in a shift to the left in the oxygen dissociation curve. Such a shift may decrease oxygen delivery to the tissues. A decrease in oxygen af nity results in a shift to the right in the oxygen dissociation curve, facilitating oxygen delivery to the tissues.

Heterotropic E**ff**ects—T ere are three major heterotropic (extrinsic) effectors of hemoglobin function: pH, erythrocyte 2,3-bisphosphoglycerate (2,3-BPG, formerly designated 2,3-diphosphoglycerate [2,3-DPG]) concentration, and temperature. A decrease in pH (e.g., lactic acid and carbon dioxide) lowers the affinity of hemoglobin for oxygen causing a right shif in the oxygen dissociation curve and facilitating the delivery of oxygen to tissues (Figure 11–2). As bicarbonate and carbon dioxide equilibrate in the lung, the hydrogen ion concentration decreases, which results in increased affinity of hemoglobin for oxygen and facilitated oxygen uptake.

Binding of 2,3-BPG to deoxyhemoglobin results in reduced oxygen affinity (a shif to the right of the oxygen dissociation curve), which promotes oxygen delivery to peripheral tissues. T e conformational change induced by binding of oxygen to hemoglobin alters the binding site for 2,3-BPG and results in release of 2,3-BPG from hemoglobin. T is facilitates uptake of more oxygen in the lungs for delivery to tissues. T e concentration of 2,3-BPG increases whenever there is tissue hypoxemia but may decrease in the presence of acidosis or hypophosphatemia.

T e oxygen affinity of hemoglobin decreases as the body temperature increases. T is facilitates delivery of oxygen to tissues during periods of extreme exercise and febrile illnesses associated with increased temperature. Correspondingly, oxygen affinity increases and delivery decreases during hypothermia.

TABLE 11–4 Environmental and therapeutic agents associated with methemoglobinemia.

Aminobenzenes	Nitrobenzenes
Amyl nitrate	Nitroethane
Aniline dyes and aniline derivatives	Nitroglycerin
Benzocaine	Nitrotoluenes
Beta-naphthol disulfonate	ortho-Toluidine
Butyl nitrite	para-Toluidine
Dapsone	Potassium chlorate
Flutamide	Prilocaine
Gasoline additives	Primaquine
Isobutyl nitrite	Phenacetin
Lidocaine	Phenazopyridine
Methylene blue	Quinones
Nitrates	Silver nitrate
Nitric oxide	Sulfonamide
Nitrites	Trinitrotoluene

T e respiratory function of hemoglobin may also be impaired by blocking the ligand binding site with other substances. Carbon monoxide has a relatively low rate of association with deoxyhemoglobin but shows high affinity once bound, and causes a lef shif in the oxygen dissociation curve, further compromising oxygen delivery to the tissues. Nitric oxide, an important vasodilator that modulates vascular tone, binds avidly to heme iron. Erythrocytes can influence the availability of nitric oxide in parts of the circulation because the nitric oxide is bound to erythrocyte hemoglobin.

Alterations in Erythrocyte Survival

T e normal survival of erythrocytes in the circulation is about 120 days. During this period, erythrocytes are exposed to a various oxidative injuries and must negotiate the tortuous passages of the microcirculation and the spleen. T is requires a deformable cell membrane and energy to maintain the sodium–potassium gradients and repair mechanisms. Very little protein synthesis occurs during this time, as erythrocytes are anucleate when they enter the circulation and residual mRNA is rapidly lost over the first 1 to 2 days in the circulation. Consequently, senescence occurs over time until the aged erythrocytes are removed by the spleen, where the iron is recovered for reutilization in heme synthesis. Any insult that increases oxidative injury, decreases metabolism, or alters the membrane may cause a decrease in erythrocyte concentration and a corresponding anemia.

Nonimmune Hemolytic Anemia

Microangiopathic Anemias—Intravascular fragmentation of erythrocytes gives rise to the microangiopathic hemolytic anemias. T e hallmark of this process is the presence of schistocytes (fragmented RBCs) in peripheral blood. T e formation of fibrin strands in the microcirculation is a common mechanism for RBC fragmentation. T is may occur in the setting of disseminated intravascular coagulation, sepsis, hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP). T e erythrocytes are essentially sliced into fragments by the fibrin strands that extend across the vascular lumen and impede the flow of erythrocytes through the vasculature. Excessive fragmentation can also be seen in the presence of abnormal vasculature. T e high shear associated with malignant hypertension may also lead to RBC fragmentation.

Other Mechanical Injuries—RBC destruction can also occur as a result of mechanical stress. For instance, march hemoglobinuria is an episodic anemia resulting from mechanical trauma to the feet from prolonged activity. Major thermal burns are also associated with a hemolytic process. T e erythrocyte membrane becomes unstable as temperature increases to the point where small RBC fragments break off and the membrane reseals. Consequently, these abnormal cell fragments are removed by the spleen, leading to anemia.

Infectious Diseases—Infectious diseases may be associated with significant hemolysis, by either direct effect on the erythrocyte or an immune-mediated hemolytic process. Erythrocytes parasitized in malaria and babesiosis may undergo destruction. Clostridial infections are associated with release of hemolytic toxins that enter the circulation and lyse erythrocytes.

Oxidative Hemolysis—Molecular oxygen is a reactive and potentially toxic chemical species; consequently, the normal respiratory function of erythrocytes generates oxidative stress on a continuous basis. T ere are several mechanisms that protect against oxidative injury in erythrocytes including NADH-diaphorase, superoxide dismutase, catalase, and the glutathione pathway.

Xenobiotics capable of inducing oxidative injury in erythrocytes are listed in Table 11–5. T ese agents appear to potentiate the normal redox reactions and are capable of overwhelming the usual protective mechanisms. T e interaction between these xenobiotics and hemoglobin leads to the formation of free radicals that denature critical proteins, including hemoglobin, thiol-dependent enzymes, and components of the erythrocyte membrane. Significant oxidative injury usually occurs when the concentration of the xenobiotic is high enough to overcome the normal protective mechanisms, or, more commonly, when there is an underlying defect in the protective mechanisms.

T e most common enzyme defect associated with oxidative hemolysis is glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, a sex-linked disorder characterized by diminished G-6-PD activity. It is of en clinically asymptomatic until the erythrocytes are exposed to oxidative stress from the host response to infection or exposure to xenobiotics.

Nonoxidative Chemical-induced Hemolysis—Exposure to some xenobiotics is associated with hemolysis without

TABLE 11–5 Xenobiotics associated with oxidative injury.

Acetanilide	Phenacetin
Aminosalicylic acid	Phenol
Chlorates	Phenylhydrazine
Dapsone	Primaquine
Furazolidone	Phenazopyridine
Hydroxylamine	Sodium sulfoxone
Methylene blue	Sulfamethoxypyridazine
Nalidixic acid	Sulfanilamide
Naphthalene	Sulfasalazine
Nitrofurantoin	Toluidine blue
Nitrobenzene	

significant oxidative injury. For example, inhalation of gaseous arsenic hydride (arsine) can result in severe hemolysis, with anemia, jaundice, and hemoglobinuria. Several elements are known to cause hemolysis in the absence of oxidative damage, namely, lead, copper, and chromium. Additionally, significant hemolysis may occur with biologic toxins found in insect and snake venoms.

Immune Hemolytic Anemia—Immunologic destruction of erythrocytes is mediated by the interaction of IgG or IgM antibodies with antigens expressed on the surface of the erythrocyte. In the case of autoimmune hemolytic anemia, the antigens are intrinsic components of the patient's own erythrocytes.

A number of mechanisms have been implicated in xenobiotic-mediated antibody binding to erythrocytes. Some drugs, of which penicillin is a prototype, appear to bind to the surface of the cell, with the "foreign" drug acting as a hapten and eliciting an immune response. The antibodies that arise in this type of response only bind to drug-coated erythrocytes. Other drugs, of which quinidine is a prototype, bind to components of the erythrocyte surface and induce a conformational change in one or more components of the membrane. A third mechanism, for which α-methyldopa is a prototype, results in production of a drug-induced autoantibody that cannot be distinguished from the antibodies arising in idiopathic autoimmune hemolytic anemia.

TOXICOLOGY OF THE LEUKON

Components of Blood Leukocytes

The leukon consists of leukocytes, or white blood cells, including granulocytes, which may be subdivided into neutrophils, eosinophils, and basophils; monocytes; and lymphocytes. Granulocytes and monocytes are nucleated ameboid cells that are phagocytic. They play a central role in the inflammatory response and host defense. Unlike the RBC, which resides exclusively within blood, granulocytes and monocytes merely pass through the blood on their way to extravascular tissues, where they reside in large numbers.

Granulocytes are defined by the characteristics of their cytoplasmic granules as they appear on a blood smear. Neutrophils, the largest component of blood leukocytes, are highly specialized in the mediation of inflammation and the ingestion and destruction of pathogenic microorganisms. Eosinophils and basophils modulate inflammation through the release of various mediators.

Evaluation of Granulocytes

In the blood, neutrophils are distributed between circulating and marginated pools, which are of equal size in humans and in constant equilibrium. A blood neutrophil count assesses only the circulating pool, which remains remarkably constant (1800 to 7500 μL^{-1}) in a healthy adult human. During inflammation, an increased number of immature (non-segmented) granulocytes may be seen in peripheral blood. In certain conditions, neutrophils may show morphological changes indicative of toxicity.

Toxic Effects on Granulocytes

Effects on Proliferation—The high rate of proliferation of neutrophils makes their progenitor and precursor granulocyte pool particularly susceptible to inhibitors of mitosis. Such effects by cytotoxic drugs are generally nonspecific as they similarly affect cells of the dermis, gastrointestinal tract, and other rapidly dividing tissues. Agents that affect both neutrophils and monocytes pose a greater risk for toxic sequelae, such as infection. Such effects tend to be dose-related, with mononuclear phagocyte recovery preceding neutrophil recovery.

Myelotoxicity is commonly seen with cytoreductive cancer chemotherapy agents, which often act to inhibit DNA synthesis or directly attack its integrity through the formation of DNA adducts or enzyme-mediated breaks. However, this is changing, as more cancer cell-targeted, normal-tissue-sparing anticancer agents are being developed. The toxicity associated with cytotoxic drugs, however, remains important in that it is often dose-limiting (even with some of the newer drugs) with serious manifestations that include febrile neutropenia associated with life-threatening infections. While these drugs can be toxic to both resting and actively dividing cells, nonproliferating cells such as metamyelocytes, bands, and mature neutrophils are relatively resistant. Because stem cells cycle slowly, they are minimally affected by a single administration of a cytotoxic drug. Sustained exposure to drugs affecting stem cells is believed to cause more prolonged myelosuppression.

Two innovations have had a dramatic impact on cancer chemotherapy and the dose-limiting myelotoxicity associated with these drugs: (1) the development of drugs with cancer cell–specific molecular targets that are relatively bone marrow sparing, such as those that target aberrant growth factor receptor signaling, apoptosis, angiogenesis, and other metabolic, immune, inflammatory, and mutation-promoting pathways that selectively advantage tumor cells, and (2) cotreatment with hematopoietic growth factors mitigates or successfully rescues patients from the effects of myelosuppression. Cytokine-induced differentiation therapy of leukemias is another exciting treatment modality. The prospect of exaggerated pharmacology and off-target effects of these sophisticated interventions should provide the preclinical toxicologist and oncologist with interesting hematotoxicologic challenges.

Effects on Function—While there are a variety of disorders associated with defects in the parameters of neutrophil function discussed above, demonstrable in vivo effects associated with drugs and nontherapeutic chemicals are surprisingly few. Examples include ethanol and glucocorticoids, which impair phagocytosis and microbe ingestion. Iohexol and ioxaglate, components of radiographic contrast media, have also been reported to inhibit phagocytosis. Superoxide production,

required for microbial killing and chemotaxis, is reportedly reduced in patients using parenteral heroin as well as in former opiate abusers on long-term methadone maintenance. Chemotaxis is also impaired following treatment with zinc salts in antiacne preparations.

Idiosyncratic Toxic Neutropenia—Of greater concern are agents that unexpectedly damage neutrophils and granulocyte precursors and induce agranulocytosis, which is characterized by a profound depletion in blood neutrophils to less than 500 μL^{-1}. Such injury occurs in specifically conditioned individuals, and is therefore termed idiosyncratic.

Idiosyncratic xenobiotic-induced agranulocytosis may involve a sudden depletion of circulating neutrophils concomitant with exposure, which may persist as long as the agent or its metabolites persist in the circulation. Hematopoietic function is usually restored when the agent is detoxified or excreted. Toxicants affecting uncommitted stem cells induce total marrow failure, as seen in aplastic anemia. After agents that affect more differentiated precursors, surviving uncommitted stem cells eventually produce recovery, provided that the risk of infection is successfully managed during the leukopenic episodes.

Mechanisms of Toxic Neutropenia—In immune-mediated neutropenia, antigen–antibody reactions lead to destruction of peripheral neutrophils, granulocyte precursors, or both. As with RBCs, an immunogenic xenobiotic can act as a hapten, where the agent must be physically present to cause cell damage, or alternatively, may induce immunogenic cells to produce antineutrophil antibodies that do not require the drug to be present.

Non-immune-mediated toxic neutropenia often shows a genetic predisposition. Direct damage may cause inhibition of granulopoiesis or neutrophil function. Some studies suggest that a buildup of toxic oxidants generated by leukocytes can result in neutrophil damage.

Examples of agents associated with immune and nonimmune neutropenia/agranulocytosis are listed in Table 11–6.

TABLE 11–6 Examples of toxicants that cause immune and nonimmune idiopathic neutropenia.

Drugs Associated with WBC Antibodies	Drugs Not Associated with WBC Antibodies
Aminopyrine	Allopurinol
Ampicillin	Ethambutol
Aprindine	Flurazepam
Azulfidine	Hydrochlorothiazide
Chlorpropamide	Isoniazide
Clozapine	Phenothiazines
CPZ/phenothiazines	Rifampicin
Dicloxacillin	
Gold	
Levamisole	
Lidocaine	
Methimazole	
Metiamide	
Phenytoin	
Procainamide	
Propylthiouracil	
Quinidine	
Tolbutamide	

LEUKEMOGENESIS AS A TOXIC RESPONSE

Human Leukemias

Leukemias are proliferative disorders of hematopoietic tissue that are monoclonal and originate from individual bone marrow cells. Historically they have been classified as myeloid or lymphoid, referring to the major lineages for erythrocytes, granulocytes, thrombocytes, or lymphocytes, respectively. Poorly differentiated phenotypes have been designated as "acute," including acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML), whereas well-differentiated ones are referred to as "chronic" leukemias, which include chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), and the myelodysplastic syndromes (MDS).

There is considerable evidence supporting the notion that leukemogenesis is a multievent progression, which suggest that factors involved in the regulation of hematopoiesis also influence neoplastic transformation. Such factors include cellular growth factors (cytokines), proto-oncogenes, and other growth-promoting genes, as well as additional genetic and epigenetic factors that govern survival, proliferation, and differentiation.

Secondary leukemia is a term used to describe patients with AML or MDS who have a history of environmental, occupational, or therapeutic exposure to hematotoxins or radiation. It also includes patients with AML evolving from antecedent myelodysplastic or other myeloid stem cell disorders. Therapy-related AML and MDS is a term applied to the former group; both are used to distinguish from features of AML that arise de novo. Various cytogenetic findings have been associated with prognosis and response to therapy. It has been suggested that secondary leukemias be redefined as any leukemia with a specific cytogenic or molecular poor prognostic feature due to a presumed predisposing factor.

Mechanisms of Toxic Leukemogenesis

AML is the dominant leukemia associated with drug or chemical exposure, followed by MDS. T is represents a continuum of one toxic response that has been linked to cytogenetic abnormalities, particularly the loss of all or part of chromosomes 5 and 7. Remarkably, the frequency of these deletions in patients who develop MDS and/or AML af er treatment with alkylating or other antineoplastic agents ranges from 67% to 95%, depending on the study. Some of these same changes have been observed in AML patients occupationally exposed to benzene, who also show aneuploidy with a high frequency of involvement of chromosome 7. T e relatively low frequency of deletions in chromosomes 5 and 7 in de novo as compared with secondary AML suggests that these cytogenetic markers can be useful in discriminating between toxic exposures and other etiologies of this leukemia.

Leukemogenic Agents

Most alkylating agents used in cancer chemotherapy can cause MDS and/or AML. Of the aromatic hydrocarbons, only benzene has been proven to be leukemogenic. Treatment with the topoisomerase II inhibitors, etoposide and teniposide, can induce AML.

Exposure to high-dose γ- or x-ray radiation has long been associated with ALL, AML, and CML, as demonstrated in survivors of the atom bombings of Nagasaki and Hiroshima. Less clear is the association of these diseases with low-dose radiation secondary to fallout or diagnostic radiographs. Other controversial agents include 1,3-butadiene, nonionizing radiation (electromagnetic, microwave, infrared, visible, and the high end of the ultraviolet spectrum), cigarette smoking, and formaldehyde.

TOXICOLOGY OF PLATELETS AND HEMOSTASIS

Hemostasis, the stoppage of bleeding or blood flow through an organ, is a multicomponent system responsible for preventing the loss of blood from sites of vascular injury and maintaining circulating blood in a fluid state. Loss of blood is prevented by formation of stable hemostatic plugs. T e major constituents of the hemostatic system include circulating platelets, a variety of plasma proteins, and vascular endothelial cells. Alterations in these components or systemic activation of this system can lead to the clinical manifestations of deranged hemostasis, including excessive bleeding and thrombosis. T e hemostatic system is a frequent target of therapeutic intervention as well as inadvertent expression of the toxic effect of a variety of xenobiotics.

Toxic Ef ects on Platelets

The Thrombocyte—Platelets are essential for formation of a stable hemostatic plug in response to vascular injury. Platelets initially adhere to the damaged blood vessel wall through binding of von Willebrand factor (vWF) with the platelet Ib/IX/V (GP Ib/IX/V) receptor complex. Activation of a pathway of several factors permits fibrinogen and other multivalent adhesive molecules to form cross-links between nearby platelets, resulting in platelet aggregation. Xenobiotics may interfere with the platelet response by causing thrombocytopenia (low platelet levels) or interfering with platelet function.

Thrombocytopenia—Like anemia, thrombocytopenia may be due to decreased production or increased destruction of platelets. T rombocytopenia is a common side effect of intensive chemotherapy, due to the predictable effect of antiproliferative agents on hematopoietic precursors. T rombocytopenia is a clinically significant component of idiosyncratic xenobiotic-induced aplastic anemia. Indeed, the initial manifestation of aplastic anemia may be mucocutaneous bleeding secondary to thrombocytopenia.

Exposure to xenobiotics may cause increased immune-mediated platelet destruction through any one of the several mechanisms. Penicillin is an example of a drug that functions as a hapten, which is a small molecule that only produces a specific immune response if bound to a protein carrier. T e responding antibody then binds to the hapten on the platelet surface, leading to removal of the antibody-coated platelet from the circulation.

A second mechanism of immune thrombocytopenia is initiated by a change in a platelet membrane glycoprotein caused by the xenobiotic. T is elicits an antibody response, with the responding antibody binding to this altered platelet antigen in the presence of drug, resulting in removal of the platelet from the circulation by the mononuclear phagocytic system.

T rombocytopenia is an uncommon, but serious, complication of drugs that inhibit the platelet glycoprotein IIb/IIIa receptor. Inhibitors like abciximab can change the conformation of this receptor, causing exposure of certain peptides (called neoepitopes because they are newly exposed to the immune system) on the factors that react with endogenous antibodies. T is leads to phagocytosis of the platelets associated with these factors. T us, exposure of epitopes that react with naturally occurring antibodies represents a third mechanism of immune-mediated platelet destruction.

Heparin-induced thrombocytopenia (HIT) represents a fourth mechanism of immune-mediated platelet destruction. When heparin (an anticoagulant) binds to certain clotting factors, a neoepitope is exposed, and an immune response is mounted against the neoepitope. T is results in platelet activation and aggregation instead of heparin's normal function of preventing clot formation, which can lead to a risk of thrombosis (pieces of clots falling off and lodging in microvasculature, impairing circulation).

T rombotic thrombocytopenic purpura (TTP) is a syndrome characterized by the sudden onset of thrombocytopenia, a microangiopathic hemolytic anemia, and multisystem organ failure. T e syndrome tends to occur following an infectious disease but may also occur following administration of some drugs. T e pathogenesis of TTP appears to be related

to the ability of unusually large vWf multimers to activate platelets, even in the absence of significant vascular damage. Acquired TTP is associated with the development of an antibody that inhibits the protease responsible for processing very large vWf multimers into smaller multimers; the large multimers persist in circulation and inappropriately activate the platelets. T e organ failure and hemolysis in TTP is due to the formation of platelet-rich microthrombi throughout the circulation. T e development of TTP or TTP-like syndromes has been associated with drugs such as ticlopidine, clopidogrel, cocaine, mitomycin, and cyclosporine.

Hemolytic uremic syndrome (HUS) is a disorder with clinical features similar to those of TTP, but with less severe neurologic complications and predominant renal failure. Sporadic HUS cases have been linked to Escherichia coli infection, but HUS can also occur during therapy with some drugs, including mitomycin. Unlike TTP, the vWf-cleaving protease is normal and the pathogenesis is thought to be related to endothelial cell damage with subsequent platelet activation and thrombus formation.

Toxic Ef ects on Platelet Function—Platelet function is dependent on the coordinated interaction of a number of biochemical response pathways. Major drug groups that affect platelet function include nonsteroidal anti-inflammatory drugs (NSAIDs), β-lactam-containing antibiotics, cardiovascular drugs (particularly β-blockers), psychotropic drugs, anesthetics, antihistamines, and some chemotherapeutic agents.

Xenobiotics may interfere with platelet function through a variety of mechanisms. Some drugs inhibit the phospholipase A_2/cyclooxygenase pathway and synthesis of thromboxane A_2 (e.g., NSAIDs). Other agents appear to interfere with the interaction between platelet agonists and their receptors (e.g., antibiotics, ticlopidine, and clopidogrel). As the platelet response is dependent on rapid increase in cytoplasmic calcium, any agent that interferes with translocation of calcium may inhibit platelet function (e.g., calcium channel blockers). Occasionally, drug-induced antibodies will bind to a critical platelet receptor and inhibit its function.

Toxic Ef ects on Fibrin Clot Formation

Coagulation—Fibrin clot formation results from sequential activation of a series of serine proteases that culminates in the formation of thrombin. T rombin is a multifunctional enzyme that converts fibrinogen to fibrin; activates factors V, VIII, XI, XIII, protein C, and platelets; and interacts with a variety of cells (e.g., leukocytes and endothelial cells), activating cellular signaling pathways.

Decreased Synthesis of Coagulation Proteins—Most proteins involved in the coagulation cascade are synthesized in the liver. T erefore, any agent that impairs liver function may cause a decrease in production of coagulation factors. T e common tests of the coagulation cascade, the prothrombin time (PT) and activated partial thromboplastin time (aPTT), may be used to screen for liver dysfunction and a decrease in clotting factors.

TABLE 11–7 Conditions associated with abnormal synthesis of vitamin K–dependent coagulation factors.

Warfarin and analogs	Intravenous α-tocopherol
Rodenticides (e.g., brodifacoum)	Dietary deficiency
Broad-spectrum antibiotics	Cholestyramine resin
N-Methyl-thiotetrazole cephalosporins	Malabsorption syndromes

Factors II, VII, IX, and X are dependent on vitamin K for their complete synthesis. Anything that interferes with absorption of vitamin K from the intestine or with the reduction of vitamin K epoxide may lead to a deficiency of these factors and a bleeding tendency (Table 11–7).

Increased Clearance of Coagulation Factors—Idiosyncratic reactions to xenobiotics include the formation of antibodies that react with coagulation proteins, forming an immune complex that is rapidly cleared from the circulation resulting in deficiency of the factor. T e factors that are most of en affected by xenobiotics are listed in Table 11–8. In addition to causing increased clearance from the circulation, these antibodies of en inhibit the function of the coagulation factor. Other antibodies have catalytic activity, resulting in proteolysis of the target coagulation factor.

Lupus anticoagulants are antibodies that are directed against phospholipid binding proteins like prothrombin, can potentiate procoagulant mechanisms and interfere with the protein C system, increasing the risk of thrombosis. T e development of lupus anticoagulants has been seen in association with chlorpromazine, procainamide, hydralazine, quinidine, phenytoin, and viral infections.

Toxicology of Agents Used to Modulate Hemostasis

Oral Anticoagulants—Oral anticoagulants (e.g., warfarin) interfere with vitamin K metabolism by preventing the reduction of vitamin K epoxide, resulting in a functional deficiency of reduced vitamin K. T ese drugs are widely used for prophylaxis and therapy of venous and arterial thrombosis. T e therapeutic window for oral anticoagulants is relatively narrow, and there is considerable interindividual variation in the response to a given dose. A number of factors, including concurrent medications and genetics, affect the individual response to oral anticoagulants. For these reasons, therapy with these drugs must be routinely monitored to maximize both safety and efficacy. T is is routinely performed with the PT, with results expressed in terms of the international normalized ratio (INR).

A number of xenobiotics, including foods, have been found to affect the response to oral anticoagulants. Mechanisms for interference with oral anticoagulants include induction or

TABLE 11–8 Relationship between xenobiotics and the development of specific coagulation factor inhibitors.

Coagulation Factor	Xenobiotic
Thrombin	Topical bovine thrombin Fibrin glue
Factor V	Streptomycin Penicillin Gentamicin Cephalosporins Topical bovine thrombin
Factor VIII	Penicillin Ampicillin Chloramphenicol Phenytoin Methyldopa Nitrofurazone Phenylbutazone
Factor XIII	Isoniazid Procainamide Penicillin Phenytoin Practolol
von Willebrand factor	Ciprofloxacin Hydroxyethyl starch Valproic acid Griseofulvin Tetracycline Pesticides

inhibition of biotransformation; interference with absorption of warfarin from the gastrointestinal tract; displacement of warfarin from albumin in plasma, which temporarily increases the bioavailability of warfarin until equilibrium is reestablished; diminished vitamin K availability; and inhibition of the reduction of vitamin K epoxide, which potentiates the effect of oral anticoagulants. Additionally, administration of oral anticoagulants may affect the activity or the half-lives of other medications.

Oral anticoagulants have been associated with warfarin-induced skin necrosis, which is due to development of microvascular thrombosis in skin. T is uncommon effect occurs most commonly in patients deficient in proteins C or S or in patients administered high doses of warfarin too rapidly.

Vitamin K is also necessary for the synthesis of osteocalcin, a major component of bone. Long-term administration of warfarin has been associated with bone demineralization.

Administration of warfarin, particularly during the first 12 weeks of pregnancy, is associated with congenital anomalies in 25% to 30% of exposed infants. Many of the anomalies are related to abnormal bone formation. It is thought that warfarin may interfere with synthesis of additional proteins critical for normal structural development.

Heparin—Heparin is widely used for both prophylaxis and therapy of acute venous thromboembolism. T e major complication associated with heparin therapy is bleeding, which is a direct manifestation of its anticoagulant activity. T e aPTT is commonly used to monitor therapy with unfractionated heparin, a naturally occurring polysaccharide. Long-term administration of heparin is associated with an increased risk of clinically significant osteoporosis.

Fibrinolytic Agents—Fibrinolytic agents dissolve pathogenic thrombi by converting plasminogen, an inactive zymogen, to plasmin, an active proteolytic enzyme. Plasmin is normally tightly regulated and is not freely present in the circulation. However, administration of fibrinolytic agents regularly results in the generation of free plasmin leading to systemic fibrin(ogen)olysis, which is characterized by prolongation of the PT, aPTT, and thrombin time. All of these effects increase the risk of bleeding. Platelet inhibitors and heparin are commonly used in conjunction with fibrinolytic therapy to prevent recurrent thrombosis.

Streptokinase is a protein derived from group C β-hemolytic streptococci that is antigenic in humans. Allergic reactions to the protein can result from streptococcal infection or from exposure to streptokinase-containing fibrinolytic drugs. Acute allergic reactions may occur in 1% to 5% of patients exposed to streptokinase. Allergic reactions also occur with other fibrinolytic agents containing streptokinase (e.g., anisoylated plasminogen–streptokinase complex, alteplase) or streptokinase-derived peptides.

Inhibitors of Fibrinolysis—Antifibrinolytics are commonly used to control bleeding in patients with congenital abnormalities of hemostasis, such as von Willebrand disease. Tranexamic acid and ε-aminocaproic acid are small molecules that block the binding of plasminogen and plasmin to fibrin. Although relatively well tolerated, there is some evidence that administration of these chemicals may increase the risk of thrombosis due to the inhibition of the fibrinolytic system.

Aprotinin is a naturally occurring polypeptide inhibitor of serine protease clotting factors that is immunogenic when administered to humans.

RISK ASSESSMENT

Assessing the risk that exposure to new chemical products poses to humans—in terms of significant toxic effects on hematopoiesis and the functional integrity of blood cells and hemostatic mechanisms—is challenging. T is is due in part to the complexity of hematopoiesis and the range of important tasks that these components perform. Risk assessment includes preclinical testing of animals and clinical trials in humans. It is hoped that in preclinical trials, the test animals will react similarly to humans on exposure to the xenobiotic, and the animals are examined in detail for signs of toxicity.

TABLE 11–9 Examples of problem-driven tests used to characterize hematologic observations in preclinical toxicology.

Reticulocyte count
Heinz body preparation
Cell-associated antibody assays (erythrocyte, platelet, neutrophil)
Erythrocyte osmotic fragility test
Erythrokinetic/ferrokinetic analyses
Cytochemical/histochemical staining
Electron microscopy
In vitro hematopoietic clonogenic assays
Platelet aggregation
Plasma fibrinogen concentration
Clotting factor assays
Thrombin time
Bleeding time

Subsequent clinical trials are conducted in humans and measure myriad parameters of potential toxicity to determine the relative safety or toxicity of the test substance.

Tests used to assess blood and bone marrow in preclinical toxicology studies should provide information on the effects of single- and multiple-dose exposure on erythrocyte parameters (RBC, hemoglobin, PCV, MCV, and MCHC), leukocyte parameters (WBC and absolute differential counts), thrombocyte counts, coagulation tests (PT and aPTT), peripheral blood cell morphology, and bone marrow cytologic and histologic examinations. Additional tests should be employed in a problem-driven fashion as required to better characterize hematotoxicologic potential. Examples of these tests are listed in Table 11–9.

Patient- or population-related risk factors include pharmacogenetic variations in drug metabolism and detoxification that lead to reduced clearance of the agent or production of novel intermediate metabolites, histocompatibility antigens, interaction with drugs or other agents, increased sensitivity of hematopoietic precursors to damage, preexisting disease of the bone marrow, and metabolic defects that predispose to oxidative or other stresses associated with the agent.

A central issue in drug and nontherapeutic chemical development is the predictive value of preclinical toxicology data and the expansive but inevitably limited clinical database for the occurrence of significant hematotoxicity on broad exposure to human populations.

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Q U E S T I O N S

1. Which of the following statements is FALSE regarding true anemia?
   a. Alterations of the mean corpuscular volume are characteristic of anemia.
   b. Increased destruction of erythrocytes can lead to anemia.
   c. Decreased production of erythrocytes is not a common cause of anemia because the bone marrow is continuously renewing the red blood cell pool.
   d. Reticulocytes will live for a longer period of time in the peripheral blood when a person is anemic.
   e. T e main parameters in diagnosing anemia are RBC count, hemoglobin concentration, and hematocrit.

2. Which of the following types of anemia is properly paired with its cause?
   a. iron deficiency anemia—blood loss.
   b. sideroblastic anemia—vitamin B_{12} deficiency.
   c. megaloblastic anemia—folate supplementation.
   d. aplastic anemia—ethanol.
   e. megaloblastic anemia—lead poisoning.

3. T e inability to synthesize the porphyrin ring of hemoglobin will most likely result in which of the following?
   a. iron deficiency anemia.
   b. improper RBC mitosis.
   c. inability to synthesize thymidine.
   d. accumulation of iron within erythroblasts.
   e. bone marrow hypoplasia.

4. Which of the following will cause a right shif in the oxygen dissociation curve?
   a. increased pH.
   b. decreased carbon dioxide concentration.
   c. decreased body temperature.
   d. increased 2,3-BPG concentration.
   e. fetal hemoglobin.

5. All of the following statements regarding erythrocytes are true EXCEPT:
   a. Aged erythrocytes are removed by the liver, where the iron is recycled.
   b. Erythrocytes have a life span of approximately 120 days.
   c. Red blood cells generally lose their nuclei before entering the circulation.
   d. Reticulocytes are immature RBCs that still have a little RNA.
   e. Persons with anemia have a higher than normal reticulocyte:erythrocyte ratio.

6. All of the following statements regarding oxidative hemolysis are true EXCEPT:
   a. Reactive oxygen species are commonly generated by RBC metabolism.
   b. Superoxide dismutase and catalase are enzymes that protect against oxidative damage.
   c. Reduced glutathione (GSH) increases the likelihood of oxidative injuries to RBCs.
   d. Glucose-6-phosphate dehydrogenase deficiency is commonly associated with oxidative hemolysis.
   e. Xenobiotics can cause oxidative injury to RBCs by overcoming the protective mechanisms of the cell.

7. Which of the following sets of leukocytes is properly characterized as granulocytes because of the appearance of cytoplasmic granules on a blood smear?
   a. neutrophils, basophils, and monocytes.
   b. basophils, eosinophils, and lymphocytes.
   c. eosinophils, neutrophils, and lymphocytes.
   d. basophils, eosinophils, and neutrophils.
   e. lymphocytes, neutrophils, and basophils.

8. All of the following statements are true EXCEPT:
   a. Xenobiotics can greatly slow down the proliferation of neutrophils and monocytes, increasing the risk of infection.
   b. Ethanol and cortisol decrease phagocytosis and microbe ingestion by the immune system.
   c. Agranulocytosis is predictable and can be caused by exposure to a number of environmental toxins.
   d. Heroin and methadone abusers have reduced ability to kill microorganisms due to drug-induced reduction in superoxide production.
   e. Toxic neutropenia may be mediated by the immune system.

9. Leukemias:
   - **a.** are of en due to cytogenic abnormalities, particularly damage to or loss of chromosomes 8 and 11.
   - **b.** are rarely caused by agents used in cancer chemotherapy.
   - **c.** originate in circulating blood cells.
   - **d.** are characterized as "acute" if their effects are short-lived and severe.
   - **e.** have long been associated with exposure to x-ray radiation.

10. Regarding platelets and thrombocytopenia, which of the following statements is FALSE?
   - **a.** Platelets can be removed from the circulation through a hapten-mediated pathway that is induced by drugs or chemicals.
   - **b.** Cortisol decreases platelet activity by inhibiting thromboxane prostaglandin synthesis.
   - **c.** Toxins can induce a change in a platelet membrane glycoprotein, leading to recognition and removal of the platelet by phagocytes.
   - **d.** Heparin administration can result in platelet aggregation and cause thrombocytopenia.
   - **e.** T rombotic thrombocytopenic purpura is most commonly caused by infectious disease, but can also be associated with administration of pharmacologic agents.

CHAPTER

12

Toxic Responses of the Immune System

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THE IMMUNE SYSTEM

- Antigen Recognition
  - Immunity
  - Antigen
  - Antibody
  - Complement
  - Antigen Processing
- Innate Immunity
  - Cellular Components: Neutrophils, Macrophages, Natural Killer Cells, NKT, and γδ T Cells
  - Soluble Factors
- Acquired (Adaptive) Immunity
  - Cellular Components: APCs, B Cells, and T Cells
  - Humoral and Cell-mediated Immunity
- Inflammation
  - Cellular Components: Macrophages, Neutrophils, and T Cells
- Immune-mediated Disease
  - Hypersensitivity
  - Autoimmunity
- Developmental Immunology
- Neuroendocrine Immunology

ASSESSMENT OF IMMUNOLOGIC INTEGRITY

- Methods to Assess Immunocompetence
  - General Assessment
  - Functional Assessment
  - Molecular Biology Approaches to Immunotoxicology
  - Mechanistic Approaches to Immunotoxicology
  - Regulatory Approaches to the Assessment of Immunotoxicity
- Animal Models in Immunotoxicology
- Evaluation of Mechanisms of Action

IMMUNE MODULATION BY XENOBIOTICS

- Halogenated Aromatic Hydrocarbons
- Pesticides
- Metals
- Solvents and Related Chemicals
- Mycotoxins
- Natural and Synthetic Hormones
- Therapeutic Agents
  - Immunosuppressive Agents
  - AIDS Therapeutics
  - Biologics
  - Anti-inflammatory Agents
- Drugs of Abuse
- Inhaled Substances
- Ultraviolet Radiation

XENOBIOTIC-INDUCED HYPERSENSITIVITY AND AUTOIMMUNITY

- Hypersensitivity
  - Metals
  - Drugs
  - Latex
  - Food and Genetically Modified Organisms
  - Formaldehyde
- Autoimmunity
- Therapeutic Agents
  - Methyldopa
  - Hydralazine, Isoniazid, and Procainamide
  - Halothane
  - Vinyl Chloride
  - Mercury
  - Silica
  - Hexachlorobenzene

NEW FRONTIERS AND CHALLENGES IN IMMUNOTOXICOLOGY

KEY POINTS

- Immunity is a series of delicately balanced, complex, multicellular, and physiologic mechanisms that allow an individual to distinguish foreign material from "self" and to neutralize and/or eliminate that foreign matter.
- Innate immunity, which eliminates most potential pathogens before significant infection occurs, includes physical and biochemical barriers both inside and outside of the body as well as immune cells designed for specific responses.
- Acquired immunity involves producing a specific immune response to each infectious agent (specificity) and remembering that agent so as to mount a faster response to a future infection by the same agent (memory).
- Autoimmunity occurs when the reactions of the immune system are directed against the body's own tissues, resulting in tissue damage and disease.
- Hypersensitivity reactions require prior exposure leading to sensitization in order to elicit a reaction on subsequent challenge.
- Xenobiotics that alter the immune system can upset the balance between immune recognition and destruction of foreign invaders and the proliferation of these microbes and/or cancer cells.

Immunity is a homeostatic process, a series of delicately balanced, complex, multicellular, and physiologic mechanisms that allow an individual to distinguish foreign material from "self" and to neutralize and/or eliminate the foreign matter. Decreased immunocompetence (immunosuppression) may result in repeated, more severe, or prolonged infections as well as the development of cancer. Immunoenhancement may lead to immune-mediated diseases such as hypersensitivity responses, and if some integral bodily tissue is not identified as self, an autoimmune disease may be the end result.

THE IMMUNE SYSTEM

The immune system comprises numerous lymphoid organs and different cellular populations with a variety of functions. The bone marrow and the thymus support the production of mature T and B lymphocytes and myeloid cells, such as macrophages and polymorphonuclear cells (PMN), and are referred to as primary lymphoid organs.

Within the bone marrow, the cells of the immune system developmentally "commit" to either the lymphoid or myeloid lineages. Cells of the lymphoid lineage make a further commitment to become either T cells or B cells. T-cell precursors are programmed to leave the bone marrow and migrate to the thymus, where they differentiate further.

Mature naive or virgin lymphocytes (those T and B cells that have never undergone antigenic stimulation) are first brought into contact with exogenously derived antigens within the spleen and lymph nodes, otherwise known as the secondary lymphoid organs.

Lymphoid tissues associated with the skin and the mucosal lamina propria of the gut, respiratory tract, and genitourinary tract can be classified as tertiary lymphoid tissues. Tertiary lymphoid tissues are primarily effector sites where memory and effector cells exert immunologic and immunoregulatory functions.

Antigen Recognition

Immunity—Mammalian immunity can be classified into two functional divisions: innate immunity and acquired (adaptive) immunity. Innate immunity is a nonspecific, first-line defense response with no associated immunologic memory. The innate immune response to a foreign organism is the same for a secondary or tertiary exposure as it is for the primary exposure. Acquired immunity is characterized by both specificity and memory, resulting in a much greater immune response on secondary challenge.

Antigen—The primary determinant in either type of immune response is the ability of the immune system components to recognize self versus non-self. A broad definition of non-self is anything other than that encoded in one's own germline genome, which includes foreign DNA, RNA, protein, carbohydrates, and even mutated self-proteins. A non-self substance that can be recognized by the immune system is called an antigen, immunogen, or allergen. Antigens are usually (but not absolutely) biological molecules that can be cleaved and rearranged for presentation to other immune cells. Generally, antigens are at least 10 kDa in size. Smaller antigens are termed "haptens" and must be conjugated with carrier molecules (larger antigens) in order to elicit a specific response.

Antibody—Antibodies are produced by B cells and are functionally defined both by the antigen with which they react and their subtype, termed "isotypes" (e.g., IgM, IgG, IgE, IgD, and IgA). Antibodies of a known specificity are labeled as such, e.g., an IgM antibody against sheep red blood cells (sRBCs) is called an anti-sRBC IgM. Antibodies of unknown specificity are referred to as immunoglobulin (Ig) until they can be defined by their specific antigen.

The basic components of an Ig are the same regardless of isotype, namely, heavy chains, light chains, constant regions (Fc),

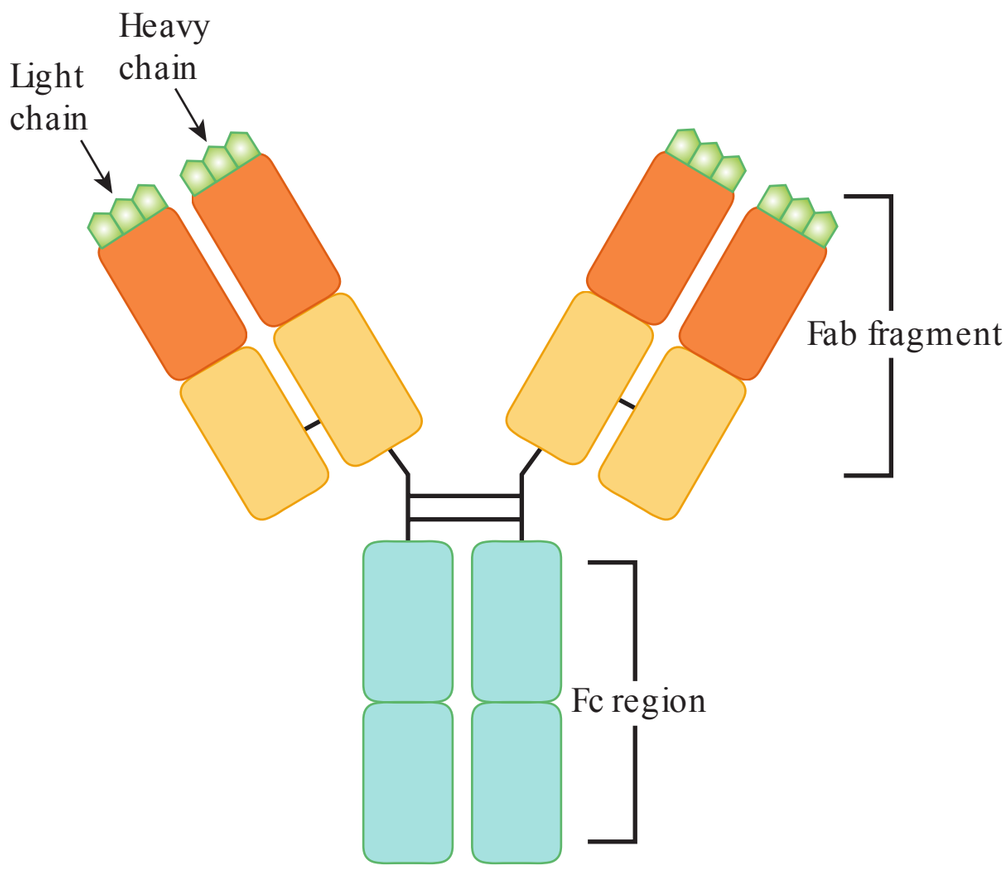

FIGURE 12–1 **Ig structure.** Igs are composed of two heavy chains and two light chains, which are connected by disulfide bonds. Orange areas are variable regions and green areas at top are antigen recognition regions.

and variable regions (Fab). The general structure of antibodies are also conserved across isotypes (Figure 12–1). There are two genes coding for light chains (V and J) and three genes coding for heavy chains (V, D, and J). Isotype is determined by which Fc of the heavy chain is transcribed and translated (heavy chain genes μ, γ, ε, δ, or α encode for the IgM, IgG, IgE, IgD, or IgA heavy chain proteins, respectively).

The immune system generates antibodies to thousands of antigens with which the host may or may not ever contact. During this process called somatic recombination, V and J segments for light chains as well as V, D, and J segments for heavy chains are combined, within the B cell, to form an Ig. The Fab regions of both the heavy and light chains determine antibody specificity and interact directly with antigen. In addition to isotype determination, the Fc region is also responsible for the various effector functions, such as complement activation (e.g., IgM and some subclasses of IgG) and phagocyte binding (via Fc receptors).

Antibodies possess several functions: (1) opsonization, which is coating of a pathogen with antibody to enhance Fc receptor-mediated endocytosis by phagocytic cells; (2) initiation of the classic pathway of complement-mediated lysis; (3) neutralization of viral infection by binding to viral particles and preventing further infection; and (4) enhancement of the specificity of effectors of cell-mediated immunity (CMI) by binding to specific antigens on target cells, which are then recognized and eliminated by effector cells such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs).

Complement—The complement system is a series of about 30 serum proteins whose primary functions are the destruction of membranes of infectious agents, opsonization to facilitate phagocytosis, and the promotion of an inflammatory response (see the "Inflammation" section). Complement activation occurs with each component sequentially acting on others, in a manner similar to the blood-clotting cascade (Figure 12–2). The final components that can enter the membrane and disrupt its integrity are termed the membrane attack complex (MAC). Three pathways have been identified in the activation of the complement cascade, the classical pathway (antibody:antigen-dependent), the alternative pathway (spontaneous activation of C3), and the mannin-binding lectin pathway (a hepatogenic molecule that binds to pathogens).

Antigen Processing—To elicit an acquired immune response to a particular antigen, that antigen must be taken up and processed by accessory cells for presentation to lymphocytes. Accessory cells that perform this function are termed antigen-presenting cells (APCs) and include macrophages, B cells, and dendritic cells (DCs). Of these, the most proficient APC is the DC. There are several subtypes of DC, including plasmacytoid DCs (pDCs), conventional DCs (cDCs), specialized DCs in the skin called Langerhans cells, and follicular DCs in germinal centers within secondary lymphoid organs.

In most tissues, DCs are in an immature state in which they capture antigens through phagocytosis, pinocytosis, or receptor-mediated endocytosis. DCs then process the antigen (intracellular denaturation and catabolism) and display fragments of it on the extracellular side of their cell membrane through direct association with special cell surface molecules (major histocompatibility complex [MHC] classes MHCI and MHCII).

Antigen presentation of the kind discussed here primarily occurs via MHCII (Figure 12–3), although certain types of antigens are processed and presented via MHCI. The pathway of presentation between these two classes have similarities, but the major differences between the MHCI and MHCII pathways are (1) antigens processed and presented via MHCI are not limited to professional APC; (2) all nucleated cells express MHCI; (3) the mechanisms by which the antigen is processed and loaded onto MHCI are slightly different than MHCII; (4) the MHCI antigenic peptides are usually smaller, often 8 to 10 amino acids in length; (5) the MHCI antigens to be processed are usually aberrantly expressed proteins, such as viral-associated proteins or mutated proteins; (6) MHCI facilitates antigen presentation to $CD8^+$ T cells, whereas MHCII facilitates antigen presentation to $CD4^+$ T cells. MHCI antigen processing and presentation is the major pathway by which virally infected cells are detected and killed by the acquired immune system.

Regardless of the MHC utilized to present antigens to lymphocytes, T cells are able to recognize antigen in the context of MHC with their T-cell receptor (TCR). Similar to Ig, the ability of T cells to specifically recognize thousands of antigens is due to somatic recombination. All TCRs are composed of two different subunits, each encoded from a distinct gene (most abundant T-cell population expresses αβ, but γδ also exist). All TCR subunits are made up of constant and variable regions. For α subunits, two separate gene segments (V and J) are combined to form the variable region, which is then joined to one constant region. For β subunits, three separate gene segments (V, D, and J) are combined to form the variable region, which

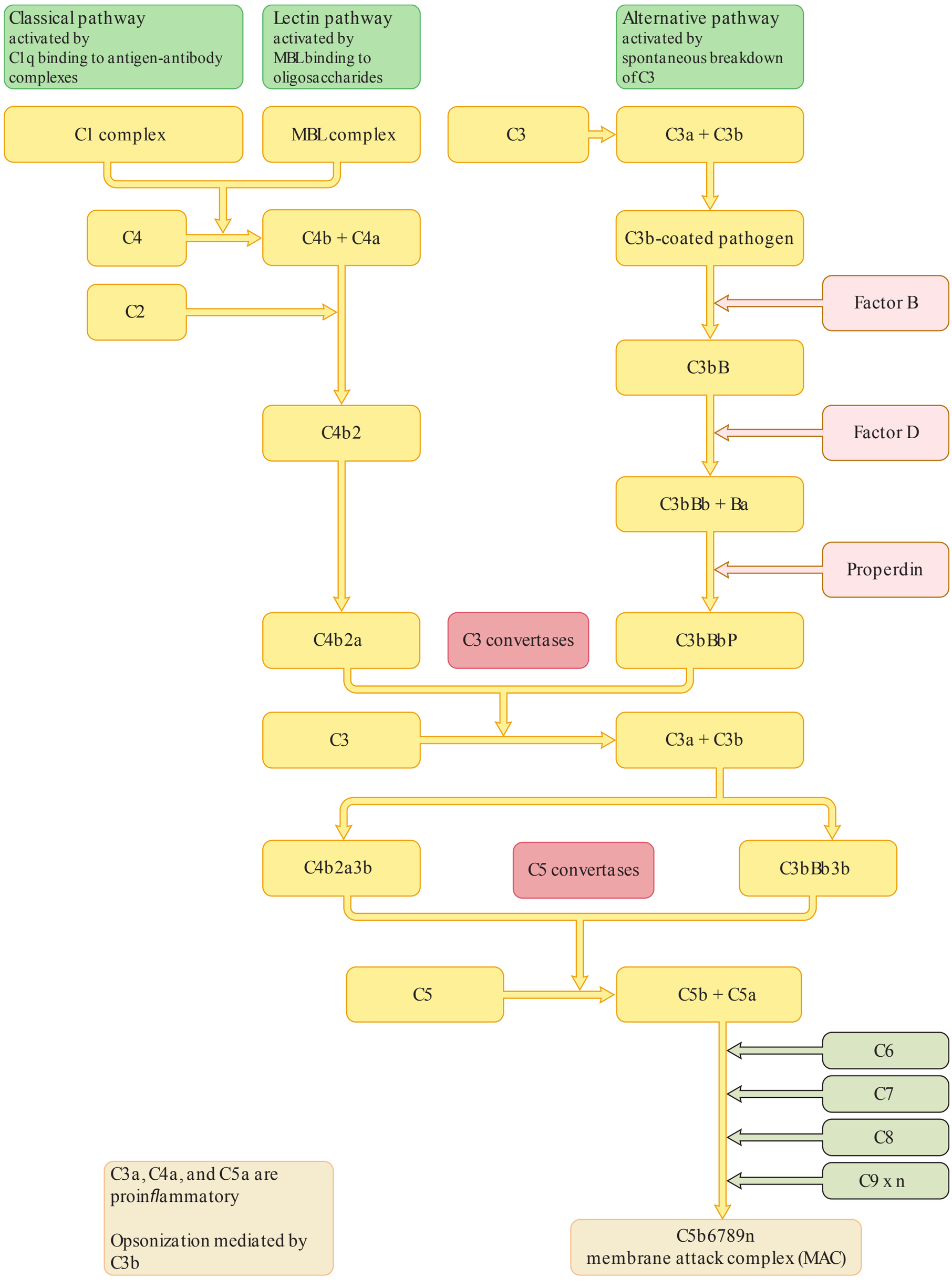

FIGURE 12–2 **The complement cascade.** The complement cascade can be activated in three dif erent ways. Cytolysis occurs via generation of the MAC. Various complement proteins generated along the pathway are either pro-inflammatory mediators (C3a, C5a) or result in opsonization (C3b).

is then joined to one of the two constant regions. Similar to the Ig genes, there are several light chain V and J genes, and several heavy chain V, D, and J genes.

With regard to T and B cells, key events that occur following antigen encounter are (1) specific antigen recognition either in the context of MHCI or MHCII for T cells or through the Ig receptor for B cells, (2) cellular activation and initiation of intracellular signaling cascades that contribute to production and release of cytokines and other cellular mediators, (3) clonal expansion (proliferation) of antigen-specific cells, and (4) dif-ferentiation of antigen-stimulated lymphocytes into effector and memory cells.

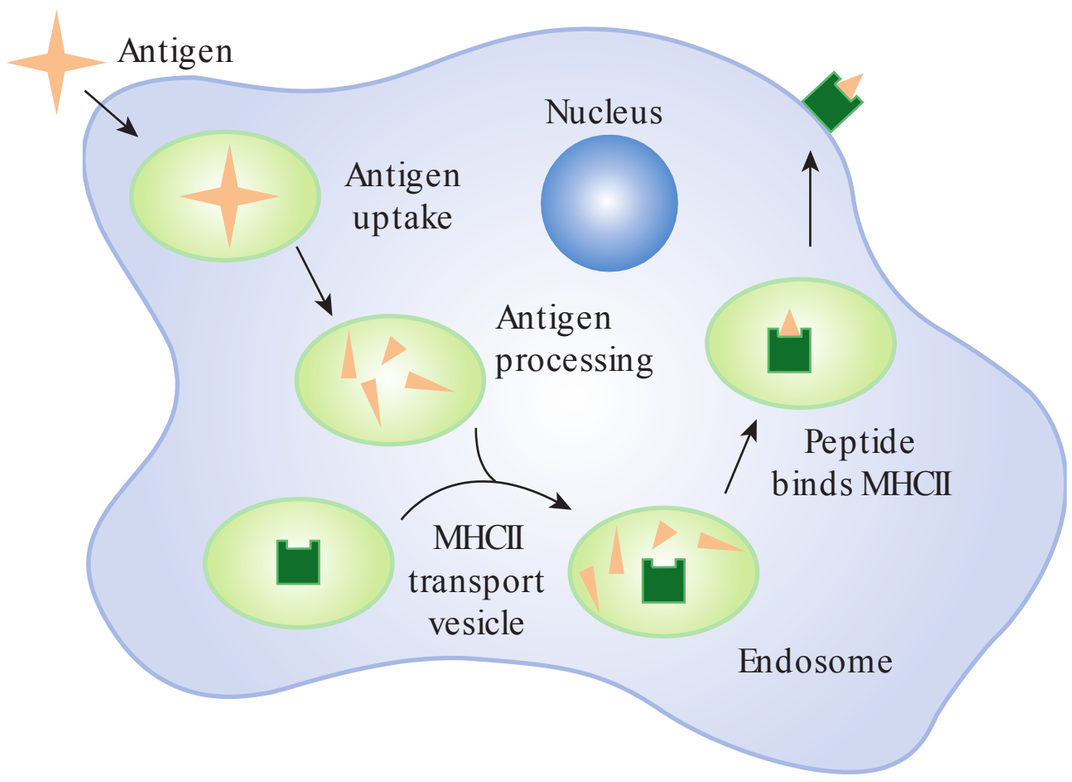

FIGURE 12–3 **Antigen processing by the MHCII pathway.** Antigen is engulfed by an APC (DC, macrophage, or B cell), degraded, and loaded onto MHCII. The MHCII–peptide complex is then expressed on the surface of the APC for presentation to $CD4^+$ Th cells.

Innate Immunity

Innate immunity acts as a first line of defense against infectious agents, eliminating most potential pathogens before significant infection occurs. It includes physical and biochemical barriers both inside and outside of the body as well as immune cells designed for specific responses. There is no immunologic memory associated with innate immunity.

Externally, the skin provides an effective barrier, as most organisms cannot penetrate intact skin. Most infectious agents enter the body through the respiratory system, gut, or genitourinary tract. Innate defenses present to combat infection from pathogens entering through the respiratory system include mucus secreted along the nasopharynx, the presence of lysozyme in most secretions, and cilia lining the trachea and main bronchi. In addition, reflexes such as coughing, sneezing, and elevation in body temperature are also a part of innate immunity. Pathogens that enter the body via the digestive tract are met with severe changes in pH (acidic) within the stomach and a host of microorganisms living in the intestines.

Cellular Components: Neutrophils, Macrophages, Natural Killer Cells, NKT, and **γδ** T Cells—Neutrophils (also known as polymorphonuclear cells [PMNs]) are phagocytic cells that develop from the myeloid lineage of HSCs. They enter the bloodstream where they circulate for about 10 h, after which they enter tissues and perform effector functions for one or two days. Neutrophils are a primary line of defense because of their ability to pass between the endothelial cells of blood vessels (termed extravasation) and access peripheral tissues. They are excellent phagocytic cells and can eliminate most microorganisms through the release of various reactive oxygen species (ROS), such as superoxide, singlet oxygen, ozone, hydrogen peroxide, and hydroxyl radicals. Their phagocytic activity is greatly enhanced by the presence of complement and antibody deposited on the surface of the foreign target. They are also important in the induction of an inflammatory response.

Macrophages are terminally differentiated monocytes that are developed from the myeloid lineage of HSCs. Upon exiting the bone marrow, monocytes circulate within the bloodstream for about one day. At that time, they begin to distribute to the various tissues where they can then differentiate into macrophage subsets. Tissue-specific macrophages include Kupffer cells (liver), alveolar macrophages (lung), microglial cells (CNS), and peritoneal and splenic macrophages. Macrophages can be classified as classically activated macrophages (M1), which are pro-inflammatory and participate in antigen presentation, or alternatively activated macrophages (M2), which do not present antigen well, but are efficient in apoptotic cell removal. Based on this classification, M1 macrophages are also APCs.

Like other immune cells, natural killer (NK) cells are derived from a HSC. The two major functions of NK cells are cytokine (soluble protein messenger) production and cytolysis. NK cells are the predominant producer of the cytokine, IFN-γ, which promotes DC maturation. In this way, NK cells serve as a bridge between innate and adaptive immunity. NK cells also mediate both antibody-independent and antibody-dependent cellular cytotoxicity (ADCC) using a variety of mechanisms, including perforin and granzyme, Fas L, TRAIL, and TNF-α. Antigen-dependent cell-mediated cytotoxicity occurs via the FcγRIIIA, which is present on most NK cell subsets. NKT cells are one such subset of NK cells that express both NK- and T-cell markers, which allows the NKT cell to present self and exogenous antigenic glycolipids.

Another cell type that has recently been shown to facilitate the innate-adaptive immunity bridge is the γδ T cell. These cells migrate predominantly to "exposed" tissues, including skin, lung, gut, and reproductive organs, and are also expressed highly in the liver. In part through the expression of TLRs, γδ T cells can acquire effector functions similar to those of NK cells. There is also a subpopulation of B cells that also bridge innate and adaptive immunity. Unlike the conventional B-2 cells, B-1 cells predominate in embryonic life and are later found mostly in the peritoneal and pleural cavities. B-1 cells are self-renewing and spontaneously produce polyspecific IgM antibodies (i.e., natural antibodies) independent of T-cell help.

Historically, innate immunity was defined as nonspecific. It is now clear that innate cells express receptors that respond to soluble components (e.g., Fc or complement receptors) or to certain antigenic motifs. Pattern recognition receptors are a family of receptors that recognize pathogen-derived molecules or cell-derived molecules produced in response to cellular stress ("danger" molecules). Receptors that recognize pathogen-associated molecular patterns (PAMPs) are toll-like receptors (TLRs); receptors that recognize danger-associated molecular patterns (DAMPs) include NOD-like receptors (NLR) and RIG-like receptors (RLR). TLRs are expressed both extracellularly and intracellularly in endosomes, which confers the ability to respond to a variety of pathogenic components, such as bacterial cell wall lipids, single- and double-stranded nucleic acids, or fungal and parasitic products. Functional

consequences of TLR engagement on cells include expression of adhesion molecules, chemokines, or cytokines to stimulate T- or B-cell differentiation, enhance phagocytosis, or facilitate maturation of DCs.

Soluble Factors—A common effector mechanism for many immune cells, is cytokine, chemokine, or interferon (IFN) production. T e primary function of cytokines, chemokines, and IFNs include cellular activation, initiation or termination of intracellular signaling events, proliferation, differentiation, migration, traf cking, or effector functions (Table 12–1). Although some of these molecules might be constitutively expressed, most are inducible in response to antigens, cellular stressors, or other cytokines. T us, many cytokines, chemokines, and IFNs are not stored in the cell, but rather are tightly regulated, often at the transcriptional level, so that they are quickly generated on demand. Also, many cytokines share common receptor subunits. T is means if a particular subunit of one receptor is affected by an immunotoxic agent, all others that share this subunit are also affected.

TABLE 12–1 Cytokines: sources and functions in immune regulation.

Cytokine	Source	Physiologic Actions
IL-1	Macrophages Epithelial cells	Activation and proliferation of T cells Pro-inflammatory Induces fever and acute-phase proteins Induces synthesis of pro-inflammatory cytokines
IL-2	T cells	Primary T-cell growth factor Growth factor for B and NK cells
IL-4	Th2 cells Mast cells	Proliferation of activated T cells and B cells B-cell dif erentiation and IgE isotype switching functions Antagonizes IFN-γ Inhibits Th1 responses
IL-5	Th2 cells Mast cells	Proliferation and dif erentiation of eosinophils
IL-6	Macrophages Th2 cells B cells Endothelial cells	Enhances B-cell dif erentiation and immunoglobulin secretion Induction of acute-phase proteins by liver Pro-inflammatory Proliferation of T cells and increased IL-2 receptor expression Synergizes with IL-4 to induce secretion of IgE
IL-10	Tregs Bregs	Inhibits T-cell and macrophage responses
IL-12	DCs Macrophages	Activates B K cells Induces TH1 responses
IL-13	Th2 cells	Stimulates B-cell growth Inhibits Th1 responses
IL-17	Th17 cells NK cells γδ T cells Neutrophils	Pro-inflammatory Inhibits Tregs
Interferon-α/β (IFN-α/β) (type 1 IFN)	Leukocytes DCs Fibroblasts	Induction of MHC class I expression Antiviral activity Stimulation of NK cells
Interferon-γ (IFN-γ)	T cells NK cells	Induction of MHC I and MHC II Activates macrophages
Transforming growth factor-β (TGF-β)	Macrophages Megakaryocytes T cells	Enhances monocyte/macrophage chemotaxis Enhances wound healing: angiogenesis, fibroblast proliferation, deposition of extracellular matrix Inhibits T- and B-cell proliferation Inhibits antibody secretion Primary inducer of isotype switch to IgA
GM-CSF	T cells Macrophages Endothelial cells Fibroblasts	Stimulates growth and dif erentiation of monocytes and granulocytes

Other soluble components of innate immunity include the complement cascade (discussed earlier), acute-phase proteins, granzyme and perforin, and various cytokines, chemokines and IFNs. Complement is important in innate immunity because of its activation through the mannin-binding lectin pathway. Furthermore, C3a and C5a, which are chemokines generated during the cascade, recruit phagocytic cells to the site of complement activation. Acute-phase proteins, such as serum amyloid A, serum amyloid P, and C-reactive protein, participate in an acute-phase response to infection by binding bacteria and facilitating complement activation. Granzyme and perforin work in conjunction, with perforin disrupting the target cell membrane, allowing granzyme to enter and mediate cell lysis by several mechanisms.

Acquired (Adaptive) Immunity

If the primary defenses against infection (innate immunity) are breached, the acquired arm of the immune system is activated and produces a specific immune response to each infectious agent. T is branch of immunity can protect the host from future infection by the same agent. Two key features that distinguish acquired immunity are specificity and memory. Acquired immunity may be further subdivided into humoral and cell-mediated immunity (CMI). Humoral immunity is directly dependent on the production of antigen-specific antibody by B cells and involves the coordinated interaction of APCs, T cells, and B cells. CMI is that part of the acquired immune system in which effector cells, such as phagocytic cells, helper T cells (T cells), T-regulatory cells (Tregs), APCs, CTLs, or T memory cells, play the critical role(s) without antibody involvement.

Cellular Components: APCs, B Cells, and T Cells—APCs, which have been discussed previously in the "Antigen Processing" section, include professional APCs such as B cells, macrophages, and DCs. Although all cells may act as APCs with internal antigen processing through the MHCI pathway, what distinguishes a professional APC from the others is the ability to internalize external antigens and process them through the MHCII pathway for presentation to T cells.

Besides serving as APCs, B lymphocytes are also the effector cells of humoral immunity, producing a number of Ig isotypes with varying specificities and af nities. Upon antigen binding to surface Ig (part of the B-cell receptor [BCR]), the mature B cell becomes activated and, after proliferation, undergoes differentiation into either a memory B cell or an antibody-forming cell ([AFC]; also known as a plaque-forming cell [PFC]) that actively secretes antigen-specific antibody.

T-cell precursors migrate from the bone marrow to the thymus where, in a manner analogous to B cells, they begin to rearrange their TCRs. T ese immature cells then undergo positive and negative selection to (1) eliminate cells that do not produce a functional TCR or produce TCRs with no af n-ity for self-MHC (positive selection) or (2) eliminate cells that strongly bind MHC plus self-peptide (negative selection). T is rigorous selection process produces T cells that can recognize MHC plus foreign peptides and eliminates autoreactive T cells.

Additionally, expression of CD4 or CD8 will determine to which MHC class the αβ TCR will bind. CD4 will facilitate binding to MHCII expressed on APCs; T cells expressing CD4 (helper T cells, T) help activate other cells of the adaptive immune response. CD8 will facilitate binding to MHCI, which is expressed on all nucleated cells; generally, T cells expressing CD8 mediate cell killing (CTL). T e γδ T cells do not express CD4 or CD8 and therefore do not interact with MHC and do not undergo positive or negative selection. Since γδ T cells are not negatively selected for autoreactivity, these cells may be associated with the development of hypersensitivity and autoimmunity (see subsequent sections).

Mature T cells are found in the lymph nodes, spleen, and peripheral blood. Upon binding of the TCR to MHC plus antigen, the mature T cell becomes activated and, after proliferation, undergoes differentiation into either an effector cell or a memory T cell. T ere are many subsets of effector T cells and two subsets, T 1 and T 2, dictate whether CMI or humoral immunity will predominate, respectively. T 1 cells predominantly express IL-2, IFN-γ, and lymphotoxin, which promote CMI and humoral defense against intracellular invaders. T 2 cells predominantly express IL-3, IL-4, IL-5, IL-6, IL-10, and IL-13, which promote humoral defense against extracellular invaders. Although the two populations are not mutually exclusive, they do negatively regulate each other, such that a strong T 1 response suppresses a T 2 response and vice versa.

T e ability of APCs, B cells, and T cells to communicate with each other is dependent on a variety of receptor–ligand interactions between cell types. T ese interactions also help dictate the type of immune response (i.e., humoral versus CMI) and the magnitude of the immune response. T e duration and extent of an acquired immune response is also controlled by specialized regulatory cells found in both the T-cell and B-cell lineages.

For the T-cell lineage, there is a small population of CD4+ cells that develop into T-regulatory cells (Tregs), which help to control various immune responses, including those directed against self. T e mechanisms by which Tregs suppress immune responses involve direct Treg-cell contact. Several subsets of regulatory B cells have already been identified, but more are being discovered recently. T ese cells generally play a suppressive role in hypersensitivity and autoimmune diseases. T e regulatory T- and B-cell subsets also appear to reciprocally activate or suppress each other and may cooperatively control immune responses.

Humoral and Cell-mediated Immunity—Humoral immunity is that part of the acquired immune system in which antibody is involved, and CMI is that part of the immune system in which various effector cells perform a wide variety of functions to eliminate invaders. T ese two branches are often coordinated, as depicted in Figure 12–4.

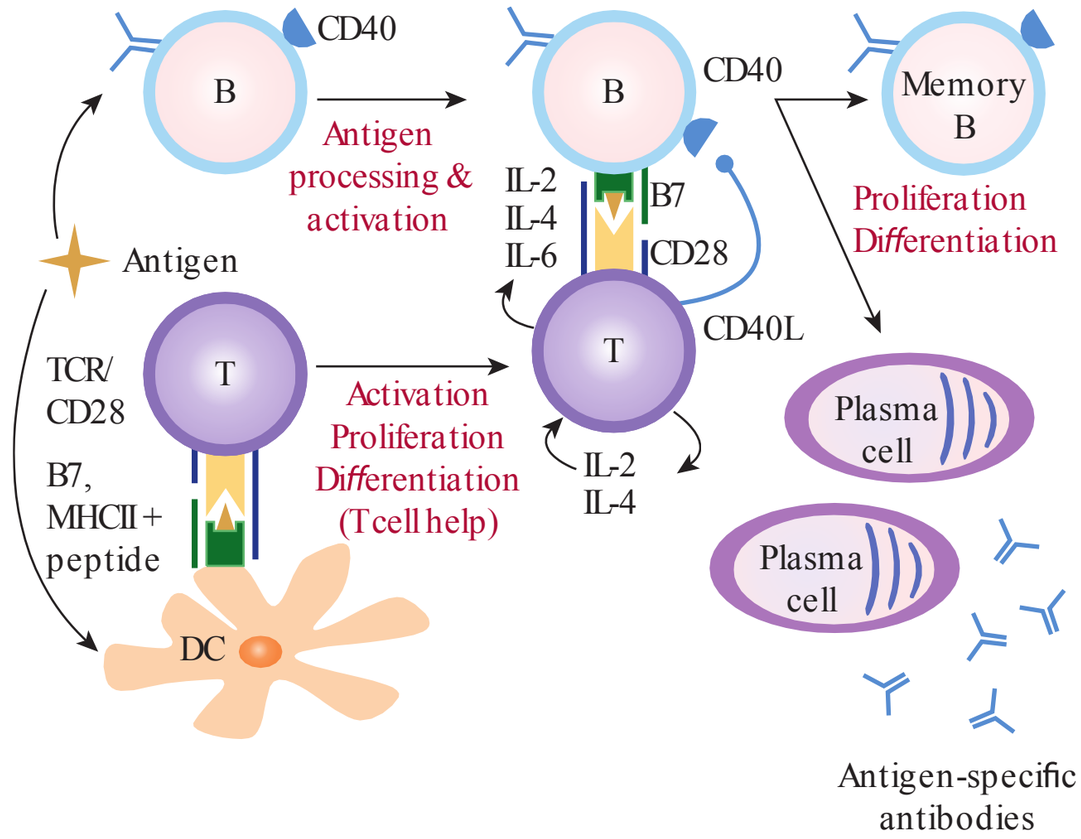

FIGURE 12–4 **Cellular interactions in the humoral immune response.** Antigen is engulfed by an APC (usually DC) and the antigenic peptide is presented to $CD4^+$ Tcells in the context of MHCII. $CD4^+$ Tcells then become activated, proliferate, and dif erentiate into Th cells, which release cytokines to help Bcells that had also encountered the same antigen. Bcells then become activated, proliferate, and dif erentiate into memory Bcells or antigen-producing plasma cells.

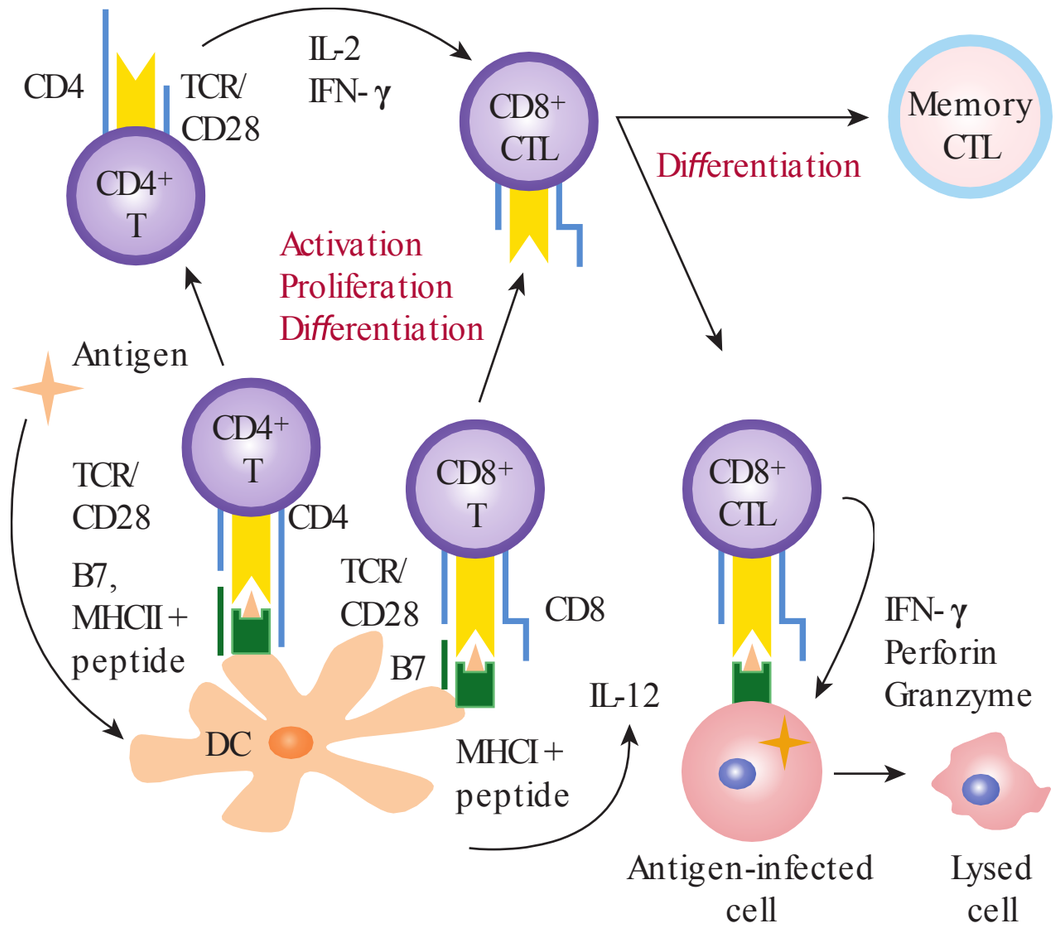

FIGURE 12–5 **Cellular interactions in the CTLresponse.** Antigen is engulfed by an APC (usually DC) and the antigenic peptide is presented to $CD4^+$ and $CD8^+$ Tcells in the context of MHCII and I, respectively. $CD4^+$ Tcells then become activated, proliferate, and dif erentiate into Th cells, which release cytokines to help $CD8^+$ Tcells that had also encountered the same antigen. Especially in the presence of IL-12 produced by the DC, $CD8^+$ Tcells become activated, proliferate, and dif erentiate into CTLthat can kill other antigen-infected cells.

In general, B cells produce antibodies specific to an antigen, which may act to opsonize or neutralize the invader, or the antibodies act to recruit other factors, such as the complement cascade. T e production of antigen-specific IgM requires 3 to 5 days after the primary (initial) exposure to antigen. Upon secondary antigenic challenge, the B cells undergo isotype switching, producing primarily IgG antibody, which is of higher af nity for the activating antigen. In addition, there is a higher serum antibody titer associated with a secondary antibody response.

CMI functions include delayed-type hypersensitivity (DTH) and cell-mediated cytotoxicity. Cell-mediated cytotoxicity responses may occur in numerous ways: (1) MHC-dependent recognition of specific antigens (such as viral particles or tumor proteins) by CTL (Figure 12–5); (2) indirect antigen-specific recognition by the binding of Fc receptors on NK cells to antibodies coating target cells; and (3) receptor-mediated recognition of complement-coated foreign targets by macrophages.

In cell-mediated cytotoxicity, the CTL or NK effector cell binds in a specific manner to the target cell. T e majority of CTLs express CD8 and recognize either foreign MHCI on the surface of allogeneic cells or antigen in association with self-MHCI (e.g., viral particles). Once the CTL or NK cells interact with the target cell, the effector cell releases granules containing perforin and other enzymes. T is degranulation damages the target cell, after which the effector cell can release and attack other target cells In addition, CTLs induce the target to undergo apoptosis through activation of the Fas and cytotoxic cytokine (i.e., TNF and lymphotoxin) pathways.

Inflammation

Inflammation refers to a complex reaction to injury, irritation, or foreign invaders characterized by pain, swelling, redness, and heat. Inflammation involves various stages, including release of chemotactic factors following the insult, increased blood flow, increased capillary permeability allowing for cellular infiltration, followed by either an acute resolution of tissue damage or persistence of the response that might contribute to fibrosis or subsequent organ failure. It is important to emphasize that while inflammation is a natural reaction to repair tissue damage or attack foreign invaders, the process often results in destruction of adjacent cells and/or tissues. T us, there is overwhelming evidence that inflammation plays a critical role in many diseases, including asthma, multiple sclerosis, cardiovascular disease, Alzheimer's disease, bowel disorders, and cancer. In addition, inflammation exacerbates idiosyncratic reactions to drugs and other chemicals.

Cellular Components: Macrophages, Neutrophils, and T Cells—Many of the cellular components described in the sections above are critical to initiation and maintenance of an inflammatory response. Major cellular contributors to an inflammatory response are macrophages, neutrophils, and T cells. Neutrophils are often the first, and most numerous, responders to sites of insult. In response to either host- or pathogen-derived signals, neutrophils secrete chemotactic factors to recruit other pro-inflammatory cells, such as macrophages, to the area.

Macrophages can be activated by a variety of mechanisms at the site of insult, such as activation via toll-like receptor, pro-inflammatory cytokines, or recognition of opsonized particles by Fc receptors or complement receptors. Macrophages and neutrophils also induce apoptosis of cells in the insult area through the release of nitric oxide and other ROS, resulting in disruption of extracellular structures that compromise tissue structure and function. Both neutrophils and macrophages are phagocytic cells and can contribute to clearing of apoptotic cells.

Later in the inflammatory response, T cells are critical for generating an adaptive immune response. T cells are attracted to the insult area by adhesion molecules and integrins, and are activated in response to antigen presented in the context of MHC, often by a DC. Depending on the signals that the T cell receives from the cytokine milieu, distinct subpopulations of T cells are induced.

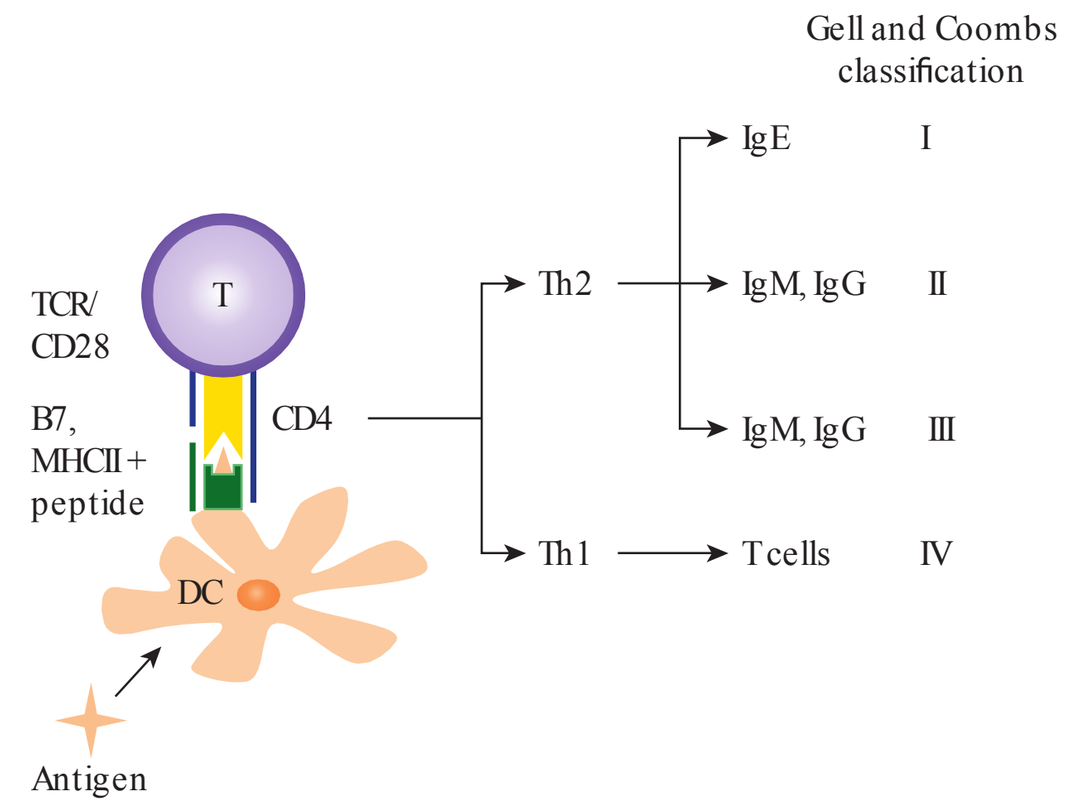

FIGURE 12–6 **Overview of classif cation of hypersensitivity reactions.** Hypersensitivity reactions are mediated via T cells and antibody production.

Immune-mediated Disease

While the primary purpose of the immune system is to preserve the integrity of the individual from disease states, situations arise in which the individual's immune system responds in a matter producing tissue damage, resulting in self-induced disease. T ese disease states fall into two categories: (1) hypersensitivity, or allergy, and (2) autoimmunity.

Hypersensitivity

Classi*fi*cation o*f* Hypersensitivity Reactions—T ere are four types of hypersensitivity reactions as classified by Coombs and Gell. One characteristic common to all four types of hypersensitivity reactions is the necessity of prior exposure leading to sensitization in order to elicit a reaction upon subsequent challenge. In the case of types I, II, and III, prior exposure to antigen leads to the production of allergen-specific antibodies (IgE, IgM, or IgG) and, in the case of type IV, the generation of allergen-specific memory T cells. Figure 12–6 illustrates the mechanisms of hypersensitivity reactions.

Type I (Immediate or IgE-mediated Hypersensitivity): Using penicillin as an example, Figure 12–7 depicts the major events involved in a type I hypersensitivity reaction (what most people think of as "allergy" and is clinically referred to as "atopy"). Sensitization occurs as the result of dermal exposure to antigens or by exposure to antigens through the respiratory or gastrointestinal tract. Most people would mount an IgM, IgG, or IgA immune response to these antigens and clear them without causing any allergic symptoms. It is unclear why these antigens become allergens in certain individuals who respond by mounting an IgE immune response instead, but appears to involve genetic and/or environmental determinants and likely some type of triggering event (e.g., acute pathogen exposure and emotional stress).

Once produced, soluble IgE not only binds to local tissue mast cells, but also enters the circulation, where it binds to circulating mast cells, basophils, and tissue mast cells at distant sites. Once an individual is sensitized, reexposure to the antigen results in binding to IgE on local mast cells and degranulation with the release of preformed mediators and cytokines which recruit and activate circulating eosinophils, basophils, macrophages, and neutrophils leading to the synthesis and release of more cytokines and of leukotrienes and thromboxanes. T ese mediators promote vasodilation, bronchial constriction, and inflammation. Clinical manifestations can vary from urticarial skin reactions (wheals and flares) to signs of hay fever, including rhinitis and conjunctivitis, to more serious diseases, such as asthma and potentially life-threatening anaphylaxis.

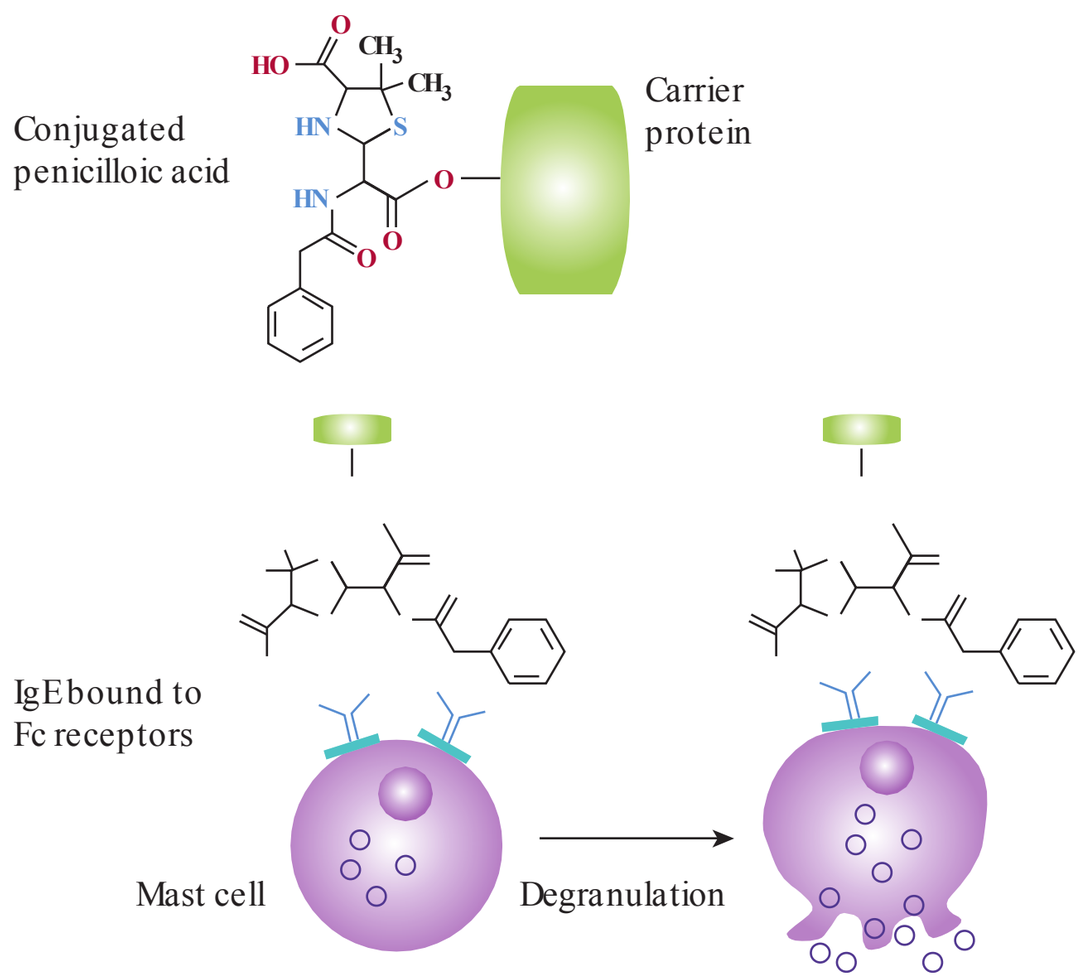

FIGURE 12–7 **Type I hypersensitivity reaction.** Metabolized penicillin is a hapten that conjugates with a protein. The conjugated hapten cross-links IgE antibodies on mast cells. IgE cross-linking causes mast-cell degranulation and releasing histamine and other pro-inflammatory mediators.

T ese responses may begin within minutes of reexposure to the offending antigen; therefore, type I hypersensitivity is often referred to as immediate hypersensitivity.

Type II (Antibody-dependent Cytotoxic Hypersensitivity): Type II hypersensitivity is IgG- or IgM-mediated. T e antibody response may be mediated by a foreign antigen attached to the surface of a cell or tissue. Conversely, an antibody response could be mediated by an autoantibody due to a breakdown in tolerance and the resulting response would be part of an autoimmune disease (e.g., autoimmune hemolytic anemias and Goodpasture's syndrome). Figure 12–8 shows the mechanisms of action for complement-independent and complement-dependent cytotoxic reactions. Tissue damage may result from the direct action of cytotoxic cells, such as macrophages, neutrophils, or eosinophils, linked through the Fc receptor to antibody-coated target cells (complement-independent) or by antibody-induced activation of the classic complement pathway.

Type III (Immune Complex-mediated Hypersensitivity): Type III hypersensitivity reactions also involve IgG or IgM. T e distinguishing feature of type III is that, unlike type II, in which Ig production is against specific cellular or tissue-associated antigen, Ig production is against soluble antigen in the serum (Figure 12–9). T is allows for the formation of circulating immune complexes composed of a lattice of antigen and Ig, which may result in widely distributed tissue damage in areas where immune complexes are deposited. T e most common location is the vascular endothelium in the lung, joints, and kidneys. T e skin and circulatory systems may also be involved. Pathology results from the inflammatory response initiated by the activation of complement. Macrophages, neutrophils, and platelets attracted to the deposition site contribute to the tissue damage. As with type II hypersensitivity, responses similar to type III hypersensitivity can be induced in autoimmune diseases due to autoantibodies directed against soluble antigens such as double-stranded DNA or small nuclear proteins as seen with systemic lupus erythematosus (SLE).

Autoimmunity—Autoimmune disease occurs when the reactions of the immune system are directed against the body's own tissues and is characterized by a genetic susceptibility. T ese diseases can be either tissue-specific or nonspecific, and the targets from the perspective of the primary sites of tissue damage in autoimmune disease are many and varied. Both humoral immunity and CMI can be involved as effector mechanisms in causing the damage in autoimmune conditions.

Although the resulting pathology may be the same for autoimmune reactions and hypersensitivity, mechanisms of true autoimmune disease are distinguished from hypersensitivity. In cases of autoimmunity, self-antigens are the target, and in the case of chemical-induced autoimmunity, the disease state is induced by a modification of host tissues or immune cells by the chemical and not the chemical acting as an antigen/hapten as in hypersensitivity reactions.

Mechanisms *of* Autoimmunity—T e rearrangement and recombination of the genes that comprise Ig and TCR result

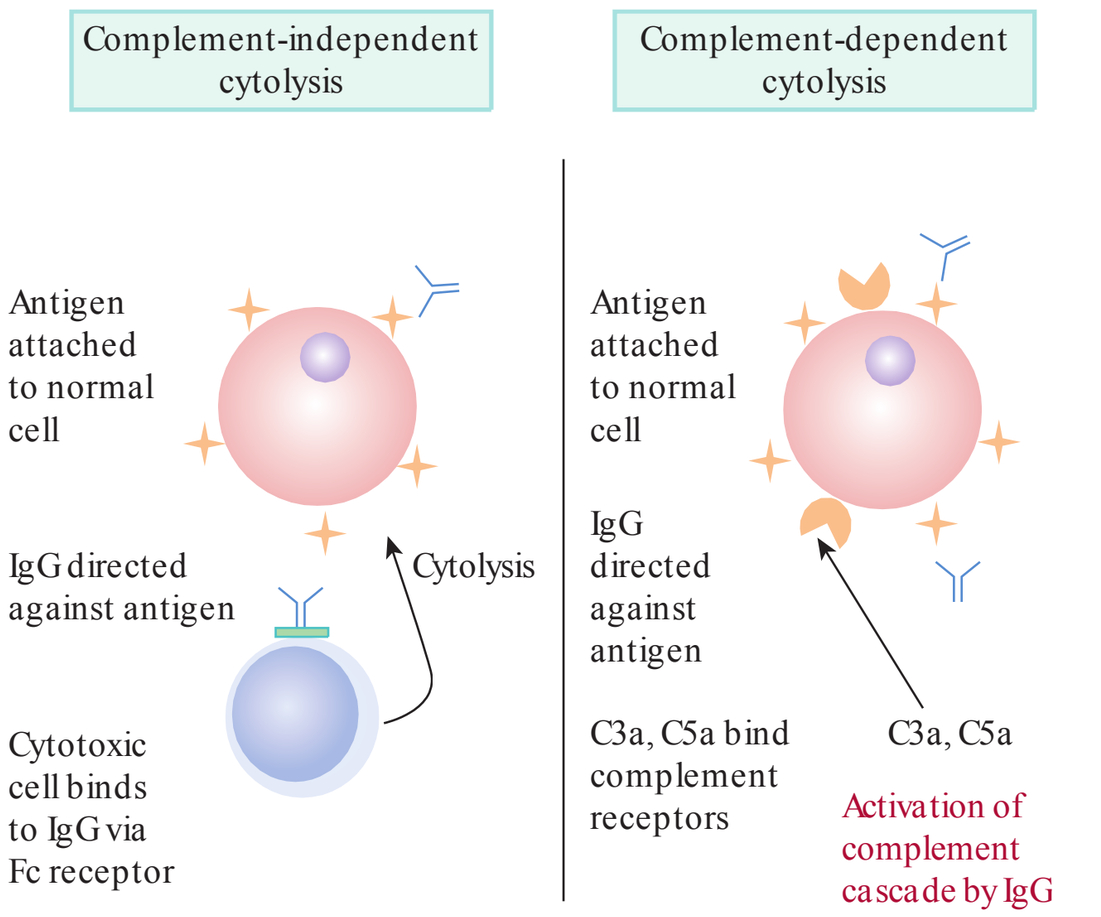

FIGURE 12–8 **Type II hypersensitivity reactions.** In complement-independent cytolysis, antigen becomes attached to a normal cell, which can be recognized by IgG. A cell capable of cytolysis (CTL, NK cell) binds to IgG via its Fc receptor and kills the antigen-coated cell. In complement-dependent cytolysis, antigen becomes attached to a normal cell, which can be recognized by IgG. Complement gets activated by the classical pathway (antigen–antibody complexes) and C3a and C5a bind complement receptors.

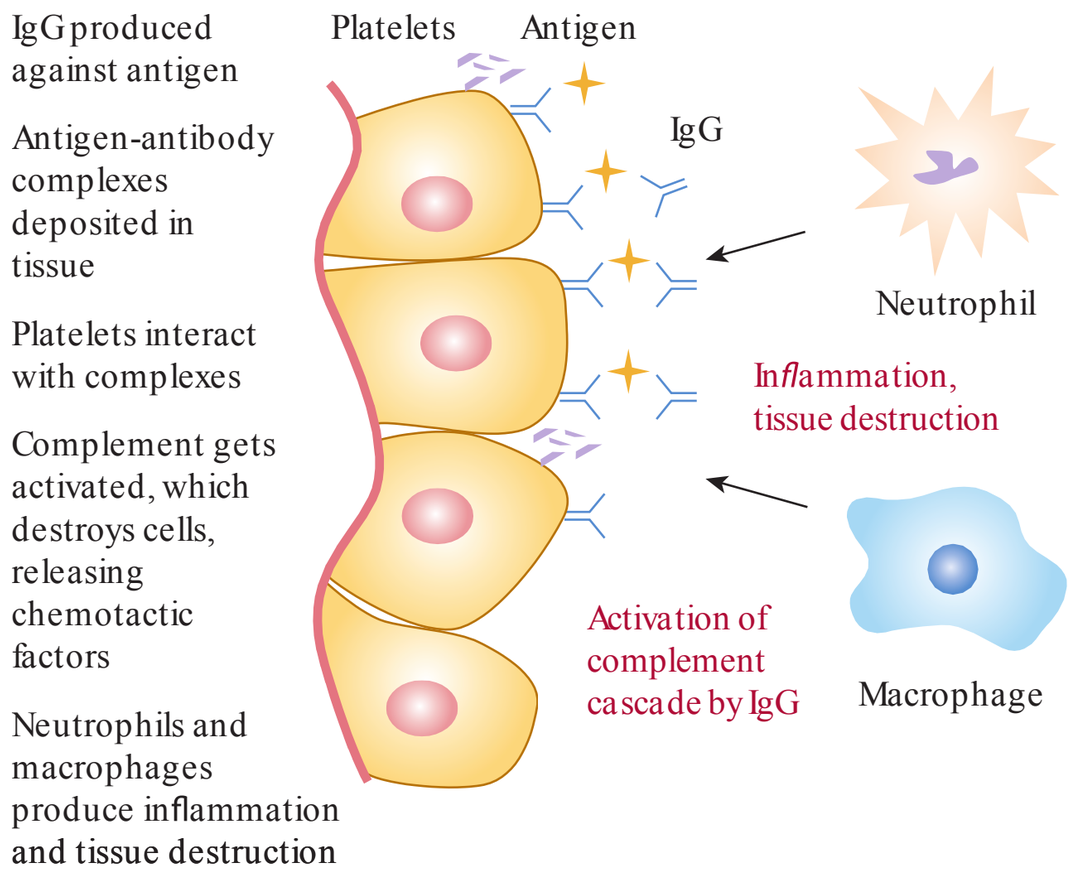

FIGURE 12–9 **Type III hypersensitivity reactions.** IgG is produced against an antigen and antigen–antibody complexes form, which can become deposited in tissue. Complement gets activated by the classical pathway (antigen–antibody complexes), and platelets also interact with complexes. Following complement-mediated cytolysis, released chemotactic factors attract neutrophils and macrophages, causing additional inflammation and tissue damage.

in tremendous diversity in the potential antigen recognition of B cells and T cells, respectively. Ideally, during development those lymphocytes recognizing self-antigens will largely be deleted by negative selection as central tolerance is established. Autoreactive clones that escape central tolerance and migrate to the periphery are normally controlled by peripheral tolerance mediated by various mechanisms that ultimately induce anergy or clonal deletion. For autoimmune disease to occur, an autoreactive clone must escape central tolerance, pass into the periphery, and bind with specificity to its self-antigen, then mechanisms of peripheral tolerance must fail and the autoreactive clone must induce a detrimental immunological response.

Autoimmunity is multifaceted and has been associated with several mechanisms primarily related to insuf cient peripheral tolerance, while defects in central tolerance are rarely encountered. Several mechanisms have been associated with the breakdown of peripheral tolerance and prime events for the onset of autoimmune disease. T ese mechanisms include inflammation; molecular mimicry by pathogen antigens; inherent defects in T or B cells including regulatory subsets, APCs, cytokines, or complement; and epitope spreading.

Effector mechanisms in autoimmune disease can be the same as those described earlier for types II and III hypersensitivity or, in the case of pathology associated with solid tissues, they may involve $CD8^+$ cytotoxic T lymphocytes. Tissue damage associated with CTL may be the result of direct cell membrane damage and lysis, or the result of cytokines produced and released by the T cell. TNF-β has the ability to kill susceptible cells, and IFN-γ may increase the expression of MHCI on cell surfaces, making them more susceptible to $CD8^+$ cells. Cytokines may also be chemotactic for macrophages, which can cause tissue damage directly or indirectly through the release of pro-inflammatory cytokines. As is the case with hypersensitivity reactions, autoimmune disease is often the result of more than one mechanism working simultaneously.

Developmental Immunology

A sequential series of carefully timed and coordinated developmental events, beginning early in embryonic/fetal life and continuing through the early postnatal period, is required to establish a functional immune system in all mammals. T e immune system develops initially from a population of pluripotent HSCs that gives rise to all circulating blood cell lineages, including cells of the immune system. T e bone marrow and thymus are the primary sites of lymphopoiesis and appear to be unique in providing the microenvironment factors necessary for the development of functionally competent immune cells.

Immune system development does not cease at birth, but continues to develop until 5 to 12 years of age in humans. After birth, immunocompetent cells continue to be produced from proliferating progenitor cells in the bone marrow and thymus. T ese cells subsequently migrate via the blood to the secondary immune organs: spleen, lymph nodes, and mucosal lymphoid tissues. T e onset of functional immune competence depends on the specific parameter being measured, and varies across species with striking differences noted between rodents and humans.

Exposure to specific antigens during the perinatal period results in a rapidly expanding accumulation of lymphocyte specificities in the pool of memory cells in secondary lymphoid tissues. As thymic function wanes and thymocytes are no longer produced in that tissue, it is this pool of memory B and T cells that maintains immune competence for the life of the individual. Senescence of immunity is associated with reductions in both innate and acquired immune responses to antigens during the last quartile of life. T is failure of the immune response is due, in part, to a continual reduction in the production of newly formed cells, and to the decreased survival of long-lived memory cells in lymphoid tissues.

One feature of the developing immune system that clearly distinguishes it from the mature immune system, especially during gestation, is the role played by organogenesis. Defects in the development of the immune system due to heritable changes in the lymphoid elements have provided clinical and experimental examples of the devastating consequences of impaired immune development. T erefore, the effects of chemicals on the genesis of critical immune organs in the developing fetus may be more important than effects on these tissues after having been populated by hematopoietic and lymphoid cells. Interestingly, immune organs, such as the thymus, spleen, and/or bone marrow, are not typically assessed in routine developmental and reproductive toxicology studies.

Neuroendocrine Immunology

Cytokines, neuropeptides, neurotransmitters, and hormones (as well as their receptors) are an integral and interregulated part of the central nervous system, the endocrine system, and the immune system. Because receptors for neuropeptides, neurotransmitters, and hormones are present on lymphoid cells, some chemicals may exert their immunomodulatory effects indirectly on the immune system by modulating the activity of the nervous or endocrine systems. In addition, immune cells are capable of secreting peptide hormones and neurotransmitters, which can have autocrine (immune system) and paracrine (endocrine and nervous systems) effects.

ASSESSMENT OF IMMUNOLOGIC INTEGRITY

Xenobiotics can have significant effects on the immune system. Among the unique features of immune cells is their ability to be removed from the body and to function in vitro. T is unique quality offers the toxicologist an opportunity to comprehensively evaluate the actions of xenobiotics on the immune system.

Many medical devices may have intimate and prolonged contact with the body. Possible immunologic consequences of this contact could be envisioned to include immunosuppression, immune stimulation, inflammation, and sensitization.

Methods to Assess Immunocompetence

General Assessment—All studies of immunocompetence should include toxicologic studies (such as organ weights, serum characteristics, hematologic parameters, and bone marrow function) to investigate the effects of immune modulation on other body organs. Histopathology of lymphoid organs also may provide insight into potential immunotoxicants. Moreover, use of fluorescently labeled monoclonal antibodies to cell surface markers in conjunction with a flow cytometer enables accurate enumeration of lymphocyte subsets and whether the xenobiotic may affect maturation.

Functional Assessment

Innate Immunity—Innate immunity encompasses all those immunologic responses that do not require prior exposure to an antigen and that are nonspecific in nature. These responses include recognition of tumor cells by NK cells, phagocytosis of pathogens by macrophages, and the lytic activity of the complement system.

To evaluate phagocytic activity, macrophages are placed in culture plates and incubated with radiolabeled red blood cells. Those cells that are not bound by the macrophages are removed, as are the cells that are bound but not phagocytized. The macrophages are then lysed to determine the amount of cells that were phagocytized. This test provides information about both the binding and phagocytizing activity of the macrophages and can also be performed in vivo by measuring the uptake of the radiolabeled red blood cells by certain tissue macrophages.

Another method to evaluate phagocytosis in vitro is to evaluate the uptake of latex spheres by macrophages. Evaluation of the ability of NK cells to lyse tumor cells is achieved by incubating radiolabeled target cells with NK cells and measuring the amount of radioactivity released into solution from the target cells.

Acquired Immunity: Humoral—The plaque (antibody)-forming cell (PFC or AFC) assay tests the ability of the host to mount an antibody response to a specific antigen, which requires the coordinated interaction of several different immune cells: macrophages, T cells, and B cells. Therefore, an effect on any of these cells (e.g., antigen processing and presentation, cytokine production, proliferation, or differentiation) can have a profound impact on the ability of B cells to produce antigen-specific antibody.

A standard PFC assay involves immunizing mice with sRBC. The antigen is taken up in the spleen and an antibody response occurs. Four days after immunization, spleens are removed and splenocytes are mixed with RBCs, complement, and agar, the mixture plated, and incubated until the B cells secrete anti-sRBC IgM antibody. This antibody then coats the surrounding sRBCs, and areas of hemolysis (plaques) can be seen.

The PFC assay can be evaluated in vivo using serum from peripheral blood of immunized mice and an enzyme-linked immunosorbent assay (ELISA). Serum from mice immunized with sRBCs is incubated in microtiter plates that have been coated with sRBC membranes to serve as the antigen for sRBC-specific IgM or IgG to bind. After incubation, an enzyme-conjugated monoclonal antibody (the secondary antibody) against IgM (or IgG) is added. This antibody recognizes the IgM (or IgG) and binds specifically to that antibody. Then, the enzyme substrate (chromogen) is added. When the substrate comes into contact with the enzyme on the secondary antibody, a color change occurs that can be detected by measuring absorbance with a plate reader.

Acquired Immunity: Cell-mediated—Of numerous assays of CMI, three routinely performed tests are the cytotoxic T lymphocyte (CTL) assay, the delayed hypersensitivity response (DHR), and the T-cell proliferative responses to antigens.

The CTL assay measures the in vitro ability of splenic T cells to recognize allogeneic or antigenically distinct target cells by evaluating the ability of the CTLs to proliferate and then lyse the target cells. CTLs are incubated with target cells that have been treated so that they cannot themselves proliferate. CTLs recognize the target cells and proliferate until they are harvested. Then, they are incubated with radiolabeled target cells. CTLs that have acquired memory recognize the foreign MHC class I on target cells and lyse them.

The DHR evaluates the ability of memory T cells to recognize foreign antigen, proliferate and migrate to the site of the antigen, and secrete cytokines in vivo. Mice are sensitized by a subcutaneous injection of the chemical. Radiolabeled iodine is allowed to be incorporated into the mouse's mononuclear cells by injecting it into the mouse's bloodstream. Then, some of the sensitizing chemical is injected into the ear, and, after euthanizing the mouse, the ear is evaluated for the presence of radiolabeled mononuclear cells.

Several mechanisms exist to evaluate proliferative capacity of T cells in CMI. The mixed lymphocyte response (MLR) measures the ability of T cells to recognize foreign MHC class I and undergo proliferation.

Flow Cytometric Analysis—Flow cytometry employs light scatter, fluorescence, and absorbance measurements to analyze large numbers of cells on an individual basis. Usually, fluorochrome-conjugated monoclonal antibodies raised against a specific protein are employed for detection. This is approach can be used to provide insight into which specific T-cell subsets are targeted after exposure to a xenobiotic, and to identify putative effects on T-cell maturation.

Molecular Biology Approaches to Immunotoxicology—Proteomics (the study of all expressed proteins in a particular cell, and thus the functional expression of the genome) and genomics (the study of all genes encoded by an organism's DNA), combined with bioinformatics, facilitate the evaluation of xenobiotic-induced alterations in the pathways and signaling networks of the immune system.

Mechanistic Approaches to Immunotoxicology—Once an agent has been identified as being an immunotoxicant, it may be necessary to further characterize its mechanism.

A general strategy involves the following steps: (1) identifying the cell type(s) targeted by the agent, (2) determining whether the effects are mediated by the parent compound or by a metabolite of the parent, (3) determining whether the effects are mediated directly or indirectly by the xenobiotic, and (4) elucidating the molecular events responsible for altered leukocyte function.

Regulatory Approaches to the Assessment of Immunotoxicity

T e N*T*P *T*ier Approach—T e National Toxicology Program screens for potential immunotoxic agents using a tier approach. Tier I provides assessment of general toxicity (immunopathology, hematology, and body and organ weights) as well as endline functional assays (proliferative responses, PFC assay, and NK assay). Tier II was designed to further define an immunotoxic effect and includes tests for CMI (CTL and DHR), secondary antibody responses, enumeration of lymphocyte populations, and host resistance models.

Health Ef ects *T*est Guidelines—Guidelines for functional immunotoxicity assessments in regulatory studies recommend conduct of three tests. Assessment of immunotoxicity begins by exposure for a minimum of 28 days to the chemical followed by assessment of humoral immunity (PFC assay or anti-sRBC ELISA). If the chemical produces significant suppression of the humoral response, surface marker assessment by flow cytometry may be performed. If the chemical produces no suppression of the humoral response, an assessment of innate immunity (NK assay) may be performed.

Animal Models in Immunotoxicology

Rats and mice have been the animals of choice for studying the actions of xenobiotics on the immune system because (1) there is a vast database available on the immune system, (2) rodents are less expensive to maintain than larger animals, and (3) a wide variety of reagents (cytokines, antibodies, etc.) are available. Many reagents that are available for studying the human immune system can also be used in rhesus and cynomolgus monkeys. Chicken and fish are being used to evaluate the immunotoxicity of xenobiotics as alternative animal models with heightened environmental consciousness.

T e manipulation of the embryonic genome, creating transgenic and knockout mice, may allow complex immune responses to be dissected into their components. In this way, the mechanisms by which immunotoxicants act can be better understood. Severe combined immunodeficient (SCID) mice have been used to study immune regulation, hematopoiesis, hypersensitivity, and autoimmunity.

Evaluation of Mechanisms of Action

Direct effects on the immune system may include chemical effects on immune function, structural alterations in lymphoid organs or on immune cell surfaces, or compositional changes in lymphoid organs or in serum. Xenobiotics may exert an indirect action on the immune system as well. T ey may be metabolically activated to their toxic metabolites, and may also have effects on other organ systems (e.g., liver damage) that then impact the immune system.

IMMUNE MODULATION BY XENOBIOTICS

T e expansive and versatile nature of the immune system renders it susceptible to modulation by a wide variety of xenobiotics (Table 12–2). Many xenobiotics exhibit immunosuppressive actions, whereas some are immunomodulatory, meaning they might produce immune suppression and immune enhancement. Regardless of the end effect (immune suppression, immune enhancement, hypersensitivity, or autoimmunity) of a particular xenobiotic on the immune system, several common themes exist regarding the mechanisms by which these chemicals act. First, the mechanisms by which a xenobiotic affects immune function are likely to be multifaceted, involving several proteins, signaling cascades, or receptors. In fact, there is evidence to suggest that immune system effects for some xenobiotics are both xenobiotic-specific receptor-dependent and independent. Second, whether a xenobiotic produces a particular immune effect might depend on the concentration or dose of the xenobiotic, the mode and/or magnitude of cellular stimulation, and the kinetic relationship between exposure to the xenobiotic and exposure to the immune stimulant (i.e., antigen, mitogen, and pharmacological agent). T ird, xenobiotic exposures rarely occur in one chemical at a time; thus, the effects and/or mechanisms observed might be attributable to several chemicals or classes of chemicals. Finally, determination of immune system effects and/or mechanisms by xenobiotics in humans might be further confounded by the physiological or immunological state of the individual.

Halogenated Aromatic Hydrocarbons

Few classes of xenobiotics have been as extensively studied for immunotoxicity as the halogenated aromatic hydrocarbons (HAHs). T e majority of the biochemical and toxic effects produced by the HAHs are mediated via HAH binding to the cytosolic aromatic hydrocarbon receptor (AHR). Binding of HAH to AHR ultimately results in upregulation of certain proteins with a net immunosuppressive effect. Interestingly, the degree of immunosuppression is positively correlated with the binding af nity of the HAH for the AHR.

Pesticides

Pesticides include all xenobiotics whose specific purpose is to kill another form of life, including insects (insecticides), small rodents (rodenticides), or even vegetation (herbicides). Exposure to pesticides occurs most often in occupational settings, in which manufacturers, those applying the pesticides, or those harvesting treated agricultural products, are exposed.

TABLE 12–2 Xenobiotics capable of immunosuppression.

Halogenated aromatic hydrocarbons
Polychlorinated biphenyls
Polybrominated biphenyls
Polychlorinated dibenzodioxins
Polychlorinated dibenzofurans
Polycyclic aromatic hydrocarbons
Nitrosamines
Pesticides
Organophosphate pesticides
Organochlorine pesticides
Organotin pesticides
Carbamate pesticides
Pyrethroids
Metals
Arsenic
Beryllium
Cadmium
Chromium
Cobalt
Gold
Lead
Mercury
Nickel
Platinum
Inhaled substances
Asbestos
Ethylenediamine
Formaldehyde
Silica
Tobacco smoke
Urethane
Oxidant gases
Ozone (O_3)
Nitrogen dioxide (NO_2)
Sulfur dioxide (SO_2)
Phosgene
Aromatic hydrocarbons
Carbon tetrachloride
Ethylene glycol monomethyl ether
2-Methoxyethanol
Mycotoxins
Aflatoxin
Ochratoxin
Tricothecenes
Vomitoxin
Natural and synthetic hormones
Estrogens
Androgens
Glucocorticoids
Therapeutics
AIDS therapeutics
Biologics
Anti-inflammatory agents
Immunosuppressive drugs
Azathioprine
Cyclophosphamide
Cyclosporin A
Leflunomide
Rapamycin
Stavudine (2′,3′-didehydro-2′,3′-dideoxythymidine)
Videx (2′,3′-dideoxyinosine; ddI)
Zalcitabine (2′,3′-dideoxycytidine; ddC)
Zidovudine (3′-azido-3′-deoxythymidine; AZT)
Drugs of abuse
Cannabinoids
Cocaine
Ethanol
Opioids: heroin and morphine

Pesticides act through a variety of mechanisms and can be both immunosuppressive and immunoenhancing (see Chapter 22).

Metals

Generally speaking, metals target multiple organ systems and exert their toxic effects via an interaction of the free metal with targets, such as enzyme systems, membranes, or cellular organelles. In considering their immunotoxicity, metals at high concentrations usually exert immunosuppressive effects; however, at lower concentrations, immune enhancement is often observed. Furthermore, as with most immunotoxicants, exposures to metals are likely not single exposures, although one metal might dominate depending on the exposure conditions (e.g., high levels of mercury in fish or high levels of lead from paint). Many metals are immunotoxic and the interested reader is referred to Chapter 23.

Solvents and Related Chemicals

T ere is limited, but substantive, evidence that exposure to organic solvents and their related compounds can produce immune suppression. Chemicals in this category are aromatic hydrocarbons, such as benzene, haloalkanes and haloalkenes, glycols and glycol ethers, and nitrosamines. T e interested reader is referred to Chapter 24.

Mycotoxins

Mycotoxins are structurally diverse secondary metabolites of fungi (see Chapter 26). T is class of chemicals comprises such

toxins as aflatoxin, ochratoxin, and the trichothecenes, notably T-2 toxin and deoxynivalenol (vomitoxin). As a class, these toxins can produce cellular depletion in lymphoid organs, alterations in T- and B-lymphocyte function, suppression of antibody responses, suppression of NK cell activity, decreased DTH responses, and an apparent increase in susceptibility to infectious disease.

Natural and Synthetic Hormones

It is well established that a sexual dimorphism exists in the immune system. Females have higher levels of circulating Igs, a greater antibody response, and a higher incidence of autoimmune disease than males. Males appear to be more susceptible to the development of sepsis and the mortality associated with soft tissue trauma and hemorrhagic shock. Specific natural sex hormones in this dichotomy have been implicated. Immune effects of androgens and estrogens appear to be very tightly controlled within the physiological range of concentrations, and profound changes in immune activity can result from very slight changes in concentrations of hormones. T e interested reader is referred to Chapters 20 and 21 for more detailed discussion of estrogens, androgens, and glucocorticoids.

Therapeutic Agents

Historically speaking, very few drugs used today as immunosuppressive agents were actually developed for that purpose. In fact, nearly all therapeutic agents possess some degree of immunomodulatory activity at some dose.

Immunosuppressive Agents—Several immunosuppressive drugs are ef cacious simply due to their ability to impair cellular proliferation, since proliferation is required for lymphocyte clonal expansion and, subsequently, differentiation. Other drugs inhibit specific intracellular proteins that are critical in the activation of the immune response.

AIDS Therapeutics—Traditionally, antiviral therapies have not been extremely successful in their attempt to rid the host of viral infection because these pathogens target the DNA of the host. Eradication of the infection means killing infected cells, which for HIV are primarily $CD4^+$ T cells. Numerous strategies have been developed to combat HIV, including targeting viral reverse transcriptase (nucleoside and nonnucleoside reverse transcriptase inhibitors), viral protease, viral fusion and entry, virus–T-cell interaction proteins, and stimulating immune responses. T e multidrug therapy used currently is referred to as highly active antiretroviral therapy (HAART). However, eradication of this virus, and subsequently AIDS, remains a challenge because the very nature of the infection has significant immunosuppressive consequences. In addition, some of the current therapies also exhibit immunosuppressive actions. One such antiviral drug is zidovudine (Retrovir).

Biologics—Biologics refers to those therapies that are derived in some manner from living organisms and include monoclonal antibodies, recombinant proteins, and adoptive cell therapies. By its very nature, the immune system is often both the intended therapeutic target and unintended toxicological target of various biologics. Overall, manifestations of toxicity may include exaggerated pharmacology, effects due to biochemical cross-talk, and disruptions in immune regulation by cytokine networks. Monoclonal antibodies can bind normal as well as targeted tissues, and any foreign protein may elicit the production of neutralizing antibodies against the therapeutic protein (i.e., the therapeutic protein may be immunogenic).

Anti-in ammatory Agents—Anti-inflammatory agents include nonselective and selective nonsteroidal anti-inflammatory drugs (NSAIDs), which suppress the production of pro-inflammatory soluble factors, such as prostaglandins and thromboxanes. Nonselective NSAIDs are a large class of drugs that reversibly inhibit both isoforms of cyclooxygenase (COX-1 and COX-2). T e COX-2 enzyme, in particular, is induced in response to inflammatory cytokines and mediators and, therefore, represents an attractive target to combat inflammatory diseases. Aspirin, like nonselective NSAIDs, inhibits COX-1 and COX-2 enzymes, but inhibition is irreversible due to covalent binding of aspirin by acetylation to a serine residue in the COX enzyme. Aspirin is an especially effective antiplatelet agent since platelets possess little biosynthesizing capacity and, therefore, aspirin will inhibit COX for the life of the platelet (8–11 days).

Drugs of Abuse

Drug abuse is a social issue with extensive pathophysiological effects on the abuser. Drugs of abuse exhibit immunosuppressive actions, and in fact it has been suggested that in addition to the direct risk of HIV contraction via needle sharing or judgment lapses, abuse of some drugs has been associated with disease progression to AIDS. Several classes of drugs are included in this category, such as cannabinoids, opioids, cocaine, methamphetamine, and ethanol.

Inhaled Substances

Pulmonary defenses against inhaled gases and particulates are dependent on both physical and immunological mechanisms. Immune mechanisms primarily involve the complex interactions between neutrophils and alveolar macrophages and their abilities to phagocytize foreign material and produce cytokines, which not only act as local inflammatory mediators, but also serve to attract other cells into the airways.

Ultraviolet Radiation

Ultraviolet radiation (UVR), especially midrange UVB (290–340 nm), is an important environmental factor affecting human health with both beneficial effects including vitamin D

production, tanning, and adaptation to UV, and adverse effects including sunburn, skin cancer, and ocular damage. It is important to emphasize that all humans encounter lifetime exposure to this ubiquitous environmental immunotoxicant. While UV-induced immunomodulation has been shown to have some beneficial effects on some skin diseases, such as psoriasis, and has been demonstrated to impair some allergic and autoimmune diseases in both animals and humans. UV-induced immunomodulation can also lead to several adverse health consequences, including a pivotal role during the process of skin carcinogenesis.

XENOBIOTIC-INDUCED HYPERSENSITIVITY AND AUTOIMMUNITY

When an individual's immune system responds in a manner producing tissue damage, it could result in hypersensitivity or autoimmunity, which could be exacerbated, or even induced, by another xenobiotic.

Hypersensitivity

Numerous xenobiotics illicit hypersensitivity reactions. Polyisocyanates, and toluene diisocyanate in particular, used in the production of adhesives and coatings are known to induce the full spectrum of hypersensitivity responses, types I to IV, as well as nonimmune inflammatory and neuroreflex reactions in the lung. Inhaled acid anhydrides, which are used in the manufacturing of paints, varnishes, coating materials, adhesives, and casting and sealing materials, may conjugate with serum albumin or erythrocytes leading to type I, II, or III hypersensitivity reactions on subsequent exposure.

Metals—Metals and metallic substances, including metallic salts, are responsible for producing contact and pulmonary hypersensitivity reactions. Platinum, cobalt, chromium, nickel, and beryllium are commonly implicated.

Drugs—Hypersensitivity responses to drugs are among the major types of unpredictable drug reactions. Drugs are designed to be reactive in the body and multiple treatments are common. T is type of exposure is conducive to producing an immunologic reaction. Immunologic mechanisms of hypersensitivity reactions to drugs include types I to IV. Penicillin is the most common agent involved in drug allergy.

Latex—Natural rubber latex is used in the manufacture of over 40 000 products from balloons to surgical gloves. Allergic reactions to natural rubber latex products have become an important occupational health concern with increased use of universal precautions, particularly latex gloves, to combat the spread of bloodborne pathogens. Hypersensitivity to latex usually occurs via a type I or type IV reaction. Dermatologic reactions to latex include irritant dermatitis and contact dermatitis.

Food and Genetically Modif ed Organisms—Awareness of hypersensitivity reactions to foods and genetically modified organisms (or crops; GMOs) has increased in the last several years. T e most common food allergens are milk, egg, peanuts and other tree nuts, fish, shellfish, soy, and wheat. Hypersensitivity to peanuts occurs primarily via a type I reaction, and the IgE responses may include shortness of breath, asthma, and anaphylaxis.

Formaldehyde—Formaldehyde is used as a preservative, sterilant, and fumigant. Additional exposures come from the textile industry, where it is used to improve wrinkle resistance, and in the furniture, auto upholstery, and resins industries. Occupational exposure to formaldehyde has been associated both with the occurrence of asthma and increased respiratory allergic responses to other stimuli.

Autoimmunity

T ere are numerous reports of xenobiotics that have been associated with autoimmunity; however, firm evidence for their involvement is dif cult to obtain. Presently, there are very few instances of human autoimmune diseases for which an environmental trigger has been definitely identified. T ese relationships may be causative through direct mechanisms, or they may be indirect, acting as an adjuvant. In the area of xenobiotic-induced autoimmunity, exact mechanisms of action are not always known. Chemical exposure may also serve to exacerbate a preexisting autoimmune state.

Therapeutic Agents

Methyldopa—Methyldopa is a centrally acting sympatholytic drug that has been widely used for the treatment of essential hypertension, but with the advent of newer antihypertensive drugs, the use of methyldopa has declined. Platelets and erythrocytes are targeted by the immune system in individuals treated with this drug, resulting in thrombocytopenia and hemolytic anemia.

Hydralazine, Isoniazid, and Procainamide—Hydralazine is a direct-acting vasodilator drug used in the treatment of hypertension. Isoniazid is an antimicrobial drug used in the treatment of tuberculosis. Procainamide is a drug that selectively blocks sodium channels in myocardial membranes, making it useful in the treatment of cardiac arrhythmias. All three drugs produce autoimmunity, which is manifested as a systemic lupus erythematosus-like syndrome.

Halothane—Halothane, one of the most widely studied of the drugs inducing autoimmunity, is an inhalation anesthetic that can induce autoimmune hepatitis. T e pathogenesis of the hepatitis results from the chemical altering a specific liver protein to such a degree that the immune system recognizes the altered protein and antibodies are produced.

Vinyl Chloride—Vinyl chloride is used in the plastics industry as a refrigerant and in the synthesis of organic

chemicals. Although the exact mechanism whereby this chemical produces autoimmunity is unclear, it is presumed that vinyl chloride acts as an amino acid and is incorporated into protein. Because this would produce a structurally abnormal protein, which would be antigenic, an immune response would be directed against tissues with the modified protein present.

Mercury—T is widely used metal is known to have several target systems, including the CNS and renal system. Mercury also has two different actions with respect to the immune system. T e first action is direct injury, and the second action is mercury-induced autoimmune disease that is manifested as glomerular nephropathy. Antibodies produced to laminin are believed to be responsible for damage to the basement membrane of the kidney.

Silica—Crystalline silica (silicon dioxide) is a primary source of elemental silicon and is used commercially in large quantities as a constituent of building materials, ceramics, concretes, and glasses. Experimental animals as well as humans exposed to silica may have perturbations in the immune system. Depending on the length of exposure, dose, and route of administration of silica, it may kill macrophages or may act as an immunostimulant. Silica has been shown to be associated with an increase in scleroderma in silica-exposed workers. Adjuvancy as a mechanism of causing autoimmunity has been implicated with a number of other chemicals, including paraf n and silicones.

Hexachlorobenzene—Hexachlorobenzene is a low molecular weight compound that was used in the past as a fungicide for seed grains. After exposure to hexachlorobenzene, its deposition can directly induce cell damage or elicit damage by interfering with the integrity of cell membranes due to its lipophilic nature. Ultimately, hexachlorobenzene exposure triggers pro-inflammatory mediators, such as TNF-α, IL-1, IL-6, ROS, and chemokines. T ese pro-inflammatory mediators serve as adjuvant signals that induce a systemic inflammatory response with influxes of neutrophils and macrophages into various nonimmune and immune organs. Subsequently, this leads to polyclonal activation of T and B cells, eosinophilia, and eventually to visible clinical effects.

NEW FRONTIERS AND CHALLENGES IN IMMUNOTOXICOLOGY

T ere are several specific areas within the subdiscipline of immunotoxicology that are currently on the forefront, but will likely see significant advancements and changes. T e first will be the continued evolution and application of human primary leukocytes in mechanistic studies of immunotoxicology. In spite of the similarities between the human immune system and that of other animal species, there is an increasing appreciation that often subtle but potentially important differences exist. Advancements in technology, especially flow cytometry, have already and will continue to greatly facilitate the application of human primary leukocytes in studies of immunotoxicology.

A second area that will see significant changes will be the application of human-derived cell lines, and validation of assays using these cell lines for the purpose of evaluating and screening potential immunotoxicants. A significant driver for broader employment of cell lines in immunotoxicity testing are cost, ethical considerations to reduce the use of animals in toxicity testing, and a fundamental belief that toxicants which alter one or more of the major signaling pathways regulating cell function can be identified in this manner.

T e third area of emphasis in immunotoxicology will be the application of computational biology to better understand and describe the underlying molecular mechanisms by which an immunotoxicant alters immune function. Computational biology has tremendous potential in estimating the potential risk from exposure to immunotoxicants as well as predicting the risk associated with exposure to multiple immunotoxicants simultaneously.

T e last area on the forefront of immunotoxicology is increased use of transcriptome analysis. T is change will be primarily driven by major advancements and applications of next generation sequencing which will likely make microarrays obsolete due to the significantly greater sensitivity of this technology, decreased cost, and open platform (i.e., capable of quantifying the entire transcriptome and not restricted to the DNA tiled on a chip). Moreover, the applications of next generation sequencing beyond studies of the transcriptome are considerable and span uses such as identification of single-nucleotide polymorphisms associated with sensitive subpopulations and applications in personalized medicine to identification and analysis of DNA methylation for studies of epigenetics.

In spite of these advances, significant challenges remain to be addressed within the discipline of immunotoxicology and include (1) how to interpret the significance of minor or moderate immunotoxic effects in animal models in relation to human risk assessment; (2) how to better integrate a consideration of exposure, especially to multiple agents simultaneously, into immunotoxicologic risk assessment; (3) how to design better human studies to assess the impact on the immune system in the species of greatest interest in the context of risk assessment; (4) how to identify and establish sensitive human biomarkers of immunotoxicity; and (5) how to gain a better understanding of the role of genetics in identifying sensitive subpopulations to immune-altering agents.

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QUESTIONS

1. Which of the following cells or substances is NOT part of the innate immune system?
   a. lysozyme.
   b. monocytes.
   c. complement.
   d. antibodies.
   e. neutrophils.

2. Myeloid precursor stem cells are responsible for the formation of all of the following EXCEPT:
   a. platelets.
   b. lymphocytes.
   c. basophils.
   d. erythrocytes.
   e. monocytes.

3. When an Rh− mother is exposed to the blood of an Rh+ baby during childbirth, the mother will make antibodies against the Rh factor, which can lead to the mother attacking the next Rh+ fetus. T is is possible because of which antibody's ability to cross the placenta.
   a. IgM.
   b. IgE.
   c. IgG.
   d. IgA.
   e. IgD.

4. Which of the following statements is FALSE regarding important cytokine function in regulating the immune system?
   a. IL-1 induces inflammation and fever.
   b. IL-3 is the primary T-cell growth factor.
   c. IL-4 induces B-cell differentiation and isotype switching.
   d. Transforming growth factor-β (TGF-β) enhances monocyte/macrophage chemotaxis.
   e. Interferon gamma (IFN-gamma) activates macrophages.

5. Which of the following is NOT a step performed during an enzyme-linked immunosorbent assay (ELISA)?
   a. A chromogen is added and color is detected.
   b. T e antigen of interest is fixed to a microtiter plate.
   c. Radioactively labeled cells are added to the solution.
   d. Enzyme-tagged secondary antibodies are added.
   e. Test sera are added.

6. T e delayed hypersensitivity response (DHR) test does NOT:
   a. evaluate memory T-cells' ability to recognize a foreign antigen.
   b. evaluate memory T-cells' ability to secrete cytokines.
   c. evaluate memory T-cells' ability to proliferate.
   d. evaluate memory T-cells' ability to lyse foreign target cells.
   e. evaluate memory T-cells' ability to migrate to the site of foreign antigen.

7. T e number of alveolar macrophages in smokers is greatly increased relative to nonsmokers. What is a characteristic of the alveolar macrophages found in smokers?
   a. T ey are in an inactive state.
   b. T ey are far larger than normal.
   c. T ey have increased phagocytic activity.
   d. T ey are incapable of producing cytokines.
   e. T ey have decreased bactericidal activity.

8. Which of the following is NOT characteristic of a type I hypersensitivity reaction?
   a. It is mediated by IgE.
   b. It involves immune complex deposition in peripheral tissues.
   c. It involves mast-cell degranulation.
   d. Anaphylaxis is an acute, systemic, and very severe type I hypersensitivity reaction.
   e. It is usually mediated by preformed histamine, prostaglandins, and leukotrienes.

9. Which of the following types of hypersensitivity is NOT mediated by antibodies?
   a. type I.
   b. type II.
   c. type III.
   d. type IV.
   e. type V.

10. Which of the following is NOT a common mechanism of autoimmune disorders?
    a. subjection to positive selection in the thymus.
    b. anergic T cells become activated.
    c. interference with normal immunoregulation by $CD8^+$ suppressor T cells.
    d. lack of subjection to negative selection in the thymus.
    e. decreased self-tolerance.

CHAPTER

13

Toxic Responses of the Liver

Hartmut Jaeschke

INTRODUCTION

LIVER PHYSIOLOGY

- Hepatic Functions
- Structural Organization
- Bile Formation

LIVER PATHOPHYSIOLOGY

- Mechanisms and Types of Toxicant-induced Liver Injury
  - Cell Death
  - Canalicular Cholestasis
  - Bile Duct Damage
  - Sinusoidal Damage
  - Disruption of the Cytoskeleton
  - Fatty Liver
  - Fibrosis and Cirrhosis
  - Tumors
- Critical Factors in Toxicant-induced Liver Injury
  - Uptake and Concentration
  - Bioactivation and Detoxification
  - Regeneration
  - Inflammation and Immune Responses
  - Activation of Sinusoidal Cells
  - Mitochondrial Damage
  - Idiosyncratic Liver Injury

FUTURE DIRECTIONS

KEY POINTS

- The liver's strategic location between intestinal tract and the rest of the body facilitates its maintenance of metabolic homeostasis in the body.
- The liver extracts ingested nutrients, vitamins, metals, drugs, environmental toxicants, and waste products of bacteria from the blood for catabolism, storage, and/or excretion into bile.
- Formation of bile is essential for uptake of lipid nutrients from the small intestine, protection of the small intestine from oxidative insults, and excretion of endogenous and xenobiotic compounds.
- Cholestasis is either a decrease in the volume of bile formed or an impaired secretion of specific solutes into bile, which results in elevated serum levels of bile salts and bilirubin.
- Hepatocytes have a rich supply of phase I enzymes that often convert xenobiotics to reactive electrophilic metabolites and of phase II enzymes that add a polar group to a molecule and thereby enhance its removal from the body. The balance between phase I and phase II reactions determines whether a reactive metabolite will initiate liver cell injury or be safely detoxified.

INTRODUCTION

T e liver is the main organ where exogenous chemicals are metabolized and eventually excreted. As a consequence, liver cells are exposed to significant concentrations of these chemicals, which can result in liver dysfunction, cell injury, and even organ failure. T e liver, with its multiple cell types and numerous functions, can respond in many different ways to acute and chronic insults. To recognize potential liver cell dysfunction and injury, it is necessary to have a general knowledge of basic liver functions, the structural organization of the liver, the processes involved in hepatic excretory functions, and mechanisms of cell and organ injury.

LIVER PHYSIOLOGY

Hepatic Functions

T e liver's strategic location between intestinal tract and the rest of the body facilitates the performance of its enormous task of maintaining the metabolic homeostasis of the body. Venous blood from the stomach and intestines flows into the portal vein, through the liver, and then enters the systemic circulation. T e liver is the first organ to encounter ingested nutrients, vitamins, metals, drugs, and environmental toxicants as well as waste products of bacteria that enter portal blood. Efficient scavenging or uptake processes extract these absorbed materials from the blood for catabolism, storage, and/or excretion into bile.

All of the major functions of the liver can be detrimentally altered by acute or chronic exposure to toxicants (Table 13–1). When toxicants inhibit or otherwise impede hepatic transport and synthetic processes, dysfunction can occur without appreciable cell damage. Loss of function also occurs when toxicants kill a considerable number of cells and when chronic insult leads to replacement of cell mass by nonfunctional scar tissue.

Structural Organization

Two concepts exist for organization of the liver into operational units, namely, the lobule and the acinus. Classically, the liver is divided into hexagonal lobules oriented around terminal hepatic venules (also known as central veins). At the corners of the lobule are the portal triads (or portal tracts), containing a branch of the portal vein, a hepatic arteriole, and a bile duct (Figure 13–1). Blood entering from the portal vein and hepatic artery mixes in the penetrating vessels, enters the sinusoids, percolates along the cords of parenchymal cells (hepatocytes), flows into terminal hepatic venules, and exits the liver via the hepatic vein. T e lobule is divided into three regions known as centrolobular, midzonal, and periportal. T e preferred concept of a functional hepatic unit is the acinus. T e base of the acinus is formed by the terminal branches of the portal vein and hepatic artery, which extend out from the portal tracts. T e acinus has three zones: zone 1 is closest to the entry of blood, zone 3 abuts the terminal hepatic vein, and zone 2 is intermediate. T e three zones of the acinus roughly coincide with the three regions of the lobule (Figure 13–1).

Acinar zonation is of considerable functional consequence regarding gradients of components both in blood and in hepatocytes. Blood entering the acinus consists of oxygen-depleted blood from the portal vein (60% to 70% of hepatic blood flow) plus oxygenated blood from the hepatic artery (30% to 40%). En route to the terminal hepatic venule, oxygen rapidly leaves the blood to meet the high metabolic demands of the parenchymal cells. Hepatocytes in zone 3 are exposed to substantially lower concentrations of oxygen than hepatocytes in zone 1. In comparison to other tissues, zone 3 is hypoxic.

TABLE 13–1 Major functions of liver and consequences of impaired hepatic functions.

Type of Function	Examples	Consequences of Impaired Functions
Nutrient homeostasis	Glucose storage and synthesis Cholesterol uptake	Hypoglycemia, confusion Hypercholesterolemia
Filtration of particulates	Products of intestinal bacteria (e.g., endotoxin)	Endotoxemia
Protein synthesis	Clotting factors Albumin Transport proteins (e.g., very low-density lipoproteins)	Excess bleeding Hypoalbuminemia, ascites Fatty liver
Bioactivation and detoxification	Bilirubin and ammonia Steroid hormones Xenobiotics	Jaundice, hyperammonemia-related coma Loss of secondary male sex characteristics Diminished drug metabolism Inadequate detoxification
Formation of bile and biliary secretion	Bile acid–dependent uptake of dietary lipids and vitamins Bilirubin and cholesterol Metals (e.g., Cu and Mn) Xenobiotics	Fatty diarrhea, malnutrition, vitamin E deficiency Jaundice, gallstones, hypercholesterolemia Mn-induced neurotoxicity Delayed drug clearance

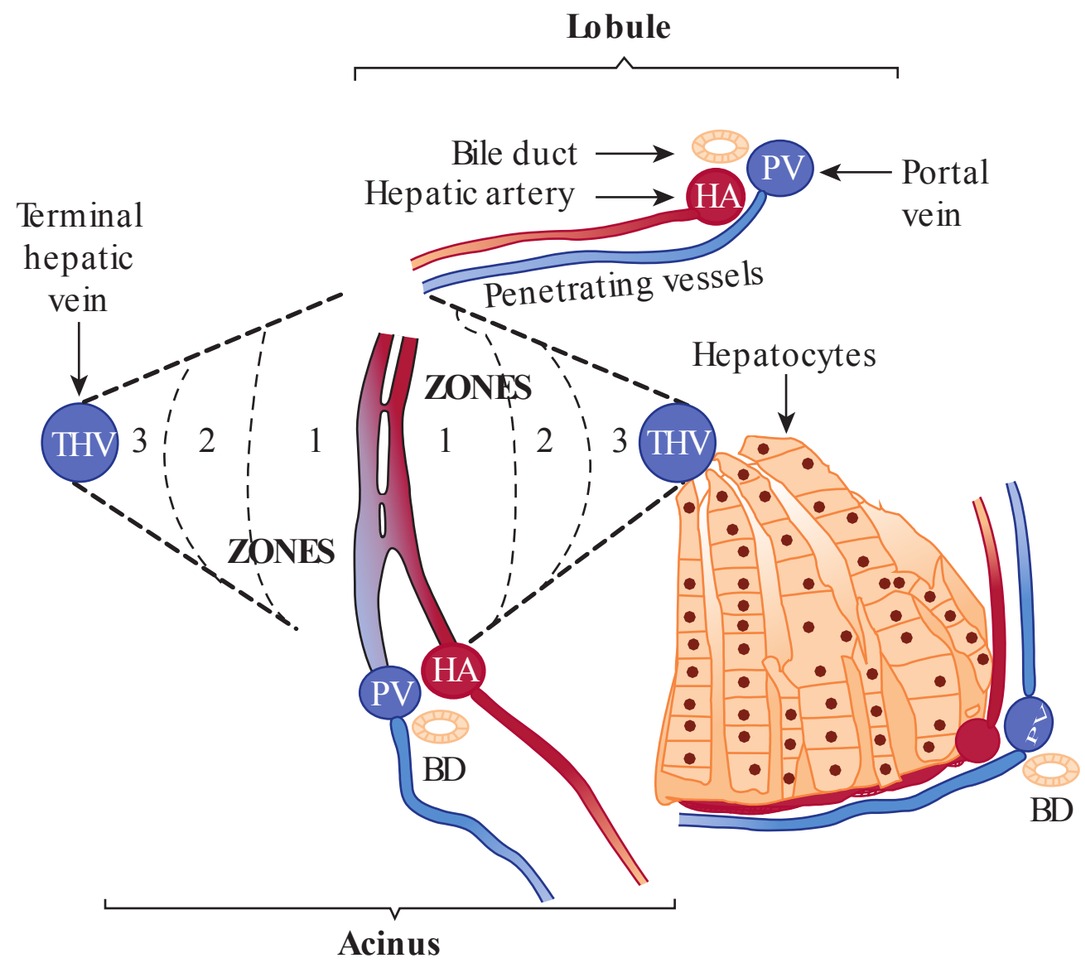

FIGURE 13–1 **Schematic of liver operational units, the classic lobule and the acinus.** The lobule is centered around the terminal hepatic vein (central vein), where the blood drains out of the lobule. The acinus has as its base the penetrating vessels, where blood supplied by the portal vein and hepatic artery flows down the acinus past the cords of hepatocytes. Zones 1, 2, and 3 of the acinus represent metabolic regions that are increasingly distant from the blood supply.

Well-documented acinar gradients exist for bile salts, bilirubin, and many organic anions as well.

Heterogeneities in protein levels of hepatocytes along the acinus generate gradients of metabolic functions. Hepatocytes in the mitochondria-rich zone 1 are predominant in fatty acid oxidation, gluconeogenesis, and ammonia detoxification to urea. Gradients of enzymes involved in the bioactivation and detoxification of xenobiotics have been observed along the acinus by immunohistochemistry (exploiting the immune system's specificity to stain tissue). Notable gradients for hepatotoxicants are the higher levels of glutathione in zone 1 and the greater amounts of cytochrome P450 proteins in zone 3, particularly the CYP2E1 isozyme inducible by ethanol.

Hepatic sinusoids are the channels between cords of hepatocytes where blood percolates on its way to the terminal hepatic vein. The three major types of cells in the sinusoids are endothelial cells, Kupffer cells, and stellate (Ito) cells. Sinusoids are lined by thin, discontinuous endothelial cells with numerous fenestrae (or pores) that allow molecules smaller than 250 kDa to cross the interstitial space (known as the space of Disse) between the endothelium and hepatocytes. The numerous fenestrae and the lack of basement membrane facilitate exchanges of fluids and molecules, such as albumin, between the sinusoid and hepatocytes, but hinder movement of particles larger than chylomicron remnants.

Kupffer cells, the resident macrophages of the liver, constitute approximately 80% of the fixed macrophages in the body. Kupffer cells are situated within the lumen of the sinusoid. The primary function of Kupffer cells is to ingest and degrade particulate matter. Also, Kupffer cells are a major source of cytokines and eicosanoids and can act as antigen-presenting cells (APCs). Ito cells (also known as fat-storing cells and stellate cells) are located between endothelial cells and hepatocytes. Ito cells synthesize collagen and are the major storage site for vitamin A in the body.

Bile Formation

Bile contains bile acids, glutathione, phospholipids, cholesterol, bilirubin, and other organic anions, proteins, metals, ions, and xenobiotics. Formation of this fluid is a specialized function of the liver. Adequate bile formation is essential for uptake of lipid nutrients from the small intestine (Table 13–1), protection of the small intestine from oxidative insults, and excretion of endogenous and xenobiotic compounds. Hepatocytes begin the process by transporting bile acids, glutathione, and other solutes including xenobiotics and their metabolites into the canalicular lumen (the space formed by specialized regions of the plasma membrane between adjacent hepatocytes). The canaliculi are separated from the intercellular space between hepatocytes by tight junctions, which form a barrier permeable only to water, electrolytes, and to some degree to small organic cations. These canaliculi form channels between hepatocytes that connect to a series of larger and larger channels or ducts within the liver. The large extrahepatic bile ducts merge into the common bile duct. Bile can be stored and concentrated in the gallbladder before its release into the first segment of the small intestine, the duodenum.

The major driving force of bile formation is the active transport of bile salts and other osmolytes into the canalicular lumen. Most conjugated bile acids (taurine and glycine conjugates) and some unconjugated bile acids are transported into hepatocytes by sodium-dependent transporters. Sodium-independent uptake of conjugated and unconjugated bile acids is performed by members of the organic anion-transporting polypeptides (OATPs). OATPs also transport numerous drugs and hepatotoxicants. Lipophilic cationic drugs, estrogens, and lipids are exported by the canalicular multiple-drug resistance (MDR) P-glycoproteins, one of which is exclusive for phospholipids. Conjugates of glutathione, glucuronide, and sulfate are exported by multidrug resistance–associated protein 2 (MRP2). The many different transporters are shown in Figure 13–2.

Metals are excreted into bile by a series of processes that include (1) uptake across the sinusoidal membrane by facilitated diffusion or receptor-mediated endocytosis; (2) storage in binding proteins or lysosomes; and (3) canalicular secretion via lysosomes, a glutathione-coupled event, or use of a specific canalicular membrane transporter, e.g., MRP2. Biliary excretion is important in the homeostasis of metals, notably copper, manganese, cadmium, selenium, gold, silver, and arsenic. Inability to export Cu into bile is a central problem in Wilson's disease, a rare genetic disorder characterized by accumulation of Cu in the liver and then in other tissues.

Canalicular lumen bile is propelled forward into larger channels by dynamic, ATP-dependent contractions of the

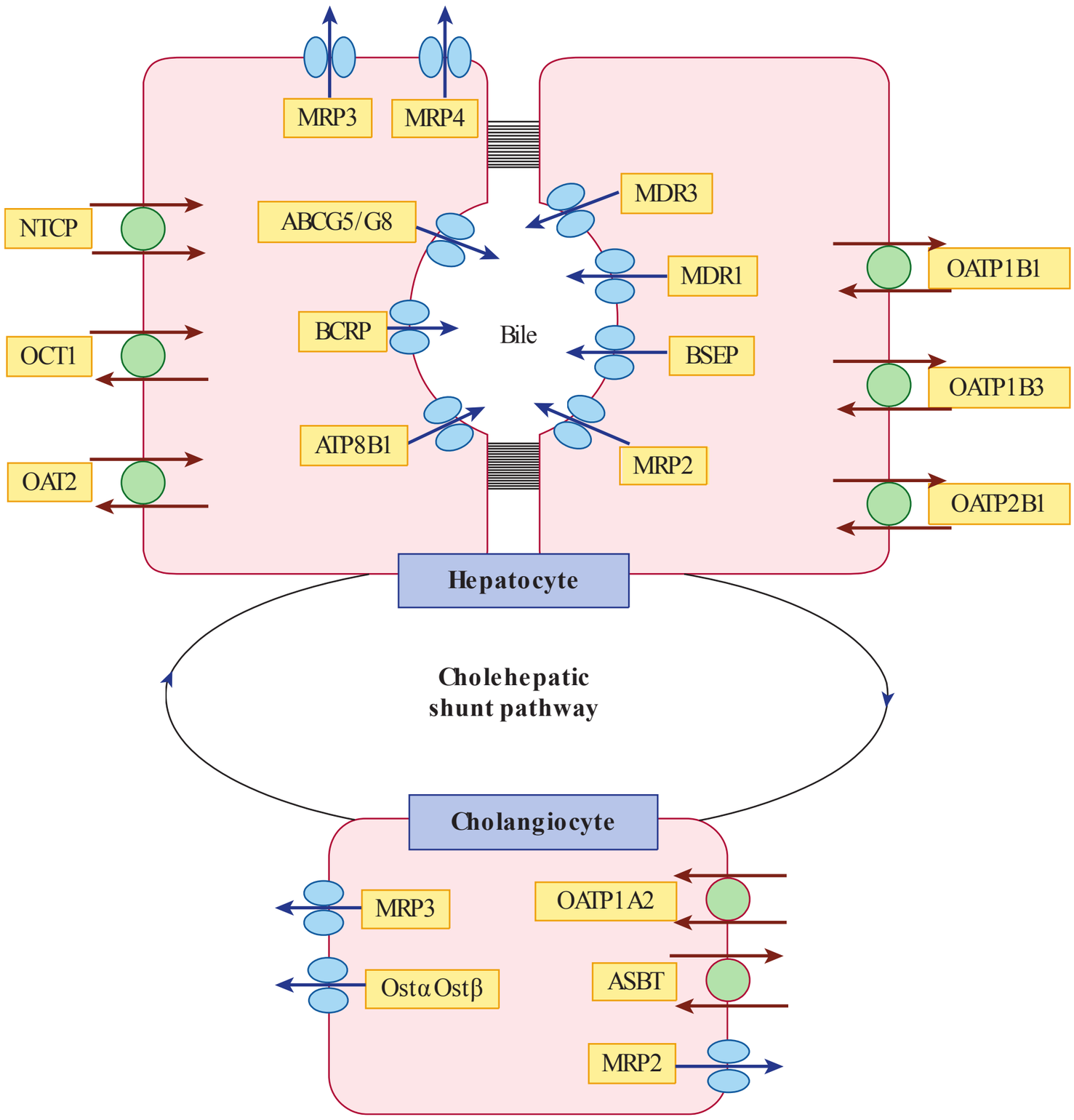

FIGURE 13–2 **Transport proteins in human hepatocytes and cholangiocytes.** Efflux transporters (blue ovals with blue arrows): BSEP, bile salt export pump; MDR, multidrug resistance protein; MRP, multidrug resistance-associated protein; ABCG5/8, heterodimeric ATP binding cassette transporter G5/G8; BCRP, breast cancer resistance protein; Ostα/Ostβ, heterodimeric organic solute transporter alpha and beta. Uptake transporters (green circles with red arrows): ASBT, apical sodium–dependent bile salt transporter; NTCP, sodium taurocholate cotransporting polypeptide; OATP, organic anion-transporting polypeptide; OCT, organic cation transporter; OAT, organic anion transporter. Transporters localized to the sinusoidal membrane extract solutes from the blood. Exporters localized to canalicular membrane move solutes into the lumen of the canaliculus. Exporters of particular relevance to canalicular secretion of toxic chemicals and their metabolites are the canalicular multiple organic anion transporter (MOAT) system and the family of multiple-drug resistant (MDR) P-glycoproteins. MDR3 (ABCB4) flops phosphotidylcholine from the inner to the outer leaflet of the canalicular membrane. ATP8B1 flips phosphatidylserine from the outer to inner membrane to maintain the lipid asymmetry of the canalicular membrane. (Reproduced with permission from Pauli-Magnus C, Meier PJ: Hepatobiliary transporters and drug-induced cholestasis, Hepatology, 2006 Oct;44(4):778–787.)

pericanalicular cytoskeleton. Bile ducts modify bile by absorption and secretion of solutes. Biliary epithelial cells also express a variety of phase I and phase II enzymes, which may contribute to the biotransformation of toxicants present in bile.

Secretion into biliary ducts is usually, but not always, a prelude to toxicant clearance by excretion in feces or urine. Exceptions occur when compounds are repeatedly delivered into the intestinal lumen via bile, efficiently absorbed from the intestinal lumen, and then redirected to the liver via portal blood, a process known as enterohepatic cycling.

Toxicant-related impairments of bile formation are more likely to have detrimental consequences in populations with other conditions where biliary secretion is marginal. For example, neonates exhibit delayed development of bile salt synthesis and the expression of sinusoidal and canalicular transporters. Neonates are more prone to develop jaundice when treated with drugs that compete with bilirubin for biliary clearance.

LIVER PATHOPHYSIOLOGY

Mechanisms and Types of Toxicant-induced Liver Injury

Hepatic response to insults by chemicals (Table 13–2) depends on the intensity of the insult, the population of cells affected, and whether the exposure is acute or chronic.

TABLE 13–2 Types of hepatobiliary injury.

Type of Injury or Damage	Representative Toxins
Fatty liver	Amiodarone, CCl_4, ethanol, fialuridine, tamoxifen, valproic acid
Hepatocyte death	Acetaminophen, allyl alcohol, Cu, dimethylformamide, ethanol
Immune-mediated response	Diclofenac, ethanol, halothane, tienilic acid
Canalicular cholestasis	Chlorpromazine, cyclosporin A, 1,1-dichloroethylene, estrogens, Mn, phalloidin
Bile duct damage	Alpha-naphthylisothiocyanate, amoxicillin, methylene dianiline, sporidesmin
Sinusoidal disorders	Anabolic steroids, cyclophosphamide, microcystin, pyrrolizidine alkaloids
Fibrosis and cirrhosis	CCl_4, ethanol, thioacetamide, vitamin A, vinyl chloride
Tumors	Aflatoxin, androgens, arsenic, thorium dioxide, vinyl chloride

Cell Death—Based on morphology, liver cells can die by two different modes, necrosis or apoptosis. Necrosis is characterized by cell swelling, leakage, nuclear disintegration (karyolysis), and an influx of inflammatory cells. When necrosis occurs in hepatocytes, the associated plasma membrane leakage can be detected biochemically by assaying plasma (or serum) for liver cytosol-derived enzymes such as aspartate or alanine aminotransferases (AST or ALT) or γ-glutamyltranspeptidase (GGT). In contrast, apoptosis is characterized by cell shrinkage, nuclear fragmentation, formation of apoptotic bodies, and a lack of inflammation. It is always a single cell event with the main purpose of removing cells no longer needed during development or eliminating aging cells.

Hepatocyte death can occur in a focal, zonal, or panacinar (widespread) pattern. Focal cell death is characterized by the randomly distributed death of single hepatocytes or small clusters of hepatocytes. Zonal necrosis is death to hepatocytes in certain functional regions. Panacinar necrosis is massive death of hepatocytes with only a few or no remaining survivors.

Mechanisms of toxicant-induced injury to liver cells include lipid peroxidation, binding to cell macromolecules, mitochondrial damage, disruption of the cytoskeleton, and massive calcium influx. Independent of the initial insult, the mitochondrial membrane permeability transition pore opens causing collapse of the membrane potential and depletion of cellular ATP, and necrotic cell death. The loss of ATP inhibits the ion pumps in the plasma membrane, which results in the loss of cellular ion homeostasis and causes the characteristic swelling of oncotic necrosis.

Canalicular Cholestasis—Defined physiologically as a decrease in the volume of bile formed or an impaired secretion of specific solutes into bile, cholestasis is characterized biochemically by elevated serum levels of compounds normally concentrated in bile, particularly bile salts and bilirubin. When biliary excretion of the yellowish bilirubin pigment is impaired, this pigment accumulates in the skin and eyes, producing jaundice, and spills into urine, which becomes bright yellow or dark brown. Toxicant-induced cholestasis can be transient or chronic; when substantial, it is associated with cell swelling, cell death, and inflammation. Many different types of chemicals cause cholestasis (Table 13–2).

The molecular mechanisms of cholestasis are related to expression and function of transporter systems in the basolateral and canalicular membranes. An increased hepatic uptake, decreased biliary excretion, and increased biliary reabsorption (cholehepatic shunting) of a drug may contribute to its accumulation in the liver.

Bile formation is vulnerable to toxicant effects on the functional integrity of sinusoidal transporters, canalicular exporters, cytoskeleton-dependent processes for transcytosis, and the contractile closure of the canalicular lumen (Figure 13–3). Changes that weaken the junctions that form the structural barrier between the blood and the canalicular lumen allow solutes to leak out of the canalicular lumen. These paracellular junctions provide a size and charge barrier to the diffusion of solutes between the blood and the canalicular lumen while water and

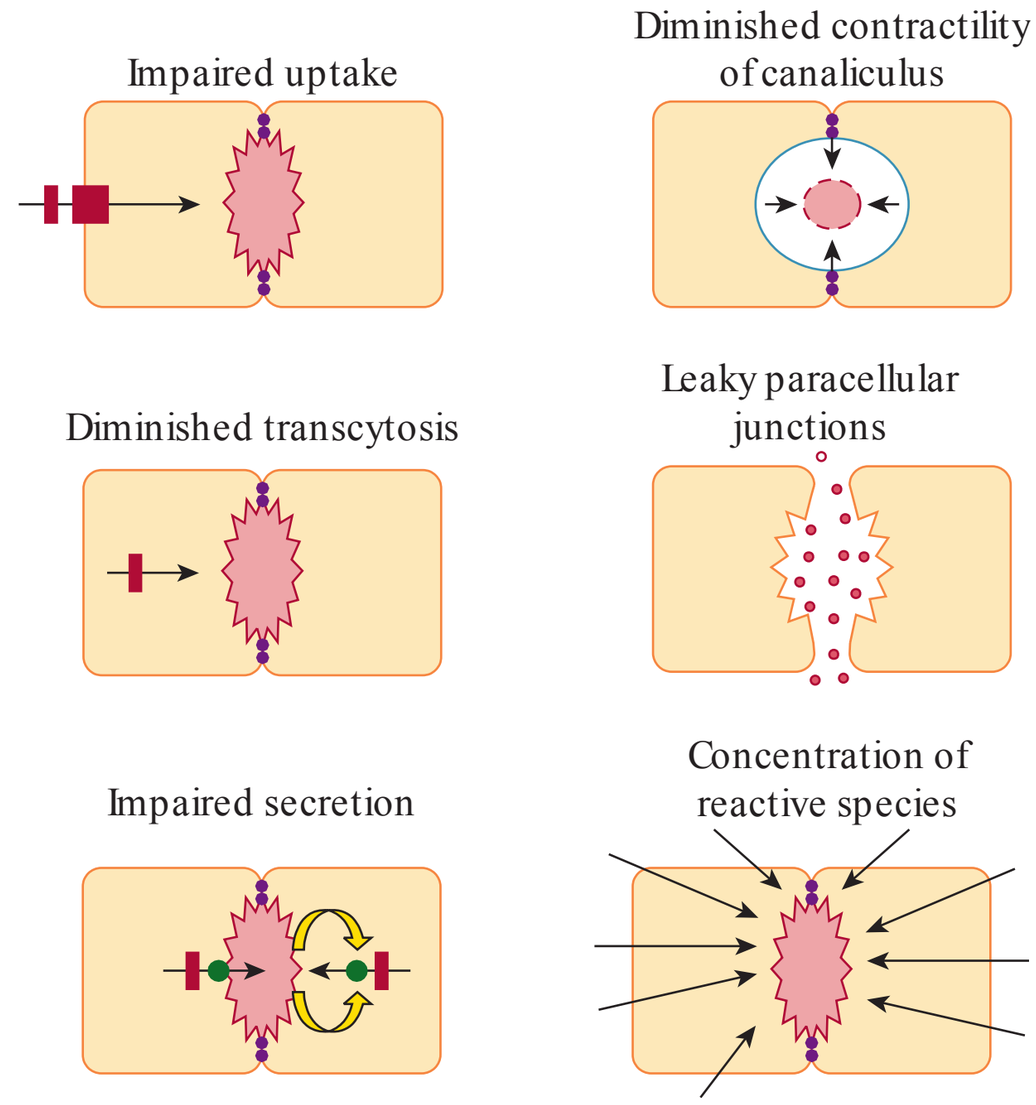

FIGURE 13–3 **Schematic of six potential mechanisms for cholestasis.** Inhibited uptake, diminished transcytosis, impaired secretion, diminished canalicular contractility, leakiness of the junctions that seal the canalicular lumen from the blood, and detrimental consequences of high concentrations of toxic entities in the pericanalicular area are possible. Note that impaired secretion across the canalicular membrane can result from inhibition of a transporter or retraction of a transporter away from the canalicular membrane.

small ions diffuse across these junctions. One hepatotoxicant that causes tight-junction leakage is α-napthylisothiocyanate.

Compounds that produce cholestasis do not necessarily act by a single mechanism or at just one site. Chlorpromazine impairs bile acid uptake and canalicular contractility. Multiple alterations have been well documented for estrogens, a well-known cause of reversible canalicular cholestasis. Estrogens and progestins decrease bile salt uptake by effects at the sinusoidal membrane including a decrease in the Na^+,K^+-ATPase necessary for Na-dependent transport of bile salts across the plasma membrane and changes in lipid component of this membrane. At the canalicular membrane, estrogens diminish the transport of glutathione conjugates and reduce the number of bile salt transporters.

An additional mechanism for canalicular cholestasis is concentration of reactive forms of chemicals in the pericanalicular area (Figure 13–3). Most chemicals that cause canalicular cholestasis are excreted in bile. T erefore, the proteins and lipids in the canalicular region encounter a high concentration of these chemicals. Observations consistent with this concentration mechanism have been reported for Mn, reactive thioether glutathione conjugates of 1,1-dichloroethylene, and sporidesmin.

Although no case of drug toxicity has been reported in response to modifications of basolateral uptake, OATPs can contribute to the liver injury potential of toxicants. T e hepatotoxicity of phalloidin, microcystin, and amanitin is facilitated by the uptake through OATPs. Furthermore, there is a growing list of drugs including rifampicin, bosentan, and troglitazone, which are known to directly inhibit bile salt export pump (BSEP). Estrogens and progestins inhibit BSEP from the canalicular side after excretion by MRP2. A substantial inhibition of bile salt excretion can lead to accumulation of these compounds in hepatocytes and may directly cause cell injury. However, more recent findings indicate that most of the bile acids accumulating in the liver after obstructive cholestasis are nontoxic and instead of cell death cause proinflammatory gene expression in hepatocytes.

While liver injury after obstructive cholestasis is produced mainly by inflammatory cells, compensatory mechanisms within the hepatocyte itself can limit this potential injury. Bile acids are substrates for the nuclear farnesoid X receptor (FXR), down-regulate NTCP and limit bile acid uptake. In addition, FXR activation causes increased expression of transporters on the canalicular and basolateral membranes, which all work to limit the amount of bile acid accumulation.

Bile Duct Damage—Damage to the intrahepatic bile ducts (which carry bile from the liver to the GI tract) is called cholangiodestructive cholestasis. A useful biochemical index of bile duct damage is a sharp elevation in serum alkaline phosphatase activity. In addition, serum levels of bile salts and bilirubin are elevated, as observed with canalicular cholestasis. Initial lesions following a single dose of cholangiodestructive agents include swollen biliary epithelium, debris of damaged cells within lumens of the biliary tract, and inflammatory cell infiltration of portal tracts. Chronic administration of chemicals that cause bile duct destruction can lead to biliary proliferation and fibrosis resembling biliary cirrhosis. A rare response is the loss of bile ducts, a condition known as vanishing bile duct syndrome. T is persisting problem has been reported in patients receiving antibiotics, anabolic steroids, contraceptive steroids, or the anticonvulsant carbamazepine.

Sinusoidal Damage—T e sinusoid is, in effect, a specialized capillary with numerous fenestrae for high permeability. Functional integrity of the sinusoid can be compromised by dilation or blockade of its lumen or by progressive destruction of its endothelial cell wall. Dilation of the sinusoid will occur whenever ef ux of hepatic blood is impeded. Blockade will occur when red blood cells become caught in the sinusoids. Such changes have been illustrated after large doses of the drug acetaminophen. A consequence of extensive sinusoidal blockade is that the liver becomes engorged with blood cells and the rest of the body goes into shock.

Progressive destruction of the endothelial wall of the sinusoid will lead to gaps and then ruptures of its barrier integrity, with entrapment of red blood cells. T ese disruptions of the sinusoid are considered the early structural features of the vascular disorder known as veno-occlusive disease, which occurs after exposure to pyrrolizidine alkaloids, which may be found in some herbal teas and chemotherapeutic agents.

Disruption of the Cytoskeleton—Phalloidin (from a mushroom) and microcystin (from blue-green algae) disrupt the integrity of hepatocyte cytoskeleton by affecting proteins that are vital to its dynamic nature, preventing disassembly of actin filaments. Phalloidin uptake into hepatocytes leads to an accentuated actin web of cytoskeleton and the canalicular lumen dilates.

Microcystin uptake into hepatocytes leads to hyperphosphorylation of cytoskeletal proteins. Reversible phosphorylations of cytoskeletal structural and motor proteins are critical to the dynamic integrity of the cytoskeleton. As depicted in Figure 13–4, extensive hyperphosphorylation produced by large amounts of microcystin leads to marked deformation of hepatocytes due to a unique collapse of the microtubular actin scaffold into a spiny central aggregate. Lower doses of microcystin interfere with vesicle transport by hyperphosphorylating the transport protein dynein.

Dynein is a mechanicochemical protein that drives vesicles along microtubules using energy from ATP hydrolysis; central to the hydrolysis of the dynein-bound ATP is a cycle of kinase phosphorylation and phosphatase dephosphorylation. T us, hyperphosphorylation of dynein freezes this motor pump. Chronic exposure to low levels of microcystin has raised new concerns about the health effects of this water contaminant. Specifically, low levels of microcystin promote liver tumors and kill hepatocytes in the zone 3 region, where microcystin accumulates.

Fatty Liver—Fatty liver (steatosis) is defined biochemically as an appreciable increase in the hepatic lipid (mainly triglyceride) content, which is < 5 wt% in the normal human liver. Currently, the most common cause of hepatic steatosis is insulin resistance due to central obesity and sedentary lifestyle.

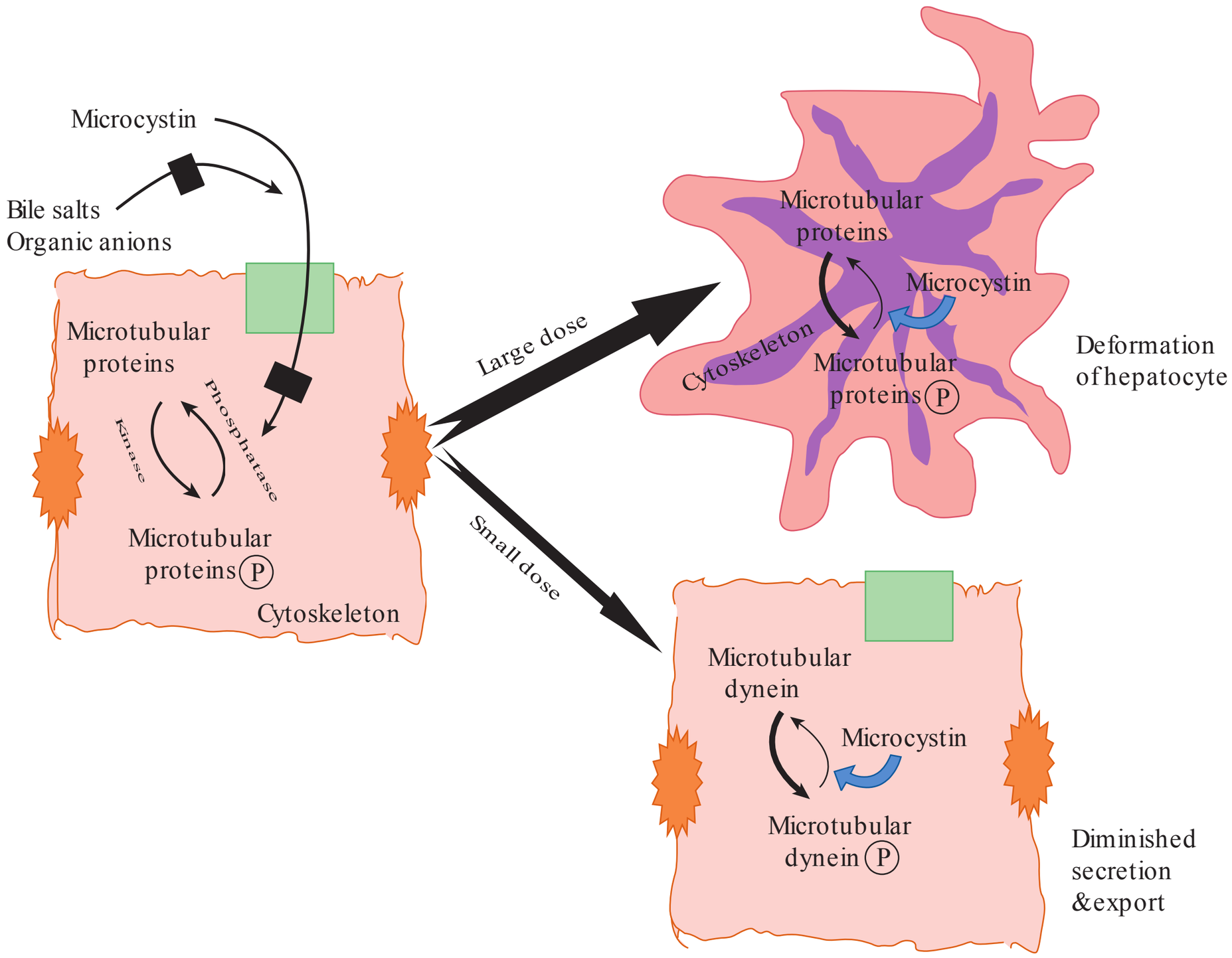

FIGURE 13–4 **Schematic of events in the mechanism by which microcystin damages the structural and functional integrity of hepatocytes.** Microcystin is taken up exclusively into hepatocytes by a sinusoidal transporter in a manner inhibitable by bile salts and organic anions. Then microcystin inhibition of protein phosphatases leads to hyperphosphorylation of cytoskeletal proteins whose dynamic functions are dependent on reversible phosphorylations. Extensive hyperphosphorylation of microtubular proteins leads to a collapse of the microtubular actin filament scaf old into a spiky aggregate that produces a gross deformation of hepatocytes. More subtle changes in microtubule-mediated transport activities have been linked to hyperphosphorylation of dynein, a cytoskeletal motor protein.

However, acute exposure to hepatotoxicants like carbon tetrachloride and some drugs can induce steatosis. Ethanol is by far the most relevant drug or chemical leading to steatosis in humans and in experimental animals.

Often, drug-induced steatosis is reversible and does not lead to death of hepatocytes. T e metabolic inhibitors ethionine, puromycin, and cycloheximide cause fat accumulation without causing cell death. Although steatosis alone may be benign, it can develop into steatohepatitis (alcoholic or nonalcoholic), which is associated with significant liver injury. Livers with steatosis can be more susceptible to additional insults such as hepatotoxicants or hepatic ischemia.

T e previously preferred hypothesis of nonalcoholic steatohepatitis (NASH) considered triglyceride accumulation in hepatocytes as the initial pathological event causing steatosis, with any additional stress (e.g., oxidant stress or lipid peroxidation) causing progression to steatohepatitis. T is thinking has recently been overturned and a new hypothesis postulates that nonalcoholic fatty liver disease (NAFLD) is mainly caused by lipotoxicity of non-triglyceride fatty metabolites. Although the specific fatty acids or their metabolites causing NAFLD in patients have not been identified, the emerging evidence suggests that the excessive burden of fatty acids in the liver from either inappropriate lipolysis in adipose tissue or synthesis in the liver may cause liver injury.

T is change, also known as steatosis, is a buildup of lipids in the hepatocyte. Fatty liver can stem from disruptions in lipid metabolism. Steatosis is a common response to acute exposure to many hepatotoxicants. Often, chemical-induced steatosis is reversible and does not lead to death of hepatocytes. Ethanol is by far the most relevant drug or chemical leading to steatosis in humans. T e metabolic inhibitors ethionine, puromycin, and cycloheximide cause fat accumulation without causing death of cells. Many other conditions besides toxicant exposure, such as insulin resistance due to central obesity, are associated with marked fat accumulation in the liver.

Fibrosis and Cirrhosis—Hepatic fibrosis (scarring) is characterized by the accumulation of extensive amounts of collagen fibers, in response to direct injury or to inflammation. With repeated chemical insults, destroyed hepatic cells are replaced by fibrotic scars. With continuing collagen deposition,

TABLE 13–3 Factors in the site-specific injury of representative hepatotoxicants.

Site	Representative Toxicants	Potential Explanation for Site Specificity
Zone 1 hepatocytes (versus zone 3)	Fe (overload)	Preferential uptake and high oxygen levels
	Allyl alcohol	Higher oxygen levels for oxygen-dependent bioactivation
Zone 3 hepatocytes (versus zone 1)	CCl_4	More P450 isozyme for bioactivation
	Acetaminophen	More P450 isozyme for bioactivation and less GSH for detoxification
	Ethanol	More hypoxic and greater imbalance in bioactivation/detoxification reactions
Bile duct cells	Methylene dianiline, sporidesmin	Exposure to the high concentration of reactive metabolites in bile
Sinusoidal endothelium (versus hepatocytes)	Cyclophosphamide, monocrotaline	Greater vulnerability to toxic metabolites and less ability to maintain glutathione levels
Kupffer cells	Endotoxin, $GdCl_3$	Preferential uptake and then activation
Stellate cells	Vitamin A	Preferential site for storage and then engorgement
	Ethanol (chronic)	Activation and transformation to collagen-synthesizing cell

the architecture of the liver is disrupted by interconnecting fibrous scars. When the fibrous scars subdivide the remaining liver mass into nodules of regenerating hepatocytes, fibrosis has progressed to cirrhosis and the liver has limited residual capacity to perform its essential functions. The primary cause of hepatic fibrosis/cirrhosis in humans worldwide is viral hepatitis. However, biliary obstruction and, in particular, alcoholic and NASH are of growing importance for the development of hepatic fibrosis. In addition, fibrosis can be induced by chronic exposure to drugs and chemicals especially ethanol and heavy metals. Cirrhosis is not reversible, has a poor prognosis for survival, and is usually the result of repeated exposure to chemical toxicants.

Tumors—Chemically induced neoplasia can involve tumors that are derived from hepatocytes, bile duct progenitor cells, the ductular "bipolar" progenitor cells, and the periductular stem cells. The rare, highly malignant angiosarcomas are derived from sinusoidal lining cells. Hepatocellular cancer has been linked to abuse of androgens, alcohol, and a high prevalence of aflatoxin-contaminated diets.

Thorotrast (radioactive thorium dioxide used as a contrast medium for radiology) accumulates in Kupffer cells and emits radioactivity throughout its very extended half-life, thus increasing the risk for developing gallbladder cancer about 14-fold and over 100-fold for liver cancers. Multiple types of liver tumors are linked to thorium dioxide exposure.

Critical Factors in Toxicant-induced Liver Injury

Why is the liver the target site for so many chemicals of diverse structure? Why do many hepatotoxicants preferentially damage one type of liver cell? Our understanding of these fundamental questions is incomplete. Influences of several factors are of obvious importance (Table 13–3). Location and specialized processes for uptake and biliary secretion produce higher exposure levels in the liver than in other tissues of the body and strikingly high levels within certain types of liver cells. Then the abundant capacity for bioactivation reactions influences the rate of exposure to proximate toxicants. Subsequent events in the pathogenesis appear to be critically influenced by responses of sinusoidal cells and the immune system.

A number of experimental systems are useful for defining factors and mechanisms of liver injury. In vitro systems using the isolated perfused liver, isolated liver cells, and cell fractions allow observations at various levels of complexity without the confounding influences of other systems. Models using cocultures or agents that inactivate a given cell type can document the contributions and interactions between cell types. Whole-animal models are essential for assessment of the progression of injury and responses to chronic insult. Application of gene transfection or repression attenuates some of these interpretive problems. Knockout animals are extremely useful models for studying complex aspects of hepatotoxicity.

Uptake and Concentration—Lipophilic drugs and environmental pollutants readily diffuse into hepatocytes because the fenestrated epithelium of the sinusoid enables close contact between circulating molecules and hepatocytes. The membrane-rich liver concentrates lipophilic compounds. Other toxicants are rapidly extracted from blood because they are substrates for sinusoidal transporters. Phalloidin and microcystin are illustrative examples of toxicants that target the liver as a consequence of extensive uptake into hepatocytes by sinusoidal transporters. Vitamin A hepatotoxicity initially affects the sinusoidal stellate cells, which actively extract and store this vitamin. Cadmium hepatotoxicity becomes manifest

when cells exceed their capacity to complex cadmium with the metal-binding protein metallothionein.

Hepatocytes contribute to the homeostasis of iron by extracting this essential metal from the sinusoid by a receptor-mediated process and maintaining a reserve of iron within the storage protein ferritin. Acute iron toxicity is most commonly observed in young children who accidently ingest iron tablets. T e cytotoxicity of free iron is attributed to its function as an electron donor for the formation of reactive oxygen species, which initiate destructive oxidative stress reactions. Accumulation of excess iron beyond the capacity for its safe storage in ferritin leads to liver damage. Chronic hepatic accumulation of excess iron in cases of hemochromatosis is associated with a spectrum of hepatic disease including a greater than 200-fold risk for liver cancer.

Bioactivation and Detoxification—Hepatocytes have very high constitutive activity of the phase I enzymes that often convert xenobiotics to reactive electrophilic metabolites. Also, hepatocytes have a rich collection of phase II enzymes that add a polar group to a molecule and thereby enhance its removal from the body. Phase II reactions usually yield stable, nonreactive metabolites. In general, the balance between phase I and phase II reactions determines whether a reactive metabolite will initiate liver cell injury or be safely detoxified. Because the expression of phase I and II enzymes and of the hepatic transporters can be influenced by genetics (e.g., polymorphism of drug-metabolizing enzymes) and lifestyle (e.g., diet, consumption of other drugs and alcohol), the susceptibility to potential hepatotoxicants can vary markedly between individuals.

Acetaminophen—One of the most widely used analgesics acetaminophen (APAP) is a safe drug when used at therapeutically recommended doses. Overdose can cause severe hepatotoxicity, and certain acquired factors (e.g., diet, drugs, diabetes, and obesity) can enhance hepatotoxicity. Typical therapeutic doses of acetaminophen are not hepatotoxic, because most of the acetaminophen gets glucuronidated or sulfated with little drug bioactivation. Injury after large doses of acetaminophen is enhanced by fasting and other conditions that deplete glutathione and is minimized by treatments with N-acetylcysteine that enhance hepatocyte synthesis of glutathione.

Alcoholics are vulnerable to the hepatotoxic effects of acetaminophen at dosages within the high therapeutic range. T is acquired enhancement has widely been attributed to accelerated bioactivation of acetaminophen to the electrophilic N-acetyl-p-benzoquinone imine (NAPQI) intermediate by ethanol induction of CYP2E1. Inducers of CYP3A including many drugs and dietary chemicals potentially influence acetaminophen toxicity.

Although many of the details of the mechanism for APAP-induced hepatotoxicity remain to be elucidated, newly gained insight into signaling events in response to APAP overdose suggests two fundamentally new developments. First, necrotic cell death is in most cases not caused by a single catastrophic event but can be the result of a cellular stress, which is initiated by metabolic activation and triggers sophisticated signaling mechanisms culminating in cell death (Figure 13–5). Second, the multitude of events following the initial stress offers many opportunities for therapeutic interventions at later time points. Because these events are not occurring in all cells to the same degree and at the same time, delayed interventions may not completely prevent cell damage but limit the area of necrosis enough to prevent liver failure.

Ethanol—Morbidity and mortality associated with the consumption of alcohol is mainly caused by the toxic effects of ethanol on the liver. T is targeted toxicity is due to the fact that > 90% of a dose of ethanol is metabolized in the liver. T ree principal pathways of ethanol metabolism are known. In the primary pathway, ethanol is bioactivated by alcohol dehydrogenase to acetaldehyde, a reactive aldehyde, which is subsequently detoxified to acetate by aldehyde dehydrogenase. Both enzymes exhibit genetic polymorphisms that result in higher concentrations of acetaldehyde—a "fast" activity isozyme of alcohol dehydrogenase [ALD2*2] and a physiologically very "slow" mitochondrial isozyme of aldehyde dehydrogenase [ALDH2*2]. Approximately 50% of Asian populations but virtually no Caucasians have the slow aldehyde dehydrogenase; alcohol consumption by people with this slow polymorphism leads to uncomfortable symptoms of flushing and nausea due to high systemic levels of acetaldehyde.

T e second major pathway involves the alcohol-inducible enzyme CYP2E1, which oxidizes ethanol to acetaldehyde. T e enzyme is located predominantly in hepatocytes of the centrilobular region and requires oxygen and NADPH. Due to the nature of the enzyme, this reaction is most relevant for high doses of ethanol and for chronic alcoholism. T e third pathway involves catalase in peroxisomes. In this reaction, ethanol functions as an electron donor for the reduction of hydrogen peroxide to water. T us, the capacity of this pathway is limited due to the low levels of hydrogen peroxide. It is estimated that < 2% of an ethanol dose is metabolized through this pathway.

Allyl Alcohol—An industrial chemical used in the production of resins, plastics, and fire retardants, allyl alcohol is also used as a model hepatotoxicant due to its preferential periportal (zone 1) hepatotoxicity. T e alcohol is metabolized by alcohol dehydrogenase to acrolein, a highly reactive aldehyde, which is then further oxidized by aldehyde dehydrogenase to acrylic acid. T e fact that the toxicity depends on depletion of hepatic glutathione levels is prevented by inhibitors of alcohol dehydrogenase but enhanced by inhibitors of aldehyde dehydrogenase suggests that acrolein formation is the critical event in liver injury. Age and gender differences in allyl alcohol hepatotoxicity can be explained by variations in the balance between alcohol dehydrogenase and aldehyde dehydrogenase expression. T e preferential occurrence of allyl alcohol injury in zone 1 hepatocytes is caused by the predominant uptake of allyl alcohol in the periportal region and the oxygen dependence of the toxicity. Although protein binding of the reactive metabolite

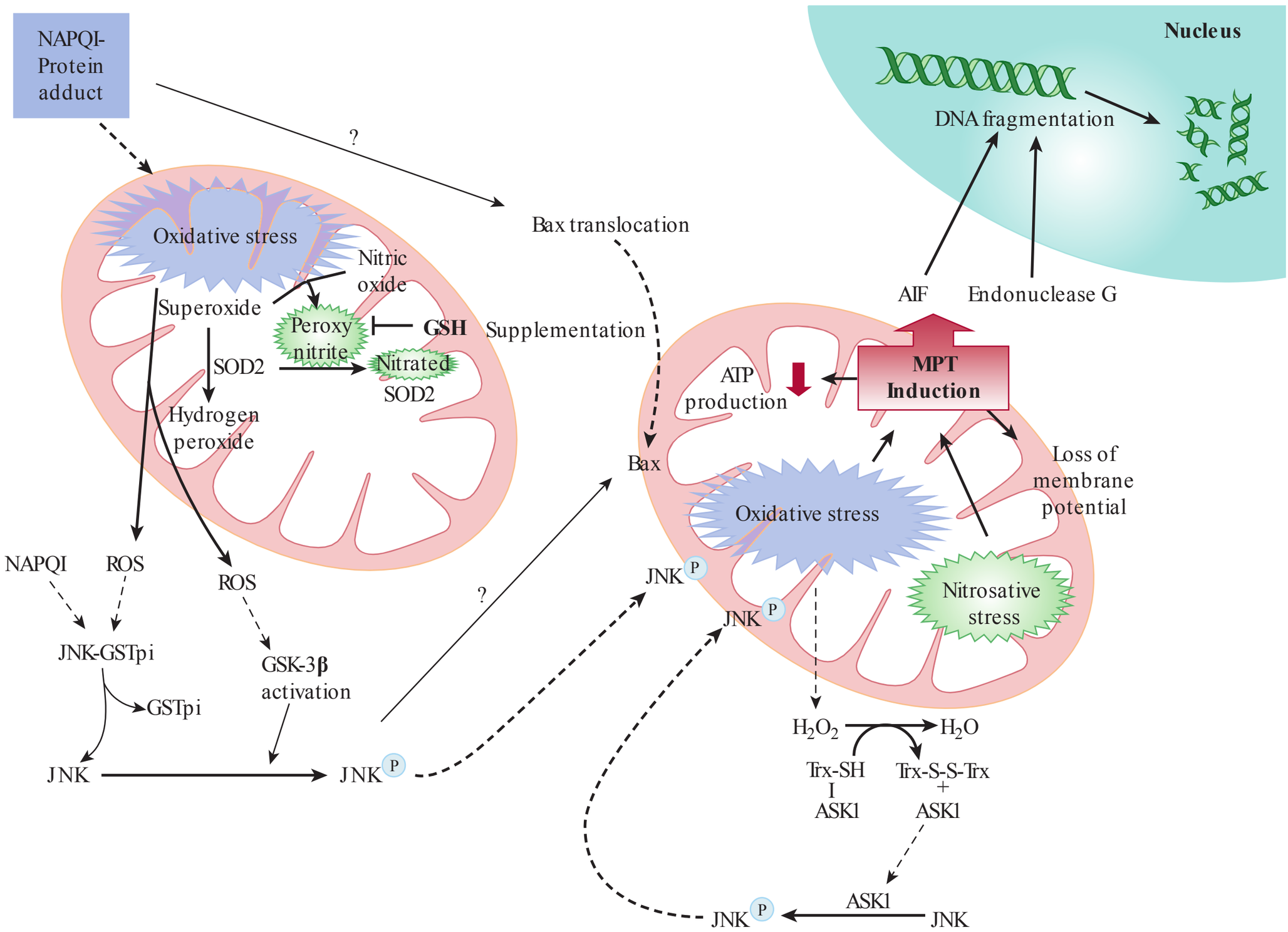

FIGURE 13–5 **Acetaminophen-induced mitochondrial oxidant stress and its influence on cellular signaling.** Metabolism of APAP results in the generation of the reactive intermediate, NAPQI, which forms protein adducts and induces mitochondrial oxidative stress. The increased generation of superoxide and its reaction with NO results in the production of peroxynitrite. The superoxide can be scavenged by SOD2 and converted into hydrogen peroxide, although the generation of peroxynitrite can interfere in this process by the nitration of SOD2. Mitochondrial oxidative stress and hydrogen peroxide can also activate the mitogen-activated protein kinase, JNK, by multiple pathways, resulting in its phosphorylation and translocation to the mitochondria. This then amplifies the mitochondrial oxidant stress, which, subsequently, leads to activation of the mitochondrial permeability transition, and translocation of mitochondrial proteins, such as AIF and endonuclease G, to the nucleus. This results in DNA fragmentation and, finally, oncotic necrosis. (Reproduced with permission from Jaeschke H, McGill MR, Ramachandra A: Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity, Drug Metab Rev, 2012 Feb;44(1):88–106.)

acrolein and subsequent adduct formation appears to be the main cause of liver cell death, lipid peroxidation can become a relevant mechanism of cell injury under conditions of a compromised antioxidant status. Lipid peroxidation is caused by a reductive stress where the excessive NADH formation leads to mobilization of redox-active iron from storage proteins.

Carbon Tetrachloride—Cytochrome P450–dependent conversion of CCl_4 to $\bullet CCl_3$ and then to $CCl_3OO\bullet$ is the classic example of xenobiotic bioactivation to a free radical that initiates lipid peroxidation by abstracting a hydrogen atom from the polyunsaturated fatty acid of a phospholipid. Metabolic activation of CCl_4 primarily involves CYP2E1. CCl_4-induced lipid peroxidation increases the permeability of the plasma membrane to Ca^{2+}, leading to severe disturbances of the calcium homeostasis and necrotic cell death. Recent research indicates that CCl_4 also induces significant mitochondrial damage, which is dependent on lipid peroxidation events and on CYP2E1 activity. In addition, the $\bullet CCl_3$ radical can directly bind to tissue macromolecules and some of the lipid peroxidation products are reactive aldehydes, e.g., 4-hydroxynonenal, which can form adducts with proteins. These events also cause the immune system to be involved, which can contribute to liver injury. Conditions in which cytochrome P450 is depleted lead to decreased liver damage when exposed to CCl_4.

Regeneration—The liver has a high capacity to restore lost tissue and function by regeneration. Loss of hepatocytes due to hepatectomy or cell injury triggers proliferation of all mature liver cells. This process is capable of restoring the original liver mass. However, regeneration is not just a response

to cell death, but a process that actively determines the final injury after exposure to hepatotoxic chemicals. Stimulation of repair by exposure to a moderate dose of a hepatotoxicant strongly attenuates tissue damage of a subsequent high dose of the same chemical. Tissue repair is dose–responsive up to a threshold, after which the injury is too severe and cell proliferation is inhibited.

Inf ammation and Immune Responses—T e activation of resident macrophages (Kupffer cells), NK and NKT cells, and the migration of activated neutrophils, lymphocytes, and monocytes into regions of damaged liver are a well-recognized feature of the hepatotoxicity produced by many chemicals. T e main reason for an inflammatory response is to remove dead and damaged cells. However, under certain circumstances, these inflammatory cells can aggravate the existing injury by release of directly cytotoxic mediators or by formation of pro- and anti-inflammatory mediators (Figure 13–6).

In addition to the activation of an inflammatory response, immune-mediated reactions may also lead to severe liver injury. Drugs and chemicals that have been suggested to cause immune-mediated injury mechanisms in the liver include halothane, tienilic acid, and dihydralazine. A delay in onset of the injury or the requirement for repeated exposure to the drug and the formation of antibodies against drug-modified hepatic proteins are characteristic features of immune reactions, but the mechanisms are not well understood. Two proposed mechanisms of immune-mediated liver injury are the hapten hypothesis and the danger hypothesis (Figure 13–7).

T e hapten hypothesis assumes that a reactive metabolite covalently binds to cellular proteins and the drug-modified protein is taken up by APCs, cleaved to peptide fragments, which are then presented within the major histocompatibility complex (MHC) to T cells. T is hypothesis does not explain, however, why other drugs (e.g., APAP), which also form reactive metabolites and drug-modified proteins, do not trigger an immune response. T e danger hypothesis (Figure 13–8) postulates that damaged cells release danger signals, which induce the upregulation of a peripheral protein B7 on activated antigen presenting cells (APCs), which when paired with CD28 on T cells generates a costimulatory signal. A cytotoxic immune response occurs only when the T-cell receptor stimulation with the antigen is accompanied by an independent costimulation of the T cell. In the absence of this costimulatory signal, the antigens derived from drug-modified proteins induce immune tolerance.

Activation of Sinusoidal Cells—Four kinds of observations, collectively, indicate roles for sinusoidal cell (immune cells present in the liver sinusoids) activation as primary or secondary factors in toxicant-induced injury to the liver:

1. Kupffer and Ito cells exhibit an activated morphology after acute and chronic exposure to hepatotoxicants.
2. Pretreatments that activate or inactivate Kupffer cells appropriately modulate the extent of damage produced by classic toxicants. Kupffer cell activation by vitamin A profoundly enhances the acute toxicity of carbon tetrachloride; this enhancement did not occur when animals were also given an inactivator of Kupffer cells.
3. Activated Kupffer cells secrete appreciable amounts of soluble cytotoxins, including reactive oxygen and nitrogen species.
4. Acute and chronic exposure to alcohol directly or indirectly affects sinusoidal cells.

Figure 13–8 summarizes information presented in this and earlier sections of this chapter about the multiplicity of toxicant-induced interactions with and between various liver cells. T e effect on a given cell type can be direct or may result from a cascade of signals and responses between cell types.

Mitochondrial Damage—Mitochondrial DNA codes for several proteins in the mitochondrial electron transport chain. Nucleoside analog drugs for the therapy of hepatitis B and AIDS infections cause mitochondrial DNA damage directly, when incorporation of the analog base leads to miscoding or early termination of polypeptides. T e severe hepatic mitochondrial injury produced by the nucleoside analog fialuridine is attributed to its higher affinity for the polymerase responsible for mitochondrial DNA synthesis than for the polymerases responsible for nuclear DNA synthesis. Mitochondrial DNA is also more vulnerable to miscoding (mutation) due to its limited capacity for repair.

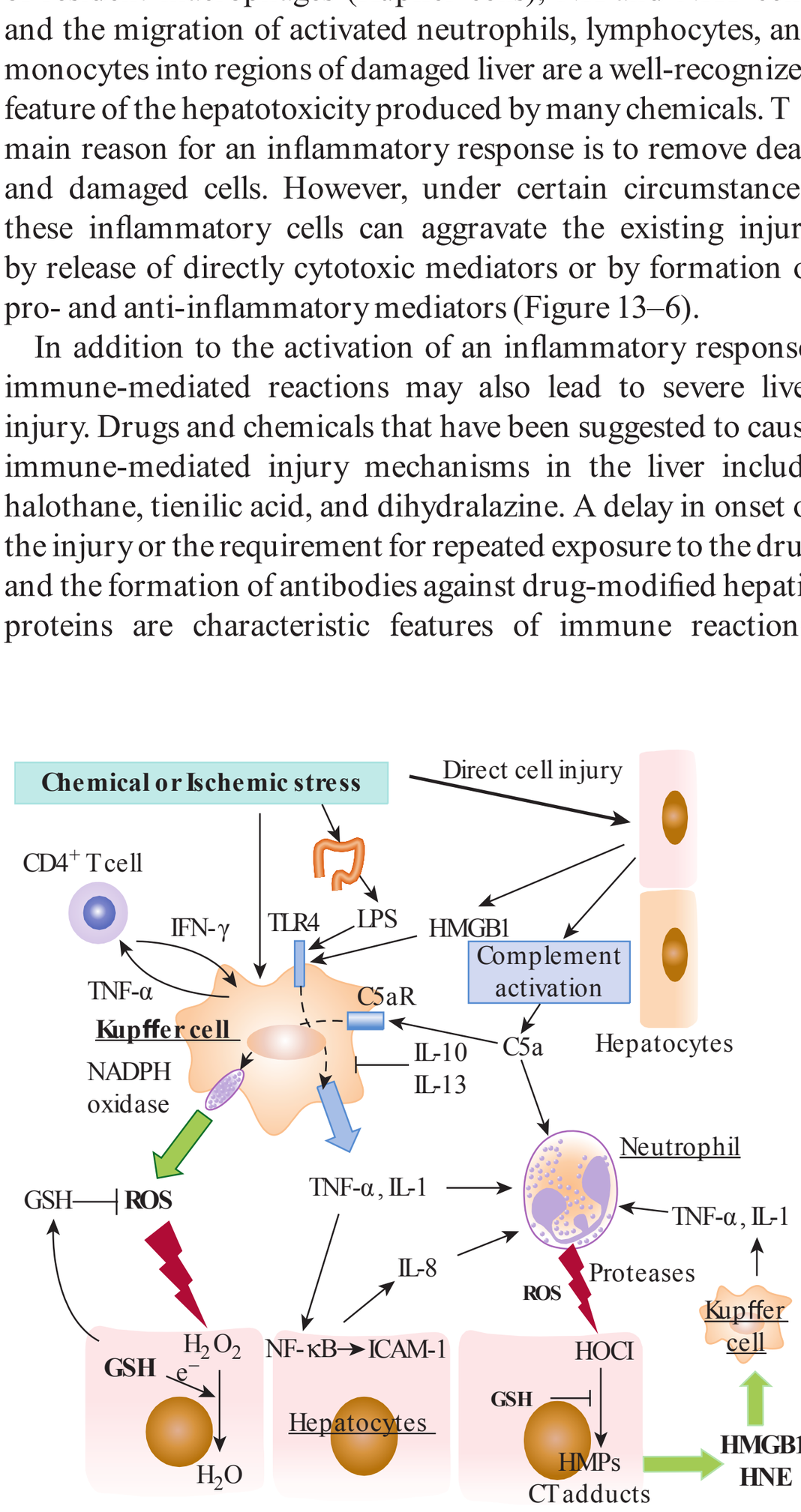

FIGURE 13–6 **Self-perpetuating inf ammatory response after chemical or ischemic stress.** C5aR, C5a complement receptor; CT, chlorotyrosine protein adducts; GSH, reduced glutathione; HMGB1, high-mobility group box-1; HMPs, hypochlorous acid modified proteins; HNE, hydroxynonenal; HOCl, hypochlorous acid; ICAM-1, intercellular adhesion molecule-1; IFN-γ, interferon-γ; IL-1, interleukin-1, LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; ROS, reactive oxygen species; TLR4, toll-like receptor-4; TNF, tumor necrosis factor.

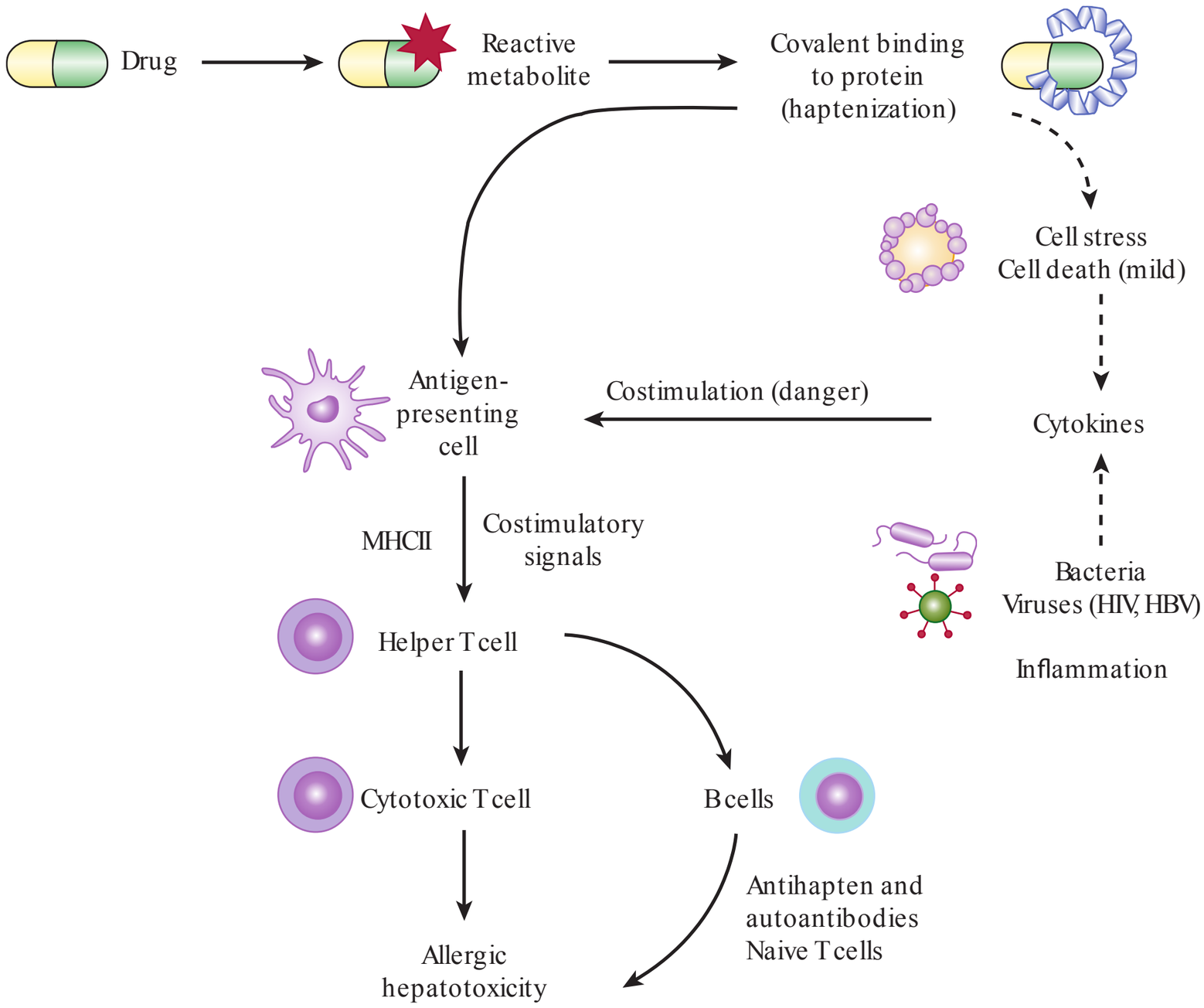

FIGURE 13–7 **The danger hypothesis for immune-mediated idiosyncratic hepatotoxicity.** Hapten formation leading to major histocompatibility complex class II (MHCII) presentation of haptenized peptide by antigen-presenting cells (APCs) along with costimulation of APC signaling molecules by mild injury, inflammation, or infection promotes helper T-cell activation leading to T-cell responses to the antigen. The cytotoxic T cells are then targeted against hepatocytes that express haptenized protein or MHCI presentation of haptenized peptides on the cell surface. Antibody to haptenized protein or concomitant autoantibodies could theoretically mediate and promote antibody-dependent cell-mediated hepatotoxicity. (Reproduced with permission from Kaplowitz N: Idiosyncratic drug hepatotoxicity. Nat Rev Drug Discov, 2005 June;4(6):489–499.)

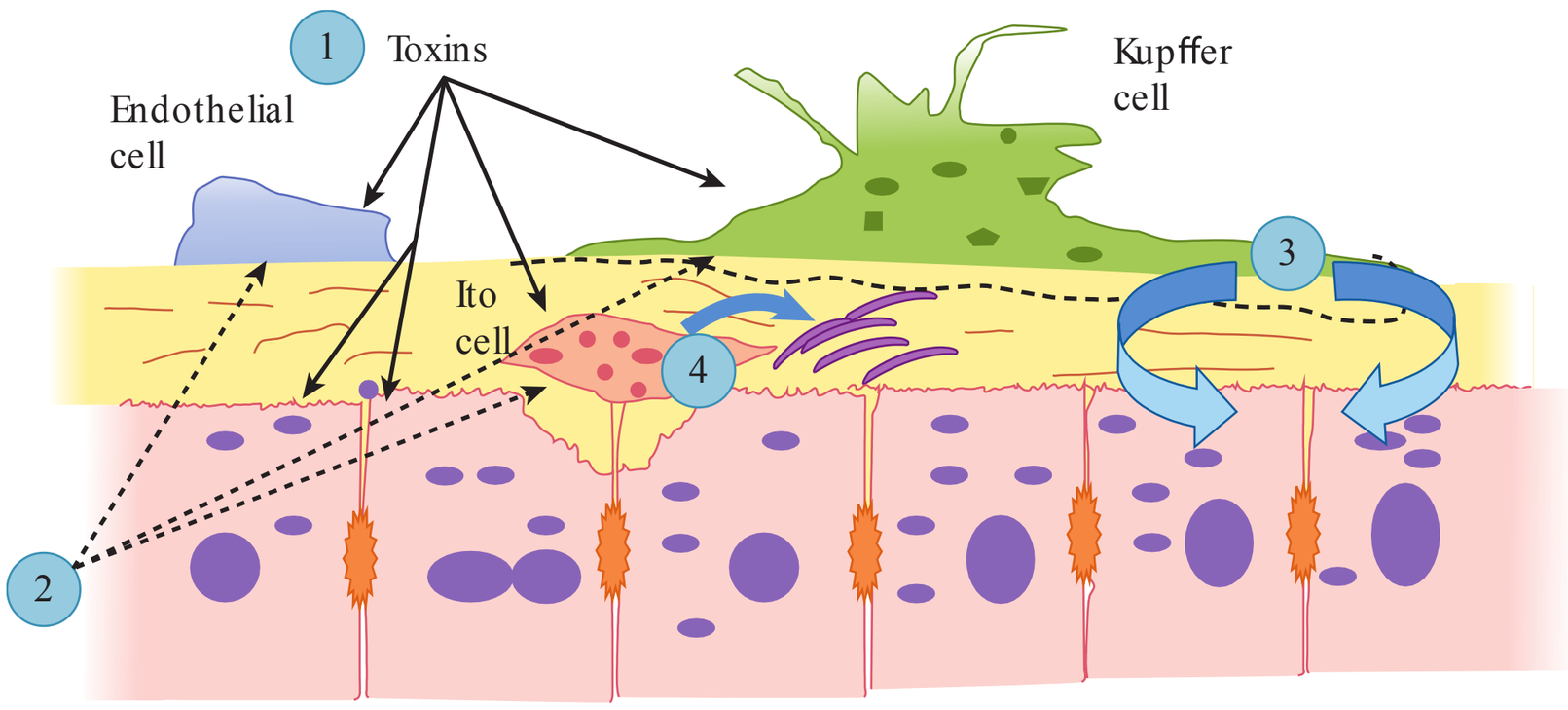

FIGURE 13–8 **Schematic depicting the complex cascade of toxicant-evoked interactions between hepatocytes and sinusoidal cells.** Sinusoidal cell responses to toxicants can lead to either injury or activation. A scenario could involve (1) toxicant injury to hepatocytes, (2) signals from the injured hepatocyte to Kupffer and Ito cells, followed by (3) Kupffer cell release of cytotoxins, and (4) Ito cell secretion of collagen. Activation of Kupffer cells is an important factor in the progression of injury evoked by many toxicants. Stimulation of collagen production by activated Ito cells is a proposed mechanism for toxicant-induced fibrosis.

TABLE 13–4 Examples of drugs with known idiosyncratic hepatotoxicity.

A. Immune-mediated (allergic) idiosyncratic hepatotoxicity
• Diclofenac (analgesic)
• Halothane (anesthetic)
• Nitrofurantoin (antibiotic)
• Phenytoin (anticonvulsant)
• Tienilic acid (diuretic)
B. Nonimmune-mediated (nonallergic) idiosyncratic hepatotoxicity
• Amiodarone (antiarrhythmic)
• Bromfenac (analgesic)—withdrawn from market
• Diclofenac (analgesic)
• Disulfiram (alcoholism)
• Isoniazid (antituberculosis)
• Ketoconazole (antifungal)
• Rifampicin (antimicrobial)
• Troglitazone (antidiabetes)—withdrawn from market
• Valproate (anticonvulsant)

Alcohol abuse causes mitochondrial injury by shifting the bioactivation/detoxification balance for ethanol, leading to an accumulation of its reactive acetaldehyde metabolite within mitochondria, because mitochondrial aldehyde dehydrogenase is the major enzymatic process for detoxification of acetaldehyde. Bioactivation of large amounts of ethanol by alcohol dehydrogenase hampers the detoxification reaction, since the two enzymes require the common, depletable cofactor nicotinamide adenine dinucleotide (NAD). Any type of ethanol-induced change that enhances the leakiness of the mitochondrial transport chain would lead to an increased release of reactive oxygen species capable of attacking nearby mitochondrial constituents.

Idiosyncratic Liver Injury—Idiosyncratic drug hepatotoxicity is a rare but potentially serious adverse event, which is not clearly dose-dependent, is at this point unpredictable, and affects only very few of the patients exposed to a drug or other chemicals. However, idiosyncratic toxicity is a leading cause for failure of drugs in clinical testing and it is the most frequent reason for posting warnings, restricting use, or even withdrawal of the drug from the market (Table 13–4). In addition, idiosyncratic hepatotoxicity is observed after consumption of herbal remedies and food supplements. Because idiosyncratic hepatotoxicity is a rare event for most drugs, it is likely that a combination of gene defects and adverse events need to be present simultaneously in an individual to trigger the severe liver injury. A detailed genomic analysis of patients with idiosyncratic responses to drug exposure may give additional insight as to what gene expression profile renders a patient susceptible.

FUTURE DIRECTIONS

Continued progress in the understanding of drug- and chemical-induced hepatotoxicity will depend on the use of relevant in vivo and in vitro models including human hepatocytes and analysis of human liver tissue. Traditional mechanistic investigations in combination with genomic and proteomic approaches have the greatest potential to yield important new insight into pathophysiologic mechanisms. Progress in the understanding of the liver's response to known hepatotoxicants and other adverse conditions will not only aid in the development of therapies to limit and reverse acute and chronic liver injury, but also improve the predictability of the potential hepatotoxicity of new drugs and other chemicals.

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QUESTIONS

1. T e impairment of hepatic function can have numerous negative consequences. Which of the following is likely NOT caused by impaired hepatic function?
   a. jaundice.
   b. hypercholesterolemia.
   c. hyperammonemia.
   d. hyperglycemia.
   e. hypoalbuminemia.

2. All of the following statements regarding the liver are true EXCEPT:
   a. T e major role of the liver is to maintain metabolic homeostasis of the body.
   b. T e liver encounters ingested nutrients before the heart does.
   c. Hepatic triads contain a branch of the hepatic portal vein, a branch of the hepatic artery, and a bile ductule.
   d. T e liver manufactures and stores bile.
   e. T e large fenestrae of hepatic sinusoids facilitate exchange of materials between the sinusoid and the hepatocyte.

3. Activation of which of the following cell types can result in increased secretion of collagen scar tissue, leading to cirrhosis?
   a. hepatocyte.
   b. Ito cell.
   c. Kupffer cell.
   d. endothelial cell.
   e. β-cell.

4. Wilson's disease is a rare genetic disorder characterized by the failure to export which of the following metals into bile?
   a. iron.
   b. zinc.
   c. silver.
   d. lead.
   e. copper.

5. Which of the following is NOT characteristic of apoptosis?
   a. cell swelling.
   b. nuclear fragmentation.
   c. lack of inflammation.
   d. programmed death.
   e. chromatin condensation.

6. A patient suffering from canalicular cholestasis would NOT be expected to exhibit which of the following?
   a. increased bile salt serum levels.
   b. jaundice.
   c. increased bile formation.
   d. dark brown urine.
   e. vitamin A deficiency.

7. Which of the following statements regarding liver injury is FALSE?
   a. Large doses of acetaminophen have been shown to cause a blockade of hepatic sinusoids.
   b. Hydrophilic drugs readily diffuse into hepatocytes because of the large sinusoidal fenestrations.
   c. T ere are sinusoidal transporters that take toxicants up into hepatocytes.
   d. Hepatocellular cancer has been associated with androgen abuse.
   e. In cirrhosis, excess collagen is laid down in response to direct injury or inflammation.

8. T e inheritance of a "slow" aldehyde dehydrogenase enzyme would result in which of the following after the ingestion of ethanol?
   a. high ethanol tolerance.
   b. little response to low doses of ethanol.
   c. low serum levels of acetaldehyde.
   d. nausea.
   e. increased levels of blood ethanol compared to an individual with a normal aldehyde dehydrogenase.

9. Which of the following is not a common mechanism of hepatocellular injury?
   a. deformation of the hepatocyte cytoskeleton.
   b. mitochondrial injury.
   c. cholestasis.
   d. interference with vesicular transport.
   e. increased transcytosis between hepatocytes.

10. Ethanol is not known to cause which of the following types of hepatobiliary injury?
    a. fatty liver.
    b. hepatocyte death.
    c. fibrosis.
    d. immune-mediated responses.
    e. canalicular cholestasis.

CHAPTER

14

Toxic Responses of the Kidney

Rick G. Schnellmann

FUNCTIONAL ANATOMY

- Renal Vasculature and Glomerulus
- Proximal Tubule
- Loop of Henle
- Distal Tubule and Collecting Duct

PATHOPHYSIOLOGIC RESPONSES OF THE KIDNEY

- Acute Kidney Injury
- Adaptation Following Toxic Insult
- Chronic Kidney Disease

SUSCEPTIBILITY OF THE KIDNEY TO TOXIC INJURY

- Incidence and Severity of Toxic Nephropathy
- Reasons for the Susceptibility of the Kidney to Toxicity
- Site-Selective Injury
- Glomerular Injury
- Proximal Tubular Injury
- Loop of Henle/Distal Tubule/Collecting Duct Injury
- Papillary Injury

ASSESSMENT OF RENAL FUNCTION

BIOCHEMICAL MECHANISMS/MEDIATORS OF RENAL CELL INJURY

- Cell Death
- Mediators of Toxicity
- Cellular/Subcellular and Molecular Targets

SPECIFIC NEPHROTOXICANTS

- Heavy Metals
  - Mercury
  - Cadmium
  - Chemically Induced α_{2u}-Globulin Nephropathy
- Halogenated Hydrocarbons
  - Chloroform
  - Tetrafluoroethylene
  - Bromobenzene
- Mycotoxins
- Therapeutic Agents
  - Acetaminophen
  - Nonsteroidal Anti-inflammatory Drugs
  - Aminoglycosides
  - Amphotericin B
  - Cyclosporine
  - Cisplatin
  - Radiocontrast Agents

KEY POINTS

- T e kidney contributes to total body homeostasis via its role in the excretion of metabolic wastes, the synthesis and release of renin and erythropoietin, and the regulation of extracellular fluid volume, electrolyte composition, and acid–base balance.
- Xenobiotics in the systemic circulation will be delivered to the kidney in relatively high amounts.
- T e processes that concentrate urine also serve to concentrate potential toxicants in the tubular fluid.
- Renal transport, accumulation, and biotransformation of xenobiotics contribute to the susceptibility of the kidney to toxic injury.
- Numerous nephrotoxicants cause mitochondrial dysfunction via compromised respiration and ATP production, or some other cellular process, leading to either apoptosis or necrosis.

T e functional integrity of the mammalian kidney is vital to total body homeostasis because the kidney plays a principal role in the excretion of metabolic wastes and in the regulation of extracellular fluid volume, electrolyte composition, and acid–base balance. In addition, the kidney synthesizes and releases hormones, such as renin and erythropoietin, and metabolizes vitamin D_3 to the active 1,25-dihydroxyvitamin D_3 form. A toxic insult to the kidney therefore could disrupt any or all of these functions and could have profound effects on total body metabolism.

FUNCTIONAL ANATOMY

Gross examination of a sagittal section of the kidney reveals three clearly demarcated anatomical areas: the cortex, medulla, and papilla (Figure 14–1). T e cortex constitutes the major portion of the kidney and receives a disproportionately higher percentage (90%) of blood flow compared with the medulla (~6% to 10%) or papilla (1% to 2%). T us, when a bloodborne toxicant is delivered to the kidney, a high percentage of the material will be delivered to the cortex and will have a greater opportunity to influence cortical rather than medullary or papillary functions. However, medullary and papillary tissues are exposed to higher luminal concentrations of toxicants for prolonged periods of time, a consequence of the more concentrated tubular fluid and the more sluggish flow of blood and filtrate in these regions.

T e functional unit of the kidney, the nephron, may be considered in three portions: the vascular element, the glomerulus, and the tubular element.

Renal Vasculature and Glomerulus

T e renal artery branches successively into interlobar, arcuate, interlobular arteries and afferent arterioles that supply the glomerulus (Figure 14–1). Blood then leaves the glomerular capillaries via the efferent arterioles. Both the afferent and efferent arterioles control glomerular capillary pressure and glomerular plasma flow rate. T ese arterioles are innervated by the sympathetic nervous system and respond to nerve stimulation, angiotensin II, vasopressin (also called arginine vasopressin [AVP], anti-diuretic hormone [ADH]), endothelin, adenosine, and norepinephrine. T e efferent arterioles draining the cortical glomeruli branch into a peritubular capillary network, whereas those draining the juxtamedullary glomeruli form a capillary loop, called the vasa recta (literally, straight vessels), supplying the medullary structures. T ese postglomerular capillary loops provide delivery of nutrients to the postglomerular tubular structures, delivery of wastes to the tubule for excretion, and return of reabsorbed electrolytes, nutrients, and water to the systemic circulation.

T e glomerulus is a complex, specialized capillary bed composed primarily of endothelial cells that are characterized by an attenuated and fenestrated cytoplasm, visceral epithelial cells characterized by a cell body (podocyte) from which many trabeculae and pedicles (foot processes) extend, and a glomerular basement membrane (GBM), which is a trilamellar structure sandwiched between the endothelial and epithelial cells (Figure 14–2). A portion of the blood entering the glomerular capillary network is fractionated into a virtually protein-free and cell-free ultrafiltrate, which passes through Bowman's space and into the tubular portion of the nephron. T e formation of such an ultrafiltrate is the net result of the balance between transcapillary hydrostatic pressure and colloid oncotic pressure. An additional determinant of ultrafiltration is the effective hydraulic permeability of the glomerular capillary wall, in other words, the ultrafiltration coefficient (K_f), which is determined by the total surface area available for filtration and the hydraulic permeability of the capillary wall. Consequently, chemically induced decreases in glomerular filtration rate (GFR) may be related to decreases in transcapillary hydrostatic pressure and glomerular plasma flow due to increased afferent arteriolar resistance or to decreases in the surface area available for filtration, resulting from decreases in the size and/or number of endothelial fenestrae or detachment or effacement of foot processes.

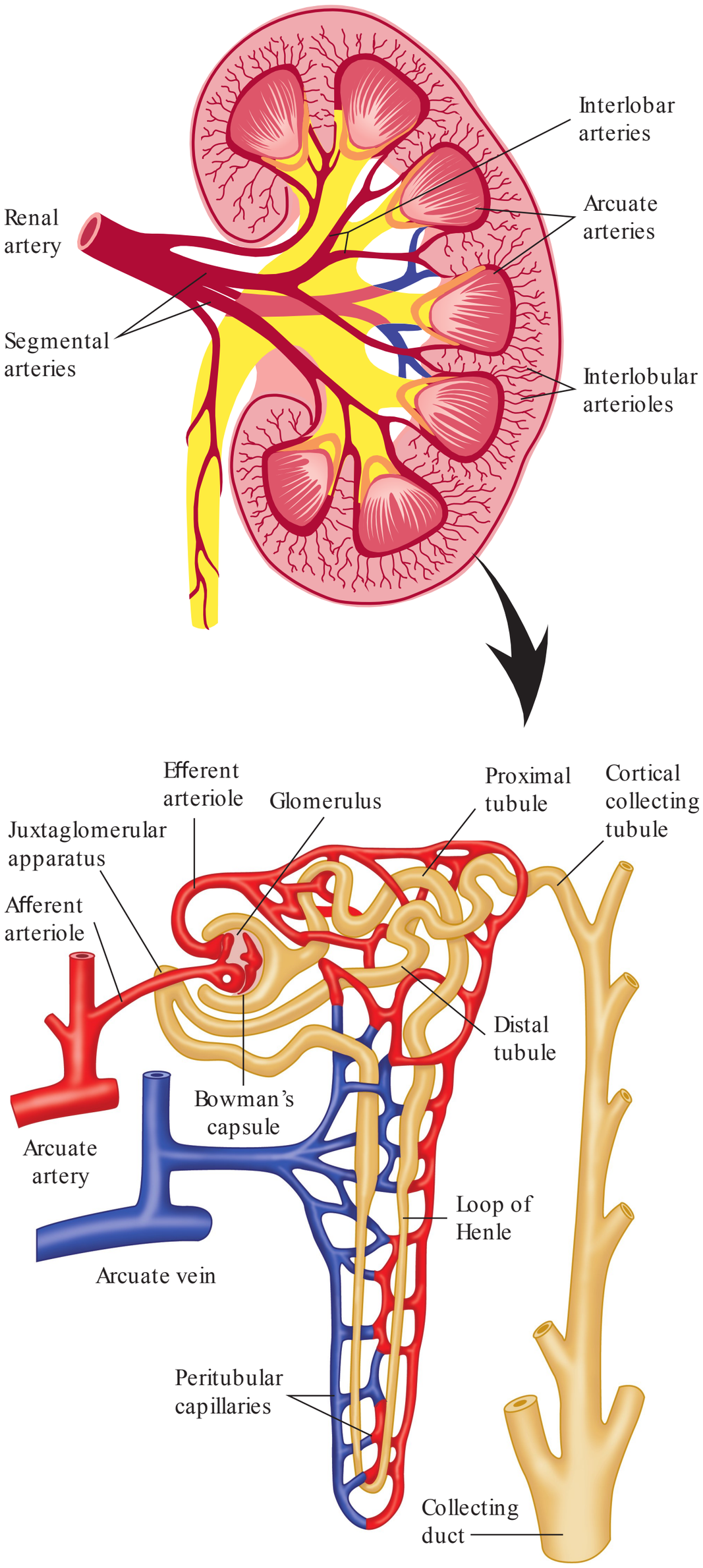

FIGURE 14–1 **Schematic of the human kidney showing the major blood vessels and the microcirculation and tubular components of each nephron.** (Reproduced with permission from Guyton AC, Hall JE: Textbook of Medical Physiology. 9th edition. Philadelphia, PA: Saunders/Elsevier; 1996.)

Although the glomerular capillary wall permits a high rate of fluid filtration (~20% of blood entering the glomerulus is filtered in a single pass), it provides a significant barrier to the transglomerular passage of macromolecules. T us, small molecules, such as inulin (MW ~5 500), are freely filtered, whereas large molecules, such as albumin (MW 56 000–70 000), are restricted. Filtration of anionic molecules tends to be restricted compared to that of neutral or cationic molecules of the same size; this is primarily due to the charge-selective properties of the GBM (Figure 14–2).

Proximal Tubule

T e proximal tubule consists of three discrete segments: the S_1 (pars convoluta), S_2 (transition between pars convoluta and pars recta), and S_3 (pars recta) segments. T e formation of urine is a highly complex and integrated process in which the volume and composition of the glomerular filtrate is progressively altered as fluid passes through each of the different tubular segments. T e proximal tubule is the workhorse of the nephron, as it reabsorbs approximately 60% to 80% of solute and water filtered at the glomerulus, mostly by numerous transport systems capable of driving the concentrative transport of many metabolic substrates. Toxicant-induced injury to the proximal

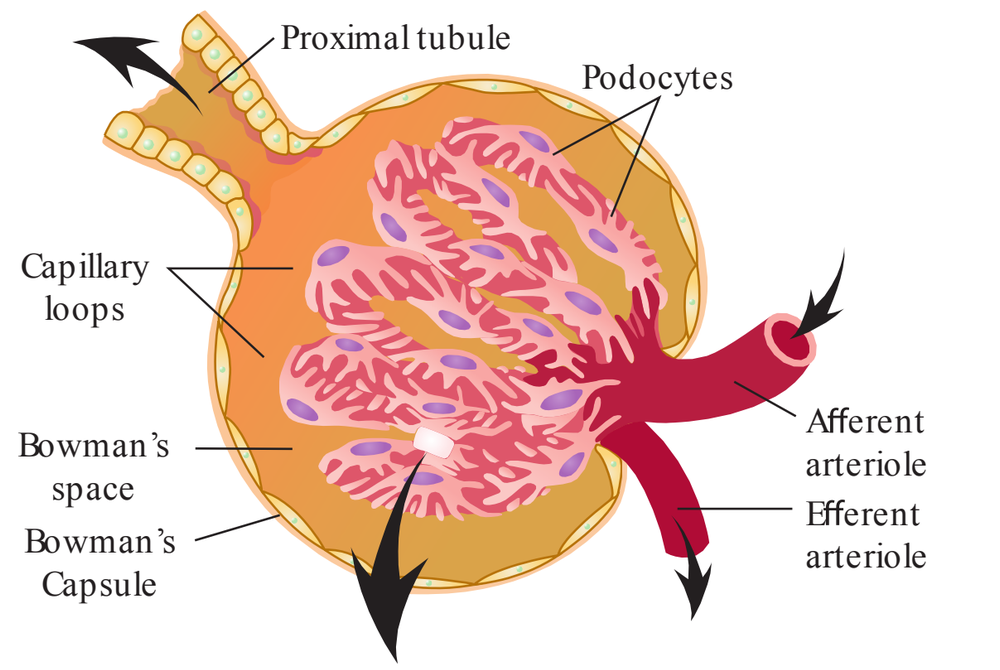

FIGURE 14–2 **Schematic of the ultrastructure of the glomerular capillary (A); cross section of the glomerular capillary membrane with the capillary epithelium, basement membrane and epithelium podocytes.** (Reproduced with permission from Guyton AC, Hall JE: Textbook of Medical Physiology. 9th edition. Philadelphia, PA: Saunders/Elsevier; 1996)

tubule therefore will have major consequences to water and solute balance.

T e proximal tubule also reabsorbs virtually all the filtered low-molecular-weight proteins by specific endocytotic protein reabsorption processes. An important excretory function of the proximal tubule is secretion of weak organic anions and cations by specialized transporters that drive concentrative movement of these ions from postglomerular blood into proximal tubular cells, followed by secretion into tubular fluid. Toxicant-induced interruptions in the production of energy for any of these active transport mechanisms or the function of critical membrane-bound enzymes or transporters can profoundly affect proximal tubular and whole-kidney function.

Loop of Henle

T e thin descending and ascending limbs and the thick ascending limb of the loop of Henle are critical to the processes involved in urinary concentration (Figure 14–1). Approximately 25% of the filtered Na^+ and K^+ and 20% of the filtered water are reabsorbed by the segments of the loop of Henle. T e tubular fluid entering the thin descending limb is iso-osmotic to the renal interstitium; water is freely permeable and solutes, such as electrolytes and urea, may enter from the interstitium. In contrast, the thin ascending limb is relatively impermeable to water and urea, and Na^+ and Cl^- are reabsorbed by passive diffusion. T e thick ascending limb is impermeable to water, and electrolytes are reabsorbed by the active $Na^+/K^+/2Cl^-$ cotransport mechanism, with the energy provided by the Na^+,K^+-ATPase.

Distal Tubule and Collecting Duct

T e macula densa comprises specialized cells located between the end of the thick ascending limb and the early distal tubule, in close proximity to the afferent arteriole (Figure 14–1). Under normal physiologic conditions, increased solute delivery or concentration at the macula densa triggers a signal resulting in afferent arteriolar constriction leading to decreases in GFR (and hence decreased solute delivery). T us, increases in fluid/solute out of the proximal tubule, due to impaired tubular reabsorption, will activate this feedback system, referred to as tubuloglomerular feedback (TGF) and resulting in decreases in the filtration rate of the same nephron. T is regulatory mechanism is a volume-conserving mechanism, designed to decrease GFR and prevent massive losses of fluid/electrolytes due to impaired tubular reabsorption. Humoral mediation of TGF by the renin–angiotensin system has been proposed, and evidence suggests that other substances may be involved. T e early distal tubule reabsorbs most of the remaining intraluminal Na^+, K^+, and Cl^- but is relatively impermeable to water.

T e late distal tubule, cortical collecting tubule, and medullary collecting duct perform the final regulation and fine-tuning of urinary volume and composition. T e remaining Na^+ is reabsorbed in conjunction with K^+ and H^+ secretion in the late distal tubule and cortical collecting tubule. T e combination of medullary and papillary hypertonicity generated by countercurrent multiplication and the action of ADH serves to enhance water permeability of the medullary collecting duct. Chemicals that interfere with ADH action impair the concentrating ability of the distal nephron.

PATHOPHYSIOLOGIC RESPONSES OF THE KIDNEY

Acute Kidney Injury

One of the most common manifestations of nephrotoxic damage is acute renal failure (ARF) or acute kidney injury (AKI). AKI is characterized by an abrupt decline in GFR with resulting azotemia, or a buildup of nitrogenous wastes in the blood. AKI describes the entire spectrum of the disease and is defined as a complex disorder that comprises multiple causative factors with clinical manifestations ranging from minimal elevation in serum creatinine to anuric renal failure.

Any decline in GFR is complex and may result from prerenal factors (renal vasoconstriction, intravascular volume depletion, and insufficient cardiac output), postrenal factors (ureteral or bladder obstruction), and intrarenal factors (glomerulonephritis, tubular cell injury, death, and loss resulting in back-leak; renal vasculature damage; interstitial nephritis). Figure 14–3 illustrates the pathways that lead to diminished GFR following chemical exposure. Table 14–1 provides a partial list of chemicals that produce AKI through different mechanisms.

T e maintenance of tubular integrity is dependent on cell-to-cell and cell-to-matrix adhesion (Figure 14–4). It has been hypothesized that after a chemical or hypoxic insult, adhesion of nonlethally damaged, apoptotic, and oncotic cells to the basement membrane is compromised, leading to gaps in the epithelial cell lining, potentially resulting in back-leak of filtrate and diminished GFR. T ese detached cells may aggregate in the tubular lumen (cell-to-cell adhesion) and/or adhere or reattach to adherent epithelial cells downstream, resulting in tubular obstruction.

Extensive evidence supports the idea that inflammatory cells play a role in ischemia-induced AKI. Injury to the renal vasculature endothelium results in chemokine and proinflammatory cytokine production and neutrophil adhesion, but the specific role of each inflammatory cell remains to be elucidated.

Adaptation Following Toxic Insult

T e kidney has a remarkable ability to compensate for a loss in renal functional mass. Following a unilateral nephrectomy, GFR of the remnant kidney increases by approximately 40% to 60%. Compensatory increases in single-nephron GFR are accompanied by proportionate increases in proximal tubular water and solute reabsorption; glomerulotubular balance (i.e., constant fractional reabsorption of GFR by all segments of the nephron) is therefore maintained and overall renal function

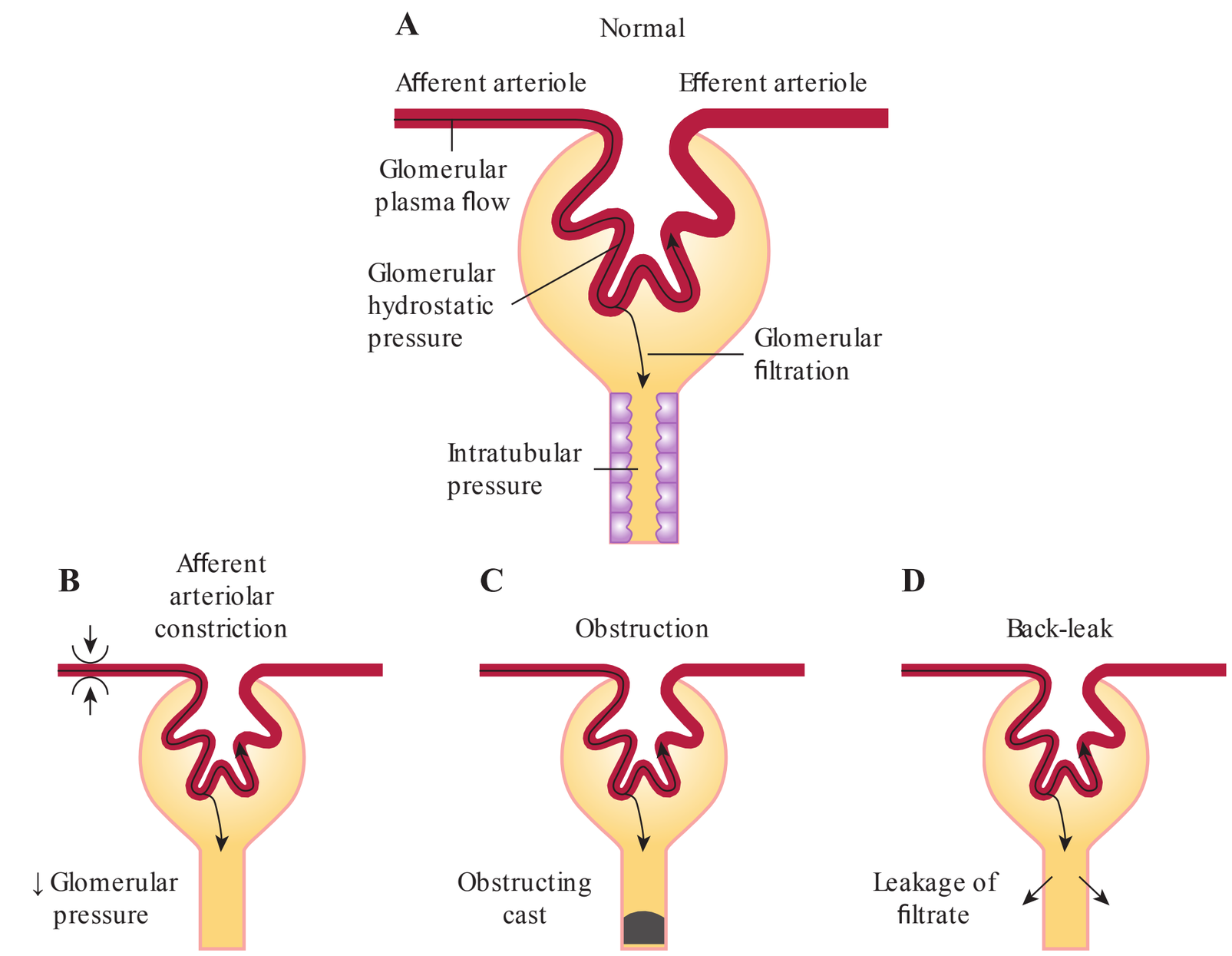

FIGURE 14–3 **Mechanisms of reduction of GFR. (A)** GFR depends on adequate blood flow to the glomerulus, adequate glomerular filtration pressure, glomerular permeability and low intratubular pressure. **(B)** Af erent arteriolar constriction decreases GFR by reducing blood flow, resulting in diminished capillary pressure. **(C)** Obstruction of the tubular lumen by cast formation increases tubular pressure; when tubular pressure exceeds glomerular capillary pressure, filtration decreases or ceases. **(D)** Back-leak occurs when the paracellular space between cells increases and the glomerular filtrate leaks into the extracellular space and bloodstream. (Reproduced with permission from Schrier RW: Atlas of Diseases of the Kidney. Philadelphia, PA: Current Medicine; 1999.)

appears normal by standard clinical tests. Consequently, chemically induced changes in renal function may not be detected until these compensatory mechanisms are overwhelmed by significant nephron loss and/or damage.

T ere are a number of cellular and molecular responses to a nephrotoxic insult. After a population of renal cells are exposed to a toxicant, a fraction of the cells will be severely injured and undergo cell death by apoptosis or oncosis (necrotic cell death). T ose cells with nonlethal injuries may undergo cell repair and/or adaptation, which contribute to the structural and functional recovery of the nephron (Figure 14–5). In addition, there is a population of uninjured cells that may undergo compensatory hypertrophy, cellular adaptation, and cellular proliferation. Tubular epithelial cells are primarily responsible for the structural and functional recovery of the nephron following injury by replacing dead and detached cells through de-differentiation, proliferation, migration, and re-differentiation.

TABLE 14–1 Mechanisms of chemically induced acute kidney injury.

Prerenal	Vasoconstriction	Crystalluria	Tubular Toxicity	Endothelial Injury	Glomerulopathy	Interstitial Nephritis
Diuretics Angiotensin receptor antagonists Angiotensin converting enzyme inhibitors Antihypertensive agents	Nonsteroidal anti-inflammatory drugs Radiocontrast agents Cyclosporine Tacrolimus Amphotericin B	Sulfonamides Methotrexate Acyclovir Triamterene Ethylene glycol Protease inhibitors	Aminoglycosides Cisplatin Vancomycin Pentamidine Radiocontrast agents Heavy metals Haloalkane- and Haloalkene-cysteine conjugates	Cyclosporine Mitomycin C Tacrolimus Cocaine Conjugated estrogens Quinine	Gold Penicillamine Nonsteroidal anti-inflammatory drugs	Antibiotics Nonsteroidal anti-inflammatory drugs Diuretics

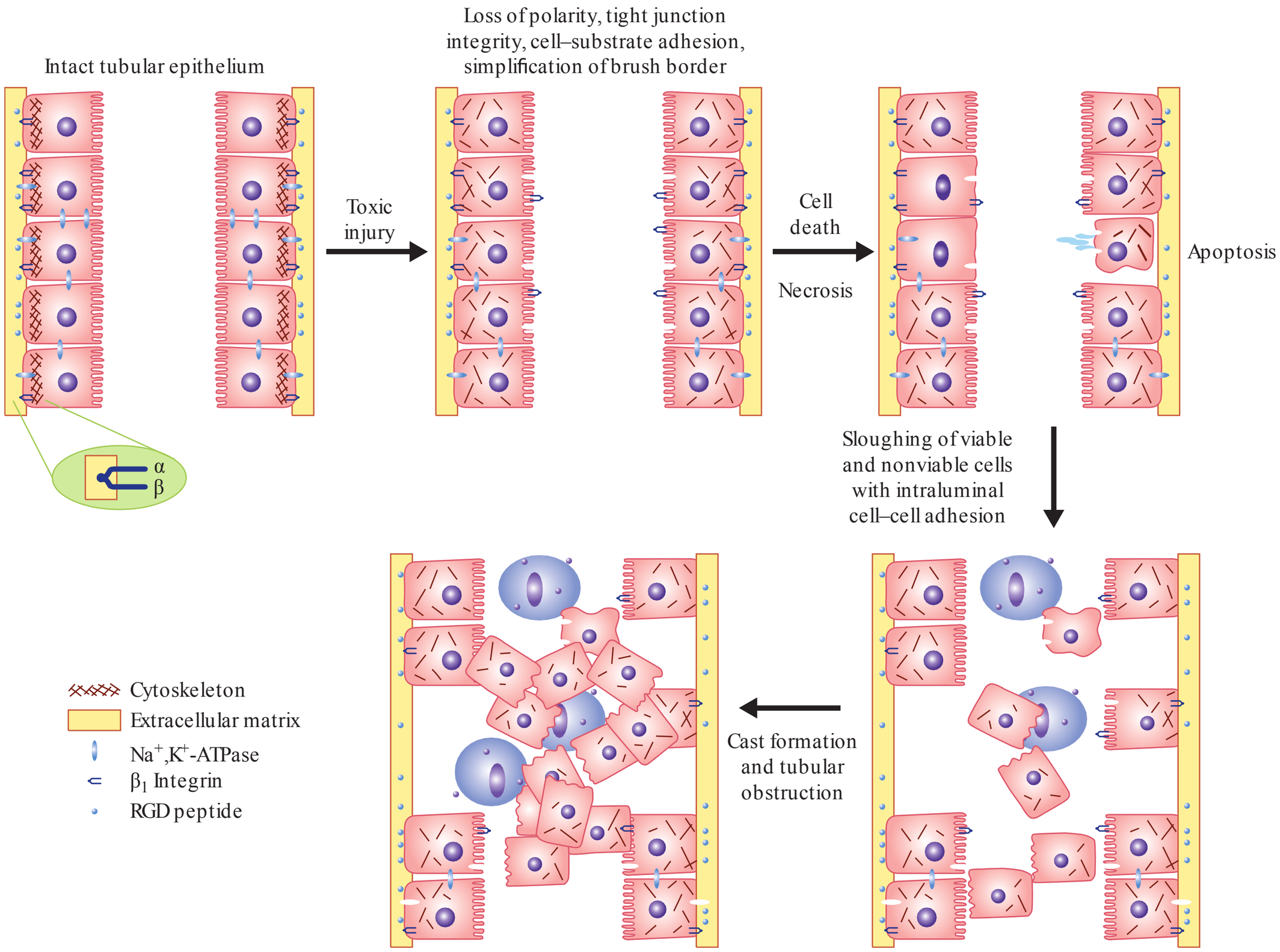

FIGURE 14–4 **After injury, alterations can occur in the cytoskeleton and in the normal distribution of membrane proteins such as Na^+,K^+-ATPase, and β1 integrins in sublethally injured renal tubular cells.** These changes result in loss of cell polarity, tight-junction integrity, and cell–substrate adhesion. Lethally injured cells undergo oncosis or apoptosis, and both dead and viable cells may be released into the tubular lumen. Adhesion of released cells to other released cells and to cells remaining adherent to the basement membrane may result in cast formation, tubular obstruction, and further compromise the GFR. (Reproduced with permission from Schrier RW: Atlas of Diseases of the Kidney. Philadelphia, PA: Current Medicine; 1999.)

Two of the most notable cellular adaptation responses are metallothionein induction and stress protein induction. T e distribution of individual heat-shock proteins (Hsps) and glucose-regulated proteins (Grps) are two examples of stress protein families that are induced in response to a number of pathophysiologic states such as heat shock, anoxia, oxidative stress, toxicants, heavy metal exposure, and tissue trauma. T e distribution of Hsps and Grps varies between different cell types in the kidney and within subcellular compartments. T ese proteins play important roles in protein folding, translocation of proteins across organelle membranes, prevention of aggregation of damaged proteins, and repair and degradation of damaged proteins, and thereby provide a defense mechanism against toxicity and/or for the facilitation of recovery and repair.

Chronic Kidney Disease

Progressive deterioration of renal function may occur with long-term exposure to various chemicals (e.g., analgesics, lithium, and cyclosporine). Following nephron loss, adaptive increases in glomerular pressures and flows increase the single-nephron GFR of remnant viable nephrons, which serve to maintain whole-kidney GFR. With time, these alterations are maladaptive, and focal glomerulosclerosis eventually develops that may lead to tubular atrophy and interstitial fibrosis. Compensatory increases in glomerular pressures and flows of the remnant glomeruli may result in mechanical damage to the capillaries, leading to altered permeabilities.

SUSCEPTIBILITY OF THE KIDNEY TO TOXIC INJURY

Incidence and Severity of Toxic Nephropathy

A wide variety of drugs, environmental chemicals, and metals can cause site-specific nephrotoxicity (Table 14–1). T e

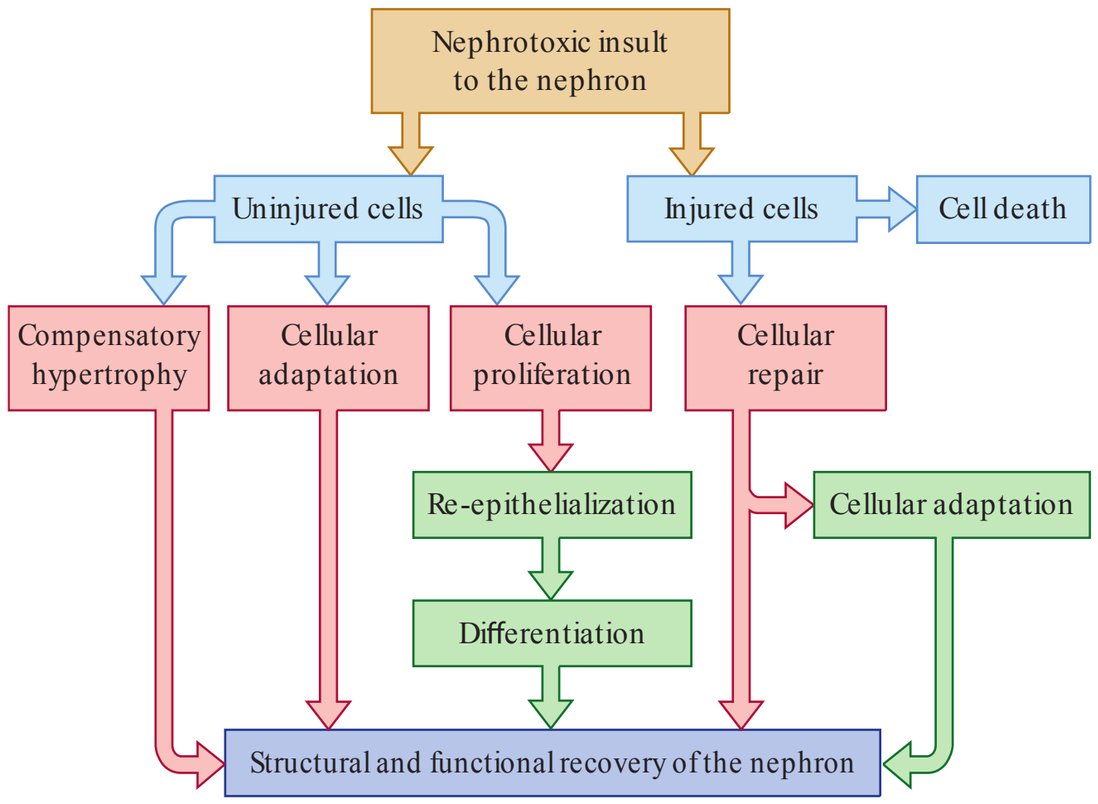

FIGURE 14–5 **The response of the nephron to a nephrotoxic insult.** After a population of cells is exposed to a nephrotoxicant, the cells respond; ultimately the nephron recovers function or, if cell death and loss are extensive, nephron function ceases. Terminally injured cells undergo cell death through oncosis or apoptosis. Cells injured sublethally undergo repair and adaptation in response to the nephrotoxicant. Cells not injured and adjacent to the injured area may undergo dedif erentiation, proliferation, migration or spreading, and dif erentiation. Cells not injured may also undergo compensatory hypertrophy in response to the cell loss and injury. Finally the uninjured cells also may undergo adaptation in response to a nephrotoxicant exposure. (Reproduced with permission from Schrier RW: Atlas of Diseases of the Kidney. Philadelphia, PA: Current Medicine; 1999.)

consequences of AKI vary from recovery to permanent renal damage, which may require dialysis or renal transplantation.

Reasons for the Susceptibility of the Kidney to Toxicity

Although the kidneys constitute only 0.5% of total body mass, they receive about 20% to 25% of the resting cardiac output. Consequently, any drug or chemical in the systemic circulation will be delivered to these organs in relatively high amounts. T e processes involved in forming concentrated urine also serve to concentrate potential toxicants in the tubular fluid, thereby driving passive diffusion of toxicants into tubular cells. T erefore, a nontoxic concentration of a chemical in the plasma may reach toxic concentrations in the kidney and its tubules. Finally, renal transport, accumulation, and metabolism of xenobiotics contribute significantly to the susceptibility of the kidney to toxic injury.

In addition to intrarenal factors, the incidence and/or severity of chemically induced nephrotoxicity may be related to the sensitivity of the kidney to circulating vasoconstrictors (angiotensin II or ADH), whose actions are normally counterbalanced by the actions of increased vasodilatory prostaglandins. When prostaglandin synthesis is suppressed by nonsteroidal anti-inflammatory drugs (NSAIDs), renal blood flow (RBF) declines markedly and AKI ensues due to the unopposed actions of vasoconstrictors. Another example of predisposing risk factors relates to the clinical use of angiotensin converting enzyme (ACE) inhibitors. Glomerular filtration pressure is dependent on angiotensin II–induced efferent arteriolar constriction. ACE inhibitors block this vasoconstriction, resulting in a precipitous decline in filtration pressure and AKI.

Site-Selective Injury

Many nephrotoxicants have their primary effects on discrete segments or regions of the nephron. T e reasons underlying this site-selective injury are complex but can be attributed in part to site-specific differences in blood flow, transport and accumulation of chemicals, physicochemical properties of the epithelium, reactivity of cellular/molecular targets, balance of bioactivation/detoxification reactions, cellular energetics, and/or regenerative/repair mechanisms.

Glomerular Injury

T e glomerulus is the initial site of chemical exposure within the nephron, and a number of nephrotoxicants produce structural injury to this segment. In certain instances, chemicals alter glomerular permeability to proteins by altering the size- and charge-selective functions.

Cyclosporine, amphotericin B, and gentamicin impair glomerular ultrafiltration without significant loss of structural integrity and decrease GFR. Amphotericin B decreases GFR by causing renal vasoconstriction and decreasing the glomerular capillary ultrafiltration coefficient (K_f). Gentamicin interacts with the anionic sites on the endothelial cells, decreasing K_f and GFR. Finally, cyclosporine not only causes renal vasoconstriction and vascular damage, but is also injurious to the glomerular endothelial cell.

Chemically induced glomerular injury may also be mediated by extrarenal factors. Circulating immune complexes may be trapped within the glomeruli (as could be the case in a type 3 hypersensitivity reaction). Neutrophils and macrophages are commonly observed within glomeruli in membranous glomerulonephritis, and the local release of cytokines and reactive oxygen species (ROS) may contribute to glomerular injury. Heavy metals, hydrocarbons, penicillamine, and captopril can produce this type of glomerular injury. A chemical may function as a hapten attached to some native protein or as a complete antigen and elicit an antibody response. Antibody reactions with cell-surface antigens (e.g., GBM) lead to immune deposit formation within the glomeruli, mediator activation, and subsequent injury to glomerular tissue.

Proximal Tubular Injury

T e proximal tubule is the most common site of toxicant-induced renal injury. T e reasons for this relate in part to the selective accumulation of xenobiotics into this segment of the nephron. T e proximal tubule has a leaky epithelium, favoring

the flux of compounds into proximal tubular cells. More importantly, tubular transport of organic anions and cations, low-molecular-weight proteins and peptides, GSH conjugates, and heavy metals is localized primarily if not exclusively to the proximal tubule. T us, transport of these molecules will be greater in the proximal tubule than in other segments, resulting in proximal tubular accumulation and toxicity. Although correlations between proximal tubular transport, accumulation, and toxicity suggest that the site of transport is a crucial determinant of the site of toxicity, transport is unlikely to be the sole criterion.

In addition to segmental differences in transport, segmental differences in cytochrome P450 and cysteine conjugate β-lyase activity also are contributing factors to the enhanced susceptibility of the proximal tubule. Both enzyme systems are localized almost exclusively in the proximal tubule, with negligible activity in the glomerulus, distal tubules, or collecting ducts. T us, nephrotoxicity requiring P450 and β-lyase-mediated bioactivation will most certainly be localized in the proximal tubule.

Finally, proximal tubular cells appear to be more susceptible to ischemic injury than distal tubular cells. T erefore, the proximal tubule will likely be the primary site of toxicity for chemicals that interfere with RBF, cellular energetics, and/or mitochondrial function.

Loop of Henle/Distal Tubule/Collecting Duct Injury

Functional abnormalities at distal nephron sites manifest primarily as impaired concentrating ability and/or acidification defects. Amphotericin B, cisplatin, and methoxyflurane induce an ADH-resistant polyuria, suggesting that the concentrating defect occurs at the level of the medullary thick ascending limb and/or the collecting duct.

Papillary Injury

T e renal papilla is susceptible to the chronic injurious effects of abusive consumption of analgesics. T e initial target of abusive consumption of analgesics is the medullary interstitial cells, followed by degenerative changes in the medullary capillaries, loops of Henle, and collecting ducts. High papillary concentrations of potential toxicants and inhibition of vasodilatory prostaglandins compromise RBF to the renal medulla/papilla and result in tissue ischemia.

ASSESSMENT OF RENAL FUNCTION

Both in vivo and in vitro methods are available for evaluation of the effects of a chemical on kidney function. Initially, nephrotoxicity can be assessed by evaluating serum and urine chemistries following treatment with the chemical in question. T e standard battery of noninvasive tests includes measurement of urine volume and osmolality, pH, and urinary composition (e.g., electrolytes, glucose, and protein). Although specificity is often lacking in such an assessment, urinalysis provides a relatively easy and noninvasive assessment of overall renal functional integrity and can provide some insight into the nature of the nephrotoxic insult. T e simultaneous analysis of cellular metabolites in sera and urine using nuclear magnetic analysis (metabonomics) has matured over the past few years and may provide an additional technology to identify and monitor nephrotoxicity.

Chemically induced increases in urine volume accompanied by decreases in osmolality may suggest an impaired concentrating ability, possibly via a defect in ADH synthesis, release, and/or action. Glucosuria may reflect chemically induced defects in proximal tubular reabsorption of sugars or be secondary to hyperglycemia. Urinary excretion of high-molecular-weight proteins, such as albumin, is suggestive of glomerular damage, whereas excretion of low-molecular-weight proteins, such as β_2-microglobulin, suggests proximal tubular injury. Urinary excretion of enzymes localized in the brush border (e.g., alkaline phosphatase and γ-glutamyl transferase) may reflect brush-border damage, whereas urinary excretion of other enzymes (e.g., lactate dehydrogenase) may reflect more generalized cell damage. Enzymuria is often a transient phenomenon, as chemically induced damage may result in an early loss of most of the enzyme available. T us, the absence of enzymuria does not necessarily reflect an absence of damage.

GFR can be measured directly by determining creatinine or inulin clearance, both of which are essentially freely filtered and not reabsorbed or secreted. T erefore, the clearance of creatinine or inulin is about the same as the GFR. Creatinine is an endogenous compound released from skeletal muscle. Inulin is an exogenous compound. Creatinine or inulin clearance is determined by the following formula:

$$\text{Inulin clearance (mL/ min)} = \frac{\text{Inulin concentration in urine (mg/ L)} \times \text{Urine volume (mL/ min)}}{\text{Inulin concentration in serum (mg/ L)}}$$

Indirect markers of GFR are serial blood urea nitrogen (BUN) and serum creatinine concentrations. However, a 50% to 70% decrease in GFR must occur before increases in serum creatinine and BUN develop. Chemically induced increases in BUN and/or serum creatinine may not necessarily reflect renal damage, but rather may be secondary to dehydration, hypovolemia, and/or protein catabolism. Serum cystatin C levels may be more sensitive as a marker of mildly impaired GFR.

Histopathologic evaluation of the kidney following treatment is crucial in identifying the site, nature, and severity of the nephrotoxic lesion. Assessment of chemically induced nephrotoxicity therefore should include urinalysis, serum clinical chemistry, and histopathology to provide a reasonable profile of the functional and morphologic effects of a chemical on the kidney. Site-specific biomarkers for common nephrotoxicants are shown in Figure 14–6.

Various in vitro techniques may be used to elucidate underlying mechanisms of chemically induced nephrotoxicity.

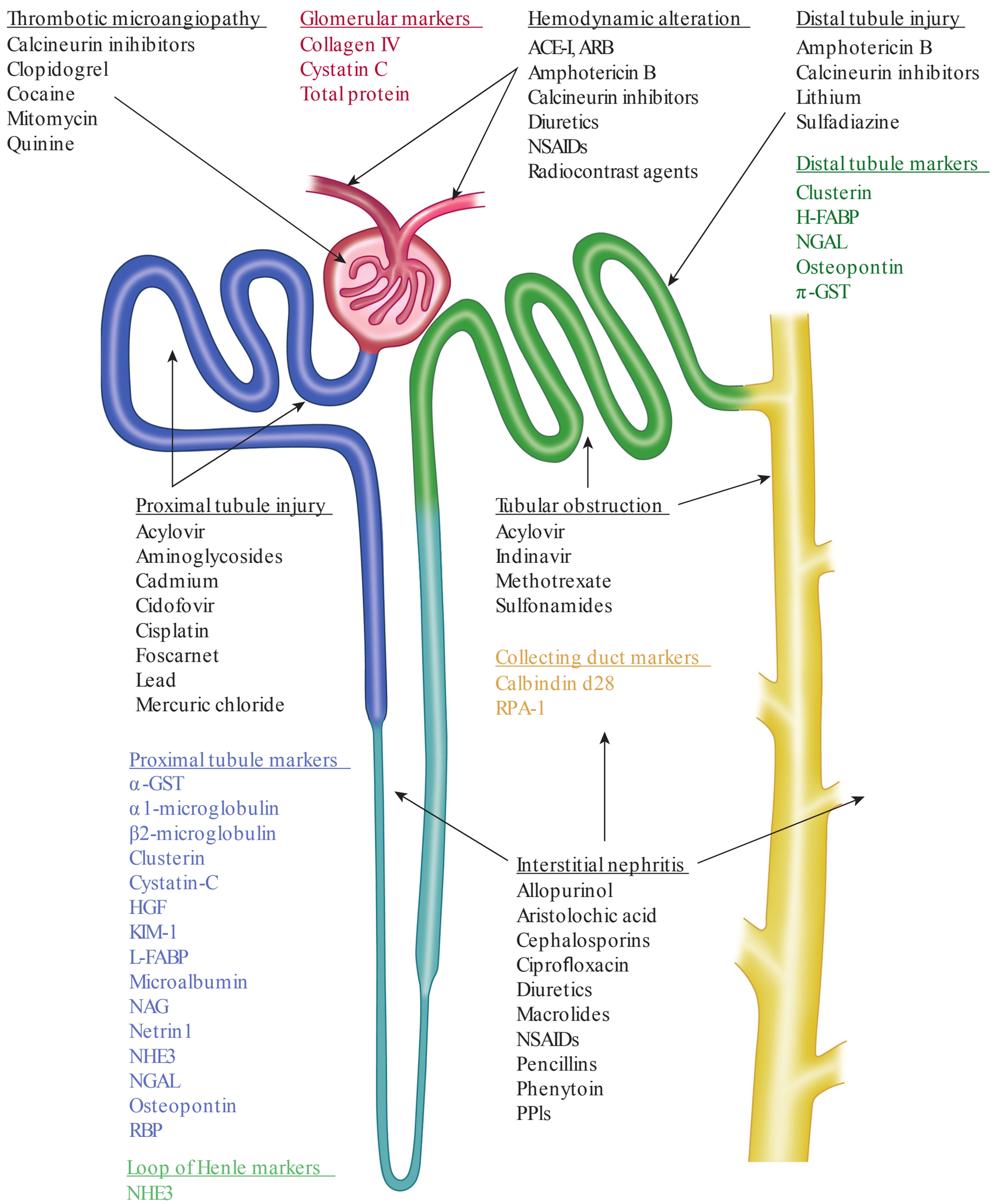

FIGURE 14–6 **Site-specif c biomarkers, common nephrotoxicants, and mechanisms of injury.** (Reproduced with permission from McQueen CA, Schnellmann (eds): Comprehensive Toxicology. Oxford, UK: Elsevier; 2010.)

Freshly prepared isolated perfused kidneys, kidney slices, and renal tubular suspensions and cells exhibit the greatest degree of differentiated functions and similarity to the in vivo situation, but these models have limited life spans of 2 to 24 h. In contrast, primary cultures of renal cells and established renal cell lines exhibit longer life spans (> 2 weeks). Once a mechanism has been identified in vitro, the postulated mechanism must be tested in vivo. T us, appropriately designed in vivo and in vitro studies should provide a complete characterization of the biochemical, functional, and morphologic effects of a chemical on the kidney and an understanding of the underlying mechanisms in the target cell population(s).

BIOCHEMICAL MECHANISMS/ MEDIATORS OF RENAL CELL INJURY

Cell Death

Cell death may occur through either oncosis or apoptosis. Apoptosis is a tightly controlled, organized process that usually affects scattered individual cells, which break into small fragments that are phagocytosed by adjacent cells or macrophages without producing an inflammatory response. In contrast, oncosis often affects many contiguous cells; the cells rupture, releasing cellular contents and inflammation follows. With

many toxicants, lower but injurious concentrations produce cell death through apoptosis. As the concentration of the toxicant increases, oncosis plays a predominant role.

Mediators of Toxicity

A chemical can initiate cell injury by various mechanisms (Figure 14–5). T e chemical may initiate toxicity due to its intrinsic reactivity with cellular macromolecules, may require renal or extrarenal bioactivation to a reactive intermediate, or may initiate injury indirectly by inducing oxidative stress via increased production of ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. ROS and reactive nitrogen species from nitric oxide, such as peroxynitrite ($ONOO^-$), can attack proteins, lipids, and DNA to induce cellular injury and death.

Cellular/Subcellular and Molecular Targets

A number of cellular targets have been identified to play a role in cell death. It is generally thought that an intracellular interaction (e.g., an alkylating agent or ROS with a macromolecule) initiates a sequence of events that leads to cell death. In the case of oncosis, a "point of no return" is reached in which the cell will die regardless of any intervention. T e idea of a single sequence of events is probably simplistic for most toxicants, given the extensive number of targets available for alkylating species and ROS. Rather, multiple pathways, with both distinct and common sequences of events, may lead to cell death.

Many cellular processes depend on mitochondrial ATP and, thus, become compromised simultaneously with inhibition of respiration. Conversely, mitochondrial dysfunction may be a consequence of some other cellular process altered by the toxicant. Numerous nephrotoxicants cause mitochondrial dysfunction in different ways. Whether toxicants target mitochondria directly or indirectly, it is clear that mitochondria play a critical role in determining whether cells die by apoptosis or oncosis. It is thought that the mitochondrial permeability transition (MPT) occurs during cell injury and ultimately progresses to apoptosis if sufficient ATP is available, or to oncosis if ATP is depleted. Further, the release of apoptotic proteins, such as apoptosis inducing factor, cytochrome c, Smac/Diablo, Omi, and Endonuclease G following MPT play a key role in activating downstream caspases and executing apoptosis.

Ca^{2+} is a second messenger and plays a critical role in a variety of cellular functions. Sustained elevations or abnormally large increases in cytosolic free Ca^{2+} can exert a number of detrimental effects on the cell. For example, an increase in cytosolic free Ca^{2+} can activate a number of degradative Ca^{2+}-dependent enzymes, such as phospholipases and proteinases (e.g., calpains), and can produce aberrations in the structure and function of cytoskeletal elements. Release of endoplasmic reticulum (ER) Ca^{2+} stores may be a key step in initiating the injury process and increasing cytosolic free Ca^{2+} concentrations, because prior depletion of ER Ca^{2+} stores protects renal proximal tubules from extracellular Ca^{2+} influx and cell death produced by mitochondrial inhibition and hypoxia. Mitochondria are known to accumulate Ca^{2+} in lethally injured cells through a low-affinity, high-capacity Ca^{2+} transport system. Although this system plays a minor role in normal cellular Ca^{2+} regulation, under injurious conditions the uptake of Ca^{2+} may facilitate ROS formation and damage.

Signaling kinases such as protein kinase C, mitogen-activated protein kinases (e.g., ERK1/2, p38, JNK/SAPK), protein kinase B (Akt), src, and phosphoinositide-3-kinase phosphorylate other proteins and, thereby, alter their activity, expression, or localization. Numerous recent studies reveal critical roles for signaling kinases in renal cell death and in the recovery of renal cells after toxicant injury.

Cell volume and ion homeostasis are tightly regulated and are critical for the reabsorptive properties of the tubular epithelial cells. Toxicants generally disrupt cell volume and ion homeostasis by either increasing ion permeability or inhibiting energy production. Loss of ATP results in the inhibition of membrane transporters that maintain the internal ion balance.

SPECIFIC NEPHROTOXICANTS

Heavy Metals

Many metals—including cadmium, chromium, lead, mercury, platinum, and uranium—are nephrotoxic. T e nature and severity of metal nephrotoxicity varies with respect to its form. In addition, different metals have different primary targets within the kidney. Metals may cause renal cellular injury through their ability to bind to sulf ydryl groups of critical proteins within the cells and thereby inhibit their normal function.

Mercury—Humans and animals are exposed to elemental mercury vapor, inorganic mercurous and mercuric salts, and organic mercuric compounds through the environment. Administered elemental mercury is rapidly oxidized in erythrocytes or tissues to inorganic mercury, and thus the tissue distribution of elemental and inorganic mercury is similar. Due to its high affinity for sulf ydryl groups, virtually all of the Hg^{2+} found in blood is bound to albumin, other sulf ydryl-containing proteins, glutathione, and cysteine.

T e kidneys are the primary target organs for accumulation of Hg^{2+}, and the S_3 segment of the proximal tubule is the initial site of toxicity, but the S_1 and S_2 segments may become affected as dose or duration increases. Renal uptake of Hg^{2+} is very rapid with as much as 50% of a nontoxic dose of Hg^{2+} found in the kidneys within a few hours of exposure. Considering the fact that virtually all of the Hg^{2+} found in blood is bound to an endogenous ligand, it is likely that the luminal and/or basolateral transport of Hg^{2+} into the proximal tubular epithelial cell is through cotransport of Hg^{2+} with an endogenous ligand such as glutathione, cysteine, or albumin, or through some plasma membrane Hg^{2+}-ligand complex.

T e acute nephrotoxicity induced by $HgCl_2$ is characterized by proximal tubular necrosis and AKI within 24 to 48 h after administration. Early markers of $HgCl_2$-induced renal

dysfunction include an increase in the urinary excretion of brush-border enzymes such as alkaline phosphatase and γ-glutamyl transferase. Subsequently, when tubular injury becomes severe, intracellular enzymes, such as lactate dehydrogenase and aspartate aminotransferase, increase in the urine. As injury progresses, tubular reabsorption of solutes and water decreases and there is an increase in the urinary excretion of glucose, amino acids, albumin, and other proteins. Also associated with the increase in injured proximal tubules is a decrease and progressive decline in the GFR.

Changes in mitochondrial morphology and function are very early events following $HgCl_2$ administration, supporting the hypothesis that mitochondrial dysfunction is an early and important contributor to inorganic mercury-induced cell death along the proximal tubule.

Cadmium—Chronic exposure of nonsmoking humans and animals to cadmium is primarily through food and results in nephrotoxicity. In the workplace, inhalation of cadmium-containing dust and fumes is the major route of exposure. Cadmium has a half-life of greater than 10 years in humans and thus accumulates in the body over time. Approximately 50% of the body's burden of cadmium can be found in the kidney. Cadmium produces proximal tubule dysfunction (S_1 and S_2 segments) and injury that may progress to a chronic interstitial nephritis.

Chemically Induced α_{2u}-Globulin Nephropathy

A diverse group of chemicals, including unleaded gasoline, jet fuels, d-limonene, 1,4-dichlorobenzene, decalin, tetrachloroethylene, and lindane, causes α_{2u}-globulin nephropathy or hyaline droplet nephropathy in male rats. Binding to α_{2u}-globulin decreases lysosomal proteases breakdown of α_{2u}-globulin. Chronic exposure to these compounds results in progression of these lesions and ultimately in chronic nephropathy.

Humans are not at risk because (1) humans do not synthesize α_{2u}-globulin; (2) humans secrete less proteins in general and in particular less low-molecular-weight proteins in urine than the rat; (3) the low-molecular-weight proteins in human urine are either not related structurally to α_{2u}-globulin, do not bind to compounds that bind to α_{2u}-globulin, or are similar to proteins in female rats, male Black Reiter rats, rabbits, or guinea pigs that do not exhibit α_{2u}-globulin nephropathy; and (4) mice excrete a low-molecular-weight urinary protein that is 90% homologous to α_{2u}-globulin, but they do not exhibit α_{2u}-globulin-nephropathy and renal tumors following exposure to α_{2u}-globulin-nephropathy-inducing agents.

Halogenated Hydrocarbons

Halogenated hydrocarbons are a diverse class of compounds and are used extensively as chemical intermediates, solvents, and pesticides. Consequently, humans are exposed to these compounds not only in the workplace but also through the environment. Numerous toxic effects have been associated with acute and chronic exposure to halogenated hydrocarbons, including nephrotoxicity.

Chloroform—The primary cellular target of chloroform is the proximal tubule, with no primary damage to the glomerulus or the distal tubule. Proteinuria, glucosuria, and increased BUN levels are all characteristic of chloroform-induced nephrotoxicity. The nephrotoxicity produced by chloroform is linked to its metabolism by renal cytochrome P450, which biotransforms chloroform to trichloromethanol, which is unstable and releases HCl to form phosgene, which injuriously reacts with cellular macromolecules.

Tetrafluoroethylene—Tetrafluoroethylene is conjugated with glutathione in the liver, and the GSH conjugate is secreted into the bile and small intestine where it is degraded to the cysteine S-conjugate (TFEC), reabsorbed, and transported to the kidney. Although several metabolites are formed, the cysteine S-conjugate is the penultimate nephrotoxicant. Following transport into the proximal tubule, the cysteine S-conjugate is a substrate for the cytosolic and mitochondrial forms of the enzyme cysteine conjugate β-lyase. The products of the reaction are ammonia, pyruvate, and a reactive thiol that is capable of binding covalently to cellular macromolecules causing cellular damage. Functionally, increases in urinary glucose, protein, cellular enzymes, and BUN are noted.

Bromobenzene—Biotransformation of bromobenzene and other halogenated benzenes is critical for their nephrotoxicity. Hepatic cytochrome P450 metabolizes bromobenzene and conjugates it to glutathione, and releases it as a form that can cause nephrotoxicity. The diglutathione conjugate of the hydroquinone is approximately 1000-fold more potent than bromobenzene in producing nephrotoxicity, producing the same pathologic changes in the S_3 segment, and increasing the amount of protein, glucose, and cellular enzymes in the urine.

Mycotoxins

Mycotoxins are products of molds and fungi, and a number of mycotoxins produce nephrotoxicity. Three examples of nephrotoxic mycotoxins will be discussed. Citrinin nephrotoxicity is characterized by decreased urine osmolality, GFR and RBF, glycosuria, and increased urinary enzyme excretion. Interestingly, the location of citrinin-induced tubular vacuolization and necrosis (proximal, distal) varies among species. Whereas the mechanism of citrinin toxicity to the tubules remains unresolved, citrinin enters the cells through the organic anion transporter and causes mitochondrial dysfunction.

Fumonisins B_1 and B_2 are commonly found on corn and corn products and they are known to produce nephrotoxicity in rats and rabbits. Histologic examination of the kidney revealed disruption of the basolateral membrane, mitochondrial swelling, increased numbers of clear and electron-dense vacuoles, and apoptosis in proximal tubular cells at the junction of the cortex

and medulla. Changes in renal function included increased urine volume, decreased osmolality, and increased excretion of low- and high-molecular-weight proteins. T e toxicity of fumonisins may be through increased sphinganine, reactive oxygen species, and apoptosis.

Aristolochic acids (AAs) and aristolactams are natural products found in the Aristolochia and Asarum genera. Despite the extensive use of Aristolochia as a herbal remedy for thousands of years, recent reports of its human toxicity include tubular dysfunction, proteinuria, and interstitial fibrosis. AAs form covalent DNA adducts, and are genotoxic and carcinogenic. Renal uptake of the penultimate toxicant, AA-I, involves mOat-mediated transport, and it is bioactivated through nitroreduction to produce DNA and protein adducts.

Therapeutic Agents

Acetaminophen—Acetaminophen (APAP) nephrotoxicity is characterized by proximal tubular necrosis with increases in BUN and plasma creatinine, decreases in GFR and clearance of para-aminohippurate, increases in the fractional excretion of water, sodium, and potassium, and increases in urinary glucose, protein, and brush-border enzymes. Although renal cytochrome P450 plays a role in APAP activation and nephrotoxicity, glutathione conjugates of APAP may also contribute to APAP-induced nephrotoxicity.

Nonsteroidal Anti-inf ammatory Drugs—NSAIDs such as aspirin, ibuprofen, naproxen, indomethacin, and cyclooxygenase-2 inhibitors (e.g., celecoxib) are extensively used as analgesics and anti-inflammatory drugs and produce their therapeutic effects through the inhibition of prostaglandin synthesis. At least three different types of nephrotoxicity have been associated with NSAID administration. AKI may occur within hours of a large dose of a NSAID, is usually reversible on withdrawal of the drug, and is characterized by decreased RBF and GFR and by oliguria. When normal production of vasodilatory prostaglandins (e.g., PGE_2, PGI_2) is inhibited by NSAIDs, vasoconstriction induced by circulating catecholamines and angiotensin II is unopposed, resulting in decreased RBF and ischemia.

In contrast, chronic consumption of NSAIDs and/or APAP (> 3 years) results in an often irreversible nephrotoxicity that is known as analgesic nephropathy. T e primary lesion is papillary necrosis with chronic interstitial nephritis. T e mechanism by which NSAIDs produce analgesic nephropathy is not known but may result from chronic medullary/papillary ischemia, secondary to renal vasoconstriction, or genesis of a reactive intermediate that, in turn, initiates an oxidative stress or binds covalently to critical cellular macromolecules.

T e third albeit rare type of nephrotoxicity associated with NSAIDs is an interstitial nephritis. Patients normally present with elevated serum creatinine and proteinuria. If NSAIDs are discontinued, renal function improves in 1 to 3 months.

Aminoglycosides—T e aminoglycoside antibiotics are so named because they consist of two or more amino sugars joined in a glycosidic linkage to a central hexose nucleus. Although they are drugs of choice for many gram-negative infections, their use is primarily limited by their nephrotoxicity. Renal dysfunction by aminoglycosides is characterized by a nonoliguric renal failure with reduced GFR, an increase in serum creatinine and BUN, and polyuria. Within 24 h, increases in urinary brush-border enzymes, glucosuria, aminoaciduria, and proteinuria are observed. Histologically, lysosomal alterations are noted initially, followed by damage to the brush border, ER, mitochondria, and cytoplasm, ultimately leading to tubular cell necrosis. Interestingly, proliferation of renal proximal tubule cells can be observed early after the onset of nephrotoxicity.

T e earliest lesion observed following clinically relevant doses of aminoglycosides is an increase in the size and number of lysosomes, which contain phospholipids. T e renal phospholipidosis produced by the aminoglycosides is thought to occur through their inhibition of lysosomal hydrolases, such as sphingomyelinase and phospholipases.

Amphotericin B—Amphotericin B is an effective antifungal agent, causing nephrotoxicity characterized by ADH-resistant polyuria, renal tubular acidosis, hypokalemia, and either acute or chronic renal failure. T e functional integrity of the glomerulus and of the proximal and distal portions of the nephron is impaired, leading to decreases in RBF and GFR secondary to renal arteriolar vasoconstriction or activation of tubuloglomerular feedback.

Cyclosporine—Cyclosporine is an important immunosuppressive agent and is widely used to prevent graft rejection in organ transplantation. Cyclosporine is a fungal cyclic polypeptide and acts by selectively inhibiting cyclophylin and, in turn, calcineurin and T-cell activation. Nephrotoxicity is a critical side effect of cyclosporine, with nearly all patients who receive the drug exhibiting some form of nephrotoxicity. Cyclosporine-induced nephrotoxicity may manifest as (1) acute reversible renal dysfunction, (2) acute vasculopathy, and (3) chronic nephropathy with interstitial fibrosis. Acute renal dysfunction is characterized by dose-related decreases in RBF and GFR and increases in BUN and serum creatinine. T e decrease in RBF and GFR is related to marked vasoconstriction induced by cyclosporine.

Acute vasculopathy or thrombotic microangiopathy following cyclosporine treatment affects arterioles and glomerular capillaries, without an inflammatory component. T e lesion consists of fibrin–platelet thrombi and fragmented red blood cells occluding the vessels.

Long-term treatment with cyclosporine can result in chronic nephropathy with interstitial fibrosis and tubular atrophy. Modest elevations in serum creatinine and decreases in GFR occur along with hypertension, proteinuria, and tubular dysfunction. Histologic changes are profound; they are characterized by arteriolopathy, global and segmental glomerular sclerosis, striped interstitial fibrosis, and tubular atrophy. T ese lesions may not be reversible if cyclosporine therapy is discontinued and may result in end-stage renal disease.

Cisplatin—Cisplatin is a valuable drug in the treatment of solid tumors, with nephrotoxicity limiting its clinical use. The kidney is not only responsible for the majority of cisplatin excreted but is also the primary site of accumulation. Cisplatin nephrotoxicity includes acute and chronic renal failure, renal magnesium wasting, and polyuria. Patients treated with cisplatin regimens permanently lose 10% to 30% of their renal function.

The nephrotoxicity of cisplatin can be grouped as (1) tubular toxicity, (2) vascular damage, (3) glomerular injury, and (4) interstitial injury. Early effects of cisplatin are decreases in RBF and polyuria that is concurrent with increased electrolyte excretion. GFR is produced by vasoconstriction and is followed by tubular injury with enzymuria. Although the primary cellular target associated with AKI is the proximal tubule S_3 segment in the rat, in humans the S_1 and S_2 segments, distal tubule, and collecting ducts can also be affected. Cisplatin may produce nephrotoxicity through its ability to inhibit DNA synthesis as well as transport functions. In addition, cisplatin is known to induce mitochondrial dysfunction and activates numerous pathways in the mitogen-activated protein kinase family.

Radiocontrast Agents—Iodinated contrast media used for the imaging of tissues have a very high osmolality (> 1200 mOsm/L) and are potentially nephrotoxic, particularly in patients with existing renal impairment, diabetes, or heart failure or who are receiving other nephrotoxic drugs. The newer nonionic contrast agents (e.g., iotrol and iopamidol) have lower nephrotoxicity. The nephrotoxicity of these agents is due to both hemodynamic alterations (vasoconstriction) and tubular injury (via ROS).

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QUESTIONS

1. T e kidney is responsible for all of the following EXCEPT:
   a. synthesis of renin.
   b. acid–base balance.
   c. reabsorption of electrolytes.
   d. regulation of extracellular fluid.
   e. release of angiotensin.

2. Which of the following does NOT contribute to filtrate formation in the nephron?
   a. capillary hydrostatic pressure.
   b. positive charge of glomerular capillary wall.
   c. hydraulic permeability of glomerular capillary wall.
   d. colloid oncotic pressure.
   e. size of filtration slits.

3. Which of the following is NOT a characteristic of the loop of Henle?
   a. T ere is reabsorption of filtered Na^+ and K^+.
   b. Tubular fluid in the thin descending limb is isosmotic to the renal interstitium.
   c. Water is freely permeable in the thin ascending limb.
   d. Na^+ and Cl^- are reabsorbed in the thin ascending limb.
   e. T e thick ascending limb is impermeable to water.

4. Although the kidneys constitute 0.5% of total body mass, approximately how much of the resting cardiac output do they receive?
   a. 0.5% to 1%.
   b. 5%.
   c. 10%.
   d. 20% to 25%.
   e. 50% to 60%.

5. Which of the following is most likely to occur after a toxic insult to the kidney?
   a. GFR will decrease in the unaffected kidney.
   b. Tight-junction integrity will increase in the nephron.
   c. T e unaffected cells will undergo atrophy and proliferation.
   d. Clinical tests will likely show normal renal function.
   e. Glomerulotubular balance is lost.

6. Chronic renal failure does not typically result in:
   a. decrease in GFR of viable nephrons.
   b. glomerulosclerosis.
   c. tubular atrophy.
   d. increased glomerular pressures.
   e. altered capillary permeability.

7. All of the following statements regarding toxicity to the kidney are true EXCEPT:
   a. Concentration of toxins in tubular fluid increase the likelihood that the toxin will diffuse into tubular cells.
   b. Drugs in the systemic circulation are delivered to the kidneys at relatively high amounts.
   c. T e distal convoluted tubule is the most common site of toxicant-induced renal injury.
   d. Immune complex deposition within the glomeruli can lead to glomerulonephritis.
   e. Antibiotics and/or antifungal drugs affect the functioning of the nephron at multiple locations.

8. Which of the following test results is NOT correctly paired with the underlying kidney problem?
   a. increased urine volume—defect in ADH synthesis.
   b. glucosuria—defect in reabsorption in the proximal convoluted tubule.
   c. proteinuria—glomerular damage.
   d. proteinuria—proximal tubular injury.
   e. brush-border enzymuria—glomerulonephritis.

9. Renal cell injury is NOT commonly mediated by which of the following mechanisms?
   a. loss of membrane integrity.
   b. impairment of mitochondrial function.
   c. increased cytosolic Ca^{2+} concentration.
   d. increased Na^+,K^+-ATPase activity.
   e. caspase activation.

10. Which of the following statements is FALSE with respect to nephrotoxicants?
    a. Mercury poisoning can lead to proximal tubular necrosis and acute renal failure.
    b. Cisplatin may cause nephrotoxicity because of its ability to inhibit DNA synthesis.
    c. Chronic consumption of NSAIDs results in nephrotoxicity that is reversible with time.
    d. Amphotericin B nephrotoxicity can result in ADH-resistant polyuria.
    e. Acetaminophen becomes nephrotoxic via activation by renal cytochrome P450.

CHAPTER

15

Toxic Responses of the Respiratory System

George D. Leikauf

RESPIRATORY TRACT STRUCTURE AND FUNCTION

- Oronasal Passages
  - Structure
  - Sensory Functions
  - Irritant, Thermosensory, and Mechanosensory Functions
- Conducting Airways
  - Structure
  - Mucociliary Clearance and Antimicrobial Functions
- Gas Exchange Region
  - Structure
  - Function

BIOTRANSFORMATION IN THE RESPIRATORY TRACT

GENERAL PRINCIPLES IN THE PATHOGENESIS OF LUNG DAMAGE CAUSED BY CHEMICALS

- Toxic Inhalants, Gases, and Dosimetry
- Regional Particle Deposition
- Deposition Mechanisms
- Particle Clearance
  - Nasal Clearance
  - Tracheobronchial Clearance
  - Alveolar Clearance

ACUTE RESPONSES OF THE LUNG TO INJURY

- Trigeminally Mediated Airway Reflexes
- Bronchoconstriction, Airway Hyperreactivity, and Neurogenic Inflammation
- Acute Lung Injury (Pulmonary Edema)

CHRONIC RESPONSES OF THE LUNG TO INJURY

- Chronic Obstructive Pulmonary Disease
- Lung Cancer
- Asthma
- Pulmonary Fibrosis

AGENTS KNOWN TO PRODUCE LUNG INJURY IN HUMANS

EVALUATION OF TOXIC LUNG DAMAGE

- Human Studies
- Animal Studies
  - Inhalation Exposure Systems
  - Pulmonary Function Tests in Experimental Animals
  - Morphological Techniques
  - Pulmonary Lavage and Pulmonary Edema
- In Vitro Studies
  - Isolated Perfused Lung
  - Airway Microdissection and Organotypic Tissue Culture Systems
  - Lung Cell Culture

KEY POINTS

- Inhaled xenobiotics can affect lung tissues directly or distant organs after absorption.
- Water solubility is a decisive factor in determining how deeply a given gas penetrates into the lung.
- Particle size is usually the critical factor that determines the region of the respiratory tract in which a particle or an aerosol will deposit.
- T e lung contains most of the enzymes involved in xenobiotic biotransformation that have been identified in other tissues.
- Asthma is characterized by increased reactivity of the bronchial smooth muscle in response to exposure to irritants.
- In emphysema, destruction of the gas-exchanging surface area results in a distended, hyperinf ated lung that no longer effectively exchanges oxygen and carbon dioxide.

Toxic substances can disrupt the respiratory system and distant organs after chemicals enter the body by means of inhalation. Pathological changes in the respiratory tract also can be a target of blood-borne agents. Inhalation toxicology refers to the route of exposure, whereas respiratory toxicology refers to target organ toxicity. Lung tissue can be injured directly or secondarily by metabolic products from organic compounds. However, the most important effect of many toxic inhalants is to place an undue oxidative burden on the lungs.

RESPIRATORY TRACT STRUCTURE AND FUNCTION

Oronasal Passages

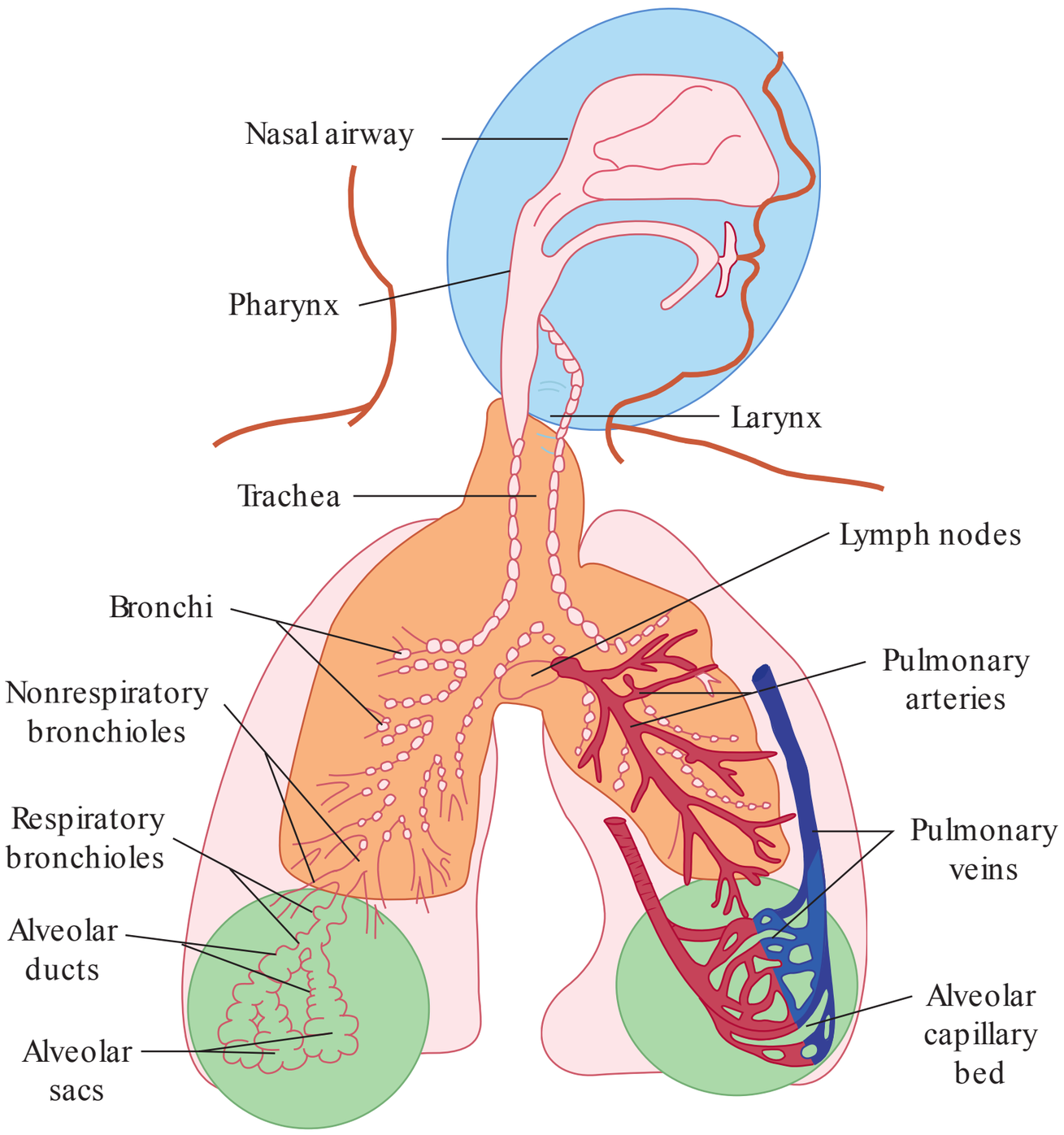
Structure—T e respiratory tract is divided into the upper respiratory tract (extrathoracic airway passages above the neck) and lower respiratory track (airway passages and lung parenchyma below the pharynx) (Figure 15–1). T e upper respiratory track reaches from the nostril or mouth to the pharynx and functions to conduct, heat, humidify, filter, and chemosense incoming air. Leaving the nasal passage, air is warmed to about 33°C and humidified to about 98% water saturation. Air is filtered in the nasal passages with highly water-soluble gases being absorbed efficiently. T e nasal passages also filter particles, which may be deposited by impaction or diffusion on the nasal mucosa.

Sensory Functions—In addition to conducting, conditioning, and filtering air to the lower respiratory tract, a major function of the oronasal passage is chemosensory. Nasal epithelia can metabolize many foreign compounds by cytochrome P450 and other enzymes. Humans can distinguish between more than 5 000 odors. T e detection of odor can be protective and can induce avoidance behaviors. Odorant can be added to the otherwise colorless and almost odorless gas used by consumers (e.g., mercaptans to methane), to assist in detecting leaks and thereby preventing fires or explosions.

Chemosensory function of the nasal passages is accomplished by a wide variety of specialized receptors in major subtypes including (1) olfactory receptors, (2) trace amine–associated receptors (TAARs), (3) membrane guanylyl cyclase GC-D receptors, (4) vomeronasal receptors, and (5) formyl peptide receptors (FPRs).

The olfactory epithelium contains specialized chemosensory olfactory neurons located in the superior portion of the nasal passage. Airflow in this region of the nasal passage is typically low, thus sniffing can increase perception. TAARs detect trace amines, with fishy or putrid odor, that are found in foods and can also be generated during fermentation or decay. GC-D receptors are located in the cilia of olfactory sensory neurons and detect the natriuretic peptides, uroguanylin (found in urine) and guanylin. In rodents, these receptors detect carbon dioxide, which is odorless in humans and other primates. Vomeronasal receptors are separate from, but adjacent to, olfactory neurons. They can detect higher molecular weight stimuli, including nonvolatile chemicals. FPRs are also a part of the vomeronasal system and detect bacterial or mitochondrial formylated peptides, which are thought to identify pathogens or pathogenic states.

Irritant, Thermosensory, and Mechanosensory Functions—In addition to the detection of odor, the detection of irritant chemicals, cold and hot temperatures, or mechanical stress can be a protective mechanism that may limit exposure. T e main nerve endings that perceive irritants, the chemical nociceptors also discern temperature and mechanical stress. Two protein families, the transient receptor potential (TRP) channels and the taste (TAS) receptors, perform these functions in the upper respiratory tract. TRP channels are ion channels that are permeable to cations, including calcium, magnesium, and sodium. T ese receptors are sensitive to a variety of natural ingredients, pain stimuli, and heat. Taste buds, which contain TAS, determine salt, sour, sweet, umami (glutamate and nucleotides), and bitter.



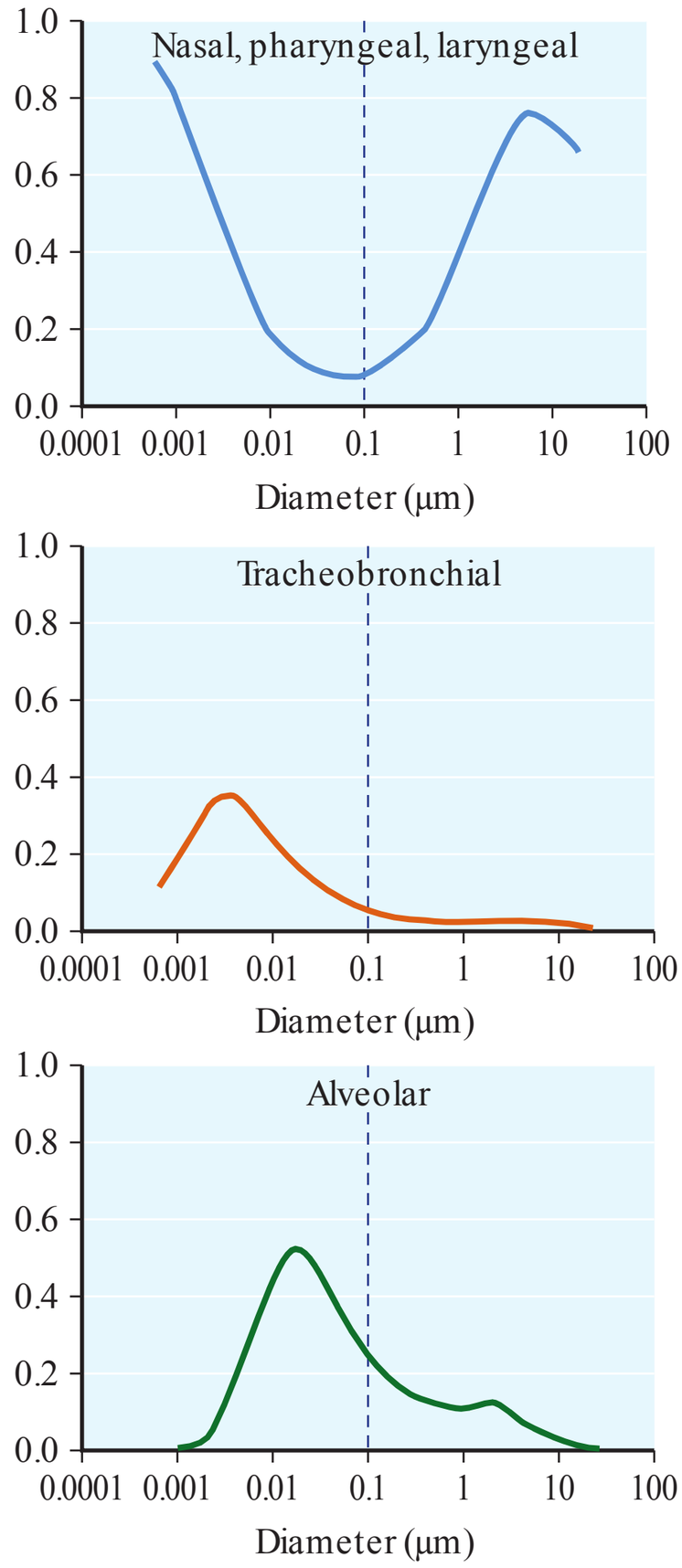


FIGURE 15–1 **Predicted fractional deposition of inhaled particles in the nasopharyngeal, tracheobronchial, and alveolar region of the human respiratory tract during nose breathing.** (Used with permission of J. Harkema.) (Reproduced with permission from Oberdorster G, Oberdorster E, Oberdorster J: Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles, Environ Health Perspect, 2005 Jul;113(7):823–839.)

Conducting Airways

Structure—At the beginning of the lower respiratory track is the larynx, which is responsible for speech (phonation). T e conducting airways of the lower respiratory tract can be divided into proximal (trachea and bronchi) and distal regions (bronchioles). Conducting airways have a bifurcating structure, with successive airway generations containing about twice the number of bronchi progressively decreasing in internal diameter. Eventually a transition zone is reached where cartilaginous bronchi give way to noncartilaginous bronchioles, which in turn give way to gas exchange regions, respiratory bronchioles, and alveoli. In the bronchiolar epithelium, mucus-producing cells and glands give way to bronchiolar secretoglobin cells (BSCs). One way in which airf ow is altered is by smooth muscle that surrounds the airways and is under autonomic innervation via the vagus nerve.

Mucociliary Clearance and Antimicrobial Functions—In humans, the proximal airway and a portion of the nasal passage are covered by a pseudostratified respiratory epithelium that contains a number of specialized cells including ciliated, mucous, and basal cells (Figure 15–2). T ese cells work together to form a mucous layer that traps and removes inhaled material via mucociliary clearance. For mucociliary clearance in the airways to function optimally, regulation of ion transport, f uid, and mucus must be coordinated. Chloride ion channels and the cystic fibrosis transmembrane regulator are needed to move f uid into the airway lumen and sodium channels are needed to move water out of the lumen.

Ciliated cells have microtubule-based protrusions, cilia, of which there are two types: motile and primary. Motile cilia exert mechanical force through continuous motion to propel harmful inhaled material out of the nose and lung. Motile cilia

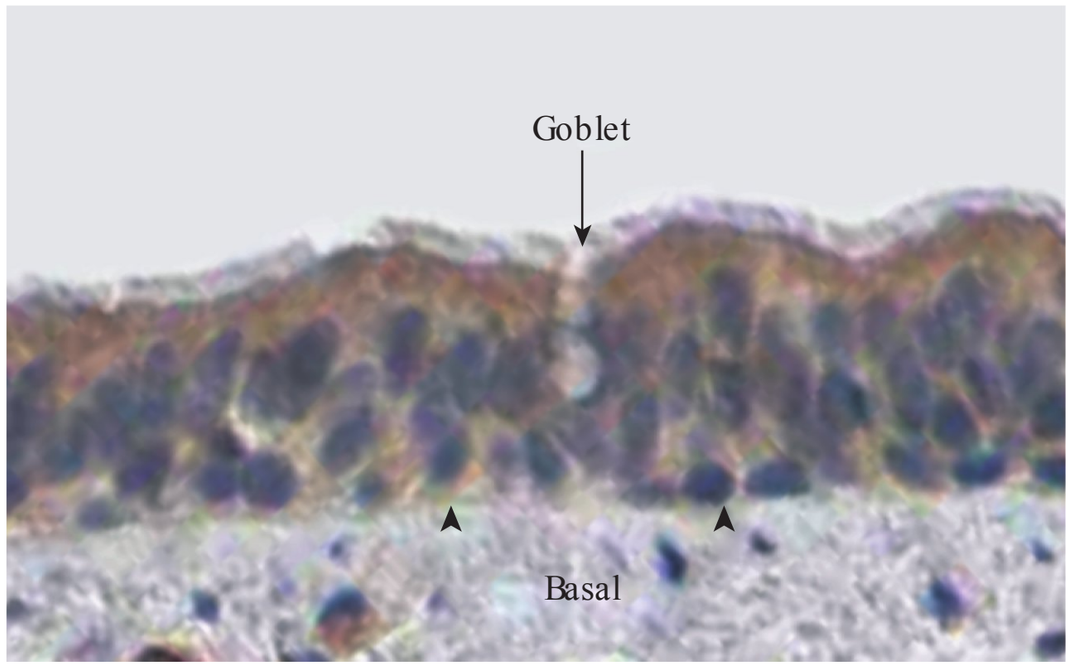


FIGURE 15–2 **Pseudo stratified respiratory epithelium lines the nasal cavity, trachea, and bronchi.** The surface includes mainly ciliated epithelial cells that may or may not touch the basement membrane, (arrow) surface mucous (goblet) cell, and (arrowhead) basal cells. (Modified with permission from the Human Protein Atlas (www.proteinatlas.org).)

also exhibit mechanosensory and chemosensory functions and can respond to mechanical stress, heat, acidic pH, and endogenous and synthetic agonists. Primary cilia often serve as sensory organelles.

Serous cells contain and secrete a less viscous f uid, and are also enriched in antimicrobial proteins including lysozyme and lactotransferin. T ese cells also contain the antimicrobial protein, BPIF2 (aka SPLUNC2). Secretory leukocyte proteinase inhibitor (SLPI) is a serine proteinase inhibitor that is produced locally in the lung by cells of the submucosal bronchial glands and by nonciliated epithelial cells. T e main function of SLPI is the inhibition of neutrophil elastase and other proteinases, and may also have antimicrobial functions.

Another airway secretory cell is the bronchiolar secretoglobin cell (BSC), previously called the Clara cell. T e role of secretoglobins is not fully understood in the lung, but they are known to inhibit phospholipase A2 and limit inf ammation. In humans, BSCs are found mainly in the distal airways and can act as tissue stem cells.

Gas Exchange Region

Structure—T e gas exchange region consists of terminal bronchioles, respiratory bronchioles, alveolar ducts, alveoli, blood vessels, and lung interstitium (Figure 15–3). Gas exchange occurs in the alveoli, which comprise □85% of the total parenchymal lung volume. Adult human lungs contain an estimated 300 to 500 million alveoli. Capillaries, blood plasma, and formed blood elements are separated from the air space by a thin layer of tissue formed by epithelial, interstitial, and endothelial components.

T e alveolar epithelium consists of two cells, the alveolar type I and type II cell (Figure 15–4). Alveolar type I cells cover

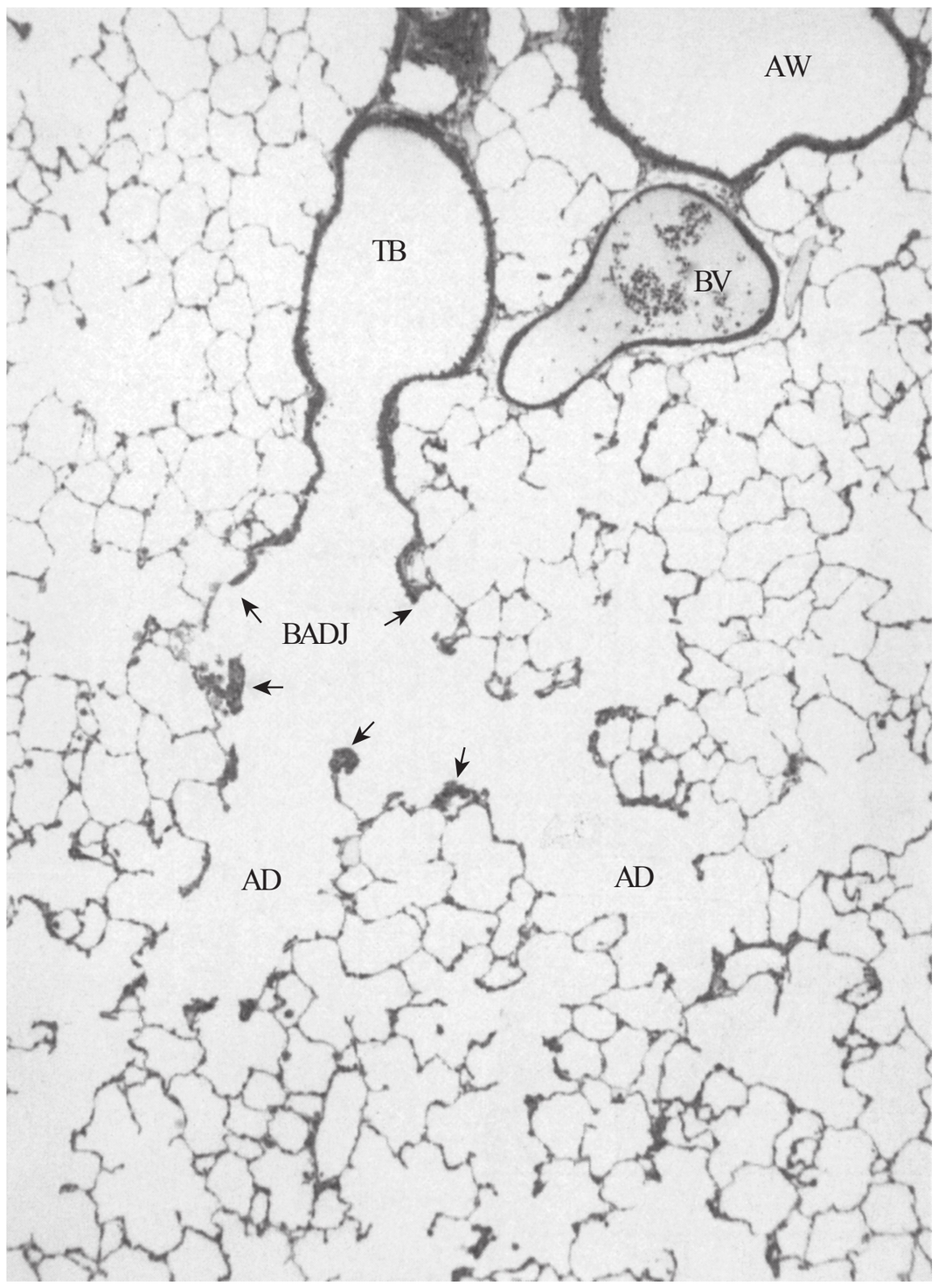


FIGURE 15–3 **Centriacinar region (ventilatory unit) of the lung.** An airway (AW) and a blood vessel (BV) (arteriole) are in close proximity to the terminal bronchiole (TB). The terminal bronchiole leads to the bronchiole–alveolar duct junction (BADJ) and the alveolar duct (AD). A number of the (arrows) alveolar septal tips close to the BADJ are thickened after a brief (4-h) exposure to asbestos fibers, indicating localization of fiber deposition. Other inhalants, such as ozone, produce lesions in the same locations. (Used with permission of Dr Kent E. Pinkerton, University of California, Davis.)

□95% of the alveolar surface and therefore are susceptible to damage by noxious agents that penetrate to the alveolus. Alveolar type I cells have an attenuated cytoplasm to enhance gas exchange. Alveolar type II cells produce and secrete surfactant, a mixture of lipids, and four surfactant associated proteins and can undergo mitotic division and replace damaged type I cells. Surfactant protein B and C are amphipathic and aid in spreading secreted lipids which form a monolayer that reduces surface tension. Surfactant proteins A1, A2, and D are members of the subfamily of C-type lectins called collectins, which defend against pathogens. Surfactant proteins A1 and A2 do not alter lipid structure but do bind lipopolysaccharides (LPS) and various microbial pathogens, enhancing their clearance from the lung. Surfactant protein D is also necessary in the suppression of pulmonary inf ammation and in host defense against viral, fungal, and bacterial pathogens.

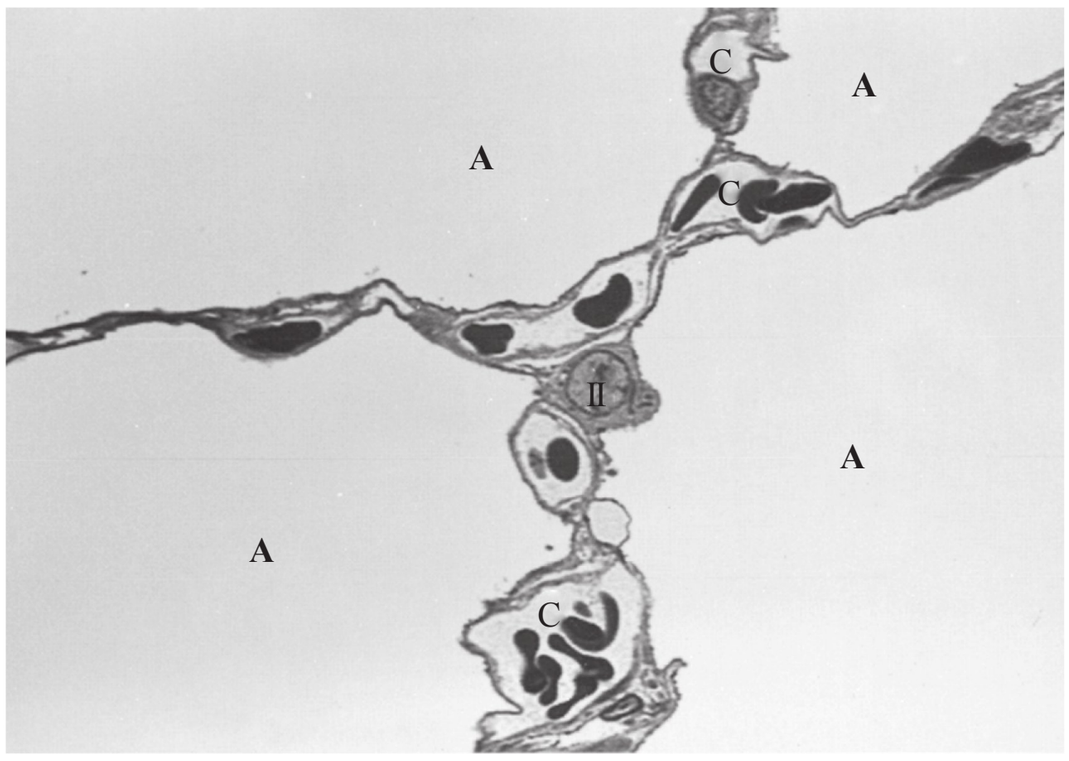


FIGURE 15–4 **Alveolar region of the lung.** The (A) alveolus is separated by the thin air-to-blood tissue barrier of the alveolar septal wall, which is composed of flat alveolar type I cells and occasional rounded (II) alveolar type II cells. A small interstitial space separates the epithelium and endothelium that form the (C) capillary wall. During lung injury the interstitial space enlarges and interferes with gas exchange. (Used with permission of Dr Kent E. Pinkerton, University of California, Davis.)

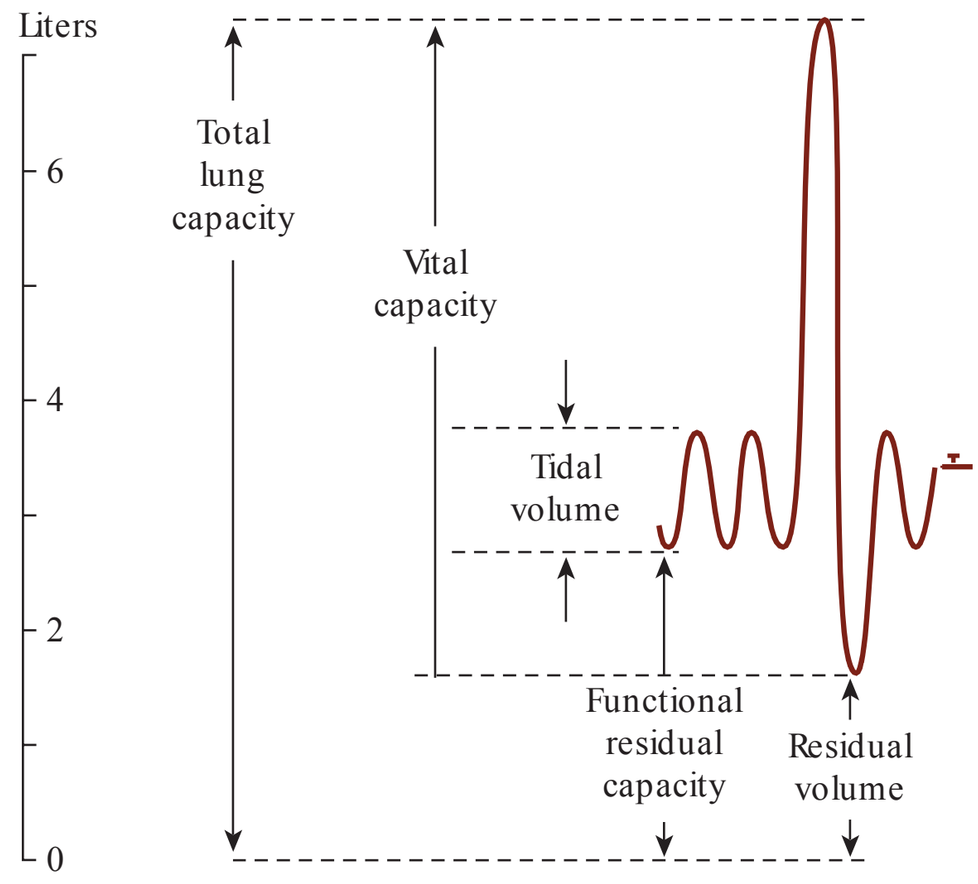


FIGURE 15–5 **A spirometer reading of lung volumes.** The total lung capacity is the total volume of air in an inflated human lung. After a maximum expiration, the lung retains a small volume of air, which is the residual volume. The air volume moved into and out of the lung during maximal inspiratory and expiratory movement, which is called the vital capacity. The tidal volume is typically moved into and out of the lung during each breathe. The functional residual capacity and residual volume cannot be measured with spirometer.

Function

Ventilation—The principal function of the lung is gas exchange, which consists of ventilation, perfusion, and diffusion. During inhalation, fresh air is moved into the lung through the upper respiratory tract and conducting airways and into the terminal respiratory units when the thoracic cage enlarges and the diaphragm moves downward; the lung passively follows this expansion. After diffusion of oxygen into the blood and that of CO_2 from the blood into the alveolar spaces, the air (now enriched in CO_2) is expelled by exhalation. Relaxation of the chest wall and diaphragm diminishes the internal volume of the thoracic cage, the elastic fibers of the lung parenchyma recoil, and air is expelled from the alveolar zone through the airways. Any interference with the elastic properties of the lung, e.g., the alteration of elastic fibers that occurs in emphysema, adversely affects ventilation, as do the decrease in the diameters of, or blockage of, the conducting airways, as in asthma.

Lung function changes with age and disease and can be measured with a spirometer (Figure 15–5). The total lung capacity (TLC) is the total volume of air in an inflated human lung, 4 to 5 L (women) and 6 to 7 L (men). After a maximum expiration, the lung retains 1.1 L (women) and 1.2 L (men), which is the residual volume (RV). The vital capacity is the air volume moved into and out of the lung during maximal inspiratory and expiratory movement and typical is 3.1 L (women) and 4.8 L (men). Only a small fraction of the VC, the tidal volume (TV), is typically moved into and out of the lung during quiet breathing. In resting humans, the TV measures ~0.5 L with each breath. The respiratory frequency is 12 to 20 breaths per minute (thus the resting ventilation is about 6–8 L/min).

Spirometry is a test in which an individual inhales maximally and then exhales as rapidly as possible. The volume of air expired in one second, called the forced expiratory volume 1 second (FEV1), and the total amount expired, forced vital capacity (FVC), and the ratio of FEV1/FVC, are good measures of the recoil capacity and airway obstruction of the lung. In a healthy individual the FEV1/FVC = ~80%.

Perfusion—The lung receives the entire output from the right ventricle, ~75 mL of blood per heartbeat. Blood with high CO_2 and low O_2 travels to the lung via the pulmonary artery and leaves the lung with high O_2 and low CO_2 via the pulmonary vein. The bronchi also have independent circulation with O_2-enriched blood supplied by an artery. Substantial amounts of toxic chemicals carried in the blood can be delivered to the lung. A chemical placed onto or deposited under the skin (subcutaneous injection) or introduced directly into a peripheral vein (intravenous injection) travels through the venous system to the right ventricle and comes into contact with the lungs before distribution to other organs or tissues in the body.

Diffusion—Gas exchange takes place across the entire alveolar surface, meaning contact with an airborne toxic chemical occurs over an area of ~140 m^2. This surface area is second only to the small intestine (~250 m^2) and is considerably larger than the skin (~2 m^2). Oxygen normally diffuses, unhindered, across the pulmonary capillary and into erythrocytes. Acute events that can disrupt this process may include collection of liquid in the alveolar or interstitial space and disruption of the pulmonary surfactant system. Chronic toxicity can impair

diffusion due to abnormal alveolar architecture or abnormal formation and deposition of extracellular substances such as collagen in the interstitium.

BIOTRANSFORMATION IN THE RESPIRATORY TRACT

Often overlooked as an organ involved in metabolism of chemicals, in favor of the liver, the lung has substantial capabilities for biotransformation (see Chapter 6). Total lung cytochrome P450 (CYP) activity is roughly one-tenth to one-third of that in the liver. However, when specific activity in a few cell types is considered, the difference is only twofold for many enzymes, and in the case of nasal mucosa, higher enzyme activity is reported per cell. Metabolic competence in the lung and nasal tissues is concentrated in a few cell types and these have a defined, and sometimes limited, distribution in the respiratory tract that can vary substantially by species.

T e CYP monooxygenase system is concentrated into a few lung cells: BSCs, alveolar type II cells, macrophages, and endothelial cells. Of these cell types, BSCs have the most CYP, followed by the type II cells. T e total amount of total lung CYP contributed by BSCs is species-dependent, as are the CYP isoforms present and their location along the respiratory tract. Most species have CYPs in nasal tissue and some are predominantly expressed in the olfactory mucosa, which may play a role in providing or preventing access of inhalants directly to the brain.

Phase II enzymes include glutathione S-transferases (GSTs) (alpha, mu, and pi), glucuronosyl transferases, and sulfotransferases (SULTs). GSTs (and glutathione) play a major role in the modulation of both acute and chronic chemical toxicity in the lung. T ese enzyme systems work in concert with one another and it is the combined action of all these enzymes that determines toxicity. T e regulation of many of these enzymes is under coordinated control of the transcription factor nuclear factor, erythroid-derived 2, -like 2 (aka Nrf2).

A major determinant of the potential for detoxification may also be the cellular localization of, and the ability to synthesize, glutathione in the lung. T e distribution of GST isoforms varies by lung region and their activity is 5% to 15% of that in the rodent liver and about 30% of that in the human liver. Polymorphisms in glutathione transferase genes have been associated with a possible increase in risk of developing lung cancer, particularly in smokers.

GENERAL PRINCIPLES IN THE PATHOGENESIS OF LUNG DAMAGE CAUSED BY CHEMICALS

Toxic Inhalants, Gases, and Dosimetry

In inhalation toxicology, exposure is measured as a concentration (compound mass per unit of air). Typically highly toxic compounds can produce adverse effects in a concentration of mg/m^3 or $\mu g/m^3$. For reference, $1\,m^3$ is 1 000 L. For gases, concentration may also be expressed as volume to volume of air, that is, parts per million (ppm) or parts per billion (ppb). T is can be calculated from the mass per unit air by using the ideal gas law to determine the gas's volume. It is important to note that exposure does not equate to dose (compound mass per unit), which requires a measure of organ, cell, or subcellular target.

T e sites of deposition of gases in the respiratory tract define the pattern of toxicity of those gases. Solubility, diffusivity, and metabolism/reactivity in respiratory tissues and breathing rate are the critical factors in determining how deeply a given gas penetrates into the lung. Highly soluble gases such as SO_2 or formaldehyde do not penetrate farther than the nose (during nasal breathing) unless doses are very high, and are therefore relatively nontoxic to the lung of rats (which are obligatory nasal breathers). Relatively insoluble gases such as ozone and NO_2 penetrate deeply into the lung and reach the smallest airways and the alveoli (centriacinar region), where they can elicit toxic responses. Very insoluble gases such as CO and H_2S efficiently pass through the respiratory tract and are taken up by the pulmonary blood supply to be distributed throughout the body. Mathematical models of gas entry and deposition in the lung predict sites of lung lesions fairly accurately. T ese models may be useful for extrapolating findings made in laboratory animals to humans.

Regional Particle Deposition

Particle size is a critical factor in determining the region of the respiratory tract in which a particle will be deposited. In respiratory toxicology, aerosols (solid or liquid particles dispersed into air) include dusts, fumes, smoke, mists, fog, or smog (ranging from $\geq 1.0\,\mu m$ for dusts to ≥ 0.01–$50\,\mu m$ for smog). Smaller aerosols include submicrometer particles, nanometer particles or nanoparticles. All these distinguishing forms are included in the term "aerosol" or "particle."

T e upper respiratory tract is very efficient in removing particles that are very large ($> 10\,\mu m$) or very small ($< 0.01\,\mu m$) (Figure 15–1). During nasal breathing, 1 to $10\,\mu m$ particles are usually deposited in the upper nasopharyngeal region or the first five generations of large conducting airways. During oral breathing, deposition of these particles can increase in the tracheobronchial airways and alveolar region. Smaller particles (0.001–$0.1\,\mu m$) can also be deposited in the tracheobronchial region. Particles ranging from 0.003 to $5\,\mu m$ can be transported to the smaller airways and deposited in the alveolar region.

Patterns of breathing can change the site of deposition of a particle of a given size. T e size of a particle may change during inspiration before deposition in the respiratory tract. Materials that are hygroscopic (i.e., those that readily absorb moisture), such as sodium chloride, sulfuric acid, and glycerol, take on water and grow in size in the warm, saturated atmosphere of the upper and lower respiratory tract.

Deposition Mechanisms

In the respiratory tract, particles deposit by impaction, interception, sedimentation, diffusion, and electrostatic deposition (for positively charged particles only) (Figure 15–6). Impaction occurs in the upper respiratory tract and large proximal airways where the airf ow is faster than in the small distal airways because the cumulative diameter is smaller than in the proximal airways. In humans, most > 10 µm particles are deposited in the nose or oral pharynx and cannot penetrate tissues distal to the larynx. For 2.5 to 10 µm particles, impaction continues to be the mechanism of deposition in the first generations of the tracheobronchial region.

Interception occurs when the trajectory of a particle brings it near enough to a surface so that an edge of the particle contacts the airway surface. Although fiber diameter determines the probability of deposition by impaction and sedimentation, interception is dependent on fiber length. T us, a fiber with a diameter of 1 µm and a length of 200 µm will be deposited in the bronchial tree primarily by interception rather than impaction. Interception is also important for submicrometer particles in the tracheobronchial region where inertial airf ow directs a disproportionately large fraction of the f ow volume toward the surface of small airway bifurcations.

Sedimentation controls deposition in the smaller bronchi, the bronchioles, and the alveolar spaces, where the airways are small and the velocity of airf ow is low. Air resistance and buoyancy act on a particle in an upward direction while gravity acts on a particle in a downward direction. T ese forces eventually balance as a particle travels through air, causing the particle to settle. Sedimentation is not a significant route of particle deposition when the aerodynamic diameter is ≤ 0.5 µm. Sedimentation is dependent on the time a particle is in a compartment (i.e., an alveolus) and can be increased by breath holding.

Diffusion of a particle within the air is an important factor in the deposition of submicrometer particles. T e distance a particle travels within a gas depends upon the ratio of the particle's mass to the momentum of the colliding gas molecules such that large particles are hardly moved and nanoparticles are moved extensively. Diffusion is an important deposition mechanism in the nose, airways, and alveoli for particles ≤ 0.5 µm. Nanometer particles (0.1 µm and smaller) are also trapped relatively efficiently in the upper airways by diffusion. Particles that penetrate beyond the upper airways are available to be deposited in the bronchial region and the deep-lying airways.

Electrostatic deposition is a minor deposition mechanism for positively charged particles. T e surface of the airways is negatively charged and attracts positively charged particles. Freshly fractured mineral dust particles and laboratory-generated aerosols from evaporation of aqueous droplets can have substantial electrostatic mobilities.

During quiet breathing, in which the TV is only two to three times the volume of the anatomic dead space (i.e., the volume of the conducting airways where gas exchange does not occur), a large proportion of the inhaled particles may be exhaled. During exercise, when larger volumes are inhaled at higher velocities, impaction in the large airways and sedimentation and diffusion in the smaller airways and alveoli increase. Breath holding also increases deposition from sedimentation and diffusion. Cigarette smoke is hydroscopic aerosol of nicotine-laden particles that grow to a median diameter of about 0.5 to 1.0 µm. T us, a smoker's respiratory pause at the end of inhalation increases alveolar sedimentation and thereby nicotine delivery to the alveolar surface and to the blood upon absorption.

Factors that modify the diameter of the conducting airways can alter particle deposition. In patients with chronic bronchitis or pneumonia, the airway lining f uid can greatly thicken and may partially block the airways in some areas. Sonic jets (e.g., during wheezing and rales) formed by high air f owing through such partially occluded airways have the potential to increase the deposition of particles by impaction and diffusion in the small airways. Irritant materials that produce bronchoconstriction tend to increase the proximal tracheobronchial deposition of particles.

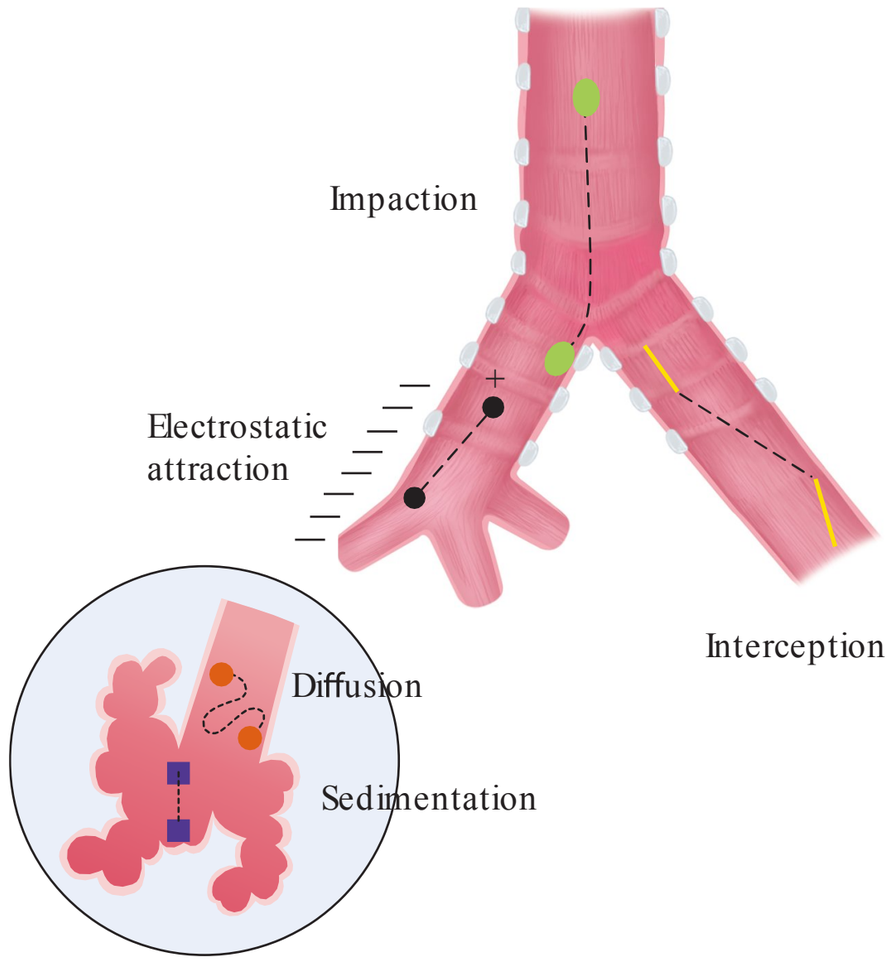


FIGURE 15–6 **Mechanism of particle deposition in the respiratory tract.** Impaction occurs in the upper respiratory tract and large proximal airways where fast airflow imparts momentum to the inhaled particle. The particle's inertia causes it to continue to travel along its original path and deposit on the airway surface. Interception occurs when the trajectory of a particle brings it near enough to a surface so that an edge of the particle contacts the airway surface. Sedimentation controls deposition in the smaller bronchi, the bronchioles, and the alveolar spaces, where the airways are small and the velocity of airflow is low. Sedimentation is dependent on the time a particle is in a compartment (i.e., an alveolus) and can be increased by breath holding. Dif usion is an important factor in the deposition of submicrometer particles. Electrostatic deposition is a minor deposition mechanism for positively charged particles. The surface of the airways is negatively charged and attracts positively charged particles. (Adapted with permission from Lippmann M (ed): Environmental toxicants human exposures and their health ef ects. 3rd edition. New York, NY: Wiley; 2009.)

Particle Clearance

Lung defense is dependent on particle clearance, wherein rapid removal lessens the time available to cause damage to the pulmonary tissues or permit local absorption. However, it is important to remember that particle clearance is not equivalent to clearance from the body.

Nasal Clearance—Particles deposited in the anterior portion of the nose are removed by extrinsic actions such as wiping and blowing. Particles deposited in the posterior portion of the nose are removed by mucociliary clearance that propels mucus toward the glottis, after which the particles are swallowed. Soluble particles may dissolve and enter the epithelium and/or blood before they can be mechanically removed.

Tracheobronchial Clearance—Particles deposited in the tracheobronchial tree are also removed by mucociliary clearance. In addition to deposited particles, particle-laden macrophages are also moved upward to the oropharynx, where they are swallowed.

Alveolar Clearance—Particles deposited in the alveolar region are removed by specialized cells, the alveolar macrophage. Lung defense involve both the innate and adaptive and immune systems (see Chapter 12). Macrophages are the primary effector of innate lung immunity and their ability to accomplish phagocytosis depends on the recognition of foreign or damage cells by a variety of macrophage surface macromolecules and receptors. Phagocytosis requires (1) particle binding to the membrane specifically via recognition molecule–receptor interactions or nonspecifically by electrostatic forces (inert materials), (2) receptor activation that initiates cell signaling, (3) actin polymerization and coordinated cytoskeletal movements that leads to extension of membranes, and (4) vesicular membrane closure closely apposed to the particle or the fiber ingested forming a phagosome shaped by the material ingested.

ACUTE RESPONSES OF THE LUNG TO INJURY

Trigeminally Mediated Airway Reflexes

Certain gases and vapors stimulate nerve endings in the nose, particularly those of the trigeminal nerve. The result is holding of the breath or changes in breathing patterns, to avoid or reduce further exposure. Transient receptor potential channel receptors may be activated by many irritants causing tickling, itching, and painful nasal sensations. Subfamily A receptors are activated by several irritants including acrolein, allyl isothiocyanate (wabasi), allicin (garlic), cinamaldehyde, chlorine, ozone, and hydrogen peroxide.

If continued exposure cannot be avoided, many acidic or alkaline irritants produce cell necrosis and increased permeability of the alveolar walls. Other inhaled agents can be more insidious; inhalation of high concentrations of HCl, NO_2, NH_3, or phosgene may at first produce very little apparent damage in the respiratory tract. The epithelial barrier in the alveolar zone, after a latency period of several hours, begins to leak, flooding the alveoli and producing a delayed pulmonary edema that is often fatal.

A different pathogenetic mechanism is typical of highly reactive molecules such as ozone. It is unlikely that ozone as such can penetrate beyond the layer of fluid covering the cells of the lung. Instead, ozone lesions are propagated by a cascade of secondary reaction products and by reactive oxygen species arising from free radical reactions.

Bronchoconstriction, Airway Hyperreactivity, and Neurogenic Inflammation

Large diameter airways are surrounded by bronchial smooth muscles, which help maintain airway tone and diameter during expansion and contraction of the lung. Bronchial smooth muscle tone is normally regulated by the autonomic nervous system. Bronchoconstriction can be provoked by irritants (acrolein), cigarette smoke, air pollutants, cholinomimetic drugs (acetylcholine), histamine, various prostaglandins and leukotrienes, substance P, and nitric oxide. Bronchoconstriction causes a decrease in airway diameter and a corresponding increase in resistance to airflow. Characteristic symptoms include wheezing, coughing, a sensation of chest tightness, and dyspnea. Exercise potentiates these problems. Because the major component of airway resistance usually is contributed by large bronchi, inhaled chemicals that cause reflex bronchoconstriction are generally irritant gases with moderate solubility.

Acute Lung Injury (Pulmonary Edema)

Acute lung injury (adult or infant respiratory distress syndrome) is marked by alveolar epithelial and endothelial cell perturbation and inflammatory cell influx that leads to surfactant disruption, pulmonary edema, and atelectasis. Toxic pulmonary edema represents an acute, exudative phase of lung injury that alters ventilation–perfusion relationships and limits diffusive transfer of O_2 and CO_2 even in otherwise structurally normal alveoli. Acrolein, HCl, NO_2, NH_3, or phosgene may compromise alveolar barrier function several hours after exposure to low concentrations, and immediate alveolar damage and death with high concentrations.

CHRONIC RESPONSES OF THE LUNG TO INJURY

Chronic Obstructive Pulmonary Disease

Characterized by a progressive airflow obstruction, chronic obstructive pulmonary disease involves airway (bronchitis) and alveolar pathology. Chronic bronchitis is defined by the presence of sputum production and cough for at least three months. In emphysema, destruction of the gas-exchanging surface area results in a distended, hyperinflated lung that no

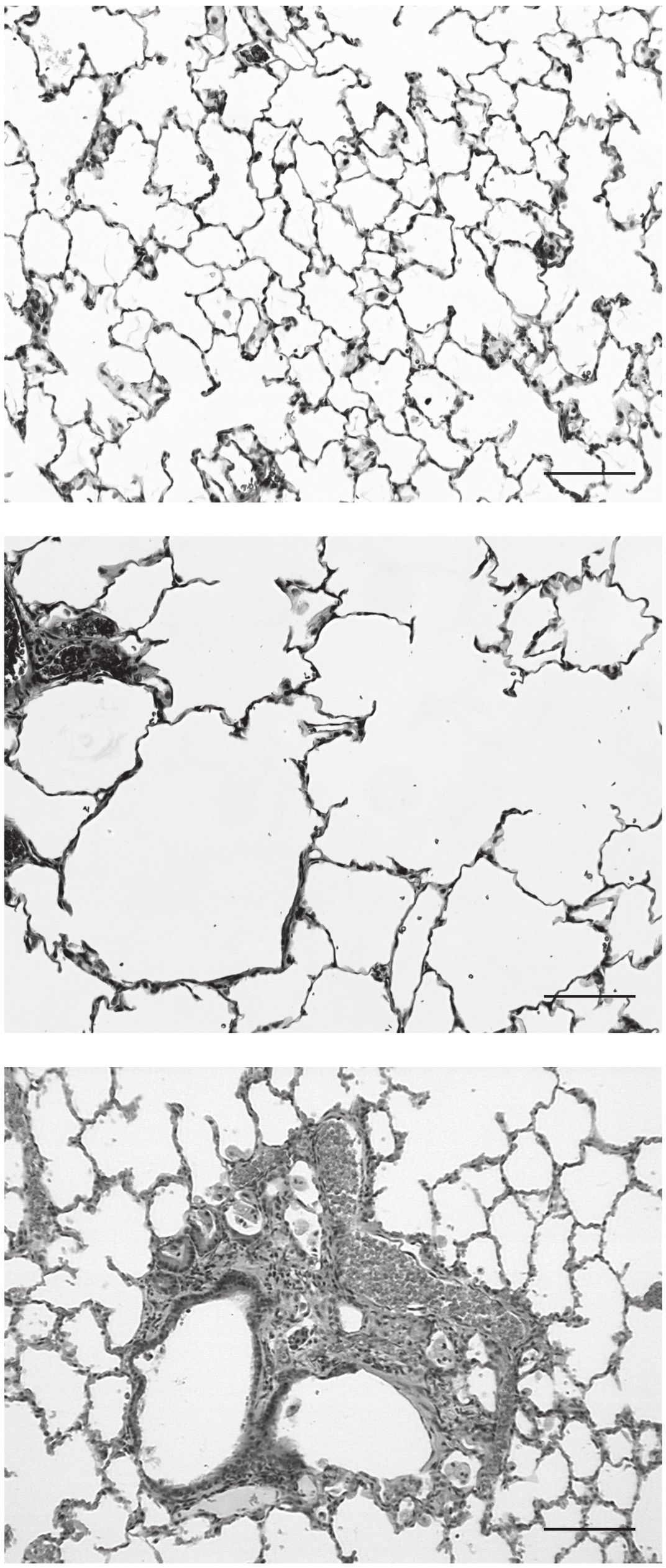


FIGURE 15–7 **Airspace enlargement induced by tobacco smoke and pulmonary fibrosis induced by asbestos in rat lung.** Top panel: Normal rat lung. Middle panel: Extensive distention of the alveoli (emphysema) in rat lung following inhalation of tobacco smoke (90 mg/m^3 of total suspended particulate material). Bottom panel: Lung of a rat one year after exposure to chrysotile asbestos. Note accumulation of connective tissue around blood vessel and airways (fibrosis). Bar length: 100 µm. (Used with permission of Dr Kent E. Pinkerton, University of California, Davis.)

longer effectively exchanges oxygen and carbon dioxide as a result of both loss of tissue and air trapping (Figure 15–7). The major cause of human emphysema is, by far, cigarette smoke inhalation, although other toxicants also can elicit this response. A feature of toxicant-induced emphysema is severe or recurrent inflammation.

The pathogenesis of emphysema involves a proteinase–antiprotease imbalance that leads to the remodeling of the supportive connective tissue in the parenchyma and separate lesions that coalesce to destroy lung tissue. Alpha$_1$-antiprotease (also called alpha$_1$-antitrypsin) is one of the body's main defenses against uncontrolled proteolytic digestion by this class of elastolytic enzymes, which includes elastase. Studies in smokers led to the hypothesis that neutrophil (and perhaps alveolar macrophage) elastases can break down lung elastin and thus cause emphysema; these elastases usually are kept in check by alpha$_1$-antiprotease that diffuses into the lung from the blood. As the individual ages, an accumulation of random elastolytic events can cause the emphysematous changes in the lungs that are normally associated with aging. Toxicants that cause inflammatory cell influx and thus increase the burden of neutrophil elastase can accelerate this process.

Lung Cancer

Lung cancer is now the leading cause of death from cancer among men and women. Retrospective and prospective epidemiologic studies unequivocally show an association between tobacco smoking and lung cancer. Average smokers have a 10-fold and heavy smokers a 20-fold increased risk of developing lung cancer compared with nonsmokers. Many other agents also cause lung cancer (see Table 15–1).

Human lung cancers may have a latency period of 20 to 40 years, making the relationship to specific exposures difficult to establish. Two major forms are non-small-cell lung cancer, which accounts for about 85% of all lung cancers, and may be characterized as squamous cell carcinoma, adenocarcinoma, and large-cell lung cancer. Small-cell lung cancers account for about 15% of lung cancers. Compared with cancer in the lung, cancer in the upper respiratory tract is less common.

The potential mechanisms of lung carcinogenesis center on damage to DNA. An activated carcinogen or its metabolic product may interact with DNA. DNA damage caused by active oxygen species is another potentially important mechanism. Ionizing radiation leads to the formation of superoxide. Cigarette smoke contains high quantities of active oxygen species and other free radicals. Critical genetic and epigenetic changes include DNA mutations, loss of heterozygosity, and promoter methylation. Global transcriptome changes can include stimulation of mitogenic pathways and suppression of apoptosis.

Asthma

Asthma is characterized clinically by attacks of shortness of breath, which is caused by narrowing of the large conducting

TABLE 15–1 Agents that produce lung injury and disease.

Toxicant	Disease	Exposure	Acute Effect	Chronic Effect
Acrolein	Acute lung injury, chronic obstructive pulmonary disease	Biomass or hot oil cooking, fire fighters, environmental tobacco smoke, biocide water treatment	Cough, shortness of breath, extreme oronasal irritation, pulmonary edema, airway hyperreactivity	Chronic obstructive pulmonary disease, possibly asthma or lung cancer
Aluminum abrasives	Shaver disease, corundum smelter's lung, bauxite lung	Abrasives manufacturing, smelting	Alveolar edema	Interstitial fibrosis, emphysema
Aluminum dust	Aluminosis	Aluminum, firework, ceramic, paint, electrical good, and abrasive manufacturing	Cough, shortness of breath	Interstitial fibrosis
Ammonia		Farming, refrigeration operations, ammonia, fertilizer, chemical, and explosive manufacturing	Oronasal and bronchial irritation, pulmonary edema	Acute lung injury, chronic bronchitis
Arsenic		Pesticide, pigment, glass, and alloy manufacturing	Bronchitis	Laryngitis, bronchitis, and lung cancer
Asbestos	Asbestosis	Mining, construction, shipbuilding, brake repair, vermiculite contaminant		Fibrosis, pleural calcification, lung cancer, mesothelioma
Aspergillus	Framer lung, composte lung, malt worker's lung	Working with moldy hay, compost, or barley	Bronchoconstriction, cough, chest tightness	Extrinsic allergic alveolitis (hypersensitivity pneumonitis)
Avian protein	Bird fancier's lung	Bird handling and farming with exposure to bird droppings	Bronchoconstriction, cough, chest tightness	Extrinsic allergic alveolitis (hypersensitivity pneumonitis)
Beryllium	Berylliosis	Mining, alloy, and ceramic manufacturing, Milling beryllium	Pulmonary edema, pneumonia	Interstitial granulomatosis, progressive dyspnea, cor pulmonarle, fibrosis, and lung cancer
Cadmium		Welding, smelting, and electrical equipment, battery, alloy, and pigment manufacturing	Cough, pneumonia	Emphysema, cor pulmonale
Carbides of tungsten, titanium, or tantalum	Hard metal disease	Metal cutting and manufacturing	Bronchial epithelial hyper- and metaplasia	Peribronchial and perivascular fibrosis
Chlorine		Paper, plastics, chlorinated product manufacturing	Cough, hemoptysis, dyspnea, bronchitis, pneumonia	
Chromium (VI)		Chromium compound, paint, pigment, chromite ore reduction manufacturing	Oronasal and bronchial irritation	Fibrosis, lung cancer
Coal dust	Coal worker's pneumoconiosis	Coal mining		Fibrosis with emphysema
Cotton dust	Byssinosis	Textile manufacturing	Chest tightness, wheezing, dyspnea	Restrictive lung disease, chronic bronchitis
Hydrogen fluoride		Chemical, photograph film, solvent and plastic manufacturing	Airway irritation, hemorrhagic pulmonary edema	

TABLE 15–1 Agents that produce lung injury and disease. (Continued)

Toxicant	Disease	Exposure	Acute Effect	Chronic Effect
Iron oxides	Siderotic lung disease, silver finisher's lung, hematite miner's lung, arc welder's lung	Welding, steel and jewelry manufacturing, foundry work, hematite mining	Cough	Silver finisher's lung with subpleural and perivascular macrophage aggregates; hematite miner's lung with diffuse fibrosis-like pneumoconiosis; arc welder's lung with bronchitis
Isocyanates		Auto painting, and plastic and chemical manufacturing	Airway irritation, cough, dyspnea	Asthma
Kaolin	Kaolinosis	Pottery making		Fibrosis
Manganese	Manganese pneumonia	Chemical and metal manufacturing	Acute pneumonia (often fatal)	Recurrent pneumonia
Nickel		Nickel mining, smelting, electroplating, battery manufacturing, fossil fuel combustion	Delayed pulmonary edema, skin allergy	Acute lung injury, chronic bronchitis, non- small-cell lung cancer, nasal cancer
Nitrogen oxides	Silo-filler's diseases	Silo filling, welding, explosive manufacturing	Immediate or delayed pulmonary edema	Bronchiolitis obliterans, emphysema in experimental animals
Nontuberculous mycobacteria	Metalworking fluid hypersensitivity	Working with metal cutting fluid contain water and contaminated with mycobacteria	Bronchoconstriction, cough, chest tightness	Extrinsic allergic alveolitis (hypersensitivity pneumonitis)
Organic (sugar cane) dust (possibly contaminated with thermophilic actinomycete)	Bagassosis	Sugarcane and molasses manufacturing (bagasse is the fibrosis residue from sugar extraction)	Bronchoconstriction, cough, chest tightness	Extrinsic allergic alveolitis (hypersensitivity pneumonitis)
Ozone		Welding, photocopying, bleaching flour, water treatment, deordorizing	Substernal pain, exacerbation of asthma, bronchitis, pulmonary edema	Fibrosis (including airways)
Perchloroethylene		Dry cleaning, metal degreasing, grain fumigation	Edema	Hepatic and lung cancer
Phosgene		Plastic, pesticide, and chemical manufacturing	Severe pulmonary edema	Bronchitis and fibrosis
Silica	Silicosis, pneumoconiosis	Mining, stone cutting, sand blasting, farming, quarry mining, tunneling	Acute silicosis (inflammation)	Fibrosis, silicotuberculosis
Sulfur dioxide		Chemical manufacturing, refrigeration, bleaching, fumigation	Bronchoconstriction, cough, chest tightness	Chronic bronchitis
Talc	Talcosis	Mining, rubber manufacturing, cosmetics	Cough	Fibosis
Thermophilic actinomycete	Farmer's lung, mushroom worker's lung, penguin humidifier lung	Farming (hay or grain degradation)	Bronchoconstriction, cough, chest tightness	Extrinsic allergic alveolitis (hypersensitivity pneumonitis)
Tin		Mining, tin processing		Widespread mottling in chest X-ray often without clinical impairment
Vanadium		Metal cutting and manufacturing, specialty steel manufacturing	Airway irritation and mucus production	Chronic bronchitis

airways (bronchi). T e clinical hallmark of asthma is increased airway reactivity of the bronchial smooth muscle in response to exposure to irritants. T ere may be common mechanisms between asthma and pulmonary fibrosis, with regard to the role of recurrent or chronic inf ammation in disease pathogenesis. Agents that can induce asthma are listed in Table 15–1.

Pulmonary Fibrosis

Fibrotic lungs from humans with acute or chronic pulmonary fibrosis contain increased amounts of collagen. In lungs damaged by toxicants, the response resembles adult or infant respiratory distress syndrome. Excess lung collagen is usually observed not only in the alveolar interstitium, but also throughout the alveolar ducts and respiratory bronchioles (Figure 15–7).

Types I and III collagen are major interstitial components and are found in an approximate ratio of 2:1. T ere is an increase in type I collagen relative to type III collagen in patients with idiopathic pulmonary fibrosis and patients dying of acute respiratory distress syndrome. It is not known whether shifts in collagen types, compared with absolute increases in collagen content, account for the increased stiffness of fibrotic lungs. Because type III collagen is more compliant than type I, increasing type I relative to type III collagen may result in a stiffer lung. Changes in collagen cross-linking in fibrotic lungs also may contribute to the increased stiffness.

AGENTS KNOWN TO PRODUCE LUNG INJURY IN HUMANS

T ere are over 7 900 unique chemicals that are commonly used in industry, many of which represent hazards to the respiratory tract. Exposure prevention is one of the most effective approaches to prevent lung injury and disease, and many values and exposure limits exist to aid prevention. Nonetheless, given the large morbidity and mortality associated with current acute and chronic lung disease, a great need exists to develop additional preventative and therapeutic strategies based on the knowledge of the cellular and molecular events that determine lung injury and repair. Table 15–1 lists a portion of the respiratory toxicants that can produce acute and chronic lung injury in humans.

EVALUATION OF TOXIC LUNG DAMAGE

Human Studies

Although the lung is susceptible to multiple toxic injuries, it is also amenable to a number of tests that allow evaluation of proper functioning. Commonly used tests include measurement of FEV1, FVC, and airway resistance. Additional tests evaluate the maximal f ow rates and different lung volumes, diffusion capacity, oxygen, and carbon dioxide content of the arterial and venous blood, distribution of ventilation, and lung and chest wall compliance.

Diffusion defects (i.e., defects in gas exchange across the pulmonary capillary) can be evaluated by measuring the arterial partial pressure of both oxygen and CO_2. In general, blood gas analysis is a comparatively insensitive assay for disturbed ventilation because of the organisms' buffering and reserve capacities, but may be a useful tool in clinical medicine. Measurement of diffusion capacity with CO, a gas that binds with 250 times higher affinity to hemoglobin than does oxygen, is more sensitive. Proper lung function in humans can be evaluated with several additional techniques, including computed tomography (CT), molecular content analysis, fiberoptic bronchoscopy.

Animal Studies

T e toxicology of inhaled materials has been and continues to be extensively studied in experimental animals. Obviously, selecting animals with a respiratory system similar to that of humans is particularly desirable (e.g., monkey). However, rodents are widely used despite fundamental differences to combat cost and ethical considerations.

Inhalation Exposure Systems—In inhalation studies, animals are kept within a chamber that is ventilated with a defined test atmosphere. Generation of such an atmosphere is comparatively easy for gases that are available in high purity in a compressed tank (e.g., SO_2, O_2, NO_2). Gas concentration within the chamber is measured continuously, and is usually within 5% of the targeted concentration. More challenging is the generation of particles or complex mixtures (e.g., tobacco smoke, diesel, and gasoline exhaust or residual oil f y ash), particularly because of the possibility of interactions between individual mixture constituents and the possibility of formation of artifacts.

Pulmonary Function Tests in Experimental Animals—Conducting pulmonary function tests in experimental animals poses distinct challenges, especially in small rodents. Experimental animals cannot be made to maximally inhale or exhale at the investigator's will, for instance. Analysis of pressure–volume curves, which provides an indication of lung compliance, is comparatively easy to perform in animals in that it does not require a specialized apparatus. Another pulmonary function test is the analysis of airway resistance, which can be measured via restrained plethysmography, unrestrained video-assisted plethysmography, or unrestrained acoustic plethysmography. Analysis of breathing pattern can also be used and may differentiate between upper airway and lower airway irritants. In rodents, upper airway ("sensory") irritants produce a breathing pattern of decreased respiratory frequency with increased tidal volume, whereas lower airway ("pulmonary") irritants produce a breathing pattern of increased respiratory frequency and decreased minute volume (i.e., the total volume of air breathed in 1 minute).

Morphological Techniques—The pathology of acute and chronic injury may be examined by gross inspection and under the microscope and should include the nasal passages, larynx, major bronchi, and the lung parenchyma.

Regional distribution of lesions in nasal passages can be assessed after fixation and decalcification. Various regions of the nasal passages can then be examined by obtaining cross sections at multiple levels, staining the tissue to highlight particular structures, and examining the tissue under a microscope. This permits semiquantitative or quantitative measurements to be made.

Additional tools for the study of toxic lung injury include immunohistochemistry, in situ hybridization, and analysis of cell kinetics. Transcriptome, proteome, and metabolome profiling are additional valuable tools to assess the lung in health and disease.

Pulmonary Lavage and Pulmonary Edema—Pulmonary edema and/or pulmonary inflammation are early events in acute and chronic lung injury. The fluid lining the pulmonary epithelium can be recovered by the medical procedure, bronchoalveolar lavage. Analysis of the lavage fluid is a useful tool to detect respiratory tract toxicity. Influx of neutrophils or other leukocytes such as lymphocytes or eosinophils into the lavage fluid is the most sensitive sign of inflammation. Measurements of lung injury include total protein and/or albumin. Additional measurements include secretory products of macrophages and epithelial cells include fibronectin, chemokines, and other cytokines (e.g., TNF or IL1B). Reduced glutathione levels may be an indicator of oxidative stress. Lactate dehydrogenase activity (and its substituent isozymes), N-acetylglucosaminidase, acid or alkaline phosphatase, other lysosomal hydrolases, and sialic acid add additional information. In addition pulmonary edema can be assessed by determining lung wet:dry ratio or injection of Evan blue dye albumin.

In Vitro Studies

In vitro systems with materials originally obtained from either human tissues or experimental animals are particularly suited for the study of mechanisms that cause lung injury. The methods include isolated perfused lung, microdissection/organotypic tissue culture systems, and cell type–specific cell culture.

Isolated Perfused Lung—The isolated perfused lung method is applicable to lungs from many laboratory species (e.g., mouse, rat, guinea pig, or rabbit). The lung is perfused with blood or a blood substitute through the pulmonary arterial bed. At the same time, the lung is actively (through rhythmic inflation–deflation cycles with positive pressure) or passively (by creating negative pressure with an artificial thorax in which the lung is suspended) ventilated. Toxic agents can be introduced into the perfusate or the inspired air. Repeated sampling of the perfusate allows one to determine the rate of metabolism of drugs and the metabolic activity of the lung.

Airway Microdissection and Organotypic Tissue Culture Systems—Many inhalants act in specific regions of the respiratory tract. Microdissection of the nasal passage and airways consists of stripping away surrounding tissue or parenchyma while maintaining the airway structure and exposing the epithelium. Microdissected airways can be studied in culture for up to one week, can be used to study site-specific gene expression, morphological changes in toxicant injury and repair, or can be used for biochemical analyses including enzyme activity measurements and determination of antioxidant concentrations (such as glutathione).

Tissue culture systems have been developed in which epithelial cells maintain their polarity, differentiation, and normal function similar to what is observed in vivo. Epithelial cell surfaces are exposed to air (or a gas phase containing an airborne toxic agent), while the basal portion is bathed by a tissue culture medium.

Lung Cell Culture—Many lung-specific cell types have been isolated and can be maintained as cell culture. Human and animal alveolar or interstitial macrophages can be obtained from lavage or lung tissue. Their function can be examined in vitro with or without exposure to appropriate toxic stimuli. Type II alveolar epithelial cells can be isolated and primary cell cultures maintained in culture for short periods. Direct isolation of type I epithelial cells has also been successful.

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QUESTIONS

1. Which of the following statements is FALSE regarding the role of mucus in the conducting airways?
   a. Pollutants trapped by mucus can be eliminated via expectoration or swallowing.
   b. Mucus is of a basic pH.
   c. T e beating of cilia propels mucus out of the lungs.
   d. Mucus plays a role promoting oxidative stress.
   e. Free radical scavenging is believed to be a role of mucus.

2. Respiratory distress syndrome sometimes affects premature neonates due to lack of surfactant production by which of the following cell types?
   a. lung fibroblasts.
   b. type II pneumocytes.
   c. endothelial cells.
   d. alveolar macrophages.
   e. type I pneumocytes.

3. In a situation where there is an increased metabolic demand for oxygen, which of the following volume measurements will greatly increase?
   a. total lung capacity (TLC).
   b. residual volume (RV).
   c. functional residual capacity (FRC).
   d. tidal volume (TV).
   e. vital capacity (VC).

4. T e free radicals that inf ict oxidative damage on the lungs are generated by all of the following EXCEPT:
   a. tobacco smoke.
   b. neutrophils.
   c. ozone.
   d. monocytes.
   e. SO_2.

5. Which of the following gases would most likely pass all the way through the respiratory tract and diffuse into the pulmonary blood supply?
   a. O_3 (ozone).
   b. NO_2.
   c. H_2O.
   d. CO.
   e. SO_2.

6. All of the following statements regarding particle deposition and clearance are true EXCEPT:
   a. One of the main modes of particle clearance is via mucociliary escalation.
   b. Diffusion is important in the deposition of particles in the bronchial regions.
   c. Larger volumes of inspired air increase particle deposition in the airways.
   d. Sedimentation results in deposition in the bronchioles.
   e. Swallowing is an important mechanism of particle clearance.

7. Which of the following is not a common location to which particles are cleared?
   a. stomach.
   b. lymph nodes.
   c. pulmonary vasculature.
   d. liver.
   e. GI tract.

8. Pulmonary fibrosis is marked by which of the following?
   a. increased type I collagen.
   b. decreased type III collagen.
   c. increased compliance.
   d. elastase activation.
   e. decreased overall collagen levels.

9. Activation of what enzyme(s) is responsible for emphysema?
   a. antitrypsin.
   b. epoxide hydrolase.
   c. elastase.
   d. hyaluronidase.
   e. nonspecific proteases.

10. Which of the following measurements would NOT be expected from a patient with restrictive lung disease?
    a. decreased FRC.
    b. decreased RV.
    c. increased VC.
    d. decreased FEV_1.
    e. impaired ventilation.

CHAPTER

16

Toxic Responses of the Nervous System

Virginia C. Moser, Michael Aschner, Rudy J. Richardson, and Martin A. Philbert

OVERVIEW OF THE NERVOUS SYSTEM

- Blood–Brain Barrier
- Energy Requirements
- Axonal Transport
- Axonal Degeneration
- Myelin Formation and Maintenance
- Neurotransmission
- Development of the Nervous System
- Factors Relevant to Neurodegenerative Diseases

FUNCTIONAL MANIFESTATIONS OF NEUROTOXICITY

MECHANISMS OF NEUROTOXICITY

- Neuronopathies
  - Doxorubicin
  - Methyl Mercury
  - Trimethyltin
- Axonopathies
  - Gamma-diketones
  - Carbon Disulfide
  - β,β′-Iminodipropionitrile (IDPN)
  - Acrylamide
  - Organophosphorus Compounds
  - Pyridinethione
  - Microtubule-associated Neurotoxicity
- Myelinopathies
  - Hexachlorophene
  - Tellurium
  - Lead
- Astrocytes
  - Ammonia
  - Nitrochemicals
  - Methionine Sulfoximine
  - Fluoroacetate and Fluorocitrate
- Neurotransmission-associated Neurotoxicity
  - Nicotine
  - Cocaine and Amphetamines
  - Excitatory Amino Acids
- Models of Neurodegenerative Disease
  - MPTP
  - Manganese
- Developmentally Neurotoxic Chemicals

CHEMICALS THAT INDUCE DEPRESSION OF NERVOUS SYSTEM FUNCTION

IN VITRO AND OTHER ALTERNATIVE APPROACHES TO NEUROTOXICOLOGY

KEY POINTS

- The central nervous system (CNS) is protected from the adverse effects of many potential toxicants by an anatomical blood–brain barrier.
- Neurons are highly dependent on aerobic metabolism because this energy is needed to maintain proper ion gradients.
- Individual neurotoxic compounds typically target the neuron, the axon, the myelinating cell, or the neurotransmitter system.
- Neuronopathy is the toxicant-induced irreversible loss of neurons, including its cytoplasmic extensions, dendrites, and axons, and the myelin ensheathing the axon.
- Neurotoxicants that cause axonopathies cause axonal degeneration, and loss of the myelin surrounding that axon; however, the neuron cell body remains intact.
- Numerous naturally occurring toxins as well as synthetic chemicals may interrupt the transmission of impulses, block or accentuate transsynaptic communication, block reuptake of neurotransmitters, or interfere with second-messenger systems.

OVERVIEW OF THE NERVOUS SYSTEM

Several generalities that allow a basic understanding of the actions of neurotoxicants include (1) the privileged status of the nervous system (NS) with the maintenance of a biochemical barrier between the brain and the blood; (2) the importance of the high energy requirements of the brain; (3) the spatial extensions of the NS as long cellular processes and the requirements of cells with such a complex geometry; (4) the maintenance of an environment rich in lipids; (5) the transmission of information across extracellular space at the synapse; (6) the distances over which electrical impulses must be transmitted, coordinated, and integrated; and (7) development and regenerative patterns of the NS.

Blood–Brain Barrier

The NS is protected from the adverse effects of many potential toxicants by an anatomical barrier between the blood and the brain, or a "blood–brain barrier" (BBB). Most of the brain, spinal cord, retina, and peripheral NS (PNS) maintain this barrier with the blood, with selectivity similar to the interface between cells and the extracellular space. To gain entry to the NS, molecules must pass into the cell membranes of endothelial cells of the brain rather than between endothelial cells, as they do in other tissues (Figure 16–1). The principal basis of the blood–brain barrier is thought to be specialized endothelial cells in the brain's microvasculature, aided, at least in part, by interactions with glia. In addition to this interface with blood, the

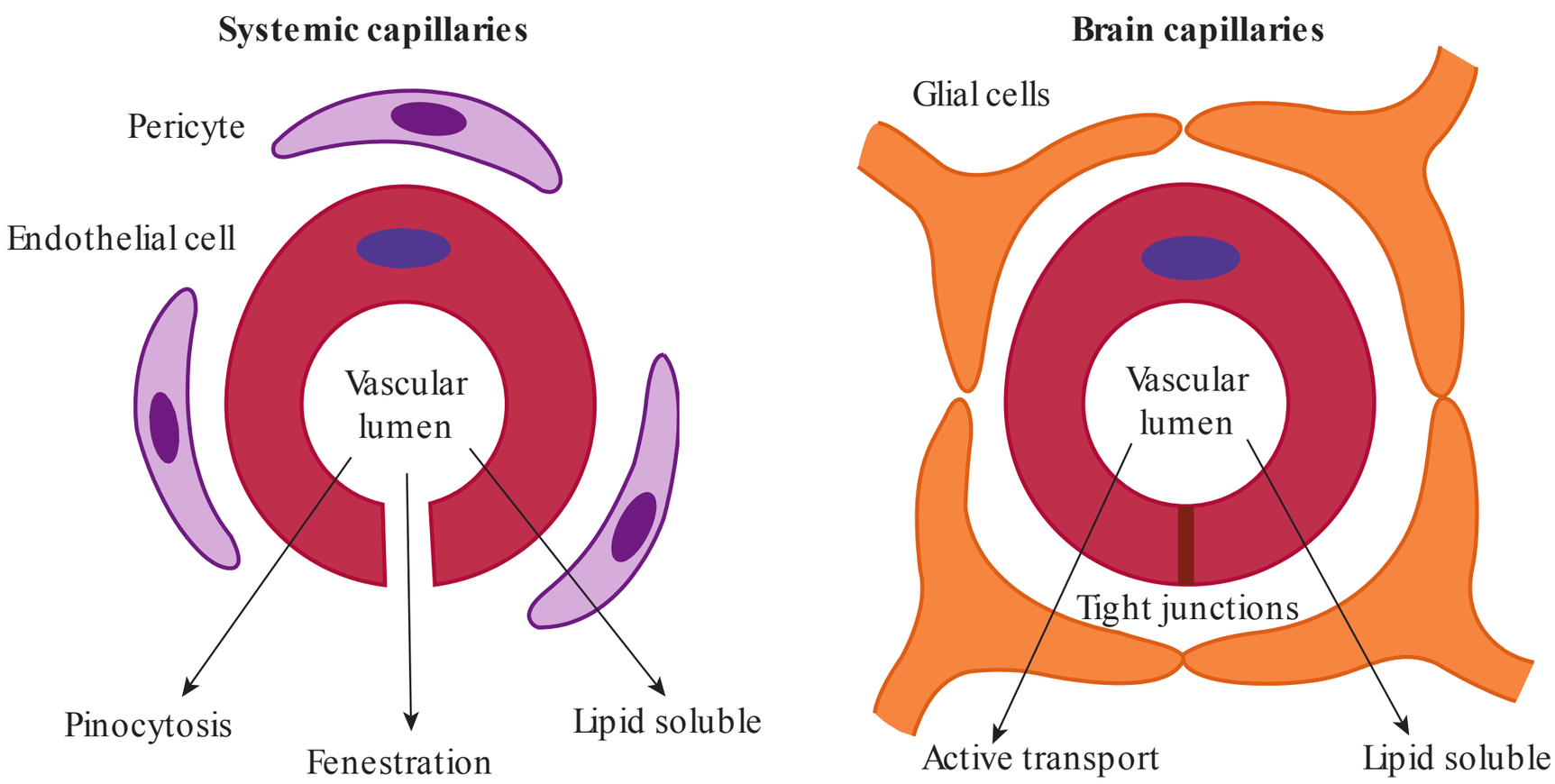

FIGURE 16–1 **Schematic diagram of the blood–brain barrier.** Systemic capillaries are depicted with intercellular gaps, or fenestrations, which permit the passage of molecules incapable of crossing the endothelial cell. There is also more abundant pinocytosis in systemic capillaries, in addition to the transcellular passage of lipid-soluble compounds. In brain capillaries, tight junctions between endothelial cells and the lack of pinocytosis limit transport to compounds with active transport mechanisms or those that pass through cellular membranes by virtue of their lipid solubility.

brain, spinal cord, and peripheral nerves are also completely covered with a continuous lining of specialized cells that limits the entry of molecules from adjacent tissue. In the brain and spinal cord, this is the meningeal surface; in peripheral nerves, each fascicle of nerve is surrounded by perineurial cells. Among the unique properties of endothelial cells in the NS is the presence of tight junctions between cells. T us, molecules must pass through membranes of endothelial cells, rather than between them, as they do in other tissues.

T e blood–brain barrier also contains xenobiotic transporters that transport some xenobiotics that have diffused through endothelial cells back into the blood. If not actively transported into the brain, the penetration of toxicants or their metabolites is largely related to their lipid solubility and to their ability to pass through the plasma membranes of cells forming the barrier. However, spinal ganglia, autonomic ganglia, and a small number of other sites within the brain are not protected by blood–tissue barriers. T is discontinuity of the barrier is the basis for the selective neurotoxicity of some compounds. T e blood–brain barrier is incompletely developed at birth and even less so in premature infants. T is predisposes the premature infant to brain injury by toxicants that are excluded from the NS later in life.

Energy Requirements

Neurons (and cardiac myocytes) are highly dependent on aerobic metabolism because they must use this energy to maintain proper ion gradients. T e brain is extremely sensitive to even brief interruptions in the supply of oxygen or glucose. Exposure to toxicants that inhibit aerobic respiration (e.g., cyanide) or to conditions that produce hypoxia (e.g., CO poisoning) leads to early signs of neuronal dysfunction. Damage to the NS under these conditions is a combination of direct toxic effects on neurons and secondary damage from systemic hypoxia or ischemia.

Axonal Transport

Impulses are conducted over great distances at rapid speed, providing information about the environment to the organism in a coordinated manner that allows an organized response to be carried out at a specific site. However, the intricate organization of such a complex network places an unparalleled demand on the cells of the NS. Single cells, rather than being spherical and a few micrometers in diameter, are elongated and may extend over 1 m in length. Two immediate demands placed on the neuron are the maintenance of a much larger cellular volume, requiring more protein synthesis, and the transport of intracellular materials over great distances using various mechanisms. T ese demands require ATP.

Axonal transport moves protein products from the cell body to the appropriate site in the axon. Fast axonal transport carries a large number of proteins from their site of synthesis in the cell body into the axon. Many proteins associated with vesicles migrate through the axon at a rate of 400 mm/day (Figure 16–2). T is process is dependent on microtubule-associated ATPase activity and the microtubule-associated motor proteins (kinesin and dynein) that provide both the mechanochemical force in the form of a microtubule-associated ATPase and the interface between microtubules as the track and vesicles as the cargo. Vesicles are transported rapidly in an anterograde direction by kinesin, and they are transported in a retrograde direction by dynein. T is mechanism of cytoplasmic transport is amplified within the NS, compared with other cells, by the distances encompassed by the axonal extensions of neurons.

T e transport of some organelles, including mitochondria, constitutes an intermediate component of axonal transport, moving at 50 mm/day. T e slowest component of axonal transport represents the movement of the cytoskeleton itself (Figure 16–2). T e cytoskeleton is composed of microtubules formed by the association of tubulin subunits and

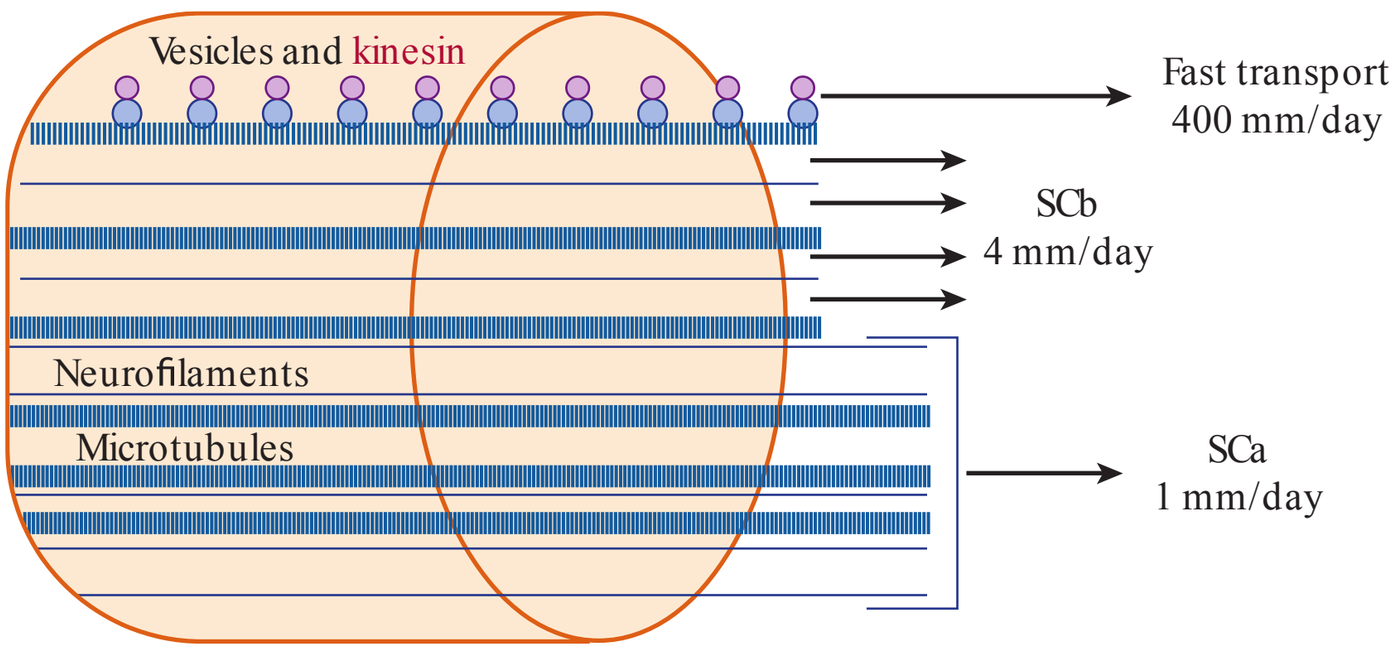

FIGURE 16–2 **Schematic diagram of axonal transport.** Fast axonal transport is depicted as spherical vesicles moving along microtubules with intervening microtubule-associated motors. The slow component A (SCa) represents the movement of the cytoskeleton, composed of neurofilaments and microtubules. Slow component B (SCb) moves at a faster rate than SCa and includes soluble proteins, which are apparently moving between the more slowly moving cytoskeleton.

neurofilaments formed by the association of three neurofilament protein subunits.

Neurofilaments and microtubules move at a rate of approximately 1 mm/day and make up the majority of SCa, which is the slowest moving component of axonal transport. Moving at only a slightly more rapid rate of 2 to 4 mm/day in an anterograde direction is SCb, which is composed of many proteins. Included in SCb are several structural proteins, such as the component of microfilaments (actin) and several microfilament-associated proteins (M2 protein and fodrin), as well as clathrin and many soluble proteins.

T is continual transport of proteins from the cell body through the various components of anterograde axonal transport is the mechanism through which the neuron provides the distal axon with its complement of functional and structural proteins. Some vesicles are also moving in a retrograde direction and undoubtedly provide the cell body with information concerning the status of the distal axon.

Axonal Degeneration

When the neuronal cell body has been lethally injured, it degenerates, in a process called neuronopathy. T is is characterized by the loss of the cell body and all of its processes, with no potential for regeneration. However, when the injury is at the level of the axon, the axon may degenerate while the neuronal cell body continues to survive, a condition known as an axonopathy. In this setting, there is a potential for regeneration and recovery from the toxic injury as the axonal stump sprouts and regenerates (Figure 16–3).

T e result of axotomy (transection of an axon) is that the distal axon is destined to degenerate, a process known as axonal degeneration, which is unique to the NS. T e cell body of the neuron responds to the axotomy as well and undergoes a process of chromatolysis. T e sequence of events that occurs in the distal stump of an axon following transection is referred to as Wallerian degeneration. Because the axonal degeneration associated with chemicals and some disease states is thought to occur through a similar sequence of events, it is often referred to as Wallerian-like axonal degeneration.

Following axotomy, there is degeneration of the distal nerve stump, followed by generation of a microenvironment supportive of regeneration and involving the distal axon, ensheathing glial cells and the blood nerve barrier. Initially there is a period during which the distal stump survives and maintains relatively normal structural, transport, and conduction properties. T e duration of survival is proportional to the length of the axonal stump, and this relationship appears to be maintained across species.

Terminating the period of survival is an active proteolysis that digests the axolemma and axoplasm, leaving only a myelin sheath surrounding a swollen degenerate axon. Digestion of the axon appears to be an all-or-none event effected through endogenous proteases that are activated through increased levels of intracellular free Ca^{2+}.

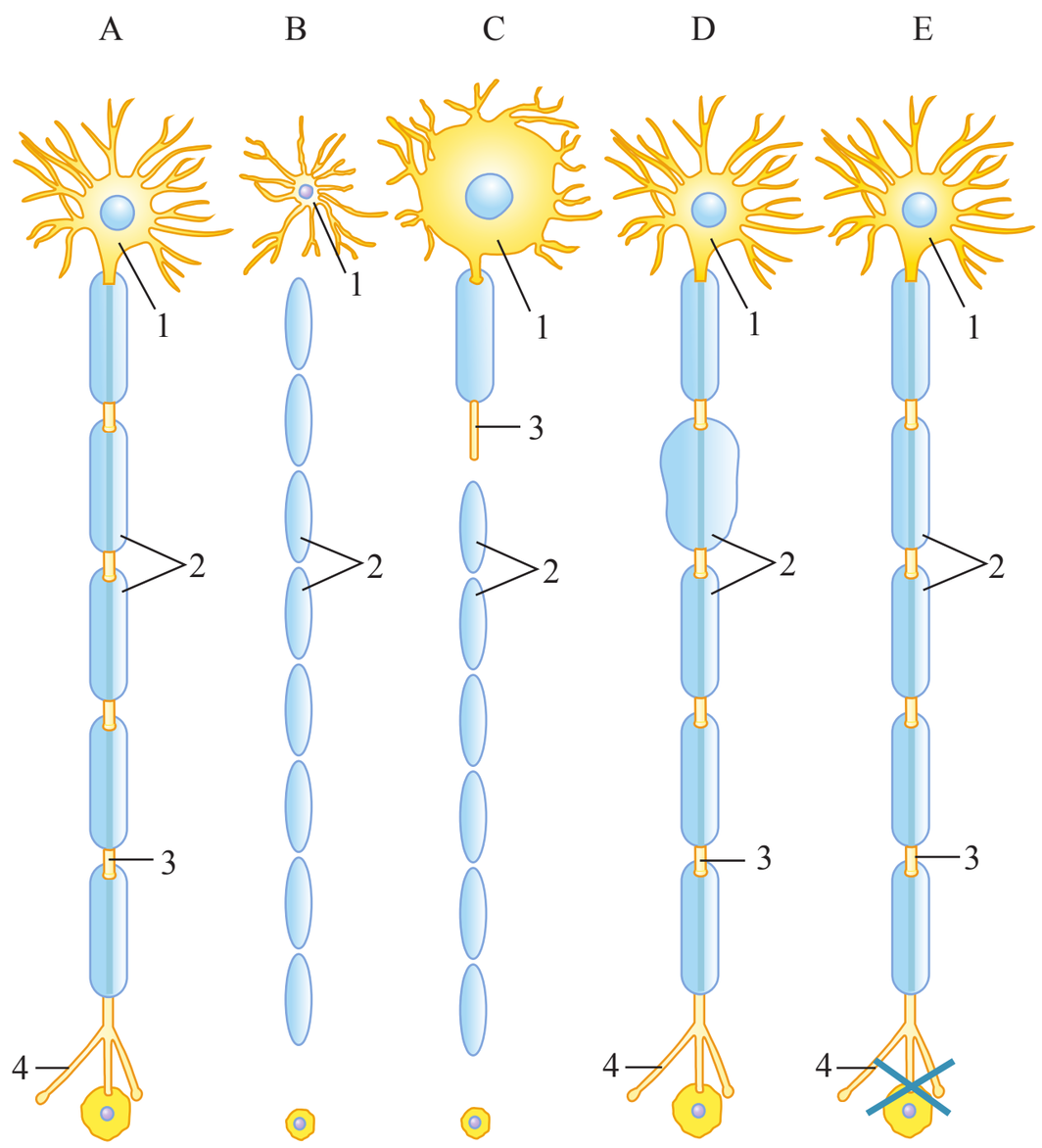

FIGURE 16–3 **Patterns of neurotoxic injury.** (**A**) Normal neuron showing (1) cell body and dendrites, (2) myelinating cells, encircling the (3) axon, and (4) synapse. (**B**) A neuronopathy resulting from the death of the entire neuron. Astrocytes often proliferate in response to the neuronal loss, creating both neuronal loss and gliosis. (**C**) An axonopathy occurs when the axon is the primary site of injury, the axon degenerates, and the surviving neuron shows only chromatolysis with margination of its Nissl substance and nucleus to the cell periphery. (**D**) Myelinopathy resulting from disruption of myelin or selective injury to the myelinating cells. To prevent cross-talk between adjacent axons, myelinating cells divide and cover the denuded axon rapidly; however, the process of remyelination is much less effective in the CNS than in the PNS. (**E**) Some forms of toxicity are due to interruption of the process of neurotransmission, either through blocking excitation or by excessive stimulation, rather than actual cell death.

In the PNS, Schwann cells respond to loss of axons by decreasing synthesis of myelin lipids, down-regulating genes encoding myelin proteins, and dedifferentiating to a premyelinating mitotic Schwann cell phenotype. T e proliferating Schwann cells create a tubular structure around the axon (referred to as a band of Bungner), providing physical guidance for regenerating axons. T ese tubes also provide trophic support from nerve growth factor (NGF), brain-derived neurotrophic factor, insulin-like growth factor, and corresponding receptors produced by the associated Schwann cells. Resident macrophages distributed along the endothelium within the endoneurium and the denervated Schwann cells assist in clearing myelin debris, but the recruitment of hematogenous macrophages accounts for the removal of the majority of myelin. Another essential role of recruited, circulating macrophages is the production of interleukin-1 (IL-1), which is responsible for stimulating production of NGF by Schwann cells.

A critical difference exists between axonal degeneration in the CNS compared with that in the PNS: peripheral axons can regenerate, whereas central axons cannot. Main factors contributing to the inability of the CNS to regenerate include inhibitory factors secreted by oligodendrocytes, astrocyte scarring, and glial interference. Interestingly, experiments involving cellular transplants of Schwann cells to the CNS or CNS neurons to the PNS show that the regenerative capability of CNS neurons depends on both the microenvironment and the properties of mature neurons.

Wallerian degeneration was long thought to be a passive process that proceeded inexorably after separating the axon from the trophic support provided by the cell body. However, we now know from several lines of evidence that Wallerian degeneration is an active process mediated by the axon itself, and that it is possible to slow or even halt its progression. Moreover, although axonal degeneration can be initiated by many different means, including physical, genetic, or toxic, the mechanisms of degeneration converge into common regulated pathways that are potentially subject to pharmacological intervention.

Myelin Formation and Maintenance

Myelin is formed in the CNS by oligodendrocytes and in the PNS by Schwann cells. Both of these cell types form concentric layers of lipid-rich myelin by the progressive wrapping of their cytoplasmic processes around the axon in successive loops (Figure 16–4). T ese cells exclude cytoplasm from the inner surface of their membranes to form the major dense line of myelin. In a similar process, the extracellular space is reduced on the extracellular surface of the bilayers, and the lipid membranes stack together.

T e maintenance of myelin is dependent on a number of membrane-associated proteins and on metabolism of specific lipids present in myelin bilayers. Some toxic compounds interfere with this complex process of the maintenance of myelin and result in the toxic "myelinopathies" (Figure 16–3). In general, the loss of myelin with the preservation of axons is referred to as demyelination.

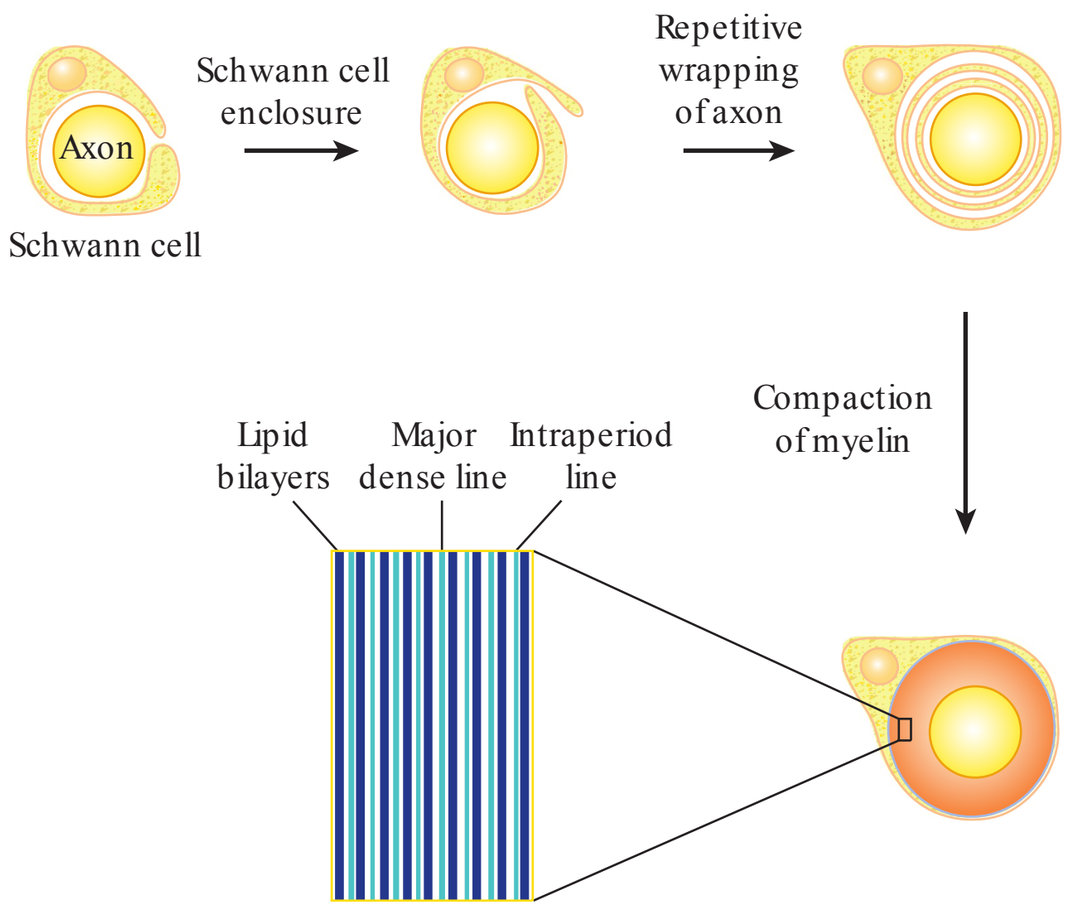

FIGURE 16–4 **Process of myelination.** Myelination begins when a myelinating cell encircles an axon, either Schwann cells in the peripheral nervous system or oligodendrocytes in the CNS. Simple enclosure of the axon persists in unmyelinated axons. Myelin formation proceeds by a progressive wrapping of multiple layers of the myelinating cell around the axon, with extrusion of the cytoplasm and extracellular space to bring the lipid bilayers into close proximity. The intracellular space is compressed to form the major dense line of myelin, and the extracellular space is compressed to form the intraperiod line.

Neurotransmission

Intercellular communication is achieved in the NS through the synapse. Neurotransmitters released from one neuron act as the first messenger. Binding of the transmitter to the postsynaptic receptor is followed by modulation of an ion channel or activation of a second-messenger system, leading to changes in the responding cell. Various therapeutic drugs and toxic compounds impact the process of neurotransmission.

Neurotoxicity expresses itself in terms of altered conduction and propagation of nerve impulses and changes in functions such as behavior, performance, and conditioning. Chemicals acting on neurotransmission may interrupt the transmission of impulses, block or accentuate transsynaptic communication, block reuptake of neurotransmitters or precursors, or interfere with second-messenger systems.

In terms of toxicity, many side effects of neurological drugs may be viewed as short-term interactions that are reversible with time or that may be counteracted by the use of appropriate antagonists. However, some of the toxicity associated with long-term exposures may be irreversible. Excessive stimulation of neurotransmitter systems may also have long-term consequences; e.g., excitatory system (e.g., glutamate) produces excitotoxicity that is manifest as CNS diseases and nerve cell death.

Development of the Nervous System

T e NS begins development during gestation and continues through adolescence. Proliferation, migration, differentiation, synaptogenesis, apoptosis, and myelination are the basic processes that underlie development of the NS, and these occur in a tightly choreographed sequence that depends on the region, cell type, and neurotrophic signals. T e proliferation and migration of neurons and glia occur in waves that are specific for brain regions, but in general, the brain develops in a caudal to rostral direction (with cerebellar development being a notable exception). During differentiation (phenotype expression) and synaptogenesis (formation of functional synaptic connections), the circuitry of the NS is established. Chemicals such as nerve growth factors, adhesive molecules, and neurotransmitters serve as morphogenic signals; neurotransmitter developmental signals are separate from their synaptic transmission function. Selected cells are also removed during ontogeny via

apoptosis (programmed cell death), which results in the appropriate cell types in the correct regions. T e glial supportive cells develop last, and myelination is protracted.

T e immature NS is especially vulnerable to certain agents and there are several factors that make the developing NS uniquely susceptible. Cell sensitivity differs with the developmental stage, leading to critical windows of vulnerability. Chemicals that alter the timing and formation of neural connections could result in permanent malformations, the consequences of which may be quite unlike the chemical's effects in the adult NS. Furthermore, while synaptogenesis can continue throughout life, proliferation cannot; therefore, the CNS is unique in that damaged neural cells are not readily replaced. Finally, there are physiological and kinetic differences in the developing organism that may profoundly influence its sensitivity, including the slow formation of the blood–brain barrier and lack of key metabolic enzymes to protect the brain and eliminate toxicants.

Factors Relevant to Neurodegenerative Diseases

A classic example of toxicant-induced neurodegeneration is exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is a by-product of the opioid analgesic, MPPP. Exposure to a sufficient amount of MPTP can lead to immediate parkinsonism, a disease in which dopaminergic neurons of the substantia nigra are lost. Exposure to an amount of MPTP insufficient to cause immediate parkinsonism leads to early signs of the disease years later. It does not seem likely that an early sublethal injury to dopaminergic neurons later becomes lethal. Rather, smaller exposures to MPTP may cause a decrement in the population of dopaminergic neurons and leave the individual vulnerable to further loss of dopaminergic neurons.

Epidemiological studies also implicate exposure to herbicides, pesticides, and metals as risk factors for Parkinson's disease (PD). Several studies suggest that dithiocarbamates also play an important role. Interestingly, some studies suggest that cigarette smoking may have a protective effect against both Alzheimer's disease and PD.

Environmental chemicals may cause heritable alterations in gene expression in the absence of changes in genome sequences. T e study of epigenetics has established two categories of mechanisms affecting gene expression: DNA methylation and histone posttranslational modifications. In most instances, methylation of the promotor region results in transcriptional repression of the gene. Histone posttranslational modifications are characterized by lysine acetylation, arginine and lysine methylation, serine phosphorylation, lysine ubiquitylation, etc.

Finally, it is necessary to recognize that microRNAs (miRNAs) provide regulatory control over gene expression. mRNAs can control developmental timing, cell proliferation, cell death, and patterning of the NS, thus providing extensive regulatory networks with a complexity comparable to that of transcription factors. More than 250 miRNAs have been already identified, but their mRNA targets and functions have yet to be fully appreciated. Emerging studies also suggest that miRNAs may be targeted by neurotoxicants, thus potentially affecting a broad spectrum of functions, encompassing cell differentiation and migration, neurogenesis, as well as synaptic function, to name a few.

FUNCTIONAL MANIFESTATIONS OF NEUROTOXICITY

Functions of the NS include motor, sensory, autonomic, and cognitive capabilities. Functional assessment uses a battery of tests as a means for screening potentially neurotoxic compounds. Specific behavioral methods include functional observational batteries (FOBs), Irwin screens, tests of motor activity, and expanded clinical observations. T ese tests have the advantage over biochemical and pathological measures in that they permit evaluation of a single animal over longitudinal studies to determine the onset, progression, duration, and reversibility of a neurotoxic injury.

Some functional tests are more specific than observations and motor activity, and many of these functions have a clinical or behavioral correlate in humans. Electrophysiological tests provide sensory-specific information on nerve conduction velocity and integrity, and have been used to complement behavioral evaluations. Measures of sensory function tap specific neuronal pathways that govern stimuli-dependent reflexes. Autonomic function includes evaluations of cardiovascular status and cholinergic/adrenergic balance.

Deficits in cognitive function, especially in the context of developmental toxicity, represent an end point of great public concern and rhetoric. In most cases, deficits in human cognitive function may be detected in laboratory animals as well, although the affected cognitive domain may vary. Ultimately, neurotoxicants identified by behavioral methods are also evaluated at a cellular and molecular level to provide an understanding of the events in the NS that cause the neurological dysfunction.

MECHANISMS OF NEUROTOXICITY

Individual neurotoxic compounds typically have one of four targets: the neuron, the axon, the myelinating cell, or the neurotransmitter system.

Neuronopathies

Certain toxicants are specific for neurons, resulting in their injury or death. Neuron loss is irreversible and includes degeneration of all of its cytoplasmic extensions, dendrites and axons, and the myelin ensheathing the axon (Figure 16–3). Unique features of the neuron that place it at risk for the action of cellular toxicants include a high metabolic rate, a long cellular process that is supported by the cell body, and an excitable membrane that is rapidly depolarized and repolarized.

Although a large number of compounds are known to result in toxic neuronopathies (Table 16–1), all of these toxicants

TABLE 16–1 Chemicals associated with neuronal injury (neuronopathies).

Neurotoxicant	Neurologic Findings	Cellular Basis of Neurotoxicity
Aluminum	Dementia, encephalopathy (humans), learning deficits	Spongiosis cortex, neurofibrillary aggregates, degenerative changes in cortex
6-Amino-nicotinamide	Not reported in humans; hind limb paralysis (experimental animals)	Spongy (vacuolar) degeneration in spinal cord, brainstem, cerebellum; axonal degeneration of the peripheral nervous system (PNS)
Arsenic	Encephalopathy (acute), peripheral neuropathy (chronic)	Brain swelling and hemorrhage (acute); axonal degeneration in PNS (chronic)
Azide	Insuf cient data (humans); convulsions, ataxia (primates)	Neuronal loss in cerebellum and cortex
Bismuth	Emotional disturbances, encephalopathy, myoclonus	Neuronal loss, basal ganglia, and Purkinje cells of cerebellum
Carbon monoxide	Encephalopathy, delayed parkinsonism/dystonia	Neuronal loss in cortex, necrosis of globus pallidus, focal demyelination; blocks oxygen-binding site of hemoglobin and iron-binding sites of brain
Carbon tetrachloride	Encephalopathy (secondary to liver failure)	Enlarged astrocytes in striatum, globus pallidus
Chloramphenicol	Optic neuritis, peripheral neuropathy	Neuronal loss (retina), axonal degeneration (PNS)
Cyanide	Coma, convulsions, rapid death; delayed parkinsonism/dystonia	Neuronal degeneration, cerebellum, and globus pallidus; focal demyelination; blocks cytochrome oxidase/ATP production
Doxorubicin	Insuf cient data (humans); progressive ataxia (experimental animals)	Degeneration of dorsal root ganglion cells, axonal degeneration (PNS)
Ethanol	Mental retardation, hearing deficits (prenatal exposure)	Microcephaly, cerebral malformations
Lead	Encephalopathy (acute), learning deficits (children), neuropathy with demyelination (rats)	Brain swelling, hemorrhages (acute), axonal loss in PNS (humans)
Manganese	Emotional disturbances, parkinsonism/dystonia	Degeneration of striatum, globus pallidus
Mercury, inorganic	Emotional disturbances, tremor, fatigue	Insuf cient data in humans (may affect spinal tracts; cerebellum)
Methanol	Headache, visual loss or blindness, coma (severe)	Necrosis of putamen, degeneration of retinal ganglion cells
Methylazoxymethanol acetate (MAM)	Microcephaly, retarded development (rats)	Developmental abnormalities of fetal brain (rats)
Methyl bromide	Visual and speech impairment; peripheral neuropathy	Insuf cient data
Methyl mercury (organic mercury)	Ataxia, constriction of visual fields, paresthesias (adult) Psychomotor retardation (fetal exposure)	Neuronal degeneration, visual cortex, cerebellum, ganglia Spongy disruption, cortex, and cerebellum
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)	Parkinsonism, dystonia (acute exposure) Early onset parkinsonism (late effect of acute exposure)	Neuronal degeneration in substantia nigra Neuronal degeneration in substantia nigra
3-Nitropropionic acid	Seizures, delayed dystonia/grimacing	Necrosis in basal ganglia
Phenytoin (diphenyl-hydantoin)	Nystagmus, ataxia, dizziness	Degeneration of Purkinje cells (cerebellum)
Quinine	Constriction of visual fields	Vacuolization of retinal ganglion cells
Streptomycin (aminoglycosides)	Hearing loss	Degeneration of inner ear (organ of Corti)
Thallium	Emotional disturbances, ataxia, peripheral neuropathy	Brain swelling (acute), axonal degeneration in PNS
Trimethyltin	Tremors, hyperexcitability (experimental animals)	Loss of hippocampal neurons, amygdala pyriform cortex

share certain features. Each toxic condition is the result of a cellular toxicant that has a predilection for neurons. The initial injury to neurons is followed by apoptosis or necrosis, leading to permanent loss of the neuron. These agents tend to be diffuse in their action, although they may show some selectivity in the degree of injury of different neuronal subpopulations. The expression of these cellular events is often a diffuse encephalopathy, with global dysfunctions.

Doxorubicin—Doxorubicin (Adriamycin), a quinone-containing anthracycline antibiotic, is one of the most effective antimitotics in cancer chemotherapy. Unfortunately, clinical application of doxorubicin is greatly limited by its acute and chronic cardiotoxicity. Doxorubicin injures neurons in the PNS, specifically those of the dorsal root ganglia and autonomic ganglia by intercalating with DNA and interfering with transcription. Other important mechanisms of action of doxorubicin include its interaction with topoisomerase II, which forms a DNA-cleavable complex and generation of reactive oxygen species (ROS) by enzymatic electron reduction of doxorubicin by variety of oxidases, reductases, and dehydrogenases. The vulnerability of sensory and autonomic neurons appears to reflect the lack of protection of these neurons by a blood–tissue barrier within ganglia.

Methyl Mercury—Methyl mercury (MeHg) exposure occurs primarily from eating fish in which the substance has accumulated. In addition, mercury is a common pollutant in hazardous waste sites in the United States. The clinical picture of MeHg poisoning varies with both the severity of exposure and the age of the individual at the time of exposure. In adults, the most dramatic sites of injury are the neurons of the visual cortex and the small internal granular cell neurons of the cerebellar cortex, whose massive degeneration results in blindness and marked ataxia. In children, developmental disabilities, retardation, and cognitive deficits occur. It has been suggested that these differences are caused by an immature blood–brain barrier causing a more generalized distribution of mercury in the developing brain. Recent studies in rats show that the neurons that are most sensitive to the toxic effects of MeHg are those that reside in the dorsal root ganglia, perhaps again reflecting the vulnerability of neurons not shielded by blood–tissue barriers. The mechanism of MeHg toxicity has been the subject of intense investigation and it remains unknown whether the ultimate toxicant is MeHg itself or the liberated mercuric ion. A variety of aberrations in cellular function have been noted, including impaired glycolysis, nucleic acid biosynthesis, aerobic respiration, protein synthesis, and neurotransmitter release. In addition, there is evidence for enhanced oxidative injury and altered calcium homeostasis. Exposure to MeHg leads to widespread neuronal injury and subsequently to a diffuse encephalopathy.

Trimethyltin—Organotins are used industrially as plasticizers, antifungal agents, or pesticides. Intoxication with trimethyltin has been associated with a potentially irreversible limbic-cerebellar syndrome in humans and similar behavioral changes in primates. Trimethyltin gains access to the NS where, by an undefined mechanism, it leads to diffuse neuronal injury. Several hypotheses are suggested for the mechanism of trimethyltin neurotoxicity, however, including energy deprivation and excitotoxic damage.

Axonopathies

The neurotoxic disorders termed axonopathies are those in which the primary site of toxicity is the axon itself. The axon degenerates, and with it the myelin surrounding that axon; however, the neuron cell body remains intact (Figure 16–5). The toxicant results in a "chemical transection" of the axon at some point along its length, and the axon distal to the transection degenerates.

A critical difference exists in the significance of axonal degeneration in the CNS compared with that in the PNS: peripheral axons can regenerate, whereas central axons cannot. In the PNS, glial cells and macrophages support axonal regeneration. In the CNS, release of inhibitory factors from damaged myelin and astrocyte scarring actually interferes with regeneration. The clinical relevance of the disparity between the CNS and PNS is that partial to complete recovery can occur after axonal degeneration in the PNS, whereas the same event is irreversible in the CNS.

Axonopathies can be considered to result from a chemical transection of the axon. The number of axonal toxicants is large and increasing in number (Table 16–2). As the axons degenerate, sensations and motor strength are first impaired in the most distal extent of the axonal processes (e.g., the hands and feet), resulting in a "glove-and-stocking" neuropathy. With time and continued injury, the deficit progresses to involve more proximal areas of the body and the long axons of the spinal cord.

Gamma-diketones—Humans develop a progressive sensorimotor distal axonopathy when exposed to high concentrations of a simple alkane, n-hexane, day after day in work settings or after repeated intentional inhalation of hexane-containing glues. An identical axonopathy can be produced by methyl-n-butyl ketone (2-hexanone).

The ω-1 oxidation of n-hexane results in the γ-diketone, 2,5-hexanedione (HD), which reacts with amino groups in all tissues to form pyrroles that derivatize and cross-link neurofilaments, leading to development of neurofilament aggregates of the distal, subterminal axon (Figure 16–5). The neurofilament-filled axonal swellings distort nodal anatomy and impair axonal transport. The pathologic processes of neurofilament accumulation and degeneration of the axon are followed by the emergence of a clinical peripheral neuropathy.

Carbon Disulfide—The most significant exposures of humans to CS_2 have occurred in the vulcan rubber and viscose rayon industries. High-level exposures of humans to CS_2 cause a distal axonopathy that is identical pathologically to that caused by hexane. Covalent cross-linking of neurofilaments also occurs and it is known that CS_2 is itself the ultimate toxicant.

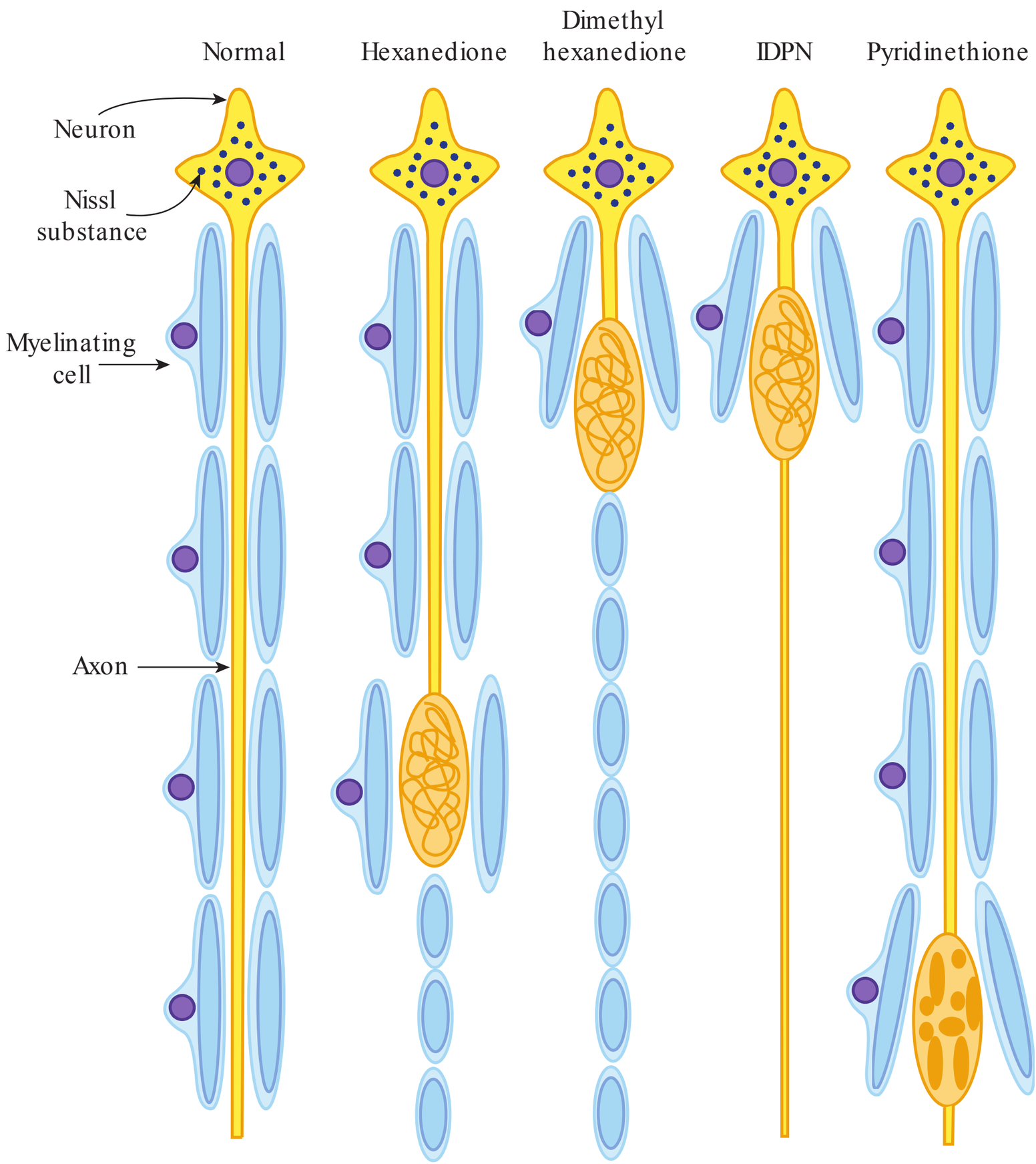

FIGURE 16–5 **Diagram of axonopathies.** Whereas 2,5-hexanedione results in the accumulation of neurofilaments in the distal regions of the axon, 3,4-dimethyl-2,5-hexanedione results in identical accumulation within the proximal segments. These proximal neurofilamentous swellings are quite similar to those that occur in the toxicity of β,β′-iminodipropionitrile (IDPN), although the distal axon does not degenerate in IDPN axonopathy but becomes atrophic. Pyridinethione results in axonal swellings that are distended with tubulovesicular material, followed by distal axonal degeneration.

The clinical effects of exposure to CS_2 in the chronic setting are very similar to those of hexane exposure, with the development of sensory and motor symptoms occurring initially in a glove-and-stocking distribution. In addition to this chronic axonopathy, CS_2 can also lead to aberrations in mood and signs of diffuse encephalopathic disease.

β,β′-Iminodipropionitrile (IDPN)—IDPN is a synthetic, bifunctional nitrile that causes a bizarre "waltzing syndrome" in rats and other mammals, although human exposure has never been documented. Features of this syndrome include excitement, circling, head twitching, and overalertness, which appears to result from degeneration of the vestibular sensory hair cells. In addition, administration of IDPN leads to accumulation of neurofilaments in the proximal axon, leading to swelling without degeneration in most animals. These neurofilament swellings are similar to those observed in carbon disulfide or γ-diketone toxicity.

Repeated exposure to IDPN leads to demyelination and onion bulb formation (Figure 16–5), and eventually can produce distal axonal atrophy due to a reduction in anterograde neurofilament transport to the distal axon. This impairment of axonal transport results from the disruption of the association between microtubules and neurofilaments by IDPN, causing neurofilament accumulation. This leads to complete disturbance of the cytoskeleton of the axon.

Acrylamide—Acrylamide is a man-made vinyl monomer used widely in water purification, paper manufacturing, mining, and waterproofing. It is also used extensively in

TABLE 16–2 Chemicals associated with axonal injury (axonopathies).

Neurotoxicant	Neurologic Findings	Basis of Neurotoxicity
Acrylamide	Peripheral neuropathy (often sensory)	Axonal degeneration, axon terminal affected in earliest stages
p-Bromophenylacetylurea	Peripheral neuropathy	Axonal degeneration in the peripheral nervous system (PNS) and central nervous system (CNS)
Carbon disulfide	Psychosis (acute), peripheral neuropathy (chronic)	Axonal degeneration, early stages include neurofilamentous swelling
Chlordecone (Kepone)	Tremors, incoordination (experimental animals)	Insufficient data (humans); axonal swelling and degeneration
Chloroquine	Peripheral neuropathy, weakness	Axonal degeneration, inclusions in dorsal root ganglion cells; also vacuolar myopathy
Clioquinol	Encephalopathy (acute), subacute myelooptic neuropathy (subacute)	Axonal degeneration, spinal cord, PNS, optic tracts
Colchicine	Peripheral neuropathy	Axonal degeneration, neuronal perikaryal filamentous aggregates; vacuolar myopathy
Dapsone	Peripheral neuropathy, predominantly motor	Axonal degeneration (both myelinated and unmyelinated axons)
Dichlorophenoxyacetate	Peripheral neuropathy (delayed)	Insufficient data
Dimethylaminopropionitrile	Peripheral neuropathy, urinary retention	Axonal degeneration (both myelinated and unmyelinated axons)
Ethylene oxide	Peripheral neuropathy	Axonal degeneration
Glutethimide	Peripheral neuropathy (predominantly sensory)	Insufficient data
Gold	Peripheral neuropathy (may have psychiatric problems)	Axonal degeneration, some segmental demyelination
n-Hexane	Peripheral neuropathy, severe cases have spasticity	Axonal degeneration, early neurofilamentous swelling, PNS, and spinal cord
Hydralazine	Peripheral neuropathy	Insufficient data
β,β′-Iminodipropionitrile	No data in humans; excitatory movement disorder (rats)	Proximal axonal swellings, degeneration of olfactory epithelial cells, vestibular hair cells
Isoniazid	Peripheral neuropathy (sensory), ataxia (high doses)	Axonal degeneration
Lithium	Lethargy, tremor, ataxia (reversible)	Insufficient data
Methyl n-butyl ketone	Peripheral neuropathy	Axonal degeneration, early neurofilamentous swelling, PNS, and spinal cord
Metronidazole	Sensory peripheral neuropathy, ataxia, seizures	Axonal degeneration, mostly affecting myelinated fibers; lesions of cerebellar nuclei
Misonidazole	Peripheral neuropathy	Axonal degeneration
Nitrofurantoin	Peripheral neuropathy	Axonal degeneration
Organophosphorus compounds (NTE inhibitors)	Abdominal pain (acute); peripheral neuropathy	Axonal degeneration
Paclitaxel (taxoids)	Delayed peripheral neuropathy (motor), spasticity	Axonal degeneration (delayed after single exposure), PNS, and spinal cord
Platinum (cisplatin)	Peripheral neuropathy	Axonal degeneration; microtubule accumulation in early stages
Pyridinethione (pyrithione)	Movement disorders (tremor, choreoathetosis)	Axonal degeneration (variable)
Vincristine (vinca alkaloids)	Cranial (most often trigeminal) neuropathy Peripheral neuropathy, variable autonomic symptoms	Insufficient data Axonal degeneration (PNS), neurofibrillary changes (spinal cord, intrathecal route)

biochemical laboratories, and is present in many foods prepared at high temperatures. Although it can be dangerous if not handled carefully, most toxic events in humans have been observed as peripheral neuropathies in factory workers exposed to high doses.

Studies of acrylamide neuropathy revealed a distal axonopathy characterized by multiple axonal swellings. A single large dose is sufficient to produce these swellings; however, repeated dosing results in a more proximal axonopathy, in a "dying back" process. T ese changes are caused by accumulations of neurofilaments at the nerve terminal. Recently it has been observed that nerve terminal degeneration occurs prior to development of axonopathy, suggesting that this degeneration is the primary lesion.

Organophosphorus Compounds—Organophosphorus (OP) compounds are used as insecticides, chemical warfare agents, chemical intermediates, flame retardants, fuel additives, hydraulic fluids, lubricants, pharmaceuticals, and plasticizers. T e OP insecticides and nerve agents are designed to inhibit AChE, thereby causing accumulation of acetylcholine in cholinergic synapses resulting in cholinergic toxicity and death. Some OP compounds, such as tri-o-cresyl phosphate (TOCP), can cause a severe sensorimotor central peripheral distal axonopathy called OP compound–induced delayed neurotoxicity (OPIDN) without inducing cholinergic poisoning.

Many OP compounds are lipophilic and readily enter the NS, where they can phosphorylate neural target proteins. When the principal target is AChE, cholinergic toxicity can ensue, either because of suprathreshold levels of inhibition or inhibition plus aging. When aging of inhibited AChE also occurs (i.e., net loss of a ligand from the phosphorus of the OP-enzyme conjugate, leaving a negatively charged phosphoryl moiety attached to the active site), the qualitative nature of the toxicity does not change. Instead, the inhibited AChE becomes intractable to reactivation. When the principal target is neuropathy target esterase (neurotoxic esterase, NTE), OPIDN can result only if both suprathreshold (> 70%) inhibition occurs and the inhibited enzyme undergoes aging. T us, in the case of NTE and OPIDN, inhibition alone is insufficient to precipitate toxicity. Neuropathic (aging) inhibitors of NTE include compounds from the phosphate, phosphonate, and phosphoramidate classes of OP compounds.

Axonal degeneration does not commence immediately after acute exposure to a neuropathic OP compound but is delayed for at least eight days between the acute high-dose exposure and clinical signs of axonopathy. Some effective regeneration of axons occurs in the PNS while axonal degeneration is progressive and persistent in the long tracts of the spinal cord.

Human cases of OPIDN are now rare and usually arise from intentional ingestion of massive doses of OP insecticides in suicide attempts. Nevertheless, the fact remains that OPIDN is a debilitating and incurable condition. While the preceding discussion was limited to organic compounds of pentacovalent phosphorus, organic compounds of trivalent phosphorous also produce axonal degeneration in the CNS and PNS albeit in a different form than classical OPIDN.

Pyridinethione—T is compound is a chelating agent that is usually encountered as the zinc complex, called zinc pyridinethione (ZPT), which has antibacterial and antifungal properties and is a component of shampoos that are effective in the treatment of seborrhea and dandruff. Although the compound is applied to the human scalp in antidandruff shampoos, dermal absorption of ZPT is minimal and exposure primarily occurs orally. Only the pyridinethione moiety is absorbed following ingestion, with the majority of zinc eliminated in the feces. Pyridinethione appears to interfere with the fast axonal transport systems, impairs the turnaround of rapidly transported vesicles, and slows the retrograde transport of vesicles. Aberration of the fast axonal transport systems most likely contributes to the accumulation of tubular and vesicular structures in the distal axon (Figure 16–5). As these materials accumulate in one region of the axon, the axon degenerates in its more distal regions beyond the accumulated structures. T e earliest signs are diminished grip strength and changes of the axon terminal, leading to a peripheral neuropathy.

Microtubule-associated Neurotoxicity—A number of plant alkaloids alter the assembly and depolymerization of microtubules in nerve axons, causing neurotoxicity. T e oldest known of these are colchicine and the vinca alkaloids, which bind to tubulin and cause depolymerization of microtubules. Colchicine is an alkaloid pharmaceutical used in the treatment of gout, familial Mediterranean fever, and other disorders. Vincristine and vinblastine are two vinca alkaloids used as chemotherapeutic agents. Both colchicine and the vinca alkaloids produce a similar peripheral axonal neuropathy. Hallmarks of this neuropathy include paresthesia (tingling) of the fingers, generalized weakness, and clumsiness.

Paclitaxel (Taxol), another plant alkaloid, has become a popular chemotherapeutic drug used to treat a variety of neoplasms. However, side effects include a predominantly sensory neuropathy, beginning in the hands and feet. Like colchicine and the vinca alkaloids, paclitaxel binds to tubulin; however, instead of leading to depolymerization, it promotes the formation of microtubules. Once formed, these microtubules remain stabilized by paclitaxel even in conditions that normally lead to dissociation of tubulin subunits, including cold temperatures or the presence of calcium.

T e pathologies of the axon induced by these drugs are different. Although colchicine leads to atrophy of the axon and a decrease in the number of microtubules, paclitaxel causes the aggregation to form a matrix that may inhibit fast axonal transport, which has been demonstrated with both colchicine and paclitaxel.

Myelinopathies

Myelin provides electrical insulation of neuronal processes, and its absence leads to a slowing of conduction and aberrant

conduction of impulses between adjacent processes. Toxicants exist that result in the separation of the myelin lamellae, termed intramyelinic edema, and in the selective loss of myelin, termed demyelination. Intramyelinic edema may be caused by alterations in the transcript levels of myelin basic protein mRNA, and early in its evolution is reversible. Demyelination may result from progressive intramyelinic edema or from direct toxicity to the myelinating cell. Remyelination in the CNS occurs to only a limited extent after demyelination. However, Schwann cells in the PNS are capable of remyelinating the axon. All the compounds in Table 16–3 lead to a myelinopathy.

Hexachlorophene—Hexachlorophene, or 2,2′-methylenebis-(3,4,6-trichlorophenol), caused neurotoxicity when newborn infants were bathed with the compound to avoid staphylococcal skin infections. Following skin absorption of this hydrophobic compound, hexachlorophene enters the NS and results in intramyelinic edema, which leads to the formation of vacuoles creating a "spongiosis" of the brain. Hexachlorophene causes intramyelinic edema that leads to segmental demyelination. Swelling of the brain causes increased intracranial pressure, axonal degeneration, along with degeneration of photoreceptors in the retina. Humans exposed acutely to hexachlorophene may have generalized weakness, confusion, and seizures. Progression may occur, to include coma and death.

Tellurium—Although exposures have not been reported in humans, the neurotoxicity of tellurium in young rats alters the synthesis of myelin lipids in Schwann cells, because of various lipid abnormalities. As biochemical changes occur, lipids accumulate in Schwann cells, which eventually lose their ability to maintain myelin in the PNS.

Lead—Lead exposure in animals results in a peripheral neuropathy with prominent segmental demyelination. In young children, acute massive exposures to lead result in severe cerebral edema, perhaps from damage to endothelial cells. Children absorb lead more readily, and the very young do not have the protection of the blood–brain barrier. Chronic lead intoxication in adults results in peripheral neuropathy, gastritis, colicky abdominal pain, anemia, and the prominent deposition of lead in particular anatomical sites, creating lead lines in the gums and in the epiphyses of long bones in children. Lead in the peripheral nerve of humans slows nerve conduction. T e basis of lead encephalopathy is unclear, although an effect on the membrane structure of myelin and myelin membrane fluidity has been shown.

Astrocytes

Astrocytes perform and regulate a wide range of physiologic functions in the CNS. T e astrocyte appears to be a primary means of defense in the CNS following exposure to neurotoxicants, as a spatial buffering system for osmotically active ions, and as a depot for the sequestration and metabolic processing of endogenous molecules and xenobiotics.

Ammonia—At high CNS concentrations, ammonia produces seizures, resulting from its depolarizing action on cell membranes, whereas at lower concentrations, ammonia produces

TABLE 16–3 Chemicals associated with injury of myelin (myelinopathies).

Neurotoxicant	Neurologic Findings	Basis of Neurotoxicity
Acetylethyltetramethyl tetralin (AETT)	Not reported in humans; hyperexcitability, tremors (rats)	Intramyelinic edema; pigment accumulation in neurons
Amiodarone	Peripheral neuropathy	Axonal degeneration and demyelination; lipid-laden lysosomes in Schwann cells
Cuprizone	Not reported in humans; encephalopathy (experimental animals)	Status spongiosis of white matter, intramyelinic edema (early stages); gliosis (late)
Disulfiram	Peripheral neuropathy, predominantly sensory	Axonal degeneration, swellings in distal axons
Ethidium bromide	Insuf cient data (humans)	Intramyelinic edema, status spongiosis of white matter
Hexachlorophene	Irritability, confusion, seizures	Brain swelling, intramyelinic edema in CNS and PNS, late axonal degeneration
Lysolecithin	Effects only on direct injection into PNS or CNS (experimental animals)	Selective demyelination
Perhexilene	Peripheral neuropathy	Demyelinating neuropathy, membrane-bound inclusions in Schwann cells
Tellurium	Hydrocephalus, hind limb paralysis (experimental animals)	Demyelinating neuropathy, lipofuscinosis (experimental animals)
Triethyltin	Headache, photophobia, vomiting, paraplegia (irreversible)	Brain swelling (acute) with intramyelinic edema, spongiosis of white matter

stupor and coma, consistent with its hyperpolarizing effects. Ammonia intoxication is associated with astrocytic swelling and morphological changes. Increased intracellular ammonia concentrations have also been implicated in the inhibition of neuronal glutamate precursor synthesis, resulting in diminished glutamatergic neurotransmission, changes in neurotransmitter uptake (glutamate), and changes in receptor-mediated metabolic responses of astrocytes to neuronal signals.

Nitrochemicals—Organic nitrates are used for peripheral vasodilatation and reduction of blood pressure (nitroglycerine) in treatment of cardiovascular disease. T e dinitrobenzenes are important synthetic intermediates in the industrial production of dyes, plastics, and explosives. T e neurotoxic compound, 1,3-dinitrobenzene (DNB), produces gliovascular lesions that specifically target astrocytes in the periaqueductal gray matter of the brainstem and deep cerebellar roof nuclei. Metronidazole, a 5-nitroimidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], is an antimicrobial, antiprotozoal agent that is commonly used for the treatment of a wide variety of infections. Prolonged treatment with metronidazole is associated with a peripheral neuropathy characterized by paraesthesias, dysaesthesias, headaches, glossitis, urticaria, and pruritus in addition to other somatosensory disorders.

Methionine Sulfoximine—Methionine sulfoximine (MSO) is an irreversible inhibitor of the astrocyte-specific enzyme, glutamine synthase. Ingestion of large amounts of MSO leads to neuronal cell loss in the hippocampal fascia dentata and pyramidal cell layer, in the short association fibers and lower layers of the cerebral cortex, and in cerebellar Purkinje cells. MSO also leads to large increases of glycogen levels, primarily within astrocytic cell bodies, as well as swollen and damaged astrocytic mitochondria.

Fluoroacetate and Fluorocitrate—T e Krebs cycle inhibitor fluorocitrate (FC) and its precursor fluoroacetate (FA) are preferentially taken up by glia. FA occurs naturally in a number of plants, and is available commercially as a rodenticide (Compound 1080). Exposure to FA may also occur via exposure to the anti-cancer drug 5-fluorouracil. Ingestion of large amounts of FA results in ionic convulsions, with onset of seizures within minutes of consumption; those surviving these episodes frequently die later on due to respiratory arrest or heart failure. T e actions of FC and FA have been attributed both to the disruption of carbon flux through the Krebs cycle and to impairment of ATP production.

Neurotransmission-associated Neurotoxicity

A wide variety of naturally occurring toxins, as well as synthetic chemicals, alter specific mechanisms of intercellular communication (Table 16–4). Although neurotransmitter-associated

TABLE 16–4 Chemicals associated with neurotransmitter-associated toxicity.

Neurotoxicant	Neurologic Findings	Basis of Neurotoxicity
Amphetamine and methamphetamine	Tremor, restlessness (acute); cerebral infarction and hemorrhage; neuropsychiatric disturbances	Bilateral infarcts of globus pallidus, abnormalities in dopaminergic, serotonergic, cholinergic systems Acts at adrenergic receptors (PNS)
Atropine	Restlessness, irritability, hallucinations	Blocks cholinergic receptors (anticholinergic)
Cocaine	Increased risk of stroke and cerebral atrophy (chronic users); increased risk of sudden cardiac death; movement and psychiatric abnormalities, especially during withdrawal Decreased head circumference (fetal exposure)	Infarcts and hemorrhages; alteration in striatal dopamine neurotransmission Structural malformations in newborns
Domoic acid	Headache, memory loss, hemiparesis, disorientation, seizures	Neuronal loss, hippocampus and amygdala, layers 5 and 6 of neocortex Kainate-like pattern of excitotoxicity
Kainate	Insuf cient data in humans; seizures in animals (selective lesioning compound in neuroscience)	Degeneration of neurons in hippocampus, olfactory cortex, amygdala, thalamus Binds AMPA/kainate receptors
β-N-Methylamino-l-alanine (BMAA)	Weakness, movement disorder (monkeys)	Degenerative changes in motor neurons (monkeys) Excitotoxic probably via NMDA receptors
Muscarine (mushrooms)	Nausea, vomiting, headache	Binds muscarinic receptors (cholinergic)
Nicotine	Nausea, vomiting, convulsions	Binds nicotinic receptors (cholinergic) low-dose stimulation; high-dose blocking
β-N-Oxalylamino-l-alanine (BOAA)	Seizures	Excitotoxic probably via AMPA class of glutamate receptors

actions may be well understood for some agents, the specificity of the mechanisms should not be assumed.

Nicotine—Widely available in tobacco products and in certain pesticides, nicotine has diverse pharmacological actions and may be the source of considerable toxicity. Nicotine exerts its effects by binding to a subset of nicotinic cholinergic receptors. Smoking and "pharmacologic" doses of nicotine accelerate heart rate, elevate blood pressure, and constrict blood vessels within the skin as a result of stimulation of the ganglionic sympathetic NS.

The rapid rise in circulating levels of nicotine after acute overdose leads to excessive stimulation of nicotinic receptors, a process that is followed rapidly by ganglionic paralysis. Initial nausea, rapid heart rate, and perspiration are followed shortly by marked slowing of heart rate with a fall in blood pressure. Somnolence and confusion may occur, followed by coma; if death results, it is often the result of paralysis of the muscles of respiration.

Acute poisoning with nicotine fortunately is uncommon; however, exposure to lower levels for longer duration is very common. In humans, it has been difficult to separate the effects of nicotine from those of other components of cigarette smoke. The complications of smoking include cardiovascular disease, cancers (especially malignancies of the lung and upper airway), chronic pulmonary disease, and attention deficit disorders in children of women who smoke during pregnancy.

An increased propensity for platelets to aggregate is seen in smokers, and this platelet abnormality correlates with the level of nicotine. Nicotine also places an increased burden on the heart through its acceleration of heart rate and blood pressure, suggesting that nicotine may play a role in the onset of myocardial ischemia. In addition, nicotine also inhibits apoptosis and may play a direct role in tumor promotion and tobacco-related cancers.

Cocaine and Amphetamines—Cocaine blocks the reuptake of dopamine (DA), norepinephrine (NE), and serotonin (5-HT) at the nerve terminal in the CNS, and also causes release of DA from storage vesicles. The primary event responsible for the addictive properties and euphoric feeling when intoxicated is a block on the DA reuptake transporter (DAT).

Cocaine abuse also puts individuals at risk for cerebrovascular defects, cerebral atrophy, stroke, and intracranial hemorrhage. Cerebrovascular resistance has also been found to be higher in cocaine abusers. In chronic cocaine users, neurodegenerative disorders have been observed, similar to those observed with amphetamine use.

Amphetamines affect catecholamine neurotransmission in the CNS and have the potential to damage monoaminergic cells directly. Amphetamines, including methylenedioxymethamphetamine (MDMA, or "ecstasy"), have become popular with young adults in recent decades due to the belief that it is a "safe" drug, and its ability to increase energy and sensation in adults. Similar to cocaine, the most pronounced effect of amphetamines is on the DAergic neurons, but they can also damage 5-HT axons and axon terminals. The result is a distal axotomy of DA and 5-HT neurons.

The exact mechanism of amphetamine neurotoxicity is still unknown, but it seems that oxidative stress plays a key role. DA is oxidized to produce free radicals, and chronic use can affect superoxide dismutase (SOD) and catalase balance in rodents. In support of this hypothesis, studies have shown amphetamine neurotoxicity is attenuated by antioxidants.

Excitatory Amino Acids—Glutamate and certain other acidic amino acids are excitatory neurotransmitters. The toxicity of glutamate can be blocked by certain glutamate antagonists, and the concept has emerged that the toxicity of excitatory amino acids may be related to such conditions as hypoxia, epilepsy, and neurodegenerative diseases.

Glutamate is the main excitatory neurotransmitter of the brain, and its effects are mediated by several subtypes of receptors (Figure 16–6) called excitatory amino acid receptors (EAARs). The two major subtypes of glutamate receptors are those that are ligand-gated directly to ion channels (ionotropic) and those that are coupled with G proteins (metabotropic). Ionotropic receptors may be further subdivided by their specificity for binding kainate, quisqualate, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), and N-methyl-d-aspartate (NMDA). The entry of glutamate into the CNS is regulated at the blood–brain barrier, and glutamate exerts its effects in the circumventricular organ of the brain in which the blood–brain barrier is least developed. Within this site of limited access, glutamate injures neurons, apparently by opening glutamate-dependent ion channels, ultimately leading to neuronal swelling and neuronal cell death. The only known related human condition is the "Chinese restaurant syndrome,"

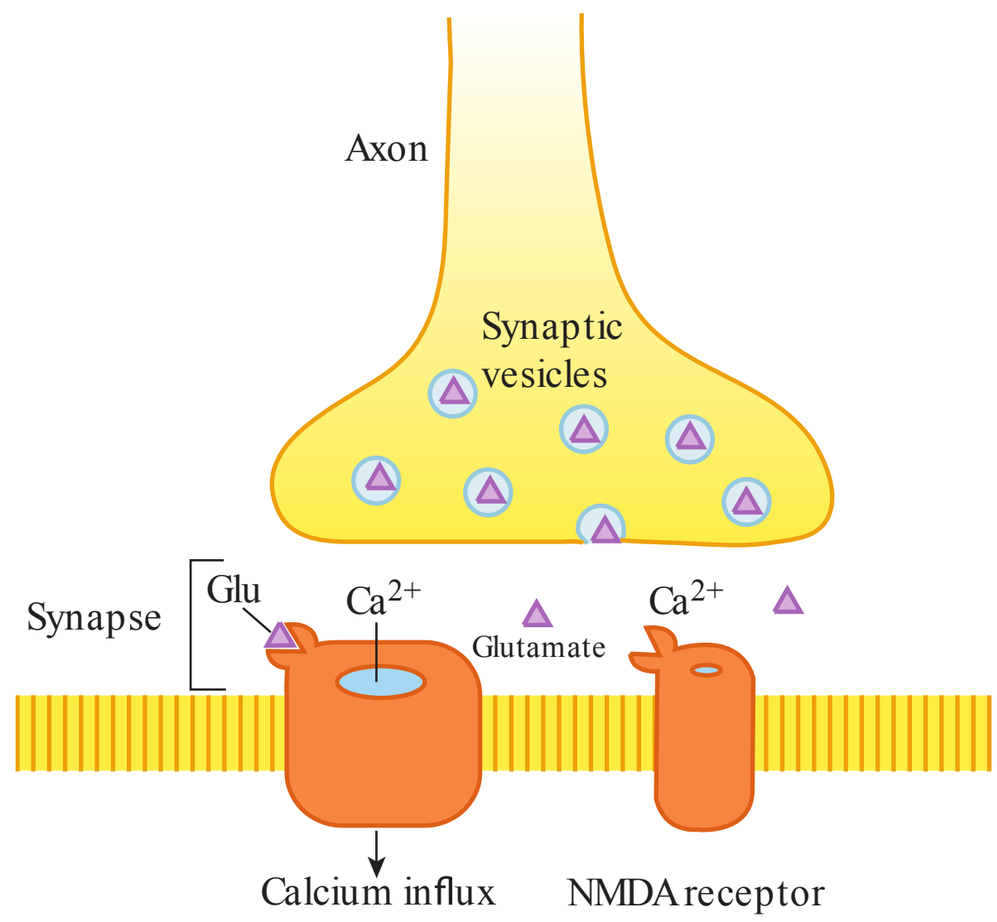

FIGURE 16–6 **Schematic diagram of a synapse.** Synaptic vesicles are transported to the axonal terminus, and released across the synaptic cleft to bind to the postsynaptic receptors. Glutamate, as an excitatory neurotransmitter, binds to its receptor and opens a calcium channel, leading to the excitation of the postsynaptic cell.

in which consumption of large amounts of monosodium glutamate (MSG) as a seasoning may lead to a burning sensation in the face, neck, and chest.

T e cyclic glutamate analog kainate, isolated from a seaweed in Japan, is extremely potent as an excitotoxin, being a 100-fold more toxic than glutamate, and is selective at a molecular level for the kainate receptor. Like glutamate, kainate selectively injures dendrites and neurons and shows no substantial effect on glia or axons. Injected into a region of the brain, it can destroy the neurons of that area without disrupting all of the fibers that pass through the same region. Kainate has become a tool for neurobiologists to explore the anatomy and function of the NS. Kainate, through its selective action on neuronal cell bodies, has provided a greater understanding of the functions of cells within a specific region of the brain, whereas previous lesioning techniques addressed only regional functions. T is void in understanding and the epidemiologic evidence that some neurodegenerative diseases may have environmental contributors inspire a heightened desire to appreciate more fully the effects of elements of our environment on the NS.

Development of permanent neurologic deficits occurred in individuals accidentally exposed to high doses of the EAAR agonist domoic acid, an analog of glutamate. T e acute illness most commonly presented as gastrointestinal disturbance, severe headache, and short-term memory loss. A subset of the more severely af icted patients had chronic memory deficits and motor neuropathy. Neuropathologic investigation of patients who died within 4 months of intoxication showed neurodegeneration that was most prominent in the hippocampus and amygdala.

Models of Neurodegenerative Disease

MPTP—A contaminant formed during meperidine synthesis, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Figure 16–7), produces over hours to days the signs and symptoms of irreversible Parkinson's disease. Autopsy studies have demonstrated marked degeneration of dopaminergic neurons in the substantia nigra, with degeneration continuing many years after exposure. It appears that MPTP is metabolized by two 2-electron oxidation reactions to the pyridinium ion, MPP+ , which enters the dopaminergic neurons of the substantia nigra, resulting in their deaths by blocking mitochondrial respiration at complex I. Although not identical, MPTP neurotoxicity and Parkinson's disease produce symptomatology of masked facies, difficulties in initiating and terminating movements, resting "pill-rolling" tremors, rigidity, and bradykinesias.

Manganese—As an essential trace metal that is found in all tissues, manganese (Mn) is required for normal metabolism of amino acids, proteins, lipids, and carbohydrates, acting as a cofactor of synthesis enzymes. Excessive exposure to Mn produces neurotoxicity. T e most common commercial sources of Mn include the fuel additive methylcyclopentadienyl

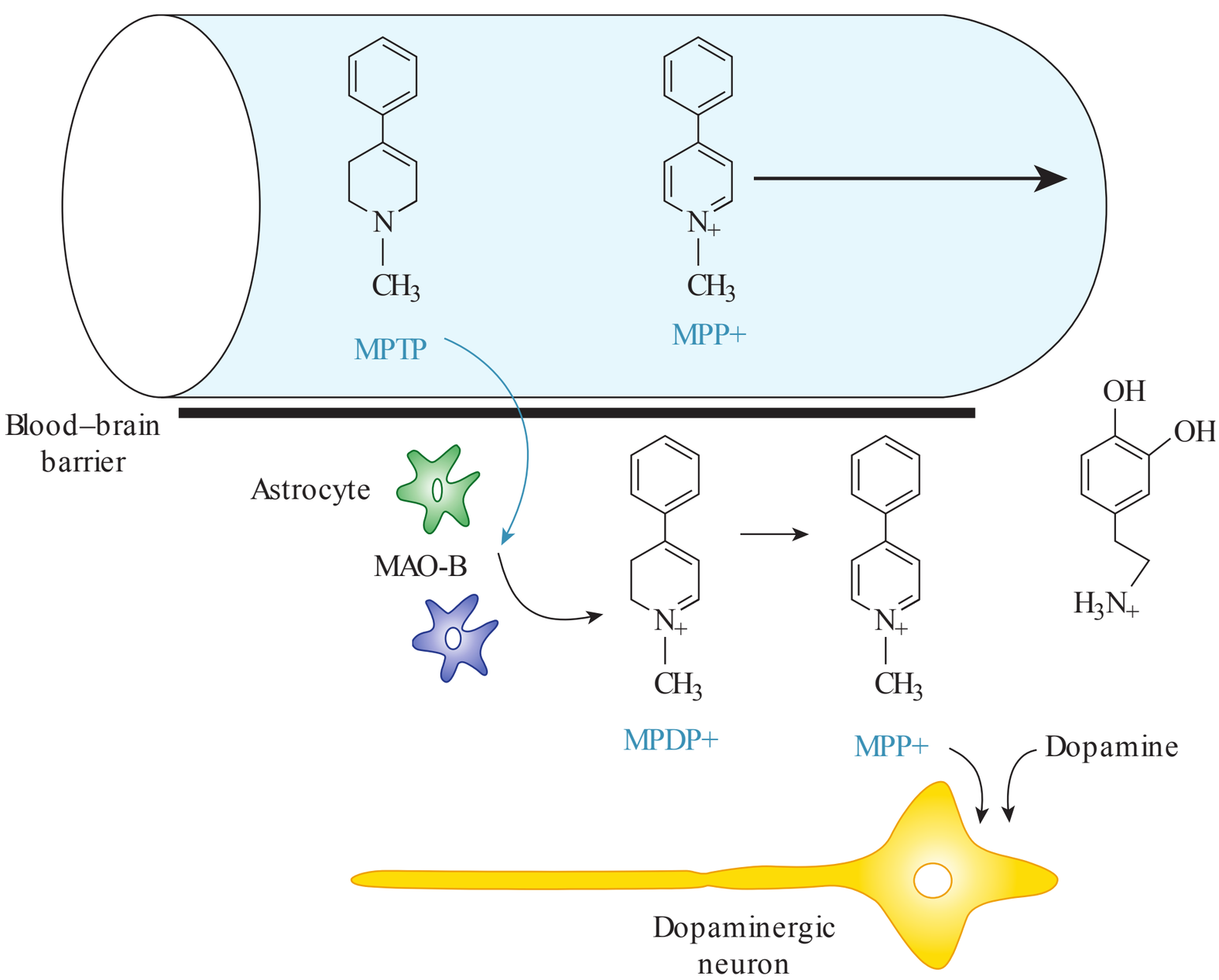

FIGURE 16–7 **MPTP toxicity.** MPP^+, either formed elsewhere in the body following exposure to MPTP or injected directly into the blood, is unable to cross the blood–brain barrier. In contrast, MPTP gains access and is oxidized in situ to $MPDP^+$ and MPP^+. The same transport system that carries dopamine into the dopaminergic neurons also transports the cytotoxic MPP^+.

manganese tricarbonyl (MMT), pesticides such as Maneb, steel factories, welding, and mining plants. Occupational exposure to toxic levels of Mn in industrial workers results in psychologic and neurologic disturbances, including delusions, hallucinations, depression, disturbed equilibrium, compulsive or violent behavior, weakness, and apathy, followed by extrapyramidal motor system defects such as tremors, muscle rigidity, ataxia, bradykinesia, and dystonia. Mn toxicity causes a loss of DA neurons in the substantia nigra, and as in Parkinson's disease, oxidative stress appears to play a significant role in the disorder.

Developmentally Neurotoxic Chemicals

Replication, migration, differentiation, myelination, and synapse formation are the basic processes that occur in specific spatial and temporal patterns and underlie development of the NS. T ere are a variety of insults known to disrupt NS development, the outcomes of which may be very different depending on the time of exposure, including exposures to certain metals, solvents, antimetabolites, persistent organic pollutants, pesticides, pharmaceuticals, and ionizing radiation. Multiple mechanisms of action may be present, producing a wide array of effects in the offspring. T e impact on the developing NS may be very different, and often cannot be predicted, from effects observed in adults. A number of neurodevelopmental disorders have been, at least partially, attributed to exposures to neurotoxicological agents during the fetal, infant, or childhood periods.

Ethanol exposure during pregnancy can result in abnormalities in the fetus, including abnormal neuronal migration and facial development, and diffuse abnormalities in the development of neuronal processes, especially the dendritic spines. T e clinical result of fetal alcohol exposure is often mental retardation, with malformations of the brain and delayed myelination of white matter.

MeHg exposure leads to developmental disabilities, including cerebral palsy, mental retardation, and seizures, in many children at birth. Children exposed to MeHg in utero show widespread neuronal loss, disruption of cellular migration, profound mental retardation, and paralysis.

T ere is considerable evidence that chronic exposure to nicotine has effects on the developing fetus. Along with decreased birth weights, attention deficit disorders are more common in children whose mothers smoke cigarettes during pregnancy, and nicotine has been shown to lead to analogous neurobehavioral abnormalities in animals exposed prenatally to nicotine.

Cocaine is able to cross the placental barrier and the fetal blood–brain barrier, and also causes reduced blood flow in the uterus. In severe events at large doses taken by the mother, the fetus may develop hypoxia, leading to a higher rate of birth defects. Maternal cocaine use is associated with low–birth weight and behavioral defects, including a decreased awareness of the surroundings and altered response to stress and pain sensitivity.

Several epidemiological studies have reported deficits in neurodevelopment and psychological performance in children exposed to polychlorinated biphenyls (PCBs) and/or dioxins. T ese persistent pollutants produce endocrine disruptions, cognitive deficits, and changes in activity levels in exposed offspring; however, the specific outcomes depend on the congener or mixture tested as well as the timing of exposure. Changes in estrogen or thyroid hormone, neurotransmitter function, and second messenger systems have been proposed as cellular bases for PCB toxicity. Another persistent class of hydrocarbons, polybrominated diphenyl ethers (PBDEs), have shown similarities in altering thyroid hormone metabolism and cholinergic function, and it has thus been proposed that this chemical class would also be developmentally neurotoxic.

CHEMICALS THAT INDUCE DEPRESSION OF NERVOUS SYSTEM FUNCTION

Generalized depression of CNS function is produced by a variety of volatile solvents, including ethanol, organics, and anesthetics. T ese solvents include several chemical classes—aliphatic and aromatic hydrocarbons, halogenated hydrocarbons, ketones, esters, alcohols, and ethers—that are small, lipophilic molecules. T ey are widely found in industry, medicine, and commercial products. Human exposure ranges from chronic low level to occupational to high levels occurring with solvent abuse. Recent research has implicated interactions with ligand-gated ion channels as well as voltage-gated calcium channels as the mechanism of generalized depression.

IN VITRO AND OTHER ALTERNATIVE APPROACHES TO NEUROTOXICOLOGY

T e goal for future studies of neurotoxicology is to replace standard in vivo assessments with high-throughput in vitro assays and quantitative structure–activity relationships (QSARs) to predict adverse outcomes. T e use of tiered testing schemes has been proposed, where the first tiers rely on high-throughput methods that test for chemical actions on key biological receptors that initiate pathways of changes that lead to adverse outcomes, in order to identify chemicals for future testing. Second tier tests could involve the use of alternative species, such as small fish or invertebrate species, that will allow more moderate throughput, but in an intact or developing NS. Chemicals identified as having neurotoxic properties could then be tested in intact mammalian models as necessary. T e extraordinary conservation of both genomic/epigenomic elements and differentiation processes between mammals and nonmammals, which has been revealed during the last two decades, makes more feasible the use of these alternative models.

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QUESTIONS

1. Which of the following statements regarding axons and/or axonal transport is FALSE?
   a. Single nerve cells can be over 1 m in length.
   b. Fast axonal transport is responsible for movement of proteins from the cell body to the axon.
   c. Anterograde transport is accomplished by the protein kinesin.
   d. T e motor proteins, kinesin and dynein, are associated with microtubules.
   e. A majority of the ATP in nerve cells is used for axonal transport.

2. Which of the following statements is not characteristic of Schwann cells in Wallerian degeneration?
   a. Schwann cells provide physical guidance needed for the regrowth of the axon.
   b. Schwann cells release trophic factors that stimulate growth.
   c. Schwann cells act to clear the myelin debris with the help of macrophages.
   d. Schwann cells increase synthesis of myelin lipids in response to axonal damage.
   e. Schwann cells are responsible for myelination of axons in the peripheral nervous system.

3. Prenatal exposure to ethanol can result in mental retardation and hearing deficits in the newborn. What is the cellular basis of the neurotoxicity?
   a. neuronal loss in cerebellum.
   b. acute cortical hemorrhage.
   c. microcephaly.
   d. loss of hippocampal neurons.
   e. degeneration of the basal ganglia.

4. Which of the following characteristics is LEAST likely to place a neuron at risk of toxic damage?
   a. high metabolic rate.
   b. ability to release neurotransmitters.
   c. long neuronal processes supported by the soma.
   d. excitable membranes.
   e. large surface area.

5. T e use of meperidine contaminated with MPTP will result in a Parkinson's disease-like neurotoxicity. Where is the most likely site in the brain that MPTP exerts its toxic effects?
   a. cerebellum.
   b. cerebral cortex.
   c. brainstem.
   d. substantia nigra.
   e. hippocampus.

6. Which of the following statements regarding the PNS and the CNS is TRUE?
   a. Nerve impulse transduction is much faster in the CNS than in the PNS.
   b. PNS axons can regenerate, whereas CNS axons cannot.
   c. Remyelination does not occur in the CNS.
   d. Oligodendrocytes perform remyelination in the PNS.
   e. In the CNS, oligodendrocyte scarring interferes with axonal regeneration.

7. Platinum (cisplatin) results in which of the following neurologic problems?
   a. peripheral neuropathy.
   b. trigeminal neuralgia.
   c. spasticity.
   d. gait ataxia.
   e. tremor.

8. Which of the following is NOT characteristic of axonopathies?
   a. T ere is degeneration of the axon.
   b. T e cell body of the neuron remains intact.
   c. Axonopathies result from chemical transaction of the axon.
   d. A majority of axonal toxicants cause motor deficits.
   e. Sensory and motor deficits are first noticed in the hands and feet following axonal degeneration.

9. All of the following statements regarding lead exposure are true EXCEPT:
   a. Lead exposure results in peripheral neuropathy.
   b. Lead slows peripheral nerve conduction in humans.
   c. Lead causes the transection of peripheral axons.
   d. Segmental demyelination is a common result of lead ingestion.
   e. Lead toxicity can result in anemia.

10. Regarding excitatory amino acids, which of the following statements is FALSE?
    a. Glutamate is the most common excitatory amino acid in the CNS.
    b. Excitotoxicity has been linked to conditions such as epilepsy.
    c. Overconsumption of monosodium glutamate (MSG) can result in a tingling or burning sensation in the face and neck.
    d. An ionotropic glutamate receptor is coupled to a G protein.
    e. Glutamate is toxic to neurons.

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CHAPTER

17

Toxic Responses of the Ocular and Visual System[1]

Donald A. Fox and William K. Boyes

INTRODUCTION TO OCULAR AND VISUAL SYSTEM TOXICOLOGY

EXPOSURE TO THE EYE AND VISUAL SYSTEM

- Ocular Pharmacodynamics and Pharmacokinetics
- Nanoparticles and Ocular Drug Delivery
- Ocular Drug Metabolism
- Central Visual System Pharmacokinetics
- Light and Phototoxicity

EVALUATING OCULAR TOXICITY AND VISUAL FUNCTION

- Evaluation of Ocular Irritancy and Toxicity
- Ophthalmologic Evaluations
- Electrophysiologic Techniques
- Behavioral and Psychophysical Techniques
- Color Vision Testing

TARGET SITES AND MECHANISMS OF ACTION: CORNEA

- Acids
- Bases or Alkalies
- Organic Solvents
- Surfactants

TARGET SITES AND MECHANISMS OF ACTION: LENS

- Corticosteroids
- Naphthalene
- Phenothiazines

TARGET SITES AND MECHANISMS OF ACTION: RETINA

- Retinotoxicity of Systemically Administered Therapeutic Drugs
  - Cancer Chemotherapeutics
  - Chloroquine and Hydroxychloroquine
  - Digoxin and Digitoxin
  - Indomethacin
  - Sildenafil Citrate
  - Tamoxifen
  - Vigabatrin
- Retinotoxicity of Known Neurotoxicants
  - Inorganic Lead
  - Methanol
  - Organic Solvents

TARGET SITES AND MECHANISMS OF ACTION: OPTIC NERVE AND TRACT

- Acrylamide
- Carbon Disulfide
- Ethambutol

TARGET SITES AND MECHANISMS OF ACTION: THE CENTRAL VISUAL SYSTEM

- Lead
- Methyl Mercury

[1]This chapter has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. EPA, and approved for publication.

KEY POINTS

- Toxic chemicals and systemic drugs can affect all parts of the eye, including cornea, iris, ciliary body, lens retina, and optic nerve.
- Ophthalmologic procedures for evaluating the health of the eye include routine clinical screening evaluations using a slit-lamp biomicroscope and ophthalmoscope, and an examination of the pupillary light reflex.
- Most electrophysiologic or neurophysiologic procedures for testing visual function after toxicant exposure involve stimulating the eyes with visual stimuli and electrically recording potentials generated by visually responsive neurons.

INTRODUCTION TO OCULAR AND VISUAL SYSTEM TOXICOLOGY

Environmental and occupational exposure to toxic chemicals, gases, and vapors as well as side effects resulting from therapeutic drugs frequently result in structural and functional alterations in the eye and central visual system. T e retina and central visual system are especially vulnerable to toxic insult.

EXPOSURE TO THE EYE AND VISUAL SYSTEM

Ocular Pharmacodynamics and Pharmacokinetics

Toxic chemicals and systemic drugs can affect all parts of the eye (Figure 17–1; Tables 17–1 and 17–2). Factors determining whether a chemical can reach a particular ocular site of action include physiochemical properties of the chemical, concentration and duration of exposure, and movement across ocular compartments and barriers. T e cornea, conjunctiva, and eyelids are often exposed directly to chemicals, gases, drugs, and particles. T e first site of action is the tear film, a three-layered structure with both hydrophobic and hydrophilic properties. T e outermost thin tear film layer is secreted by the meibomian (sebaceous) glands. T is superficial lipid layer protects the underlying thicker aqueous layer that is produced by the lacrimal glands. T e third layer is the very thin mucoid layer that is secreted by the goblet cells of the conjunctiva and acts as an interface between the hydrophilic layer of the tears and the hydrophobic layer of the corneal epithelial cells.

T e avascular cornea is considered the external barrier to the internal ocular structures. Greater systemic absorption occurs through contact with the vascularized conjunctiva (Figure 17–2). T e human cornea has several distinct layers through which a chemical must pass in order to reach the anterior chamber. T e first is the corneal epithelium of stratified squamous, nonkeratinized cells with tight junctions. T e permeability of the corneal epithelium is low and only lipid-soluble chemicals readily pass through this layer. Bowman's membrane separates the epithelium from the stroma. T e corneal stroma comprises 90% of the corneal thickness and is composed of water, collagen, and glycosaminoglycans, which permits hydrophilic chemicals to easily dissolve in this thick layer. T e inner edge of the corneal stroma is bounded by a thin basement membrane, called Descemet's membrane, which is secreted by the corneal endothelium. T e innermost layer of the cornea, the corneal endothelium, is composed of a single layer of cells that are surrounded by lipid membranes. T e permeability of the corneal endothelial cells to ionized chemicals is relatively low.

T ere are two separate vascular systems in the eye: (1) the uveal blood vessels, which include the vascular beds of the iris, ciliary body, and choroid, and (2) the retinal vessels. In the anterior segment of the eye, there is a blood–aqueous barrier that has relatively tight junctions between the endothelial cells of the iris capillaries and nonpigmented cells of the ciliary epithelium. T e major function of the ciliary epithelium is to produce aqueous humor from the plasma filtrate present in the stroma of the ciliary processes.

In humans and several widely used experimental animals (e.g., monkeys, pigs, dogs, rats, and mice), the retina has a dual circulatory supply: choroidal and retinal. T e retina consists of the outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), and ganglion cell layer (GCL). T e endothelial cells of capillaries of the retinal vessels have tight junctions forming the blood–retinal barrier. However, at the level of the optic disk, the blood–retinal barrier is lacking and thus hydrophilic molecules can enter the optic nerve (ON) head by diffusion from the extravascular space and cause selective damage at this site of action. T e outer or distal retina, which consists of the retinal pigment epithelium (RPE), rod and cone photoreceptor outer segments (ROS and COS) and inner segments (RIS and CIS), and the photoreceptor outer nuclear layer (ONL) are avascular. T ese areas of the retina are supplied by the choriocapillaris: a dense, one-layered network of fenestrated vessels formed by the short posterior ciliary arteries and located next to the RPE. Consistent with their known structure and function, these capillaries have loose endothelial junctions and abundant fenestrae; they are highly permeable to large proteins.

Following systemic exposure to drugs and chemicals by the oral, inhalation, dermal, or parenteral route, these compounds are distributed to all parts of the eye by the blood in the uveal blood vessels and retinal vessels (Figure 17–3). Most chemicals rapidly equilibrate with the extravascular space of the

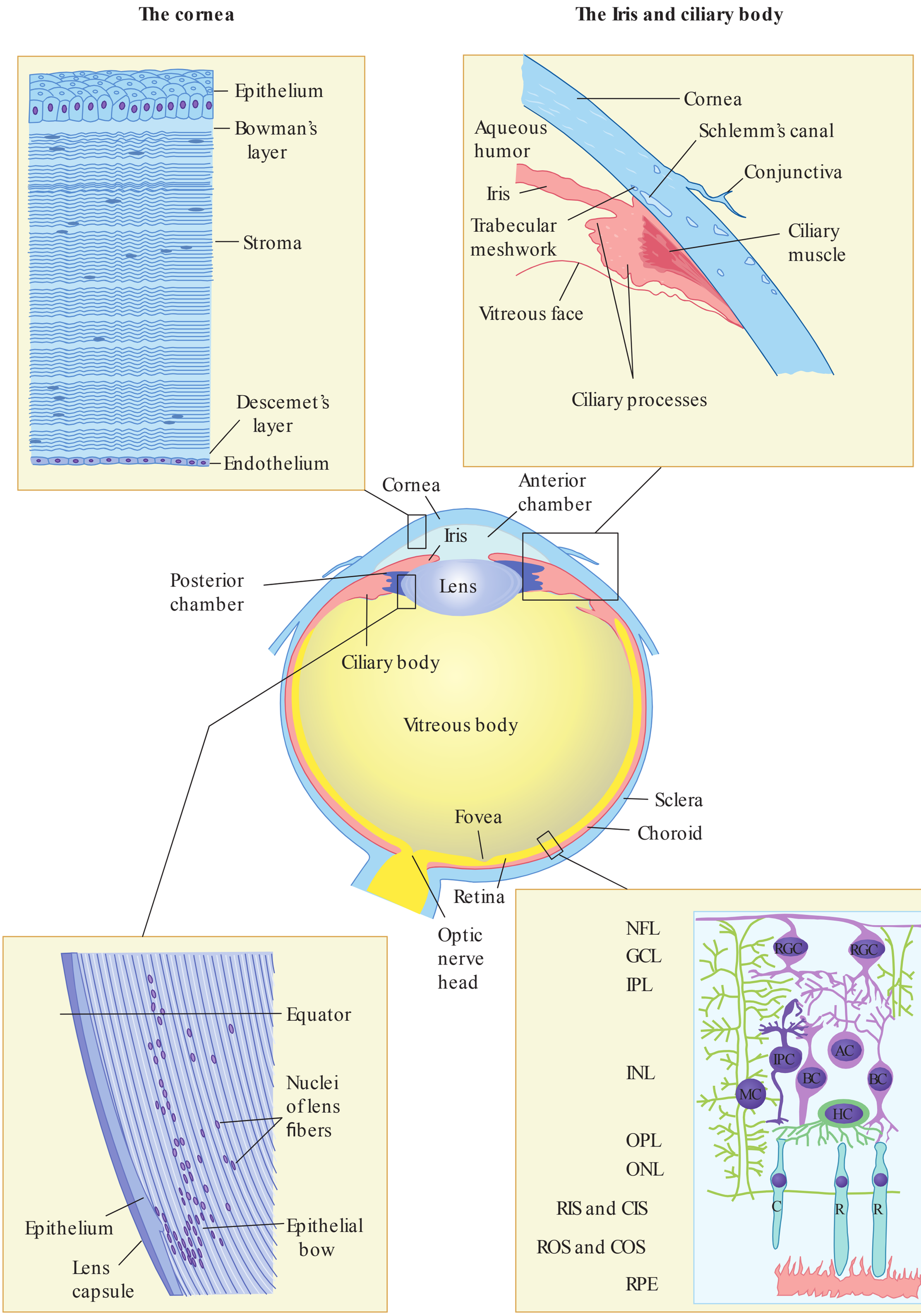

FIGURE 17–1 **Diagrammatic horizontal cross-section of the eye, with medium-power enlargement of details for the cornea, iris and ciliary body, lens, and retina.** The morphologic features, their role in ocular pharmacodynamics, pharmacokinetics, drug metabolism, and the adverse effects of drugs and chemical agents on these sites are discussed in the text.

TABLE 17–1 Ocular and central visual system sites of action of selected xenobiotics following systemic exposure.

Xenobiotic	Cornea	Lens	Outer Retina: RPE	Outer Retina: Rods and Cones	Inner Retina: BCs, ACs, IPCs	RGCs, Optic Nerve or Tract	LGN, Visual Cortex
Acrylamide				–	–	++	++
Amiodarone	+	+				+	
Carbon disulfide				+	–	++	+
Chloroquine	+		+	+		+	
Chlorpromazine	+	+	+	+			
Corticosteroids		++				+	
Digoxin and digitoxin	+	+	+	++		+	+
Ethambutol				+		++	
Hexachlorophene				+		+	+
Indomethacin	+		+	+			
Isotretinoin	+						
Lead	+		+	++	+	+	+
Methanol			+	++	–	++	+
Methyl mercury, mercury				+	–	–	++
n-Hexane			+	+		+	
Naphthalene		+		+			
Organic solvents				+			+
Organophosphates		+		+		+	+
Styrene				+			
Tamoxifen	+			+		+	
Vigabatrin	–	–	–	+	+	+	–

RPE, retinal pigment epithelium; BC, bipolar cell; AC, amacrine cell; IPC, interplexiform cell; RGC, retinal ganglion cell; LGN, lateral geniculate nucleus.
"+ "and "– "indicate that this site of action was cited as being positively affected or not affected by the exposure to the toxicant.

choroid where they are separated from the retina and vitreous body by the RPE and endothelial cells of the retinal capillaries, respectively. Hydrophilic molecules with molecular weights less than 200 to 300 Da can cross the ciliary epithelium and iris capillaries and enter the aqueous humor. T us, the corneal endothelium—the cells responsible for maintaining normal hydration and transparency of the corneal stroma—could be exposed to chemical compounds by the aqueous humor and limbal capillaries. Similarly, the anterior surface of the lens also can be exposed as a result of its contact with the aqueous humor. T e most likely retinal target sites following systemic drug and chemical exposure appear to be the RPE and photoreceptors, because the endothelial cells of the choriocapillaris are permeable to proteins smaller than 50 to 70 kDa. However, the cells of the RPE are joined on their basolateral surface by tight junctions that limit the passive penetration of large molecules into the neural retina.

Intraocular melanin plays a special role in ocular toxicology. First, it is found in several different locations in the eye: pigmented cells of the iris, ciliary body, RPE, and uveal tract. Second, it has a high binding af nity for polycyclic aromatic hydrocarbons, electrophiles, calcium, and toxic heavy metals such as aluminum, iron, lead, and mercury. Although this initially may play a protective role, the excessive accumulation, long-term storage, and slow release of numerous drugs and chemicals from melanin can influence toxicity.

Nanoparticles and Ocular Drug Delivery

T e main ocular target sites of importance for disease treatment and neuroprotection are the anterior segment and posterior retina. As noted above, there are numerous barriers that restrict bioavailability, decrease therapeutic ef cacy, and increase side effects. Development of nanoscale preparations for drug delivery is a new

TABLE 17–2 Common signs and symptoms of visual system dysfunction.

Common Signs and Symptoms	Possible Pathophysiological Basis	Examples of Chemicals Producing this Effect
Cornea		
Pigment deposits in corneal epithelium (verticillate keratopathy)	Intralysosomal accumulation of lipids	Amiodarone, chloroquine, clofazimine, phenothiazines, suramin
Lens		
Cataracts: anterior cortical (AC), posterior subcapsular (PSC)	Chemical deposition, photochemical oxidation	Long-term systemic use of phenothiazine (AC), corticosteroids (PSC)
Pupil		
Pupil constriction (miosis)	Anticholinesterases	Organophosphate and carbamate insecticides, nerve gas agents such as sarin, soman, and tabun
Pupil dilation (mydriasis) and photophobia	Cholinergic antagonists	Atropine or belladonna alkaloids
	Adrenergic agonists	Amphetamine, cocaine, phenylephrine
Ocular motility		
Diplopia (double vision), nystagmus	Oculomotor impairment, damage or dysfunction in vestibular/oculomotor ref ex pathways	Acute alcohol intoxication, barbiturate toxicity, acute solvent exposure
Retinal pigment epithelium		
Loss of central vision (central scotoma)	Degeneration of retinal pigment epithelium and underlying photoreceptors	Carbon disulfide, chloroquine
Retina		
Poor night (scotopic) vision and impaired dark adaptation	Damage to and apoptosis of rod photoreceptors	Lead, methyl mercury, vigabatrin
	Acetylcholinesterase inhibitors	Organophosphate and carbamate insecticides, nerve gas agents
Altered color perception, central scotoma	Inhibition of cone photoreceptor sodium-pumps	Digitalis/digitoxin
Altered color perception	Inhibition of cone photoreceptor cGMP-phosphodiesterase	Sildenafil and tadalafil
Impaired color discrimination (blue/yellow)	Damage to cone photoreceptors and inner retina	Chronic exposure to styrene and organic solvents, trimethadione, chronic high-dose antibiotics
Impaired color discrimination (red/green)	Acquired damage to cone photoreceptors, neural retina, and/or afferent visual pathway	Higher level chronic exposure to organic solvents, carbon disulfide or hexane, chronic carbon monoxide, chronic alcoholism, ethambutol
Loss of peripheral vision (tunnel vision, peripheral scotoma, visual field constriction)	Degeneration of peripheral retina and nerve fiber layer	Methyl mercury, vigabatrin
Reduced contrast sensitivity and visual acuity	Degeneration of the retinal ganglion cells and optic tract, microaneurysms and retinal vasculopathy	Acrylamide, carbon disulfide
Optic nerve and optic tract		
Reduced contrast sensitivity and visual acuity	Optic neuritis and/or degeneration of the optic tract, generally affecting mitochondrial ATP production	Higher level chronic exposure to organic solvents such as carbon disulfide or hexane, ethambutol, ethylene glycol, isoniazid, linezolid and chloramphenicol, methanol, vigabatrin
Monocular and/or binocular visual loss	Nonarteretic anterior ischemic optic neuropathy	Amiodarone, sildenafil and tadalafil
Lateral geniculate and visual cortex		
Central scotoma	Degeneration of calcarine fissure of visual cortex	Methyl mercury
Visuomotor deficits and reduced contrast sensitivity	Visual and motor cortex dysfunction	Lead, chronic exposure to carbon disulfide, hexane and other solvents, toluene

Inclusion in this table indicates that this drug, chemical, or toxicant was cited in one or more case reports, review articles, or clinical or animal studies. The pathophysiological causes and chemicals listed are provided as examples and are not exhaustive.

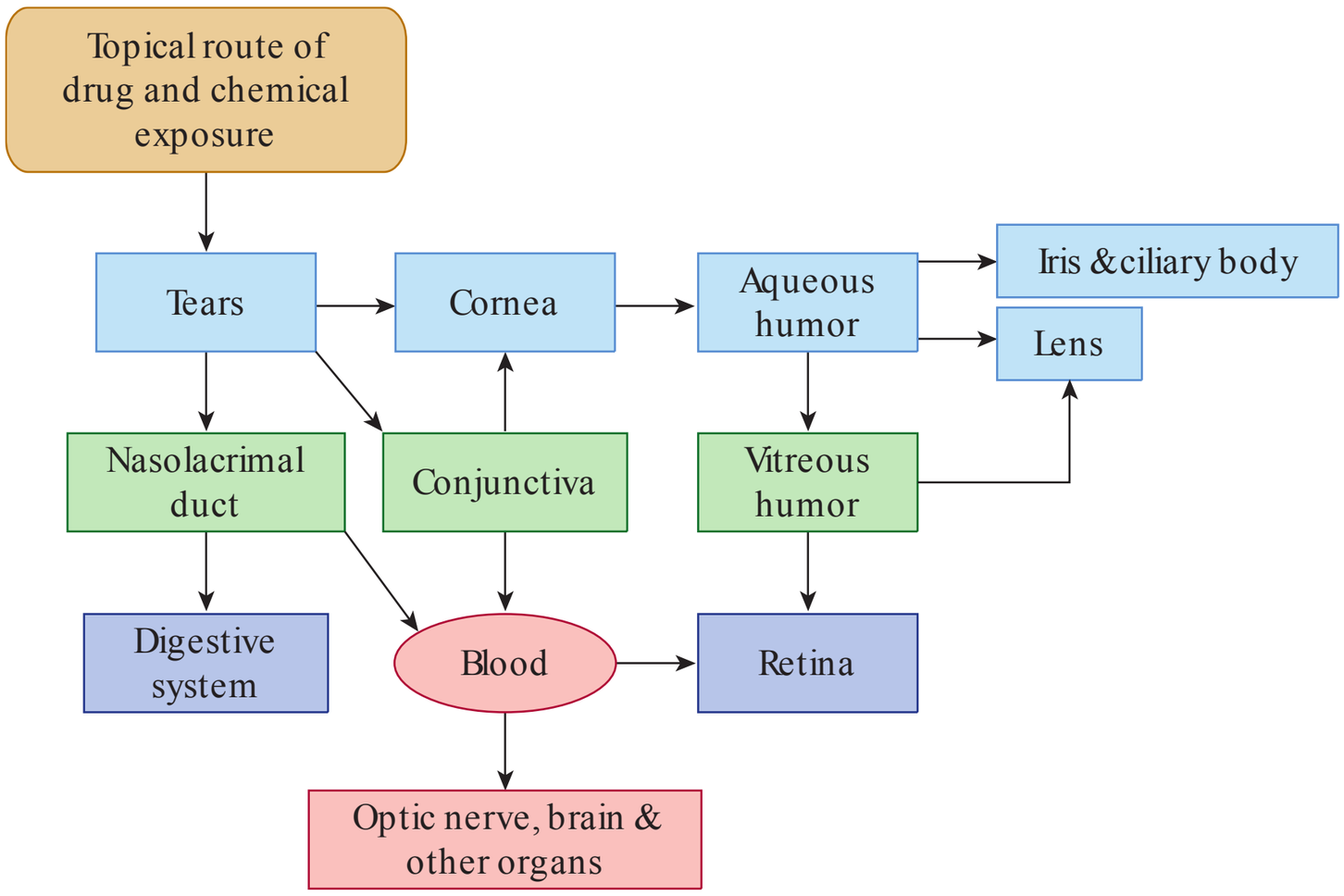

FIGURE 17–2 **Ocular absorption and distribution of drugs and chemicals following the topical route of exposure.** The details for movement of drugs and chemicals between compartments of the eye and subsequently to the optic nerve, brain, and other organs are discussed in the text.

approach to drug delivery which can substantially enhance penetration from the cornea, deliver a wide variety of drugs and molecules, and increase the concentration and contact time of drugs with these tissues. A wide variety of nanoformulations have been considered including solid lipid nanoparticles containing lipids, phospholipids, and/or metals; liposomes; nanosuspensions; and emulsions; and the use of biocompatible coatings such as chitosan. Metallic particles that enable remote magnetic targeting of drug delivery also are under development.

Ocular Drug Metabolism

Metabolism of xenobiotics occurs in all compartments of the eye by well-known phase I and II xenobiotic-biotransforming enzymes. Drug-metabolizing enzymes that are present in the tears, iris–ciliary body, choroid, and retina of many different species are listed in Table 17–3. Whereas the activity of these enzymes varies between species and ocular tissues, the whole lens has low biotransformational activity.

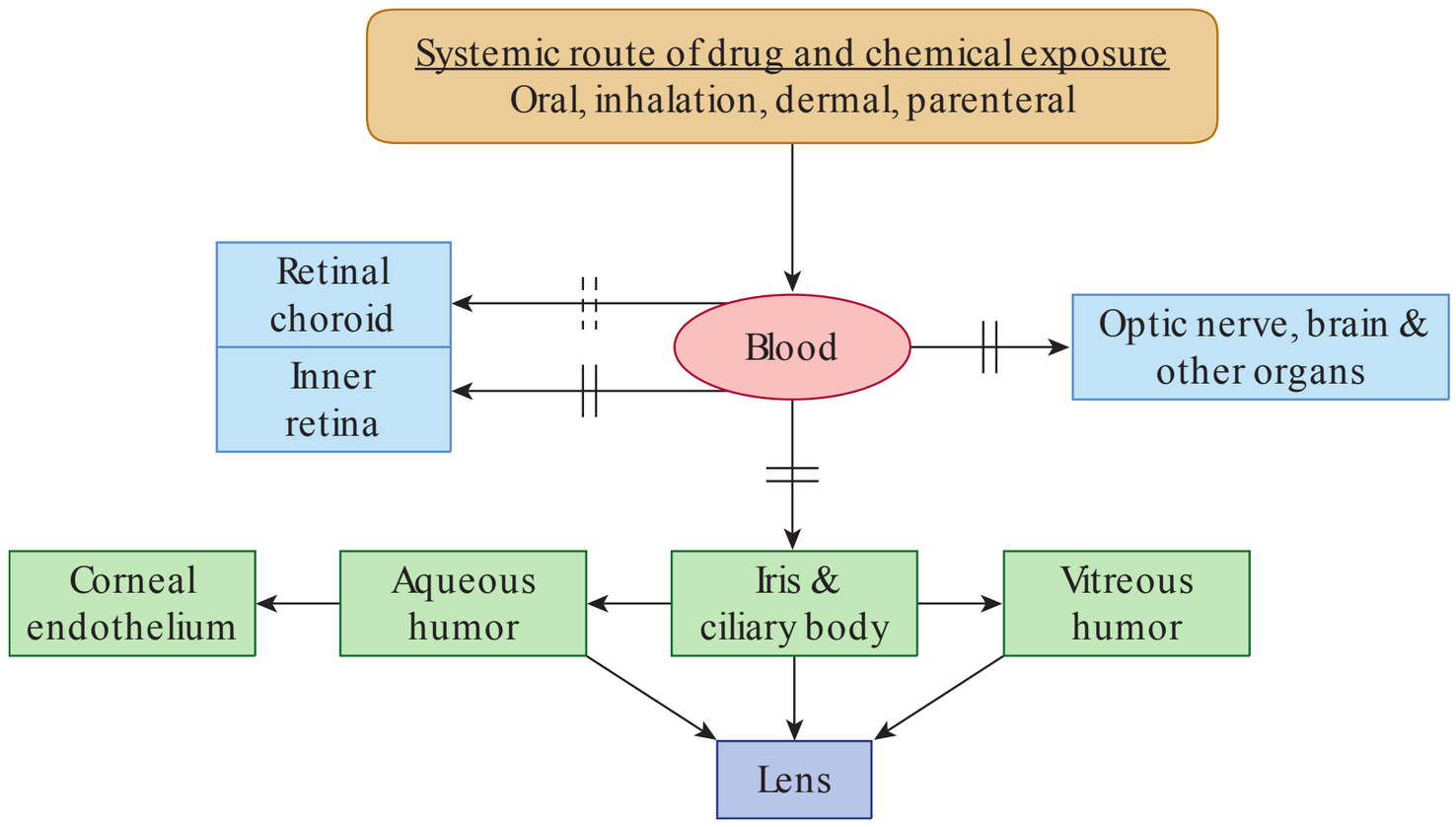

FIGURE 17–3 **Distribution of drugs and chemicals in the anterior and posterior segments of the eye, optic nerve, brain, and other organs following the systemic route of exposure.** The details for movement of drugs and chemicals between compartments of the eye are discussed in the text. The solid and dotted double lines represent the different blood–tissue barriers present in the anterior segment of the eye, retina, optic nerve, and brain. The solid double lines represent tight endothelial junctions, whereas the dotted double lines represent loose endothelial junctions.

TABLE 17–3 Distribution of ocular xenobiotic-biotransforming enzymes.

Enzymes	Tears	Cornea	Iris/Ciliary	Lens	Retina	Choroid
Phase I reactions						
Acetylcholinesterase (AChE)	+		+		+	+
Alcohol dehydrogenase		+		–	+	+
Aldehyde dehydrogenase		+		+	+	+
Aldehyde reductase		+	+	+	+	+
Aldose reductase		+		+	+	
Carboxylesterase	+	+	+		+	+
Catalase	–	+	+	+	+	+
Cu/Zn superoxide dismutase	+	+		– /+	+	
CYP1A1 or CYP1A2	+	+	+	–	+	+
CYP1B1		+	+	+	+	
CYP2B1 or CYP2B2				+	+	
CYP2C11				+		
CYP3A1				+	+	
CYP4A1 or CYP4B2		+	+		+	
CYP27A1					+	
MAO-A or MAO-B	+		+		+	+
Phase II reactions						
Glutathione peroxidase	–	+	+	+	+	+
Glutathione reductase		+		+	+	
Glutathione S-transferase		+	+	+	+	
Sulfotransferases				+	+	
UDP-glucuronosyltransferases				+	+	
N-Acetyltransferase		+	+	+	+	+

"+ "and "– "indicate that the enzyme was present (localized by immunohistochemistry, immunogold electron microscopy, Western blot, or gene expression) or absent, respectively, in human, monkey, or rodent tissues.

Central Visual System Pharmacokinetics

T e penetration of potentially toxic compounds into visual areas of the central nervous system (CNS) is governed by the blood–brain barrier (Figure 17–3), which is differentially permeable to compounds depending on their size, charge, and lipophilicity. Compounds that are large, highly charged, or otherwise not very lipid soluble tend to be excluded from the brain, whereas smaller, uncharged, and lipid-soluble compounds more readily penetrate into the brain tissue. In some cases, toxic compounds may be actively transported into the brain by mimicking the natural substrates of active transport systems. One area of the brain lacking a blood–brain barrier is the ON near the lamina cribrosa, which could cause this part of the central visual system to be vulnerable to exposures that do not affect much of the remainder of the brain.

Light and Phototoxicity

T e most important oxidizing agents are visible light and UV radiation, particularly UV-A (320 to 400 nm) and UV-B (290 to 320 nm), and other forms of electromagnetic radiation. Light- and UV-induced photooxidation leads to generation of reactive oxygen species (ROS), and oxidative damage that can accumulate over time. Higher energy UV-C (100 to 290 nm) is even more damaging. At sea level,

the atmosphere filters out virtually all UV-C and all but a small fraction of UV-B derived from solar radiance. The cornea absorbs about 45% of light with wavelengths below 280 nm, but only about 12% between 320 and 400 nm. The lens absorbs much of the light between 300 and 400 nm and transmits 400 nm and above to the retina. Absorption of light energy in the lens triggers a variety of photoreactions, including the generation of fluorophores and pigments that lead to the yellow-brown coloration of the lens. Sufficient exposure to infrared radiation, as occurs to glassblowers, or microwave radiation will also produce cataracts through direct heating of the ocular tissues.

Drugs and other chemicals can mediate photo-induced toxicity in the cornea, lens, or retina. This occurs when the chemical structure allows absorption of light energy and the subsequent generation of activated intermediates, free radicals, and ROS. The propensity of chemicals to cause phototoxic reactions can be predicted using photophysical and in vitro procedures.

The phototoxic properties of chemicals are being exploited for photodynamic therapies where photoactive chemicals are delivered to pathological tissues. Wavelength-specific light is introduced to the tissue causing the photoactive chemical to activate thereby initiating a free-radical cascade that kills the pathological tissues. These agents also are being developed to utilize long wavelengths near the red/infrared end of the spectrum where the irradiation penetrates deeper into the tissue.

EVALUATING OCULAR TOXICITY AND VISUAL FUNCTION

Evaluation of Ocular Irritancy and Toxicity

The so-called Draize test, with some additions and revisions, has formed the basis of standard procedures employed for evaluating ocular irritation and safety evaluations. Traditionally, albino rabbits are the subjects evaluated in the Draize test. The procedure involves instillation of 0.1 mL of a liquid or 100 mg of a solid into the conjunctival sac of one eye and then gently holding the eye closed for 1 s. The untreated eye serves as a control. Both eyes are evaluated at 1, 24, 48, and 72 h after treatment. If there is evidence of damage in the treated eye at 72 h, the examination time may be extended. The cornea, iris, and conjunctiva are evaluated and scored according to a weighted scale. The cornea is scored for both the degree of opacity and area of involvement, with each measure having a potential range from 0 (none) to 4 (most severe). The iris receives a single score (0 to 2) for irritation, including degree of swelling, congestion, and degree of reaction to light. The conjunctiva is scored for the redness (0 to 3), chemosis (swelling 0 to 4), and discharge (0 to 3). The individual scores are then multiplied by a weighting factor: 5 for the cornea, 2 for the iris, and 5 for the conjunctiva. The results are summed for a maximum total score of 110. In this scale, the cornea accounts for 73% of the total possible points, in accordance with the severity associated with corneal injury.

The Draize test has been criticized on several grounds, including high interlaboratory variability, the subjective nature of the scoring, poor predictive value for human irritants, and for causing undue pain and distress to the tested animals. These criticisms have spawned development of alternative methods or strategies to evaluate compounds for their potential to cause ocular irritation.

Ophthalmologic Evaluations

There are many ophthalmologic procedures for evaluating the health of the eye. Procedures available range from fairly routine clinical screening evaluations to sophisticated techniques for targeted purposes. Examination of the adnexa includes evaluating the eyelids, lacrimal apparatus, and palpebral (covering the eyelid) and bulbar (covering the eye) conjunctiva. The anterior structures or anterior segment include the cornea, iris, lens, and anterior chamber. The posterior structures, referred to as the ocular fundus, include the retina, retinal vasculature, choroid, ON, and sclera. The adnexa and surface of the cornea can be examined initially with the naked eye, a hand-held light, or a slit-lamp biomicroscope, using a mydriatic drug (which causes pupil dilation) if the lens is to be observed. The width of the reflection of a thin beam of light projected from the slit-lamp is an indication of the thickness of the cornea and may be used to evaluate corneal edema. Lesions of the cornea can be better visualized with the use of fluorescein dye, which is retained where there is an ulceration of the corneal epithelium. Examination of the fundus requires use of a mydriatic drug and a direct or an indirect ophthalmoscope.

An examination of the direct pupillary reflex involves shining a bright light into the eye and observing the reflexive pupil constriction in the same eye. The consensual pupillary reflex is observed in the eye not stimulated. Both the direct and consensual pupillary light reflexes are dependent on function of a reflex arc involving cells in the retina, which travel through the ON, optic chiasm, and optic tract (OT) to project to neurons in the pretectal area. The absence of a pupillary reflex is indicative of damage somewhere in the reflex pathway, and differential impairment of the direct or consensual reflexes can indicate the location of the lesion. The presence of a pupillary light reflex, however, is not synonymous with normal visual function. Pupillary reflexes can be maintained even with substantial retinal damage. In addition, lesions in visual areas outside of the reflex pathway, such as in the visual cortex, may also leave the reflex function intact.

Electrophysiologic Techniques

Most electrophysiologic or neurophysiologic procedures for testing visual function in a toxicologic context involve stimulating the eyes with visual stimuli and electrically recording potentials generated by visually responsive neurons. The most commonly used procedures are the flash-evoked electroretinogram (ERG), visual-evoked potentials (VEPs), and, less often, the electrooculogram (EOG).

ERGs are typically elicited with a brief flash of light and recorded from an electrode placed in contact with the cornea. A typical ERG waveform includes an a-wave that reflects the activation of photoreceptors and a b-wave that reflects the activity of retinal bipolar cells (BC) and associated membrane potential changes in Müller cells (MC). A standard set of ERG procedures includes the recording of (1) a response reflective of only rod photoreceptor function in the dark-adapted eye, (2) the maximal response in the dark-adapted eye, (3) a response developed by cone photoreceptors, (4) oscillatory potentials, and (5) the response to rapidly flickered light.

Flash-elicited VEPs are recorded from electrodes overlying visual (striate) cortex, and they reflect the activity of the retinogeniculostriate pathway and the activity of cells in the visual cortex. Pattern-elicited VEPs (PEPs), which are widely used in human clinical evaluations, have diagnostic value.

T e EOG is generated by a potential difference between the front and back of the eye, which originates primarily within the RPE. T e magnitude of the EOG is a function of the level of illumination and health status of the RPE. Electrodes placed on the skin on a line lateral or vertical to the eye measure potential changes correlated with eye movements as the relative position of the ocular dipole changes. T us, the EOG finds applications in assessing both RPE status and measuring eye movements. T e EOG is also used in monitoring eye movements during the recording of other brain potentials, so that eye movement artifacts are not misinterpreted as brain-generated electrical activity.

Behavioral and Psychophysical Techniques

Behavioral testing procedures typically vary the parameters of the visual stimulus and then determine whether the subject can discriminate or perceive the stimulus. Contrast sensitivity refers to the ability to resolve small differences in luminance contrast, such as the difference between subtle shades of gray or a series of visual patterns that differ in pattern size, or the luminance changes across the pattern in a sinusoidal profile. Contrast sensitivity functions depend primarily on the neural as opposed to the optic properties of the visual system. T e assessment of visual acuity and contrast sensitivity has been recommended for field studies of humans potentially exposed to neurotoxic substances.

Color Vision Testing

Color vision deficits are either inherited or acquired. Hereditary red–green color deficits occur in about 8% of males (X-linked) whereas only about 0.5% of females show similar congenital deficits. Inherited color deficiencies take two common forms: protan, a red–green confusion caused by abnormality or absence of the long-wavelength (red) sensitive cones (L-type cones); and deutan caused by abnormality or absence of the middle-wavelength sensitive (green) cones (M-type cones). Dutanopes demonstrate a concomitant confusion of red–green and blue–yellow due to the lack of M-type cones. Congenital loss of short-wavelength cones, resulting in a blue–yellow confusion (tritanopia, or type III), is extremely rare. Most acquired color vision deficits, such as those caused by drug and chemical exposure, begin with a reduced ability to perform blue–yellow discriminations. With increased or prolonged low-level exposure, the color confusion can progress to the red–green axis as well. Because of the rarity of inherited tritanopia, it is generally assumed that blue–yellow deficits, when observed, are acquired deficits. Generally, disorders of the outer retina produce blue–yellow deficits, whereas disorders of the inner retina and ON produce red–green perceptual deficits. Bilateral lesions in the visual cortex can also lead to color blindness.

Assessment of color vision in human toxicologic evaluations includes the Farnsworth–Munson 100 Hue (FM-100) test and the simplified 15-chip tests using either the saturated hues of the Farnsworth D-15 or the desaturated hues of the Lanthony Desaturated Panel D-15. T e Farnsworth–Munson procedure involves arrangement of 85 chips in order of progressively changing color. T e relative chromatic value of successive chips induces those with color perception deficits to abnormally arrange the chips. T e pattern is indicative of the nature of the color perception anomaly. T e FM-100 is considered more diagnostically reliable but takes considerably longer to administer than the similar but more ef cient Farnsworth and Lanthony tests. T e desaturated hues of the Lanthony D-15 are designed to better identify subtle acquired color vision deficits.

TARGET SITES AND MECHANISMS OF ACTION: CORNEA

T e cornea provides three essential functions. First, it provides a clear refractive surface and the curvature of the cornea must be correct for the visual image to be focused at the retina. Second, the cornea provides tensile strength to maintain the appropriate shape of the globe. T ird, the cornea protects the eye from external factors, including potentially toxic chemicals.

T e cornea is transparent to wavelengths of light ranging between 310 nm (UV) and 2 500 nm (IR). Exposure to UV light below this range can damage the cornea. It is most sensitive to wavelengths of approximately 270 nm. Excessive UV exposure leads to photokeratitis and corneal pathology, the classic example being welder's-arc burns. Also, the cornea can be damaged by topical or systemic exposure to chemicals.

Direct chemical exposure to the eye requires emergency medical attention. Products at pH extremes $\leq$ 2.5 or $\geq$ 11.5 can cause severe ocular damage and permanent loss of vision. Damage that extends to the corneal endothelium is associated with poor repair and recovery. T e most important therapy is immediate and adequate irrigation with large amounts of water or saline. T e extent of damage to the eye and the ability to achieve a full recovery depend on the nature of the chemical, the concentration and duration of exposure, and the speed and magnitude of the initial irrigation.

Acids

Among the most significant acidic chemicals in terms of the tendency to cause clinical ocular damage are hydrofluoric acid, sulfurous acid, sulfuric acid, and chromic acid, followed by hydrochloric and nitric acid and finally acetic acid. Injuries may be mild if contact is with weak acids or with dilute solutions of strong acids. Compounds with a pH between 2.5 and 7 produce pain or stinging, but with only a brief contact, they will cause no lasting damage. Following mild burns, the corneal epithelium may become turbid as the corneal stroma swells (chemosis). Mild burns are typically followed by rapid regeneration of the corneal epithelium and full recovery. In more severe burns, the epithelium of the cornea and conjunctiva become opaque and necrotic and may disintegrate over the course of a few days. In severe burns, there may be no sensation of pain because the corneal nerve endings are destroyed.

Acid chemical burns of the cornea occur through hydrogen ion–induced denaturing and coagulation of proteins. As epithelial cell proteins coagulate, glycosaminoglycans precipitate and stromal collagen fibers shrink causing the cornea to become cloudy. T e protein coagulation and shrinkage of the collagen is protective in that it forms a barrier and reduces further penetration of the acid. T e collagen shrinkage, however, contracts the eye and can lead to a dangerous acute increase in intraocular pressure.

Bases or Alkalies

Compounds with a basic pH are potentially more damaging to the eye than are strong acids. Among the compounds of clinical significance in terms of frequency and severity of injuries are ammonia or ammonium hydroxide, sodium hydroxide (lye), potassium hydroxide (caustic potash), calcium hydroxide (lime), and magnesium hydroxide. One reason that caustic agents are so dangerous is their ability to rapidly penetrate the ocular tissues. T e toxicity of these substances is a function of their pH, being more toxic with increasing pH values. Rapid and extensive irrigation after exposure and removal of particles, if present, is the immediate therapy of choice.

Caustic burns differ from acid burns in that two phases of injury may be observed with caustic burns. T ere is an acute phase from time of exposure up to 1 week. Depending on the extent of injury, direct damage from exposure is observed in the cornea, adnexia, and possibly the iris, ciliary body, and lens. Strong alkali substances attack membrane lipids, causing necrosis, hydration of the collagen matrix, and corneal swelling. Intraocular pressure may increase. Conversely, if the alkali burn extends to involve the ciliary body, the intraocular pressure may decrease due to reduced formation of aqueous humor. T e acute phase of damage is typically followed by initiation of corneal repair. T e repair process may involve corneal neovascularization along with regeneration of the corneal epithelium. Approximately 2 to 3 weeks after alkali burns, however, damaging ulceration of the corneal stroma often occurs as a result of inflammatory infiltration of polymorphonuclear leukocytes and fibroblasts and the release of proteolytic enzymes. Stromal ulceration usually stops when the corneal epithelium is restored.

Organic Solvents

When organic solvents are splashed into the eye, the result is typically a painful immediate reaction. Exposure of the eye to solvents should be treated rapidly with abundant water irrigation. Highly lipophilic solvents can damage the corneal epithelium and produce swelling of the corneal stroma. Most organic solvents cause minimal chemical burns to the cornea. In most cases, the corneal epithelium will be repaired over the course of a few days and there will be no residual damage. Exposure to solvent vapors may produce small transparent vacuoles in the corneal epithelium, which may be asymptomatic or associated with moderate irritation and tearing.

Surfactants

T ese compounds have water-soluble (hydrophilic) properties at one end of the molecule and lipophilic properties at the other end that help to dissolve fatty substances in water and also serve to reduce water surface tension. T e widespread use of these agents in soaps, shampoos, detergents, cosmetics, and similar consumer products leads to abundant opportunities for exposure to ocular tissues. Many of these agents may be irritating or injurious to the eye. In general, cationic surfactants tend to be stronger irritants and more injurious than the other types, and anionic compounds more so than neutral ones. Because these compounds are soluble in both aqueous and lipid media, they readily penetrate the sandwiched aqueous and lipid barriers of the cornea.

TARGET SITES AND MECHANISMS OF ACTION: LENS

T e lens of the eye plays a critical role in focusing the visual image on the retina. T e lens is a biconvex transparent body, encased in an elastic capsule, and located between the pupil and the vitreous humor (Figure 17–1). T e mature lens has a dense inner nuclear region surrounded by the lens cortex. T e high transparency of the lens to visible wavelengths of light is a function of its chemical composition, approximately two-thirds water and one-third protein, and the special organizational structure of the lenticular proteins. Nutrients provided from the aqueous and vitreous fluids are transported into the lens substance through a system of intercellular gap-type junctions. T e lens is a metabolically active tissue that maintains careful electrolyte and ionic balance. T e lens continues to grow throughout life, with new cells added to the epithelial margin of the lens as the older cells condense into a central nuclear region. T e dramatic growth of the lens is illustrated by its increasing weight, from approximately 150 mg at 20 years of age to approximately 250 mg at 80 years of age.

Cataracts are decreases in the optic transparency of the lens that ultimately can lead to functional visual disturbances. Cataracts can occur at any age; they can also be congenital. Risk factors for the development of cataracts include aging, diabetes, low antioxidant levels, and exposure to a variety of environmental factors, including exposure to UV radiation and visible light, trauma, smoking, and exposure to a large variety of topical and systemic drugs and chemicals.

Several different mechanisms have been hypothesized to account for the development of cataracts. T ese include the disruption of lens energy metabolism, hydration and/or electrolyte balance, oxidative stress due to the generation of free radicals and ROS, and the occurrence of oxidative stress due to a decrease in antioxidant defense mechanisms such as glutathione, superoxide dismutase, catalase, ascorbic acid, or vitamin E. T e generation of ROS leads to oxidation of lens membrane proteins and lipids. A critical pathway is oxidation of protein thiol groups, particularly in methionine or cysteine amino acids, leading to the formation of polypeptide links through disulfide bonds, and in turn, high-molecular-weight protein aggregates. T ese large aggregations of proteins can attain a size suf cient to scatter light, thus reducing lens transparency. Oxidation of membrane lipids and proteins may also impair membrane transport and permeability.

Corticosteroids

T ere are two proposed mechanisms by which systemic treatment with corticosteroids may cause cataracts. Corticosteroids alter lens epithelium electrolyte balance, which disrupts the normal lens epithelial cell structure causing gaps to appear between the lateral epithelial cell borders. Another theory is that corticosteroid molecules react with lens crystallin proteins, producing corticosteroid–crystallin adducts that would be light-scattering complexes.

Naphthalene

Accidental exposure to naphthalene results in cortical cataracts and retinal degeneration. T e metabolite 1,2-dihydro-1,2-dihydroxynaphthalene (naphthalene dihydrodiol) is the cataract-inducing agent instead of naphthalene itself. Subsequent studies showed that aldose reductase in the rat lens is the enzyme responsible for the formation of naphthalene dihydrodiol, and that treatment with aldose reductase inhibitors prevents naphthalene-induced cataracts.

Phenothiazines

Schizophrenics receiving phenothiazine drugs develop pigmented deposits in their eyes and skin. T e phenothiazines combine with melanin to form a photosensitive product that reacts with sunlight, causing formation of the deposits in lens and cornea. T e amount of pigmentation is related to the dose of the drug, with the annual yearly dose being the most predictive dose metric. More recent epidemiologic evidence demonstrates a dose-related increase in the risk of cataracts from use of nonantipsychotic phenothiazines.

TARGET SITES AND MECHANISMS OF ACTION: RETINA

T e adult mammalian retina is a highly differentiated tissue containing eight distinct layers plus the RPE, 10 major types of neurons, and a Müller glial cell ([MGC] Figure 17–1). T e eight layers of the neural retina, which originate from the cells of the inner layer of the embryonic optic cup, are the nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), rod and cone photoreceptor inner segment layer (RIS, CIS), and the rod and cone photoreceptor outer segment layer (ROS, COS). T e retinal pigment epithelium (RPE) is a single layer of cuboidal epithelial cells that lies on Bruch's membrane adjacent to the vascular choroid. Between the RPE and photoreceptor outer segments lies the subretinal space, which is similar to the brain ventricles. T e 10 major types of neurons are the rod and cone photoreceptors, (depolarizing) ON-rod and ON-cone bipolar cells, (hyperpolarizing) OFF-cone bipolar cells, horizontal cells, numerous subtypes of amacrine cells, an interplexiform cell, and ON-RGCs and OFF-RGCs. T e MGC is the only glial cell in the retina. T e somas of the MGCs are in the INL. T e end feet of the MGCs in the proximal or inner retina along with a basal lamina form the internal limiting membrane of the retina, which is similar to the pial surface of the brain. In the distal retina, the MGC end feet join with the photoreceptors and zonula adherens to form the external limiting membrane, which is located between the ONL and RIS/CIS.

T e mammalian retina is highly vulnerable to toxicant-induced structural and/or functional damage due to (1) the highly fenestrated choriocapillaris that supplies the distal or outer retina as well as a portion of the inner retina; (2) the very high rate of oxidative mitochondrial metabolism, especially that in the photoreceptors; (3) high daily turnover of rod and cone outer segments; (4) high susceptibility of the rod and cones to degenerate due to inherited retinal dystrophies as well as associated syndromes and metabolic disorders; (5) presence of specialized ribbon synapses and synaptic contact sites; (6) presence of numerous neurotransmitter and neuromodulatory systems, including extensive glutamatergic, GABAergic, and glycinergic systems; (7) presence of numerous and highly specialized gap junctions used in the information signaling process; (8) presence of melanin in the choroid and RPE and also in the iris and pupil; (9) a very high choroidal blood flow rate, as high as 10 times that of the gray matter of the brain; and (10) the additive or synergistic toxic action of certain chemicals with ultraviolet and visible light.

Each of the retinal layers can undergo specific or general toxic effects. T ese alterations and deficits include, but are not limited to, visual field deficits, scotopic vision deficits such as night blindness and increases in the threshold for

dark adaptation, cone-mediated (photopic) deficits such as decreased color perception, decreased visual acuity, macular and general retinal edema, retinal hemorrhages and vasoconstriction, and pigmentary changes.

Retinotoxicity of Systemically Administered Therapeutic Drugs

Cancer Chemotherapeutics—Ocular toxicity is a common side effect of cancer chemotherapy, resulting in blurred vision, diplopia, decreased color vision and visual acuity, optic/retrobular neuritis, transient cortical blindness, and demyelination of the ONs. The retina, due to its high metabolic activity and choroidal circulation (vide infra), appears to be particularly vulnerable to numerous cytotoxic drugs such as the alkylating agents cisplatin, carboplatin, and carmustine; the antimetabolites cytosine arabinoside, 5-fluorouracil, and methotrexate; and the mitotic inhibitors such as docetaxel. The ocular toxicity of different drugs is dependent upon the dose, duration of dosage, and route of administration. If not detected at an early stage of toxicity, the ocular complications are often irreversible even after chemotherapy is discontinued.

Chloroquine and Hydroxychloroquine—Chloroquine (Aralen) and hydroxychloroquine (Plaquenil) are 4-aminoquinoline derivatives used as antimalarial and anti-inflammatory drugs that can cause irreversible loss of retinal function. Chloroquine, its major metabolite desethylchloroquine, and hydroxychloroquine have high affinity for melanin, which results in these drugs accumulating in the choroid and RPE, ciliary body, and iris during and following drug administration. Prolonged exposure of the retina to these drugs, especially chloroquine, may lead to an irreversible retinopathy. Doses of hydroxychloroquine less than 400 mg/day appear to produce little or no retinopathy even after prolonged therapy.

The clinical findings accompanying chloroquine retinopathy can be divided into early and late stages. The early changes include (1) the pathognomonic "bull's-eye retina" visualized as a dark, central pigmented area involving the macula, surrounded by a pale ring of depigmentation, which, in turn, is surrounded by another ring of pigmentation; (2) a diminished EOG; (3) possible granular pigmentation in the peripheral retina; and (4) visual complaints such as blurred vision and problems discerning letters or words. Late-stage findings, which can occur during or even following cessation of drug exposure, include (1) a progressive scotoma, (2) constriction of the peripheral fields commencing in the upper temporal quadrant, (3) narrowing of the retinal artery, (4) color and night blindness, (5) absence of a typical retinal pigment pattern, and (6) very abnormal EOGs and ERGs. These late-stage symptoms are irreversible.

Digoxin and Digitoxin—The cardiac glycosides digoxin and digitoxin are used in the treatment of congestive heart disease and in certain cardiac arrhythmias. Digitalis-induced visual system abnormalities include decreased vision, flickering scotomas, and altered color vision. Digoxin produces more toxicity than digitoxin due to its greater volume of distribution and plasma protein binding. The most frequent visual complaints are color vision impairments and hazy or snowy vision, although complaints of flickering light, colored spots surrounded by bright halos, blurred vision, and glare sensitivity also are reported. Photoreceptors are the primary site of toxicity, with cone photoreceptors being more susceptible to the effects than rod photoreceptors. The retina has the highest number of Na^+,K^+-ATPase sites of any ocular tissue, which are potently inhibited by digoxin and digitoxin.

Indomethacin—Indomethacin is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties that is frequently used for the management of arthritis, gout, and musculoskeletal discomfort. Chronic administration of 50 to 200 mg/day of indomethacin for 1 to 2 years has been reported to produce corneal opacities, discrete pigment scattering of the RPE perifoveally, paramacular depigmentation, decreases in visual acuity, altered visual fields, increases in the threshold for dark adaptation, blue–yellow color deficits, and decreases in ERG and EOG amplitudes. Decreases in the ERG a- and b-wave amplitudes, with larger changes observed under scotopic dark-adapted than light-adapted conditions, have been reported. On cessation of drug treatment, the ERG waveforms and color vision changes return to near normal, although the pigmentary changes are irreversible. The mechanism of retinotoxicity is unknown; however, it appears likely that the RPE is a primary target site.

Sildenafil Citrate—Sildenafil citrate (Viagra) is a cGMP-specific phosphodiesterase (PDE) type 5 inhibitor that is utilized in the treatment of erectile dysfunction. Sildenafil is also a weak cGMP PDE type 6 inhibitor, which is present in rod and cone photoreceptors. Transient visual symptoms such as a blue tinge to vision, increased brightness of lights and blurry vision, as well as alterations in scotopic and photopic ERGs have been reported.

Tamoxifen—Tamoxifen (Nolvadex, Tamoplex), a triphenylethylene derivative, is a nonsteroidal antiestrogenic drug that competes with estrogen for its receptor sites. It is a highly effective antitumor agent used for the treatment of metastatic breast carcinoma in postmenopausal women. Chronic high-dose therapy (180–240 mg/day for ~2 years) produces widespread axonal degeneration in the macular and perimacular areas. Clinical symptoms include a permanent decrease in visual acuity and abnormal visual fields, as the axonal degeneration is irreversible. Chronic low-dose tamoxifen (20 mg/day) can result in a small increase in the incidence of keratopathy, with minimal alterations in visual function. Following cessation of low-dose tamoxifen therapy, most of the keratopathy and retinal alterations, except the corneal opacities and retinopathy, are reversible.

Vigabatrin—Vigabatrin, an inhibitor of GABA-transaminase, is used to treat refractory complex partial seizures and infantile spasms. Retinopathy induced by vigabatrin is characterized by irreversible bilateral, concentric peripheral visual constriction, and decreased retinal nerve fiber thickness. Onset of the visual field loss has been observed after six weeks of exposure, but generally requires a couple of years. Rod and cone ERGs as well as flicker responses are altered, indicating that retinal damage also occurs. T is drug is now recommended only for epileptic patients with no alternative choices.

Retinotoxicity of Known Neurotoxicants

Inorganic Lead—Lead poisoning (mean blood lead [BPb] ≥ 80 μg/dL) in humans produces amblyopia, blindness, optic neuritis or atrophy, peripheral and central scotomas, paralysis of eye muscles, and decreased visual function. Moderate- to high-level lead exposure produces scotopic and temporal visual system deficits in occupationally exposed factory workers, and developmentally lead-exposed monkeys and rats. T is lead exposure dosage produces irreversible retinal deficits in the experimental animals.

Occupational lead exposure produces concentration- and time-dependent alterations in the retina such that higher levels of lead directly and adversely affect both the retina and ON, whereas lower levels of lead appear to primarily affect the rod photoreceptors and the rod pathway. T ese retinal and oculomotor alterations are, in most cases, correlated with blood lead levels and occurred in the absence of observable ophthalmologic changes, CNS symptoms, and abnormal performance test scores. T us, these measures of temporal visual function may be among the most sensitive for the early detection of the neurotoxic effects of inorganic lead.

Methanol—Methanol is a low-molecular-weight (32 Da), colorless, and volatile liquid that is readily and rapidly absorbed from all routes of exposure (dermal, inhalation, and oral), easily crosses all membranes, and thus is uniformly distributed to organs and tissues in direct relation to their water content. Following different routes of exposures, the highest concentrations of methanol are found in the blood, aqueous, and vitreous humors, and bile as well as the brain, kidneys, lungs, and spleen. In the liver, methanol is oxidized sequentially to formaldehyde by alcohol dehydrogenase in human and nonhuman primates or by catalase in rodents and then to formic acid. It is excreted as formic acid in the urine or oxidized further to carbon dioxide and then excreted by the lungs. Formic acid is the toxic metabolite of methanol that mediates the metabolic acidosis as well as the retinal and ON toxicity observed in humans, monkeys, and rats with a decreased capacity for folate metabolism.

Human and nonhuman primates are highly sensitive to methanol-induced neurotoxicity due to their limited capacity to oxidize formic acid. T e toxicity occurs in several stages. It first occurs as a mild CNS depression, followed by an asymptomatic 12 to 24 h latent period, followed by a syndrome consisting of formic acidemia, uncompensated metabolic acidosis, ocular and visual toxicity, coma, and possibly death. Acute methanol poisoning results in profound and permanent structural alterations in the retina and ON, and visual impairments ranging from blurred vision to decreased visual acuity and light sensitivity to blindness. Formate is directly toxic to Müller glial cell function as well as rod and cone photoreceptors. T e mechanism of formate toxicity appears to involve a disruption in oxidative phosphorylation in photoreceptors, Müller glial cells, and ON.

Organic Solvents—Organic solvents produce structural alterations in rods and cones as well as functional alterations such as color vision deficits, decreased contrast sensitivity, and altered visuomotor performance. Dose–response color vision loss and decreases in the contrast sensitivity function occur in workers exposed to organic solvents such as trichlorethylene, alcohols, xylene, toluene, n-hexane, styrene, mixtures of these, and others. Adverse effects usually occur only at concentrations above the occupational exposure limits.

TARGET SITES AND MECHANISMS OF ACTION: OPTIC NERVE AND TRACT

T e ON consists primarily of RGC axons carrying visual information from the retina to several distinct anatomical destinations in the CNS. Disorders of the ON may be termed optic neuritis, optic neuropathy, or ON atrophy, referring to inflammation, damage, or degeneration, respectively, of the ON. Retrobulbar neuritis refers to inflammation or involvement of the orbital portion of the ON posterior to the globe. Among the symptoms of ON disease are reduced visual acuity, contrast sensitivity, and color vision. Toxic effects observed in the ON may originate from damage to the ON fibers themselves or to the RGC somas that provide axons to the ON. A number of toxic and nutritional disorders can adversely affect the ON. Deficiency of thiamine, vitamin B_{12}, or zinc results in degenerative changes in ON fibers. A condition referred to as alcohol–tobacco amblyopia or simply as toxic amblyopia is observed in habitually heavy users of these substances and is associated with nutritional deficiency.

Acrylamide

Acrylamide monomer is used in a variety of industrial and laboratory applications, where it serves as the basis for the production of polyacrylamide gels and other polyacrylamide products. Exposure to acrylamide produces a distal axonopathy in large-diameter axons of the peripheral nerves and spinal cord that is well documented in humans and laboratory animals. In contrast, middle diameter axons of optic tract are affected, specifically, RGCs that project to the parvocellular layers of the LGN. Why the axons of the optic nerve and tract show a different size-based pattern of vulnerability than do axons of the peripheral nerve and spinal cord is not understood.

Carbon Disulfide

Carbon disulfide (CS_2) is used in industry to manufacture viscose rayon, carbon tetrachloride, and cellophane. CS_2 damages both the PNS and CNS, and has profound effects on vision. In the visual system, workers exposed to CS_2 experience loss of visual function accompanied by observable lesions in the retinal vasculature. Central scotoma, depressed visual sensitivity in the peripheral visual field, optic atrophy, pupillary disturbances, blurred vision, and disorders of color perception have all been reported. The retinal and ON pathologies produced by CS_2 are likely a direct neuropathologic action and not the indirect result of vasculopathy.

Ethambutol

The dextro isomer of ethambutol is widely used as an antimycobacterial drug for the treatment of tuberculosis. Ethambutol produces dose-related alterations in the visual system, such as blue–yellow and red–green dyschromatopsias, decreased contrast sensitivity, reduced visual acuity, and visual field loss. The earliest visual symptoms appear to be a decrease in contrast sensitivity and color vision. Impaired red–green color vision is the most frequently observed and reported complaint. The symptoms are primarily associated with one of the two forms of retrobulbar neuritis (i.e., optic neuropathy). The most common form, seen in almost all cases, involves the central ON fibers and typically results in a central or paracentral scotoma in the visual field and is associated with impaired red–green color vision and decreased visual acuity, whereas the second form involves the peripheral ON fibers and typically results in a peripheral scotoma and visual field loss.

TARGET SITES AND MECHANISMS OF ACTION: THE CENTRAL VISUAL SYSTEM

Many areas of the cerebral cortex are involved in the perception of visual information. The primary visual cortex (V1), Brodmann area 17, or striate cortex receives the primary projections of visual information from the lateral geniculate nucleus (LGN) and also from the superior colliculus. Neurons from the LGN project to the visual cortex maintaining a topographic representation of the receptive field origin in the retina. The receptive fields in the left and right sides of area 17 reflect the contralateral visual world and representations of the upper and lower regions of the visual field are separated below and above, respectively, the calcarine fissure. Cells in the posterior aspects of the calcarine fissure have receptive fields located in the central part of the retina. Cortical cells progressively deeper in the calcarine fissure have retinal receptive fields that are located more and more peripherally in the retina. The central part of the fovea has tightly packed photoreceptors for resolution of fine detailed images, and the cortical representation of the central fovea is proportionately larger than the peripheral retina in order to accommodate a proportionately larger need for neural image processing. The magnocellular and parvocellular pathways project differently to the histologically defined layers of primary striate visual cortex and then to extrastriate visual areas. The receptive fields of neurons in the visual cortex are more complex than the circular center-surround arrangement found in the retina and LGN. Cortical cells respond better to lines of a particular orientation than to simple spots. The receptive fields of cortical cells are thought to represent computational summaries of a number of simpler input signals. As the visual information proceeds from area V1 to extrastriate visual cortical areas, the representation of the visual world reflected in the receptive fields of individual neurons becomes progressively more complex.

Lead

In addition to the retinal effects of lead (see above), lead exposure during adulthood or perinatal development produces structural, biochemical, and functional deficits in the visual cortex of humans, nonhuman primates, and rats. Quantitative morphometric studies in monkeys exposed to high levels of lead from birth or infancy to 6 years of age revealed a decrease in visual cortex (areas V1 and V2), cell volume density, and a decrease in the number of initial arborizations among pyramidal neurons. These alterations could partially contribute to the alterations in the amplitude and latency measures of the flash-evoked and pattern-reversal-evoked potentials in lead-exposed children, workers, monkeys, and rats, and the alterations in tasks assessing visual function in lead-exposed children.

Methyl Mercury

Methyl mercury–poisoned individuals experience a striking and progressive constriction of the visual field (peripheral scotoma). The narrowing of the visual world gives impression of looking through a long tunnel, hence the term tunnel vision. The damage is most severe in the regions of primary visual cortex subserving the peripheral visual field, with relative sparing of the cortical areas representing the central vision. Methyl mercury–poisoned individuals also experience poor night vision that is also attributable to peripheral visual field losses.

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QUESTIONS

1. In which of the following locations would one NOT find melanin?
   a. iris.
   b. ciliary body.
   c. retinal pigment epithelium (RPE).
   d. uveal tract.
   e. sclera.

2. Systemic exposure to drugs and chemicals is most likely to target which of the following retinal sites?
   a. RPE and ganglion cell layer.
   b. optic nerve and inner plexiform layer.
   c. RPE and photoreceptors.
   d. photoreceptors and ganglion cell layer.
   e. inner plexiform layer and RPE.

3. Which of the following structures is NOT part of the ocular fundus?
   a. retina.
   b. lens.
   c. choroid.
   d. sclera.
   e. optic nerve.

4. Drugs and chemicals in systemic blood have better access to which of the following sites because of the presence of loose endothelial junctions at that location?
   a. retinal choroid.
   b. inner retina.
   c. optic nerve.
   d. iris.
   e. ciliary body.

5. All of the following statements regarding ocular irritancy and toxicity are true EXCEPT:
   a. T e Draize test involves instillation of a potentially toxic liquid or solid into the eye.
   b. T e effect of the irritant in the Draize test is scored on a weighted scale for the cornea, iris, and conjunctiva.
   c. T e Draize test usually uses one eye for testing and the other as a control.
   d. T e Draize test has strong predictive value in humans.
   e. T e cornea is evaluated for opacity and area of involvement in the Draize test.

6. Which of the following statements regarding color vision deficits is FALSE?
   a. Inheritance of a blue–yellow color deficit is common.
   b. Bilateral deficits in the visual cortex can lead to color blindness.
   c. Disorders of the outer retina produce blue–yellow deficits.
   d. Drug and chemical exposure most commonly results in blue–yellow color deficits.
   e. Disorders of the optic nerve produce red–green deficits.

7. A substance with which of the following pH values would be most damaging to the cornea?
   a. 1.0.
   b. 3.0.
   c. 7.0.
   d. 10.0.
   e. 12.0.

8. Which of the following statements concerning the lens is FALSE?
   a. UV radiation exposure is a common environmental risk factor for developing cataracts.
   b. Cataracts are opacities of the lens that can occur at any age.
   c. T e lens continues to grow throughout one's life.
   d. Naphthalene and organic solvents both can cause cataracts.
   e. Topical treatment with corticosteroids can cause cataracts.

9. Which of the following is NOT a reason why the retina is highly vulnerable to toxicant-induced damage?
   a. presence of numerous neurotransmitter systems.
   b. presence of melanin in the RPE.
   c. high choroidal blood flow rate.
   d. high rate of oxidative mitochondrial metabolism.
   e. lack of gap junctions.

10. A deficiency in which of the following vitamins can result in degeneration of optic nerve fibers?
    a. vitamin A.
    b. vitamin B_3.
    c. vitamin C.
    d. vitamin B_{12}.
    e. vitamin E.

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CHAPTER 18

Toxic Responses of the Heart and Vascular System

Y. James Kang

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Environmental Pollutants and Industrial Chemicals
- Carbon Monoxide
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- 1,3-Butadiene
- Metals and Metalloids
- Aromatic Hydrocarbons
- Particulate Air Pollution

KEY POINTS

- Typical chemical-induced disturbances in cardiac function consist of effects on heart rate (chronotropic), contractility (inotropic), conductivity (dromotropic), and/or excitability (bathmotropic).
- Cardiomyopathy includes morphologic and functional alterations induced by toxic exposure, leading to decreased cardiac output and peripheral tissue hypoperfusion.
- Concentric cardiac hypertrophy is an increased size of cardiac myocytes in which new contractile-protein units are assembled in parallel, resulting in a relative increase in the width of individual cardiac myocytes.
- Eccentric cardiac hypertrophy is an increased size of cardiac myocytes in which new contractile-protein units are assembled in series, resulting in a relatively greater increase in the length than in the width of individual myocytes.
- Heart failure is the inability of the heart to maintain cardiac output sufficient to meet the metabolic and oxygen demands of peripheral tissues, including changes in systolic and diastolic function that reflect specific alterations in ventricular function and abnormalities in a variety of subcellular processes.
- Acute cardiac toxicity occurs after a single exposure to a high dose of cardiotoxic chemicals and may be manifested by arrhythmia and can involve apoptosis.
- Chronic cardiac toxicity, which results from long-term exposure to chemicals, is often manifested by cardiac hypertrophy and the transition to heart failure.
- Any xenobiotic that disrupts ion movement or homeostasis may induce a cardiotoxic reaction composed principally of disturbances in heart rhythm.
- All toxicants absorbed into the circulatory system contact vascular cells before reaching other sites in the body.
- Common mechanisms of vascular toxicity include (1) alterations in membrane structure and function, (2) redox stress, (3) vessel-specific bioactivation of protoxicants, and (4) preferential accumulation of the active toxin in vascular cells.

INTRODUCTION

Cardiovascular toxicology is concerned with the adverse effects of extrinsic and intrinsic stresses on the heart and vascular system. Extrinsic stress involves exposure to therapeutic drugs, natural products, and environmental toxicants. Intrinsic stress refers to exposure to toxic metabolites derived from nontoxic compounds such as those found in food additives and supplements. The intrinsic exposures also include secondary neurohormonal disturbance such as overproduction of inflammatory cytokines derived from pressure overload of the heart and counter-regulatory responses to hypertension. These toxic exposures result in alterations in biochemical pathways, defects in cellular structure and function, and pathogenesis of the affected cardiovascular system.

This chapter is divided into two parts: the heart and the vascular system. The manifestations of toxicologic response of the heart include cardiac arrhythmia, hypertrophy, and overt heart failure. The responses of the vascular system include changes in blood pressure and lesions in blood vessels in the form of atherosclerosis, hemorrhage, and edema.

OVERVIEW OF THE HEART

Overview of Cardiac Structural and Physiologic Features

The main purpose of the heart is to pump blood to the lungs and the systemic arteries so as to provide oxygen and nutrients to all body tissues. Figure 18–1 illustrates the basic anatomy of the heart.

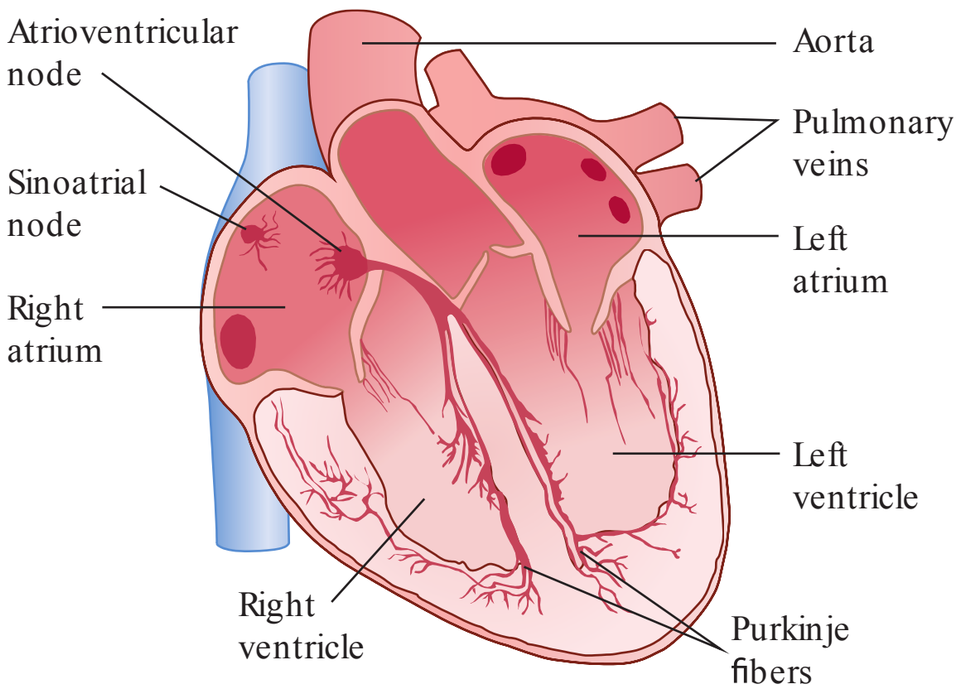

FIGURE 18–1 **Diagram illustrating the basic anatomy of the heart.**

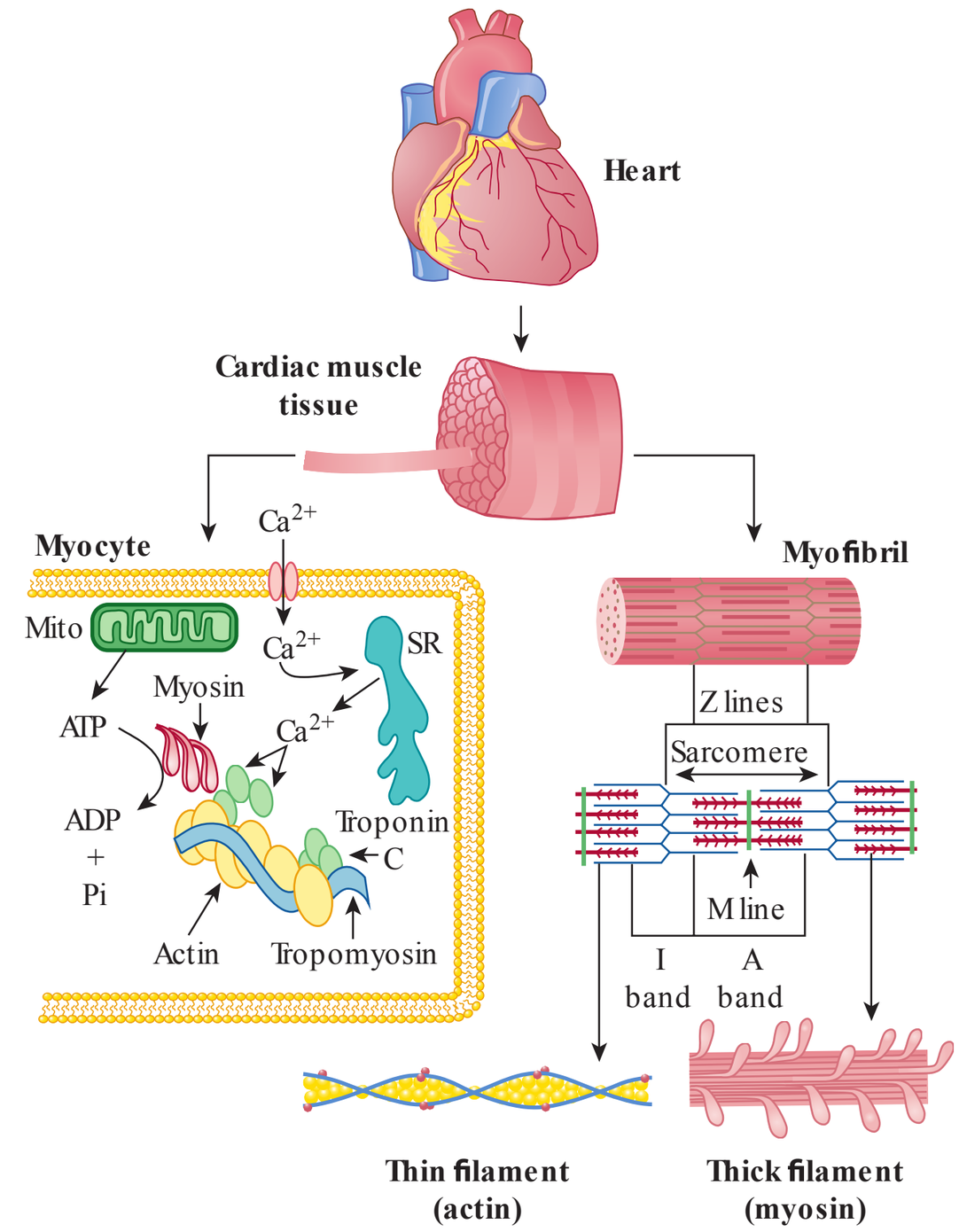

FIGURE 18–2 **Structural organization of cardiac muscle tissue.**

Review of Cardiac Structure—The primary contractile unit within the heart is the cardiac muscle cell, or cardiac myocyte. Cardiac myocytes are composed of several major structural features and organelles, as illustrated in Figure 18–2. A primary component is the contractile elements known as the myofibril. Each myofibril consists of a number of smaller filaments (the thick and thin myofilaments). The thick filaments are special assemblies of the protein myosin, whereas the thin filaments are made up primarily of the protein actin.

Cardiac and skeletal muscle share many similarities, including contractile elements and sarcomere structure. However, a major difference lies in the organization of cardiac myocytes into a functional syncytium; cardiac myocytes are joined end-to-end by dense structures known as intercalated disks. Within these, there are tight gap junctions that facilitate action potential propagation and intercellular communication.

Cardiac myocytes are the largest cells in the heart and contribute to the majority of cardiac mass. However, cardiac myocytes are only about 25% of the total number of cells. Approximately 90% of the non-muscle cells are cardiac fibroblasts, with vascular cells, Purkinje cells, and other connective tissue cells comprising the remaining 10%.

The heart normally undergoes significant increase in size and mass throughout organism growth, primarily through enlargement (hypertrophy) of the preexisting cardiac myocytes. Pathologic conditions, including exposure to toxicants, also results in hypertrophy of surviving cardiac myocytes. Cardiac fibroblasts may continue to proliferate after birth, but promote fibrosis and scarring of injured cardiac tissue in response to myocardial injuries. The limited proliferative capacity of cardiac myocytes and propensity of scar formation by cardiac fibroblasts make the heart vulnerable to injury.

Electrophysiology—Bioelectricity is the result of charge generated from the movement of positively and negatively charged ions in tissues. In cardiac myocytes, three major positively charged ions make a significant contribution to the bioelectricity of the heart: calcium (Ca^{2+}), sodium (Na^{+}), and potassium (K^{+}). Each of the ions has specific channels and transporters (pumps) on the membrane of cardiac myocytes. Through the movement of these ions across the cell membrane, an action potential is generated and propagated from one cell to another, so that electric conductance is produced in the heart.

Action Potential—Cardiac myocytes produce an action potential when activated by pacemaker cells and other stimuli. A sudden depolarization changes the membrane potential from negative inside to positive inside, followed by a repolarization to reset the resting potential. The process of an action potential from depolarization to the completion of repolarization is divided into five phases in cardiac Purkinje fibers as shown in Figure 18–3. Phase 0 represents a rapid depolarization due to the inward current of Na^{+}. Phase 1 is associated with an immediate rapid repolarization, during which the Na^{+} inward current is inactivated and a transient K^{+} outward current is activated, followed by an action potential plateau or phase 2, which is dominated by slowly decreasing inward Ca^{2+} current and a slow activation of an outward K^{+} current. Phase 3 reflects a fast K^{+} outward current and inactivation of the plateau Ca^{2+} inward current, and phase 4 is the diastolic interval for the resetting of the resting potential.

Automaticity—A group of specialized cells in the heart are capable of repetitively spontaneous self-excitation, which generate and distribute each impulse through the heart in a highly

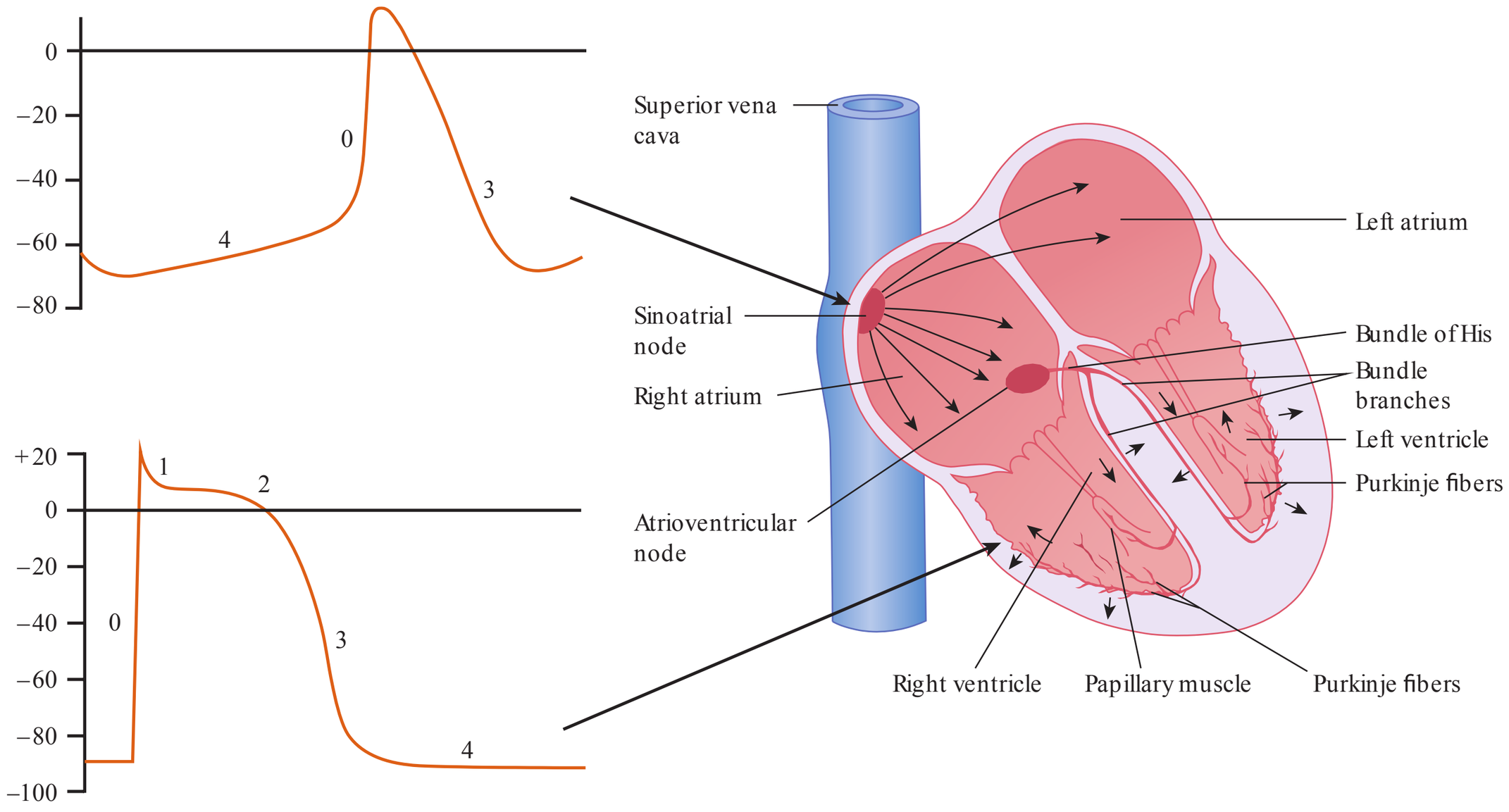

FIGURE 18–3 **Characteristic cardiac action potential recorded from sinoatrial node and Purkinje fibers as indicated.** (Reproduced with permission from Berne RM, Levy MN (eds): Physiology. St. Louis, MO: Mosby/Elsevier; 1983.)

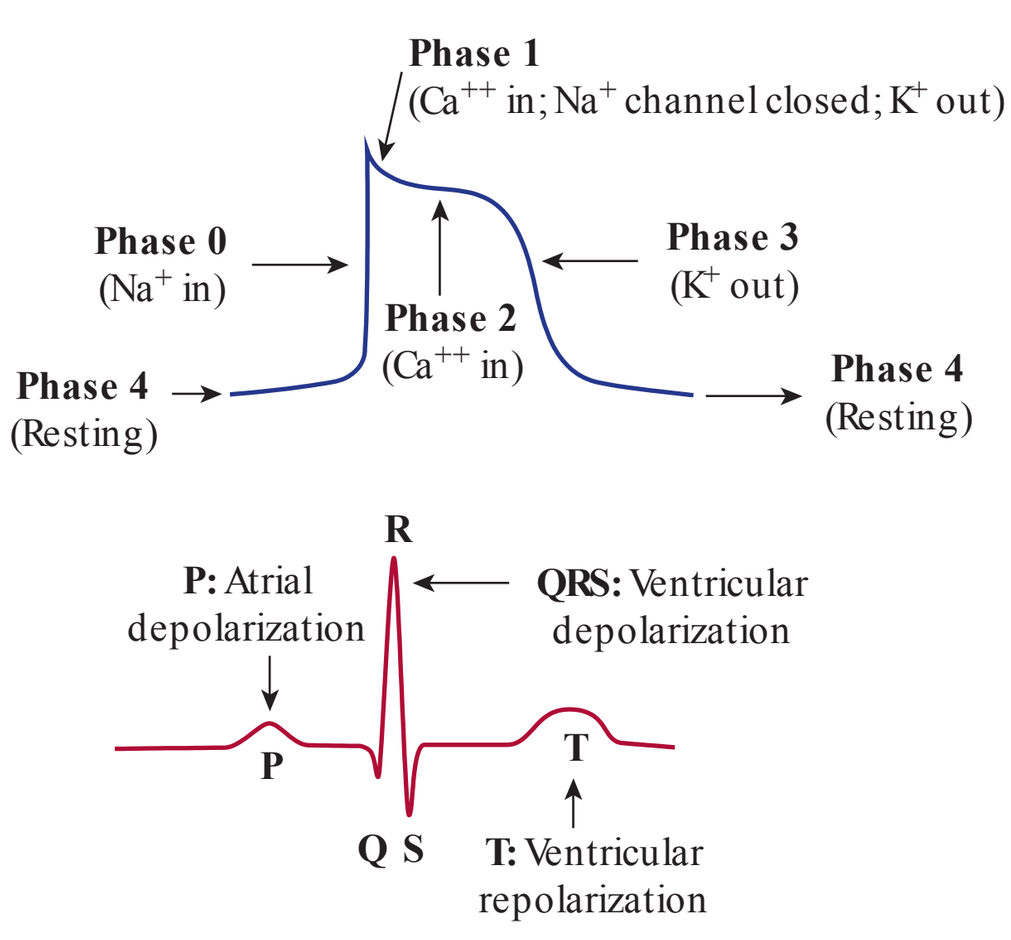

FIGURE 18–4 **Characteristic cardiac action potential and electrocardiogram (ECG).**

coordinated manner to control the normal heartbeat. The sinus node P cells or pacemaker cells have three distinct phases of action potential (Figure 18–4): phase 0, rapid depolarization; phase 3, plateau and repolarization; and phase 4, slow depolarization or often referred to as pacemaker potential. It is the pacemaker potential that brings the membrane potential to a level near the threshold for activation of the inward Ca^{2+} current, which triggers the phase 0 rapid depolarization and makes the pacemaker cells of automaticity. In pacemaker cells, phase 0 is mediated almost entirely by increased conductance of Ca^{2+} ions.

Contractility—Cardiac myocytes, like other muscle cells, have a unique functional feature called contractility. Myocyte contraction occurs when an action potential causes the release of Ca^{2+} from the sarcoplasmic reticulum as well as the entry of extracellular Ca^{2+} into the cell. This action potential-triggered Ca^{2+} increase in the plasma and myocyte contraction is called excitation–contraction coupling. The strength of contraction is directly proportional to the concentration of Ca^{2+} ions such that a large amount of ions will cause a strong contraction.

An increase in intracellular Ca^{2+} concentrations allows Ca^{2+} to bind to troponin C, which moves tropomyosin thereby exposing a site of interaction between actin and myosin. Binding of ATP to the myosin head and its subsequent hydrolysis causes the myosin head to bend in a ratchet-like fashion. This action increases the overlap of the actin and myosin filaments, resulting in shortening of the sarcomeres and contraction of the myocardium.

Electrotonic Cell-to-Cell Coupling—Myocardium as a whole has to synchronize the contraction and relaxation of individual myocytes in order to perform its pump function. This is achieved by a special structural feature of cell-to-cell interaction, electrotonic cell-to-cell coupling via the gap junction. Through the gap junction, major ionic fluxes between adjacent cardiomyocytes are spread, thus allowing electrical synchronization of contraction.

Electrocardiogram—The electrocardiogram (ECG) records electrical currents generated during depolarization and repolarization. On the ECG shown in Figure 18–5, deflections (or waves) are recorded that correspond to atrial depolarization (P wave), ventricular depolarization (QRS complex), and ventricular repolarization (T wave); however, atrial repolarization is not normally observed on the ECG because it is obscured by the large QRS complex.

Useful intervals noted on the ECG include the following. The PR interval corresponds primarily to the speed of conduction through the AV node. The QRS complex represents ventricular depolarization. The ST segment is the interval during which the entire ventricular myocardium is depolarized. The QT interval corresponds to ventricular depolarization and repolarization, which reflects the action potential duration. The QT interval prolongation is recognized as a major life-threatening factor of drug cardiac toxicity.

Neurohormonal Regulation—Although the heartbeat is governed by the automaticity of the sinus node P cells, neurohormonal regulation of cardiac electrophysiology and contraction controls cardiac function under normal and abnormal conditions. Toxicants often exert their effects on the cardiac system through interference with neurohormonal regulation, and there are many neurohormonal systems that have significant impact on the heart.

Cardiac Output—The primary indicator of cardiac function is cardiac output, which is the volume of blood pumped by the ventricles per minute. Cardiac output is dependent on heart rate and stroke volume (the amount of blood ejected by the ventricles during systole). Normal cardiac output at rest is approximately 5 L/min in an average adult human, and this value may increase three- to fourfold during strenuous exercise. Toxicants may alter cardiac output through numerous mechanisms and effects on the heart, vasculature, and/or nervous system. Cardiac arrhythmia, hypertrophy, and heart failure reflect myocardial functional alterations resulting from both acute and chronic cardiac toxicity.

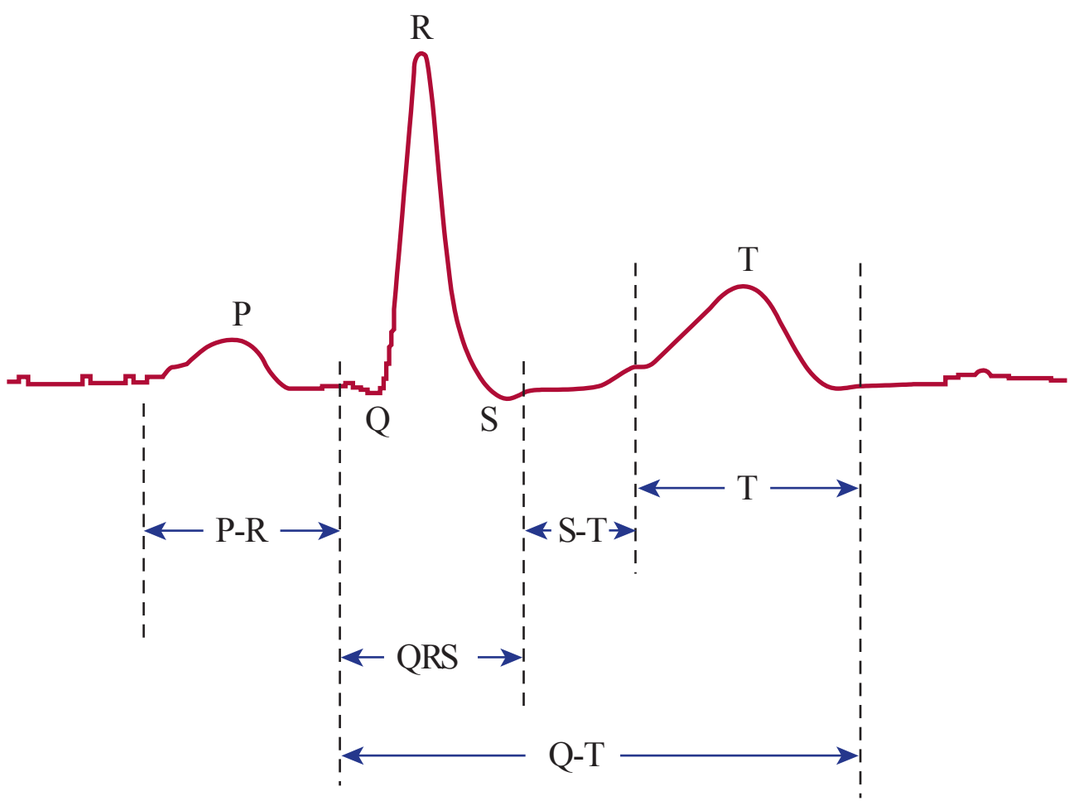

FIGURE 18–5 **A typical electrocardiogram (ECG) with the illustration of important deflections and intervals.**

CARDIAC TOXIC RESPONSES

Basic Concepts and Definitions

The interplay between environmental factors, genetic susceptibility, and myocardial pathogenesis is critical in the study of cardiac toxicity. A triangle model of cardiac toxicity is presented in Figure 18–6, which highlights the complexity of the interaction between environmental stresses and the heart, and the balance between myocardial protection and deleterious dose and time effects are considered. First, it is important to recognize that chemicals can lead to heart failure without heart hypertrophy. Second, a chemical can lead to activation of both protective and destructive responses in the myocardium. Third, long-term toxicologic responses often result in maladaptive hypertrophy, which primes the heart for malignant arrhythmia, leading to sudden cardiac death or transition to heart failure.

Myocardial Degeneration and Regeneration

Myocardial degeneration is the ultimate response of the heart to toxic exposure, which can be measured by both morphologic and functional degenerative phenotypes. The heart was previously considered incapable of regenerating. However, evidence now indicates myocardial regeneration and recovery from cardiomyopathy is possible in some instances. Cardiac toxic responses or damage are now divided into reversible and irreversible.

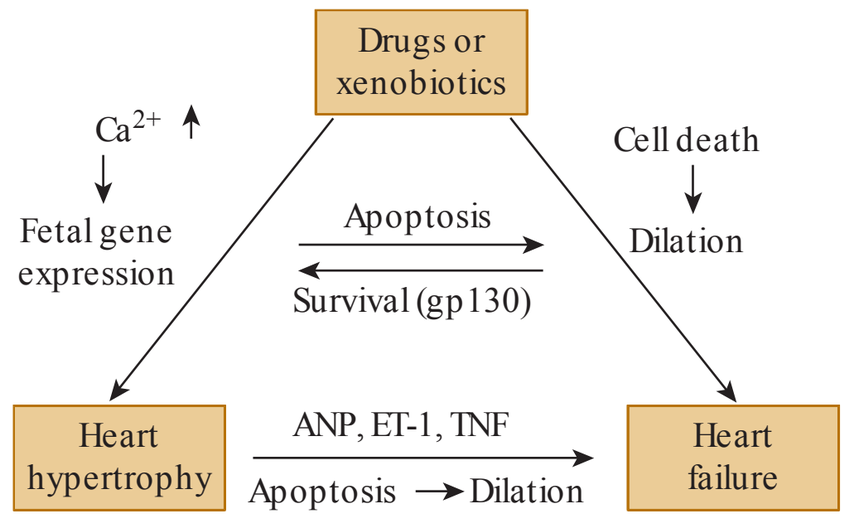

FIGURE 18–6 **Triangle analytical model of cardiac responses to drugs and xenobiotics.** Drugs or xenobiotics can directly cause both heart failure and heart hypertrophy. Under severe acute toxic insults, myocardial cell death becomes the predominant response leading to cardiac dilation and heart failure. In most cases, myocardial survival mechanisms can be activated so that myocardial apoptosis is inhibited. The survived cardiomyocytes often become hypertrophy through activation of calcium-mediated fetal gene expression and other hypertrophic program. If toxic insult continues, the counter-regulatory mechanisms against heart hypertrophy such as activation of cytokine-medicated pathways eventually lead to myocardial cell death through apoptosis or necrosis, dilated cardiomyopathy, and heart failure.

Myocardial Degenerative Responses—Myocardial cell death, fibrosis (scar tissue formation), and contractile dysfunction are considered as degenerative responses, which can result in cardiac arrhythmia, hypertrophy, and heart failure. If acute cardiac toxicity does not affect the capacity of myocardial regeneration, the degenerative phenotype is reversible. Both acute and chronic toxic stresses can lead to irreversible degeneration, depending on whether or not the cardiac repair mechanisms are overwhelmed. Cell death is the most common phenotype of myocardial degeneration. Both apoptosis and necrosis occur in the process of myocardial cell death. Myocardial cell death is also accompanied by hypertrophy of the remaining cardiac myocytes.

Myocardial fibrosis results from excess accumulation of extracellular matrix (ECM), which is mainly composed of collagens. T e net accumulation of ECM results from enhanced synthesis or diminished break down of the matrix, or both. Collagens, predominately types I and III, are the major fibrous proteins in ECM and their synthesis may increase in response to toxic insults. Degradation of ECM is dependent on the activity of matrix metalloproteinases (MMPs), which fall into five categories based on substrate specificity and organ. T e activity of MMPs is altered under toxic stress conditions such that enhanced fibrogenesis and excess collagen accumulation (i.e., fibrosis) occurs.

Toxic Effect on Myocardial Regeneration—T e recent discovery of cardiac progenitor cells has challenged the view that all myocardial degeneration is permanent. T ese cells possess the fundamental properties of stem cells and can make myocytes and vascular structures. Myocardial vascularization is required for myocardial regeneration. Many toxic insults affect the capacity of angiogenesis in the myocardium, so that cardiac ischemia occurs. T e combination of cardiac ischemia and the direct toxic insults to cardiomyocytes constitute synergistic damage to the heart. During regeneration, coronary arterioles and capillary structures are formed to bridge the dead tissue (scar tissue) and supply nutrients for the survival of the regenerated cardiomyocytes. T ere is an orderly organization of myocytes within the myocardium and a well-defined relationship between the myocytes and the capillary network. T is proportion is altered under cardiac toxic conditions.

Myocardial Cell Death and Signaling Pathways

Apoptosis and Necrosis—Toxic insults trigger a series of reactions in cardiac cells leading to measurable changes. Mild injuries can be repaired. However, severe injuries will lead to cell death in the modes of apoptosis and necrosis. If the cell survives the insults, structural and functional adaptations will take place.

Apoptosis is an important mode of myocardial cell loss that has been demonstrated in heart failure and myocardial infarction patients. Necrosis is also important in patients with myocardial infarction and the cardiomyopathy induced by environmental toxicants and pollutants.

Mitochondrial Control of Cell Death—Mitochondrial control of cell death is an important topic of apoptotic research. Factors affecting mitochondrial control of cell death are covered in Chapter 3.

Death Receptors and Signaling Pathways—T e death receptor-mediated apoptotic signaling pathway can be triggered by cytokines (see Chapter 12) and is one focus of cardiotoxicity research. Tumor necrosis factor-α (TNF-α) is the most studied cytokine in myocardial cell death signaling pathways. T is pathway is mediated by TNF receptors (TNFR1 and TNFR2).

Briefly, binding of TNF-α to TNFRs leads to activation of caspase 8, which in turn cleaves BID, a BH3 domain-containing pro-apoptotic Bcl2 family member. T e truncated BID is translocated from cytosol to mitochondria, inducing first the clustering of mitochondria around the nuclei and release of cytochrome c, and then the loss of mitochondrial membrane potential, cell shrinkage, and nuclear condensation, that is, apoptosis. Caspase 8 also directly activates caspase-3, leading to apoptosis. Fas ligand is also able to induce apoptosis of cardiomyocytes through the death receptor–mediated signaling pathway.

Mitochondrial Dynamics and Autophagy

T e importance of mitochondria in cardiac response to toxic insults and in the process of toxicologic cardiomyopathy is related not only to the control of cell death, but also to autophagy, the tightly regulated cellular "housekeeping" process responsible for the degradation of damaged and dysfunctional cellular organelles and protein aggregates.

Autophagy occurs in all eukaryotic cells under the stress of starvation, hypoxia, and toxic insults, as well as under physiologic stimulation such as hormones and developmental signals. Selective autophagy of mitochondria is termed mitophagy, which is triggered by mitochondrial permeability transition pore opening and loss of mitochondrial membrane potential. In cardiomyocytes and other terminally differentiated cells, mitophagy is a continuous process of mitochondrial turnover, but the rate of this turnover is influenced by stresses that make a critical contribution to myocardial pathogenesis. Nonselective autophagy has been observed in response to nutrient starvation; the degradation of cytosolic components including mitochondria via autophagy provides amino acids and lipid substrates for intermediate metabolism.

Cardiac Hypertrophy and Heart Failure

Adaptive and Maladaptive Responses—Myocardial adaptation refers to the general process by which the ventricular myocardium changes in structure and function. T is

process is often referred to as "remodeling." In response to pathologic stimuli, such as exposure to environmental toxicants, myocardial remodeling is adaptive in the short term, but is maladaptive in the long term, and often results in further myocardial dysfunction. The central feature of myocardial remodeling is an increase in myocardial mass associated with a change in the shape of the ventricle.

At the cellular level, the increase in myocardial mass is reflected by cardiac myocyte hypertrophy, which is characterized by enhanced protein synthesis, heightened organization of the sarcomere, and the eventual increase in cell size. At the molecular level, the phenotypic changes in cardiac myocytes are associated with reintroduction of the so-called fetal gene program, characterized by the patterns of gene expression mimicking those seen during embryonic development. These cellular and molecular changes are observed in both adaptive and maladaptive responses, thus distinguishing adaptive from maladaptive responses is difficult.

There are both physiologic hypertrophy and pathologic hypertrophy of the heart. Physiologic hypertrophy is considered an adaptive response, which is an adjustment of cardiac function for an increased demand of cardiac output. One example of adaptive hypertrophy is the increase in cardiac mass in response to exercise. The heart often increases its mass in response to toxicologic stresses, but this is generally viewed as maladaptive. An important distinction between adaptive and maladaptive hypertrophy is whether the hypertrophy is necessary for the compensatory function of the heart under physiologic and pathologic stress conditions. Cardiac hypertrophy in response to extrinsic and intrinsic stresses is not a compensatory response and actually increases the risk for malignant arrhythmia and heart failure.

Hypertrophic Signaling Pathways—Extrinsic and intrinsic stresses activate signaling transduction pathways leading to fetal gene program activation, enhanced protein synthesis of adult cardiomyocytes, and the eventual hypertrophic phenotype. The signaling pathways include several components: G-protein-coupled receptors, protein kinases including MAPK, PKC, and AMPK, calcium and calcineurin, and phosphoinositide 3-kinase (PI3K)/glycogen synthase kinase 3β (GSK3β), and transcription factors. Activation of each of the components is sufficient to induce myocardial hypertrophic growth. These components also affect each other through cross-talk.

Transition from Cardiac Hypertrophy to Heart Failure—The critical cellular event of the transition from cardiac hypertrophy to heart failure is myocardial apoptosis triggered by inflammatory cytokines, such as TNF-α. This transition can also be triggered by neurohormonal factors, such as atrial natriuretic peptide (ANP), which leads to dilated cardiomyopathy and deterioration of cardiac function. Toxicologic exposures may cause dilated cardiomyopathy or heart failure without an intermediate hypertrophic stage. Myocardial cell death also plays an essential role in direct cardiac dilation pathogenesis.

Alterations of biochemical reactions in the myocardium are often seen soon after exposure to environmental toxicants. These include alterations in ionic homeostasis, such as changes in intracellular calcium concentrations, which occur in most exposures to environmental toxicants. Aberrant energy metabolism is another early response to environmental toxicants in the heart, resulting in decreased production and/or enhanced consumption of ATP. Alterations in enzymatic reactions are also often observed in cardiac toxic responses.

Physiologic alterations occur both as early responses to environmental toxicants and as subsequent events in the late development of cardiomyopathy. The most obvious myocardial dysfunction that occurs in the early responses to toxicants is cardiac arrhythmia. Arrhythmia often results from the changes in intracellular calcium concentrations and other biochemical alterations, leading to miscommunication between cells and misconduction of electricity.

Changes in myocardial morphology take place when extensive toxic insults are imposed on the heart and/or toxic exposures persist. Cardiac hypertrophy is often observed as a consequence of long-term toxic insults. From cardiac hypertrophy to heart failure, activation of compensatory mechanisms, including the sympathetic nervous system and the renin-angiotensin system, occurs. The compensatory response in turn activates counter-regulatory mechanisms such as upregulation of ANP expression and increases in cytokines, such as TNF-α production. Extensive biochemical, physiologic, and molecular changes result in myocardial remodeling and remarkable cell death, ultimately leading to heart failure.

QT Prolongation and Sudden Cardiac Death

A simple definition for QT prolongation is that the length of QT interval observed from a typical electrocardiogram is prolonged. Clinically, long QT syndrome is defined when the QT interval is longer than 460 ms. However, torsades de pointes (TdP) occurs with an average increase in QT interval by approximately 200 ms (a normal QT interval is about 300 ms). In general, the long QT syndrome can be divided into two classes: congenital and acquired. Congenital long QT syndrome is rare and acquired is the major concern of drug cardiac toxicity in pharmaceutical discovery and development.

Molecular Basis of QT Prolongation—The longer QT interval on the electrocardiogram is caused by prolongation of the action potential of ventricular myocytes. The duration of the QT interval is related to the length of the ventricular action potentials. A reduction in net outward current and/or an increase in inward current are potential contributors to the prolongation of cardiac action potential, thereby QT prolongation on the electrocardiogram. Although many channels are potentially involved in the prolongation of the cardiac action potential, current studies have identified sodium inward

channels and potassium outward channels (I_{Kr} and I_{Ks}) as important players in the plateau phase (phase 2) of the cardiac action potential.

Torsade De Pointes and Sudden Cardiac Death—T e trigger for arrhythmia in the long QT syndrome is believed to be spontaneous secondary depolarization that arises during or just following the plateau of the action potential. T is small action potential is the so-called "early afterdepolarization." When the spontaneous depolarization is accompanied by a marked increase in dispersion of repolarization, the likelihood to trigger an arrhythmia is increased. Once triggered, the arrhythmia is maintained by a regenerative circuit of electrical activity around relatively inexcitable tissue, a phenomenon known as reentry. T e development of multiple reentrant circuits within the heart causes ventricular arrhythmia, or TdP, leading to sudden cardiac death. Drugs causing TdP are considered severe cardiac toxic agents. Several drugs that were removed from the market due to their TdP effect include the cyclooxygenase-2 (COX-2) inhibitors rofecoxib (Vioxx) and valdecoxib (Bextra).

Parameters A**ff**ecting QT Prolongation and Torsadogenesis—Many factors affect the clinical manifestations of QT prolongation and torsadogenesis. Genetic polymorphisms and female gender are two distinct risk factors. T e mechanism of the polymorphisms and the rationale for high susceptibility of females to QT prolongation and torsadogenesis are yet to be determined.

Drugs and Environmental *T*oxicants—Drug-induced QT prolongation is a major acquired long QT syndrome. Selective blockers of potassium channels, including the so-called class III antiarrhythmic drugs, have been developed for the treatment of various atrial arrhythmias. Environmental exposure to particulate matter in air is a risk factor for QT prolongation in the elderly, children, and individuals with compromised hearts.

Disturbances in Ion Homeostasis—Hypokalemia in combination with torsadogenic drugs is a recognized risk factor for QT prolongation and TdP. It is also known that sodium supplementation can diminish the long QT syndrome due to the gain-of-function mutations in sodium channels. Stress-induced Ca^{2+} overload in myocardial cells increases the likelihood of arrhythmia.

Abnormal Gap Junction—Gap junction–mediated intercellular communication is essential in the propagation of electrical impulses in the heart. Under normal conditions, the gap junction electrotonic current flow attenuates the differences in action potential duration of myocardial cells. Toxicologic exposures cause damage to constituents of gap junctions, leading to disruption of electrotonic cell-to-cell coupling.

Myocardial Ischemic Injury—Acute myocardial ischemia can cause immediate arrhythmia due to disturbance in ionic homeostasis. Acute ischemia can also induce myocardial infarction that can lead to the block of cardiac conductance. After the myocardial infarction, the areas separated by the scar tissue would be uncoupled, making the differences in the duration of action potential of myocardial cells in different regions apparent.

Cardiac Hypertrophy—T e normal distribution of Purkinje fibers in the myocardium is proportional to the mass of the heart. Cardiac hypertrophy would lead to unbalanced distribution of Purkinje fibers in the remodeling heart. T e conduction of pacemaker potentials would thus be interrupted.

Myocardial Fibrosis—Dilated cardiomyopathy in alcoholics often involves myocardial fibrosis, which simulates the effect of myocardial infarction on the electrical conduction in the heart and block of cardiac conductance.

Heart Failure—Most individuals with failing hearts die suddenly of cardiac arrhythmias. In human heart failure, selective down-regulation of two potassium channels, I_{to1} and I_{K1}, has been shown to be involved in action potential prolongation. T e I_{to1} current is involved in phase 1 of the action potential and opposes the depolarization. T e increase in depolarization may be adaptive in the short term because it provides more time for excitation–contraction coupling, mitigating the decrease in cardiac output. However, down-regulation of potassium channels becomes maladaptive in the long term because it predisposes the individual to early afterdepolarization, inhomogeneous repolarization, and polymorphic ventricular tachycardia.

Biomarkers for Cardiac Toxicity

Myocardial injury can be divided into two major classes: structural and nonstructural injuries. T e structural damage of the heart includes cell death and the associated histopathologic changes such as myocardial infarction. Functional deficits often accompany the structural injury. Nonstructural damage represents functional deficits without apparent structural alterations. Myocardial structural changes and functional alterations can be indirectly measured by echocardiography and electrocardiogram in combination with stress testing. T ese measurements can be considered in a broad sense as biomarkers. However, in clinical practice and experimental approach, biomarkers are referred to as indexes of myocardial injury measured from blood samples. T e fundamental principle of the biomarkers is that molecules that are released from the myocardium under various injury conditions are readily detectable from blood samples. Biomarkers that are currently available in a clinical setting are listed in Table 18–1.

TABLE 18–1 Biomarkers for cardiac toxicity.

Biomarker	Tissue Location	Proposed Cardiac Abnormality Indicated by Elevated Levels
Creatine kinase		
CK-MM	Skeletal muscle, myocardium	—
CK-BB	Brain, kidney	—
CK-MB	Myocardium	Acute myocardial infarction; peak values observed 18–24 h after infarction
Myoglobin	All muscle types, including myocardium	Acute myocardial infarction; peak values observed 1–4 h after infarction
B-type natriuretic peptide (BNP)	Ventricular myocardium	Volume pressure overload; ventricular wall tension; chronic heart failure
C-reactive protein (CRP)	Liver	Systemic and vascular inflammation
Cardiac troponins	Cardiomyocytes	Irreversible myocardial injury (i.e., myocardial infarction)

CARDIAC TOXIC CHEMICALS

Many substances can cause cardiac toxic responses directly or indirectly. However, only chemicals that primarily act on the heart or whose cardiac toxicity is the primary concern should be categorized as cardiotoxic chemicals.

Alcohol and Alcoholic Cardiomyopathy

Clinically, the most recognized toxicologic cardiomyopathy is often referred to as alcoholic cardiomyopathy (ACM), which is characterized by an increase in myocardial mass, dilation of the ventricles, wall thinning, ventricular dysfunction, and heart failure. While ACM has been recognized for a long time, its pathogenesis incompletely understood. However, the duration of heavy alcohol use in patients is a critical factor. Clinical data have shown that ACM typically is seen after a long term of consistent consumption of at least 80 g of alcohol per day. Also, a combination of multiple factors is involved, including malnutrition, cigarette smoking, systemic hypertension, and beverage additives, in addition to a long-term consumption of alcohol in the ACM patients. The generation of reactive oxidative metabolites from the biotransformation of ethanol has been suggested to be a major contributing factor for ACM, because these metabolites lead to lipid peroxidation of cardiac myocytes or oxidation of cytosolic and membraneous protein thiols.

Pharmaceutical Chemicals

Cardiotoxicity of pharmaceutical chemicals is a major problem in drug development and their clinical application. The pharmaceutical chemicals that cause cardiac toxic responses can be simply classified as drugs that are used to treat cardiac disease, and others that are used to treat noncardiac disease. For drugs used to treat cardiac disease, cardiac toxicity is often produced by overexpression of the principal pharmaceutical effects. Although overdosing of these drugs can be a major factor for untoward effects, cardiac toxicity is often inevitable for this group of drugs. Table 18–2 summarizes key pharmaceutical agents with their prominent cardiotoxic effects and proposed mechanisms of toxicity.

Drugs used to treat cardiac disease such as digitalis, quinidine, and procainamide often cause acute cardiac toxicity in the form of arrhythmia, which is reversible upon cessation of their use. Other cardiac drugs may cause cardiotoxicity by mechanisms different from that of the therapeutic action. For instance, catecholamines may cause cardiac toxicity through oxidative stress, rather than by their pharmaceutical action on the sympathetic nervous system.

The other category is noncardiac drugs that produce cardiac toxicity. For instance, anthracyclines, such as adriamycin, are effective anticancer drugs, but their ability to produce severe cardiac toxicity limits their use in cancer patients.

Natural Products

Natural products include naturally occurring catecholamines, hormones, and cytokines, as well as animal and plant toxins. Many of these products have been shown to cause cardiac toxic responses. It is difficult to define whether or not the cardiac toxicity results directly from the action of these products in vivo, although these products cause deleterious effects on cultured cardiomyocytes. However, Table 18–3 summarizes the cardiotoxicity of various naturally occurring substances, and proposed mechanisms of toxicity.

Environmental Pollutants and Industrial Chemicals

There are many chemicals classified in this category that cause cardiac toxicity. Metals and metalloids can be found both in environmental pollutants and industrial chemicals. Some heavy metals, such as cadmium, block calcium channels that affect cardiac rhythm leading to arrhythmia, others such as arsenic have high affinity for sulfydryl groups, and interfere with sulfydryl-containing proteins, such as receptors, regulatory proteins, and transporters. During the last decade, epidemiologic and experimental studies have identified an association of air pollution of particulate matter and cardiac toxicity; however, mechanistic insights into cardiac toxicity induced by particulate matter remain elusive. Table 18–4 provides a summary of selected industrial agents with their prominent cardiotoxic effects and proposed mechanisms of cardiotoxicity.

TABLE 18–2 Cardiotoxicity of key pharmaceutical agents.

Agents	Cardiotoxic Manifestations	Proposed Mechanisms of Cardiotoxicity
Ethanol	↓ Conductivity (acute) Cardiomyopathy (chronic)	Acetaldehyde (metabolite) Altered $[Ca^{2+}]_i$ homeostasis Oxidative stress Mitochondrial injury
Antiarrhythmic drugs		
Class I (disopyramide, encainide, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, propafenone, quinidine, tocainide)	↓ Conduction velocity Proarrhythmogenic	Na^+ channel blockade
Class II (acebutolol, esmolol, propranolol, sotalol)	Bradycardia, heart block	β-Adrenergic receptor blockade
Class III (amiodarone, bretylium, dofetilide, ibutilide, quinidine, sotalol)	↑ Action potential duration QTc interval prolongation Proarrhythmogenic	K^+ channel blockade
Class IV (diltiazem, verapamil)	↓ AV conduction Negative inotropic effect Negative chronotropic effect Bradycardia	Ca^{2+} channel blockade
Inotropic drugs and related agents		
Cardiac glycosides (digoxin, digitoxin)	Action potential duration AV conduction Parasympathomimetic (low doses) Sympathomimetic (high doses)	Inhibition of Na^+, K^+-ATPase, ↓$[Ca^{2+}]_i$
Ca^{2+}-sensitizing agents (adibendan, levosimendan, pimobendan)	↓ Diastolic function? Proarrhythmogenic	↓ Ca^{2+} sensitivity Inhibition of phosphodiesterase
Other Ca^{2+}-sensitizing agents (allopurinol, oxypurinol)	?	Inhibition of xanthine oxidase
Catecholamines (dobutamine, epinephrine, isoproterenol, norepinephrine)	Tachycardia Cardiac myocyte death	β_1-Adrenergic receptor activation Coronary vasoconstriction Mitochondrial dysfunction ↓ $[Ca^{2+}]_i$ Oxidative stress Apoptosis
Bronchodilators (albuterol, bitolterol, fenoterol, formeterol, metaproterenol, pirbuterol, procaterol, salmeterol, terbutaline)	Tachycardia	Nonselective activation of β_1-adrenergic receptors
Nasal decongestants (ephedrine, ephedrine alkaloids, ma huang, phenylephrine, phenylpropanolamine, pseudoephedrine)	Tachycardia	Nonselective activation of α_1-adrenergic receptors
Appetite suppressants (amphetamines, fenfluramine, phentermine)	Tachycardia Pulmonary hypertension Valvular disease	↓ Serotonin? Na^+ channel blockade?
Antineoplastic drugs		
Anthracyclines (daunorubicin, doxorubicin, epirubicin)	Cardiomyopathy Heart failure	Altered $[Ca^{2+}]_i$ homeostasis Oxidative stress Mitochondrial injury Apoptosis
5-Fluorouracil	Proarrhythmogenic	Coronary vasospasm?
Cyclophosphamide	Cardiac myocyte death	4-Hydroxycyclophosphamide (metabolite) Altered ion homeostasis
Antibacterial drugs		
Aminoglycosides (amikacin, gentamicin, kanamycin, netilmicin, streptomycin, tobramycin)	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Macrolides (azithromycin, clarithromycin, dirithromycin, erythromycin)	↓ Action potential duration QTc interval prolongation Proarrhythmogenic	K^+ channel blockade

TABLE 18–2 Cardiotoxicity of key pharmaceutical agents. (Continued)

Agents	Cardiotoxic Manifestations	Proposed Mechanisms of Cardiotoxicity
Fluoroquinolones (grepafloxacin, moxifloxacin, sparfloxacin)	↓ Action potential duration QTc interval prolongation Proarrhythmogenic	K^+ channel blockade
Tetracycline	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Chloramphenicol	Negative inotropic effect	↓ $[Ca^{2+}]_i$
Antifungal drugs		
Amphotericin B	Negative inotropic effect	Ca^{2+} channel blockade? Na^+ channel blockade? ↓ Membrane permeability?
Flucytosine	Proarrhythmogenic Cardiac arrest	5-fluorouracil metabolite Coronary vasospasm?
Antiviral drugs		
Nucleoside analog reverse transcriptase inhibitors (stavudine, zalcitabine, zidovudine)	Cardiomyopathy	Mitochondrial injury Inhibition of mitochondrial DNA polymerase Inhibition of mitochondrial DNA synthesis Inhibition of mitochondrial ATP synthesis
Centrally acting drugs		
Tricyclic antidepressants (amitriptyline, desipramine, doxepin, imipramine, protriptyline)	ST segment elevation QTc interval prolongation Proarrhythmogenic Cardiac arrest	Altered ion homeostasis Ca^{2+} channel blockade Na^+ channel blockade K^+ channel blockade
Selective serotonin reuptake inhibitors (fluoxetine)	Bradycardia Atrial fibrillation	Ca^{2+} channel blockade Na^+ channel blockade
Phenothiazine antipsychotic drugs (chlorpromazine, thioridazine)	Anticholinergic effects Negative inotropic effect QTc interval prolongation PR interval prolongation	Ca^{2+} channel blockade?
Other antipsychotic drugs (clozapine)	Blunting of T waves ST segment depression	
General inhalational anesthetics (enflurane, desflurane, halothane, isoflurane, methoxyflurane, sevoflurane)	Negative inotropic effect Decreased cardiac output Proarrhythmogenic	Ca^{2+} channel blockade Altered Ca^{2+} homeostasis β-Adrenergic receptor sensitization
Other general anesthetics (propofol)	Negative inotropic effect	Ca^{2+} channel blockade Altered Ca^{2+} homeostasis β-Adrenergic receptor sensitization
Local anesthetics		
Cocaine	Sympathomimetic effects Ischemia/myocardial Proarrhythmogenic Cardiac arrest Cardiac myocyte death	Na^+ channel blockade Coronary vasospasm, infarction Altered Ca^{2+} homeostasis Mitochondrial injury Oxidative stress Apoptosis
Other local anesthetics (bupivacaine, etidocaine, lidocaine, procainamide)	Decreased excitability ↓ Conduction velocity Proarrhythmogenic	Na^+ channel blockade
Antihistamines (astemizole, terfenadine)	↓ Action potential duration QTc interval prolongation Proarrhythmogenic	K^+ channel blockade
Immunosuppressants (rapamycin, tacrolimus)	Cardiomyopathy Heart failure	Altered Ca^{2+} homeostasis

(continued)

TABLE 18–2 Cardiotoxicity of key pharmaceutical agents. (Continued)

Agents	Cardiotoxic Manifestations	Proposed Mechanisms of Cardiotoxicity
Miscellaneous drugs		
Cisapride	↓ Action potential duration QTc interval prolongation Proarrhythmogenic	K^+ channel blockade
Methylxanthines (theophylline)	↓ Cardiac output Tachycardia Proarrhythmogenic	Altered Ca^{2+} homeostasis Inhibition of phosphodiesterase
Sildenafil	?	Inhibition of phosphodiesterase
Radiocontrast agents (diatrizoate meglumine, iohexol)	Proarrhythmogenic Cardiac arrest	Apoptosis?

TABLE 18–3 Cardiotoxicity of naturally occurring substances.

Agents	Cardiotoxic Manifestations	Proposed Mechanisms of Cardiotoxicity
Estrogens		
Natural estrogens (17β-estradiol, estrone, estriol) Synthetic estrogens (diethylstilbestrol, equilin, ethinyl estradiol, mestranol, quinestrol) Nonsteroidal estrogens (bisphenol A, diethylstilbestrol, DDT, genistein)	QTc interval prolongation? Cardioprotection?	Gender differences in K^+ channel expression? Antiapoptotic effects? Antioxidant activity? ↑ Na^+,K^+-ATPase activity? Ca^{2+} channel blockade? Other mechanisms?
Progestins (desogestrel, hydroxyprogesterone, medroxyprogesterone, norethindrone, norethynodrel, norgestimate, norgestrel, progesterone)	Enhanced toxicity of cocaine?	Mechanisms?
Androgens		
Natural androgens (androstenedione, dehydroepiandrosterone, dihydrotestosterone, testosterone) Synthetic androgens (boldenone, danazol, fluoxymesterone, methandrostenolone, methenolone, methyltestosterone, nandrolone, oxandrolone, oxymetholone, stanozolol)	Myocardial infarction Cardiac hypertrophy	Mitochondrial injury? Altered Ca^{2+} homeostasis? Other mechanisms?
Glucocorticoids		
Natural glucocorticoids (corticosterone, cortisone, hydrocortisone) Synthetic glucocorticoids (e.g., dexamethasone, methylprednisolone, prednisolone, prednisone)	Cardiac hypertrophy Cardiac fibrosis	Increased collagen expression Other mechanisms?
Mineralocorticoids (aldosterone)	Cardiac fibrosis Heart failure	Increased collagen expression Other mechanisms?
Thyroid hormones (thyroxine, triiodothyronine)	Tachycardia Positive inotropic effect Increased cardiac output Cardiac hypertrophy Proarrhythmogenic	Altered Ca^{2+} homeostasis
Cytokines		
Interleukin-1β	Negative inotropic effect Cardiac myocyte death	↑ Nitric oxide synthase expression Apoptosis
Interleukin-2	Negative inotropic effect	↑ Nitric oxide synthase expression
Interleukin-6	Negative inotropic effect	↑ Nitric oxide synthase expression
Interferon-γ	Cardiomyopathy Proarrhythmogenic	↑ Nitric oxide synthase expression Altered ion homeostasis
Tumor necrosis factor-α	Negative inotropic effect Cardiac myocyte death	↑ Nitric oxide synthase expression ↑ Sphingosine production ↓ Ca^{2+} transients Apoptosis

TABLE 18–4 Cardiotoxicity of selected industrial agents.

Agents	Cardiotoxic Manifestations	Proposed Mechanisms of Cardiotoxicity
Solvents Toluene (paint products)	Proarrhythmogenic	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis
Halogenated hydrocarbons (carbon tetrachloride, chloroform, chloropentafluoroethane, 1,2-dibromotetra-fluoromethane, dichlorodifluoromethane, cis-dichloroethylene, trans-dichloroethylene, dichlortetrafluorethane, difluoroethane, ethyl bromide, ethyl chloride, fluorocarbon 502, heptafluoro-1-iodo-propane, 1,2-hexafluoroethane, isopropyl chloride, methyl bromide, methyl chloride, methylene chloride, monochlorodifluoroethane, monochlorodifluoromethane, octafluorocyclobutane, propyl chloride, 1,1,1-trichloroethane, trichloroethane, trichloroethylene, trichlorofluoromethane, trichloromonofluoroethylene, trichlorotrifluoroethane, trifluoroiodomethane, trifluorobromomethane)	Proarrhythmogenic Negative inotropic effect Decreased cardiac output	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis Altered coronary blood flow
Ketones (e.g., acetone, methyl ethyl ketone)	Proarrhythmogenic	↓ Parasympathetic activity ↑ Adrenergic sensitivity Altered ion homeostasis
Heavy metals (Cadmium, cobalt, lead) (Barium, lanthanum, manganese, nickel)	Negative inotropic effect Cardiac hypertrophy Proarrhythmogenic Proarrhythmogenic	Complex formation Altered Ca^{2+} homeostasis Ca^{2+} channel blockade

OVERVIEW OF VASCULAR SYSTEM

Vascular Physiology and Structural Features

The vascular system consists of blood vessels of varying size and different cellular composition. Blood vessels can be divided into arterial, venous, and capillary systems. In addition, the lymphatic system belongs to the vascular system, but it only carries plasma. The main function of the vascular system is to provide oxygen and nutrients to and remove carbon dioxide and metabolic products from organ systems (Figure 18–7). In addition, the vascular system is a conduit that delivers hormones and cytokines to target organs. The vascular system also has regulatory functions to manipulate organ system responses under certain toxicologic conditions.

Arterial System and Physiologic Function—The arterial system is composed of the aorta, major arteries, and small arterioles. The aorta and major arteries are thick-walled structures with vascular smooth muscle, elastic, and connective tissues (Figure 18–8). Blood flow within the arterial system is initiated by contraction of the heart and begins at the ascending aorta. The ascending aorta receives all of the output of the heart with the exception of the coronary blood flow. Blood is distributed to the organ systems of the body through the major arteries

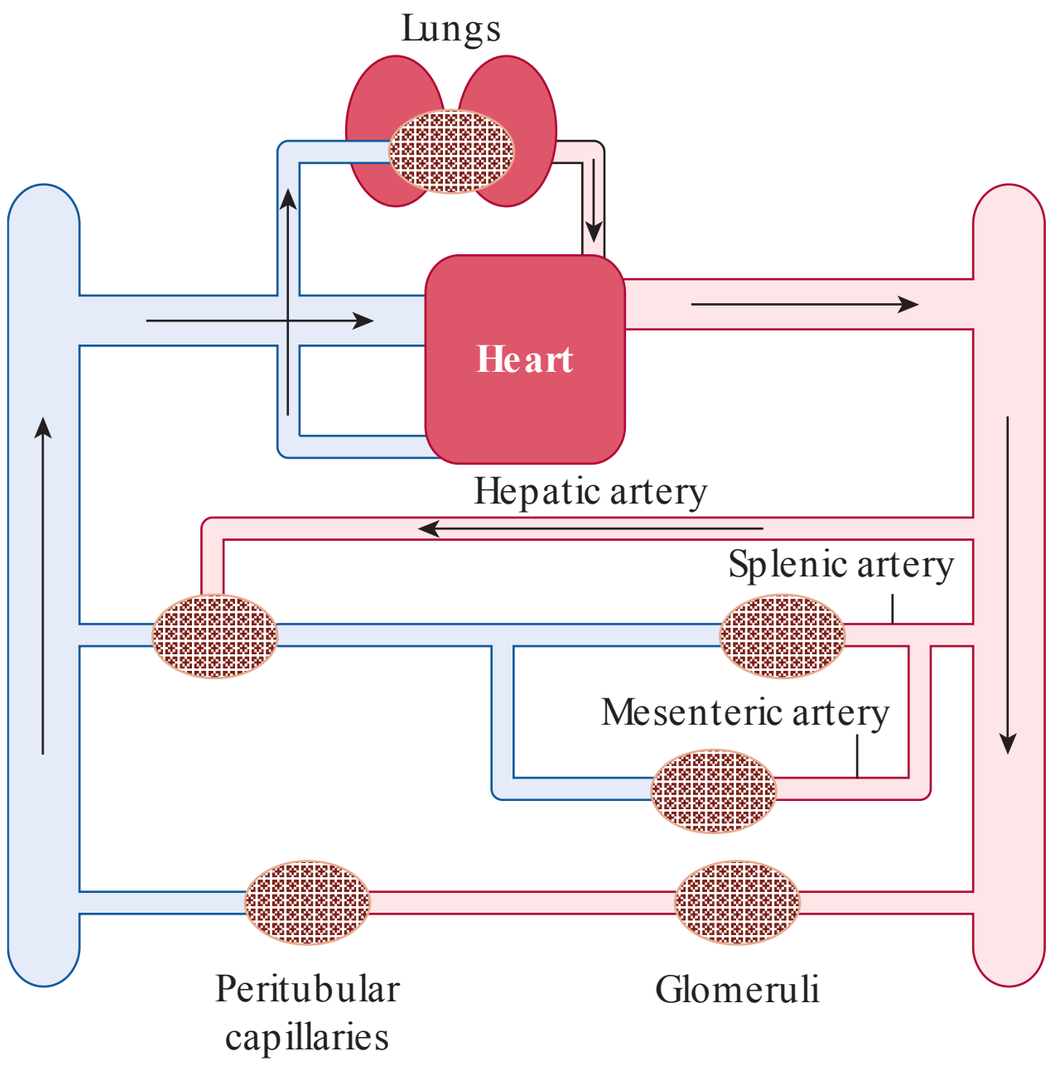

FIGURE 18–7 **Schematic diagram of vascular supply to selected organs.** The capillary beds are represented by a meshwork connnecting the arteries (right) with the veins (left); the distribution of the vasculature in several organs (liver, kidney, lung) indicates the importance of the vascular system in toxicology.

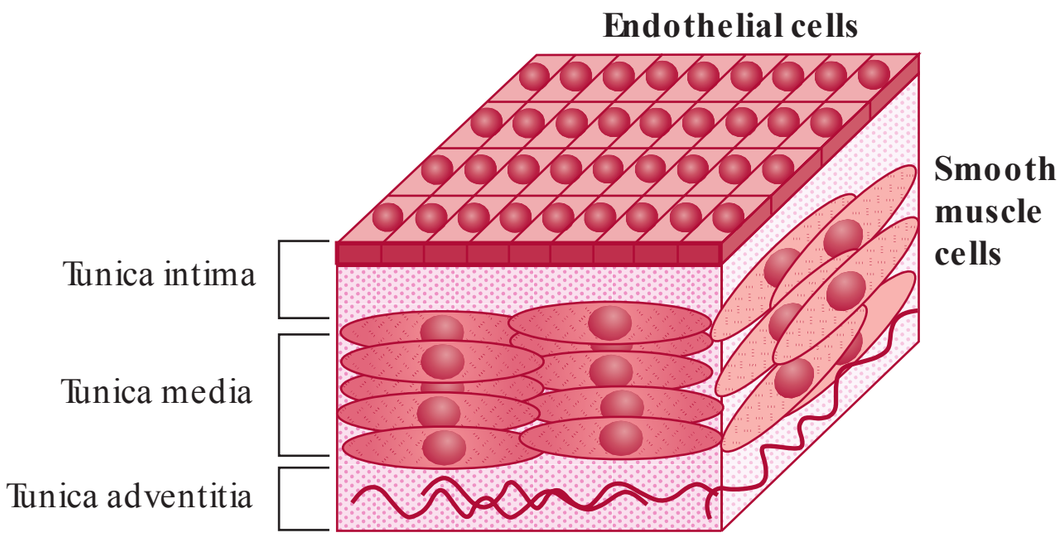

FIGURE 18–8 **Cross-sectional representation of the vascular wall of large- and medium-size blood vessels.** The tunica intima is composed of endothelial cells, facing the vessel lumen, which rest on a thin basal lamina. The tunica media consists mainly of vascular smooth muscle cells interwoven with collagen and elastin. The tunica adventia is a layer of fibroblasts, collagen, elastin, and glycosaminoglycans.

that branch from the aorta. All these arteries further branch to give rise to smaller arteries and become arterioles that connect to capillaries for the delivery of oxygen and nutrients to target tissues. The arterioles are composed of a tube of endothelial cells, surrounded by connective tissue basement membrane, a single or double layer of vascular smooth muscle cells, and a thin outer adventitial layer. The vascular smooth muscle cells are critical for the regulation of vascular resistance and, therefore, blood pressure.

Capillaries and Microcirculation—Capillaries directly connect to the distal portion of the arterioles serving as the communication site between blood and tissues, and constitute the major part of the microcirculation where nutrients, water, gases, hormones, cytokines, and waste products are exchanged between blood and tissues. Capillaries are only one cell layer thick. The passage of molecules through the capillary wall can occur both between and through the endothelial cells. Lipid-soluble molecules such as oxygen and carbon dioxide readily pass through the endothelial cell membranes. Water-soluble molecules diffuse between endothelial cells.

Venous System and Physiologic Function—Blood flow in the venous system starts from the thin-walled venules that have a relatively large surface area facilitating the reabsorption of filtered plasma from the tissue. The venules merge to veins and eventually drain into the vena cava, returning blood to the heart. The important physiologic function of the venous system is collecting blood from organ systems of the body and returning the blood to the heart. Large veins contain vascular smooth muscle cells which can increase return of blood to the heart by constricting. Xenobiotics can exert adverse effects on the vascular smooth muscle cells and compromise this function.

Lymphatic System and Physiologic Function—Lymphatic vessels are endothelial tubes within tissues. This is a low-pressure system that collects excess tissue water and plasma proteins that have not been reabsorbed by the venous system. In general, in all organ systems with the exception of the CNS, more fluid is filtered than reabsorbed by the venous system. Therefore, removal of the excess fluid as well as plasma proteins that diffuse into the interstitial spaces by the lymphatic system is essential. All the lymphatics ultimately drain into the vena cava. Toxic insults to the lymphatic system can lead to elevated interstitial pressures and subsequent edema.

Regulatory Mechanisms of the Vascular System

The vascular system includes conduits and microcirculation. The mechanisms controlling vascular physiology can be divided into neural, hormonal, and local controls that function in an integrated way as each of the three mechanisms affects the other two.

Neurohormonal Regulation—Most arteries, arterioles, venules, and veins, with the exception of those of the external genitalia, receive sympathetic innervation only. The catecholamine, norepinephrine, is the usual neurotransmitter and binding to receptors on vascular smooth muscle cells causes their contraction. Epinephrine is another catecholamine which acts on vascular smooth muscle cells to cause relaxation and vasodilation. In addition, the blood vessels of skeletal muscles receive sympathetic cholinergic innervation in addition to their sympathetic adrenergic innervation, whose activation leads to vascular smooth muscle relaxation and vasodilation. There are many other hormones that control the vascular system, including renin–angiotensin–aldosterone, antidiuretic hormone (ADH), and atrial natriuretic peptide (ANP).

The Renin–Angiotensin–Aldosterone System—Renin is released from the kidney in response to reduced arterial pressure and volume and catalyzes the conversion of a plasma protein angiotensinogen to angiotensin I. Angiotensin I is further converted to angiotensin II by an angiotensin-converting enzyme. Angiotensin II is a powerful arteriolar vasoconstrictor and also causes the release of aldosterone from the adrenal cortex. Aldosterone reduces renal sodium excretion, resulting in retention of water and increased blood volume.

ADH—ADH is a vasoconstrictor released from the posterior pituitary gland in response to volume-depleting conditions, such as hemorrhage. ADH increases water retention by the kidney, and thus increases blood volume. Atrial natriuretic peptide (ANP), a hormone with actions opposing ADH, is released from atrial muscle cells in volume-overload states and results in increased excretion of sodium and water, thereby decreasing blood volume.

Local Metabolic Regulation—The local regulation of the vascular system is primarily referred to as the control of microcirculation. Oxygen is a major regulator of microcirculation which must be replenished constantly from the blood flow.

T erefore, a change in the metabolic rate of an organ requires a parallel change in oxygen supply. While vascular smooth muscle cells cannot respond to oxygen tension under normal conditions, reduced oxygen tension causes the release of adenine nucleotides, free adenosine, and Krebs cycle intermediates; all these cause vasodilation.

Nitric oxide (NO) is an important mediator of local microcirculation regulation. NO is generated from arginine by nitric oxide synthase (NOS), and ultimately leads to relaxation of vascular smooth muscle cells, suppression of platelet activation, and reduction of leukocyte adhesion.

VASCULAR SYSTEM TOXIC RESPONSES

Mechanisms of Vascular Toxicity

All chemicals, after absorption, contact the vascular system. Vascular endothelial cells are the immediate targets of the chemicals and are of the most frequent risk for toxic insults. T ese cells are the major component of the microcirculation system.

Responses of Vascular Endothelial Cells to Toxic Insults—Vascular endothelial cells play a critical role in both vascular protection from toxic insults and triggering detrimental cascade in response to toxic insults. In response to toxic insults, production of NO and reactive oxygen species (ROS) increases in endothelial cells. Substances mimicking agonists activate the receptors on the endothelial cells and trigger intracellular signaling transduction, leading to activation of nuclear factor kappa-B (NFκB) and MAPK activity. T e downstream signaling transduction pathways triggered by NFκB, MAPK, NO, and ROS then activate gene expression and regulate post-translational modification of proteins leading to cytoprotective action against toxic insults, or the production of cytokines, chemokines, and adhesion molecules to protect the circulatory system and the affected organ systems.

Angiogenesis is an adaptive response to damages that follow toxic insults. Vascular endothelial cells are both central to initiating and promoting the formation of new blood vessels and essential for blood vessel formation by forming initial tube-like structures. Xenobiotics can both promote and suppress angiogenesis, and the primary target is the vascular endothelial cell. Apoptosis is a major mechanism for cell death of the vascular endothelial cells and mechanisms and molecular signaling pathways leading to apoptosis are basically the same as described for cardiomyocytes.

Lesions to endothelial cells can result in atherosclerosis. Injury to endothelial cells results in increased production of endothelin-1 (ET-1) and increased release of prostacyclins. ET-1 secreted by endothelial cells is a major mediator of vascular toxicity and also contributes to the pathogenesis of myocardial disease. ET-1 is a potent vasoconstrictor that plays an important role in the maintenance of vascular tone and blood pressure in healthy subjects.

Endothelial cells are also involved in the recruitment of inflammatory cells to the lesion site. Activated lymphocytes secrete cytokines, such as TGF-β, which lead to a cascade of signaling transduction and a series of injurious responses including deposition of collagen.

Responses of Smooth Muscle Cells to Toxic Insults—T e consequence of damage to vascular smooth muscle cells involves changes in the vascular tone and atherosclerosis. Activation of receptors localized in the plasma membrane of smooth muscle cells leads to increased intracellular calcium, which initiates contraction of the affected vessels. Toxic substances can also influence calcium homeostasis in other ways, including disruption of calcium-binding proteins and calcium-activated proteins.

Proliferation and migration of medial smooth muscle cells are primarily responsible for the formation of sclerosis. Under certain circumstances, smooth muscle cells lose most of their contractility. In most cases, this transformation is reversible. T is new form of smooth muscle cells synthesizes collagen, accumulates low-density lipoproteins, and decreases the number of myofilaments. T is phenotypic transformation of smooth muscle cells occurs in atherosclerosis.

Oxidative Stress and Vascular Injury—Both endothelial and smooth muscle cells are capable of producing ROS and subsequent oxidative injury by enzymatic and nonenzymatic mechanisms. Enzymes involved in the generation of ROS in vascular cells include amine oxidase, cytochrome P450 monooxygenases, and prostaglandin synthetase. T ese enzymes use a diversity of substrates to produce ROS. T e nonenzymatic reaction involves free iron and copper in the circulation system, which catalyze the Fenton reaction to produce ROS.

Inflammatory Lesions—Inflammatory lesions of the vascular system, termed vasculitis, are a common response of the vascular system. T e causes of many types of vasculitis are still unknown despite much research on the subject. T e initial injury to endothelial cells and the release of chemicals from the injured cells are responsible for the initiation of the inflammatory response, including recruitment of inflammatory cells to the injured site. Cytokines released from the activated inflammatory cells further propagate the inflammatory response leading to the eventual lesion or vasculitis.

Toxic Responses of Blood Vessels

Hypertension and Hypotension—Vasculature pressure change is a major phenotype of vascular injury. Hypertension results from excessive constriction of the arterial vasculature and/or increased resistance of the microcirculation system. However, the primary problem of sustained hypertension is an elevated vascular resistance in all organs. Once hypertension is established, it becomes a disease of the microvasculature, particularly the arteriolar microvasculature. An increased incidence of temporary or, in some cases, permanent closure of small arterioles is associated with increased resistance of the end organs. T e vascular smooth muscle cells become

hypertrophied, and vascular smooth muscle cells become exceptionally responsive to norepinephrine.

Toxic substances may directly or indirectly affect the sympathetic nervous system or alter the turnover of catecholamines in the circulation, resulting in hypertension. However, sustained hypertension by xenobiotics may involve more complicated metabolic changes in the end organs and thus changes in microcirculation also take place. For example, chemicals may enhance the renin-angiotensin system as well as renal toxicity, which may cause hypertension.

Hypotension is practically defined as the symptoms caused by low blood pressure. Baroreceptors, volume receptors, chemoreceptors, and pain receptors are all involved in the integrated regulatory action to maintain adequate blood pressure. During chemical exposure, these mechanisms may be affected individually or jointly resulting in a disturbance in the integration of the regulatory mechanisms. Both transient and sustained hypotension can be produced by xenobiotics. The most common adverse effect of antihypertensive drugs is hypotension. Other major causes of hypotension include hemorrhage and alcohol overdose.

Atherosclerosis—The most frequent vascular structural injury is atherosclerosis. The classic definition of atherosclerotic plaque is a combination of changes in the intima of arteries consisting of local accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissue, and calcium deposits. However, some advanced atherosclerotic plaques can invade the media and produce bulging or enlarged arteries, cellular infiltration, and neovascularization. The primary problem is the mechanical occlusion of the blood vessels so that blood flow is inadequate for the metabolic demands of the organs.

Activation of vascular smooth muscle cells is critically involved in atherosclerosis. Once stimulated, the vascular smooth muscle cells proliferate, migrate to the lesion site, undergo phenotype transformation and increase the production of type I and II collagen, dermatan sulfate, proteoglycan, and stromelysins. In addition, the smooth muscle cells produce cytokines including macrophage colony–stimulating factor, TNF-α, and monocyte chemoattractant protein-1. The recruitment of inflammatory cells to the lesion site is the perpetuation process of atherosclerosis.

Hemorrhage—A direct mechanical injury to blood vessels causes hemorrhage (i.e., bleeding), while chemical-induced hemorrhages are seen when damage to capillaries takes place. Toxic effects on blood clotting also increase the probability of hemorrhage.

Edema—Edema is defined as excess fluid in the interstitial space. The capillary exchange of fluid is bidirectional, meaning the balance between hydrostatic and colloid osmotic pressure can drive fluid both out of and into a capillary. Under normal physiologic conditions, more fluid is filtered than reabsorbed. This excess fluid is removed via the lymphatic system, which ultimately drains into the vena cava. Xenobiotics can change the pressure gradients such that there is even more filtration than reabsorption than normal. Further, toxic insults to the lymphatic system can lead to elevated interstitial pressures and subsequent tissue edema.

VASCULAR SYSTEM TOXIC CHEMICALS

Like cardiac toxicants, those that cause vascular toxicity can include pharmaceutical chemicals, natural products, and environmental pollutants and industrial chemicals. Although blood vessels are the primary target of these chemicals, some affect the heart as well. For instance, blood vessels in the heart belong to the vascular system, so that the toxicity of vascular toxic chemicals may express their toxicity in the form of cardiac toxic manifestations. Endothelial cells are major target cells of the chemicals affecting the vascular system, which are also found in the heart and make a contribution to cardiac toxicity. This same principle applies to other organ systems. Due to the distribution of the vascular system in the end organs, vascular toxicity affects the organs in which the vessels are localized and is often accompanied with functional defects of the organ.

Pharmaceutical Chemicals

Sympathomimetic Amines—The sympathomimetic amines, including epinephrine, norepinephrine, dopamine, and isoproterenol, can damage the arterial vasculature by various mechanisms. Large or repeated doses of catecholamines produce toxic effects on the endothelium, such as unusual endothelial cytoarchitecture and atherosclerotic lesions in several animal species. Thus, the formation of arteriosclerotic lesions in certain forms of hypertension may be initiated and/or potentiated by high levels of circulating catecholamines.

Nicotine—Nicotine is an alkaloid found in various plants that mimics the actions of acetylcholine at nicotinic receptors throughout the body. At pharmacologic concentrations, nicotine increases heart rate and blood pressure as a result of stimulation of sympathetic ganglia and the adrenal medulla. Epidemiologic and experimental studies have suggested that nicotine is a causative or aggravating factor in myocardial and cerebral infarction, gangrene, and aneurysm.

Cocaine—The central actions of cocaine are to increase the circulating levels of catecholamines and cause a generalized state of vasoconstriction. Hypertension and cerebral strokes are common vascular complications. In pregnant women, cocaine-induced vascular changes have been associated with abortions and abruptio placentae. Cocaine also enhances leukocyte migration across the cerebral vessel wall during inflammatory conditions. This effect is exerted through a cascade of augmented expression of inflammatory cytokines and endothelial adhesion molecules and may in fact underlie the cerebrovascular complications associated with cocaine abuse.

Psychotropic Agents—Trifluoperazine and chlorpromazine have been shown to cause intracellular cholesterol accumulation in cultured cells of the aortic intima. Aside from the atherogenic effects, postural hypotension has been identified as the most common cardiovascular side effect of tricyclic antidepressants.

Antineoplastic Agents—T e vasculotoxic responses elicited by antineoplastic drugs range from asymptomatic arterial lesions to thrombotic microangiopathy. Pulmonary venoocclusive disease has been reported after the administration of various drugs, including 5-fluorouracil, doxorubicin, and mitomycin. Cyclophosphamide causes cerebrovascular and viscerovascular lesions, resulting in hemorrhages.

Analgesics and Nonsteroidal Anti-inflammatory Agents—Aspirin can produce endothelial damage as part of a pattern of gastric erosion. Regular use of analgesics containing phenacetin has been associated with an increased risk of hypertension and cardiovascular morbidity. NSAIDs may induce glomerular and vascular renal lesions.

Oral Contraceptives—Oral contraceptive steroids can produce thromboembolic disorders. Epidemiologic studies have shown that oral contraceptive users have an increased risk of MI relative to nonusers, a correlation that is markedly exacerbated by smoking, and increased risk of cerebral thrombosis, hemorrhage, venous thrombosis, and pulmonary embolism.

Natural Products

Natural products that cause vascular toxicity include those discussed for drugs causing cardiotoxicity. In addition, many other drugs also cause vascular lesions and toxicity such as bacterial endotoxins and homocysteine, which have unique vascular toxic effects.

Bacterial Endotoxins—Bacterial endotoxins are potent toxic agents to the vascular system. T ese toxins are known to cause thickening of endothelial cells and the formation of fibrin thrombi in small veins. T e terminal phase of the effects of endotoxin on the systemic vasculature results in marked hypotension. T e action of these agents is somehow related to oxidative stress mechanisms, as evidenced by the ability of vitamin E to prevent some of the toxin-induced damage.

Homocysteine—Moderately elevated levels of homocysteine have been associated with atherosclerosis and venous thrombosis. Toxicity may involve oxidative injury to vascular endothelial and/or smooth muscle cells, leading to deregulation of vascular smooth muscle growth, synthesis and deposition of matrix proteins, and adverse effects on anticoagulant systems.

Hydrazinobenzoic Acid—T is nitrogen–nitrogen bonded chemical is present in the cultivated mushroom Agaricus bisporus. T is hydrazine derivative causes smooth muscle cell tumors in the aorta and large arteries of mice when administered over the life span of the animals.

T-2 Toxin—Trichothecene mycotoxins, commonly classified as tetracyclic sesquiterpenes, are naturally occurring cytotoxic metabolites of Fusarium species. T ese mycotoxins, including T-2 toxin, are major contaminants of foods and animal feeds and may cause illness in animals and humans. Intravenous infusion of T-2 toxin in rats causes an initial decrease in heart rate and blood pressure, followed by tachycardia and hypertension and finally by bradycardia and hypotension. Acute T-2 toxin exposure causes extensive destruction of myocardial capillaries, while repeated dosing promotes thickening of large coronary arteries.

Vitamin D—T e toxic effects of vitamin D may be related to its structural similarity to 25-hydroxycholesterol, a potent vascular toxin. T e manifestations of vitamin D hypervitaminosis include medial degeneration, calcification of the coronary arteries, and smooth muscle cell proliferation in laboratory animals.

β-Amyloid—Accumulation of β-amyloid is a major lesion in the brain of Alzheimer's patients. Studies have shown that administration of β-amyloid produces extensive vascular disruption, including endothelial and smooth muscle damage, and adhesion and migration of leukocytes across arteries and venules. Most importantly, the vascular actions of β-amyloid appear to be distinct from the neurotoxic properties of the peptide. It appears that vascular toxicity of β-amyloid makes contributions to Alzheimer's dementia.

Environmental Pollutants and Industrial Chemicals

T e environmental pollutants and industrial chemicals discussed in the cardiotoxicity section all have toxic effects on the vascular system. T e cardiac effect of some of these agents and pollutants actually may result primarily from the vascular effect. T e by-products of vascular tissue damage or the secreted substances, such as cytokines derived from vascular injury, can affect the heart either directly because of the vascular system in the heart or indirectly through blood circulation.

Carbon Monoxide—Carbon monoxide induces focal intimal damage and edema in laboratory animals at a concentration (180 ppm) to which humans may be exposed from environmental sources such as automobile exhaust, tobacco smoke, and fossil fuels. Short-term exposure to carbon monoxide is associated with direct damage to vascular endothelial and smooth muscle cells. T e toxic effects of carbon monoxide have been attributed to its reversible interaction with hemoglobin. As a result of this interaction, carboxyhemoglobin decreases the oxygen-carrying capacity of blood, eventually leading to functional anemia. In addition, carbon monoxide interacts with cellular proteins such as myoglobin

and cytochrome c oxidase and elicits a direct vasodilatory response of the coronary circulation.

Carbon Disulfide—Carbon disulfide (dithiocarbonic anhydride) occurs in coal tar and crude petroleum and is commonly used in the manufacture of rayon and soil disinfectants. This chemical has been identified as an atherogenic agent in laboratory animals. The mechanism for carbon disulfide–atheroma production may involve direct injury to the endothelium coupled with hypothyroidism, because thiocarbamate (thiourea), a potent antithyroid substance, is a principal urinary metabolite of carbon disulfide. Carbon disulfide also modifies low-density lipoprotein in vitro and enhances arterial fatty deposits induced by a high-fat diet in mice.

1,3-Butadiene—Studies have shown that 1,3-butadiene, a chemical used in the production of styrene–butadiene, increases the incidence of cardiac hemangiosarcomas, which are tumors of endothelial origin. Although hemangiosarcomas have also been observed in the liver, lung, and kidney, cardiac tumors are a major cause of death in animals exposed to this chemical. The toxic effects of 1,3-butadiene depend on its metabolic activation by cytochrome P450 to toxic epoxide metabolites.

Metals and Metalloids—The vascular toxicity of food- and water-borne elements (selenium, chromium, copper, zinc, cadmium, lead, and mercury) as well as airborne elements (vanadium and lead) involves reactions of metals with sulfhydryl, carboxyl, or phosphate groups. Metals such as cobalt, magnesium, manganese, nickel, cadmium, and lead also interact with and block calcium channels. Intracellular calcium-binding proteins, such as calmodulin, are biologically relevant targets of heavy metals, including cadmium, mercury, and lead, although the contribution of this mechanism to the toxic effects of metals is not fully understood.

Aromatic Hydrocarbons—Aromatic hydrocarbons, including polycyclic aromatic hydrocarbons and polychlorinated dibenzopdioxins, are persistent toxic environmental contaminants. Aromatic hydrocarbons have been identified as vascular toxins that can initiate and/or promote the atherogenic process in experimental animals. The atherogenic effect is associated with cytochrome P450–mediated conversion of the parent compound to toxic metabolic intermediates, but aromatic hydrocarbons can also initiate the atherogenic process.

Particulate Air Pollution—Recent epidemiologic studies have provided a strong body of evidence that elevated levels of ambient particulate air pollution are associated with increased cardiovascular and respiratory morbidity and mortality. Vascular effects of inhaled ambient particles include endothelial dysfunction and promotion of atherosclerotic lesions. Importantly, these lesions lead to release or secretion of cytokines and chemokines, worsening cardiac complications (discussed previously).

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QUESTIONS

1. In which of the following locations would one NOT find spontaneous depolarization?
   a. SA node.
   b. myocardium.
   c. AV node.
   d. bundle of His.
   e. Purkinje fibers.

2. Which of the following scenarios would increase contractility of the myocardium?
   a. increased activity of the Na^+/K^+-ATPase.
   b. increased activity of sacroplasmic reticulum Ca^{2+} ATPase.
   c. decreased activity of sacroplasmic reticulum Ca^{2+} ATPase.
   d. decreased intracellular calcium levels.
   e. increased intracellular K^+ levels.

3. All of the following statements regarding abnormal cardiac function are true EXCEPT:
   a. Ventricular arrhythmias are generally more severe than atrial arrhythmias.
   b. Ventricular hypertrophy is a common cause of ventricular arrhythmias.
   c. Coronary artery atherosclerosis is a major cause of ischemic heart disease.
   d. Right-sided heart failure results in pulmonary edema.
   e. Tachycardia is classified as a rapid resting heart rate (> 100 beats/min).

4. Ion balance is very important in maintaining a normal cardiac rhythm. Which of the following statements is TRUE?
   a. Blockade of K^+ channels decreases the duration of the action potential.
   b. Blockade of Ca^{2+} channels has a positive inotropic effect.
   c. Inhibition of Na^+ channels increases conduction velocity.
   d. Blockage of the Na^+/K^+-ATPase increases contractility.
   e. Calcium is transported into the cell via a Ca^{2+}-ATPase.

5. Which of the following is most likely NOT a cause of myocardial reperfusion injury?
   a. cellular pH fluctuations.
   b. damage to the sarcolemma.
   c. generation of toxic oxygen radicals.
   d. Ca^{2+} overload.
   e. inhibition of the electron transport chain.

6. Which of the following statements regarding the cardiotoxic manifestations of ethanol consumption is FALSE?
   a. Acute ethanol toxicity causes decreased conductivity.
   b. Chronic alcohol consumption is associated with arrhythmias.
   c. Acute ethanol toxicity causes an increased threshold for ventricular fibrillation.
   d. Chronic ethanol toxicity can result in cardiomyopathy.
   e. Acetaldehyde is a mediator of cardiotoxicity.

7. Cardiac glycosides:
   a. increase the activity of the Na^+/K^+-ATPase.
   b. make the resting membrane potential more negative.
   c. can have sympathomimetic and parasympathomimetic effects.
   d. decrease ventricular contractility.
   e. increase AV conduction.

8. Which of the following is NOT a common cardiotoxic manifestation of cocaine abuse?
   a. parasympathomimetic effects.
   b. myocardial infarction.
   c. cardiac myocyte death.
   d. ventricular fibrillation.
   e. ischemia.

9. Using high doses of anabolic–androgenic steroids is NOT likely associated with which of the following?
   a. an increase in LDL.
   b. cardiac hypertrophy.
   c. myocardial infarction.
   d. increased nitric oxide synthase expression.
   e. a decrease in HDL.

10. Which of the following is NOT a common mechanism of vascular toxicity?
    a. membrane disruption.
    b. oxidative stress.
    c. bioactivation of protoxicants.
    d. reduction and accumulation of LDL in endothelium.
    e. accumulation of toxin in vascular cells.

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CHAPTER

19

Toxic Responses of the Skin

Robert H. Rice and T eodora M. Mauro

SKIN AS A BARRIER

Skin Histology
Percutaneous Absorption
Transdermal Drug Delivery
Measurements of Penetration
Biotransformation

CONTACT DERMATITIS

Irritant Dermatitis
Chemical Burns
Allergic Contact Dermatitis
Diagnosis and Testing

GRANULOMATOUS REACTIONS

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Adverse Responses to Electromagnetic Radiation
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Phototoxicity
Photoallergy

ACNE

Chloracne

PIGMENTARY DISTURBANCES

URTICARIA

TOXIC EPIDERMAL NECROLYSIS

SKIN CANCER

Radiation
UV-induced Skin Cancer
Polycyclic Aromatic Hydrocarbons
Mouse Skin Tumor Promotion
Arsenic

KEY POINTS

- T e skin participates directly in thermal, electrolyte, hormonal, metabolic, and immune regulation.
- Percutaneous absorption depends on the xenobiotic's hydrophobicity, which affects its ability to partition into epidermal lipid, and rate of diffusion through this barrier.
- T e cells of the epidermis and pilosebaceous units express biotransformation enzymes.
- Irritant dermatitis is a nonimmune-related response caused by the direct action of an agent on the skin.
- Allergic contact dermatitis represents a delayed (type IV) hypersensitivity reaction, whereby minute quantities of material elicit overt reactions.

SKIN AS A BARRIER

T e skin protects the body against external insults in order to maintain internal homeostasis. It participates directly in thermal, electrolyte, hormonal, metabolic, and immune regulation. Rather than merely repelling noxious physical agents, the skin may react to them with various defensive mechanisms that serve to prevent internal or widespread cutaneous damage. If an insult is severe or intense enough to overwhelm the protective function of the skin, acute or chronic injury becomes readily manifest. T e specific presentation depends on a variety of intrinsic and extrinsic factors including body site, duration of exposure, and other environmental conditions (Table 19–1).

Skin Histology

T e skin consists of two major components: the outer epidermis and the underlying dermis, which are separated by a basement membrane (Figure 19–1). T e junction ordinarily is not flat but has an undulating appearance (rete ridges). In addition, epidermal appendages (hair follicles, sebaceous glands, and eccrine glands) span the epidermis and are embedded in the dermis. In thickness, the dermis makes up approximately 90% of the skin and has largely a supportive function. Separating the dermis from underlying tissues is a layer of adipocytes, whose accumulation of fat has a cushioning action. T e blood supply to the epidermis originates in the capillaries located in the rete ridges at the dermal–epidermal junction. Capillaries also supply the bulbs of the hair follicles and the secretory cells of the eccrine (sweat) glands. T e ducts from these glands carry a dilute salt solution to the surface of the skin, where its evaporation provides cooling.

T e interfollicular epidermis is a stratified squamous epithelium consisting primarily of keratinocytes, which are tightly attached to each other and to the basement membrane. Melanocytes are distributed sparsely in the dermis, with occasional concentrations beneath the basal lamina and in the papillae of hair follicles. In the epidermis, these cells are stimulated by ultraviolet light to produce melanin granules. T e granules are extruded and taken up by the surrounding keratinocytes, which thereby become pigmented. Migrating through the epidermis are numerous Langerhans cells (LCs), which

TABLE 19–1 Factors inf uencing cutaneous responses.

Variable	Comment
Body site	
Palms/soles	Thick stratum corneum—good physical barrier Common site of contact with chemicals Occlusion with protective clothing
Intertriginous areas (axillae, groin, neck, finger webs, umbilicus, genitalia)	Moist, occluded areas Chemical trapping Enhanced percutaneous absorption
Face	Exposed frequently Surface lipid interacts with hydrophobic substances Chemicals frequently transferred from hands
Eyelids	Poor barrier function—thin epidermis Sensitive to irritants
Postauricular region	Chemical trapping Occlusion
Scalp	Chemical trapping Hair follicles susceptible to metabolic damage
Predisposing cutaneous illnesses—atopic dermatitis	Increased sensitivity to irritants Impaired barrier function
Psoriasis	Impaired barrier function
Genetic factors	Predisposition to skin disorders Variation in sensitivity to irritants Susceptibility to contact sensitization
Temperature	Vasodilation—improved percutaneous absorption Increased sweating—trapping
Humidity	Increased sweating—trapping
Season	Variation in relative humidity Chapping and wind-related skin changes

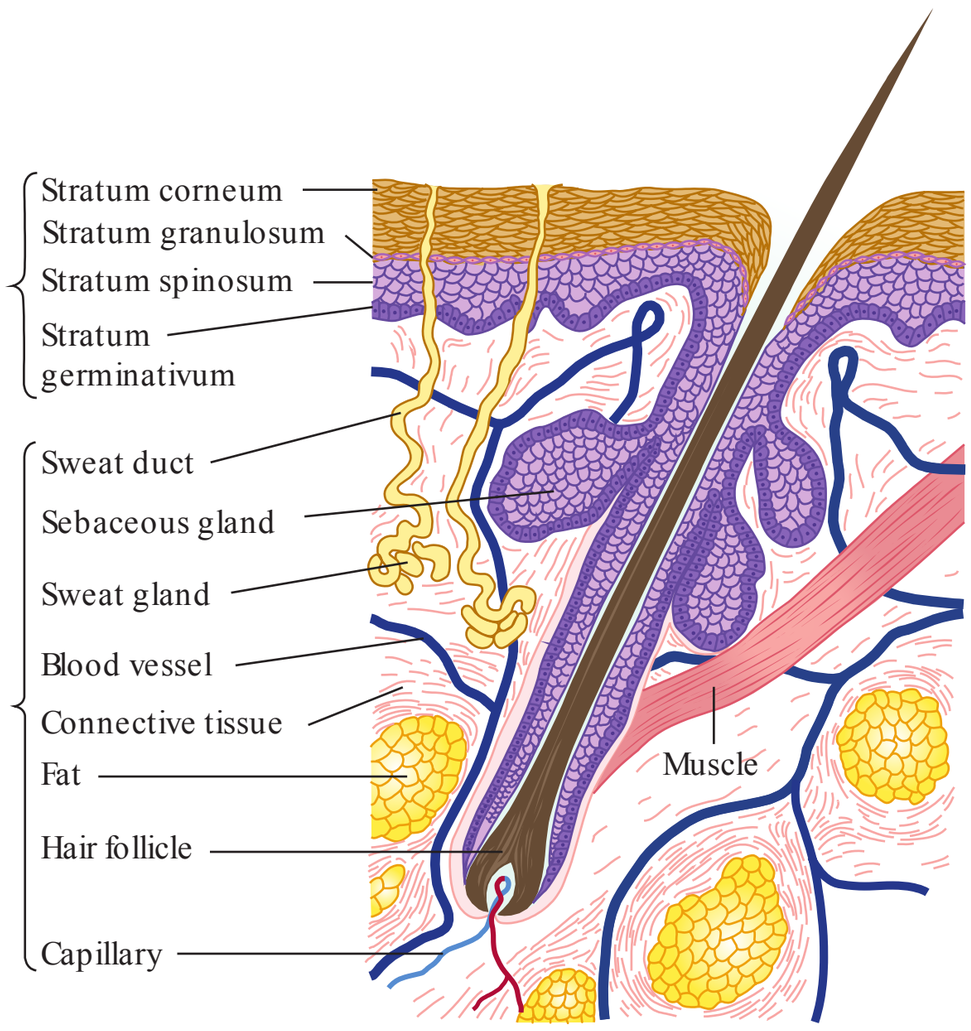

FIGURE 19–1 **Diagram of a cross-section of human skin.**

are important participants in the immune response of skin to foreign agents.

Keratinocytes of the basal layer make up the germinative compartment. When a basal cell divides, one of the progeny detaches from the basal lamina and migrates outward. As cells move toward the skin surface, they undergo a remarkable program of terminal differentiation. They gradually express new protein markers and accumulate keratin proteins. At the granular layer, the cells become flattened and increase in volume nearly 40-fold. Lipid granules fuse with the plasma membrane, replacing the aqueous environment in the intercellular space with their contents. Meanwhile, the plasma membranes of these cells become permeable and cell organelles are degraded, while a protein envelope is synthesized immediately beneath the plasma membrane. The membrane is altered characteristically by the loss of phospholipid and the addition of sphingolipid.

This program of terminal differentiation, beginning as keratinocytes leave the basal layer, produces the outermost layer of the skin, the stratum corneum. No longer viable, the mature cells (called corneocytes) are ~80% keratin in content. They are gradually shed from the surface and replaced from beneath. The process typically takes 2 weeks for basal cells to reach the stratum corneum and another 2 weeks to be shed from the surface. In instances in which the outer layer is deficient due to disease or physical or chemical trauma, the barrier to the environment that the skin provides is inferior to that provided by normal, healthy skin.

Percutaneous Absorption

The stratum corneum is the primary barrier to percutaneous absorption. Diseases (e.g., psoriasis) or other conditions (e.g., abrasion and wounding) that compromise this barrier can permit greatly increased uptake of poorly permeable substances. The viable layer of epidermis provides a much less effective barrier, because hydrophilic agents readily diffuse into the intercellular water, whereas hydrophobic agents can partition into cell membranes, and each can diffuse readily to the blood supply in the rete ridges of the dermis.

The stratum corneum prevents water loss from underlying tissues by evaporation. Its hydrophobic character reflects the lipid content of the intercellular space. The lipids, a major component being sphingolipids, have a high content of long-chain ceramides, removal of which seriously compromises barrier function as measured by transepidermal water loss. The stratum corneum is ordinarily hydrated (typically 20% water), the moisture residing in corneocyte protein, but it can take up a great deal more water on prolonged immersion, thereby reducing the effectiveness of the barrier to agents with a hydrophilic character. Indeed, occlusion of the skin with plastic wrap, permitting the retention of perspiration underneath, is a commonly employed technique to enhance uptake of agents applied to the skin surface. Penetration from the air is generally too low to be of concern.

Uptake through the skin is now incorporated in pharmacokinetic modeling to estimate potential risks from exposures. The degree of uptake depends on the details of exposure conditions, being proportional to solute concentration (assuming it is dilute), time, and the amount of skin surface exposed. In addition, two intrinsic factors contribute to the absorption rate of a given compound: its hydrophobicity, which affects its ability to partition into epidermal lipid, and its rate of diffusion through this barrier. A measure of the first property is the commonly used octanol/water partitioning ratio (K_{ow}). This is particularly relevant for exposure to contaminated water, such as occurs during bathing or swimming. However, partitioning of an agent into the skin is greatly affected by its solubility in or adhesion to the medium in which it is applied (including soil). Similarly, very hydrophobic compounds, once in the stratum corneum, may diffuse only very slowly into less hydrophobic regions below. The second property is an inverse function of molecular weight (MW) or molecular volume. Thus, hydrophobic agents of low MW permeate the skin better than those of high MW or those that are hydrophilic. For small molecules, hydrophobicity is a dominant factor in penetration.

Diffusion through the epidermis is considerably faster at some anatomical sites than others. A list in order of decreasing permeability under steady-state conditions gives the following hierarchy: foot sole > palm > scrotum > forehead > abdomen. Absorption through the epidermal appendages is generally neglected, despite the ability of agents to bypass the stratum corneum by this route, because the combined appendageal surface area is a small fraction of the total available for uptake. However, penetration through the appendages can be appreciable.

Transdermal Drug Delivery—Specially designed patches are currently in use to deliver drugs such as clonidine, estradiol, testosterone, nitroglycerin, scopolamine, fentanyl, and nicotine for therapeutic purposes. Advantages of this approach over oral dosing include providing a steady infusion for extended periods (typically 1 to 7 days) thereby avoiding large variations in plasma concentration, preventing exposure to the

acidic pH of the stomach, and avoiding biotransformation in the gastrointestinal tract or from first-pass removal by the liver.

Measurements of Penetration—For risk assessment and pharmaceutical design, the most useful subject for experimentation is human skin. Volunteers are dosed, plasma and/or urine concentrations are measured at suitable intervals, and amounts excreted from the body are estimated. For in vitro work, excised split-thickness skin can be employed in special diffusion chambers, though care is needed to preserve the viability of the living layer of epidermis. T e agent is removed for measurement from the underside by a fluid into which it partitions, thereby permitting continued penetration. A simpler setup commonly employed uses cadaver skin with the lower dermis removed. T is lacks biotransformation capability but retains the barrier function of the stratum corneum. To simplify determination of penetration kinetics, skin flaps may be employed and the capillary blood flow monitored to measure penetration. For this purpose, pig skin has particular utility. A promising variation minimizing species differences is to use skin grafts on experimental animals for these measurements. Human skin persists well on athymic mice and retains its normal barrier properties.

Biotransformation

T e ability of the skin to metabolize agents that diffuse through it contributes to its barrier function. T is influences the potential biological activity of xenobiotics and topically applied drugs, leading to their degradation or their activation as skin sensitizers or carcinogens. T e epidermis and pilosebaceous units are the major sites of such activity in the skin. Enzymes participating in biotransformation that are expressed in skin include multiple forms of cytochrome P450, epoxide hydrolase, UDP-glucuronosyltransferase, quinone reductase, and glutathione transferases. Other metabolic enzyme activities detected in human epidermal cells include sulfatases, β-glucuronidase, N-acetyltransferases, esterases, and reductases. T e intercellular region of the stratum corneum has catabolic activities (e.g., proteases, lipases, glycosidases, and phosphatase).

CONTACT DERMATITIS

Of all occupational skin diseases, contact dermatitis accounts for over 90% of reported causes. Contact dermatitis falls into the two major categories of irritant and allergic forms. Both involve inflammatory processes and can have indistinguishable clinical characteristics of erythema (redness), induration (thickening and firmness), scaling (flaking), and vesiculation (blistering) on areas directly contacting the chemical agent. Figure 19–2 shows examples of many types of contact dermatitis as a result of occupational skin toxicity.

Irritant Dermatitis

Irritant dermatitis is the condition that arises from the direct contact of agents on the skin and accounts for nearly 80% of contact dermatitis cases. A chemical in this category is anticipated to give an adverse reaction to anyone if the concentration is high enough and the exposure time long enough. Certain chemicals at suf cient concentration produce an acute irritation, sometimes called a second-degree chemical burn, that can even result in scarring in serious cases. Strong acids, alkalies, and powerful oxidizing or reducing agents can substantially disrupt the cornified layer, producing cytotoxicity directly. Contact with a variety of plants can also have irritant effects, resulting in the production of pro-inflammatory cytokines (IL1-α, IL1-β, and TNF-α) from keratinocytes. Exposure is more commonly the result of chronic cumulative irritation from repeated exposures to mild irritants such as soaps, detergents, solvents, and cutting oils. Chronic exposure in the occupational setting often elicits a process of "hardening."

Response to exposure varies depending on the sensitivity of the anatomic site. T e eyelids are quite sensitive, e.g., and the back is more sensitive than the forearm. Individuals also vary greatly in sensitivity to irritant dermatitis. Atopic individuals are the most sensitive to irritants and exhibit a propensity to produce specific IgE antibodies to allergens and typically suffer from hay fever. T ese individuals usually have a poorer prognosis than nonatopics and have a higher frequency of persistent dermatitis. T e best preventive measure for atopics and others is to avoid exposure to contact irritants.

Information on the irritancy of chemicals toward human skin may be obtained as part of differential diagnosis by patch testing for allergic response. T e skin of laboratory animals (mice, rats, rabbits, and guinea pigs) can be used for testing, but it is thinner and more sensitive than human skin to irritants. For development of new pharmaceuticals, cosmetics, and other consumer products, a great need exists for an in vitro system to determine the potential for irritant responses. Use of human epidermal cell cultures has been increasing as reconstructed epidermal and skin models come closer to the native differentiated state.

Chemical Burns

Extremely corrosive and reactive chemicals may produce immediate coagulative necrosis that results in substantial tissue damage, with ulceration and sloughing. Sometimes referred to as a third-degree chemical burn, the damage does not have a primary inflammatory component and thus may not be classified as an irritant reaction. In addition to the direct effects of the chemical, necrotic tissue can act as a chemical reservoir resulting in either continued cutaneous damage or percutaneous absorption and systemic injury after exposure. Table 19–2 lists selected corrosive chemicals that are important clinically.

Allergic Contact Dermatitis

Allergic contact dermatitis is a delayed (T-cell mediated) hypersensitive reaction (see Chapter 12). To induce sensitization, chemical haptens must penetrate the skin and become attached to carrier proteins. Complete antigens are processed by Langerhans cells and presented to type 1 T-helper cells in regional lymph nodes. Memory T cells are produced over a

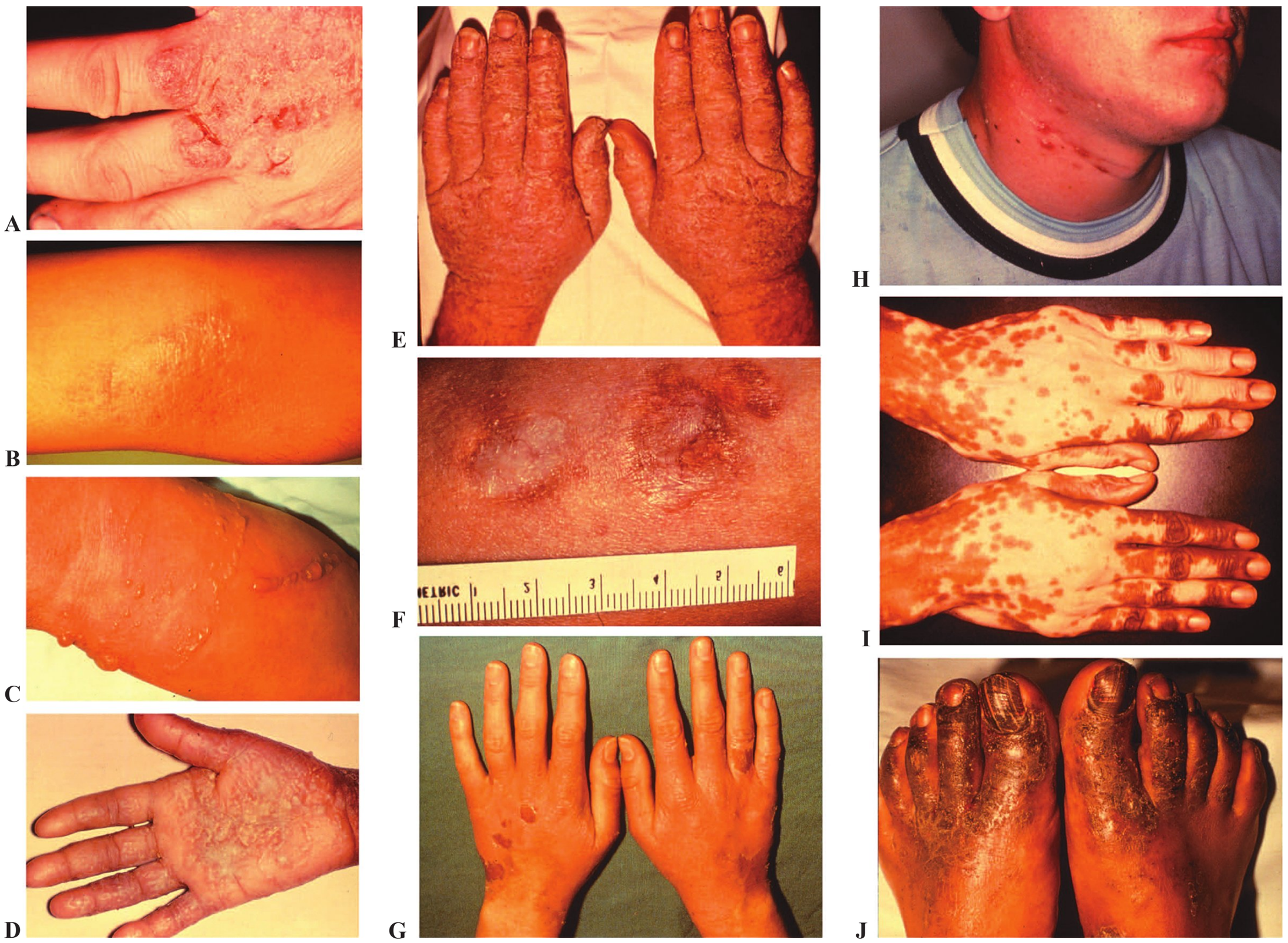

FIGURE 19–2 **Examples of occupational skin toxicity.** The panels, available at the NIOSH website (http://www.cdc.gov/niosh/topics/skin/occderm-slides/ocderm1.html), are a small selection from the 140-slide NIOSH program "Occupational Dermatoses—A Program for Physicians" prepared by Drs. E Shmunes, MM Key, JB Lucas, and JS Taylor. (A. Eczema from cutting oil. B. Atopic irritant dermatitis. C. Burn from ethylene oxide. D. Burn from alkali exposure. E. Sensitization to dichromate. F. Beryllium granulomas. G. Phototoxicity from lime juice. H. Acne from cutting oil. I. Leukoderma from rubber antioxidants. J. Hyperpigmentation from mercaptobenzothiazole.)

1- to 3-week period and enter the circulation. Subsequent exposure to the same antigen results in an amplified immune response characterized by dermal infiltration and spongiosis.

Thousands of chemicals have been reported to give rise to allergic contact dermatitis, many across a variety of occupations and consumer products (Table 19–3). Because most chemicals in the chemical universe are only weakly active or infrequently encountered, much effort has focused on finding the major allergens in the population by systematic patch testing of dermatology patients. Although not measuring sensitivity in the population at large, the results are quite useful. The panel of chemicals tested can vary with geographic location to accommodate local usage, or it can be directed to specific anatomic sites such as the foot.

Unlike contact irritants, where the response is generally proportional to the applied dose and time, contact allergens can elicit reactions at very small doses. Nevertheless, a higher dose confers a greater likelihood of sensitization and that doses below a threshold for sensitization can have a cumulative effect. In addition, the dose required to elicit a reaction is lower after sensitization with a higher dose.

Diagnosis and Testing—In order to find the responsible chemical causing allergic contact dermatitis, patch testing is commonly employed. On the washed backs of patients, patches are placed containing a small amount of a potential allergen. Diagnostic patch testing utilizes standardized concentrations of material dissolved or suspended in petrolatum or water that are placed on stainless steel chambers adhering to acrylic tape. After two to three days, during which time a maximal reaction usually develops, the patches are removed and sites of exposure are scored for degree of response. Relevance to the patient's actual environment must be considered so that exposure in daily life can be minimized to appropriate chemicals. Interpretation of the results and environmental modification should take into account the phenomenon of cross-sensitivity, where reactivity to a compound may be evident if it shares functional groups that have provoked sensitization in another

TABLE 19–2 Selected chemicals causing skin burns.

Chemical	Comment
Ammonia	Potent skin corrosive Contact with compressed gas can cause frostbite
Calcium oxide (CaO)	Severe chemical burns Extremely exothermic reaction—dissolving in water can cause heat burns
Chlorine	Liquid and concentrated vapors cause cell death and ulceration
Ethylene oxide	Solutions and vapors may burn Compressed gas can cause frostbite
Hydrogen chloride (HCl)	Severe burning with scar formation
Hydrogen fluoride (HF)	Severe, painful, slowly healing burns from high concentration Lower concentration causes delayed cutaneous injury Systemic absorption can lead to electrolyte abnormalities and death Calcium-containing topical medications and quaternary ammonium compounds are used to limit damage
Hydrogen peroxide	High concentration causes severe burns and blistering
Methyl bromide	Liquid exposure produces blistering, deep burns
Nitrogen oxides	Moist skin facilitates the formation of nitric acid causing severe yellow-colored burns
Phosphorus	White phosphorus continues to burn on skin in the presence of air
Phenol	Extremely corrosive even in low concentrations Systemic absorption through burn sites may result in cardiac arrhythmias, renal disease, and death
Sodium hydroxide	High concentration causes deep burns, readily denatures keratin
Toluene diisocyanate	Severe burns with contact Skin contact rarely may result in respiratory sensitization

compound. Common cross-reacting chemicals are listed in Table 19–4.

In animal testing, a chemical is applied to intact or abraded skin or through intradermal injection with or without adjuvant. The skin reaction to a subsequent challenge with the chemical is observed and graded, in an attempt to identify causative agents. Increasing emphasis on reducing or eliminating animal use in toxicity testing, driven in part by regulatory initiatives, has stimulated development of integrated testing strategies, where predictions of toxic effects such as skin sensitization include physical, chemical, and structural analysis and in vitro testing.

GRANULOMATOUS REACTIONS

A granulomatous reaction to a foreign body is one in which invading substances that cannot be readily removed are consequently isolated. These occur infrequently toward a variety of agents introduced into the skin through injection or after laceration or abrasion. Persistent lesions with abundant inflammatory cells can be produced, resembling chronic infectious conditions (e.g., tuberculosis, leprosy, leishmaniasis, and syphilis) and present diagnostic challenges. Many substances can produce granulomatous reactions, including silica, talc, paraffin or mineral oil, beryllium, and gadolinium. Metallic mercury and zirconium compounds, formerly used in deodorants, and tattoo dyes (containing cobalt, chromium, mercury, lead, iron, cadmium, and manganese compounds) can also induce granulomatous reactions that, in rare cases, can be induced by intense light treatment.

PHOTOTOXICOLOGY

The ultraviolet and visible spectra of solar radiation reaching the earth extend from 290 to 700 nm. Wavelengths beyond this range are either filtered by the earth's atmosphere or are insufficiently energetic to cause cutaneous pathology. Adequate doses of artificially produced UV-C (< 290 nm) or X-rays can produce profound physical and toxicological skin changes. The protective skin pigment melanin, synthesized in melanocytes, absorbs a broad range of radiation from UV-B (290 to 320 nm) through the visible spectrum. Other chromophores in the skin include amino acids, primarily tryptophan and to a lesser extent tyrosine, and their breakdown products (e.g., urocanic acid), which absorb light in the UV-B range. Biologically, the most significant chromophore is DNA, since damage from

TABLE 19–3 Common contact allergens.

Source	Common Allergens	
Topical medications/hygiene products	**Antibiotics** Bacitracin Neomycin Polymyxin Aminoglycosides Sulfonamides	**Therapeutics** Benzocaine Idoxuridine α-Tocopherol (vitamin E) Corticosteroids
	Preservatives Benzalkonium chloride Formaldehyde Formaldehyde releasers Quaternium-15 Imidazolidinyl urea Diazolidinyl urea DMDM hydantoin Methylchloroisothiazolinone	**Others** Cinnamic aldehyde Ethylenediamine Lanolin p-Phenylenediamine Propylene glycol Benzophenones Fragrances Thioglycolates
Plants and trees	Abietic acid Balsam of Peru Rosin (colophony)	Pentadecylcatechols Sesquiterpene lactone Tuliposide A
Antiseptics	Chloramine Chlorhexidine Chloroxylenol Dichlorophene Dodecylaminoethyl glycine HCl	Glutaraldehyde Hexachlorophene Thimerosal (Merthiolate) Mercurials Triphenylmethane dyes
Rubber products	Diphenylguanidine Hydroquinone Mercaptobenzothiazole p-Phenylenediamine	Resorcinol monobenzoate Benzothiazolesulfenamides Dithiocarbamates Thiurams
Leather	Formaldehyde Glutaraldehyde	Potassium dichromate
Paper products	Abietic acid Formaldehyde Nigrosine	Rosin (colophony) Triphenyl phosphate Dyes
Glues and bonding agents	Bisphenol A Epichlorohydrin Formaldehyde Acrylic monomers Cyanoacrylates	Epoxy resins p-(t-Butyl)formaldehyde resin Toluene sulfonamide resins Urea formaldehyde resins
Metals	Chromium Cobalt	Mercury Nickel

radiation can have lasting effects on the genetic information in target cells.

Adverse Responses to Electromagnetic Radiation

After exposure, the most evident acute feature of UV radiation exposure is erythema (redness or sunburn). The minimal erythema dose (MED), the smallest dose of UV light needed to induce an erythematous response, varies greatly from person to person. Vasodilation responsible for the color change is accompanied by significant alterations in inflammatory mediators released from local inflammatory cells as well as from injured keratinocytes, and may be responsible for several of the systemic symptoms associated with sunburn, such as fever, chills, and malaise. Environmental conditions that affect UV-induced injury include duration of exposure, season, altitude, body site, skin pigmentation, and previous exposure.

UV-B (290 to 320 nm) is the most effective solar band to cause erythema in human skin. A substantially greater dosage of UV-A (320 to 400 nm) reaches the earth compared with UV-B (up to 100-fold); however, its efficiency in generating erythema in humans is about 1000-fold less than that of UV-B. UV-A is likely more responsible for long-term UV effects such as wrinkling, skin atrophy, and easy bruisability. Overt pigment darkening is another typical response to UV exposure. This may

TABLE 19–4 Common cross-reacting chemicals.

Chemical	Cross-reactor
Abietic acid	Pine resin (colophony)
Balsam of Peru	Pine resin, cinnamates, benzoates
Bisphenol A	Diethylstilbestrol, hydroquinone monobenzyl ether
Canaga oil	Benzyl salicylate
Chlorocresol	Chloroxylenol
Diazolidinyl urea	Imidazolidinyl urea, formaldehyde
Ethylenediamine di-HCl	Aminophylline, piperazine
Formaldehyde	Arylsulfonamide resin, chloroallyl-hexaminium chloride
Hydroquinone	Resorcinol
Methyl hydroxybenzoate	Parabens, hydroquinone monobenzyl ether
p-Aminobenzoic acid	p-Aminosalicylic acid, sulfonamide
Phenylenediamine	Parabens, p-aminobenzoic acid
Propyl hydroxybenzoate	Hydroquinone monobenzyl ether
Phenol	Resorcinol, cresols, hydroquinone
Tetramethylthiuram disulfide	Tetraethylthiuram mono- and disulfide

be accomplished by enhanced melanin production by melanocytes or by photooxidation of melanin. Tanning or increased pigmentation usually occurs within 3 days of UV light exposure, whereas photooxidation is evident immediately. The tanning response is most readily produced by exposure to UV-B and may be induced, along with erythema and DNA repair, by DNA damage. The tanning response serves to augment the protective effects of melanin in the skin. However, the immediate pigment-darkening characteristic after exposure to UV-A and to visible light does not confer improved photoprotection.

Chronic exposure to radiation induces a variety of characteristic skin changes. For ultraviolet light, these changes accelerate or mimic aging, but the rate depends greatly on the baseline skin pigmentation of the individual. Lighter skinned people suffer from chronic skin changes with greater frequency than darker individuals. Pigmentary changes such as freckling and hypomelanotic areas, wrinkling, telangiectasias (fine superficial blood vessels), actinic keratoses (precancerous lesions), and malignant skin lesions such as basal and squamous cell carcinomas and malignant melanomas are all consequences of chronic exposure to ultraviolet light exposure.

One significant pathophysiological response of chronic exposure to ultraviolet light is the pronounced decrease of epidermal Langerhans cells. Chronically sun exposed skin may have up to 50% fewer of these compared to photoprotected areas. This decrease may result in lessened immune surveillance of neoantigens on malignant cells and thus allow such a transformation to proceed unabated. Exposures to ionizing radiation may produce a different spectrum of disease depending upon the dose delivered. Large acute exposures will result in local redness, blistering, swelling, ulceration, and pain. After a latent period or following subacute chronic exposures, characteristic changes such as epidermal thinning, freckling, telangiectasias, and nonhealing ulcerations may occur. Also, a variety of skin malignancies have been described years after skin exposure to radiation.

Aside from the toxic nature of electromagnetic radiation, natural and environmental exposures to certain bands of light are vital for survival. Ultraviolet radiation is critical for the conversion of 7-dehydrocholesterol to previtamin D_3, a required precursor for normal endogenous production of vitamin D. Blue light in the 420- to 490-nm range can photoisomerize bilirubin (a red blood cell breakdown product) in the skin, rendering urinary excretion of this neurotoxic metabolite by infants with elevated serum bilirubin. In addition, the toxic effects of UV light have been exploited for decades through artificial light sources for treatment of hyperproliferative skin disorders like psoriasis.

Photosensitivity

An abnormal sensitivity to UV and visible light, photosensitivity may result from endogenous or exogenous factors. Various genetic diseases, such as xeroderma pigmentosum, and the autoimmune disease lupus erythematosus impair the cell's ability to repair UV light-induced damage. In hereditary or chemically induced porphyrias, enzyme abnormalities disrupt the biosynthetic pathways producing heme, leading to accumulation of porphyrin precursors or derivatives throughout the body. These compounds in general fluoresce when exposed to light of 400 to 410 nm (Soret band), and in this excited state interact with cellular macromolecules or with molecular oxygen to generate toxic-free radicals. A "constitutional" sensitivity to light (porphyria cutanea tarda) can be precipitated by alcohol, estrogens, or certain antibiotics in individuals with hereditary abnormalities in porphyrin synthesis, and an "acquired" sensitivity in general by hexachlorobenzene and mixtures of polyhalogenated aromatic hydrocarbons.

Phototoxicity—Phototoxic reactions from exogenous chemicals may be produced by systemic or topical administration or exposure. In acute reactions, the skin may appear red and blister within minutes to hours after ultraviolet light exposure. Chronic phototoxic responses may result in hyperpigmentation and thickening of the affected areas. UV-A (320 to 400 nm) is the most commonly responsible; UV-B (290 to 320 nm) may occasionally be involved.

Agents most often associated with phototoxic reactions are listed in Table 19–5. These chemicals readily absorb UV light and assume a higher energy excited state. The oxygen-dependent photodynamic reaction is the most common as these excited molecules return to the ground state. Here, excited triplet-state molecules transfer their energy to oxygen, forming singlet oxygen, or become reduced and form other highly reactive free radicals. These reactive products are capable of damaging

TABLE 19–5 Selected phototoxic chemicals.

Furocoumarins
8-Methoxypsoralen
5-Methoxypsoralen
Trimethoxypsoralen
Polycyclic aromatic hydrocarbons
Anthracene
Fluoranthene
Acridine
Phenanthrene
Drugs
Tetracyclines
Sulfonamides
Sulfonylureas
Nalidixic acid
Thiazides
Phenothiazines
Nonsteroidal anti-inflammatory drugs
Dyes
Disperse blue 35
Eosin
Acridine orange
Porphyrin derivatives
Hematoporphyrin

cellular components and macromolecules and causing cell death. The resulting damage elaborates a variety of immune mediators from keratinocytes and local white blood cells that recruit more inflammatory cells to the skin, and thus yield the clinical signs of phototoxicity.

Nonphotodynamic mechanisms have been described in the pathogenesis of phototoxicity, with psoralens (furocoumarins) being prime examples. On entering the cell, psoralens intercalate with DNA. Subsequent excitation with UV-A provokes a photochemical reaction that ultimately results in a covalently linked cycloadduct between the psoralen and pyrimidine bases. This substantially inhibits DNA synthesis and repair, resulting in clinical phototoxic reactions. Psoralens may be found in sufficiently high concentrations in limes and celery to cause a significant blistering eruption called phytophotodermatitis. Psoralen-induced phototoxicity may be harnessed and controlled pharmacologically. Topically and orally administered psoralens are used therapeutically to enhance the effects of controlled delivery of UV-A. Psoralens plus UV-A (PUVA) is administered to control keratinocyte and lymphocyte hyperproliferative diseases such as psoriasis, eczema, and cutaneous T-cell lymphomas.

Photoallergy—In contrast to phototoxicity, photoallergy is a type IV delayed hypersensitivity reaction, leading typically to eczema. Hence, photoallergy requires prior sensitization to the chemical. Induction and subsequent elicitation of reactions may result from topical exposure as in photocontact dermatitis or from systemic photoallergy. Generally, the mechanisms of photocontact dermatitis and even that of systemic photoallergy are the same as that described above for allergic contact dermatitis. However, UV light is necessary to convert a potential photosensitizing chemical into a hapten that elicits an allergic response. Photoallergy generally is distinguishable from phototoxicity because the former results from delayed hypersensitivity, and amounts of chemical too low to give a toxic response still sufficient to elicit allergy.

Diagnosis is best performed by patch testing with and without light exposure of the treated surface to distinguish photocontact from contact allergy. Because the offending chemical may not be obvious from the patient history due to the delay between exposure to the chemical and sunlight and the symptoms, a panel of test chemicals may include some 50 common photoallergens as well as the patient's own sunscreen and personal care products. To assist in predicting risks of photoallergy, efforts have been made to derive important chemical features among existing photoallergens that account for their reactivity toward proteins. This information, coupled with assessment of physical properties such as aqueous/lipid partitioning, is anticipated to streamline testing of new products.

ACNE

Acne is a pleomorphic disease with a multifactorial etiology. The influence of sebum, hormones, bacteria, genetics, and environmental factors is well known. In many situations, one of these factors has an overwhelmingly greater influence in the genesis of lesions than the others.

Comedogenic chemicals induce comedone lesions, which may be open or closed (blackhead or whitehead, respectively, in the vernacular). Additionally papules, pustules, cysts, and scars may complicate the process. Hair follicles and associated sebaceous glands become clogged with compacted keratinocytes that are bathed in sebum. The pigmentary change most evident in open comedones is from melanin.

Chloracne

Chloracne, one of the most disfiguring forms of acne in humans, is caused by exposure to polyhalogenated aromatic hydrocarbons. Chloracne is a relatively rare disease; however, its recalcitrant nature and preventability make it an important occupational and environmental illness. Typically, comedones and straw-colored cysts are present behind the ears, around the eyes, and on the shoulders, back, and genitalia. In addition to acne, hypertrichosis (increased hair in atypical locations), hyperpigmentation, brown discoloration of the nail, conjunctivitis, and eye discharge may be present.

PIGMENTARY DISTURBANCES

Several factors influence pigmentation of the skin. Melanin is produced through a series of enzymatic pathways beginning with tyrosine. Errors in this pathway or exposure to tyrosine analogs may result in abnormal pigmentation. Hyperpigmentation results from increased melanin production or deposition of endogenous or exogenous pigment in the upper dermis. Exogenous hyperpigmentation can arise from deposition of metals and drugs in dermal tissue. Conversely,

TABLE 19–6 Selected causes of cutaneous pigmentary disturbances.

I. Hyperpigmentation
Ultraviolet light exposure
Postinflammatory changes (melanin and/or hemosiderin deposition)
Hypoadrenalism
Internal malignancy
Chemical exposures
Coal tar volatiles
Anthracene
Picric acid
Mercury
Lead
Bismuth
Furocoumarins (psoralens)
Hydroquinone (paradoxical)
Drugs
Chloroquine
Amiodarone
Bleomycin
Zidovudine (AZT)
Minocycline
II. Hypopigmentation/depigmentation/leukoderma
Postinflammatory pigmentary loss
Vitiligo
Chemical leukoderma/hypopigmentation
Hydroquinone
Monobenzyl, monoethyl, and monomethyl ethers of hydroquinone
p-(t-Butyl)phenol
Mercaptoamines
Phenolic germicides
p-(t-Butyl)catechols
Butylated hydroxytoluene

hypopigmentation is a loss of pigmentation from melanin loss, melanocyte damage, or vascular abnormalities. Leukoderma (vitiligo) and depigmentation denote complete loss of melanin from the skin, imparting a porcelain-white appearance. Table 19–6 lists chemicals capable of altering pigmentation.

URTICARIA

For those allergens to which IgE antibodies have been elicited by previous or ongoing exposure, subsequent contact can lead to development of utricaria (hives), typically in minutes, through an immediate type I hypersensitivity reaction (see Chapter 12). Hives are raised wheals that usually itch or sting and may appear reddish. They generally disappear within hours and rarely lasting longer than a day or two.

Food allergies and pharmaceuticals are major causes of acute urticaria, but many other causes are known. Certain food allergies (e.g., to nuts, fish, and shellfish) are capable of producing the life-threatening response, anaphylactic shock. Some agents (e.g., opiates) can bring about direct release of histamine from mast cells without antibody mediation, while others (nonsteroidal anti-inflammatories) may do so through effects on arachidonic acid metabolism or by uncertain mechanisms.

Contact urticaria in an occupational setting can arise from exposure to plant or animal proteins and appears more common in atopic individuals. Among the numerous occupations where this response occurs include hairdressers and those involving routine handling of food, plant, or animal products. Healthcare is an occupation in which allergic contact dermatitis to latex rubber is a common problem. Latex proteins have a propensity to induce immediate type I hypersensitive reactions, where the response can range from a mild skin reaction to anaphylaxis and death. Some substances that have been reported to cause contact urticaria are listed in Table 19–7.

TABLE 19–7 Selected substances reported to elicit contact urticaria.

Chemicals	Foods
Anhydrides	Animal viscera
Methylhexahydrophthalic	Apple
Hexahydrophthalic	Artichoke
Maleic	Asparagus
Antibiotics	Beef
Bacitracin	Beer
Streptomycin	Carrot
Cephalosporins	Chicken
Penicillin	Deer
Rifamycin	Egg
Benzoic acid	Fish
Cobalt chloride	Lamb
Butylhydroxyanisol (BHA)	Mustard
Butylhydroxytoluene (BHT)	Paprika
Carboxymethylcellulose	Potato
Cyclopentolate hydrochloride	Pork
Diphenyl guanidine	Rice
Epoxy resin	Strawberry
Formaldehyde	Turkey
Fragrances	
Balsam of Peru	
Cinnamic aldehyde	
Isocyanates	
Diphenylmethane-4,4-diisocyanate	
Menthol	
Plants, woods, trees, and weeds	
Latex	
Phenylmercuric acetate	
Xylene	

TOXIC EPIDERMAL NECROLYSIS

Toxic epidermal necrolysis (TEN) represents one of the most life-threatening dermatologic diseases that is caused by drugs and chemicals. At the most severe end of a spectrum, TEN

involves detachment of ≥ 30% of the epidermal surface from the dermis, commonly accompanied by severe erosions of mucous membranes, and has a fatality rate ≈ 30%. TEN commonly resembles an upper respiratory tract infection in the first several days (fever, cough, sore throat, and malaise), but prompt diagnosis when the cutaneous lesions become evident several days later improves survival chances.

Nearly 200 drugs have been reported to cause this syndrome with major contributors being anticonvulsants, nonsteroidal anti-inflammatories, antibacterial sulfonamides, allopurinol, and nevirapine. Mechanisms leading to this idiosyncratic drug reaction are under scrutiny and current hypotheses identify HLA genotype and ethnic background as contributing factors.

A characteristic feature of the syndrome is the large-scale apoptosis of epidermal keratinocytes. Candidates for mediating apoptosis through cell surface death receptors include tumor necrosis factor and FAS ligand, which appear elevated; in addition, drug-sensitized natural killer and cytotoxic T lymphocytes, secreting granulysin, and other components of the innate immune response may participate in inducing keratinocyte death. Effectiveness of treatments has been dif cult to evaluate, but promising approaches involve immunosuppression (cyclophosphamide, cyclosporine) or blockage of death receptors using intravenous immunoglobulin therapy.

SKIN CANCER

Radiation

Radiation from ionizing wavelengths to ultraviolet wavelengths has been shown to cause skin cancer. Shortly after the discovery of radioactive elements at the turn of the twentieth century, it was observed that X-rays could cause severe burns, squamous cell carcinoma, and basal cell carcinomas. X-ray-induced nonmelanoma skin cancers (NMSC) continued to be observed throughout the twentieth century, as X-rays were used therapeutically until the mid-twentieth century for a variety of skin diseases (acne, atopic dermatitis, psoriasis, and tinea). Although NMSC from X-rays are now uncommon, dermal atrophy or sclerosis still is seen as sequelae of radiodermatitis, which sometimes develops after X-ray treatment of internal malignancies.

UV-induced Skin Cancer

Most skin cancers in the United States now are UV-induced. T e most common UV-induced skin cancers are NMSC and cutaneous malignant melanoma. UV-B (290 to 320nm) induces pyrimidine dimers and 8-oxoguanine modifications, thereby eliciting mutations in critical genes. T e p53 tumor suppressor gene has been targeted in nearly all squamous cell carcinomas. Because the p53 protein arrests cell cycling until DNA damage is repaired and may induce apoptosis, its loss destabilizes the genome of initiated cells and gives them a growth advantage. UV light also has immunosuppressive effects that may help skin tumors survive. Skin cancer incidence is highest in the tropics and in pale-complexioned whites. Even when it does not cause cancer in normal individuals, sun exposure leads to premature aging of the skin. For this reason, sunbathing is discouraged and the use of sun-block lotions is encouraged.

Polycyclic Aromatic Hydrocarbons

Substances rich in polycyclic aromatic hydrocarbons (coal tar, creosote, pitch, and soot) are skin carcinogens in humans and animals. Oxidative biotransformation of polycyclic aromatic compounds produces electrophilic epoxides that can form DNA adducts. Phenols, produced by rearrangement of the epoxides, can be oxidized further to quinones, yielding active oxygen species, and they are also toxic electrophiles. Occupations at risk of skin cancer from exposure to these compounds (e.g., roofing) often involve considerable sun exposure, an additional risk factor.

Mouse Skin Tumor Promotion

Mouse skin has been developed as an important target for carcinogenicity testing. T e observed incidence of squamous cell carcinomas in mouse skin is taken as evidence of a general carcinogenic risk for humans. Much has been learned about squamous cell carcinoma pathogenesis in mouse skin that does have general applicability to human squamous cell carcinomas. An advantage of the mouse skin carcinogenesis model is the ability to separate the neoplastic process into stages of initiation, promotion, and progression depending on experimental design.

Arsenic

Arsenic is an abundant element in the earth's crust that is encountered routinely in small doses in the air, water, and food. High exposures from smelting operations and from well water derived from rock strata with a high arsenic content are associated with arsenical keratoses (premalignant lesions), blackfoot disease (a circulatory disorder reflecting endothelial cell damage), and squamous cell carcinoma of the skin and several other organs (bladder, lung, and liver). Arsenite (+ 3 oxidation state) avidly binds vicinal thiols and is thought to inhibit DNA repair, whereas arsenate (+ 5 oxidation state) can replace phosphate in macromolecules such as DNA, but the resulting esters are unstable. Arsenic also alters DNA methylation, suppresses keratinocyte differentiation markers, and enhances growth factor secretion in the epidermis. Methylation has been considered the most likely detoxification method, because the observed mono- and dimethyl arsenate isolated in urine from exposed humans and animals are indeed much less toxic.

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QUESTIONS

1. Which of the following statements is FALSE regarding skin histology?
   a. Blood supply to the epidermis originates in the epidermal–dermal junction.
   b. Melanin is made and stored by melanocytes.
   c. T e stratum corneum is made up of nonviable cells.
   d. It takes approximately 2 weeks for cells to be sloughed off from the stratum corneum.
   e. Stem cells in the basal layer replenish the keratinocytes of the layers of epidermis.

2. Transdermal drug delivery does NOT:
   a. prevent drug exposure to low pH.
   b. avoid first-pass metabolism.
   c. provide steady infusion over an extended period of time.
   d. avoid large variation in drug plasma concentration.
   e. increase safety of drug delivery.

3. Irritant and contact dermatitis are marked by all of the following characteristics EXCEPT:
   a. softness.
   b. erythema.
   c. flaking.
   d. induration.
   e. blistering.

4. Nickel is a common cause of allergic contact dermatitis, which is which type of hypersensitivity reaction?
   a. type I.
   b. type II.
   c. type III.
   d. type IV.
   e. type V.

5. All of the following statements regarding phototoxicology are true EXCEPT:
   a. Melanin is primarily responsible for the absorption of UV-B radiation.
   b. UV-A is the most effective at causing sunburn in humans.
   c. IL-1 release is responsible for systemic symptoms associated with sunburn.
   d. Melanin darkening is a common response to UV exposure.
   e. UV radiation exposure causes thickening of the stratum corneum.

6. Photoallergies:
   a. represent a form of type III hypersensitivity reaction.
   b. can occur without exposure to UV radiation.
   c. are hapten-mediated
   d. cannot be tested for as contact dermatitis allergies can.
   e. often occur on first exposure.

7. Diffusion through the epidermis would occur most slowly across skin at which of the following locations?
   a. palm.
   b. forehead.
   c. scrotum.
   d. foot sole.
   e. abdomen.

8. Which of the following statements regarding photosensitivity is FALSE?
   a. Porphyrias cause light sensitivity because of the lack of heme synthesis.
   b. Lupus patients are unable to repair damage caused by UV light.
   c. Chronic phototoxic responses often result in hyperpigmentation.
   d. Photoallergy represents a type IV hypersensitivity reaction.
   e. UV radiation causes cycloadducts between pyrimidine bases.

9. Acne is caused by all of the following EXCEPT:
   a. clogged sebaceous glands.
   b. hormones.
   c. viruses.
   d. genetics.
   e. environmental factors.

10. All of the following statements regarding urticaria are true EXCEPT:
    a. Urticaria is a delayed-type hypersensitivity reaction.
    b. Hives are mediated partly by histamine release from mast cells.
    c. Latex is a common chemical cause of urticaria.
    d. Select foods have been reported to elicit contact urticaria.
    e. Urticaria is mediated by IgE antibodies.

CHAPTER

20

Toxic Responses of the Reproductive System

Paul M.D. Foster and L. Earl Gray Jr.

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- Testing Pharmaceuticals

EVALUATION OF TOXICITY TO REPRODUCTION

KEY POINTS

- T e gonads possess a dual function: an endocrine function involving the secretion of sex hormones and a non-endocrine function relating to the production of germ cells (gametogenesis).
- Gametogenic and secretory functions of either the ovary or testes are dependent on the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary.
- T e blood–testis barrier between the lumen of an interstitial capillary and the lumen of a seminiferous tubule impedes or prevents the free exchange of chemicals/drugs between the blood and the fluid inside the seminiferous tubules.
- Xenobiotics can act directly on the hypothalamus and the adenohypophysis, leading to alterations in the secretion of hypothalamic-releasing hormones and/or gonadotropins.
- Steroid hormone biosynthesis can occur in several endocrine organs including the adrenal cortex, ovary, and the testes.
- Female reproductive processes of oogenesis, ovulation, the development of sexual receptivity, coitus, gamete and zygote transport, fertilization, and implantation of the conceptus may be sites of xenobiotic interference.
- Xenobiotics may influence male reproductive organ structure, spermatogenesis, androgen hormone secretion, and accessory organ function.

INTRODUCTION

Chemicals can adversely affect reproduction in males and females. Recent trends in human fertility point to the potential for declines in normal human reproduction and suggest that exposure to environmental chemicals and drugs may contribute to these declines. T e reproductive cycle is outlined in Figure 20–1.

THE REPRODUCTIVE CYCLE

Numerous complex processes are orchestrated in a precise and sequential order for optimal performance at different stages of the life cycle of animals and humans. Following fertilization of an egg by a sperm, the resulting zygote must be transported along the oviduct while maturing into an early embryo. T is embryo must then implant in the uterus successfully, differentiate, produce a placenta, and undergo normal embryogenesis and fetal development.

Acquisition of sexual maturity is marked by the generation of gametes by the gonads. For parental animals, once their reproductive life span has finished, the process of reproductive senescence then occurs. T ese processes all involve complex interplay between tissues and cells, under hormonal control that provides the critical signals and precise timing of these events. All these processes can be targets for the action of specific agents that can disturb events leading to adverse effects on reproduction, such that the normal production of viable offspring cannot occur.

Any description of reproductive toxicity has to be in the context of the life stage of exposure and effect. Chemicals can have different effects on reproduction at different life stages and via different modes of action/mechanisms. Indeed, it might be useful for this particular aspect of toxicity to modify the adage of Paracelsus to "It is the timing of the dose that makes the poison." T at is, the dose of the toxic chemical and its resultant effects will be dependent on when in the life stage of the organism that the chemical is administered and evaluated.

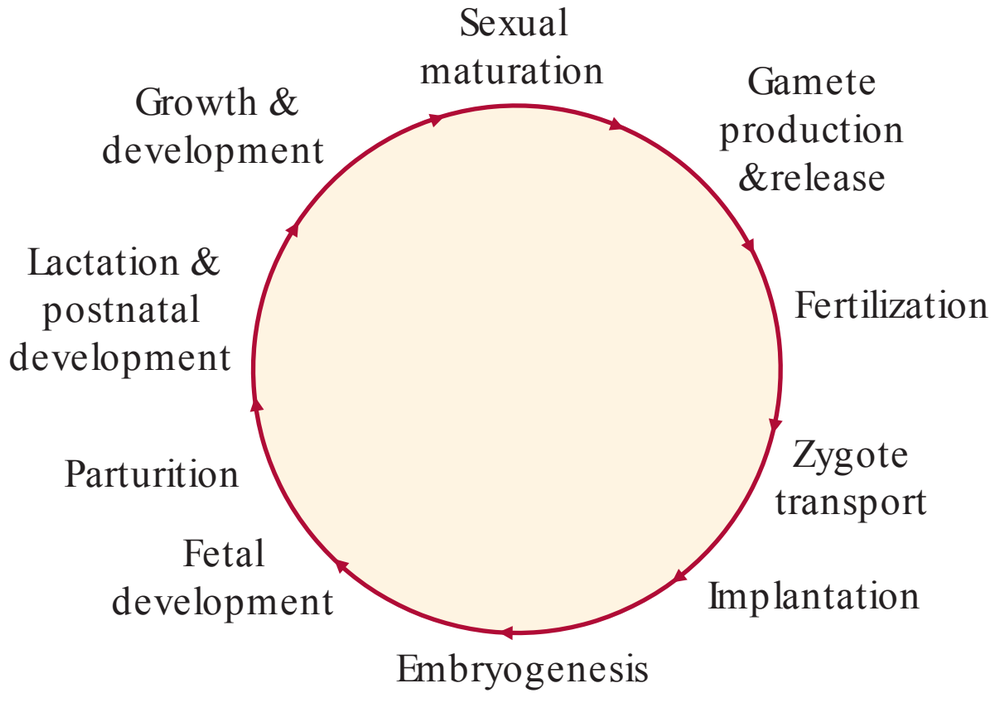

FIGURE 20–1 **The reproductive cycle.**

REPRODUCTIVE DEVELOPMENT AND SEXUAL DIFFERENTIATION

During the seventh week of human gestation, the male and female morphological characteristics begin to develop. Gonadal differentiation depends on signals from the Y chromosome, which contains the genes necessary to induce testicular morphogenesis. One of these signals is the SRY gene, which is the sex-determining region on the short arm of the Y chromosome and acts as a "switch" to initiate transcription of other genes that contribute to testicular organogenesis. In the

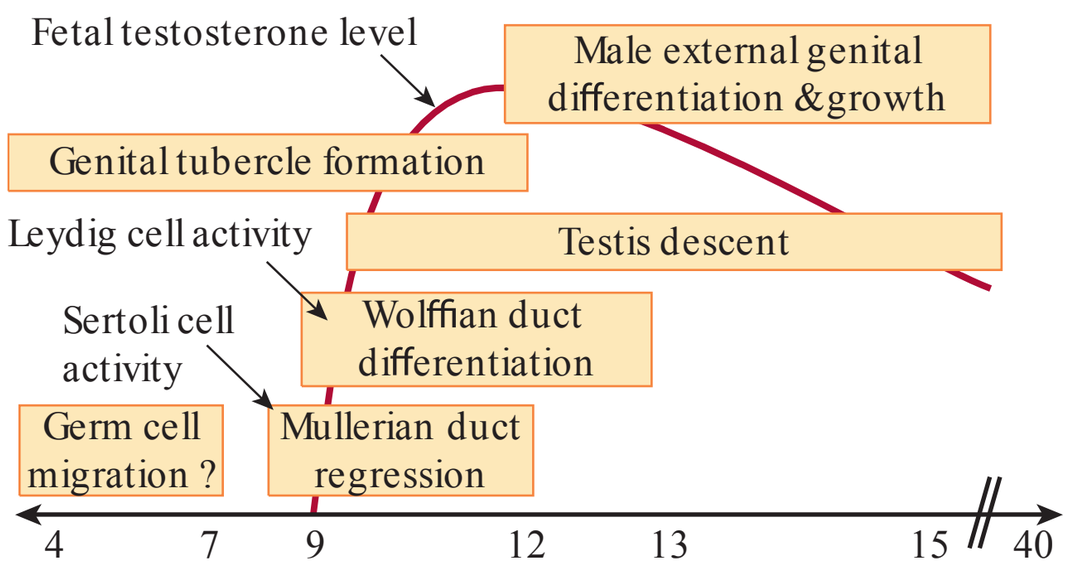

FIGURE 20–2 **Male sexual dif erentiation in humans during gestation**. (Reproduced with permission from Klonisch T, Fowler PA, Hombach-Klonisch S: Molecular and genetic regulation of testis descent and external genitalia development, Dev Biol, 2004 Jun 1;270(1):1–18.)

absence of the SRY protein, the gonad remains indifferent for a short period of time before differentiating into an ovary.

Interstitial Leydig cells produce the male sex hormone testosterone, which induces masculine differentiation of the Wolffian duct (aka mesonephric duct) and external genitalia. Figure 20–2 provides a diagrammatic representation of sexual differentiation in the human male. In rodent and human species, fetal testicular androgen production is necessary for proper testicular development, normal male sexual differentiation, and differentiation of the Wolffian ducts into the epididymides, vasa deferentia, and seminal vesicles.

Androgens derived from the Leydig interstitial cells stimulate the Wolffian ducts to form the male genital ducts, while Sertoli cells produce Müllerian-inhibiting substance (aka anti-Müllerian hormone), which suppresses development of the paramesonephric (Müllerian) ducts, or female genital ducts.

In the humans, the external genitalia are indistinguishable until the ninth week of gestation, and not fully differentiated until the 12th week of development. Development of the external genitalia coincides with gonadal differentiation. Fetal testicular androgens are responsible for the induction of masculinization of the androgynous external genitalia. T us, male, but not female, reproductive tract development is totally hormonally dependent and inherently more susceptible to endocrine disruption.

GAMETOGENESIS

T e critical feature in the production of gametes is the process of meiosis, in which there are two cell divisions with no intervening DNA replication. T is results in four daughter cells that possess half of the chromosome complement of the parent cell. T e mammalian oocyte (Figure 20–3) begins meiosis during fetal development but arrests partway through meiosis I and does not complete the first division until ovulation; the second division is completed only if the egg is fertilized. In the males, meiosis begins at puberty and is a continuous process, with spermatocytes progressing from prophase to the meiotic second division in little more than a week. T is difference in strategy has implications for the action of toxicants and critical time periods when these cells may be vulnerable to attack. Critical to this is the understanding that the complement of oocytes available to the mammalian female is complete at birth, whereas in the male there is significant stem cell (spermatogonial) renewal to maintain the significantly higher number of germ cells available in males.

NEONATAL DEVELOPMENT

Late in gestation and at birth, male rats display longer anogenital distances (AGD) than do female rats, with neonatal male AGD being more than twice as long as females. T ere are homologous sex differences in humans. In many mammalian

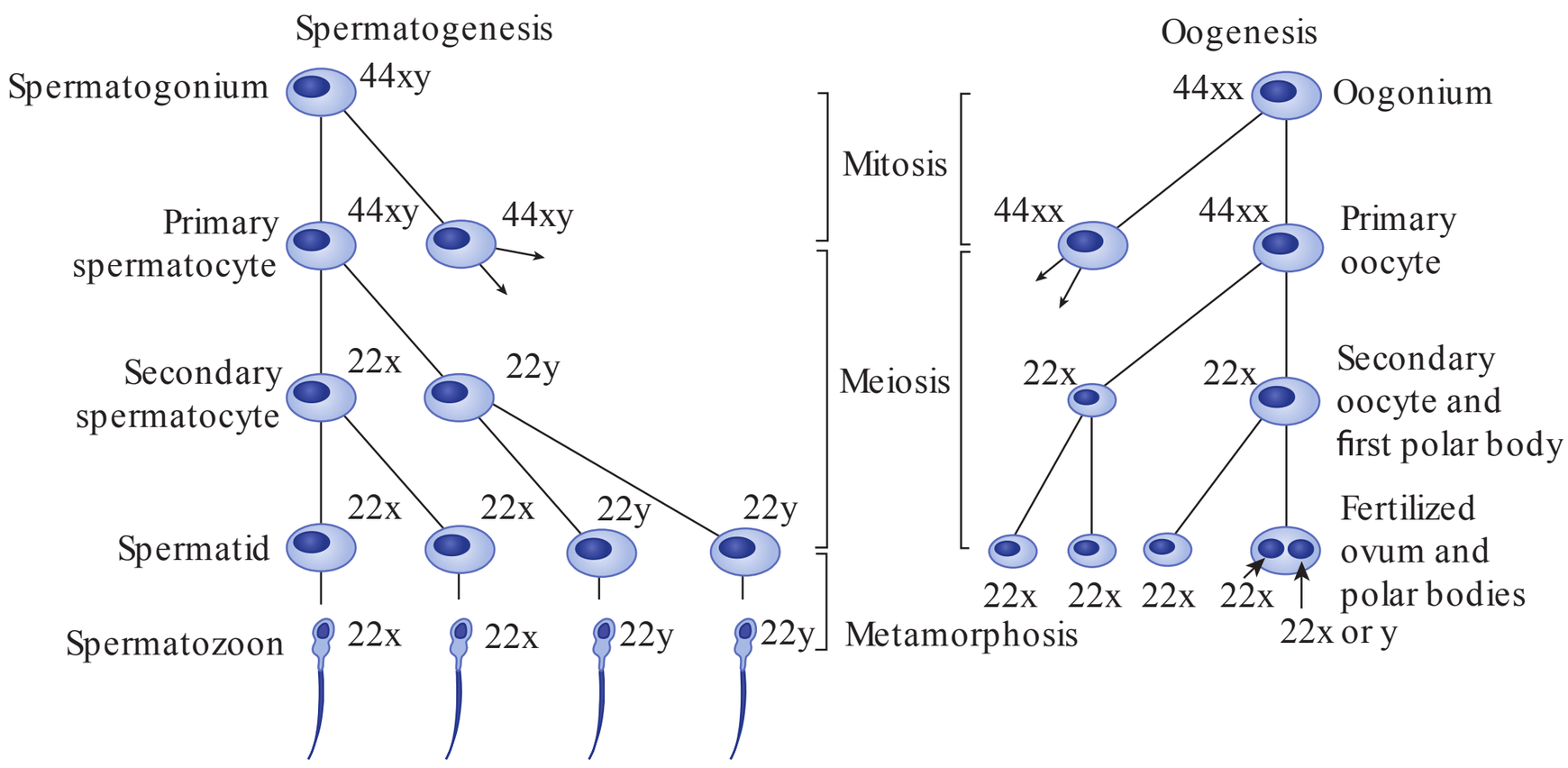

FIGURE 20–3 **Cellular replication (mitosis) and cellular reductive divisions (meiosis) involved in spermatogenesis, oogenesis, and fertilization.**

species, including humans and rats, males of the species engage in more aggressive play than do females. Both AGD and behavior can be altered by exposure to hormonal and antihormonal agents.

INFANTILE DEVELOPMENT

During the infantile period of development, emergence of the nipple buds and areolae in females as well as maturation of the hypothalamic–pituitary axis occurs. Emergence of the nipple buds is prevented in males by prenatal androgen-induced atrophy of the nipple anlagen. Prenatal androgen-treated females may display reproductive tract malformations (retained male tissues or vaginal agenesis).

PUBERTAL DEVELOPMENT

Puberty is initiated by activation of the hypothalamic–pituitary–gonadal (HPG) and hypothalamic–pituitary–adrenal (HPA) axes (see Figure 20–4). At the onset, the HPG axis releases gonadotropin-releasing hormone (GnRH) pulses with increasing frequency and amplitude that induces complementary pulsatile secretions of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. In turn, LH and FSH stimulate the gonads inducing gonadarche characterized by the onset of gonadal hormone production. In females, secretion of androgens from theca cells and estradiol from granulosa cells of maturing follicles prior to ovulation is followed by secretion of progesterone from the corpus luteum after ovulation. In males, LH stimulates testicular synthesis and secretion of androgens and insulin-like peptide 3 hormone from the Leydig cells of males.

Premature thelarche (secondary breast development) and premature adrenarche are often referred to as pseudoprecocious puberty when the full spectrum of pubertal changes does not occur. Premature thelarche in girls and gynecomastia in boys result from direct exposure to estrogen-containing personal care and "natural" products. Untoward consequences of these conditions may occur with prolonged exposure, including shortened stature due to effects of estrogens on the growth plates of the long bones and sexual–social behavior that is inappropriate for the chronological age of the child. Concerns have also been expressed that premature thelarche may enhance the likelihood of developing diseases like breast cancer and endometriosis.

T e association of pubertal alterations with environmental exposure to persistent halogenated organic chemicals such as polychlorinated biphenyls (PCBs), brominated flame retardants, dioxin, hexachlorobenzene, endosulfan, and heavy metals also has been studied but a consensus about the causative role of these chemicals in altering puberty has not been achieved.

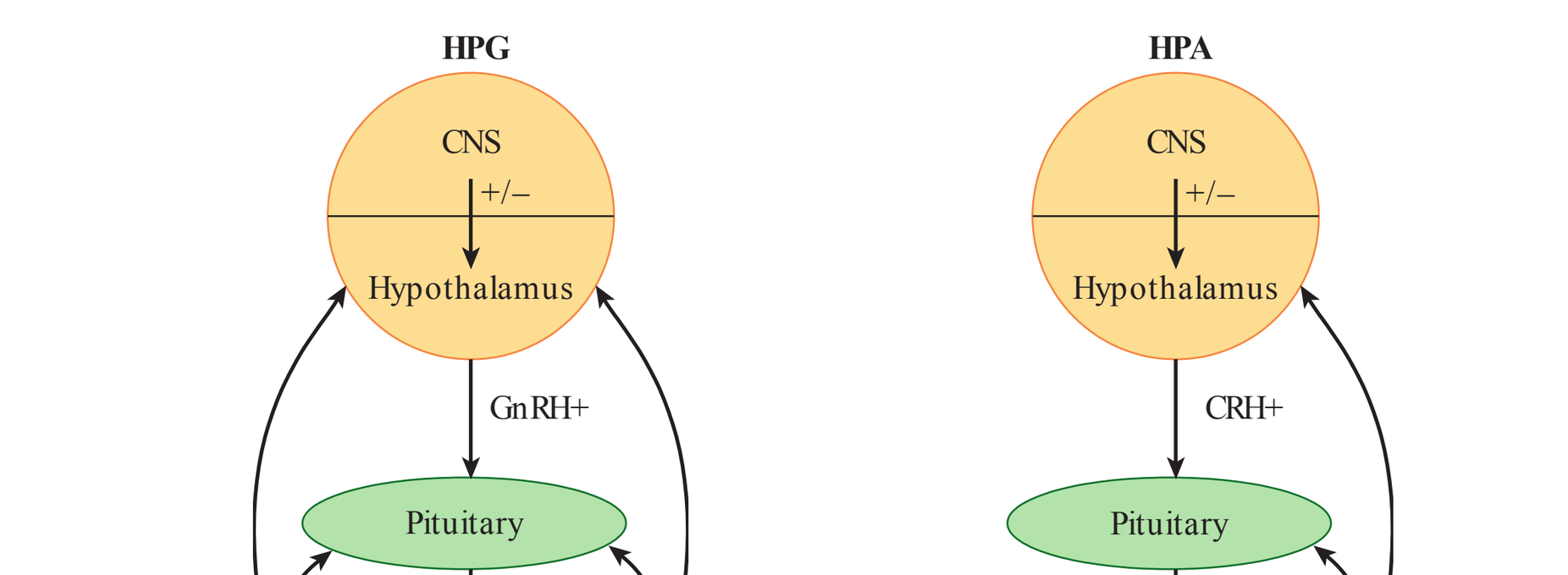

FIGURE 20–4 **Endocrine control of puberty in males and females.** Prod., production.

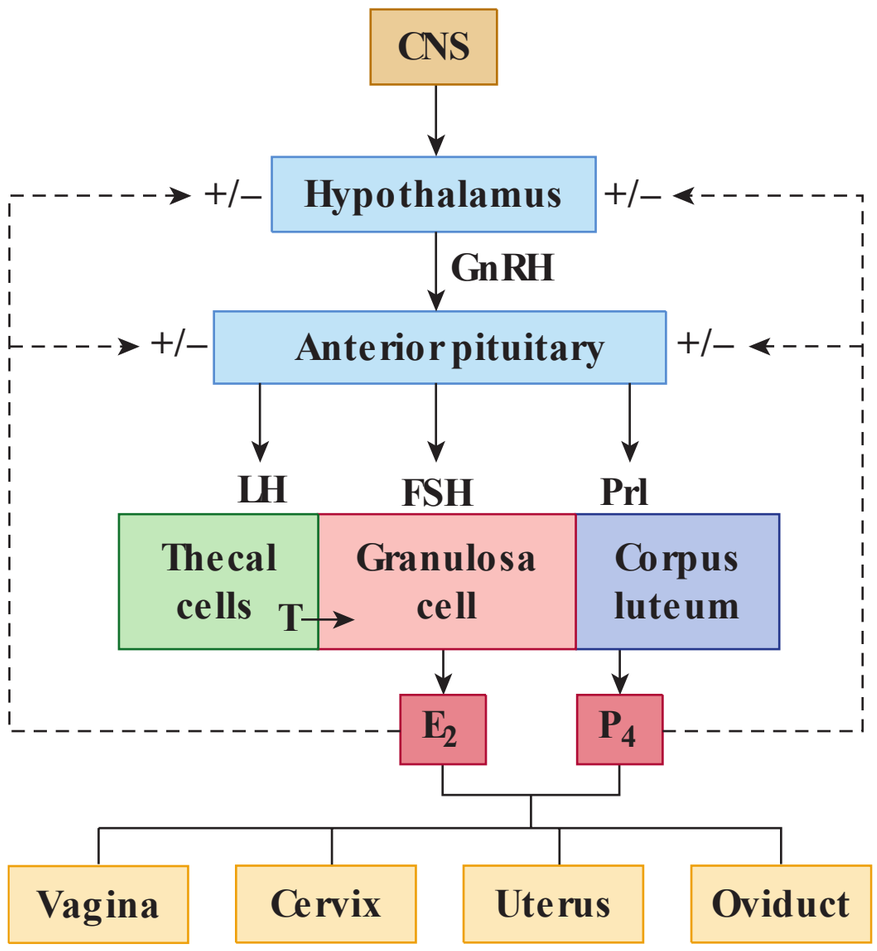

FIGURE 20–5 **Endocrine control of the female reproductive cycle.** CNS, central nervous system; GnRH, gonadotrophin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; Prl, prolactin; T, testosterone; E2, estradiol; P4, progesterone.

Rodent Models of Puberty

Rodents are important animal models in the study of the effects of toxicants on puberty. In the laboratory rats, the standard landmarks of puberty are the age of preputial separation (PPS) in the males, and the ages of vaginal opening (VO) and first estrus in females.

Onset of pubertal landmarks in rats can be altered after acute in utero and/or lactational exposures to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), busulfan, androgens, and endocrine-disrupting chemicals (EDCs). Also, onset of pubertal landmarks in rats is delayed after peripubertal exposures to antiandrogenic chemicals. T roughout puberty and into adulthood, the sex accessory glands and other androgen-dependent tissues (i.e., muscles and nervous system) continue to depend on testosterone and 5α-dihydrotestosterone for maturation and maintenance of function.

SEXUAL MATURITY

Hypothalamo-pituitary–Gonadal Axis

FSH and LH are glycoproteins synthesized and released from the anterior portion of the pituitary gland (adenohypophysis). Hypothalamic neuroendocrine neurons secrete specific releasing or release-inhibiting factors into the hypophyseal portal system, which carries them to the adenohypophysis, where they act to stimulate or inhibit the release of hormones. GnRH acts on gonadotropic cells, thereby stimulating the release of FSH and LH.

T e neuroendocrine neurons have nerve terminals containing monoamines (norepinephrine, dopamine, and serotonin) that impinge on them. Reserpine, chlorpromazine, and monoamine oxidase (MAO) inhibitors modify the content or actions of brain monoamines that affect gonadotropin production.

In females (Figure 20–5), LH acts on thecal cells of the ovary to induce steroidogenesis, particularly the production of progesterone and androgens that are transferred to the granulosa cells that can be stimulated by FSH to produce estradiol. T ese steroids provide feedback on the hypothalamus and pituitary to regulate gonadotropin production.

Similarly in the males (Figure 20–6), FSH acts primarily on the Sertoli cells, but it also appears to stimulate the mitotic activity of spermatogonia. LH stimulates steroidogenesis in the interstitial Leydig cells. A defect in the function of the testis

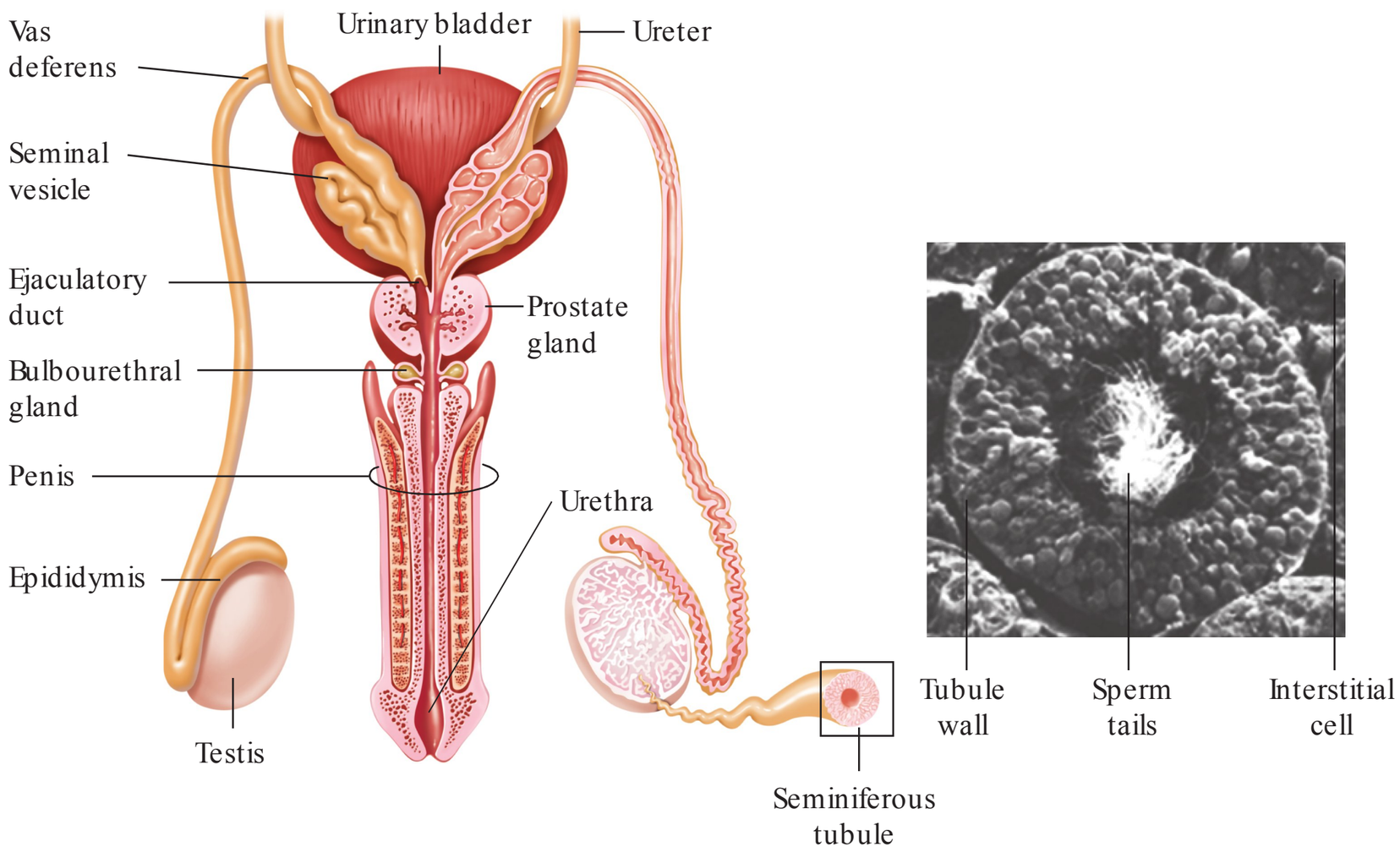

FIGURE 20–6 **Male reproductive system.**

(in the production of spermatozoa or testosterone) will tend to be reflected in increased levels of FSH and LH in serum because of the lack of the negative feedback effect of testicular hormones.

The HPG feedback system is a very delicately modulated hormonal process. Toxicants that alter the hepatic and/or renal biotransformation of endogenous sex steroids may be expected to interfere with the pituitary feedback system.

Ovarian Function

Oogenesis—About 400 000 follicles are present at birth in each human ovary. After birth, many undergo atresia (follicular death), and those that survive are continuously reduced in number. Any chemical that damages the oocytes will accelerate the depletion of the pool and can lead to reduced fertility in females. About one-half of the numbers of oocytes present at birth remain at puberty; the number is reduced to about 25 000 by 30 years of age. About 400 primary follicles will yield mature ova during a woman's reproductive life span. During the approximately three decades of fecundity, follicles in various stages of growth can always be found. After menopause, follicles are no longer present in the ovary.

Although ovarian weight does not fluctuate during the estrous cycle, ovarian weight and histology can provide very useful information about the effects of toxicants on the female reproductive system. Ovarian weight can be reduced by either depletion of oocytes or disruption of the HPG axis. Toxicants induce various ovarian lesions, including polyovular follicles, oocyte depletion, interstitial cell hyperplasia, corpora albanicans, and an absence of corpora lutea.

Case Study: Busulfan—Busulfan is an alkylating agent used to treat several diseases in humans, including chronic myelogenous leukemia, certain myeloproliferative disorders such as severe thrombocytosis, and polycythemia vera. Busulfan causes ovarian failure and prevents or delays the onset of puberty in girls.

In rodents, administration of busulfan specifically inhibits germ cell development. Offspring display permanent reproductive and CNS alterations. The most severely affected females do not display estrous cycles or spontaneous sexual behavior as a consequence of this exposure. Even though the gonads of both sexes are affected at similar dosage levels, fertility and gonadal hormone production are much more easily disrupted in female than male offspring, because the steroid-producing cells in the ovary fail to differentiate in the absence of the oocyte. A diagrammatic representation of the sites of actions of female reproductive toxicants is presented in Figure 20–7.

Ovarian Cycle

The cyclic release of pituitary gonadotropins involving the secretion of ovarian progesterone and estrogen is depicted in Figure 20–8. These female sex steroids determine ovulation and prepare the female accessory sex organs to receive the male sperm. This axis can be disrupted, resulting in infertility at any level of the endocrine system. For example, chemicals that block the LH surge transiently can prevent or delay ovulation, resulting in infertility or lower fecundity due to delayed fertilization of ova.

Postovarian Processes

Female accessory sex organs function to bring together the ovulated ovum and the ejaculated sperm. The chemical composition and viscosity of reproductive tract fluids, as well as the epithelial morphology of these organs, are controlled by ovarian (and trophoblastic) hormones.

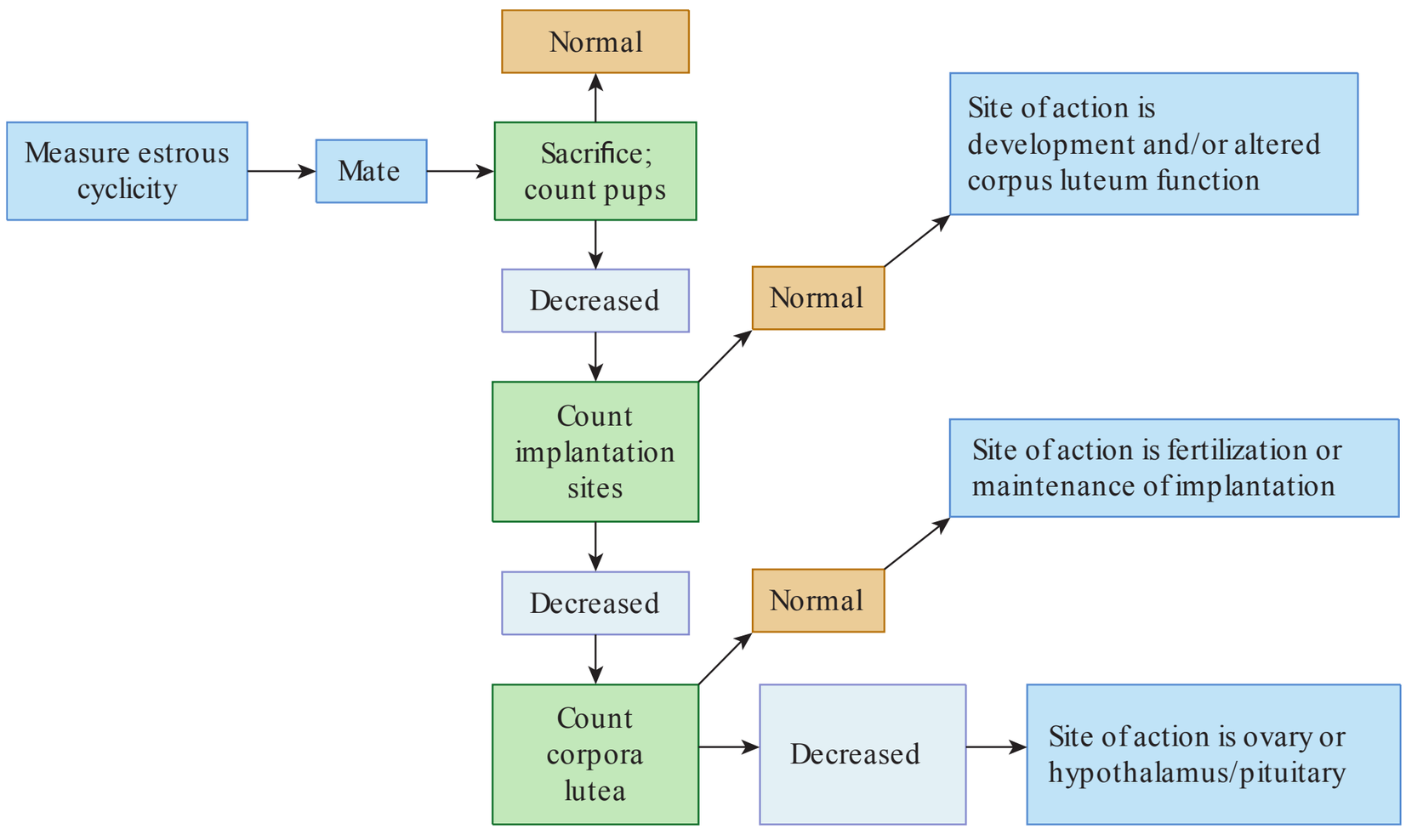

FIGURE 20–7 **Sites of action for female reproductive toxicants.**

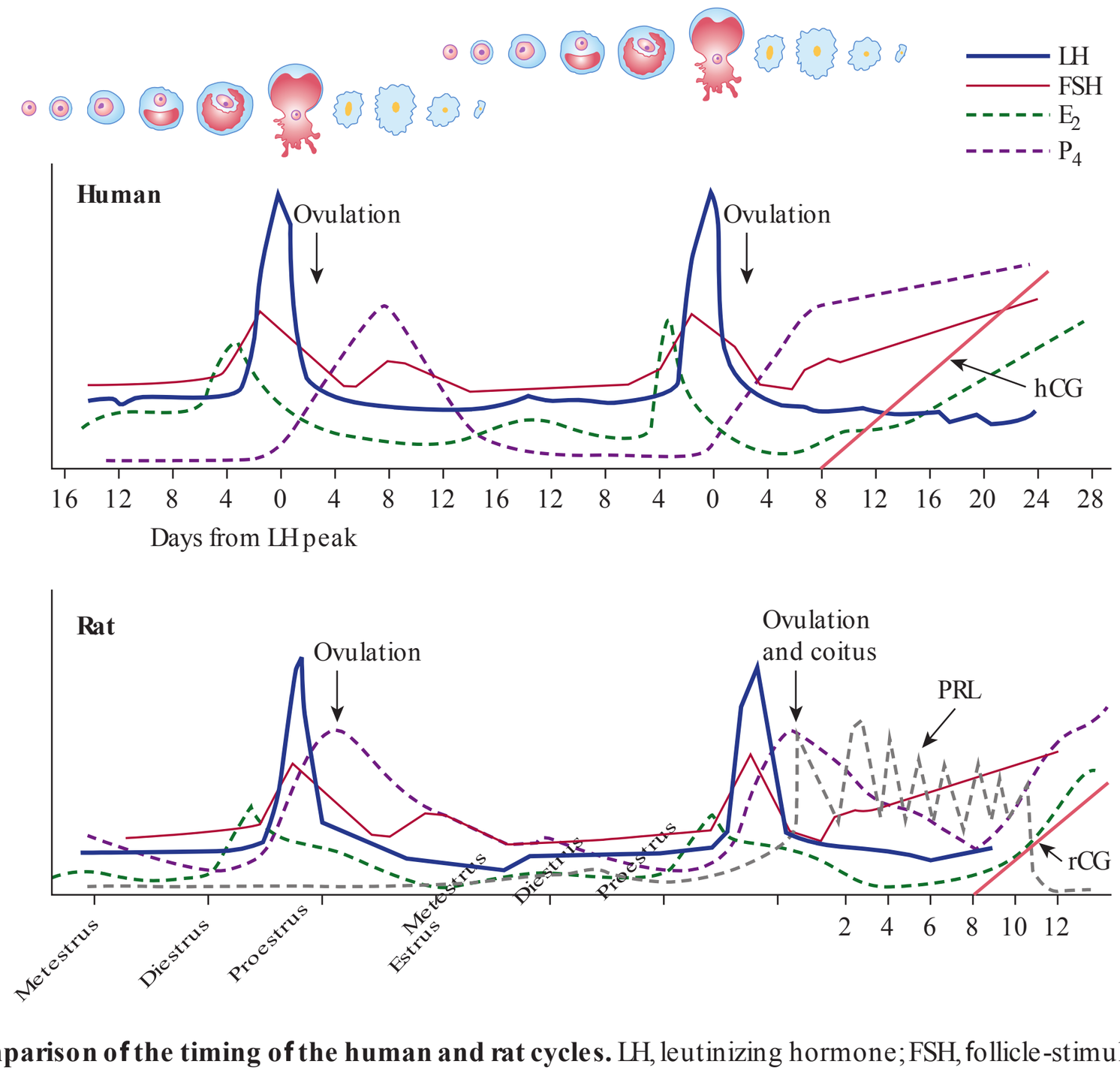

FIGURE 20–8 **Comparison of the timing of the human and rat cycles.** LH, leutinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; E_2, estradiol; P_4, progesterone; hCG, human chorionic gonadotropin; rCG, rat chorionic gonadotropin.

Oviducts—The oviducts provide conduit for, and aid the movement of, gametes. Movement of both the oviducts and fimbriae (finger-like projections of the oviduct) are under the influence of the autonomic nervous system (ANS). Therefore, drugs known to alter the ANS may alter function and fertility. Progression of fertilized eggs through the oviduct and uterus is under hormonal regulation, and chemicals such as the estrogens can stimulate oviductal transport and interfere with uterine endometrial function, precluding implantation.

Uterus—Uterine endometrium reflects the cyclicity of the ovary as it is prepared to receive the conceptus. In the proliferative stage, estrogens from the developing follicle increase the thickness of the endometrium and development of uterine glands. The secretory phase follows ovulation and is characterized by an edematous endometrium due to the action of the uterine glands. Estrogen and progesterone from the corpus luteum guide the changes of this stage. If fertilization fails to occur, the endometrium is shed (menstruation) and a new cycle begins. Uterine weight and vaginal cytology change remarkably, but in a well-defined way during the estrous cycle. These measures can be used as end points in assessment of the toxic effects of chemicals.

TESTICULAR STRUCTURE AND FUNCTION

The blood–testis barrier between the lumen of an interstitial capillary and the lumen of a seminiferous tubule impedes or prevents the free exchange of chemicals/drugs between the blood and the fluid inside the seminiferous tubules.

Targets for Toxicity

For an adult male, there are numerous potential targets for the action of chemicals on the system (Figure 20–9). These would range from the action of dopamine analogs on the hypothalamus interrupting the normal secretion of GnRH, the action of estrogens on the pituitary (and hypothalamus) to interfere with gonadotropin (LH and FSH) production through direct effects on spermatogenesis—where the vast majority of toxicants have their site of action. Perturbing the homeostasis of nutrients can lead to direct effects on spermatogenesis and subsequent issues with fertility. Similarly, chemicals that have direct effects on the liver (e.g., CCl_4) can disturb the normal metabolism of sex steroids leading to changes in clearance (predominantly of glucuronide and sulfate conjugates of hydroxytestosterones

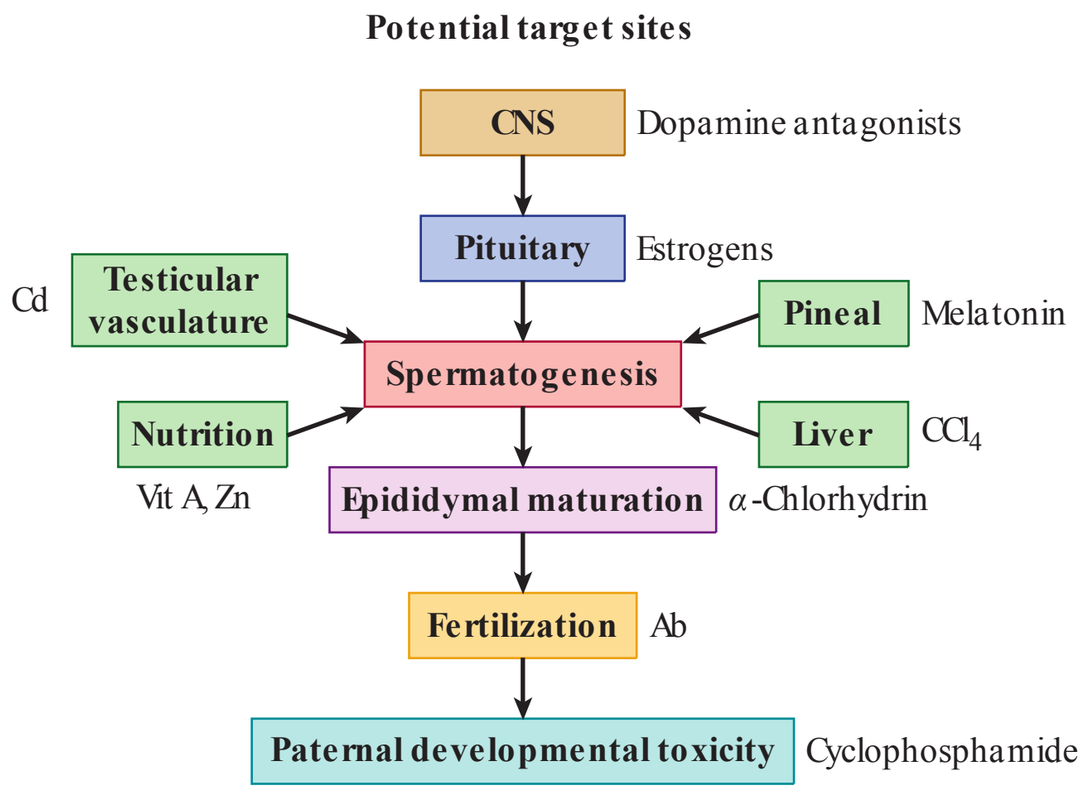

FIGURE 20–9 **Potential target sites for male reproductive toxicants.** Examples of agents shown in italics.

in the male), indirectly affecting the HPG axis and exerting effects on male reproduction.

The testis also has a finely tuned circulatory system in mammals, termed the pampiniform plexus, designed to shunt the arterial venous blood supply and aid in scrotal cooling. Some chemicals (e.g., cadmium) can actually target this structure and the testicular circulatory system to induce ischemic shock to the testes, resulting in injury and reduced fertility.

Testicular Structure and Spermatogenesis

The overwhelming number of chemicals known to affect the male reproductive system appears to do so by a direct effect on the testis and interfere with the process of spermatogenesis. Spermatogenesis is an extremely ordered process in the rat. The spermatogonia have populations that act as the stem cells for the seminiferous tubules and a proportion of these cells then undergo a series of mitotic divisions to increase numbers and move into meiotic prophase, and are then committed to becoming spermatozoa, which are released into the lumen of the seminiferous tubule.

Different biochemical events can go on during the different stages and indeed this can provide clues as to potential modes of action of chemicals that produce stage-specific lesions. Such occurrences do occur regularly with certain phthalate esters, glycol ethers, and antiandrogenic agents.

Once released into the seminiferous tubule lumen, sperm proceed to the epididymis where they can also be the target of toxicant action. Chlorosugars and epichlorohydrin have both been shown to inhibit energy metabolism in sperm, preventing them from functioning normally. The number of environmental chemicals that produce adverse responses in human males is not large. All of these have been shown to induce effects in rodents, and especially in rats, there may be differences in sensitivity based on dose.

Posttesticular Processes

Following the release of mature spermatids from the seminiferous epithelium, the extraneous cytoplasm and organelles form the residual body that is phagocytosed by the Sertoli cell and moves from the periphery of the tubule to its base. These nonmotile sperm are moved along the tubules by a peristaltic-like action of the myoepithelial cells of the tubule and eventually empty into the rete testis. Sperm are then moved into the efferent ducts that exit the testis and enter the head of the epididymis. The sperm undergo maturation in the head and body and begin to acquire motility, whereas the tail is principally used for sperm storage. Most mammals possess seminal vesicles and a prostate, which are secretory in nature and produce fluid for the ejaculation of sperm to survive within the female reproductive tract. Any disturbance in these components may have an effect on subsequent fertility.

Erection and Ejaculation

These physiological processes are controlled by the CNS but are modulated by the autonomic nervous system. Parasympathetic nerve stimulation results in dilation of the arterioles of the penis, which initiates an erection. Ejaculation is a two-stage spinal reflex involving emission and ejaculation. Emission is the movement of the semen into the urethra; ejaculation is the propulsion of the semen out of the urethra at the time of orgasm. Emission is a sympathetic response produced by contraction of the smooth muscle of the vas deferens and seminal vesicles. Semen is ejaculated out of the urethra by contraction of the bulbocavernosus muscle.

Little is known concerning the effects of chemicals on erection or ejaculation. Pesticides, particularly the organophosphates, are known to affect neuroendocrine processes involved in erection and ejaculation. Many drugs acting on the autonomic nervous system affect potency. Impotence, the failure to obtain or sustain an erection, is rarely of endocrine origin; more often, the cause is psychological.

Penile erection depends upon the relaxation of smooth muscles in the corpora cavernosa. In response to sexual stimuli, cavernous nerves and endothelial cells release nitric oxide, which stimulates the formation of cyclic guanosine monophosphate (GMP) by guanylate cyclase. Sildenafil (Viagra) selectively inhibits cGMP-specific phosphodiesterase type 5 in cavernosal smooth muscle cells, thereby restoring the natural erectile response.

Case Studies for Effects on the Male

m-Dinitrobenzene—m-Dinitrobenzene (m-DNB) has been extensively studied for its ability to produce a rapid deleterious effect on the rat testis. Testicular weight remained reduced for many weeks after the treatment period with significant dose-related effects on fertility, pregnancy rate, and implantation success. Other studies have shown abnormal sperm function and failure of fertilization in rat in vitro fertilization (IVF)

studies. Detailed electron microscopic evaluation has shown initial lesions to be present in the Sertoli cells of the testis, which results rapidly in germ cell apoptosis and death.

Ethylene Glycol Monomethyl Ether (EGME)—EGME produces testicular toxicity in various species. Sertoli cell vacuoles, swollen germ cell mitochondria, and a breakdown of the membrane between the Sertoli cell and the pachytene spermatocyte have been described. Within hours, death of (probably) those pachytene spermatocytes follows. EGME is metabolized to active intermediates methoxyacetaldehyde and methoxyacetic acid (MAA). Treating animals with MAA produces identical testicular lesions as that of the parent compound.

FERTILIZATION

Fertilization is the process whereby the genome from one generation is passed to the next to begin the development of a new organism. In mammals, the oocyte is surrounded by two layers: an outer layer of cumulus cells and an inner layer of extracellular matrix termed the zona pellucida. To reach the oocyte, the sperm must penetrate both layers requiring high motility, the release of sperm enzymes and the presence of proteins that will facilitate binding of the sperm to the oocyte. Moreover, once fertilization has occurred, mechanisms must be in place to prevent the binding of further sperm to the fertilized oocyte. Sperm possess special enzymes to facilitate these activities.

IMPLANTATION

Implantation is an intricately timed event that allows mammals to nourish and protect their young during early development and results from an intimate relationship between the developing embryo and the differentiating uterus. Implantation can only occur when the embryo reaches the blastocyst stage and gains implantation competency and the uterus, through steroid hormone-dependent changes, attains a receptive state. T is reciprocal interaction must occur between the blastocyst and uterus together with an increase in uterine vascular permeability at the site of blastocyst attachment. T ere are four stages that comprise early implantation in mammals, (1) apposition and adhesion of the blastocyst to the uterine lumen, (2) penetration of the epithelium, (3) decidualization of the stromal cells, and (4) trophoblastic invasion into the stromal vasculature. T ese four stages can vary in length and in precise order in a species-dependent manner.

PLACENTA

T e placenta plays a key role in pregnancy, mediating exchanges between the mother and fetus and maternal tolerance of antigens produced by the fetus. T ere are a huge number of different placental types across species. In humans, the pituitary gland is not required for the initiation and maintenance of pregnancy, with maintenance of the corpus luteum to produce progesterone dependent on the secretion of human chorionic gonadotropin (hCG) by the trophoblast. Sufficient progesterone is produced by the trophoblast after 8 weeks of gestation in humans to maintain pregnancy even in cases of ovariectomy.

Early in implantation the blastocyst contacts the endometrium and becomes surrounded by an outer layer (syncytiotrophoblast), which is a multinucleated mass of cells that erodes the endometrium, and the blastocyst implants. Placental circulation is then established and trophoblastic tissue differentiates into cytotrophoblast and syncytiotrophoblast cells. T e syncytiotrophoblast cells produce chorionic gonadotropin, chorionic growth hormones, placental lactogen, estrogen, and progesterone, which are needed to achieve independence from the ovary in maintaining the pregnancy. Shortly after implantation, the syncytiotrophoblast becomes bathed by maternal venous blood, which supplies nutrients and permits an exchange of gases.

Generally, the placenta is quite impermeable to chemicals/drugs with molecular weights of 1 000 Da or more. Because most medications and xenobiotics have molecular weights of 500 Da or less, molecular size is rarely a factor in denying a drug's entrance across the placenta and into the embryo/fetus. Placental permeability to a chemical is affected by placental characteristics including thickness, surface area, carrier systems, and lipid-protein concentration of the membranes. T e inherent characteristics of the chemical itself, such as its degree of ionization, lipid solubility, protein binding, and molecular size, also affect its transport across the placenta.

PREGNANCY

Because the transition from early to midpregnancy in the rat requires hormones from the feto-placental unit, if implantation or uterine decidualization is blocked by a chemical, then the female would resume her estrous cycles and the corpora lutea would regress. Chemicals that induce whole-litter loss at mid- to late pregnancy may cause abortions in some of the females, whereas others fail to deliver and appear pregnant for an unusually long period of time.

Many abortifacients induce pregnancy loss by reducing progesterone levels in the rat. Generally, reducing midpregnancy progesterone levels by half or more is sufficient to terminate pregnancy.

PARTURITION

Parturition is a complex process involving fetal, placental, and maternal signals, and the precise molecular events controlling this physiological process are not clear. Parturition is best to be thought of as a release from the inhibitory effects of pregnancy on the myometrium of the uterus rather than an active process, although the timing and order of the precise events are an active process. For most mammals, the uterus is held in a quiescent state by high levels of progesterone and it is the decrease of progesterone that provides the trigger for parturition, but this does not appear to be the case in humans.

LACTATION

T e endocrine control of lactation is one of the most complex physiological mechanisms. Mammogenesis, lactogenesis, galactopoiesis, and galactokinesis are all essential to assure proper lactation. Prolactin is the key hormone of lactation and seems to be the single most important galactopoietic (milk synthesis) hormone. Oxytocin, serotonin, opioids, histamine, substance P, and arginine–leucine modulate prolactin release by means of an autocrine/paracrine mechanism, whereas estrogen and progesterone hormones can act at the hypothalamic and adenohypophysial levels. Human placental lactogen and growth factors play an essential role to assure successful lactation during pregnancy with oxytocin being the most powerful galactokinetic (milk ejection) hormone.

SENESCENCE

Reproductive senescence is usually preceded by a dysregulation of the HPG axis. T is dysregulation leads to alterations in serum HPG hormones, accompanied an upregulation in GnRH, LH, and activin activities and a decrease in steroids in the brain. In females, reproductive senescence (menopause) is associated with a transition from regular to irregular estrus (menstrual) cycles leading to acyclicity and ultimately a loss of fertility. Perinatal exposure to toxicants with estrogenic activity can defeminize the HPG axis such that the female rats are acyclic and infertile, whereas less affected females display the "delayed anovulatory syndrome" and become anovulatory and acyclic at an early age.

In males, a decrease in androgen is noted in around 20% of fit 60-year-old men, but the value of androgen supplementation is not clear with regard to reproductive senescence.

ENDOCRINE DISRUPTION

Currently, the potential effects of endocrine disrupting chemicals (EDCs) on human health and the proven effects of EDCs on wildlife are a major focus among the scientific community. It has been suggested that in utero exposure to environmental estrogens, antiandrogens, or chemicals like phthalates or 2,3,7,8-TCDD could be responsible for the reported 50% decline in sperm counts in some areas and the apparent increase in cryptorchid testes, testicular cancer, and hypospadias.

Phthalate exposures have been associated with reduced anogenital distances (AGD) in boys and lower testosterone levels in men. In females, exposure to EDCs during development could contribute to earlier age of puberty and to increased incidences of endometriosis and breast cancer. Besides pesticides and other toxic substances in the environment, many compounds that are phytosterols, estrogens, antibiotics, beta-blockers, antiepileptics, and lipid-regulating agents have significant endocrine-disrupting activity and are capable of inducing reproductive toxicity.

In the area of wildlife toxicology and ecosystem health, it is apparent that clear-cut cause and effect relationships exist between exposure to EDCs and adverse effects in several vertebrate classes from fish to mammals.

Reports of U-shaped (nonmonotonic), ultralow dose effects and nonthreshold effects for EDCs are challenging some of the basic assumptions of risk assessment for noncancer end points. While the focus of this debate has centered on the low dose effects of bisphenol A, well-documented U-shaped dose response curves are known from many other in vitro and some in vivo studies. T us, for some EDCs the timing of exposure dictates not only the effect, but also whether the effects are adverse or beneficial. Even when administered during adult life, drugs with EDC activity can simultaneously have a beneficial effect on one tissue and an adverse effect on another.

Known Ef ects of EDCs in Humans and Animals

T e list of chemicals that are known to affect humans, domestic animals, and/or wildlife via functional developmental toxicity or endocrine mechanisms includes 2,3,7,8-TCDD, PCBs and polychlorinated dibenzofurans (PCDFs), methylmercury, ethinylestradiol, alkylphenols, plant sterols, fungal estrogens, androgens, chlordecone, DBCP, dichlorodiphenyltrichloroethane (DDT), and other organochlorine compounds. In addition to these xenobiotics, over 30 different drugs taken during pregnancy have been found to alter human development as a consequence of endocrine disruption. T ese drugs are not limited to estrogens, like diethylstilbestrol (DES). EDCs are known to alter human development via several mechanisms besides the estrogen receptor (ER), including binding to retinoic acid (RAR and RXR) receptors, and inhibiting synthesis of steroidogenic enzymes or thyroid hormones. Findings on the effects of background levels of PCBs on the neurobehavioral development of the child have contributed to the concerns about the effects of EDCs on human health via alteration of hormone function.

Ef ects of Drugs on Human Sexual Dif erentiation—Exposure to hormonally active chemicals during sex differentiation can produce pseudohermaphroditism. Androgenic drugs like danazol and methyltestosterone can masculinize human females (i.e., "female pseudohermaphroditism"). T e drug aminoglutethimide, which alters steroid hormone synthesis in a manner identical to many fungicides, also masculinizes human females following in utero exposure.

Transplacental exposure of the developing fetus to DES causes clear cell adenocarcinoma of the vagina, as well as gross structural abnormalities of the cervix, uterus, and fallopian tube. T ese DES-exposed women are more likely to have an adverse pregnancy outcome, including spontaneous abortions, ectopic pregnancies, and premature delivery. Some of the pathological effects that develop in males following fetal DES exposure appear to result from an inhibition of androgen action or synthesis (underdevelopment or absence of the vas deferens, epididymis, and seminal vesicles) and anti-Müllerian duct factor (persistence of the Müllerian ducts).

Known Effects of Plant and Fungal Products in Animals and Humans—Although most naturally occurring environmental estrogens are relatively inactive, the phytoestrogen miroestrol is almost as potent as estradiol in vitro and even more potent than estradiol when administered orally. In addition, many plant estrogens occur in such high concentrations that they induce reproductive alterations in domestic animals. "Clover disease," which is characterized by dystocia, prolapse of the uterus, and infertility, is observed in sheep that graze on highly estrogenic clover pastures. Permanent infertility can be produced in ewes by much lower amounts of estrogen over a longer time period than are needed to produce "clover disease."

Known Effects of Organochlorine Compounds in Humans—Several pesticides and toxic substances have been shown to alter human reproductive function. An accidental high-dose in utero exposure to PCBs and PCDFs has been associated with reproductive alterations in boys, increased stillbirths, low birth weights, malformations, and IQ and behavioral deficits. In addition to the effects associated with this inadvertent exposure, subtle adverse effects were seen in infants and children exposed to relatively low levels of PCBs and PCDFs.

One metabolite of DDT (mitotane, o,p′-DDD) was found to alter adrenal function with sufficient potency to be used as a drug to treat adrenal steroid hypersecretion associated with adrenal tumors. In addition, lower doses of mitotane restored menstruation in women with spanomenorrhea associated with hypertrichosis.

Occupational Exposures—Occupational exposure to pesticides and other toxic substances (i.e., chlordecone and DBCP) in the workplace has been associated with reduced fertility, lowered sperm counts, and/or endocrine alterations in male workers. Workers exposed to high levels of chlordecone, an estrogenic and neurotoxic organochlorine pesticide, displayed intoxication, severe neurotoxicity, and abnormal testicular function. Male workers involved in the manufacture of 4,4′-diaminostilbene-2,2′-disulfonic acid (DAS), a key ingredient in the synthesis of dyes and fluorescent whitening agents, had lower serum testosterone levels and reduced libido as compared with control workers. Thus, it is surprising that occupational exposures to potential EDCs at effective concentrations have not been entirely eliminated from the workplace.

Environmental Androgens

Androgenic activity has been detected in several complex environmental mixtures. Pulp and paper mill effluents (PME) include a chemical mixture that binds androgen receptors (AR) and induces androgen-dependent gene expression in vitro. This mode of action is consistent with the masculinized female mosquitofish (Gambusia holbrooki) collected from contaminated sites. Male-biased sex ratios of fish embryos have been reported in broods of eelpout (Zoarces viviparus) in the vicinity of a large kraft pulp mill on the Swedish Baltic coast, suggesting that masculinizing compounds in the effluent were affecting gonadal differentiation and skewing sex ratios. Effluents from beef-cattle concentrated animal feeding operations have been shown to display androgenicity.

Environmental Antiandrogens

Fungicides—Vinclozolin and procymidone are two members of the dicarboximide fungicide class that act as AR antagonists. These pesticides, or their metabolites, competitively inhibit the binding of androgens to AR, leading to an inhibition of androgen-dependent gene expression.

Administration of vinclozolin during sexual differentiation demasculinizes and feminizes the male rat offspring such that treated males display female-like AGD at birth, retained nipples, hypospadias, suprainguinal ectopic testes, a blind vaginal pouch, and small to absent sex accessory glands.

Procymidone induces shortening of the AGD in male pups, and older males display retained nipples, hypospadias, cryptorchidism, cleft phallus, a vaginal pouch, and reduced sex accessory gland size. Fibrosis, cellular infiltration, and epithelial hyperplasia are noted in the dorsolateral and ventral prostatic and seminal vesicular tissues in adult offspring.

Prochloraz is a fungicide that disrupts reproductive development and function by inhibiting the steroidogenic enzymes 17,20-lyase and aromatase and it is an AR antagonist. Prenatal exposure to prochloraz reduces fetal testis testosterone and increases progesterone production without affecting Leydig cell insl3 mRNA levels. Also, prenatal prochloraz treatment delayed parturition and altered reproductive development in the male offspring in a dose-related manner. Treated males displayed reduced AGD and female-like areolas and high-dose males displayed hypospadias, but the epididymides and gubernacular ligaments were relatively unaffected.

Linuron (Herbicide)—This herbicide binds rat and human AR and inhibits DHT–hAR-induced gene expression in vitro. In utero linuron exposure produces male rats displaying epididymal and testicular abnormalities. In contrast to the effects of vinclozolin and procymidone, malformed external genitalia and undescended testes were rarely displayed by linuron-exposed males. Interestingly, the syndrome of effects for linuron is atypical of an AR antagonist and more closely resembles those seen with in utero to phthalates. Also, fetal testosterone production is significantly reduced in linuron-treated fetal males.

p,p'-DDE (Pesticide Metabolite)—p,p′-DDE displays AR antagonism both in vivo and in vitro. In vitro, p,p′-DDE binds to the AR and inhibits androgen-dependent gene expression. In vivo, p,p′-DDE delays pubertal development in male rats by about 5 days at 100 mg/kg/day and inhibits androgen-stimulated tissue growth. p,p′-DDE administered male rats in utero reduces AGD, induces nipples, and permanently reduces androgen-dependent organ weights.

Phthalates (Plasticizers)—In utero, some phthalate esters alter the development of the male rat reproductive tract at

relatively low dosages. Prenatal exposures to DBP, benzyl-butyl phthalate (BBP), di-isononyl phthalate (DINP), and diethyl-hexylphthalate (DEHP) cause a syndrome of effects, including underdevelopment and agenesis of the epididymis and other androgen-dependent tissues and testicular abnormalities. T e phthalates are unique in their ability to induce agenesis of the gubernacular cords, a tissue whose development is dependent on the peptide hormone insulin-like peptide 3.

Environmental Estrogens

Methoxychlor is an estrogenic pesticide that produces estrogen-like effects. T is pesticide requires metabolic activation in order to display full endocrine activity in vitro. T e active metabolites of methoxychlor activate estrogen-dependent gene expression in vitro and in vivo in the female rats, thereby stimulating an uterotropic response, accelerating VO and inducing constant estrus, and reducing infertility. In the ovariectomized female rat, methoxychlor also induces estrogen-dependent reproductive and nonreproductive behaviors, including female sex behaviors, running wheel activity, and food consumption.

When given to the dam during pregnancy and lactation, both male and female offspring are affected. Females display irregular estrous cycles and reduced fecundity, whereas male fertility is unaffected at doses up to 200 mg/kg/day.

Ethinylestradiol is a synthetic derivative of estradiol that is in almost all modern formulations of combined oral contraceptive pills. T is drug is found in many aquatic systems contaminated by sewage ef uents, originating principally from human excretion. T us, ethinylestradiol plays a major role in causing widespread endocrine disruption in wild populations of fish species and other lower vertebrate species.

EDC Screening Programs

T e Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) proposed (1) a process to prioritize chemicals for evaluation and recommendations, for (2) screening (Tier 1), and for (3) testing (Tier 2) batteries for EDCs. T e recommended screening battery was designed to detect alterations of HPG function; estrogen, androgen, and thyroid hormone synthesis; and AR- and ER-mediated effects in mammals and other taxa.

In Vivo Mammalian Assays—EDSTAC recommended the laboratory rat as the species of choice for the endocrine screening and testing assays. T e EDSTAC proposed three short-term in vivo mammalian assays for the tier 1 screening battery: the uterotropic, Hershberger, and pubertal female rat assays.

Uterotropic Assay—Estrogen agonists and antagonists are detected in a 3-day uterotropic assay using subcutaneous administration of the test compound. T e selected uterotropic assays for estrogens and antiestrogens use either the intact juvenile or the castrated ovariectomized adult/juvenile female rat.

Hershberger Assay—T e second in vivo assay in tier 1, the Hershberger assay, detects antiandrogenic activity simply by weighing androgen-dependent tissues in the castrated male rat. In this assay, weights of the ventral prostate, Cowper's glands, seminal vesicle (with coagulating glands and fluids), glans penis, and levator ani/bulbocavernosus muscles are measured after 10 days of oral treatment with the test compound. T is assay is very sensitive for detection of androgens and antiandrogens.

Pubertal Female Rat Assay—T e third in vivo mammalian/rat assay in the screening battery is the pubertal female rat assay. Weanling female rats are dosed daily by gavage for 21 days while the age at VO (puberty) is monitored. T e females are necropsied at about 42 days of age. T is assay detects alterations in thyroid hormone status, HPG function, inhibition of steroidogenesis, estrogens, and antiestrogens, and has been found to be highly reproducible and very sensitive to certain endocrine activities including estrogenicity, inhibition of steroidogenesis, and antithyroid activity.

Alternative Screening Assays—Alternative in vivo assays were also discussed by EDSTAC and are currently being evaluated by the EPA. If they are of sufficient sensitivity, specificity, and relevance, they might replace or augment current tier 1 assays.

Pubertal Male Rat Assay—T e pubertal male rat assay detects alterations of thyroid function, HPG maturation, steroidogenesis, and altered steroid hormone function (androgen). Intact weanling males are exposed to the test substance for approximately 30 days. T e age at puberty is determined by measuring the age at PPS, and reproductive tissues are evaluated and serum taken for optional hormonal analyses.

In Utero–Lactational Assay—T e EDSTAC recommended development of utero-lactational assays due to the unique sensitivity of the fetal reproductive system to certain toxicants. One version of the assay takes about 80 days and uses approximately 10 litters per group (120–150 pups). In this protocol, androgens and antiandrogens can be detected in approximately 2 to 3 weeks, and EDCs with antithyroid activity can be detected in infant or weanling offspring after four to five weeks of maternal treatment.

TESTING FOR REPRODUCTIVE TOXICITY

Screens and Multigeneration Studies

Significant attention has focused on the development of "screens" for reproductive toxicity. T e screens currently employed have been developed to prioritize chemicals for more comprehensive testing.

T e most comprehensive assessment of reproductive toxicity would be provided by a protocol that exposes the animal model throughout the reproductive cycle (see Figure 20–1)

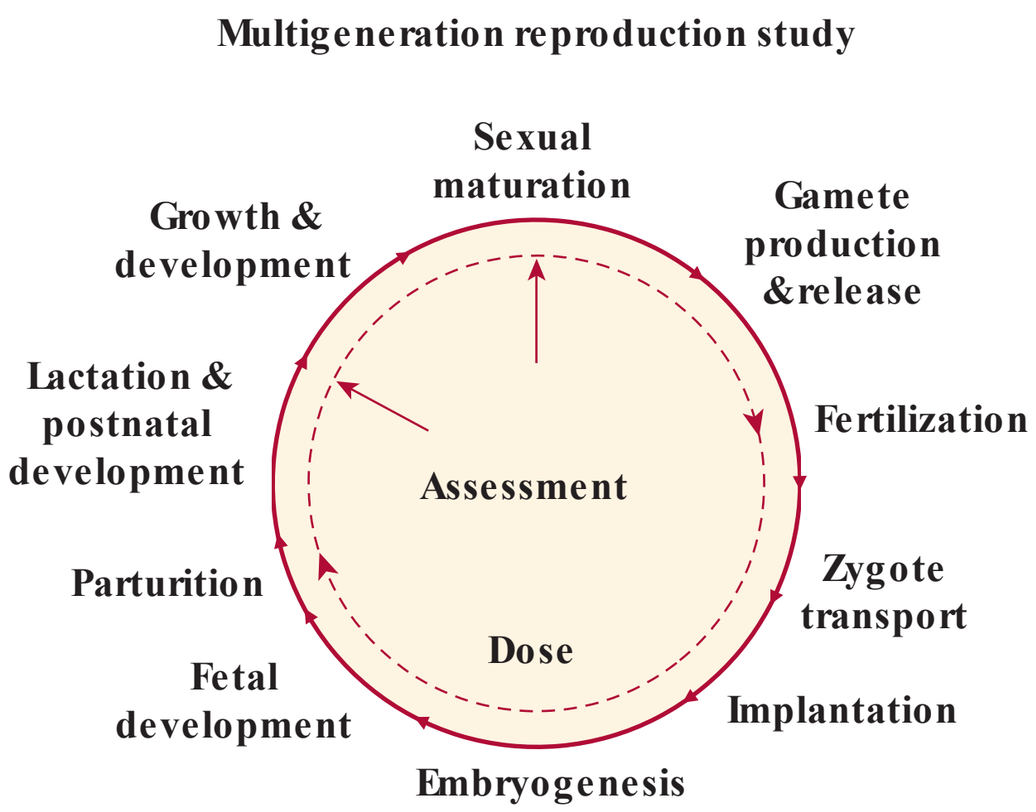

FIGURE 20–10 **Multigeneration reproduction study.**

and assesses multiple end points at different life stages during this continuous exposure. T e protocol and guideline coming closest to this ideal is the multigeneration reproduction study (Figure 20–10) used for the assessment of chemicals, pesticides, and some food additives. Multigeneration studies normally encompass detailed measurements of reproductive performance (number of pregnant females from number of pairs mated, number of females producing a litter, litter size, and number of live pups with their birth weights and sex). Measurement of growth and analysis of the reproductive organs in the F_0 parental generation is conducted. Similar measurements to those undertaken for the F_0 are made on the F_1 parents, and the offspring are examined at birth (and sexually dimorphic end points may be collected such as AGD), at weaning, and at puberty (particularly the assessment of VO and time of first estrus in females and balanopreputial separation in males) in addition to the adult measurements of reproductive performance, organ weights, histology, etc.

Testing for Endocrine-disrupting Chemicals

In the tiered screening and testing approach, only chemicals that display positive reproducible responses in tier 1 screening (T1S) would continue evaluation in full-life cycle or multigenerational tests. In tier 2 testing (T2T), issues of dose–response, relevance of the route of exposure, sensitive life stages, and adversity are resolved.

Data should be summarized in a manner that clearly delineates the proportion of animals that are affected. In teratology studies, data are typically presented and analyzed in this manner, indicating the number of malformed/number observed on an individual and litter basis, whereas multigenerational studies are frequently presented and analyzed differently, even when clear teratogenic and other developmental responses are noted after birth. Multigenerational protocols are used in T2T because only these protocols expose the animals during all critical stages of development and examine reproductive function of offspring after they mature.

Although the EPA multigenerational test provides for a comprehensive evaluation of the F_0 or parental generation, too few F_1 animals (offspring with developmental exposure) are examined after maturity to detect anything but the most profound reproductive teratogens. F_0 animals within a dose group typically respond in a similar fashion to the chemical exposure; however, the response to toxicants in utero can vary greatly even within a litter with only a few animals displaying severe reproductive malformations in the lower dosage groups.

"Transgenerational" protocols typically use fewer litters (7 to 10 per dose group) but examine all of the animals in each litter. T ese protocols actually use fewer animals but provide enhanced statistical power to detect reproductive effects in the F_1 generation. T e lifelong exposure of both males and females in the F_1 generation, which allows one to detect effects induced in utero, during lactation, or from direct exposure after puberty, can confound the identification of when the effect was induced (i.e., during adulthood versus development) or of which sex was affected.

Some EDCs disrupt pregnancy by altering maternal ovarian hormone production in F_0 dams at dosage levels that appear to be without direct effect on the offspring. In such cases, the standard EPA multigenerational protocol with minor enhancements would be recommended, or a transgenerational protocol with exposure continued after weaning. T e transgenerational or in utero lactational protocols fill a gap in the testing program for EDCs that should be used only on a case-by-case basis.

Testing Pharmaceuticals

In the case of pharmaceuticals, it is rare for multigeneration studies to be conducted, because it is not common for all the population to use a specific drug and exposure to the drug is over many different life stages, and not necessarily chronic. Typically three specific studies are undertaken:

1. **A study of fertility and early embryonic development** (see Figure 20–11). Parental adults are exposed to the test chemical for 2 weeks (females) or 4 weeks (males) prior to breeding and then during breeding. Females then continue their exposure through to implantation. Males can be necropsied for the end points noted above for the multigeneration studies after pregnancy has been confirmed, and for the pregnant females, necropsy takes place any time after midgestation. Reproductive and target organs are weighed and examined histologically, sperm parameters are assessed in males, and the uterine implantation sites and ovarian corpora lutea are counted in females, as well as live and dead embryos.
2. **A study of ef ects on pre- and postnatal development including maternal function** (see Figure 20–12). In this study, pregnant females are exposed from the time of implantation until weaning of their offspring (usually PND 21 in the rat). After cessation of exposure, selected offspring

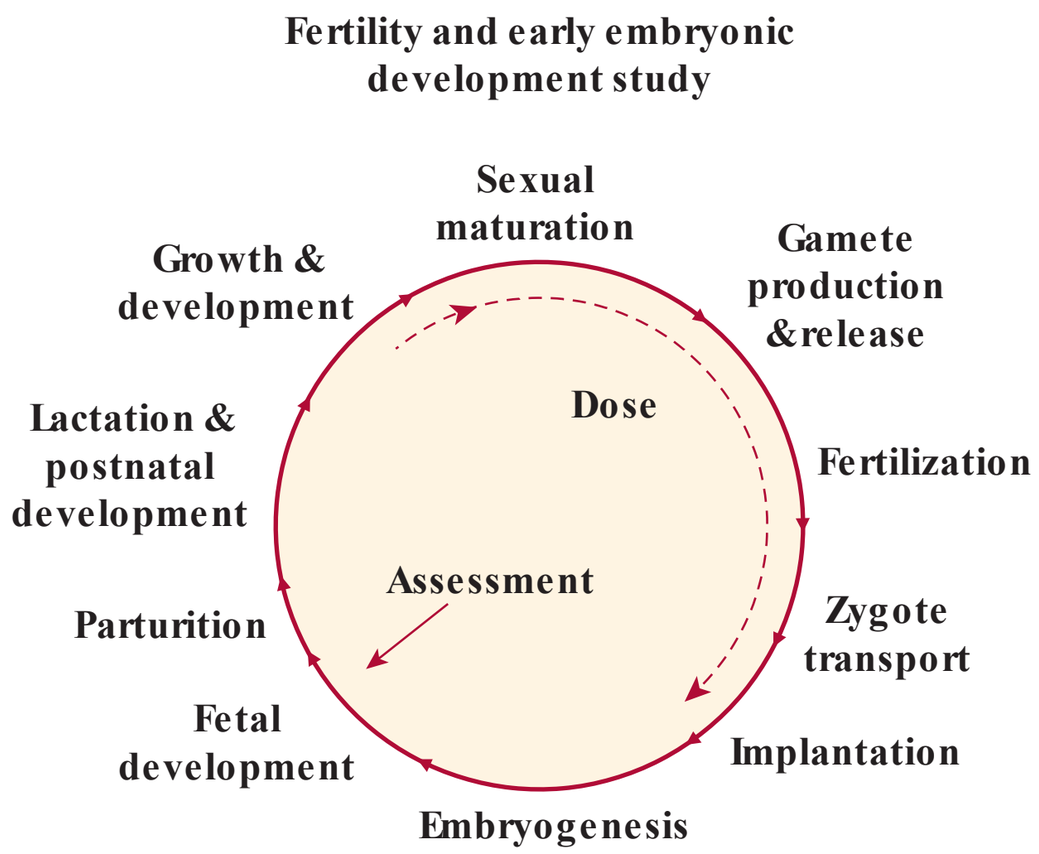

FIGURE 20–11 **Fertility and early embryonic study.**

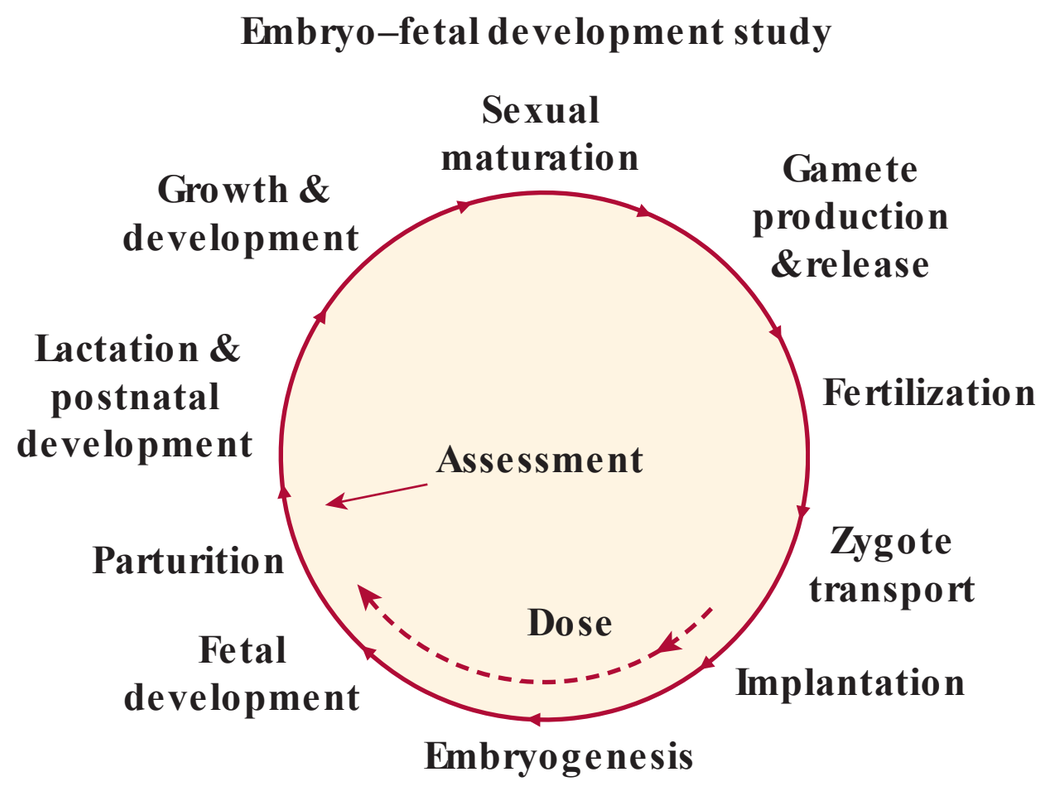

FIGURE 20–13 **Embryo–fetal developmental toxicity study as used by FDA guidelines.** Dosing starts at implantations and continues to closure of the hard palate with an assessment of fetuses just prior to parturition.

(one male and one female per litter) are raised to adulthood and then mated to assess reproductive competence. T ese animals are observed for maturation and growth (but are not exposed). Puberty indices, as employed in the multigeneration study, are measured. In addition, sensory function, reflexes, motor activity, learning, and memory are also evaluated.

3. **A study of embryo–fetal development** (see Figure 20–13). T is study tests for enhanced toxicity relative to that noted in pregnant females and, unlike the previous two studies, is normally conducted in two species (typically the rat and rabbit). Exposure occurs between implantation and closure of the hard palate and females are killed just prior to parturition. At necropsy, dams are observed for any affected organs and corpora lutea are counted. Live and dead fetuses are counted and examined for external, visceral, and skeletal abnormalities.

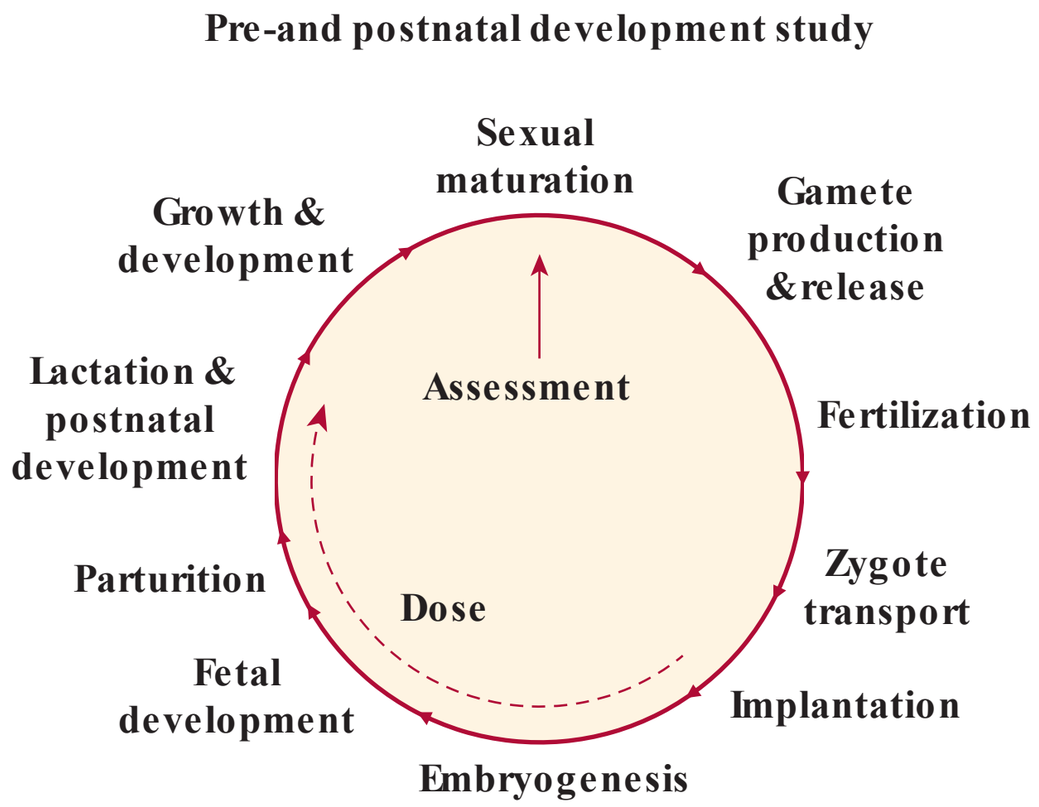

FIGURE 20–12 **Pre- and postnatal developmental toxicity study.** Dosing is from implantation until the litters are weaned.

One of the following three summary risk conclusions would be applied to the drug label: (1) the drug is not anticipated to produce reproductive and/or developmental effects above the background incidence for humans when used in accordance with the dosing information on the product label; (2) the drug may increase the incidence of adverse reproductive and/or developmental events; or (3) the drug is expected to increase the incidence of adverse reproductive and/or developmental effects in humans when used according to the product label.

An examination of the reproductive cycle in a comparison of these three most likely options for FDA studies indicates an obvious gap in the exposure regime for the complete reproductive cycle, namely exposure of weanlings through puberty to adulthood. T is exposure period has become of increasing interest to many companies developing drugs for specific administration to infants and juveniles, and "bridging-type" protocols have been developed to specifically address toxicity that may occur after exposure during this specific life stage.

EVALUATION OF TOXICITY TO REPRODUCTION

T ere are a number of general points that the investigator should note in any estimation of potential reproductive toxicity:

- Adequacy of experimental design and conduct. Was there sufficient statistical power in the evaluation(s)?
- Occurrence of common versus rare reproductive deficits. Biological versus statistical significance.
- Use of historical control data to place concurrent control data into perspective and to estimate population background incidence of various reproductive parameters and deficits.
- Known structure–activity relationships for inducing reproductive toxicity.

- Concordance of reproductive end points. Did a decrease in litter size relate to ovarian histology and changes in vaginal cytology?
- Did the reproductive deficits become more severe with increases in dose? Did histological changes at one dose level become decrements in litter size and then reductions in fertility at higher dose levels in any generation?
- Did the reproductive deficits increase in prevalence (more individuals and/or more litters) with dose level in any generation?
- Special care should be taken for decrements in reproductive parameters noted in the F_1 generation (and potentially later generations) that were not seen in the F_0 generation, which may suggest developmental, as well as reproductive toxicity. Likewise, findings in an F_1 generation animal may (or may not) be reproduced in F_2 offspring. For example, effects in the F_1 generation on reproductive parameters may have resulted in the selection out of sensitive animals in the population, thus not producing F_2 offspring for subsequent evaluation.

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Gupta RC: Reproductive and Developmental Toxicology. Burlington, MA: Academic Press, 2011.

QUESTIONS

1. Which of the following cell types secretes anti-Müllerian hormone (AMH)?
   a. spermatogonium.
   b. Leydig cell.
   c. Sertoli cell.
   d. primary spermatocyte.
   e. spermatid.

2. Penile erections are dependent on:
   a. the CNS.
   b. sympathetic nerve stimulation.
   c. helicine (penile) artery constriction.
   d. corpora cavernosa smooth muscle relaxation.
   e. a spinal reflex arc.

3. T e corpus luteum is responsible for the secretion of which of the following hormones during the first part of pregnancy?
   a. estradiol and hCG.
   b. progesterone and estradiol.
   c. progesterone and hCG.
   d. FSH and LH.
   e. FSH and progesterone.

4. All of the following statements regarding the hypothalamo-pituitary–gonadal axis are true EXCEPT:
   a. FSH increases testosterone production by the Leydig cells.
   b. FSH and LH are synthesized in the anterior pituitary.
   c. Estradiol provides negative feedback on the hypothalamus and the anterior pituitary.
   d. GnRH from the hypothalamus increases FSH and LH release from the anterior pituitary.
   e. T e LH spike during the menstrual cycle is responsible for ovulation.

5. Which of the following statements is FALSE regarding gametal DNA repair?
   a. DNA repair in spermatogenic cells is dependent on the dose of chemical.
   b. Spermiogenic cells are less able to repair damage from alkylating agents.
   c. Female gametes have base excision repair capacity.
   d. Meiotic maturation of the oocyte decreases its ability to repair DNA damage.
   e. Mature oocytes and mature sperm no longer have the ability to repair DNA damage.

6. Reduction division takes place during the transition between which two cell types during spermatogenesis?
   a. spermatogonium and primary spermatocyte.
   b. primary spermatocyte and secondary spermatocyte.
   c. secondary spermatocyte and spermatid.
   d. spermatid and spermatozoon.
   e. spermatozoon and mature sperm.

7. Which of the following cell types is properly paired with the substance that it secretes?
   a. ovarian granulosa cells—progesterone.
   b. Leydig cells—ABP.
   c. ovarian thecal cells—estrogens.
   d. Sertoli cells—testosterone.
   e. gonadotroph—LH.

8. Which of the following statements regarding male reproductive capacity is FALSE?
   a. Klinefelter's syndrome males are sterile.
   b. FSH levels are often measured in order to determine male reproductive toxicity of a particular toxin.
   c. Divalent metal ions, such as An, Hg, and Cu, act as androgen receptor antagonists and affect male reproduction.
   d. T e number of sperms produced per day is approximately the same in all males.
   e. ABP is an important biochemical marker for testicular injury.

9. Reduction of sperm production can be caused by all of the following diseases EXCEPT:
   a. hypothyroidism.
   b. measles.
   c. Crohn's disease.
   d. renal failure.
   e. mumps.

10. Of the following, which is LEAST likely to be affected by estrogen?
    a. nervous system.
    b. musculoskeletal system.
    c. digestive system.
    d. cardiovascular system.
    e. urinary system.

CHAPTER

21

Toxic Responses of the Endocrine System

Patricia B. Hoyer and Jodi A. Flaws

INTRODUCTION

PITUITARY GLAND

- Anatomy and Physiology
- Pituitary Toxicity

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- Glucocorticoids
- Adrenocortical Toxicity
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- Regulation of Thyroid Hormone Release
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ENDOCRINE PANCREAS

- Role of the Liver in Glucose Production
- Pancreatic Hormones
  - Insulin
  - Glucagon
  - Somatostatin
- Interactions of Release
- Metabolic Responses in Diabetes
- Pancreatic Toxicity
- Insulin Resistance
- In Vitro Testing

KEY POINTS

- Endocrine glands are collections of specialized cells that synthesize, store, and release their secretions directly into the bloodstream.
- Each type of endocrine cell in the adenohypophysis is under the control of a specific releasing hormone from the hypothalamus.
- Toxicants can influence the synthesis, storage, and release of hypothalamic-releasing hormones, adenohypophyseal-releasing hormones, and the endocrine gland–specific hormones.

INTRODUCTION

Higher animals have developed the ability to regulate their internal environment, independent of wide external fluctuations via the endocrine system. An endocrine system consists of an endocrine gland that secretes a hormone, the hormone itself, and a target tissue that responds to the hormone. A hormone is a chemical substance produced by a ductless endocrine gland that is secreted into the blood. T e hormone-producing glands include the pituitary, the thyroid and parathyroids, the adrenals, the gonads, and the pancreas. T ere are primarily three chemical classes of hormones: amino acid derivatives (catecholamines and thyroid hormones), peptide hormones (pancreatic), and steroids (derivatives of cholesterol). Endocrine glands are sensing and signaling devices that are capable of responding to changes in the internal and external environments and coordinating multiple activities that maintain homeostasis.

PITUITARY GLAND

Anatomy and Physiology

T e pituitary may be divided into two major subdivisions: the pars distalis and the pars nervosa (Figure 21–1). T e pars distalis, adenohypophysis or anterior pituitary, is the largest subdivision and it receives peptides from the hypothalamus through a capillary portal system (hypothalamo–hypophyseal vessels). T e pars nervosa, neurohypophysis or posterior pituitary, has its cell bodies in the hypothalamus with their axons stretching to the posterior lobe of the pituitary; therefore, functionally and anatomically, the posterior pituitary is an extension of the hypothalamus.

T e releasing and release-inhibiting hormones are synthesized by neurons in the hypothalamus, transported by axonal processes, and released into capillary plexus. T ey are transported to the adenohypophysis by the hypothalamic–hypophyseal portal system, where they interact with specific populations of trophic hormone-secreting cells to govern the rate of release of preformed hormones, such as growth hormone (GH), somatotropic hormone (STH), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyrotropic hormone (TTH), adrenocorticotropic hormone (ACTH), and melanocyte-stimulating hormone (MSH). From the pars nervosa, ADH enhances reabsorption of water by the kidney and causes contraction of vascular smooth muscle, whereas oxytocin stimulates contraction of smooth muscle for parturition and milk let-down. T ese neurohypophyseal hormones are synthetized in the cell body of hypothalamic neurons, packaged in secretory granules, transported along the axon to terminal processes in the pars nervosa for release into the blood.

To maintain appropriate homeostasis, the endocrine organ must constantly monitor systemic hormone concentrations accomplished in the form of negative feedback loops. For example, high circulating levels of cortisol will inhibit corticotrophin-releasing hormone (CRH) release from the hypothalamus, and the adrenocorticotropic hormone (ACTH) release from the pituitary.

Pituitary Toxicity

Studies consistently show that heavy metals may target pituitary gland structure or function. Cadmium inhibits prolactin, LH, and FSH secretion. Cadmium exposure increases ACTH levels in rodents exposed during puberty and decreases ACTH levels in animals exposed during adulthood. Furthermore, studies indicate that acute exposure to cadmium decreases circulating GH levels, while longer period treatment increases circulating GH levels. Lead and mercury also decrease LH and FSH.

Environmental contaminants such as polychlorinated biphenyls (PCBs) and polybrominated diphenylethers inhibit release of LH and FSH as well as TSH. T e insecticide dimethoate causes pituitary tumors in rats. Methoxychlor, dieldrin, and endosulfan increase prolactin and LH levels.

Several phytoestrogens affect pituitary cells: coumestrol reduces pulsatile LH and suppresses the pituitary response to exogenous GnRH. Acute exposure to genistein or bisphenol A alter LH secretion as well.

Industrial chemicals alter pituitary structure or function. Flame retardants tetrabromo- and tetrachlorobisphenol A stimulate

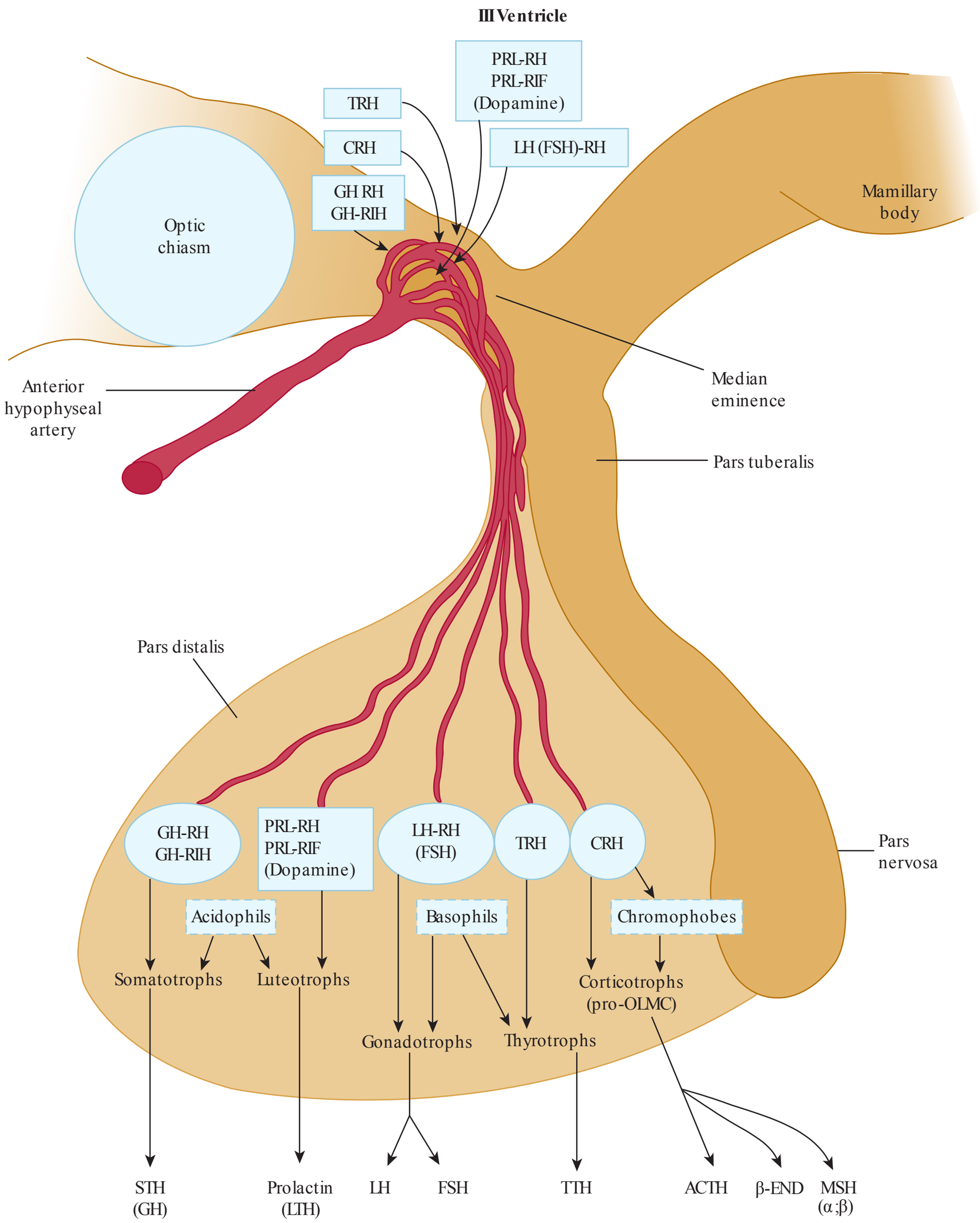


FIGURE 21–1 **Control of trophic hormone secretion from the adenohypophysis by hypothalamic-releasing hormones (RH) and release-inhibiting hormones (RIH).** The releasing and release-inhibiting hormones are synthesized by neurons in the hypothalamus, transported by axonal processes, and released into capillary plexus in the median eminence. They are transported to the adenohypophysis by the hypothalamic–hypophyseal portal system, where they interact with specific populations of trophic hormone-secreting cells to govern the rate of release of preformed hormones, such as growth hormone (GH), somatotropic hormone (STH), luteotropic hormone (LTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyrotropic hormone (TTH), adrenocorticotropic hormone (ACTH), and melanocyte-stimulating hormone (MSH). There are RIHs for those trophic hormones (e.g., prolactin and growth hormone) that do not directly influence the activity of target cells and result in production of a final endocrine product (hormone) that could exert negative feedback control.

proliferation of a pituitary cell line. 2-Mercaptobenzothiazole, which is used in rubber products, can come into contact with drinking water and cause pituitary tumors in chronically exposed rats and mice. Finally, cynamide, a chemical used in the treatment of alcoholics, increases the ACTH precursor mRNA in the anterior pituitary when co-administered with ethanol.

ADRENAL GLANDS

T e adrenals are two small glands situated on the superior poles of the kidneys. T e major physiological role of the adrenals is management of stress. Each adrenal gland is divided into two morphologically and functionally distinct regions: the outer cortex and the interior medulla. T e adrenals have not

been as widely studied in toxicology as other endocrine glands, even though it has been documented to be the most common toxicological target of all.

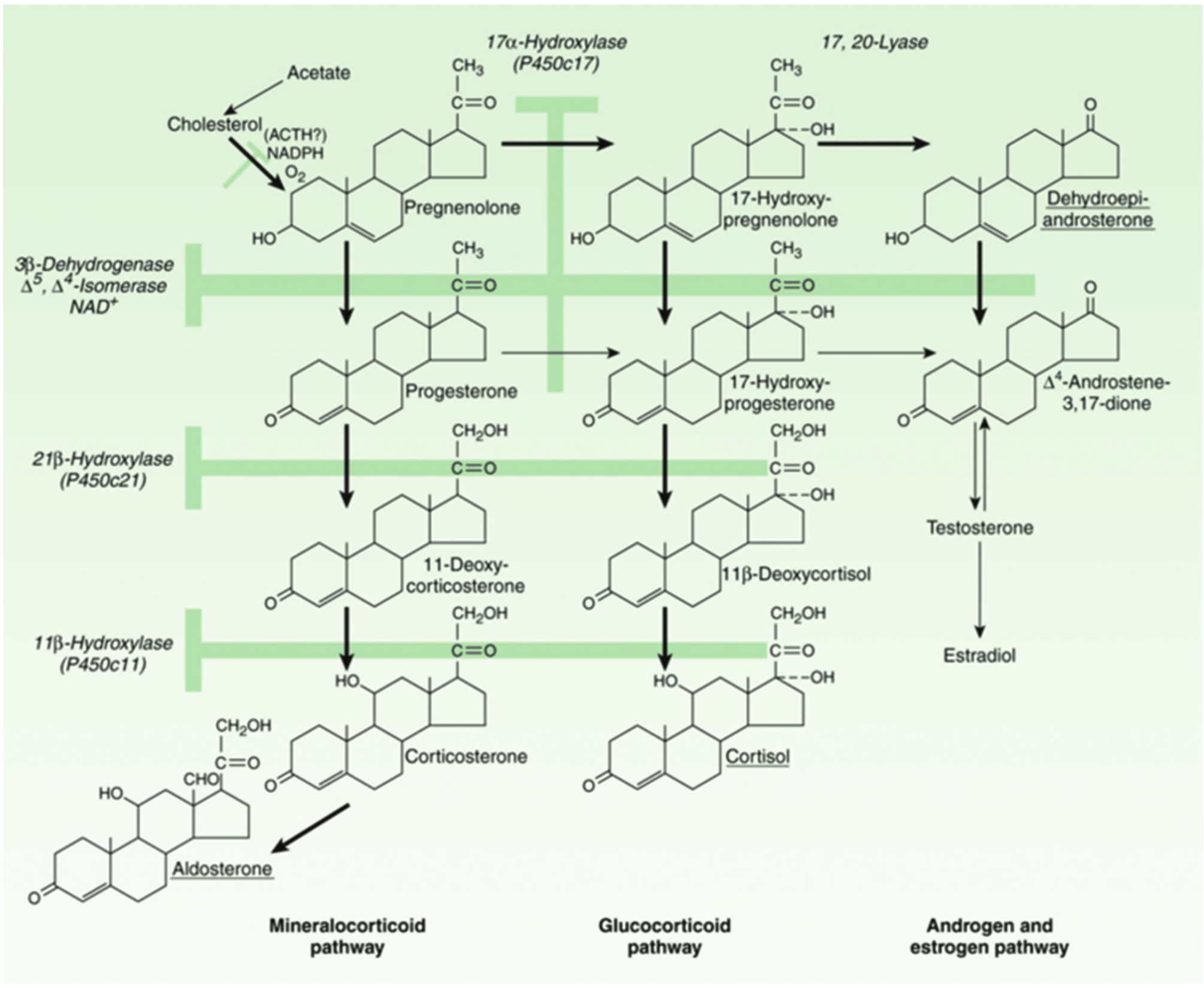
ADRENAL CORTEX

The adrenal cortex regulates many physiological functions such as the immune system, inflammation, water and electrolyte balance, carbohydrate and protein metabolism involving such target organs as the liver, kidney, heart, bone, and nervous system. The cortex is predisposed to the toxic effects of xenobiotic chemicals because many are lipophilic and the adrenal cortical cells contain large stores of lipids.

The outer region (cortex) synthesizes and secreted adrenocorticosteroid hormones. The cortex consists of three zones (Figure 21–2). The zona glomerulosa produces the mineralocorticoid aldosterone. The inner zones, fasciculata and reticularis, produce glucocorticoids, corticosterone, and cortisol, as well as adrenal androgens. The inner region, medulla, synthesizes and secretes catecholamines, epinephrine, and norepinephrine.

Steroidogenesis

Adrenal steroids are synthesized from cholesterol and through the involvement of the mitochondria and endoplasmic



Source: Katzung BG, Masters SB, Trevor AJ: Basic & Clinical Pharmacology, 12th edition: www.accessmedicine.com

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FIGURE 21–2 **Adrenocortical hormone pathway.** A series of cytochrome P450 enzymes participate in the synthesis of aldosterone (zona gomerulosa), or cortisol and adrenal androgens (zonae fasciculata and reticularis). The zona glomerulosa does not express CYP17A1; the zonae fasciculata and reticularis do not express CYP11B2. (Modified with permission from Barrett KE, Boitano S, Barman SM, et al.: Ganong's Review of Medical Physiology, 24th edition. New York, NY: The McGraw-Hill Companies, Inc; 2012.)

reticulum. The most common biosynthetic pathway from cholesterol is the formation of pregnenolone, the basic precursor for the three major classes of adrenal steroids. A series of cytochrome P450 enzymes participate in synthesis of aldosterone (zona glomerulosa) or cortisol and adrenal androgens (zona fasciculata and zona reticularis).

Glucocorticoids

The physiological effects of glucocorticoids include hepatic glucose production, gluconeogenesis, protein catabolism, fat catabolism, increased bone resorption, altered mood, and increased gastric acidity. Therapeutically, the effects of cortisol include prevention of vascular collapse during overwhelming stress, providing an anti-inflammatory effect, and invoking immunosuppression.

Adrenocortical Toxicity

The zonae fasciculata and reticularis appear to be the principal targets of xenobiotic chemicals in the adrenal cortex leading to necrosis from things such as 7,12-dimethylbenz[a] anthracene, acrylonitrile, thioacetamide, and basic polyglutamic acid. Lipidosis inducers can cause accumulations of fats which may be of sufficient quantity to cause a reduction or loss of organellar function and eventual cell destruction. Spironolactone, ketoconazole, and various PCBs directly target glucocorticoid secretion. A wide range of lesions may be produced that may be classified as follows: endothelial damage, mitochondrial damage, endoplasmic reticulum disruption, lipid aggregation, and lysosomal phospholipid aggregation.

Biologically active cationic amphiphilic compounds produce a generalized phospholipidosis that involves primarily the zonae fasciculata and reticularis and produce microscopic phospholipid-rich inclusions. The compounds that affect the functional integrity of lysosomes include chloroquin, triparanol, and chlorphentermine.

Adrenocortical toxicity can also involve increased secretion of endogenous glucocorticoids due to compounds such as ethanol, cannabinoids, cocaine, and cytotoxic anticancer drugs. Furthermore, pharmacological treatment with glucocorticoid agonists that have been widely used as anti-inflammatory agents can produce symptoms that resemble Cushing's syndrome.

In Vitro Toxicity

Of particular usefulness for in vitro testing has been the human adrenocortical carcinoma-derived NCI-H295R cell line. It has proven useful for identification of specific steroidogenic enzymes that are targeted by xenobiotics. Because it is derived from a human source, it is also worthwhile for hazard risk assessment.

Serum Binding Proteins

Cortisol and corticosterone are transported in the blood by transcortin (corticosteroid binding globulin). When bound, it is biologically inactive. Thus, a chemical affecting transcortin could alter the balance between free and bound hormone, and impact its availability in target tissues. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported to decrease its binding capacity.

Target Tissue Receptors

Adrenocortical steroids exert their effects through receptors in target tissues throughout the body that may be up- or down-regulated by the action of xenobiotic compounds. For example, spironolactone is an antisteroidal compound that competes with the receptor sites.

Neuroendocrine Regulation

The zonae fasciculata and reticularis are under tropic control by ACTH which stimulates them to produce cortisol. Increased cortisol produced then provides negative feedback; however, stress can override the negative feedback control system and stimulate cortisol secretion. Persistent exposure of the adrenal cortex to high levels of ACTH during chronic stress can result in adrenocortical hypertrophy.

A one month toxicology study of corticosterone administration in rats observed reduced body weight gain, and lower thymus, adrenal, prostate, and seminal vesicle weight. The body and thymus weight effects were attributed directly to high corticosterone, and reduced prostate and seminal vesicle weights to the inhibition of LH and testosterone by corticosterone.

Mineralocorticoids

The adrenals are essential to life, mainly because of the aldosterone promotion of sodium reabsorption and increased excretion of potassium and hydrogen ions by the kidney. Loss of mineralocorticoid production by the cortex results in a life-threatening retention of potassium and hypovolemic shock associated with excessive urinary loss of sodium, chloride, and water. Chemicals that target steroidogenic enzymes (CYP11A1, CYP21, CYP11B1) in the glucocorticoid or aldosterone pathways could affect corticosteroid and/or aldosterone production.

Fetal Adrenal

A specialized fetal adrenal cortex exists in primates during late gestation that is critical for the normal development of the fetus. After birth, there is a rapid regression, apoptosis, and lysis of the fetal cortex with dilation of cortical capillaries and

replacement by the typical three cortical zones. It is important not to misinterpret this as a lesion in neonatal primates because it represents physiological replacement of the fetal cortex with postnatal adrenal cortex.

ADRENAL MEDULLA

Because it is classified as a specialized postganglionic neuron, the adrenal medulla is a functional extension of the nervous system. It is composed of chromaffin cells, which are the site of catecholamine synthesis and secretion. T ese are true neuroendocrine cells, which provide a direct interface between the two systems. T at is, sympathetic, cholinergic stimulation of the cell bodies results in secretion of catecholamines, which behave as hormones. Chromaffin cells also contain encephalin, neuropeptide Y, substance P, vasopressin, and oxytocin.

Sympathetic Response

T e general functions of the sympathetic nervous system are to ensure reciprocity to counteract and balance the tonic effects of parasympathetic stimulation, assist in the maintenance of steady state functions, and assist in the mobilization of body reserves to meet emergency situations—"fright, fight, or flight."

Catecholamines

T e adrenal medulla is the major site of (nor)epinephrine production with a tyrosine precursor and dopamine intermediate. Release of catecholamines is stimulated by acetylcholine from cholinergic preganglionic neurons. Physiological activators of release include decreased blood pressure, decreased blood glucose, decreased oxygen availability, stress, anxiety, cold, exercise, and postural hypotension. Catecholamines affect all tissues but are most pronounced on the heart, liver, skeletal muscle, adipocytes, vascular smooth muscle, and bronchial smooth muscle.

Adrenergic Receptors

T ere are two major types of these receptors, known as alpha and beta adrenergic receptors with two subtypes of each. Beta-2 receptors bind epinephrine 10 times greater than norepinephrine. T erefore, the receptor type variation on target tissues contributes to the diversity with which the sympathetic response exerts its specific effects.

General Toxicity

Examples of specific chemicals that target chromaffin cells include toxins that block voltage-gated ion channels and bacterial toxins that block exocytosis of secretory granules, thereby preventing catecholamine release. T e most common pathological changes seen in the adrenal medulla in toxicological studies involve proliferative lesions classified as nodular hyperplasia, although degenerative changes can also occasionally be observed.

Pheochromocytoma

Large benign adrenal medullary proliferative lesions are designated pheochromocytomas. T ey are composed of chromaffin cells with variable numbers of hormone-containing secretory granules. In humans, pheochromocytomas are uncommon except in patients with inherited clinical syndromes of multiple endocrine neoplasia (MEN). In rats, these tumors do not secrete excess catecholamines, whereas in humans they secrete increased amounts leading to hypertension and other clinical disturbances.

Pheochromocytomas in rats differ from those in all other species in that they are common, often bilateral, and can be induced by many chemicals. Vitamin D is the most powerful mitogenic stimulus to cause chromaffin cell proliferation in the adrenal medulla in rats. Because the vitamin D effect has been seen in vivo, but not in vitro, it is thought to result from impaired calcium homeostasis, resulting in hypercalcemia.

T e human adrenal medulla, as in mice, has a low spontaneous incidence of proliferative lesions of chromaffin cells. Human chromaffin cells also failed to respond to a variety of mitogenic stimuli in culture. T ese findings and others suggest that the rat represents an inappropriate model to assess the potential effects of xenobiotic chemicals on chromaffin cells of the human adrenal medulla.

A relationship exists between the adenohypophyseal hormones and the development of adrenal medullary proliferative lesions. For example, the long-term administration of growth hormone is associated with an increased incidence of pheochromocytomas as well as the development of tumors at other sites. In long-term animal studies, pheochromocytomas often are accompanied by tumors or toxic effects in other organs. They are often seen in cases involving renal, lung, and hepatic toxicity, in addition to endocrine disturbances. They are also associated with hypoxia, uncoupling of oxidative phosphorylation, disturbances of calcium homeostasis, or disturbances of the hypothalamic endocrine axis.

In Vitro Testing

A commonly employed cell line used in neurobiology is the PC12 pheochromocytoma line derived from a rat adrenal medullary tumor. T e PC12 cell line has been useful in determining intracellular mechanisms at the molecular level that are involved in chromaffin cell signaling and proliferation. Substances that inhibit mitochondrial function (cyanide, rotenone) or uncouple oxidative phosphorylation (dinitrophenol) stimulate catecholamine secretion.

THYROID GLAND

General Anatomy

T e thyroid gland consists of two lobes of endocrine tissue located just below the larynx on each side of the trachea with an isthmus connecting the two lobes. T e thyroid secretes two hormones known as thyroxine (T_4) and triiodothyronine (T_3), which are produced in the thyroid follicle (Figure 21–3).

T_4 and T_3 are important regulators of overall metabolism with their primary target tissues including the liver, kidney, heart, brain, pituitary, gonads, and spleen. Some studies indicate that xenobiotics directly affect the structure of the thyroid gland. For example, heavy metals and red dye #3 are known to decrease the size of the colloid space within the follicle. T is leads to an impaired ability of the thyroid gland to synthesize and store thyroid hormones.

Thyroid Hormone Structure and Synthesis

T ryoid hormones are composed of two covalently linked tyrosine amino acids. Both T_4 and T_3 contain iodides that are derived from dietary intake and are required for biological activity. While the thyroid gland synthesizes and secretes both T_4 and T_3, it primarily releases T_4. Figure 21–3 shows the structures required to make T_3 and T_4. At the apical membrane of the follicular cells, I_2 combines with tyrosine residues on thyroglobulin (TGB) to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). Coupling between MIT and DIT occurs such that combined MIT and DIT forms T_3, whereas combined DIT and DIT forms T_4. T_4 from the thyroid gland can be peripherally converted to T_3 (active hormone) or rT_3 (inactive metabolite), then successively diodinated by the monodeiodinases.

Several studies indicate that xenobiotics can interfere with the thyroid gland function by adversely affecting the process of thyroid hormone synthesis. For example, environmental chemicals such as perchlorate, chlorate, and bromate inhibit uptake of iodide and thus decrease thyroid hormone synthesis. Other goitrogenic chemicals are indicated at the bottom of Figure 21–3.

Thyroid Hormone Binding Proteins

Once released into the blood, thyroid hormones are rapidly bound to high-affinity serum binding proteins. Less than

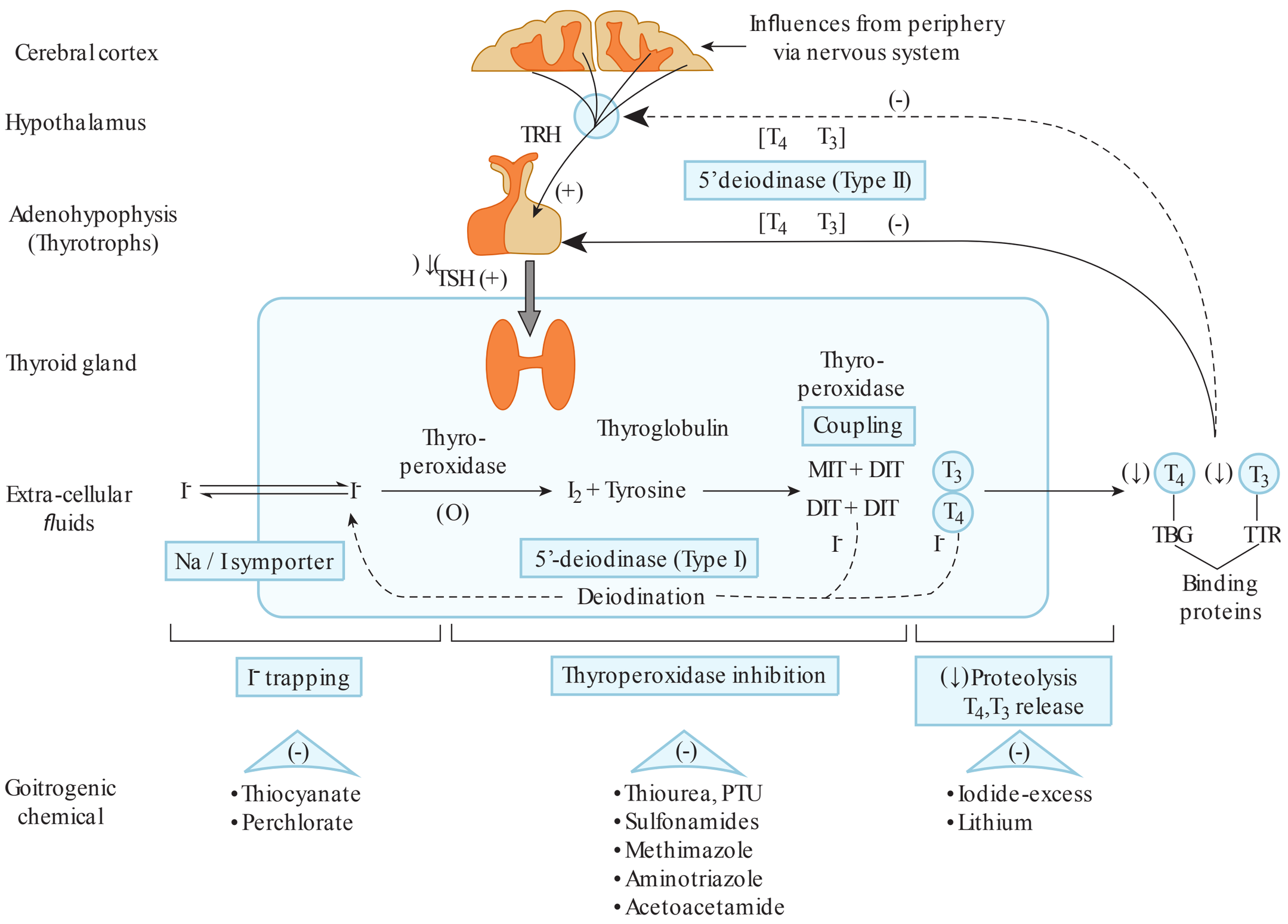

FIGURE 21–3 **Mechanism of action of goitrogenic chemicals on thyroid hormone synthesis and secretion.** (Reproduced with permission from Dunlop RH, Malbert C, Capen CC, O'Brien TD: Pathophysiology of Endocrine Homeostasis: Examples in Veterinary Pathophysiology, Blackwell Publishing, 2004.)

1% of T_3 is free in circulation. Only this small-unbound fraction has access to receptors in target cells. Environmental chemicals such as PCBs are known to displace thyroid hormones from serum binding proteins and lead to a rapid decline in serum thyroid hormone levels.

Thyroid Hormone Receptors

Thyroid hormones act by binding to the thyroid hormone receptors (TRs). Environmental chemicals can interfere with thyroid hormone binding to TRs and thyroid hormone–related transcription at multiple levels. Some can bind directly to TRs and induce either agonistic or antagonistic effects. Others interfere with the thyroid hormone binding to receptors via indirect mechanisms. There are xenobiotics that can interfere with cross-talk between TRs and other nuclear receptors.

Thyroid Hormone Clearance

The main pathway for clearance of thyroid hormones from the serum is via conjugation to glucuronic acid or sulfate (Figure 21–4). Studies indicate that some xenobiotics including coplanar and noncoplanar congeners of PCBs may increase the clearance of thyroid hormones from the serum by inducing glucuronosyltransferases and sulfotransferases. Others have shown that xenobiotics such as rifampicin and phenobarbital may decrease the transport of thyroid hormones into the brain and liver by inhibiting transporters.

Regulation of Thyroid Hormone Release

Thyroid hormone secretion is regulated by thyroid-stimulating hormone (TSH) from the anterior pituitary gland. The rate of release of TSH is under a hypothalamic–pituitary–thyroid regulatory axis involving negative feedback. The hypothalamus synthesizes and secretes thyroid-releasing hormone (TRH). TRH travels to the anterior pituitary via the portal plexus and stimulates synthesis and secretion of TSH. TSH acts on the thyroid gland to stimulate production and/or release of T_3 and T_4. These can then exert negative feedback control at the level of the anterior pituitary to inhibit further release of TSH. Chemicals such PBDEs may increase TSH levels, leading to increased levels of T_3 and T_4.

Physiological Effects

Thyroid hormones influence nearly every tissue in the body with its primary function being the determination of metabolic rate. In general, thyroid hormone stimulates both anabolic and catabolic biochemical pathways; however, its overriding effect is catabolism. Thyroid hormone also produces significant effects on growth and development of the CNS and skeleton early in life.

Thyroid Toxicity

Given the influence of thyroid hormones on numerous tissues in the body, it is not surprising that xenobiotics that affect

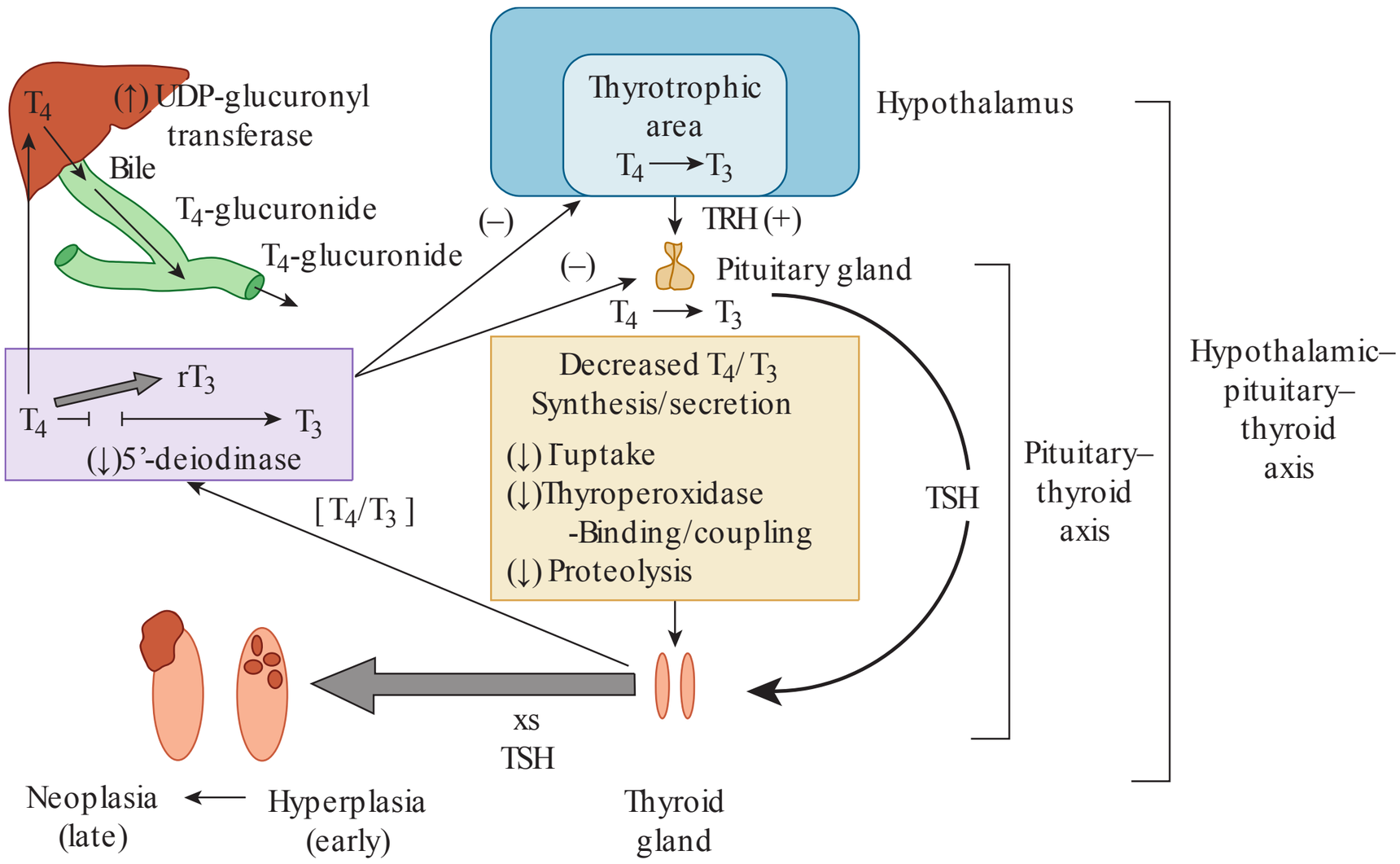

FIGURE 21–4 **Multiple sites of disruption of the hypothalamic–pituitary–thyroid axis by xenobiotic chemicals.** Chemicals can exert direct effects by disrupting thyroid hormone synthesis or secretion and indirectly influence the thyroid through an inhibition of 5′-deiodinase or by inducing hepatic microsomal enzymes (e.g., T_4–UDP-glucuronyltransferase). All of these mechanisms can lower circulating levels of thyroid hormones (T_4 and/or T_3), resulting in a release from negative feedback inhibition and increased secretion of thyroid-stimulating hormone (TSH) by the pituitary gland. The chronic hypersecretion of TSH predisposes the sensitive rodent thyroid gland to develop an increased incidence of focal hyperplastic and neoplastic lesions (adenomas) by a secondary (epigenetic) mechanism.

thyroid hormone levels often cause symptoms of hypothyroidism or hyperthyroidism, or lead to a significant impairment in brain development and function.

PCBs—PCBs are some of the best characterized thyroid disrupting chemicals. PCBs are known to interfere with the thyroid system in a manner that leads to serious neurocognitive defect. Several studies indicate that PCBs decrease the level of thyroid hormone by inhibiting synthesis and/or increasing the metabolism. Further, some studies indicate that they interfere with thyroid hormone action by inhibiting the binding of thyroid hormones to binding proteins or blocking their ability to bind to TRs.

PBDEs—Polybrominated diphenyl ethers (PBDEs) are structurally similar to that of PCBs. T us, it is not surprising that many of the toxic effects between the two are similar leading to neurocognitive defects.

Perchlorate—A few studies indicate that perchlorate exposure inhibits thyroid hormone levels, possibly leading to hypothyroid-like outcomes.

Pesticides—Pesticide mixtures containing dichlorodiphenyltrichloroethane (DDT) have been shown to increase thyroid volume and to induce antibodies that attack the thyroid gland, resulting in autoimmune thyroid disease.

Perf uorinated Chemicals—Some studies have shown that perfluorooctane sulfonate and perfluorooctanoic acid decrease T_3 and T_4 levels by potentially upregulating phase II enzymes in liver and deiodinases in the thyroid.

Bisphenol A—BPA blocks T_3 action by antagonizing the binding of T_3 to its receptor. Further, some studies have shown that BPA inhibits T_3-mediated gene expression in cell lines. It is suggested that BPA leads to symptoms of hypothyroidism or thyroid resistance syndrome in animal models.

Phthalates—To date, a few small human studies have shown that phthalate exposures may alter the levels of T_3 and T_4 in adult men and pregnant women. T ey result in low thyroid hormone levels and to symptoms of hypothyroidism.

PARATHYROID GLAND

General Anatomy

Humans have four parathyroid glands that are embedded in the surface of the thyroid gland. T ey are composed of mainly chief cells that produce parathyroid hormone (PTH). T e parathyroid glands are critical for life largely because PTH helps maintain normal plasma calcium levels (Figure 21–5). Calcium is required in optimal concentrations for processes such as fertilization, vision, locomotion-muscle contraction, nerve conduction, blood clotting, exocytosis, cell division, and the activity of a number of enzymes and hormones. When the parathyroids are removed or damaged, PTH levels drop, causing a major drop in circulating calcium levels. In turn, this can lead to tetanic convulsions and death.

Parathyroid Toxicity

Xenobiotic exposures may alter the structure of the parathyroid gland. In some cases, chemicals cause death of the parathyroid cells resulting in a reduced size and limited release of

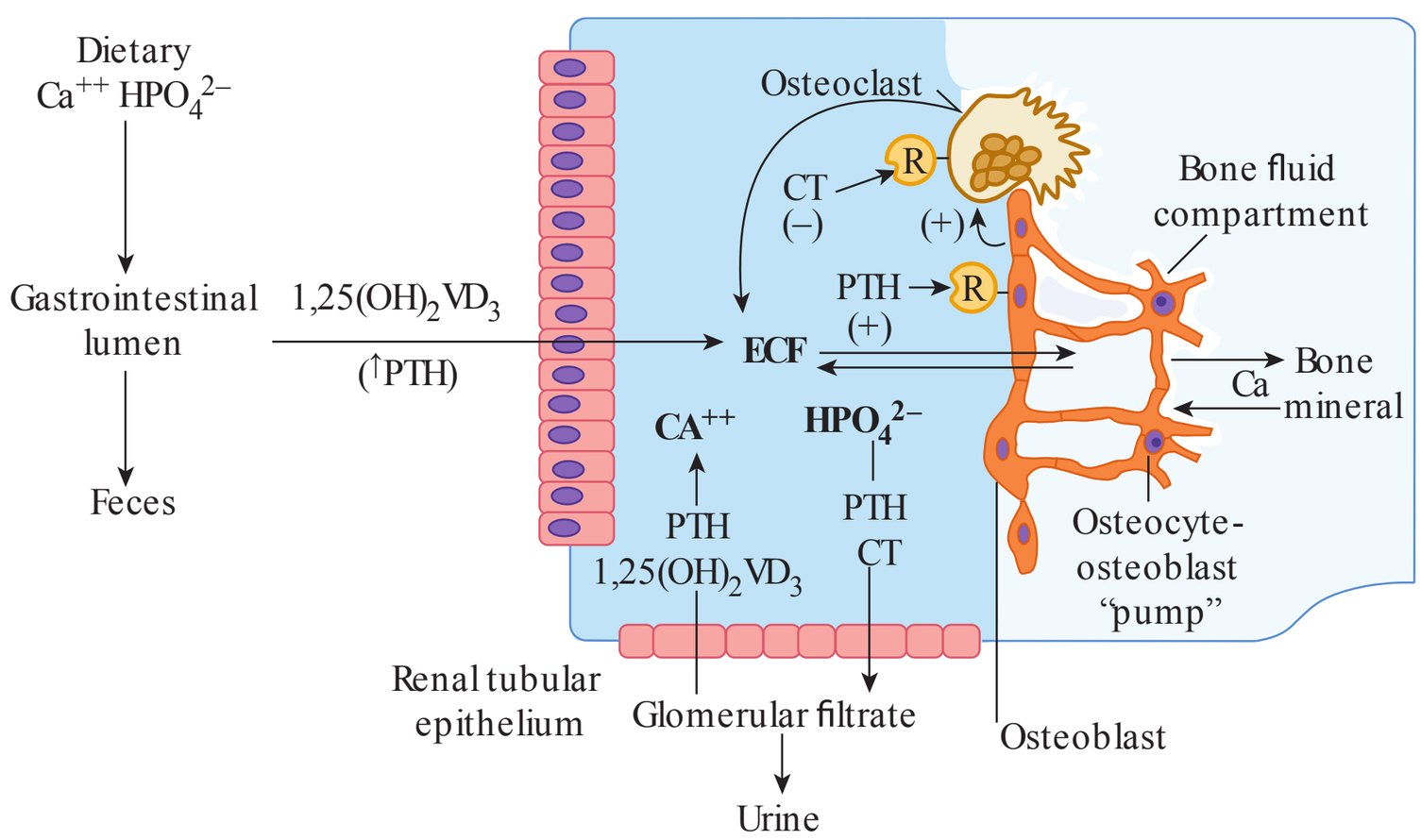

FIGURE 21–5 **Interrelationship of parathyroid hormone (PTH), calcitonin (CT), and 1,25-dihydroxycholecalciferol ($1,25(OH)_2VD_3$) in the regulation of calcium (Ca) and phosphorus in extracellular f uids.** Receptors for PTH are on osteoblasts and for CT on osteoclasts in bone. PTH and CT are antagonistic in their action on bone but synergistic in stimulating the renal excretion of phosphorous. Vitamin D exerts its action primarily on the intestine to enhance the absorption of both calcium and phosphorus.

PTH. Other xenobiotic exposures have been shown to increase the size of the parathyroid gland (lead, rotenone, malathion, hexachlorobenzene) often leading to parathyroid cancer.

PTH Structure and Synthesis

PTH is a polypeptide hormone that is derived from a precursor molecule called preproparathyroid hormone (Figure 21–6). Xenobiotics may interfere with the normal synthesis of PTH. Metals such as aluminum and cadmium have been shown to inhibit PTH secretion. Similarly, alcohol consumption has been shown to decrease PTH levels in pregnant rats. Lithium has been associated with a rise in PTH levels as well as abnormally high calcium levels.

PTH Receptors

T e PTH receptor is a single G-protein-coupled receptor called PTHR1. A study shows that xenobiotics may alter the expression of PTHR1. Specifically, studies have shown that binge alcohol drinking significantly decreases expression of PTHR1 in male rats.

Physiological Effects

T e main physiological role of the parathyroid gland is to control circulating calcium levels (Figure 21–5). PTH works in concert with calcitonin (CT) and vitamin D. PTH serves to increase circulating calcium levels by increasing the release of calcium from bone through demineralization. PTH also serves to increase calcium levels by increasing the tubular reabsorption of calcium by the kidney. Further, it inhibits the renal reabsorption of phosphate, which aids in increasing the solubility of calcium. PTH also enhances magnesium reabsorption, inhibits bicarbonate ion reabsorption, and blocks exchange of sodium ions by the tubules. T ese actions of PTH result in metabolic acidosis, which favors removal of calcium from plasma proteins and bones. In turn, this increases circulating levels of ionized calcium.

CT reduces circulating calcium levels by reversing the action of PTH on bone resorption. CT serves to prevent hypercalcemia by shutting down ef ux of calcium from bone, and it negatively regulates PTH to prevent kidney calcification. Vitamin D also serves to inhibit PTH actions and build bone. Vitamin D_3 is essential for calcium absorption in the GI tract.

Some xenobiotics such as pesticides and fungicides can cause excessive PTH secretion by the parathyroid gland and lead to hyperparathyroidism. Other xenobiotic exposures such as those to heavy metals may cause low PTH secretion and lead to hypoparathyroidism.

Regulation of PTH Release

When the calcium receptors in the parathyroid gland sense low calcium levels, they stimulate the parathyroid gland to release PTH.

ENDOCRINE PANCREAS

Scattered among the pancreatic acini are the endocrine units of the pancreas, the Islets of Langerhans. T e major physiological function of the endocrine pancreas is to serve as the primary homeostatic regulator of fuel metabolism, particularly circulating glucose. Islet cells are sensors of glucose homeostasis that respond to changes in their nutrient and hormonal environment.

Role of the Liver in Glucose Production

Energy for cellular metabolism can be derived from fatty acids or glucose in the blood. T e liver is the primary contributor to increasing blood glucose levels.

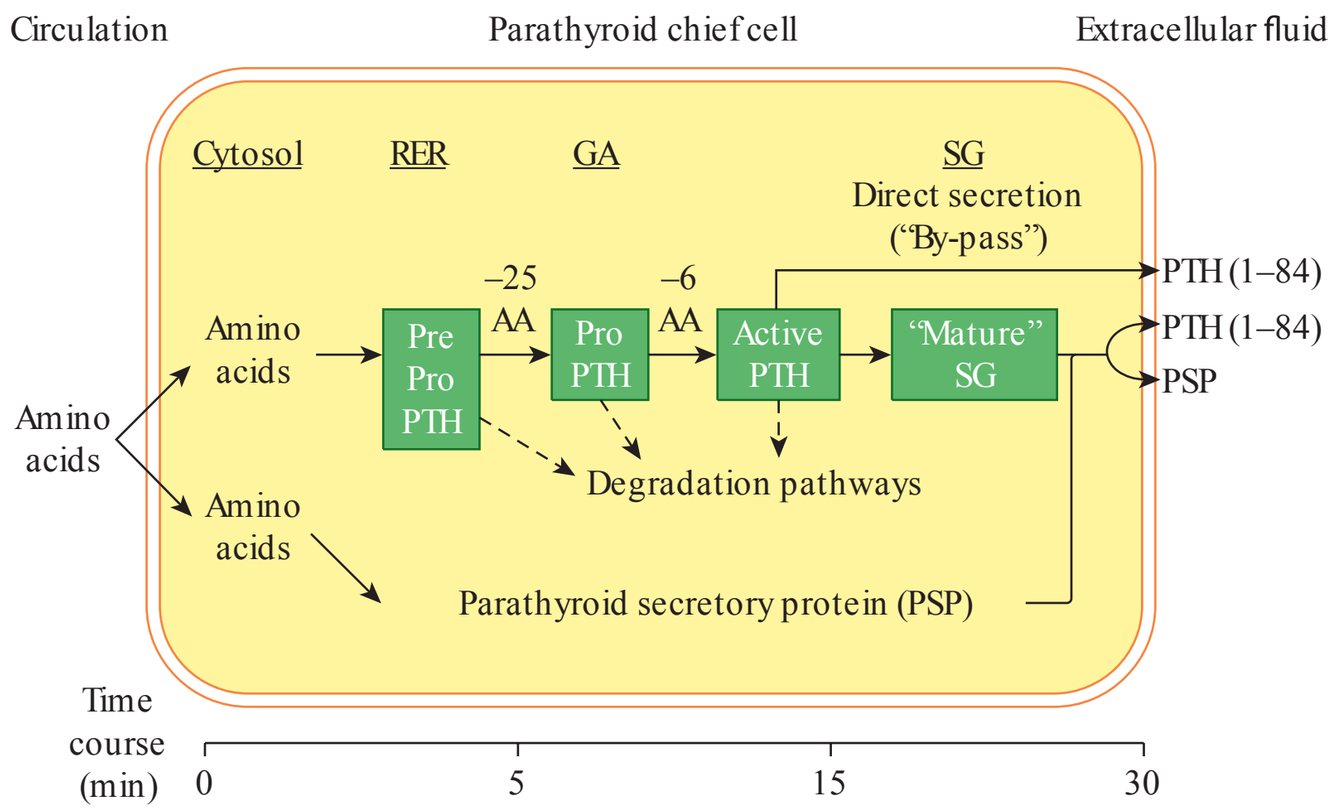

FIGURE 21–6 **Biosynthesis of PTH.** Active PTH is synthetized as a larger biosynthetic precursor (preproPTH) that undergoes rapid posttranslational processing to proPTH prior to secretion as active PTH (aminoacids 1-84) from chief cells in the parathyroid glands.

Pancreatic Hormones

Insulin—T e overall effects of insulin are to stimulate anabolic processes (energy storage). Specifically, insulin functions to lower blood levels of glucose, fatty acids, and amino acids and to promote their conversion to the storage form of each: glycogen, triglycerides, and protein, respectively.

Glucagon—Glucagon is the primary hormone with action counterregulatory to insulin, because it stimulates catabolic processes to prevent hypoglycemia. T e release of glucagon is stimulated by epinephrine and norepinephrine, and by the amino acids, arginine, leucine, and alanine. Conversely, glucagon secretion is inhibited by insulin and somatostatin.

Somatostatin—T e role of somatostatin is its role in regulation of neuroendocrine function to inhibit secretion of growth hormone in the anterior pituitary. T e generalized function of somatostatin appears to be as a hormone release inhibitor.

Interactions of Release

Although glucagon and insulin exert opposing effects on carbohydrate metabolism, they act in concert to preserve normoglycemia in the face of perturbations that might tend to elevate or lower blood glucose. Insulin and glucagon exert opposing effects on various metabolic processes. T erefore, many investigators like to think of the insulin-to-glucagon ratio in blood as an important determinant of the overall metabolic status. When there is a high ratio of insulin to glucagon, a relative anabolic state exists. When the ratio of insulin to glucagon is low, a catabolic state exists.

Metabolic Responses in Diabetes

Two major forms of diabetes mellitus result from either decreased insulin production (type 1) due to autoimmune destruction of pancreatic β cells or reduced insulin function (type 2) owing to end organ insensitivity or resistance to insulin. Insufficient insulin action leads to decreased glycogen, lipid, and protein synthesis (Figure 21–7). Reduced removal of glucose from the blood causes hyperglycemia and various metabolic alterations. Increased action of the counter-regulatory hormone glucagon stimulates glycogenolysis, lipolysis, and protein breakdown. Stimulation of glycogenolysis and gluconeogenesis increases circulating glucose.

Pancreatic Toxicity

T e insulin-secreting beta cells are particularly sensitive to chemical attack. T e clinical consequences of insulin deficiency are physiologically more severe than those that would result from glucagon deficiency because the other counterregulatory hormones that oppose insulin action can compensate for reduced glucagon regulation. Two chemicals that have been widely used to generate animal models of diabetes are alloxan and streptozotocin. A common target of these in pancreatic beta cells is DNA. T ere are data to support that DNA damage occurs, poly(ADP-ribose) synthetase is activated, polyadenylation increases, and NAD declines.

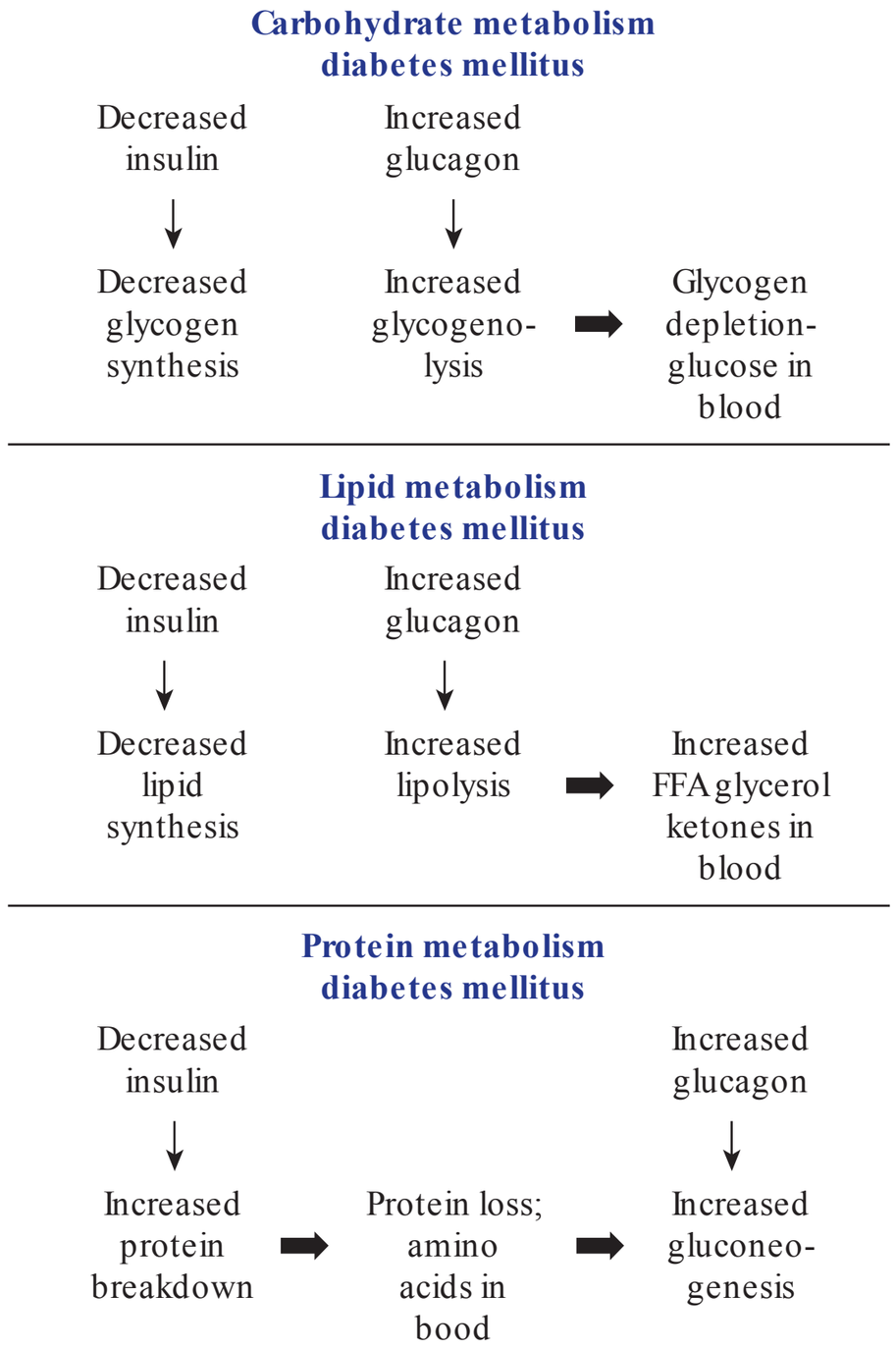

FIGURE 21–7 **Effects of diabetes mellitus on metabolism.** Decreased insulin (type 1) or insulin action (type 2) inhibits glycogen, lipid, and protein synthesis. Increased glucagon stimulates glycogenolysis, lipolysis, and protein breakdown. Glycogenolysis increases circulating glucose. Increased glycerol and amino acids serve as substrates for gluconeogenesis to further increase circulating glucose.

Insulin Resistance

Insulin resistance and defective function of pancreatic beta cells usually occur sometime before the development of type 2 diabetes. In a study investigating nondiabetic residents living near a deserted pentachlorophenol and chloralkali factory in Taiwan, insulin resistance was associated with increasing circulating levels of dioxins and mercury. In addition, BPA exposure of pregnant mice resulted in increased insulin, leptin, triglyceride, and glycerol levels.

In Vitro Testing

Several cell lines are available for testing of insulin secretion. Pancreatic beta-cell-derived RINm5F cells were exposed to a combination of the cytokines, IL-1β, TNF-α, and IFN-γ

to simulate type 1 diabetes mellitus conditions. T is study showed that hydrogen peroxide produced by these cytokines reacted in the presence of trace metal Fe^{++} with nitric oxide to form highly toxic hydroxyl radicals. RINm5F cells were also used to investigate the role of oxidative stress in inorganic arsenic exposure. A number of proapoptotic mitochondrial and cytosolic markers were investigated and found to be elevated during β-cell toxicity.

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QUESTIONS

1. The inability to release hormones from the anterior pituitary would NOT affect the release of which of the following?
   a. LH.
   b. PRL.
   c. ADH.
   d. TSH.
   e. ACTH.

2. Which of the following statements regarding pituitary hormones is TRUE?
   a. The hypothalamic–hypophyseal portal system transports releasing hormones to the neurohypophysis.
   b. Dopamine enhances prolactin secretion from the anterior pituitary.
   c. Somatostatin inhibits the release of GH.
   d. The function of chromophobes in the anterior pituitary is unknown.
   e. Oxytocin and ADH are synthesized by hypothalamic nuclei.

3. 21-Hydroxylase deficiency causes masculinization of female genitals at birth by increasing androgen secretion from which region of the adrenal gland?
   a. zona glomerulosa.
   b. zona reticularis.
   c. adrenal medulla.
   d. zona fasciculata.
   e. chromaffin cells.

4. Which of the following statements regarding adrenal toxicity is TRUE?
   a. The adrenal cortex and adrenal medulla are equally susceptible to fat-soluble toxins.
   b. Adrenal cortical cells lack the enzymes necessary to metabolize xenobiotic chemicals.
   c. Pheochromocytomas of the adrenal medulla can cause high blood pressure and clammy skin due to increased epinephrine release.
   d. Xenobiotics primarily affect the hydroxylase enzymes in the zona reticularis.
   e. Vitamin D is an important stimulus for adrenal cortex steroid secretion.

5. Chemical blockage of iodine transport in the thyroid gland:
   a. affects export of T_3 and T_4.
   b. prevents reduction to I_2 by thyroid peroxidase.
   c. decreases TRH release from the hypothalamus.
   d. interrupts intracellular thyroid biosynthesis.
   e. mimics goiter.

6. Chromaffin cells of the adrenal gland are responsible for secretion of which of the following?
   a. aldosterone.
   b. epinephrine.
   c. corticosterone.
   d. testosterone.
   e. estradiol.

7. The parafollicular cells of the thyroid gland are responsible for secreting a hormone that:
   a. increases blood glucose levels.
   b. decreases plasma sodium levels.
   c. increases calcium storage.
   d. decreases metabolic rate.
   e. increases bone resorption.

8. Parathyroid adenomas resulting in increased PTH levels would be expected to cause which of the following?
   a. hypocalcemia.
   b. hyperphosphatemia.
   c. increased bone formation.
   d. osteoporosis.
   e. rickets.

9. Which of the following vitamins increases calcium and phosphorus absorption in the gut?
   a. vitamin D.
   b. niacin.
   c. vitamin A.
   d. vitamin B_{12}.
   e. thiamine.

10. All of the following statements regarding glucose control are true EXCEPT:
    a. Glucagon stimulates glycogenolysis, gluconeogenesis, and lipolysis.
    b. Insulin stimulates glycogen synthesis, gluconeogenesis, and lipolysis.
    c. Glucagon stimulates catabolic processes (mobilizes energy) to prevent hypoglycemia.
    d. Insulin promotes storage of glucose, fatty acids, and aminoacids by their conversion to glycogen, triglycerides, and protein, respectively.
    e. Insulin and glucagon exert opposing effects on blood glucose concentrations.

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UNIT 5 TOXIC AGENTS

CHAPTER 22

Toxic Effects of Pesticides

Lucio G. Costa

INTRODUCTION

ECONOMICS AND PUBLIC HEALTH

- Use of Pesticides
- Exposure
- Human Poisoning
- Regulatory Mandate

INSECTICIDES

- Organophosphorus Compounds
  - Biotransformation
  - Signs and Symptoms of Toxicity and Mechanism of Action
  - Treatment of Poisoning
  - The Intermediate Syndrome
  - Organophosphate-induced Delayed Polyneuropathy (OPIDP)
  - Long-term Toxicity
- Carbamates
- Pyrethroids
  - Signs and Symptoms of Toxicity and Mechanism of Action
- Organochlorine Compounds
  - DDT and Its Analogs
  - Hexachlorocyclohexanes and Cyclodienes
- Other Old and New Insecticides
  - Rotenoids
  - Nicotine
  - Avermectins

INSECT REPELLENTS

- Picaridin

HERBICIDES

- Chlorophenoxy Compounds
- Bipyridil Compounds
- Chloroacetanilides
- Triazines
- Phosphonomethyl Amino Acids
  - Glyphosate
  - Glufosinate

FUNGICIDES

- Captan and Folpet
- Dithiocarbamates
- Inorganic and Organometal Fungicides

RODENTICIDES

- Fluoroacetic Acid and Its Derivatives
- Anticoagulants

FUMIGANTS

- Methyl Bromide
- 1,3-Dichloropropene
- Sulfur

KEY POINTS

- A pesticide may be defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest.
- Pesticide exposures include (1) accidental and/or suicidal poisonings; (2) occupational exposure (manufacturing, mixing/loading, application, harvesting, and handling of crops); (3) bystander exposure to off-target drift from spraying operations; and (4) the general public who consume food items containing pesticide residues.
- Chemical insecticides in use today poison the nervous systems of the target organisms.
- An herbicide is any compound that is capable of either killing or severely injuring plants.
- A fungicide is any chemical capable of preventing growth and reproduction of fungi.

INTRODUCTION

Pesticides can be defined as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating pests. Pests can be insects, rodents, weeds, and a host of other unwanted organisms. Pesticides may be more specifically identified as insecticides (insects), herbicides (weeds), fungicides (fungi and molds), rodenticides (rodents), acaricides (mites), molluscides (snails and other mollusks), miticides (mites), larvicides (larvae), and pediculocides (lice). In addition, for regulatory purposes, plant growth regulators, repellants, and attractants (pheromones) often also fall in this broad classification of chemicals.

ECONOMICS AND PUBLIC HEALTH

T e use of pesticides must consider the balance of the benefits versus the possible risks of injury to human health or degradation of environmental quality. Pesticides play a major role in the control of vector-borne diseases, which represent a major threat to the health of large human populations. When introduced in 1942, DDT appeared to hold immense promise of benefit to agriculture and public health by controlling vector-borne diseases. However, because of its bioaccumulation in the environment and its detrimental effects on bird reproduction, DDT was eventually banned in most countries by the mid-1970s. When DDT was banned in 1996 in South Africa, less than 10 000 cases of malaria were registered in that country. By 2000, the number of malaria cases had increased to 62 000, but with the reintroduction of DDT at the end of that year, cases were down to 12 500.

Excessive loss of food crops to insects or other pests contributes to economic loss and possible starvation. In developed countries, pesticides allow production of abundant, inexpensive, and attractive fruits and vegetables, as well as grains. Along with insecticides, herbicides and fungicides play a major role in this endeavor.

Use of Pesticides

In the past 20 years, use of pesticides (as amount of active ingredient) has plateaued due to the utilization of more efficacious compounds, which require less active ingredient. Pesticides are often, if not always, used as multiagent formulations, in which the active ingredient is present together with other ingredients to allow mixing, dilution, application, and stability. T ese other ingredients are lumped under the term “inert” or “other.” T ough they do not have pesticidal action, such inert ingredients may not always be devoid of toxicity.

Exposure

Exposure to pesticides can occur via the oral or dermal routes or by inhalation. High oral doses, leading to severe poisoning and death, are achieved as a result of pesticide ingestion for suicidal intent, or of accidental ingestion, commonly due to storage of pesticides in improper containers. Chronic low doses, on the other hand, are consumed by the general population as pesticide residues in food or as contaminants in drinking water. Regulations exist to ensure that pesticide residues are maintained at levels below those that would cause any adverse effects. Workers involved in the production, transport, mixing and loading, and application of pesticides, as well as in harvesting of pesticide-sprayed crops, are at the highest risk for pesticide exposure. Dermal exposure during normal handling or application of pesticides, or in case of accidental spillings, occurs in body areas not covered by protective clothing, such as the face or the hands, or by inhalation. Furthermore, pesticides deposited on clothing may penetrate the skin and/or potentially expose others, if clothes are not changed and washed on termination of exposure.

Human Poisoning

Pesticides are not always selective for their intended target species, and adverse health effects can occur in nontarget

TABLE 22–1 WHO-recommended classification of pesticides by hazard.

	LD_{50} in Rat (mg/kg Body Weight)			
	Oral		Dermal	
Class	Solids	Liquids	Solids	Liquids
Ia: Extremely hazardous	5 or less	20 or less	10 or less	40 or less
Ib: Highly hazardous	5–50	20–200	10–100	40–400
II: Moderately hazardous	50–500	200–2000	100–1000	400–4000
III: Slightly hazardous	Over 500	Over 2000	Over 1000	Over 4000
IV+ : Unlikely to present hazard in normal use	Over 2000	Over 3000	Over 4000	Over 6000

species, including humans. In the general population and in occupationally exposed workers, concerns range from acute human poisoning to a possible association between pesticide exposure and increased risk of cancer, reproductive and developmental toxicity.

With several million poisonings causing hospital admission and a couple hundred thousand deaths, the World Health Organization (WHO) has recommended a classification of pesticides by hazard, where acute oral or dermal toxicities in rats were considered (Table 22–1). As a class, insecticides are the most acutely toxic followed by herbicides and fungicides.

Regulatory Mandate

In the United States, the Environmental Protection Agency (EPA) regulates pesticide use under the Federal Insecticide, Fungicide and Rodenticide Act and the Federal Food, Drug and Cosmetic Act through registration for use and establishment of maximum allowable levels of pesticide residues (tolerances) in foods and animal feeds.

T e Food Quality Protection Act gives EPA the mandate to assess risks of pesticides to infants and children based on dietary consumption patterns of children, possible susceptibility of infants and children to pesticides, and cumulative effects of compounds that share the same mechanism of toxicity. Additional regulations concerning pesticides are present in other laws, such as the Safe Drinking Water Act or the Clean Air Act.

All pesticides sold or distributed in the United States must be registered by the EPA. To register a pesticide or a formulated product, a large number of studies (over 140) are required, a process that takes several years and costs between $50 and $100 million. T e database includes information on product and residue chemistry, environmental fate, toxicology, biotransformation/degradation, occupational exposure and reentry protection, spray drift, environmental impact on nontarget species (birds, mammals, aquatic organisms, plants, and soil), environmental persistence and bioaccumulation, as well as product performance and efficacy. Table 22–2 lists basic toxicology data needed for new pesticide registration.

Other nations, such as Canada, Japan, and most European countries, have legislated similar procedures for pesticide registration. T e European Union (EU) has created a harmonized Union-wide framework for pesticide regulation. T e WHO provides guidance, particularly with the setting of acceptable daily intake (ADI) values for pesticides.

TABLE 22–2 Basic toxicology testing requirements for pesticide registration.

Test	Animal Species*
Acute lethality (oral, dermal, inhalation)	Rat, mouse, guinea pig, rabbit
Dermal irritation	Rabbit, rat, guinea pig
Dermal sensitization	Guinea pig
Eye irritation	Rabbit
Acute delayed neurotoxicity	Hen
Genotoxicity studies (in vitro, in vivo)	Bacteria, mammalian cells, mouse, rat, Drosophila
Teratogenicity	Rabbit, rodent (mouse, rat, hamster)
2- to 4-week toxicity study (oral, dermal, inhalation)	Rat, mouse
90-Day toxicity study (oral)	Rat
Chronic toxicity study (oral; 6 months to 2 years)	Rat, dog
Oncogenicity study	Rat, mouse
Reproductive/fertility study	Rat
Developmental neurotoxicity study	Rat

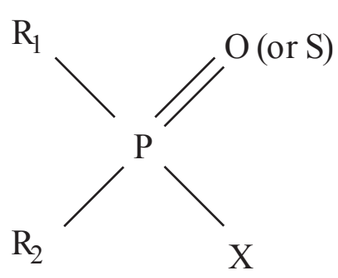
*Substantial efforts are being devoted to develop alternative nonanimal test systems. Only one in vitro test for primary irritation has been validated and accepted by regulatory bodies.

INSECTICIDES

Insecticides play a most relevant role in the control of insect pests, particularly in developing countries. All of the chemical insecticides in use today are neurotoxicants, and act by poisoning the nervous systems of the target organisms (Table 22–3). The central nervous system of insects is highly developed and not unlike that of mammals. As a class, insecticides have high acute toxicity toward nontarget species compared with other pesticides. Some of them, most notably the organophosphates, are involved in a great number of human poisonings and deaths each year.

Organophosphorus Compounds

The general structure of organophosphorus (OP) insecticides can be represented by:



where X is the so-called leaving group that is displaced when the OP phosphorylates acetylcholinesterase (AChE), and is the most sensitive to hydrolysis; R_1 and R_2 are commonly alkoxy groups (i.e., OCH_3 or OC_2H_5) or other chemical substituents; either an oxygen or a sulfur (in this case the compound should be defined as a phosphorothioate) is also attached to the phosphorus with a double bond. Based on chemical differences, OPs can be divided into several subclasses, which include phosphates, phosphorothioates, phosphoramidates, phosphonates, and others. Figure 22–1 shows the chemical structures of some commonly used OPs.

TABLE 22–3 Molecular targets of the major classes of insecticides.

Target	Insecticide	Effect
Acetylcholinesterase	Organophosphates Carbamates	Inhibition Inhibition
Sodium channels	Pyrethroids (types I and II) DDT Dihydropyrazoles	Activation Activation Inhibition
Nicotinic acetylcholine receptors	Nicotine Neonicotinoids	Activation Activation
GABA receptor–gated chloride channels	Cyclodienes Phenylpyrazoles Pyrethroids (type II)	Inhibition Inhibition Inhibition
Glutamate-gated chloride channels*	Avermectins	Activation
Octopamine receptors†	Formamidines	Activation
Mitochondrial complex I	Rotenoids	Inhibition
Ryanodine receptors	Diamides	Activation

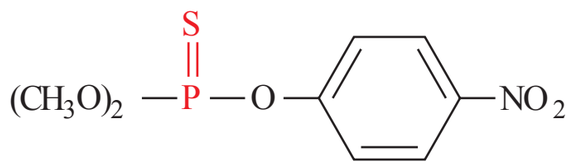
*Found only in insects. In mammals, avermectins activate $GABA_A$ receptors.
†In mammals, formamidines activate $alpha_2$-adrenoceptors.

Biotransformation—For all compounds that contain a sulfur bound to the phosphorus, a metabolic bioactivation is necessary for their biological activity to be manifest, as only compounds with a P= O moiety are effective inhibitors of AChE. Oxidative desulfuration (leads to the formation of an "oxon," or oxygen analog of the parent insecticide) and thioether oxidation (formation of a sulfoxide, S= O, followed by the formation of a sulfone, O= S= O) are catalyzed by cytochrome P450s. Catalytic hydrolysis by phosphotriesterases, known as A-esterases (which are not inhibited by OPs), plays an important role in the detoxication of certain OPs. Noncatalytic hydrolysis of OPs also occurs when these compounds phosphorylate serine esterases classified as B-esterases.

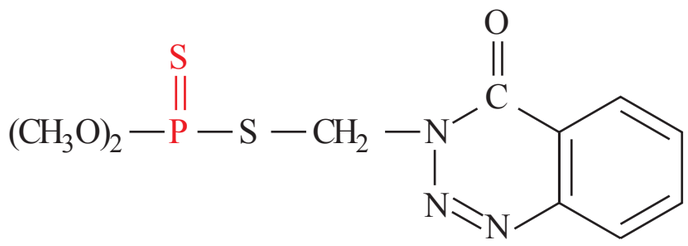
Signs and Symptoms of Toxicity and Mechanism of Action—OP insecticides have high acute toxicity, with oral LD_{50} values in rat often below 50 mg/kg. For several OPs, acute dermal toxicity is also high. Inhibition of AChE by OPs causes accumulation of acetylcholine at cholinergic synapses, with overstimulation of muscarinic and nicotinic cholinergic receptors. As these receptors are localized in most organs of the body, a "cholinergic syndrome" ensues, which includes increased sweating, salivation, bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, diarrhea, tremors, muscular twitching, and various central nervous system effects (Table 22–4). Whereas respiratory failure is a hallmark of severe OP poisoning, mild poisoning and/or early stages of an otherwise severe poisoning may display no clear-cut signs and symptoms.

OPs with a P= O moiety phosphorylate a hydroxyl group on serine in the active (esteratic) site of the enzyme, impeding its action on the physiological substrate. Phosphorylated AChE is hydrolyzed by water slowly, and the rate of "spontaneous reactivation" depends on the chemical nature of the R substituents. Reactivation of phosphorylated AChE does not occur once the enzyme-inhibitor complex has "aged," which occurs when there is loss by nonenzymatic hydrolysis of one of the two alkyl (R) groups. When phosphorylated AChE has aged, the enzyme is considered to be irreversibly inhibited, and the only means of replacing its activity is through synthesis of new enzyme, a process that may take days.

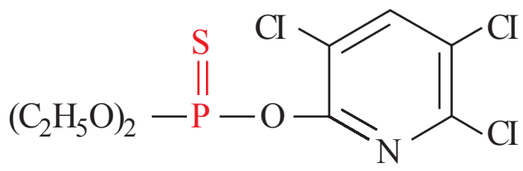
Treatment of Poisoning—Procedures aimed at decontamination and/or at minimizing absorption depend on the route of exposure. In case of dermal exposure, contaminated clothing should be removed, and the skin washed thoroughly with alkaline soap. In case of ingestion, procedures to reduce absorption from the gastrointestinal tract do not appear to be very effective. Atropine, a muscarinic receptor antagonist, prevents the action of accumulating acetylcholine on



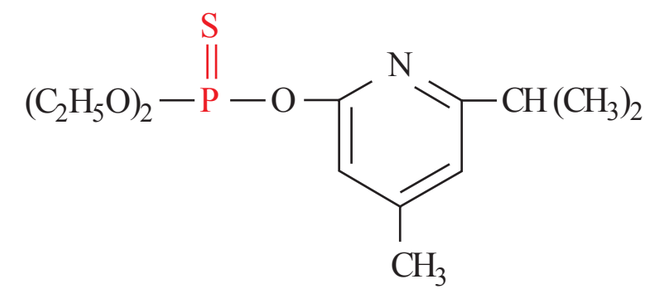
Methylparathion



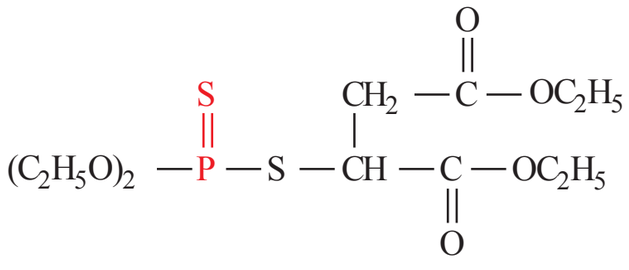
Azinphos-methyl (Guthion)



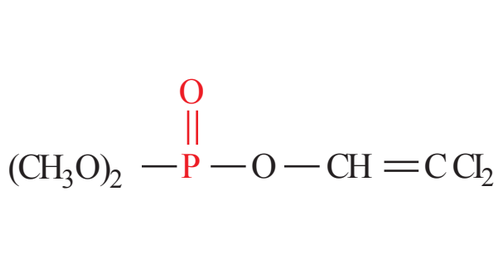
Chlorpyrifos



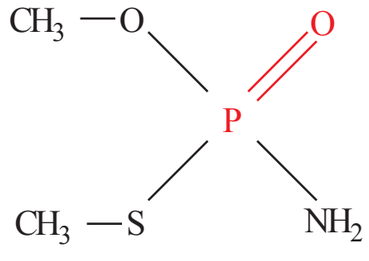
Diazinon



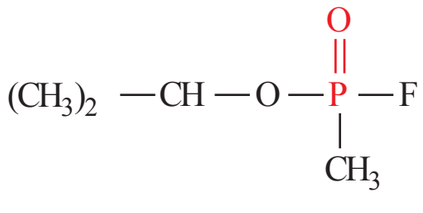
Malathion



Dichlorvos



Metamidophos



Sarin

FIGURE 22–1 **Structures of some organophosphorus insecticides and of the nerve agent sarin.** Note that most commonly used compounds are organophosphorothioates (i.e., have a P= S bond), but some, including sarin, have a P= O bond and do not require metabolic activation.

TABLE 22–4 Signs and symptoms of acute poisoning with anticholinesterase compounds.

Site and Receptor Af ected	Manifestations
Exocrine glands (M)	Increased salivation, lacrimation, perspiration
Eyes (M)	Miosis, blurred vision
Gastrointestinal tract (M)	Abdominal cramps, vomiting, diarrhea
Respiratory tract (M)	Increased bronchial secretion, bronchoconstriction
Bladder (M)	Urinary frequency, incontinence
Cardiovascular system (M)	Bradycardia, hypotension
Cardiovascular system (N)	Tachycardia, transient hypertension
Skeletal muscles (N)	Muscle fasciculations, twitching, cramps, generalized weakness, flaccid paralysis
Central nervous system (M, N)	Dizziness, lethargy, fatigue, headache, mental confusion, depression of respiratory centers, convulsions, coma

M, muscarinic receptors; N, nicotinic receptors.

these receptors. T e administration of pralidoxime (2-PAM) early after OP exposure can help prevent AChE aging, but its effectiveness is equivocal and harm may ensue. Diazepam may be used to relieve anxiety in mild cases, and to reduce muscle fasciculations and control convulsions in the more severe cases.

The Intermediate Syndrome—A second distinct manifestation of exposure to OPs is the so-called intermediate syndrome, which is seen in 20% to 50% of acute OP poisoning cases. T e syndrome develops 1 to several days after the poisoning, during recovery from cholinergic manifestations, or in some cases, when patients have completely recovered from the initial cholinergic crisis. Prominent features include a marked weakness of respiratory, neck, and proximal limb muscles. Mortality due to respiratory paralysis and complications ranges from 15% to 40%, and recovery in surviving patients takes up to 30 days. T e intermediate syndrome is not an effect of AChE inhibition, and its precise underlying mechanisms are unknown. T e hypothesis that muscle weakness may result from nicotinic receptor desensitization due to prolonged cholinergic stimulation remains the most valid.

Organophosphate-induced Delayed Polyneuropathy (OPIDP)—A few OPs may cause OPIDP. Signs and symptoms include tingling of the hands and feet, followed by sensory loss, progressive muscle weakness and f accidity of the distal skeletal muscles of the lower and upper extremities, and ataxia. T ese may occur 2 to 3 weeks after a single exposure, when signs of both the acute cholinergic and the intermediate syndromes have subsided. OPIDP can be classified as a distal sensorimotor axonopathy. Neuropathological studies in experimental OPIDP have evidenced that the primary lesion is a bilateral degenerative change in distal levels of axons and their terminals, primarily affecting larger/longer myelinated central and peripheral nerve fibers, leading to breakdown of neuritic segments and the myelin sheaths. Although several epidemics of OPIDP have occurred in the past such as Ginger–Jake paralysis with tri-ortho-cresyl phosphate in the 1930s, its occurrence in humans is now rare. Athough past studies suggested that aging of neuropathy target esterase (NTE) was involved in OPIDP, the exact mechanisms involved in phosphorylation and aging of NTE and axonal degeneration remain obscure.

Long-term Toxicity—T ere is still controversy on possible long-term effects of OPs. T e fact that acute exposure to high doses of OPs may result, in some cases, in long-lasting adverse health effects (particularly in the CNS) has been shown in animals, as well as humans. More controversial is the possibility that low exposure to OPs, at doses that produce no cholinergic signs, also may lead to long-term adverse health effects, particularly in the central and peripheral nervous systems. Chronic exposure of animals to OPs, at doses that significantly inhibit AChE but may not be associated with clinical signs, results in the development of tolerance to their cholinergic effects (which is mediated, at least in part, by down-regulation of cholinergic receptors), and has been associated with neurobehavioral abnormalities, particularly at the cognitive level.

Developmental Toxicity and Neurotoxicity—Experimental data indicate that young animals are more sensitive to the acute toxicity of OPs. T is increased sensitivity does not appear to be due to intrinsic differences in AChE, but rather due to lower detoxication abilities of young animals. Accumulating recent evidence suggests that perinatal exposure to OPs may cause developmental neurotoxicity. Studies in rodents indicate that OPs can affect various cellular processes (e.g., DNA replication, neuronal survival, neurite outgrowth) and noncholinergic pathways (e.g., serotoninergic synaptic functions, the adenylate cyclase system), and cause various behavioral abnormalities. Such effects are also seen at dose levels that produced no cholinergic signs of toxicity. T ese findings, together with results of biomonitoring studies that indicate exposure of children, particularly in inner cities and farming communities, to OPs have led to regulatory restrictions on the use of certain OPs and to concerns for their potential neurotoxic effects in children. Furthermore, specific guidelines for developmental neurotoxicity have been implemented.

Carbamates

Carbamate insecticides are derived from carbamic acid, and most are N-methylcarbamates. Acute oral toxicity ranges from moderate to low toxicity, such as carbaryl, to extremely high toxicity, such as aldicarb. Dermal skin penetration by carbamates is increased by organic solvents and emulsifiers present in most formulations. Carbamates are susceptible to a variety of enzyme-catalyzed biotransformation reactions, and the principal pathways involve oxidation and hydrolysis. T e mechanism of toxicity of carbamates is by inhibition of AChE, which is rapidly reversible.

T e signs and symptoms of carbamate poisoning include miosis, urination, diarrhea, salivation, muscle fasciculation, and CNS effects (Table 22–4). Acute intoxication by carbamates is generally resolved within a few hours. T e treatment of carbamate intoxication relies on the use of atropine. Carbamates can inhibit neuropathy target esterase (NTE), but because carbamylated NTE cannot age, they are thought to be unable to initiate OPIDP. Additionally, when given before a neuropathic organophosphate, carbamates offer protection against OPIDP, but when given after, they can promote OPIDP.

Methylcarbamates are not mutagenic, and there is no evidence of carcinogenicity. Embryotoxicity or fetotoxicity is observed only at maternally toxic doses. Limited evidence suggests that carbamates (e.g., aldicarb) may be more acutely toxic to young animals than to adults, possibly because of lower detoxication.

Pyrethroids

Pyrethrins were first developed as insecticides from extracts of the f ower heads of Chrysanthemum cinerariaefolium, whose insecticidal potential was appreciated in ancient China and Persia. Because pyrethrins decompose rapidly on exposure to light, the synthetic pyrethroid analogs were developed. Because of their high insecticidal potency, relatively low mammalian toxicity, lack of environmental persistence, and relatively low tendency to induce insect resistance, pyrethroids now account for 15% to 20% of the global insecticide market. T e pyrethroids are used widely as insecticides both in the house and in agriculture, in medicine for the topical treatment of scabies and head lice, and in tropical countries in soaked bed nets to prevent mosquito bites. Pyrethroids alter the normal function of insect nerves by modifying the kinetics of voltage-sensitive sodium channels, which mediate the transient increase in the sodium permeability of the nerve membrane that underlies the nerve action potential.

On absorption, pyrethroids are very rapidly metabolized through two major biotransformation routes: hydrolysis of the ester linkage, which is catalyzed by hepatic and plasma carboxylesterases, and oxidation of the alcohol moiety by cytochrome P450s. T ese initial reactions are followed by further oxidations, hydrolysis, and conjugation with sulfate or glucuronide.

Signs and Symptoms of Toxicity and Mechanism of Action—Based on toxic signs in rats, pyrethroids have been divided into two types (Table 22–5). Type I compounds produce a syndrome consisting of marked behavioral arousal, aggressive sparring, increased startle response, and fine body tremor progressing to whole-body tremor and prostration (type I or T syndrome). Type II compounds produce profuse salivation, coarse tremor progressing to choreoathetosis, and clonic seizures (type II or CS syndrome). T e pyrethroids disrupt voltage-gated sodium channels in mammals and insects. Pyrethroids bind to the α subunit of the sodium channel and slow the activation (opening), as well as the rate of inactivation (closing), of the sodium channel, leading to a stable hyperexcitable state. T e higher sensitivity of insects to pyrethroid toxicity, compared with mammals, is believed to result from a combination of higher sensitivity of insect sodium channels, lower body temperature (as pyrethroids show a negative temperature coefficient of action), and slower biotransformation. Type II pyrethroids bind to and inhibit $GABA_A$-gated chloride channels at higher concentrations than those sufficient to affect sodium channels (10^{-7} M versus 10^{-10} M). T is effect is believed to contribute to the seizures that accompany severe type II pyrethroid poisoning.

On occupational exposure, the primary adverse effect resulting from dermal contact with pyrethroids is paresthesia. Symptoms include continuous tingling or pricking or, when more severe, burning. T e condition reverses in about 24 h, and topical application of vitamin E has been shown to be an effective treatment. Paresthesia is presumably due to pyrethroid-induced abnormal repetitive activity in skin nerve terminals. Chronic studies with pyrethroids indicate that at high dose levels they cause slight liver enlargement often accompanied by some histopathologic changes. T ere is little evidence of teratogenicity and mutagenicity. An increased rate of lymphoma incidence in rodents has been reported for deltamethrin, but the effect was not dose-dependent.

Organochlorine Compounds

T e organochlorine insecticides include the chlorinated ethane derivatives, such as DDT and its analogs; the cyclodienes, such as chlordane, aldrin, dieldrin, heptachlor, endrin, and toxaphene; the hexachlorocyclohexanes, such as lindane; and the caged structures mirex and chlordecone. T eir acute toxicity is moderate (less than that of organophosphates), but chronic exposure may be associated with adverse health effects particularly in the liver and endocrine disruption of the reproductive system.

TABLE 22–5 Classification of pyrethroid insecticides based on toxic signs in rats.

Syndrome	Signs and Symptoms	Examples
Type I (T syndrome)	Aggressive sparring Increased sensitivity to external stimuli Whole-body tremors Prostration	Allethrin Bioallethrin Resmethrin Phenothrin
Type II (CS syndrome)	Pawing and burrowing Profuse salivation Coarse tremor Choreoathetosis Clonic seizures	Deltamethrin Fenvalerate Cypermethrin Cyhalothrin

DDT and Its Analogs—DDT is effective against a wide variety of agricultural pests, as well as against insects that transmit some of the world's most serious diseases, such as typhus, malaria, and yellow fever. DDT has a moderate oral acute toxicity and its dermal absorption is very limited. In humans, oral doses of 10 to 20 mg/kg produce illness, but doses as high as 285 mg/kg have been ingested accidentally without fatal results. Toxicity from dermal exposure in humans is also low, as evidenced by the lack of significant adverse health effects when thousands of people were liberally dusted with this compound. On absorption, DDT distributes in all tissues, and the highest concentrations are found in adipose tissue. It is excreted through the bile, urine, and milk.

Acute exposure to high doses of DDT causes motor unrest, increased frequency of spontaneous movements, abnormal susceptibility to fear, and hypersusceptibility to external stimuli (light, touch, and sound). T is is followed by the development of fine tremors, progressing to coarse tremors, and eventually tonic–clonic convulsions. Death is typically due to respiratory failure. In humans, the earliest symptom of poisoning by DDT is hyperesthesia of the mouth and lower part of the face, followed by paresthesia of the same area and of the tongue. Dizziness, tremor of the extremities, confusion, and vomiting follow; convulsions occur only in severe poisoning. Both in insects and in mammals, DDT interferes with the sodium channels in the axonal membrane by a mechanism similar to that of type I pyrethroids.

An important target for chronic DDT exposure is the liver. DDT and its breakdown product DDE increase liver weight and cause hepatic cell hypertrophy and necrosis, and they are potent inducers of cytochrome P450s, particularly CYP2B and CYP3A. Both DDE and DDD, another breakdown product, are carcinogenic in rodents, causing primarily an increase in hepatic tumors.

Hexachlorocyclohexanes and Cyclodienes—T ese two families of organochlorine insecticides comprise a large number of compounds that share a similar mechanism of neurotoxic action. Lindane is the γ isomer of benzene hexachloride (BHC; 1,2,3,4,5,6-hexachlorocyclohexane). Cyclodiene compounds include chlordane, dieldrin, aldrin (which is rapidly metabolized to dieldrin), heptachlor, and endrin. Toxaphene is a complex mixture of over 200 chlorinated bornanes and camphenes.

Lindane and cyclodienes have moderate to high acute oral toxicity (Figure 22–2). However, in contrast to DDT, these compounds are readily absorbed through the skin. T e primary target for their toxicity is the central nervous system.

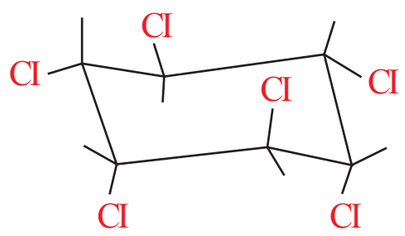
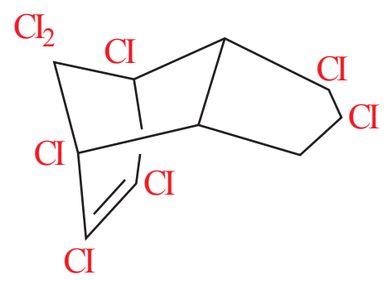
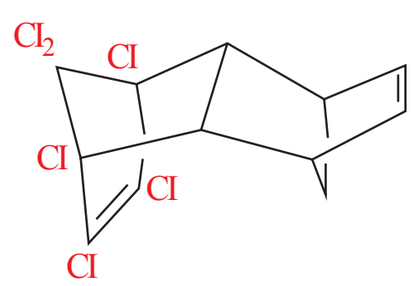
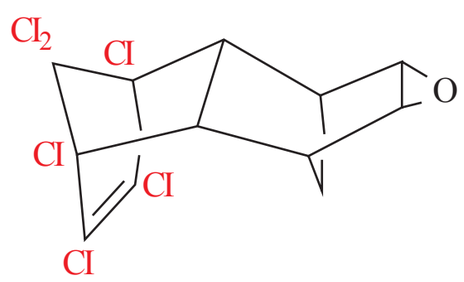
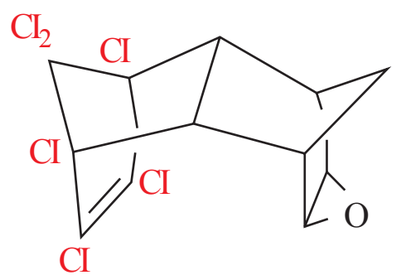
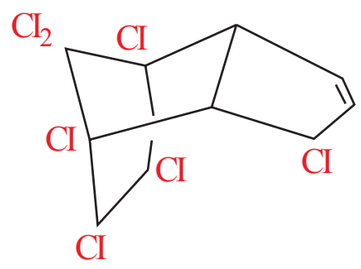
		Approximate LD_{50} (mg/kg)
Lindane (γ-BHC)		200
Chlordane		500
Aldrin		50
Dieldrin		50
Endrin		20
Heptachlor		150

FIGURE 22–2 **Structure and acute toxicity (oral LD_{50} in rat) of selected organochlorine insecticides of different chemical classes.**

Unlike DDT, tremor is essentially absent, but convulsions are a prominent aspect of poisoning. Lindane and cyclodienes bind to the picrotoxin binding site on the chloride channel, thereby blocking its opening and antagonizing the inhibitory action of GABA. Treatment of acute poisoning is symptomatic; phenobarbital and diazepam can be used as anticonvulsants.

Chlordecone—Chlordecone (Kepone) has been the most studied because of one episode that involved 148 workers in a chlordecone-producing factory in Hopewell, Virginia, between 1973 and 1975. The primary manifestation of chlordecone toxicity is the presence of tremors, which are observed in animals as well as in humans. The exact mechanism of chlordecone neurotoxicity has not been elucidated, but it is believed to involve inhibition of ATPases (both Na^+,K^+- and Mg^{2+}-ATPases), and ensuing inhibition of the uptake of catecholamines. In contrast to cyclodienes, chlordecone does not cause seizures. Furthermore, chlordecone induces hepatic drug-metabolizing enzymes, and causes hepatosplenomegaly in rats and humans.

DDT and Public Health: Risk–Benefit Considerations—The Stockholm Convention on Persistent Organic Pollutants, ratified in 2004 by 50 states, outlawed the use of 12 industrial chemicals (the "Dirty Dozen"), including DDT. Yet, an exemption clause allows malaria-endemic nations to continue utilizing DDT for indoor residual wall spraying. The United Nations Environment Program estimates that about 25 countries would use DDT under this exemption from its ban. This situation is keeping the debate on the risks and benefits of DDT usage very much alive.

Other Old and New Insecticides

Rotenoids—The roots of Derris elliptica and those of Lonchocarpus utilis and Lonchocarpus urucu in South America contain at least six rotenoid esters. The most abundant is rotenone, which is used as an agricultural insecticide/acaricide particularly in organic farming. Toxicity of rotenone in target and nontarget species is due to its ability to inhibit, at nanomolar concentrations, mitochondrial respiration by blocking electron transport at NADH–ubiquinone reductase, the energy-conserving enzyme complex commonly known as complex I. Poisoning symptoms include initial increased respiratory and cardiac rates, clonic and tonic spasms, and muscular depression, followed by respiratory depression. Rotenone may play a role in the etiology of Parkinson's disease.

Nicotine—Nicotine is an alkaloid extracted from the leaves of tobacco plants (Nicotiana tabacum and Nicotiana rustica), and is used as a free base or as the sulfate salt. Nicotine has a high acute toxicity, and the signs and symptoms of poisoning include nausea, vomiting, muscle weakness, respiratory effects, headache, lethargy, and tachycardia. Most cases of poisoning with nicotine occur after exposure to tobacco products, or gum or patches. Workers who cultivate, harvest, or handle tobacco may experience green tobacco sickness, caused by dermal absorption of nicotine.

Avermectins—The avermectins are macrocyclic lactones that are isolated from the fermentation broth of Streptomyces avermitilis. This fungus synthesizes eight individual avermectins that have antiparasitic activity. The semisynthetic derivatives of avermectin B_{1a}, emamectin benzoate, and ivermectin are used as insecticides, and for parasite control in human and veterinary medicine, respectively. Abamectin is used primarily to control mites, whereas emamectin benzoate is effective at controlling lepidopterian species in various crops and emerald ash borer in trees. Ivermectin is used as an antihelmintic and antiparasitic drug in veterinary medicine, and in humans it has proven to be an effective treatment for infection of intestinal threadworms, onchocerciasis (river blindness), and lymphatic

filariasis. In insects and nematodes, avermectins exert their toxic effects by binding to, and activating, glutamate-dependent chloride channel. Signs and symptoms of intoxication include hyperexcitability, tremors, and incoordination, followed by ataxia and coma-like sedation.

INSECT REPELLENTS

Insect-transmitted diseases remain a major source of illness and death worldwide, as mosquitoes alone transmit disease to more than 700 million persons annually. DEET (N,N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide) is very effective at repelling insects, flies, fleas, and ticks, and protection time increases with increasing concentrations. Subchronic toxicity studies in various species did not reveal major toxic effects and no significant effects of DEET were seen in mutagenicity, reproductive toxicity, and carcinogenicity studies. Acute and chronic neurotoxicity studies also provided negative results. However, in children DEET is possibly responsible for neurotoxic effects and children should only be exposed to products with up to 10% DEET.

Picaridin

Picaridin was developed as an alternative to DEET. Insect repellent formulations (cream, aerosol, wipe) containing 5% to 20% picaridin are highly effective against a variety of arthropod pests, especially mosquitoes, ticks, and flies. Its action in insects is believed to be due to the interaction with specific olfactory receptors of the arthropod. In humans it is absorbed through the skin to a limited degree, and is metabolized via hydroxylation and glucuronidation, before excretion in the urine. The toxicological profile of picaridin is unremarkable. Acute dermal toxicity is low. There is no evidence of genotoxicity, carcinogenicity, teratogenicity, reproductive toxicity, or neurotoxicity. When used as directed, picaridin-containing formulations are deemed to be safe and effective.

HERBICIDES

Herbicides are chemicals that are capable of either killing or severely injuring plants. Some of the various mechanisms by which herbicides exert their biological effects are shown in Table 22–6, together with examples for each class. Another method of classification pertains to how and when herbicides are applied. Thus, preplanting herbicides are applied to the soil before a crop is seeded, preemergent herbicides are applied to the soil before the time of appearance of unwanted vegetation, and postemergent herbicides are applied to the soil or foliage after the germination of the crop and/or weeds. Herbicides are also divided according to the manner they are applied to plants. Contact herbicides are those that affect the plant that was treated, whereas translocated herbicides are applied to the soil or to above-ground parts of the plant, and are absorbed and circulated to distant tissues. Nonselective herbicides will kill all vegetation, whereas selective compounds are those used to kill weeds without harming the crops.

TABLE 22–6 Some mechanisms of action of herbicides.

Mechanism	Chemical Classes (Example)
Inhibition of photosynthesis	Triazines (atrazine), substituted ureas (diuron), uracils (bromacil)
Inhibition of respiration	Dinitrophenols
Auxin growth regulators	Phenoxy acids (2,4-D), benzoic acids (dicamba), pyridine acids (picloram)
Inhibition of protein synthesis	Dinitroanilines
Inhibition of lipid synthesis	Aryloxyphenoxypropionates (diclofop)
Inhibition of specific enzymes • Glutamine synthetase • Enolpyruvylshikimate-3-phosphate synthetase • Acetolactate synthase	 Glufosinate Glyphosate Sulfonylureas
Cell membrane disruptors	Bipyridyl derivatives (paraquat)

A number of herbicides can cause dermal irritation and contact dermatitis, particularly in individuals prone to allergic reactions. Other compounds have generated much debate for their suspected carcinogenicity or neurotoxicity. The principal classes of herbicides associated with reported adverse health effects in humans are discussed below.

Chlorophenoxy Compounds

Chlorophenoxy herbicides are chemical analogs of auxin, a plant growth hormone, that produce uncontrolled and lethal growth in target plants. Because the auxin hormone is critical to the growth of many broad-leaved plants, but is not used by grasses, chlorophenoxy compounds can suppress the growth of weeds (e.g., dandelions) without affecting the grass. The most commonly used compound of this class is 2,4-dichlorophenoxyacetic acid (2,4-D).

Ingestion of 2,4-D has caused acute poisoning in humans, resulting in vomiting, burning of the mouth, abdominal pain, hypotension, myotonia, and CNS involvement including coma. Dermal exposure is the major route of unintentional exposure to 2,4-D in humans.

There are several case reports suggesting an association between exposure to 2,4-D and neurologic effects like peripheral neuropathy, demyelination and ganglion degeneration in the CNS, reduced nerve conduction velocity, myotonia, and behavioral alterations. 2,4-D does not appear to have genotoxic or carcinogenic properties in rats, mice, and dogs. The chlorophenoxy herbicides have attracted much attention because of an association between exposure and non-Hodgkin's lymphoma

or soft-tissue sarcoma, found in a few epidemiological studies. Nevertheless, 2,4-D is classified as a group D agent (not classifiable as to human carcinogenicity).

Bipyridil Compounds

Paraquat is a fast-acting, nonselective contact herbicide, used to control broad-leaved weeds and grasses in plantations and fruit orchards, and for general weed control. Paraquat has one of the highest acute toxicities among herbicides. On absorption, independent of the route of exposure, paraquat accumulates in the lung and the kidney. Paraquat is very poorly metabolized, and is excreted almost unchanged in the urine. It has minimal to no genotoxic activity, is not carcinogenic in rodents, has no effect on fertility, is not teratogenic, and only produces fetotoxicity at maternally toxic doses. T e major toxicologic concerns for paraquat are related to its acute systemic effects, particularly in the lung, and secondarily, the kidney.

Once paraquat enters a cell, it undergoes alternate reduction followed by reoxidation, a process known as redox cycling. Intracellular redox cycling of paraquat would also result in the oxidation of NADPH, leading to its cellular depletion, which is augmented by the detoxification of hydrogen peroxide formed in the glutathione peroxidase/reductase enzyme system to regenerate GSH (Figure 22–3).

Damage to alveolar epithelial cells occurs within 24 h after acute exposure to lethal doses of paraquat. Damage progresses in the following 2 to 4 days with loss of the alveolar epithelium, alveolar edema, extensive infiltration of inf ammatory cells into the alveolar interstitium, and finally death due to severe anoxia. Survivors of this destructive first phase show extensive proliferation of fibroblasts in the lung. T e second phase is characterized by attempts by the alveolar epithelium to regenerate and restore normal architecture, and presents as an intensive fibrosis. Individuals who survive the first phase may still die from the progressive loss of lung function several weeks after exposure.

T e herbicide diquat presents a different toxicologic profile. Acute toxicity is somewhat lower. In contrast to paraquat, diquat does not accumulate in the lung, and no lung toxicity is seen on acute or chronic exposure. On chronic exposure, target organs for toxicity are the gastrointestinal tract, the kidney, and particularly the eye. Like paraquat, diquat can be reduced to form a free radical and then reoxidized in the presence of oxygen, with the concomitant production of superoxide anion. T is process of redox cycling occurs in the eye and is believed to be the likely mechanism of cataract formation. Human clinical symptoms include nausea, vomiting, diarrhea, ulceration of mouth and esophagus, decline in renal functions, and neurologic effects, but no pulmonary fibrosis.

Chloroacetanilides

Representative compounds of this class of herbicides are alachlor, acetochlor, and metolachlor, which are used to control herbal grasses and broad-leaved weeds in a number of crops

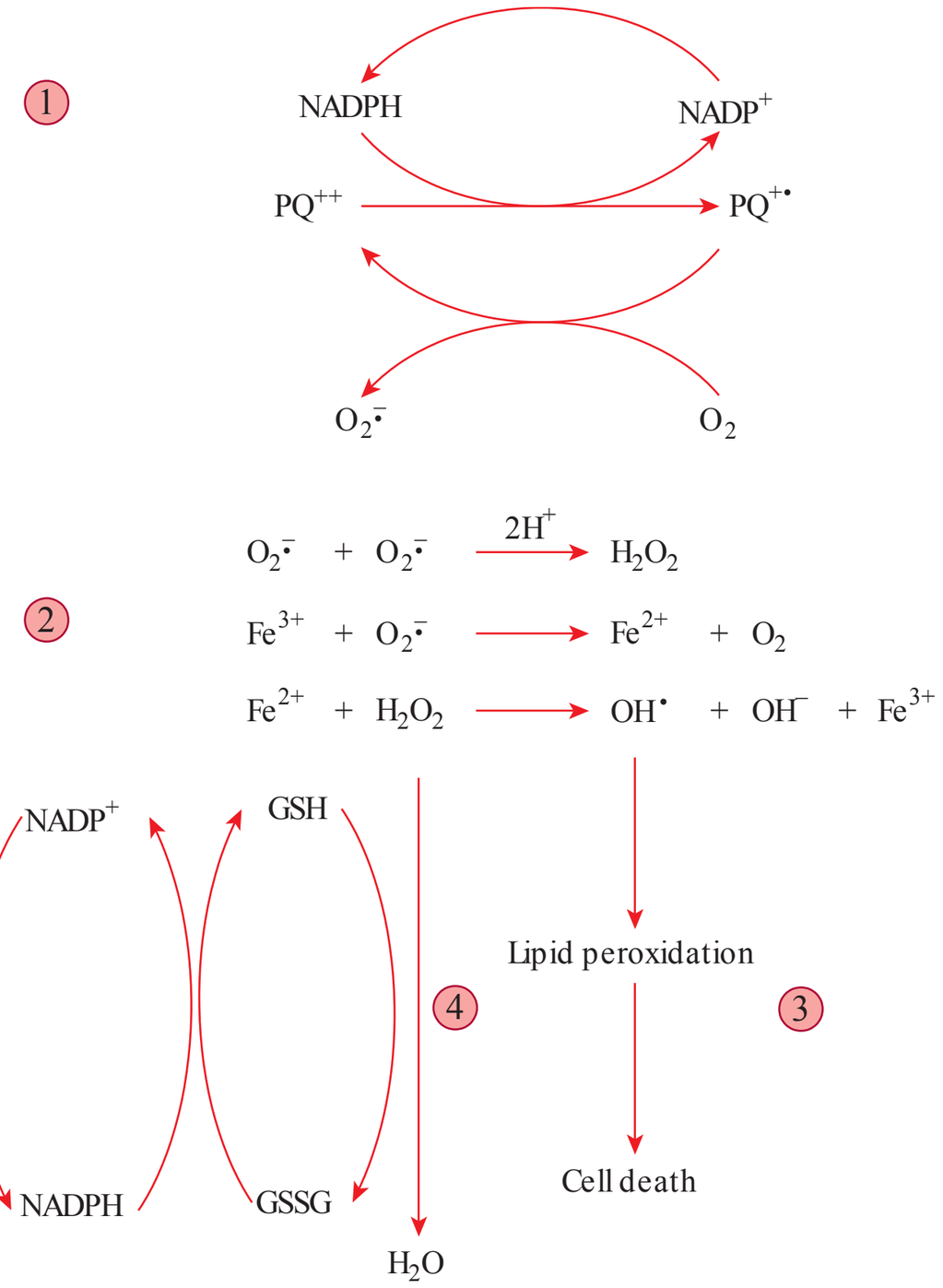


FIGURE 22–3 **Mechanism of toxicity of paraquat.** (1) Redox cycling of paraquat utilizing NADPH; (2) formation of hydroxy radicals leading to lipid peroxidation (3); (4) detoxication of H_2O_2 via glutathione reductase/peroxidase couple, utilizing NADPH. (Modif ed from Smith LL: Mechanism of paraquat toxicity in the lung and its relevance to treatment, Hum Toxicol, 1987 Jan;6(1):31–36).

(corn, soybeans, and peanuts). Alachlor, acetochlor, and butachlor are probable human carcinogens (Group B2). T e discovery of alachlor in well water led to cancellation of its registration in some countries, and to its restriction in others. Both are believed to be threshold-sensitive phenomena.

Triazines

T e family of triazine herbicides comprises several compounds (atrazine, simazine, and propazine) that are extensively used for the preemergent control of broad-leaved weeds. Triazines have low acute oral and dermal toxicity, and chronic toxicity studies indicate primarily decreased body weight gain. T ere is no evidence that triazines are teratogenic, genotoxic, or developmental or reproductive toxicants. However, a more recent study has suggested a possible clastogenic effect. T ough exposure to atrazine through residues in food commodities is very low, contamination of ground water and drinking water is common. Nevertheless, the known hormonal effects of triazines call for careful evaluation of the endocrine-disrupting effects of these herbicides.

Phosphonomethyl Amino Acids

T e two compounds of this class are glyphosate (N-phosphonomethyl glycine) and glufosinate (N-phosphonomethyl homoalanine). Both are broad-spectrum nonselective systemic herbicides used for postemergent control of annual and perennial plants. T ough both compounds contain a P= O moiety, they are organophosphonates and do not inhibit AChE.

Glyphosate—Glyphosate exerts its herbicidal action by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, responsible for the synthesis of an intermediate in the biosynthesis of various amino acids. Although important in plant growth, this metabolic pathway is not present in mammals. It has no teratogenic, developmental, or reproductive effects. Genotoxicity and carcinogenicity studies in animals were negative.

Glyphosate is one of the most widely used herbicides, and the development of transgenic crops that can tolerate glyphosate treatment has expanded its utilization. Given its widespread use, including the home and garden market, accidental or intentional exposure to glyphosate is inevitable. T e most widely used glyphosate product is Roundup®which is formulated as a concentrate containing water, 41% glyphosate (as isopropylamine salt), and 15% polyoxethyleneamine (POEA). Mild intoxication results mainly in transient gastrointestinal symptoms. Moderate or severe poisoning presents with gastrointestinal bleeding, hypotension, pulmonary dysfunction, and renal damage.

Glufosinate—Glufosinate is a nonselective contact herbicide that acts by irreversibly inhibiting glutamine synthetase. Plants die as a consequence of the increased levels of ammonia. Mammals have other metabolizing systems that can cope with the effects on glutamine synthetase activity to a certain limit. T ere is no evidence of genotoxicity or carcinogenicity, or direct effects on reproductive performance and fertility. Developmental toxic effects were found in rabbits (premature deliveries, abortions, and dead fetuses). Humans experience gastrointestinal effects, impaired respiration, neurologic disturbance, and cardiovascular effects.

FUNGICIDES

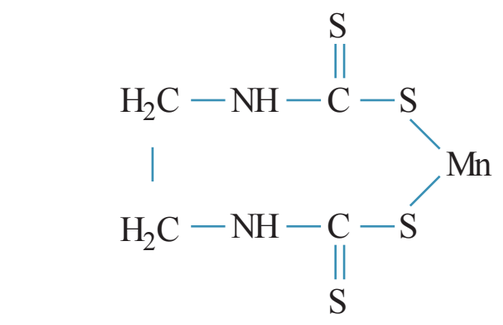
Fungal diseases are virtually impossible to control without chemical application. Fungicidal chemicals are derived from a variety of structures, ranging from simple inorganic compounds, such as copper sulfate, to complex organic compounds. Most fungicides are surface or plant protectants, and are applied prior to potential infection by fungal spores, either to plants or to postharvest crops. Other fungicides can be used therapeutically, to cure plants when an infestation has already begun. Still others are used as systemic fungicides that are absorbed and distributed throughout the plant. With a few exceptions, fungicides have low acute toxicity in mammals. Some fungicides have been associated with severe epidemics of poisoning.

Captan and Folpet

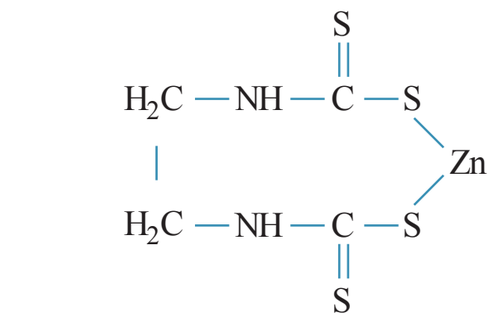
Captan and folpet are broad-spectrum protectant fungicides; together with captafol, they are called chloroalkylthio fungicides, due to the presence of side chains containing chlorine, carbon, and sulfur. T ey are potent eye irritants, but only mild skin irritants. Dermal absorption is low. Captan and folpet, as well as thiophosgene, are mutagenic in vitro tests; however, in vivo mutagenicity tests are mostly negative, possibly because of the rapid degradation of these compounds. T ese fungicides induce the development of duodenal tumors in mice, and on this basis, they are classified by the US EPA as probable human carcinogens. Because of their structural similarity to the potent teratogen thalidomide, chloroalkylthio fungicides have been extensively tested in reproductive/developmental studies in multiple species, but no evidence of teratogenicity has been found.

Dithiocarbamates

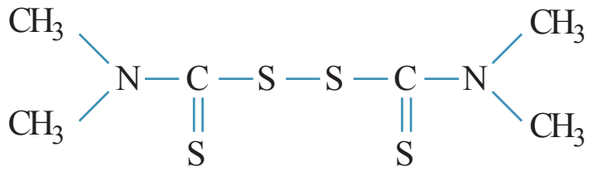
T e nomenclature of many of these compounds arises from the metal cations with which they are associated; thus, there are, e.g., Maneb (Mn), Ziram and Zineb (Zn), and Mancozeb (Mn and Zn) (Figure 22–4). T iram is an example of dithiocarbamate without a metal moiety (Figure 22–4). T e dithiocarbamates have low acute toxicity by the oral, dermal, and respiratory routes. However, chronic exposure is associated with adverse effects that may be due to the dithiocarbamate



Maneb
Manganese ethylenebisdithiocarbamate



Zineb
Zinc ethylenebisdithiocarbamate



Thiram
Bis(diethylthio-carbamoyl)disulfide

FIGURE 22–4 **Structures of three dithiocarbamate fungicides.**

acid or the metal moiety. T ese compounds are metabolized to a common metabolite, ethylenethiourea, that is responsible for the effects of dithiocarbamates on the thyroid, which include hypertrophy and hyperplasia of thyroid follicular cells that progress to adenomas and carcinomas. Similarly, dithiocarbamates alter thyroid hormone levels, and cause thyroid hypertrophy. Also, the structure of dithiocarbamate fungicides resembles that of disulfiram, which inhibits aldehyde dehydrogenase and may, after ingestion of ethanol, lead to elevated acetaldehyde levels.

Inorganic and Organometal Fungicides

Copper sulfate has overall low toxicity and remains one of the most widely used fungicides. Triphenyltin acetate is used as a fungicide, whereas tributyltin is utilized as an antifouling agent. Triphenyltin has moderate to high acute toxicity, but may cause reproductive toxicity and endocrine disruption. Organic mercury compounds, such as methylmercury, were used extensively as fungicides in the past for the prevention of seed-borne diseases in grains and cereals.

RODENTICIDES

Rats and mice can cause health and economic damages to humans. Rodents are vectors for several human diseases, including plague, endemic rickettsiosis, spirochetosis, and several others; they can occasionally bite people; they can consume large quantities of postharvest stored foods, and can contaminate foods with urine, feces, and hair. Rodenticides play an important role in rodent control. To be effective, yet safe, rodenticides must satisfy several criteria: (1) the poison must be very effective in the target species once incorporated into bait in small quantity; (2) baits containing the poison must not excite bait shyness, so that the animal will continue to eat it; (3) the manner of death must be such that survivors do not become suspicious of its cause; and (4) it should be species-specific, with considerably lower toxicity to other animals. Toxicologic problems can arise from acute accidental ingestions or from suicidal and homicidal attempts. Every year, thousands of accidental ingestions of rodenticide baits by children occur, most of which resolve without serious consequences.

Fluoroacetic Acid and Its Derivatives

Sodium fuoroacetate (Compound 1080) and fuoroacetamide are white in color and odorless. T eir high mammalian toxicity limits use to trained personnel. T e main targets of toxicity are the central nervous system and the heart. Initial gastrointestinal symptoms are followed by severe cardiovascular effects (ventricular tachycardia, fibrillation, and hypotension), as well as CNS effects (agitation, convulsions, and coma). Use of Compound 1080 in the United States is severely restricted primarily because of toxicity to nontarget animals, such as dogs.

Anticoagulants

In addition to their use as rodenticides, coumarin derivatives, including warfarin itself, are used as anticoagulant drugs and have become a mainstay for prevention of thromboembolic disease. Coumarins antagonize the action of vitamin K in the synthesis of clotting factors (factors II, VII, IX, and X). T eir specific mechanism involves inhibition of vitamin K epoxide reductase, which regenerates the reduced vitamin K necessary for sustained carboxylation and synthesis of relevant clotting factors. Human poisonings by these rodenticides are rare because they are dispersed in grain-based baits. However, there are a significant number of suicide or homicide attempts or of accidental consumption of warfarin.

FUMIGANTS

T ese agents are active toward insects, mites, nematodes, weed seeds, fungi, or rodents, and have in common the property of being in the gaseous form at the time they exert their pesticidal action. T ey can be liquids that readily vaporize (e.g., ethylene dibromide), solids that can release a toxic gas on reaction with water (e.g., phosphine released by aluminum phosphide), or gases (e.g., methyl bromide). For soil fumigation, the compound is injected directly into the soil, which is then covered with plastic sheeting, which is sealed. Compounds used as fumigants are usually nonselective, highly reactive, and cytotoxic. T ey provide a potential hazard from the standpoint of inhalation exposure, and to a minor degree for dermal exposure or ingestion, in case of solids or liquids.

Methyl Bromide

Methyl bromide is a broad-spectrum pesticide, used for soil fumigation, commodity treatment, and structural fumigation. Acute exposure results in respiratory, gastrointestinal, and neurologic symptoms; the latter include lethargy, headache, seizures, paresthesias, peripheral neuropathy, and ataxia, and are considered to be more relevant than other toxic effects for human risk assessment. Acute and chronic neurotoxicity studies in rats have demonstrated behavioral effects and morphological lesions, which were concentration- and time-dependent. Methyl bromide is positive in some genotoxicity tests, but it is listed in Group 3 as not classifiable as a human carcinogen. Methyl bromide is an odorless and colorless gas, but chloropicrin, with a pungent odor and eye irritation, is often used in conjunction with methyl bromide and other fumigant mixtures, to warn against potentially harmful exposures.

1,3-Dichloropropene

1,3-Dichloropropene is a soil fumigant, extensively utilized for its ability to control soil nematodes. It is an irritant, and can cause redness and necrosis of the skin. It is extensively metabolized, with the mercapturic acid conjugate being the

major urinary metabolite. Data on genotoxicity are contradictory, and carcinogenicity studies in rodents have found an increase in benign liver tumors in rats but not in mice, after oral administration.

Sulfur

Elemental sulfur is an effective fumigant for the control of many plant diseases, particularly fungal diseases, and represents the most heavily used crop protection chemical in the United States. Sulfur finds its major uses in grapes and tomatoes, and can be used in organic farming. The primary health effect in humans associated with the agricultural use of elemental sulfur is dermatitis. In ruminants, excessive sulfur ingestion can cause cerebrocortical necrosis (polioencephalomalacia), possibly due to its conversion by microorganisms in the rumen to hydrogen sulfide.

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QUESTIONS

1. Which of the following does NOT contribute to the environmental presence of organochlorine insecticides?
   a. high water solubility.
   b. low volatility.
   c. chemical stability.
   d. low cost.
   e. slow rate of degradation.

2. All of the following are characteristic of DDT poisoning EXCEPT:
   a. paresthesia.
   b. hypertrophy of hepatocytes.
   c. increased potassium transport across the membrane.
   d. slow closing of sodium ion channels.
   e. dizziness.

3. Anticholinesterase agents:
   a. enhance the activity of AChE.
   b. increase ACh concentration in the synaptic cleft.
   c. only target the neuromuscular junction.
   d. antagonize ACh receptors.
   e. cause decreased autonomic nervous system stimulation.

4. All of the following symptoms would be expected following anticholinesterase insecticide poisoning EXCEPT:
   a. bronchodilation.
   b. tachycardia.
   c. diarrhea.
   d. increased blood pressure.
   e. dyspnea.

5. Which of the following insecticides blocks the electron transport chain at NADH–ubiquinone reductase?
   a. nicotine.
   b. carbamate esters.
   c. nitromethylenes.
   d. pyrethroid esters.
   e. rotenoids.

6. What is the main mechanism of pyrethroid ester toxicity?
   a. blockage of neurotransmitter release.
   b. inhibition of neurotransmitter reuptake.
   c. acting as a receptor agonist.
   d. causing hyperexcitability of the membrane by interfering with sodium transport.
   e. interfering with Cl^{-} transport across the axonal membrane.

7. Which of the following herbicides is NOT correctly paired with its mechanism of action?
   a. glufosinate—inhibition of glutamine synthetase.
   b. paraquat—interference with protein synthesis.
   c. glyphosate—inhibition of amino acid synthesis.
   d. chlorophenoxy compounds—growth stimulants.
   e. diquat—production of superoxide anion through redox cycling.

8. Captan:
   a. is a herbicide that inhibits root growth.
   b. is an insecticide that targets the reproductive organs.
   c. is a fungicide that could cause duodenal tumors.
   d. is a herbicide that stimulates growth.
   e. is a fungicide that is a known teratogen.

9. What is a mechanism of action of nicotine?
   a. Nicotine antagonizes ACh at the neuromuscular junction.
   b. Nicotine decreases the rate of repolarization of the axonal membrane.
   c. Nicotine interferes with sodium permeability.
   d. Nicotine acts as an ACh agonist in the synapse.
   e. Nicotine inhibits the release of neurotransmitter.

10. Which of the following is the most characteristic of warfarin poisoning?
    a. diarrhea.
    b. cyanosis.
    c. decreased glucose metabolism.
    d. hematomas.
    e. seizures.

CHAPTER

23

Toxic Effects of Metals

Erik J. Tokar, Windy A. Boyd, Jonathan H. Freedman, and Michael P. Waalkes

INTRODUCTION
- What Is a Metal?
- Metals as Toxicants
- Movement of Metals in the Environment
- Chemical Mechanisms of Metal Toxicology
- Factors Impacting Metal Toxicity
- Biomarkers of Metal Exposure
- Molecular Responses to Metal Exposure
- Metal-binding Proteins and Metal Transporters
- Pharmacology of Metals

MAJOR TOXIC METALS
- Arsenic
  - Toxicokinetics
  - Toxicity
  - Carcinogenicity
  - Treatment
- Cadmium
  - Exposure
  - Toxicity
- Lead
  - Exposure
  - Toxicity
- Mercury
  - Global Cycling and Ecotoxicology
  - Exposure
  - Toxicity
- Nickel
  - Toxicity
  - Carcinogenicity

ESSENTIAL METALS WITH POTENTIAL FOR TOXICITY
- Copper
  - Toxicity
  - Hereditary Disease of Copper Metabolism
- Iron
  - Toxicity
- Zinc
  - Essentiality and Deficiency
  - Toxicity

METALS RELATED TO MEDICAL THERAPY
- Aluminum
  - Toxicity
- Lithium
  - Toxicokinetics
  - Toxicity
- Platinum
  - Toxicity

KEY POINTS

- Persons at either end of the life span, young children or elderly people, are more susceptible to toxicity from exposure to a particular level of metal than most adults.
- Metals that provoke immune reactions include mercury, gold, platinum, beryllium, chromium, and nickel.
- Complexation is the formation of a metal ion complex in which the metal ion is associated with a charged or uncharged electron donor, referred to as a ligand.
- Chelation occurs when bidentate ligands form ring structures that include the metal ion and the two ligand atoms attached to the metal.
- Metal–protein interactions include binding to numerous enzymes, the metallothioneins, nonspecific binding to proteins such as serum albumin or hemoglobin, and specific metal carrier proteins involved in the membrane transport of metals.

INTRODUCTION

What Is a Metal?

Metals are typically defined by physical properties of the element in the solid state. General metal properties include high reflectivity (luster), high electrical conductivity, high thermal conductivity, and mechanical ductility and strength. A toxicologically important characteristic of metals is that they may react in biological systems by losing one or more electrons to form cations.

Many metals have been reported to produce significant toxicity in humans. T ese include major toxic metals (e.g., lead and cadmium), essential metals (e.g., zinc and copper), medicinal metals (e.g., platinum and bismuth), minor toxic metals including metals in emerging technology (e.g., indium and uranium), toxic metalloids (e.g., arsenic and antimony), and certain nonmetallic elemental toxicants (e.g., selenium and fluoride). An overview of metal toxicology is shown in Figure 23–1.

Metals as Toxicants

Metals are unique among pollutant toxicants in that they are all naturally occurring and are already ubiquitous to some level within the human environment. Regardless of how safely metals are used in industrial processes or consumer products, some level of human exposure is inevitable. Metals differ from other toxic substances because, as elements, they are neither created nor destroyed by human endeavors. Human use of metals has influenced environmental levels of metals in air, water, soil, and food. Human use of metals can also alter the chemical form or speciation of an element and thereby impact toxic potential. As elemental species, metals are nonbiodegradable. T is indestructibility combined with bioaccumulation contributes to the high concern for metals as toxicants.

Movement of Metals in the Environment

Metals are redistributed naturally in the environment by both geological and biological cycles. Rainwater dissolves rocks and ores and transports materials, including metals, to rivers and underground water (e.g., arsenic), depositing and stripping materials from adjacent soil and eventually transporting these substances to the ocean to be precipitated as sediment or taken up into forming rainwater to be relocated elsewhere. Biological cycles moving metals include biomagnification by plants and animals resulting in incorporation into food cycles. Human industry greatly enhances metal distribution in the global environment by discharge to soil, water, and air. Reports of metal intoxication are common in plants, aquatic organisms, invertebrates, fish, sea mammals, birds, and domestic animals. Not all human toxicity occurs from metals deposited in the biosphere by human activity. For example, chronic arsenic poisoning from high levels of naturally occurring inorganic arsenic in drinking water is a major health issue in many parts of the world. Endemic intoxication from excess fluoride, selenium, or thallium can all occur from natural high environmental levels.

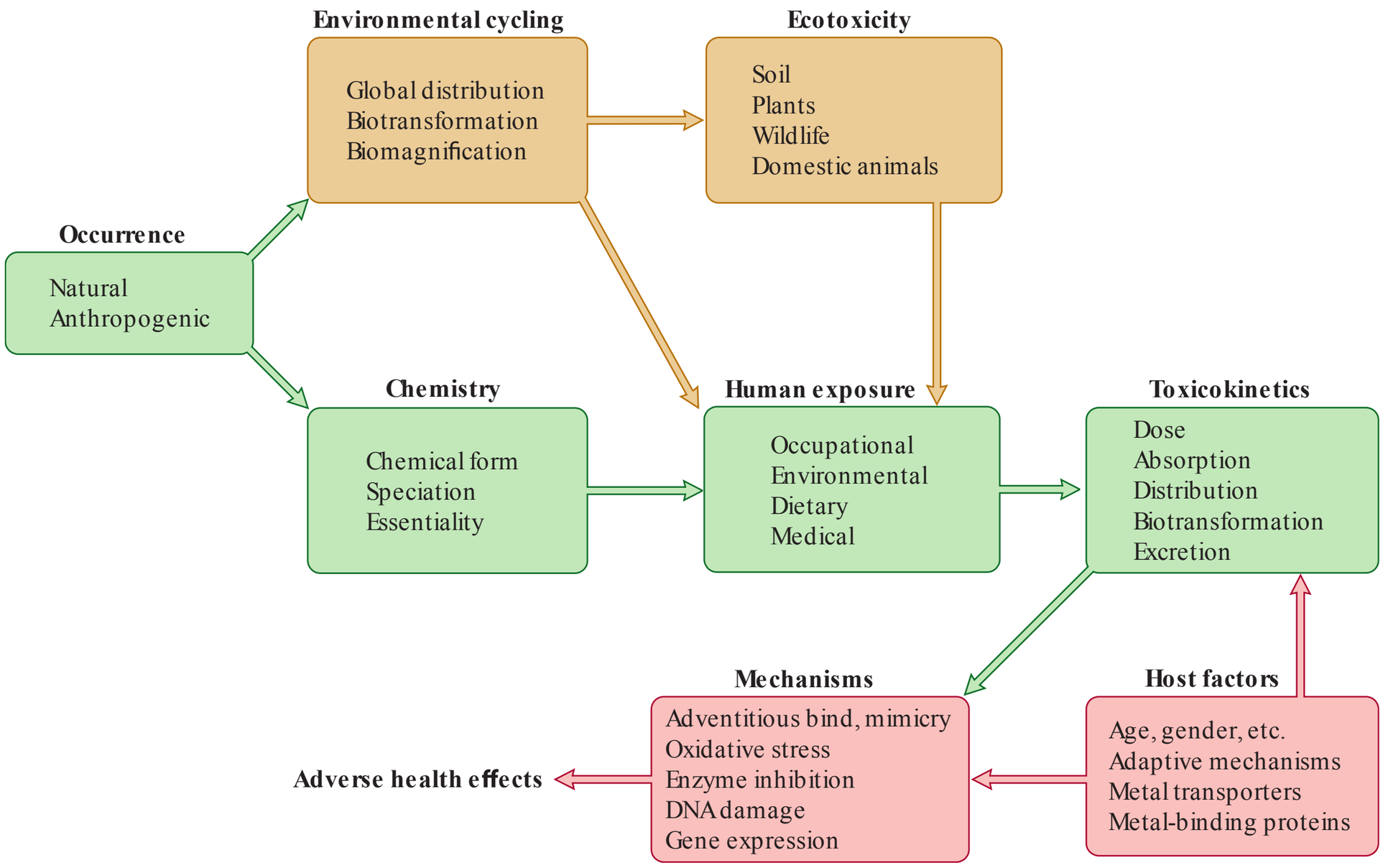

FIGURE 23–1 **Overview of metal toxicology.**

Chemical Mechanisms of Metal Toxicology

Chemically, metals in their ionic form can be very reactive and can interact with biological systems in a large variety of ways. In this regard, a cell presents numerous potential metal-binding ligands. The inhibition of biologically critical enzymes is an important molecular mechanism of metal toxicology.

The metals can show more specific forms of chemical attack through mimicry: acting as mimics of essential metals, they bind to physiological sites that normally are reserved for an essential element. For example, mimicry for, and replacement of, zinc is a mechanism of toxicity for cadmium, copper, and nickel.

Another key chemical reaction in metal toxicology is metal-mediated oxidative damage. Many metals can directly act as catalytic centers for redox reactions with molecular oxygen or other endogenous oxidants, producing oxidative modification of biomolecules such as proteins or DNA. This may be a key step in the carcinogenicity of certain metals. Besides oxygen-based radicals, carbon- and sulfur-based radicals may also occur.

Metals in their ionic form can be very reactive and form DNA and protein adducts in biological systems. They can also induce an array of aberrant gene expression, which, in turn, produces adverse effects.

Factors Impacting Metal Toxicity

Exposure-related factors include dose, route of exposure, duration, and frequency of exposure. Host-based factors that can impact metal toxicity include age at exposure, gender, and capacity for biotransformation. For instance, it is quite clear that younger subjects are often more sensitive to metal intoxication, as, e.g., with the neurotoxicity of lead in children. The major pathway of exposure to many toxic metals in children is food, and children consume more calories per pound of body weight than adults. Moreover, children have higher gastrointestinal absorption of metals, particularly lead. Elderly persons are also believed to be generally more susceptible to metal toxicity than younger adults. Lifestyle factors such as smoking or the composition of diet (i.e., alcohol ingestion) may have direct or indirect impacts on the level of metal intoxication.

Adaptive mechanisms can be critical to the toxic effects of metals, and organisms have a variety of ways in which they can adapt to otherwise toxic metal insults. Adaptation can be at the level of uptake, excretion, or long-term storage in a toxicologically inert form. Metal exposure can induce a cascade of molecular/genetic responses that may, in turn, reduce toxicity, such as with metal-induced oxidative stress responses.

Biomarkers of Metal Exposure

Biomarkers of exposure, toxicity, and susceptibility are important in assessing the level of concern with metal intoxication. Exposure biomarkers, such as concentrations in blood, urine, or hair, have long been used with metals. Techniques in molecular toxicology have greatly expanded the possibilities for biomarkers. Thus, in the case of chromium, DNA–protein complexes may serve as a biomarker of both exposure and carcinogenic potential. Also, hair can be useful in assessing variations in exposure to metals over the period of its growth.

Molecular Responses to Metal Exposure

Exposure to elevated levels of nonessential and essential metals can induce intracellular damage. This is damage includes oxidative stress, which can lead to lipid peroxidation, protein denaturation, DNA damage, and organelle dysfunction. In addition, metals can disrupt the biological function/activity of proteins by either directly binding to the protein or displacing metals within metalloproteins. The intended consequence of metal activation of gene expression is to protect the organism from metal-induced damage. Metal exposure is associated with increased expression of genes that encode proteins that (1) remove the metal from the cell via chelation or increased export, (2) reduce the level of oxidative stress, and (3) repair the metal-induced intracellular damage. However, the inappropriate activation of gene expression following metal exposure can be a contributing factor to a variety of human pathologies.

Metal-binding Proteins and Metal Transporters

Protein binding of metals is a critical aspect of essential and toxic metal metabolism. Many different types of proteins play roles in the disposition of metals in the body. Nonspecific binding to proteins, like serum albumin or hemoglobin, acts in metal transport and tissue distribution. Proteins with specific metal-binding properties play special roles in the trafficking of specific essential metals, and toxic metals may interact with these proteins through mimicry.

The metallothioneins are the best known example of metal-binding proteins. These thiol ligands provide the basis for high-affinity binding of several essential and toxic metals including zinc, cadmium, copper, and mercury.

Transferrin is a glycoprotein that binds most of the ferric iron in plasma and helps transport iron across cell membranes. The protein also transports aluminum and manganese. Ferritin is primarily a storage protein for iron. It has been suggested that ferritin may serve as a general metal detoxicant protein, because it binds a variety of toxic metals including cadmium, zinc, beryllium, and aluminum.

Ceruloplasmin is a copper-containing glycoprotein oxidase in plasma that converts ferrous iron to ferric iron, which then binds to transferrin. This protein also stimulates iron uptake by a transferrin-independent mechanism.

Pharmacology of Metals

Many metallic chemicals are valuable pharmacological tools in the treatment of human disease, as exemplified by the highly effective use of platinum compounds in cancer chemotherapy.

In addition, gallium and titanium complexes are promising metal compounds in cancer chemotherapy. Other medicinal metals used today include aluminum (antacids and buffered analgesics), bismuth (peptic ulcer and Helicobacter pylori–associated gastritis), lithium (mania and bipolar disorders), and gold (arthritis).

Treatment of metal poisoning is sometimes used to prevent, or even attempt to reverse, toxicity. T e typical strategy is to give metal chelators that will complex the metal and enhance its excretion. Such therapy can be used for many different metals including lead, mercury, iron, and arsenic.

MAJOR TOXIC METALS

Arsenic

T e most common inorganic trivalent arsenic compounds are arsenic trioxide and sodium arsenite, whereas common pentavalent inorganic compounds are sodium arsenate, arsenic pentoxide, and arsenic acid. Important organoarsenicals include arsenilic acid, arsenosugars, and several methylated forms produced as a consequence of inorganic arsenic biotransformation in various organisms, including humans. Arsine (AsH_3) is an important gaseous arsenical.

Occupational exposure to arsenic occurs in the manufacture of pesticides, herbicides, and other agricultural products. High exposure to arsenic fumes and dusts may occur in smelting industries. Environmental arsenic exposure mainly occurs from arsenic-contaminated drinking water, which is often from natural sources, and from the burning of coal containing naturally high levels of arsenic. Food, especially seafood, may contribute significantly to daily arsenic intake.

Toxicokinetics—Inorganic arsenic is well absorbed (80% to 90%) from the gastrointestinal tract, distributed throughout the body, often metabolized by methylation, and then excreted primarily in urine. Arsenic compounds of low solubility are absorbed less efficiently after oral exposure. Arsenic has a predilection for skin and is excreted by desquamation of skin and in sweat, particularly during periods of profuse sweating. It also concentrates in forming fingernails and hair. Arsenic exposure produces characteristic transverse white bands across fingernails (Mees' line), which appear about six weeks after the onset of symptoms of arsenic toxicity. Arsenic in the fingernails and hair has been used as a biomarker for exposure.

Toxicity

Acute Poisoning—Ingestion of large doses (70 to 180 mg) of inorganic arsenic can be fatal. Symptoms of acute intoxication include fever, anorexia, hepatomegaly, melanosis, cardiac arrhythmia, and, in fatal cases, eventual cardiac failure. Acute arsenic ingestion can damage mucous membranes of the gastrointestinal tract, causing irritation, vesicle formation, and even sloughing. Sensory loss in the peripheral nervous system is the most common neurological effect, appearing at 1 to 2 weeks after large doses and consisting of Wallerian degeneration of axons. Anemia and leucopenia, particularly granulocytopenia, occur a few days following high-dose arsenic exposure and are reversible.

Chronic Toxicity—T e skin is a major target organ in chronic inorganic arsenic exposure. Skin cancer is common with protracted high-level arsenical exposure. Liver injury, characteristic of long-term or chronic arsenic exposure, manifests itself initially as jaundice, abdominal pain, and hepatomegaly and may progress to cirrhosis and ascites, even to hepatocellular carcinoma.

Repeated exposure to low levels of inorganic arsenic can produce peripheral neuropathy. T is neuropathy usually begins with sensory changes, such as numbness in the hands and feet, but later may develop into a painful "pins and needles" sensation. Both sensory and motor nerves can be affected, and dying-back axonopathy with demyelination may occur. In addition, peripheral vascular disease has been observed in persons with chronic exposure to inorganic arsenic in the drinking water.

Mechanisms *of* Toxicity—T e trivalent compounds of arsenic are thiol-reactive, and thereby inhibit enzymes or alter proteins by reacting with proteinaceous thiol groups. Pentavalent arsenate is an uncoupler of mitochondrial oxidative phosphorylation, by a mechanism likely related to competitive substitution (mimicry) of arsenate for inorganic phosphate in the formation of adenosine triphosphate. Arsenic and its metabolites have been shown to produce oxidants and oxidative DNA damage, alteration in DNA methylation status and genomic instability, impaired DNA damage repair, and enhanced cell proliferation.

Carcinogenicity—Arsenic is a known human carcinogen, associated with tumors of the skin, lung, and urinary bladder, and possibly kidney, liver, and prostate. Arsenic-induced skin cancers include basal cell carcinomas and squamous cell carcinomas, both arising in areas of arsenic-induced hyperkeratosis. In humans, increased mortality occurs from lung cancer in young adults following in utero exposure to arsenic. T us, the developing fetus appears to be hypersensitive to arsenic carcinogenesis.

Treatment—For acute arsenic poisoning, treatment is symptomatic, with particular attention to fluid volume replacement and support of blood pressure. T e oral chelator penicillamine or succimer (2,3-dimercaptosuccinic acid, DMSA) is effective in removing arsenic from the body. T e best strategy for preventing chronic arsenic poisoning is by reducing exposure.

Cadmium

About 75% of cadmium produced is used in batteries, especially nickel–cadmium batteries. Because of its noncorrosive properties, cadmium has been used in electroplating or

galvanizing alloys for corrosion resistance. It is also used as a color pigment for paints and plastics. T is metal is produced as a by-product of zinc and lead smelting.

Exposure—Food is the major source of cadmium for the general population. Many plants readily accumulate cadmium from soil. Both natural and anthropogenic sources of cadmium contamination occur for soil, including fallout of industrial emissions, some fertilizers, soil amendments, and use of cadmium-containing water for irrigation, all resulting in a slow but steady increase in the cadmium content in vegetables over the years. Shellfish and animal liver and kidneys can accumulate relatively high levels of cadmium. Air can be a significant source of direct exposure or environmental contamination. Cigarette smoking is a major nonoccupational source of cadmium exposure.

Inhalation is the dominant route of exposure in occupational settings. Occupations potentially at risk from cadmium exposure include those involved with refining zinc and lead ores, iron production, cement manufacture, industries involving fossil fuel combustion, the manufacturing of paint pigments, cadmium–nickel batteries, and electroplating.

Figure 23–2 illustrates that cadmium is protein-bound in blood, is rapidly taken up by tissues, and is deposited in the liver and kidney. Cadmium stored in hepatocytes is bound to metallothionine. T is complex may be released and then travel to the kidney where the complex is reabsorbed, degraded, and free cadmium is able to induce metallothionine synthesis or cause toxicity.

Toxicity—Acute toxicity from the ingestion of high concentrations of cadmium in the form of heavily contaminated beverages or food causes severe irritation to the gastrointestinal epithelium, leading to nausea, vomiting, and abdominal pain. Inhalation of cadmium fumes or other heated cadmium-containing materials may produce acute pneumonitis with pulmonary edema.

T e major long-term toxic effects of low-level cadmium exposure are renal injury, obstructive pulmonary disease, osteoporosis, and cardiovascular disease. Cancer is primarily a concern in occupationally exposed groups. T e chronic toxic effects of cadmium are clearly a much greater concern than the rare acute toxic exposures.

Nephrotoxicity—Cadmium is toxic to tubular cells and glomeruli, markedly impairing renal function leading to proteinuria. T ese lesions consist of initial tubular cell necrosis and degeneration, progressing to an interstitial inflammation and fibrosis. Because of the potential for renal toxicity, there is considerable concern about the levels of dietary cadmium intake for the general population.

Chronic Pulmonary Disease—Cadmium inhalation is toxic to the respiratory system in a fashion related to the dose and duration of exposure. Cadmium-induced obstructive lung disease in humans can be slow in onset, and results from chronic bronchitis, progressive fibrosis of the lower airways, and accompanying alveolar damage leading to emphysema. Pulmonary function is reduced with dyspnea, reduced vital capacity, and increased residual volume.

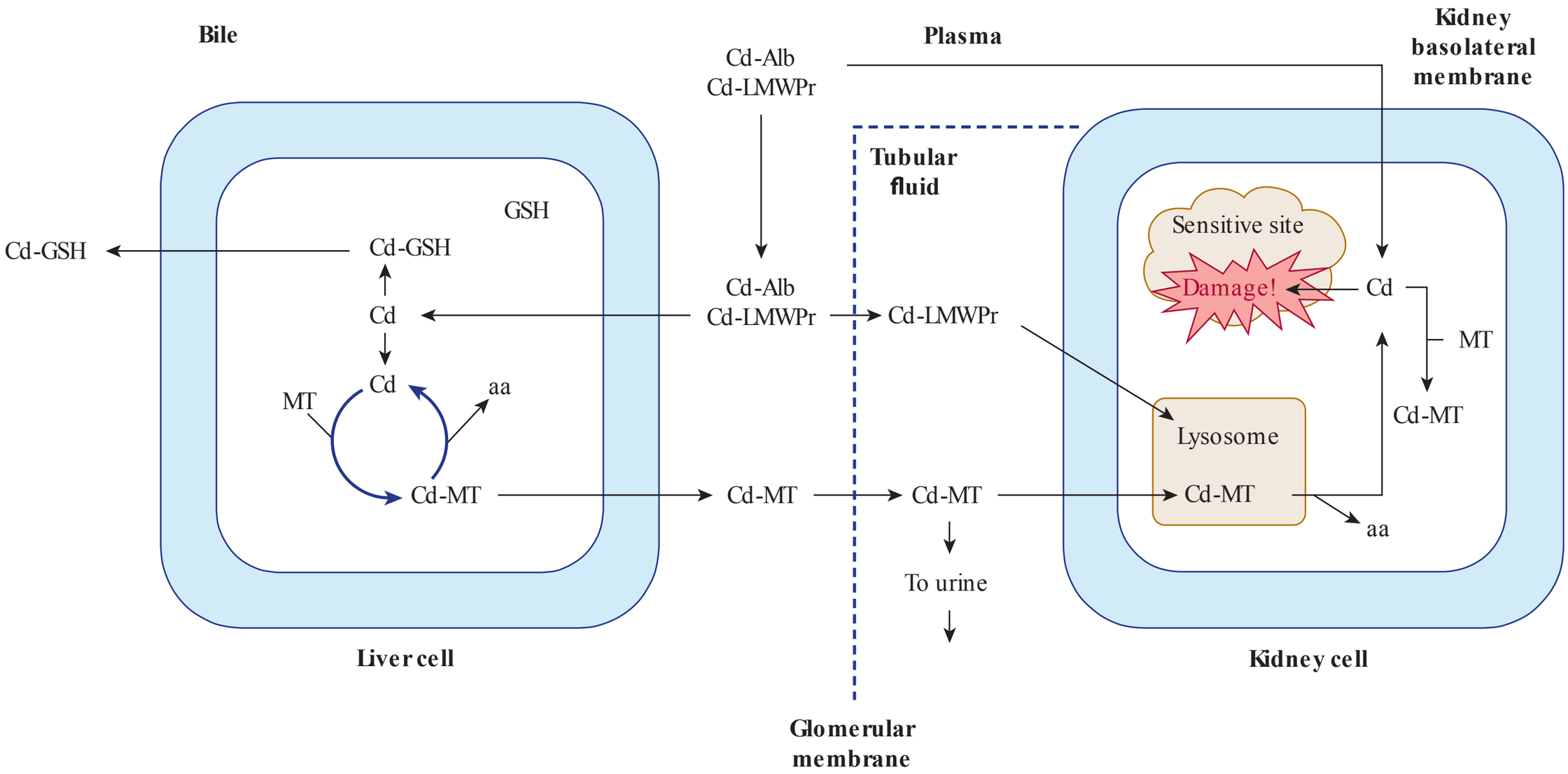

FIGURE 23–2 **Cadmium transport, protein binding, and toxicity.** GSH, glutathione; MT, metallothionine; aa, amino acids; Cd-Alb, cadmium-albumin; Cd-LMWPr, cadmium associated with low-molecular-weight proteins.

Other Toxicities—Cadmium toxicity affects calcium metabolism, and associated skeletal changes probably related to calcium loss include bone pain, osteomalacia, and/or osteoporosis. Epidemiologic studies suggest that cadmium may be an etiologic agent for hypertension. Epidemiologic studies in humans have suggested a relationship between abnormal behavior and/or decreased intelligence in children and adults exposed to cadmium. In humans, occupational respiratory exposure to cadmium has been most clearly associated with lung cancer.

Lead

Lead is a ubiquitous toxic metal and is detectable in practically all phases of the inert environment and in all biological systems. The phasing out of leaded gasoline and the removal of lead from paint, solder, and water supply pipes have significantly lowered blood lead levels in the general population. Lead exposure in children still remains a major health concern. Additionally, lead is not biodegradable and ecotoxicity of lead remains a concern.

Exposure—Lead-containing paint is a primary source of lead exposure in children. Major environmental sources of lead for infants and toddlers up to four years of age are hand-to-mouth transfer of lead-containing paint chips and dust from floors of older housing. Lead in household dust can also come from outside of the home (i.e., soil). A major route of exposure for the general population is from food and water. Dietary intake of lead has decreased dramatically in recent years. Other potential sources of lead exposure are recreational shooting, hand-loading ammunition, soldering, jewelry making, pottery making, gun smithing, glass polishing, painting, and stained glass crafting.

Toxicity—The toxic effects of lead and the minimum blood level at which an effect is likely to be observed are shown in Table 23–1. Lead can induce a wide range of adverse effects in humans depending on the dose and duration of exposure. The toxic effects range from inhibition of enzymes to the production of severe pathology or death. Children are most sensitive to effects in the central nervous system, whereas peripheral neuropathy, chronic nephropathy, and hypertension are concerns in adults. Other target tissues include the gastrointestinal, immune, skeletal, and reproductive systems. Effects on the heme biosynthesis provide a sensitive biochemical indicator even in the absence of other detectable effects.

Neurologic, Neurobehavioral, and Developmental Effects in Children—Symptoms of lead encephalopathy begin with lethargy, vomiting, irritability, loss of appetite, and dizziness, progressing to obvious ataxia, and a reduced level of consciousness, which may progress to coma and death. Recovery is often accompanied by sequelae including epilepsy, mental retardation, and, in some cases, optic neuropathy and blindness.

TABLE 23–1 Summary of lowest observed effect levels for lead-related health effects.

	Blood Lead Levels, µg/dL	
Effect	**Adult**	**Children**
Neurologic		
Encephalopathy (overt)	80–100	100–120
Hearing deficits	20	—
IQ deficits	10–15	—
In utero effects	10–15	—
Nerve conduction velocity ↓	40	40
Hematologic		
Anemia	80–100	80–100
U-ALA ↑	40	40
B-EP ↑	15	15
ALA-D inhibition	10	10
Renal		
Nephropathy	40	40–60
Vitamin D metabolism	< 30?	—

The most sensitive indicators of adverse neurologic outcomes are psychomotor tests or mental development indices, and broad measures of IQ. Lead can affect the brain by multiple mechanisms. Lead may act as a surrogate for calcium and/or disrupt calcium homeostasis. The stimulation of protein kinase C may result in alteration of the blood–brain barrier. Lead affects virtually every neurotransmitter system in the brain, including glutamatergic, dopaminergic, and cholinergic systems. All these systems play a critical role in synaptic plasticity and cellular mechanisms for cognitive function, learning, and memory.

Neurotoxic Effects in Adults—Adults with occupational exposure may demonstrate abnormalities in a number of measures in neurobehavior. Peripheral neuropathy is a classic manifestation of lead toxicity in adults. Footdrop and wristdrop may be observed in workers with excessive occupational exposure to lead. Peripheral neuropathy is characterized by segmental demyelination and possibly axonal degeneration.

Hematologic Effects—Lead has multiple hematologic effects, ranging from increased urinary porphyrins, coproporphyrins, δ-aminolevulinic acid (ALA), and zinc-protoporphyrin to anemia. The heme biosynthesis pathway and the sites of lead interference are shown in Figure 23–3. The most sensitive effects of lead are the inhibition of δ-aminolevulinic acid dehydratase (ALAD) and ferrochelatase. ALAD catalyzes the condensation of two units of ALA to form porphobilinogen

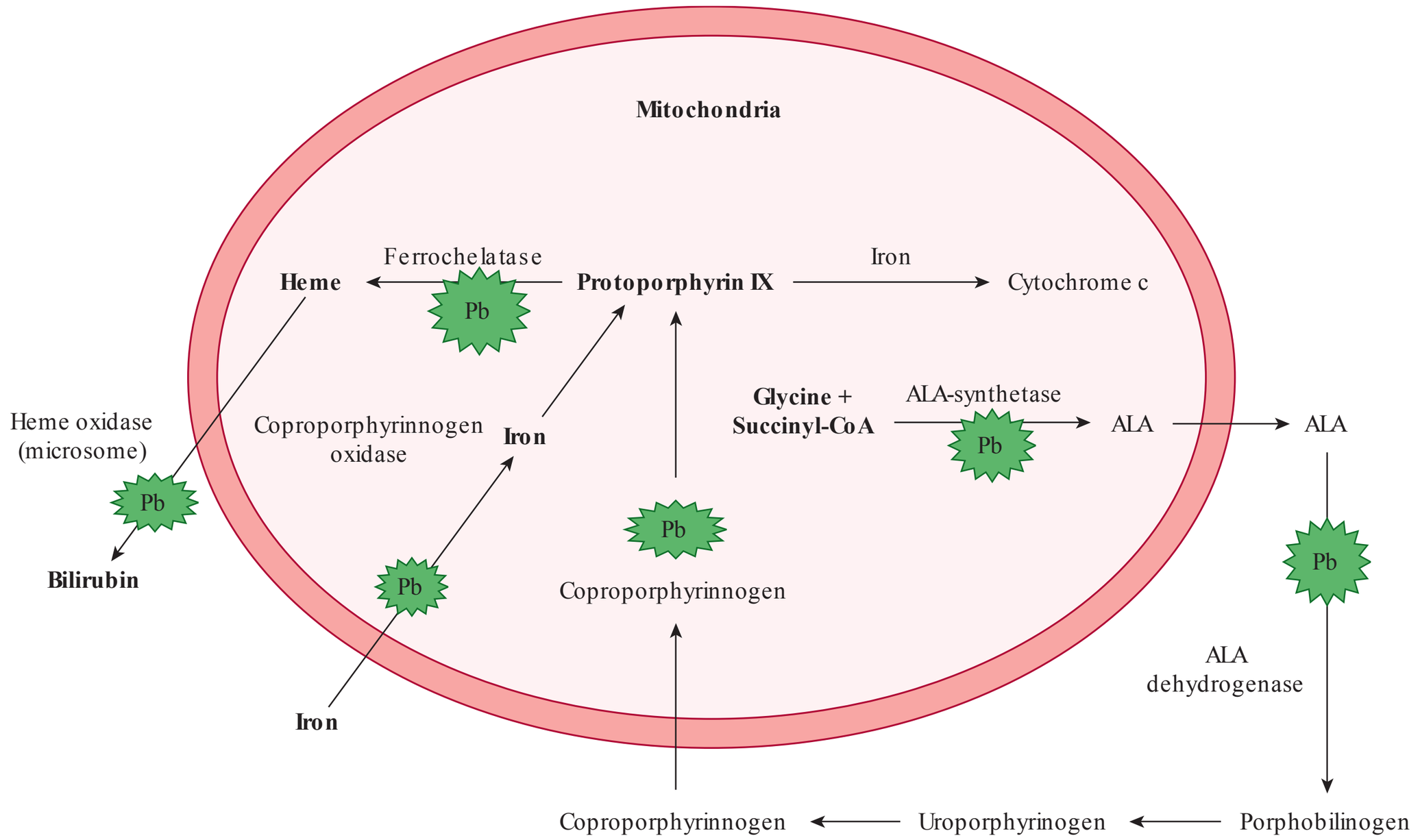

FIGURE 23–3 **Lead interruption of heme biosynthesis.** ALA, δ-aminolevulinate; Pb, sites for lead effects. The major lead inhibition sites are ALA dehydrogenase and ferrochelatase.

(PBG). Inhibition of ALAD results in accumulation of ALA. Ferrochelatase catalyzes the insertion of iron into the protoporphyrin ring to form heme. Inhibition of ferrochelatase results in accumulation of protoporphyrin IX, which takes the place of heme in the hemoglobin molecule and, as the erythrocytes containing protoporphyrin IX circulate, zinc is chelated at the site usually occupied by iron. Anemia only occurs in very marked cases of lead toxicity.

Renal Toxicity—Acute lead nephrotoxicity consists of proximal tubular dysfunction and can be reversed by treatment with chelating agents. Chronic lead nephrotoxicity consists of interstitial fibrosis and progressive nephron loss, azotemia, and renal failure. Lead nephrotoxicity impairs the renal synthesis of heme-containing enzymes in the kidney, such as heme-containing hydroxylase involved in vitamin D metabolism causing bone effects. Hyperuricemia with gout occurs more frequently in the presence of lead nephropathy. Lead nephropathy can be a cause of hypertension.

Effects on Cardiovascular System—The pathogenesis of lead-induced hypertension is multifactorial including (1) inactivation of endogenous nitric oxide and cGMP, possibly through lead-induced reactive oxygen species; (2) changes in the renin–angiotensin–aldosterone system, and increases in sympathetic activity, important humoral components of hypertension; (3) alterations in calcium-activated functions of vascular smooth muscle cells including contractility by decreasing Na^+/K^+-ATPase activity and stimulation of the Na^+/Ca^{2+} exchange pump; and (4) a possible rise in endothelin and thromboxane.

Other Toxic Effects—As an immunosuppressive agent, lead decreases immunoglobulins, alters T-cell subpopulations, and reduces polymorphonuclear leukocyte chemotactic activity. Lead can affect bone by interfering with metabolic and homeostatic mechanisms including parathyroid hormone, calcitonin, vitamin D, and other hormones that influence calcium metabolism. Lead substitutes for calcium in bone. Lead is known to affect osteoblasts, osteoclasts, and chrondrocytes and has been associated with osteoporosis and delays in fracture repair. Lead colic, although rare, is a major gastrointestinal symptom of severe lead poisoning, and is characterized by abdominal pain, nausea, vomiting, constipation, and cramps.

Mercury

Also called quicksilver, metallic mercury is in liquid state at room temperature. Mercury vapor (Hg^0) is much more hazardous than the liquid form. Mercury binds to other elements (such as chlorine, sulfur, or oxygen) to form inorganic mercurous (Hg^+) or mercuric (Hg^{2+}) salts.

Global Cycling and Ecotoxicology—Mercury exemplifies movement of metals in the environment (Figure 23–4). Atmospheric mercury, in the form of mercury vapor (Hg^0), is derived from natural degassing of the earth's crust and through volcanic eruptions as well as from evaporation from oceans

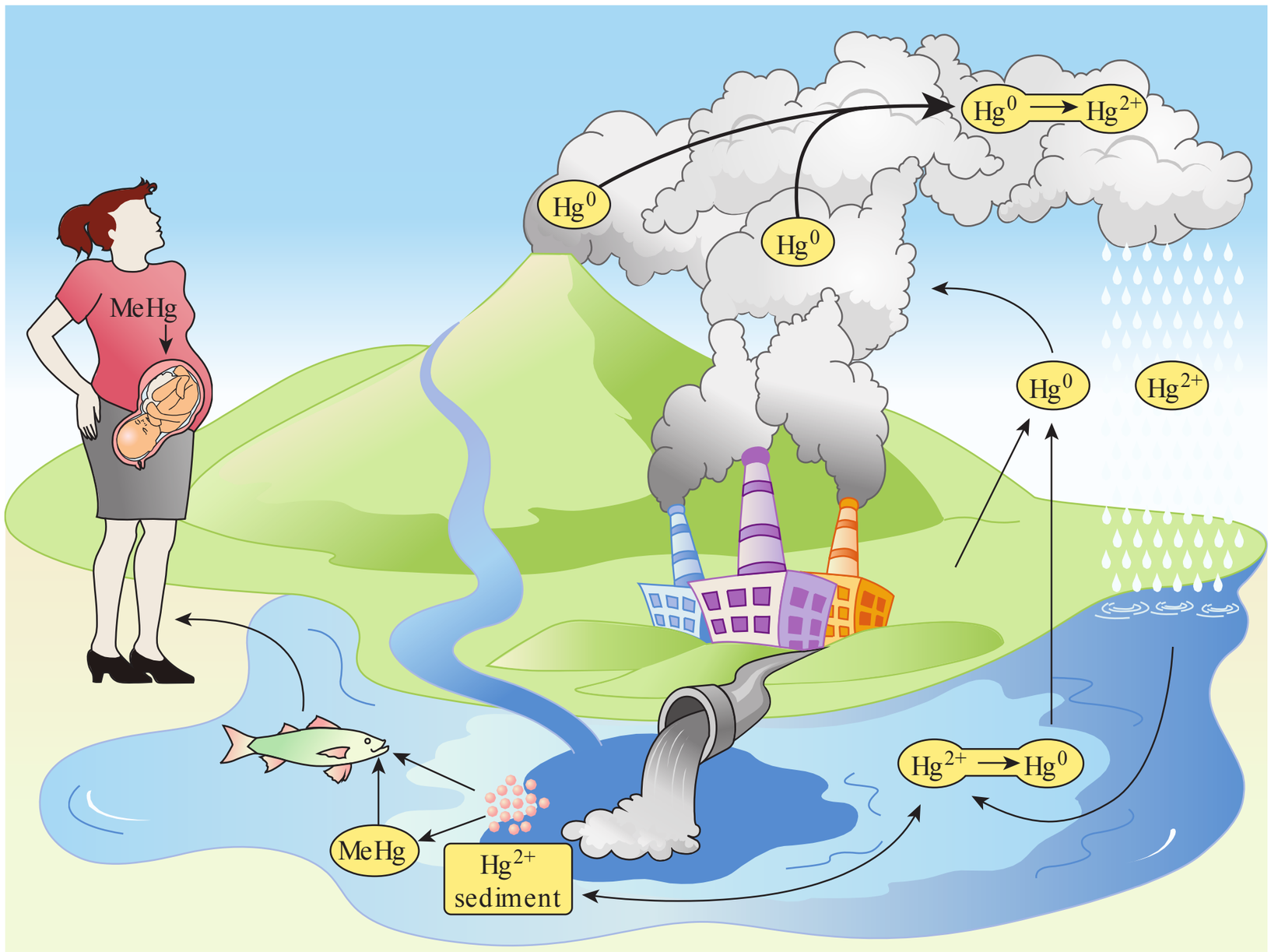

FIGURE 23–4 **The movement of mercury in the environment.** In nature, mercury vapor (Hg^0), a stable gas, evaporates from the earth's surface (both soil and water) and is emitted by volcanoes. Anthropogenic sources include emissions from coal-burning power stations and municipal incinerators. After □ 1 year, mercury vapor is converted to soluble form (Hg^{2+}) and returned to the earth by rainwater. It may be converted back to the vapor by microorganisms and reemitted into the atmosphere. Thus, mercury may recirculate for long periods. Mercury attached to aquatic sediments is subjected to microbial conversion to methylmercury, starting with plankton, then herbivorous fish, and finally ascending to carnivorous fish and sea mammals. This biomethylation and biomagnification result in human exposure to methylmercury through consumption of fish, and pose the health risk to humans, especially the developing fetus.

and soils. Anthropogenic sources have become a significant contributor to atmospheric mercury. T ese include emissions from metal mining and smelting (mercury, gold, copper, and zinc), coal combustion, municipal incinerators, and chloralkali industries. Methylmercury enters the aquatic food chain starting with plankton, then herbivorous fish, and finally ascending to carnivorous fish and sea mammals. On the top of the food chain, tissue mercury can rise to levels 1 800 to 80 000 times higher than levels in the surrounding water. T is biomethylation and bioconcentration result in human exposure to methylmercury through consumption of fish.

Exposure

Dietary Exposure—Consumption of fish is the major route of exposure to methylmercury. Inorganic mercury compounds are also found in other foods. T e source of inorganic mercurial is unknown but the amounts ingested are far below known toxic levels. Mercury in the atmosphere and in drinking water is generally so low that it does not constitute an important source of exposure to the general population.

Occupational Exposure—Inhalation of mercury vapor may occur from working in the chloralkali industry. Occupational exposure may occur during manufacture of a variety of scientific instruments and electrical control devices, in dentistry where mercury amalgams are used in tooth restoration, and in the extraction of gold.

Accidental Exposure—Elemental mercury exposure can occur from broken elemental mercury containers, medicinal devices, barometers, and melting tooth amalgam fillings to recover silver. Inhalation of large amounts of mercury vapor can be deadly.

Toxicity

Mercury Vapor—Inhalation of mercury vapor at extremely high concentrations may produce an acute, corrosive bronchitis and interstitial pneumonitis and, if not fatal, may be associated with central nervous system effects such as tremor or increased excitability. T is condition has been termed the asthenic-vegetative syndrome or micromercurialism. Identification of the syndrome requires neurasthenic symptoms and three or more of the following clinical findings: tremor, enlargement of the thyroid, increased uptake of radioiodine in the thyroid, labile pulse, tachycardia, dermographism, gingivitis, hematologic changes, or increased excretion of mercury in urine.

Inorganic Mercury—T e kidney is the major target organ for inorganic mercury. Although a high dose of mercuric chloride

is directly toxic to renal tubular cells, chronic low-dose exposure to mercury salts may induce an immunologic glomerular disease. Exposed persons may develop proteinuria that is reversible after they are removed from exposure.

Methylmercury—T e major human health effect from exposure to methylmercury is neurotoxicity. Clinical manifestations of neurotoxicity include paresthesia (a numbness and tingling sensation around the mouth and lips) and ataxia, manifested as a clumsy, stumbling gait, and difficulty in swallowing and articulating words. Other signs include neurasthenia (a generalized sensation of weakness), vision and hearing loss, and spasticity and tremor. T ese may finally progress to coma and death. T e overall acute effect is cerebral edema, but with prolonged destruction of gray matter and subsequent gliosis, cerebral atrophy results.

Mechanism o*f* Toxicity—High-affinity binding of divalent mercury to sulf ydryl groups of proteins in the cells is an important mechanism for producing nonspecific cell injury or even cell death. Other general mechanisms include an increase in genes associated with oxidative stress, reduced glutathione levels, disruption of microtubules in neuritis, damage mitochondria, and disrupt intracellular calcium homeostasis.

Nickel

Nickel is used in various metal alloys, including stainless steels, and in electroplating. Occupational exposure to nickel occurs by inhalation of nickel-containing aerosols, dusts, or fumes, or dermal contact in workers engaged in nickel production (mining, milling, refinery, etc.) and nickel-using operations (melting, electroplating, welding, nickel–cadmium batteries, etc.). Nickel is ubiquitous in nature, and the general population is exposed to low levels of nickel in air, cigarette smoke, water, and food.

Toxicity

Contact Dermatitis—Nickel-induced contact dermatitis is the most common adverse health effect from nickel exposure and is found in 10% to 20% of the general population. It can result from exposure to airborne nickel, liquid nickel solutions, or prolonged skin contact with metal items containing nickel, such as coins and jewelry.

Nickel Carbonyl Poisoning—Metallic nickel combines with carbon monoxide to form nickel carbonyl ($Ni[CO]_4$), which decomposes to nickel and carbon monoxide on heating. Nickel carbonyl is extremely toxic. Intoxication begins with headache, nausea, vomiting, and epigastric or chest pain, followed by cough, hyperpnea, cyanosis, gastrointestinal symptoms, and weakness. T e symptoms may be accompanied by fever and leukocytosis. More severe cases can progress to pneumonia, respiratory failure, and eventually to cerebral edema and death.

Carcinogenicity—Nickel is a respiratory tract carcinogen in nickel-refining industry workers. Risks are highest for lung and nasal cancers among workers heavily exposed to nickel sulfide, nickel oxide, and metallic nickel.

ESSENTIAL METALS WITH POTENTIAL FOR TOXICITY

T is group includes eight metals generally accepted as essential: cobalt, copper, iron, magnesium, manganese, molybdenum, selenium, and zinc. All can produce some target organ toxicity (Table 23–2).

Copper

Food, beverages, and drinking water are major sources of exposure in the general population. Copper exposure in industry is primarily from inhaled particulates in mining or metal fumes in smelting operations, welding, or related activities.

Toxicity—T e most commonly reported adverse health effects of excess oral copper intake are gastrointestinal distress. Nausea, vomiting, and abdominal pain have been reported shortly after drinking solutions of copper sulfate or beverages stored in containers that readily release copper. Ingestion of drinking water with > 3 mg Cu/L will produce gastrointestinal symptoms. Ingestion of large amounts of copper salts, most frequently copper sulfate, may produce hepatic necrosis and death.

Hereditary Disease o*f* Copper Metabolism

Menkes Disease—T is is a rare sex-linked genetic defect in copper metabolism resulting in copper deficiency in male infants. It is characterized by peculiar hair, failure to thrive, severe mental retardation, neurological impairment, connective tissue dysfunction, and death usually by three to five years of age. T e majority of the pathologies associated with Menkes disease can be linked to deficiencies in copper-containing proteins. Bones are osteoporotic with flared metaphases of the long bones and bones of the skull. T ere is extensive degeneration of the cerebral cortex and of white matter. T e gene responsible for Menkes disease, ATP7A, belongs to the family of ATPases and is a copper transporter. Deficiency in this copper transporter in Menkes disease blocks copper transportation across the basolateral membrane of intestinal cells into the portal circulation, resulting in accumulation of copper in the enterocytes and systemic copper deficiency in the body.

Wilson's Disease—T is is an autosomal recessive genetic disorder of copper metabolism characterized by the excessive accumulation of copper in liver, brain, kidneys, and cornea. Serum ceruloplasmin is low and serum copper not bound to ceruloplasmin is elevated. Urinary excretion of copper is high. Clinical abnormalities of the nervous system, liver, kidneys, and cornea are related to copper accumulation. Patients with Wilson's disease have impaired biliary excretion of copper, which is believed to be the fundamental cause of the copper

TABLE 23–2 Toxicity of several metals or metalloids.

Metal	CNS	GI Tract	Lung	Kidney	Liver	Heart	Blood	Skin
Aluminum	*		*					
Antimony			*			*		*
Arsenic	*	*	*	*	*		*	
Beryllium			*					*
Bismuth				*	*			*
Cadmium	*	*	*	*	*	*		
Chromium	*		*	*	*			*
Cobalt	*	*	*			*		*
Copper		*					*	
Iron	*	*	*		*		*	
Lead	*	*		*			*	*
Lithium	*	*		*		*		
Manganese	*		*					
Mercury	*	*	*	*				
Nickel	*		*					*
Selenium		*		*				*
Silver			*					*
Zinc	*						*	

overload. Reversal of abnormal copper metabolism is achieved by liver transplantation, confirming that the basic defect is in the liver. Clinical improvement can be achieved with chelation therapy.

Iron

Iron is an essential metal for erythropoiesis and a key component of hemoglobin, myoglobin, heme enzymes, metalloflavoprotein enzymes, and mitochondrial enzymes. In biological systems, iron mainly exists as the ferrous (+ 2) and ferric (+ 3) forms. Toxicologic considerations are important in terms of iron deficiency, accidental acute exposures, and chronic iron overload due to idiopathic hemochromatosis or as a consequence of excess dietary iron or frequent blood transfusions.

Toxicity—Acute iron poisoning from accidental ingestion of iron-containing dietary supplements is the most common cause of acute toxicity. Severe toxicity occurs after the ingestion of more than 0.5 g of iron or 2.5 g of ferrous sulfate. Toxicity occurs about 1 to 6 h after ingestion. Symptoms include abdominal pain, diarrhea, and vomiting. Of particular concern are pallor or cyanosis, metabolic acidosis, and cardiac collapse. Death may occur in severely poisoned children within 24 h.

Chronic iron toxicity from iron overload in adults is a relatively common problem. T ere are three basic ways in which excessive amounts of iron can accumulate in the body: (1) hereditary hemochromatosis due to abnormal absorption of iron from the intestinal tract, (2) excess intake via the diet or from oral iron preparations, and (3) repeated blood transfusions for some form of refractory anemia (transfusional siderosis). Increased body iron may play a role in the development of cardiovascular disease. It is suspected that iron may act as a catalyst to produce free radical damage resulting in artherosclerosis and ischemic heart disease. Some neurodegenerative disorders associated with aberrant iron metabolism in the brain include neuroferritinopathy, aceruloplasminemia, and manganism.

Zinc

An essential metal, zinc deficiency results in severe health consequences. However, zinc toxicity is relatively uncommon and occurs only at very high exposure levels. Zinc is present in most foodstuffs, water, and air. Occupational exposure to dusts

and fumes of metallic zinc occurs in zinc mining and smelting. T e zinc content of substances in contact with galvanized copper or plastic pipes may be high.

Essentiality and Def ciency—More than 300 catalytically active zinc metalloenzymes and 2000 zinc-dependent transcription factors exist. Zinc participates in a wide variety of metabolic processes, supports a healthy immune system, and is essential for normal growth and development during pregnancy, childhood, and adolescence. Zinc deficiency is related to poor dietary zinc intake, dietary phytate intake, chronic illness, or oversupplementation with iron or copper. Symptoms of zinc deficiency include growth retardation, appetite loss, alopecia, diarrhea, impaired immune function, cognitive impairments, dermatitis, delayed healing of wounds, taste abnormalities, and impaired sexual function. T erapeutic uses of zinc include the treatment of acute diarrhea in infants with severe zinc deficiency, the treatment of common cold by its antiviral and immunomodulatory effects, therapy for Wilson's disease to help reduce copper burden and to induce metallothionein, and the prevention of blindness in age-related macular degeneration.

Toxicity—Although uncommon, gastrointestinal distress and diarrhea have been reported following ingestion of beverages standing in galvanized cans. Following inhalation of zinc oxide the most common effect is "metal-fume fever" characterized by fever, chest pain, chills, cough, dyspnea, nausea, muscle soreness, fatigue, and leukocytosis. Acute inhalation of high levels of zinc chloride as in the military use of "smoke bombs" results in more pronounced damage to the mucous membrane including interstitial edema, fibrosis, pneumonitis, bronchial mucosal edema, and ulceration. Following long-term exposure to lower doses of zinc, symptoms generally result from a decreased dietary copper absorption, leading to early symptoms of copper deficiency, such as decreased erythrocyte number or decreased hematocrit.

Neuronal Toxicity—As an essential cofactor for numerous enzymes and proteins, zinc deficiency may alter an antioxidant enzyme resulting in excess free radicals that are damaging to cell membranes. Excess zinc released by oxidants can act as a potent neurotoxin, contributing to excitotoxic brain injury. T e release of excess, toxic free zinc could be a factor that sets the stage for the later development of Alzheimer's disease.

Pancreatic Toxicity—Because large amounts of zinc accumulate in secretory granules of pancreatic islet β-cells, zinc released under certain conditions can affect the function or survival of islet cells and cause β-cell death. Excess dietary zinc is associated with damage to exocrine pancreas. A single, high-dose injection of zinc increases plasma α-amylase activity and can produce fibrosis and necrosis of pancreatic exocrine cells, but does not affect the islets of Langerhans cells.

METALS RELATED TO MEDICAL THERAPY

Metals that are used to treat a number of human illnesses, including aluminum, bismuth, gold, lithium, and platinum, exert some toxicity (Table 23–2).

Aluminum

Chemical compounds of aluminum occur typically in the trivalent valence state (Al^{3+}). As a hard trivalent ion, aluminum binds strongly to oxygen-donor ligands such as citrate and phosphate. Human exposure to aluminum comes primarily from food and secondarily from drinking water. T e amount of aluminum in the food supply is small compared with pharmaceutical use of aluminum in antacids and buffered analgesics. Occupational exposures to aluminum occur during mining and processing, as well as in aluminum welding.

Toxicity—Most cases of aluminum toxicity in humans are observed in patients with chronic renal failure, or in persons exposed to aluminum in the workplace, with the lung, bone, and central nervous system as major target organs. Aluminum can produce developmental effects.

Lung and Bone Toxicity—Occupational exposure to aluminum dust can produce lung fibrosis in humans. Osteomalacia has been associated with excessive intake of aluminum-containing antacids in otherwise healthy individuals. T is is assumed to be due to interference with intestinal phosphate absorption. Osteomalacia also can occur in uremic patients exposed to aluminum in the dialysis fluid. In these patients, osteomalacia may be a direct effect of aluminum on bone mineralization as bone levels are high.

Neurotoxicity—Aluminum is neurotoxic to experimental animals, with wide species and age variations. In susceptible animals, such as rabbits and cats, the most prominent early pathologic change is the accumulation of neurofibrillary tangles (NFTs) in large neurons, proximal axons, and dendrites of neurons of many brain regions. T is is associated with loss of synapses and atrophy of the dendritic tree. In other species, impairment of cognitive and motor function and behavioral abnormalities are often observed.

Dialysis Dementia—A progressive, neurologic syndrome has been reported in patients on long-term intermittent hemodialysis for chronic renal failure. T e first symptom in these patients is a speech disorder followed by dementia, convulsions, and myoclonus. T e disorder, which typically arises after 3 to 7 years of dialysis treatment, may be due to aluminum intoxication. T e aluminum content of brain, muscle, and bone increases in these patients. Sources of the excess aluminum may be from oral aluminum hydroxide commonly given to these patients or from aluminum in dialysis fluid derived

from the tap water used to prepare the dialysate fluid. T e high serum aluminum concentrations may be related to increased parathyroid hormone levels that are due to low blood calcium and osteodystrophy common in patients with chronic renal disease. T e syndrome may be prevented by avoiding the use of aluminum-containing oral phosphate binders and by monitoring of aluminum in the dialysate.

Alzheimer's Disease—A possible relationship between aluminum and Alzheimer's disease has been a matter of speculation for decades. Elevated aluminum levels in Alzheimer's brains may be a consequence and not a cause of the disease. T e reduced effectiveness of the blood–brain barrier in Alzheimer's might allow more aluminum into the brain. Also, recent studies have raised the possibility that the staining methods in earlier studies may have led to aluminum contamination. T ere are conflicting conclusions from studies examining the role of aluminum in Alzheimer's disease. However, there is increasing evidence suggesting a link between aluminum in the brain and other neurodegenerative diseases.

Lithium

Lithium is used in batteries, alloys, catalysts, photographic materials, and the space industry. Lithium hydride produces hydrogen on contact with water and is used in manufacturing electronic tubes, in ceramics, and in chemical analysis. Groundwater contamination with lithium from man-made waste disposal could be a risk factor for the aquatic environment. Lithium carbonate and lithium citrate are widely used for mania and bipolar disorders.

Toxicokinetics—Lithium is readily absorbed from the gastrointestinal tract. It is distributed to total body water with higher levels in kidney, thyroid, and bone as compared with other tissues. Excretion is chiefly through the kidneys with 80% of the filtered lithium reabsorbed. Lithium can substitute for sodium or potassium on several transport proteins.

Toxicity—Except for lithium hydride, no other salts are considered hazardous, nor is the metal very toxic itself. Lithium hydride is intensely corrosive and may produce burns on the skin because of the formation of hydroxides. Intoxications related to lithium exposure are mainly related to its medicinal uses. T e toxic responses to lithium include neuromuscular changes (tremor, muscle hyperirritability, and ataxia), central nervous system disorders (blackout spells, epileptic seizures, slurred speech, coma, psychosomatic retardation, and increased thirst), cardiovascular disturbances (cardiac arrhythmia, hypertension, and circulatory collapse), gastrointestinal symptoms (anorexia, nausea, and vomiting), and renal damage (albuminuria and glycosuria).

Chronic lithium nephrotoxicity and interstitial nephritis may occur with long-term exposure even when lithium levels remain within the therapeutic range. Chronic lithium-induced neurotoxicity, nephritis, and thyroid dysfunction may occur, especially in susceptible patients with nephrogenic diabetes insipidus, older age, abnormal thyroid function, and impaired renal function. Acute lithium overdose produces neurological sequelae and cardiac toxicity, which can be fatal. T e toxicity may be treated by the administration of diuretics (amiloride) and lowering of blood levels via hemodialysis.

Platinum

Platinum compounds are used as automobile catalysts, in jewelry, in electronics, and in dental alloys. Platinum coordination complexes are very important antitumor agents.

Toxicity—Platinum can produce profound hypersensitivity reactions in susceptible individuals. T e signs of hypersensitivity include urticaria, contact dermatitis of skin, and respiratory distress, ranging from irritation to an asthmatic syndrome, following exposure to platinum dust. T e skin and respiratory changes, platinosis, are mainly confined to persons with a history of industrial exposure to soluble compounds such as sodium chloroplatinate.

Antitumor Ef ects o*f* Platinum Complexes—T e platinum-coordinated complexes are important antitumor agents, including cisplatin, carboplatin, and oxaliplatin. T ey are routinely administered, often in combination with other anticancer drugs, in the treatment of a wide spectrum of malignancies, especially advanced testicular cancer and also cancers of head and neck, bladder, esophagus, lung, and ovary.

Carcinogenic Ef ects o*f* Platinum Complexes—Although cisplatin has antitumor activity in humans, it is considered to be a probable carcinogen in humans and is clearly carcinogenic in rodents. In fact, in mice deficient in metallothionein, cisplatin can induce liver carcinoma at clinically relevant doses.

Toxicities o*f* Platinum Antitumor Complexes—Cisplatin produces proximal and distal tubular cell injury, mainly in the corticomedullary region, where the concentration of platinum is highest. Hearing loss can occur and can be unilateral or bilateral but tends to be more frequent and severe with repeated doses. Marked nausea and vomiting occur in most patients receiving the platinum complexes but can be controlled with ondansetron or high dose of corticosteroids.

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QUESTIONS

1. Which of the following is NOT a major excretory pathway of metals?
   a. sweat.
   b. urine.
   c. respiration.
   d. feces.
   e. hair.

2. Metallothioneins:
   a. are responsible for metal transport in the bloodstream.
   b. are involved in the biotransformation of metals.
   c. invoke hypersensitivity reactions.
   d. provide high-affinity binding of copper and mercury.
   e. are involved in extracellular transport of metals.

3. Which of the following metal-binding proteins is NOT correctly paired with the metal it binds?
   a. transferrin—iron.
   b. ceruloplasmin—copper.
   c. metallothioneins—zinc.
   d. ferritin—lead.
   e. albumin—nonspecific metal binding.

4. Which of the following groups is LEAST likely to chelate metals?
   a. —COOH.
   b. —Cl.
   c. —NH.
   d. —OH.
   e. —SH.

5. What is the mechanism of toxicity of arsenic (As)?
   a. inhibition of mitochondrial respiration.
   b. impairment of calcium uptake by membrane transporters.
   c. accumulation in renal corpuscle.
   d. abolition of sodium–potassium gradient.
   e. destruction of surfactant in the lungs.

6. Lead's toxicity is largely due to its ability to mimic and interfere with normal functioning of which of the following ions?
   a. Na^+.
   b. K^+.
   c. Cl^-.
   d. Fe^{2+}.
   e. Ca^{2+}.

7. Which of the following statements regarding mercury (Hg) toxicity is FALSE?
   a. A major source of environmental mercury is rainwater.
   b. Mercury vapor is much more dangerous than liquid mercury.
   c. Mercury vapor inhalation is characterized by fatigue and bradycardia.
   d. Microorganisms in bodies of water can convert mercury vapor to methylmercury.
   e. Methylmercury is the most important source of human mercury toxicity.

8. Which of the following is a common symptom of nickel exposure?
   a. renal failure.
   b. diarrhea.
   c. hepatic cirrhosis.
   d. contact dermatitis.
   e. tachycardia.

9. Which of the following statements regarding Wilson's disease is FALSE?
   a. Serum ceruloplasmin is high.
   b. Urinary excretion of copper is high.
   c. T ere is impaired biliary excretion of copper.
   d. T e disease can be treated with liver transplantation.
   e. T is is an autosomal recessive disorder.

10. Which of the following statements regarding metals and medical therapy is FALSE?
    a. T ere are elevated levels of aluminum in the brains of Alzheimer's patients.
    b. Lithium is used to treat depression.
    c. Chronic nephrotoxicity is a common result of excess aluminum exposure.
    d. Platinum is used as cancer treatment.
    e. Platinum salts can cause an allergic dermatitis.

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CHAPTER

24

Toxic Effects of Solvents and Vapors

James V. Bruckner, S. Satheesh Anand, and D. Alan Warren

INTRODUCTION

IS THERE A SOLVENT-INDUCED CHRONIC ENCEPHALOPATHY?

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  - Kidney Cancer
  - Lung Cancer
- Tetrachloroethylene
- Methylene Chloride
- Carbon Tetrachloride
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- Toluene
- Xylenes and Ethylbenzene

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- Methanol

GLYCOLS

- Ethylene Glycol
- Propylene Glycol

GLYCOL ETHERS

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- Developmental Toxicity
- Hematotoxicity

AUTOMOTIVE GASOLINE AND ADDITIVES

CARBON DISULFIDE

KEY POINTS

- T e term solvent refers to a class of liquid organic chemicals of variable lipophilicity and volatility, small molecular size, and lack of charge.
- Absorption of inhaled volatile organic compounds occurs in the alveoli, with almost instantaneous equilibration with blood in the pulmonary capillaries.
- Solvents are readily absorbed from the gastrointestinal tract and across the skin.
- Most solvents produce some degree of CNS depression.

INTRODUCTION

T e term solvent refers to a class of liquid organic chemicals of variable lipophilicity and volatility, small molecular size, and lack of charge. Solvents undergo ready absorption across the lung, skin, and gastrointestinal (GI) tract. In general, the lipophilicity of solvents increases with increasing molecular weight, while volatility decreases. Solvents are frequently used to dissolve, dilute, or disperse materials that are insoluble in water. Most solvents are refined from petroleum. Many, such as naphthas and gasoline, are complex mixtures consisting of hundreds of compounds. As such, they are widely employed as degreasers.

Solvents are classified largely according to molecular structure or functional group. Classes of solvents include aliphatic hydrocarbons, many of which are halocarbons, aromatic hydrocarbons, alcohols, ethers, esters/acetates, amides/amines, aldehydes, ketones, and complex mixtures that defy classification. T e main determinants of a solvent's inherent toxicity are (1) its number of carbon atoms; (2) whether it is saturated or has double or triple bonds between adjacent carbon atoms; (3) its configuration (i.e., straight chain, branched chain, or cyclic); (4) whether it is halogenated; and (5) the presence of functional groups. Subtle differences in chemical structure can translate into dramatic differences in solvent toxicity.

Nearly everyone is exposed to solvents during normal daily activities. Environmental exposures to solvents in air and groundwater use multiple exposure pathways (Figure 24–1). T ough not reflected in Figure 24–1, household use of solvent-contaminated water may result in solvent intake from inhalation,

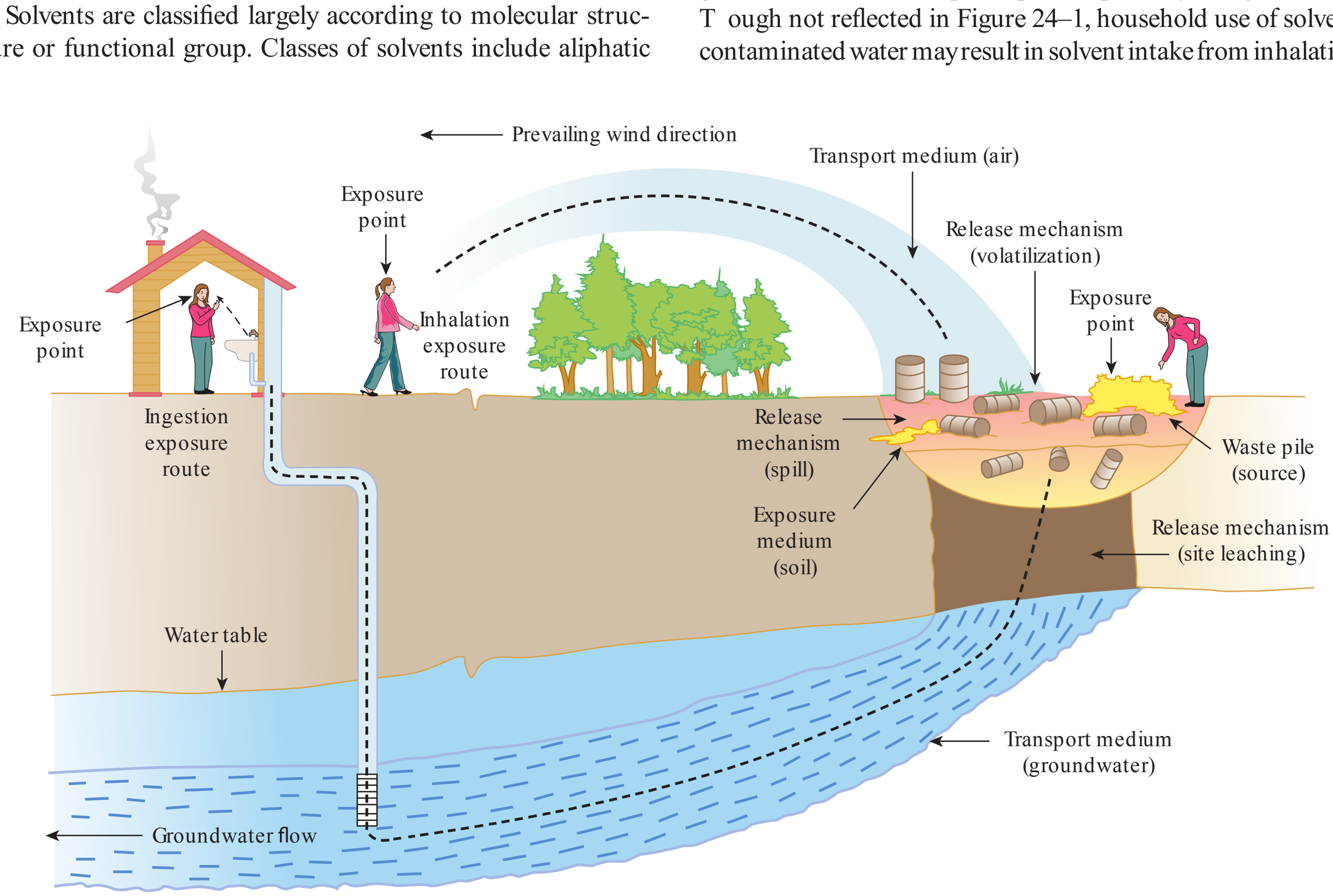

FIGURE 24–1 **Solvent exposure pathways and media.** (Reproduced with permission from EPA Risk Assessment Guidance for Superfund. Human Health Evaluation Manual Part A, Interim Final. Washington, DC: Office of Emergency and Remedial Response, 1989.)

and dermal and oral absorption. In many cases, environmental risk assessment requires that risks be determined for physiologically diverse individuals who are exposed to several solvents by multiple exposure pathways.

T e Occupational Safety and Health Administration (OSHA) has established legally enforceable Permissible Exposure Limits (PELs) for over 100 solvents. T e majority of existing PELs were adopted from the list of T reshold Limit Values (TLVs) published by the American Conference of Governmental Industrial Hygienists (ACGIH). Whereas the ACGIH's TLVs for an 8-h work day, 40-h work week are designed to be protective for a working lifetime, its Short-term Exposure Limits (STELs) and ceiling values are designed to protect against the acute effects of high-level, short-term solvent exposures. If warranted, the ACGIH will assign a skin notation to a solvent, indicating that significant dermal exposure is possible.

Most solvent exposures involve a mixture of chemicals, rather than a single compound. Whereas the assumption is frequently made that the toxic effects of multiple solvents are additive, solvents may also interact synergistically or antagonistically.

Although some solvents are less hazardous than others, all solvents can cause toxic effects. Most have the potential to induce narcosis and cause respiratory and mucous membrane irritation. As with other chemicals, whether adverse health effects occur from solvent exposure is dependent on several factors: (1) toxicity/carcinogenicity of the solvent; (2) exposure route; (3) amount or rate of exposure; (4) duration of exposure; (5) individual susceptibility; and (6) interactions with other chemicals.

IS THERE A SOLVENT-INDUCED CHRONIC ENCEPHALOPATHY?

Considerable debate has examined whether chronic, low-level exposure to virtually any solvent or solvent mixture can produce a pattern of neurologic dysfunction referred to as painter's syndrome, organic solvent syndrome, psychoorganic syndrome, and chronic solvent encephalopathy (CSE). CSE is characterized by nonspecific symptoms (e.g., headache, fatigue, and sleep disorders) with or without changes in neuropsychological function. A reversible form of CSE, the neuroasthenic syndrome, consists of symptoms only. T e "mild" and "severe" forms are accompanied by objective signs of neuropsychological dysfunction that may or may not be fully reversible. Well-designed and controlled clinical epidemiologic studies are needed to resolve this controversy of CSE.

SOLVENT ABUSE

Inhalants are volatile substances that can be inhaled to induce a psychoactive or mind-altering effect with vapor concentrations high enough to produce effects that resemble alcohol intoxication and may lead to unconsciousness. Solvent abuse is a unique exposure situation, in that participants repeatedly subject themselves to vapor concentrations high enough to produce effects as extreme as unconsciousness. T ese can be breathed in through the nose or the mouth by "sniffing" or "snorting" vapors from containers, spraying aerosols directly into the nose or mouth, "bagging" by inhaling vapors from substances inside plastic or paper bags, or "huffing" from a solvent-soaked rag stuffed into the mouth. Solvents can be addicting and are often abused in combination with other drugs. Solvents present in relatively inexpensive household and commercial products are readily available to children and adolescents. While intoxication may last only a few minutes, abusers frequently seek to prolong the "high" by inhaling repeatedly over the course of several hours. Death may occur as a result of cardiac arrhythmias, asphyxiation, and/or cachexia.

ENVIRONMENTAL CONTAMINATION

Most solvents enter the environment through evaporation (Figure 24–1). T e majority of the more volatile organic compounds (VOCs) volatilize when products containing them (e.g., aerosol propellants, paint thinners, cleaners, and soil fumigants) are used as intended. Solvent loss into the atmosphere also occurs during production, processing, storage, and transport activities, resulting in elevated concentrations in air in the proximity of point sources. Winds dilute and disperse solvent vapors across the world. Atmospheric concentrations of most VOCs are usually extremely low, though higher concentrations have been measured in urban areas, around petrochemical plants, and in the immediate vicinity of hazardous waste sites.

Solvent contamination of drinking water supplies is a major health concern. Solvents spilled onto the ground may permeate the soil and migrate until reaching groundwater or impermeable material. All solvents are soluble in water to some extent. Concentrations diminish rapidly after VOCs enter bodies of water, due primarily to dilution and evaporation. VOCs in surface waters rise to the surface or sink to the bottom, according to their density. VOCs on the surface will largely evaporate. VOCs on the bottom depend on solubilization in the water or mixing by current or wave action to reach the surface. VOCs in groundwater tend to remain trapped until the water reaches the surface.

TOXICOKINETICS

Toxicokinetic (TK) studies delineate the uptake and disposition of chemicals in the body. Toxicity is a dynamic process, in which the degree and duration of injury of a target tissue depends on the net effect of toxicodynamic (TD) and TK processes including systemic absorption, metabolism, interaction with cellular components, and tissue repair.

Volatility and lipophilicity are two important properties of solvents that govern their absorption and deposition in the body. Lipophilicity also can vary from quite water soluble (e.g., glycols and alcohols) to quite lipid soluble (e.g., halocarbons and aromatic hydrocarbons). Many solvents have a

relatively low molecular weight and are uncharged, enabling passive diffusion through membranes from areas of high to low concentration.

Absorption

Most systemic absorption of inhaled VOCs occurs in the alveoli, with some absorption occurring in the upper respiratory tract. Gases in the alveoli equilibrate almost instantaneously with blood in the pulmonary capillaries. Blood:air partition coefficients (PCs) of VOCs may be defined as the ratio of concentration of VOC achieved between two different media at equilibrium. More hydrophilic solvents have relatively high blood:air PCs, which favor extensive uptake. Because VOCs diffuse from areas of high to low concentration, increases in respiration (to maintain a high alveolar concentration) and in cardiac output/pulmonary blood flow (to maintain a large concentration gradient by removing capillary blood containing the VOC) enhance pulmonary absorption.

Solvents are well absorbed from the GI tract. Peak blood levels are observed within minutes of dosing, although the presence of food in the GI tract can delay absorption. It is usually assumed that 100% of an oral dose of most solvents is absorbed systemically. T e vehicle or diluent in which a solvent is ingested can affect the absorption and TK of the compound.

Absorption of solvents through the skin can result in both local and systemic effects. Lipophilic solvents penetrate the stratum corneum by passive diffusion. Determinants of the rate of dermal absorption of solvents include the chemical concentration, surface area exposed, exposure duration, integrity and thickness of the stratum corneum, and lipophilicity and molecular weight of the solvent.

Transport, Distribution, and Elimination

Solvents absorbed into portal venous blood from the GI tract are subject to uptake/elimination by the liver and exhalation by the lungs during their first pass through the pulmonary circulation. T ose solvents that are well metabolized and quite volatile are most efficiently eliminated before they enter the arterial blood. Hepatic first-pass elimination depends on the chemical and the rate at which it arrives in the liver. Pulmonary first-pass elimination, in contrast, is believed to be a zero-order process as a fixed percentage of the chemical is thought to exit the pulmonary blood at each pass through the pulmonary circulation.

Solvents transported by the arterial blood are taken up according to rate of tissue blood flow, mass, and the tissue:blood PC of the solvent. Relatively hydrophilic solvents solubilize to different extents in plasma. Lipophilic solvents do not bind to plasma proteins or hemoglobin, but partition into hydrophobic sites in the molecules. T ey partition into phospholipids, lipoproteins, and cholesterol that are present in the blood.

Blood levels of solvents drop rapidly during the initial elimination phase. T is redistribution phase is characterized by rapid diffusion of solvent from the blood into most tissues. Equilibration of adipose tissue is prolonged due to the small fraction of cardiac output (~3%) supplying fat depots. Body fat increases the volume of distribution and total body burden of lipophilic solvents.

Metabolism

Biotransformation can modulate the toxicities of solvents. Many solvents are poorly soluble in water and must be enzymatically converted to relatively water-soluble derivatives, which may be more readily eliminated in the largely aqueous urine and/or bile. Some solvents can undergo bioactivation to produce reactive metabolites that are cytotoxic and/or mutagenic. Toluene, benzene, 1,1,1-trichloroethylene, hexane, and carbon tetrachloride are examples of solvents that are metabolized to toxic products. In particular, CYP2E1 catalyzes the oxidation of halogenated and aromatic hydrocarbons, including benzene, styrene, chloroform, and vinyl chloride to electrophilic metabolites capable of causing cytotoxicity and/or mutagenicity.

Physiologic Modeling

Physiologically based toxicokinetic (PBTK) models are used to relate the administered dose to the tissue dose of a bioactive moiety or moieties. With knowledge of the physiology of the test animal and tissue, physiologically based toxicodynamic (PBTD) models can be developed. PBTK/TD models are well suited for species-to-species extrapolations, because human physiologic and metabolic parameter values can be entered and simulations of target tissue doses and effects in humans generated. T us, solvent exposures necessary to produce the same target organ dose in humans as that found experimentally to cause an unacceptable cancer or noncancer incidence in test animals can be determined in some cases with reasonable certainty. In the limited number of cases where there may be species differences in tissue sensitivity, PBTD models can be used to forecast toxicologically effective target organ doses.

POTENTIALLY SENSITIVE SUBPOPULATIONS

Endogenous Factors

Children—Limited information is available on the toxic potential of solvents in children. Most age-dependent differences are less than an order of magnitude, usually varying no more than two- to threefold. T e younger and more immature the subject, the more different is his or her response from that of adults.

GI absorption of solvents varies little with age, because most solvents are absorbed by passive diffusion. Systemic absorption of inhaled VOCs may be greater in infants and children than in adults owing to the relatively high cardiac output and respiratory rates despite their lower alveolar surface area. Extracellular water, expressed as percentage of body weight, is highest in newborns and gradually diminishes through childhood. Body fat content is high from ~1/2 to 3 years of age, and

then steadily decreases until adolescence, when it increases again in females. Lipophilic solvents accumulate in adipose tissue, so more body fat would result in greater body burdens and slower clearance of the chemicals.

Changes in xenobiotic metabolism during maturation may impact susceptibility to solvent toxicity. P450 isoforms develop asynchronously. Increased rates of metabolism, urinary excretion, and exhalation by children should hasten elimination and reduce body burdens of solvents. However, the net effect of immaturity on solvent disposition and toxicity is difficult to predict.

Elderly—Age influences the distribution of xenobiotics in the body as well as their metabolism and elimination. With aging, body fat usually increases substantially at the expense of lean mass and body water. Thus, relatively polar solvents tend to reach higher blood levels during exposures. Relatively lipid-soluble solvents accumulate in adipose tissue and are released slowly. Cardiac output and renal and hepatic blood flows are diminished in the elderly.

The elderly, like infants and children, may be more or less sensitive to the toxicity of solvents than young adults. Greater organ system toxicity could be due to increased inflammatory damage or to age-related dysregulation of cytokines. It must be taken into account that memory, attention, visual perception, and motor skills diminish with aging, even in the absence of chemical exposure. Other major sources of variability and complexity in geriatric populations include inadequate nutrition, the prevalence of disease states, and the concurrent use of multiple medications.

Gender—Physiologic and biochemical differences between men and women have the potential to alter tissue dosimetry and health effects of certain solvents. Whereas most predictive models suggest effects of toxicants are independent of sex, physical differences, such as the tendency of men to have more lean body mass and a larger body size, could potentially cause physiologic differences.

Genetics—Genetic polymorphisms for biotransformation occur at different frequencies in different ethnic groups. Polymorphisms for xenobiotic-metabolizing enzymes may affect the quantity and quality of enzymes and the outcomes of exposures to solvents in different racial groups. Disentangling the influences of genetic traits from those of socioeconomic status, lifestyles, and geographic setting is difficult.

Exogenous Factors

P450 Inducers and Inhibitors—Preexposure to chemicals that induce or inhibit biotransformation enzymes can potentiate or reduce the toxicity/carcinogenicity of high doses of solvents that undergo metabolism. Inhibitors would generally be anticipated to enhance the toxicity of solvents that are metabolically inactivated and protect from solvents that undergo metabolic activation.

Physical Activity—Exercise increases alveolar ventilation and cardiac output/pulmonary blood flow. Polar solvents with relatively high blood:air PCs (e.g., acetone, ethanol, and ethylene glycol [EG]) are very rapidly absorbed into the pulmonary circulation. Alveolar ventilation is rate-limiting for these chemicals. In contrast, pulmonary blood flow and metabolism are rate-limiting for uptake of more lipophilic solvents. Heavy exercise can increase pulmonary uptake of relatively polar solvents as much as fivefold in human subjects. Light exercise doubles uptake of relatively lipid-soluble solvents, but no further increase occurs at higher workloads. Blood flow to the liver and kidneys diminishes with exercise, which may diminish biotransformation of metabolized solvents and urinary elimination.

Diet—The mere presence of food in the stomach and intestines can inhibit systemic absorption of ingested chemicals by preventing contact of the chemical with the GI epithelium. VOCs in the GI tract partition into dietary lipids, largely remaining there until the lipids are emulsified and absorbed. Food intake results in increased splanchnic blood flow, which favors GI absorption, hepatic blood flow, and biotransformation. Foods may contain certain natural constituents, pesticides, and other chemicals that may enhance or reduce solvent metabolism. In addition, fasting for 1 to 3 days results in decreased detoxification of electrophilic metabolites (especially since hepatic glutathione levels are decreased) and the formation of cytotoxic, mutagenic, metabolites.

Chronic consumption of ethanol potentiates the hepatic or renal damage caused by hepatotoxic or renotoxic solvents, such as CCL_4, 1,1,1-trichloroethane, 1,1,2-trichloroethylene, or tetrachloroethylene. Many medications and nicotine and other components of tobacco smoke can induce metabolism of solvents.

Disease can have an important influence on solvent toxicity. Many illnesses impair hepatic metabolism and biliary and renal elimination. Cirrhosis, hepatitis, chronic kidney disease, diabetes mellitus, and gram-negative infections that release endotoxin may decrease solvent toxicokinetics and toxicity.

CHLORINATED HYDROCARBONS

Trichloroethylene

1,1,2-Trichloroethylene (TCE) is a widely used solvent for metal degreasing. Moderate to high doses of TCE, as with other halocarbons, are associated with a number of noncancer toxicities including autoimmune disorders, immune system dysfunction, and potentially a male reproductive toxicant. Cancer remains the dominant issue for TCE.

Metabolism and Modes of Action—Toxicities associated with TCE are predominantly mediated by metabolites rather than by the parent compound. Even the CNS-depressant effects of TCE are due in part to the sedative properties of the metabolite trichloroethanol (TCOH). After either oral or inhalational

absorption, most of the TCE undergoes oxidation via cytochrome P450s, with a small proportion being conjugated with glucuronic acid and excreted in the urine. T ese metabolic pathways are implicated in the carcinogenicity of TCE: reactive metabolite(s) of the GSH pathway in kidney tumors in rats and oxidative metabolites in liver and lung tumors in mice.

Liver Cancer—TCE induces liver cancer in B6C3F1 mice but not in rats. T is differential susceptibility is due to the greater capacity of mice to metabolize TCE to an oxidative metabolite that stimulates peroxisome proliferation. Propagation results in an increased potential for oxidative DNA damage, lipid peroxidation, and decreased gap-junctional intercellular communication, all of which have been implicated in neoplastic transformation.

Kidney Cancer—TCE exposure by inhalation or the oral route results in kidney tumors in male but not female rats. T e susceptibility of the male rat can be explained by its greater capacity for TCE metabolism via the GSH pathway. TCE-induced kidney tumors are believed to result from reactive metabolite(s) of this pathway alkylating cellular nucleophiles, including DNA. T e resulting DNA mutations lead to alterations in gene expression, which in turn lead to neoplastic transformation and tumorigenesis via a genotoxic pathway.

Alternatively, proximal tubular cell cytotoxicity and subsequent tumor formation via a nongenotoxic mode of action could be induced by reactive metabolites that cause oxidative stress, alkylation of cytosolic and mitochondrial proteins, marked ATP depletion, and perturbations in Ca^{2+} homeostasis. Tubular necrosis ensues, with subsequent reparative proliferation that can alter gene expression and, in turn, alter the regulation of cell growth and differentiation. In fact, somatic mutations in the von Hippel–Lindau (VHL) tumor suppressor gene might be a specific and susceptible target of TCE.

Chronic tubular damage may be a prerequisite to TCE-induced renal cell cancer. Reactive metabolite(s) of the GSH pathway may have a genotoxic effect on the proximal tubule of the human kidney, but full development of a malignant tumor requires a promotional effect such as cell proliferation in response to tubular damage.

Lung Cancer—Inhaled TCE is carcinogenic to the mouse lung but not to that of the rat. Oral TCE is not carcinogenic to the lung, probably due to hepatic metabolism that limits the amount of TCE reaching the organ. T e primary target of TCE within the mouse lung is the nonciliated Clara cell. Cytotoxicity to these cells is characterized by vacuolization and increases in cell replication in the bronchiolar epithelium. Clara cells of the mouse efficiently metabolize TCE to toxic metabolites, including chloral. In mouse lung, Clara cells are more numerous and have a much higher concentration of metabolizing enzymes than rat lung.

Tetrachloroethylene

Tetrachloroethylene (perchloroethylene, PERC) is commonly used as a dry cleaner, fabric finisher, degreaser, rug and upholstery cleaner, paint and stain remover, solvent, and chemical intermediate. T e highest exposures usually occur in occupational settings via inhalation. PERC is the third most frequently found chemical contaminant in groundwater at hazardous waste sites in the United States.

T e systemic disposition and metabolism of PERC and TCE are quite similar, although PERC is much less extensively metabolized. Both chemicals are well absorbed from the lungs and GI tract, distributed to tissues according to their lipid content, partially exhaled unchanged, and metabolized by P450s. PERC is oxidized by hepatic P450s to a much lesser degree than TCE, though trichloroacetic acid is a common major metabolite. GSH conjugation is a minor metabolic pathway, quantitatively, for TCE and PERC. T e extent of GSH conjugation of PERC increases when the oxidative pathway becomes saturated at high exposure levels. Metabolic products are the primary contributors to PERC-induced nephrotoxicity.

PERC-induced hepatic injury is believed to be a consequence of its intermediate metabolites: PERC oxide and trichloroacetyl chloride. T e many epidemiologic studies of cancer incidence and mortality in groups of persons occupationally exposed to PERC are equivocal and do not support a cause-and-effect relationship between either PERC or TCE and cancer. Cigarette smoking and alcohol consumption only partially account for an increased rate of esophageal cancer. Kidney, liver, and lung cancer incidences did not appear to be elevated.

Methylene Chloride

Methylene chloride (dichloromethane, MC) is used widely as a solvent in industrial processes, food preparation, degreasing agents, aerosol propellants, and agriculture. T e primary route of exposure to this very volatile solvent is inhalation.

MC is rapidly absorbed and distributed throughout the body and has limited systemic toxicity potential. High, repeated inhalation exposures produce slight, reversible changes in the livers of rodents. Persons subjected to high vapor levels manifest kidney injury occasionally. Carbon monoxide that is formed from MC binds to hemoglobin to produce dose-dependent increases in carboxyhemoglobin. Residual neurologic dysfunction in MC-exposed workers has been reported.

Occupational and environmental MC exposures are of concern primarily because of MC's carcinogenicity in rodents and its potential as a human carcinogen. Epidemiologic studies of employees exposed to MC have revealed that cancer risks from occupational exposure to MC, if any, are quite small.

Carbon Tetrachloride

Carbon tetrachloride (CCl_4) is a classic hepatotoxin, but kidney injury is often more severe in humans. It also plays a significant role in atmospheric ozone depletion.

Early signs of hepatocellular injury in rats include dissociation of polysomes and ribosomes from rough endoplasmic reticulum, disarray of smooth endoplasmic reticulum, inhibition of protein synthesis, and triglyceride accumulation. CCl_4 undergoes metabolic activation, producing lipid peroxidation, covalent binding, and inhibition of microsomal ATPase

activity. Single cell necrosis, evident 5 to 6 h postdosing, progresses to maximal centrilobular necrosis within 24 to 48 h. Cellular regeneration is maximal 36 to 48 h postdosing. T e rate and extent of tissue repair are important determinants of the ultimate outcome of liver injury.

Perturbation of intracellular calcium (Ca^{2+}) homeostasis appears to be part of CCl_4 cytotoxicity. Increased cytosolic Ca^{2+} levels may result from influx of extracellular Ca^{2+} due to plasma membrane damage and from decreased intracellular Ca^{2+} sequestration. Elevation of intracellular Ca^{2+} in hepatocytes can activate phospholipase A_2 and exacerbate membrane damage. Elevated Ca^{2+} may also be involved in alterations in calmodulin and phosphorylase activity as well as changes in nuclear protein kinase C activity. High intracellular Ca^{2+} levels activate a number of catabolic enzymes including proteases, endonucleases, and phospholipases, which kill cells via apoptosis or necrosis. Increased Ca^{2+} may stimulate the release of cytokines and eicosanoids from Kupffer cells, inducing neutrophil infiltration and hepatocellular injury. CCl_4 hepatotoxicity is obviously a complex, multifactorial process.

Chloroform

Chloroform ($CHCl_3$, trichloromethane) is used primarily in the production of the refrigerant chlorodifluoromethane (Freon 22). Measurable concentrations of $CHCl_3$ are found in municipal drinking water supplies. $CHCl_3$ is hepatotoxic and nephrotoxic. It can invoke CNS symptoms at subanesthetic concentrations similar to those of alcohol intoxication and can sensitize the myocardium to catecholamines, possibly resulting in cardiac arrhythmias.

T e metabolite phosgene covalently binds hepatic and renal proteins and lipids, which damages membranes and other intracellular structures, leading to necrosis and subsequent reparative cellular proliferation that promotes tumor formation in rodents by irreversibly "fixing" spontaneously altered DNA and clonally expanding initiated cells. T e expression of certain genes, including myc and fos, is altered during regenerative cell proliferation in response to $CHCl_3$-induced cytotoxicity.

Although a rodent carcinogen, ingestion of $CHCl_3$ in small increments, similar to drinking water patterns of humans, fails to produce sufficient cytotoxic metabolite(s) per unit time to overwhelm detoxification mechanisms. Currently, $CHCl_3$ is classified as a probable human carcinogen (group B2).

AROMATIC HYDROCARBONS

Benzene

Benzene is derived primarily from petroleum and is used in the synthesis of other chemicals and as an antiknock agent in unleaded gasoline. Inhalation is the primary route of exposure in industrial and in everyday settings. Cigarette smoke is the major source of benzene in the home. Smokers have benzene body burdens which are 6 to 10 times greater than those of nonsmokers. Passive smoke can be a significant source of benzene exposure to nonsmokers. Gasoline vapor emissions and auto exhaust are the other key contributors to exposures of the general populace.

T e hematopoietic toxicity of chronic exposure to benzene may manifest initially as anemia, leukopenia, thrombocytopenia, or a combination of these. Bone marrow depression appears to be dose-dependent in both laboratory animals and humans. Continued exposure may result in marrow aplasia and pancytopenia, an often fatal outcome. Survivors of aplastic anemia frequently exhibit a preneoplastic state, termed myelodysplasia, which may progress to myelogenous leukemia.

T ere is strong evidence from epidemiologic studies that high-level benzene exposures result in an increased risk of acute myelogenous leukemia (AML) in humans. Evidence of increased risks of other cancers in such populations is less compelling.

Various potential mechanisms require the complementary actions of benzene and several of its metabolites for toxicity. (1) A number of benzene metabolites bind covalently to GSH, proteins, DNA, and RNA. T is can result in disruption of the functional hematopoietic microenvironment by inhibition of enzymes, destruction of certain cell populations, and alteration of the growth of other cell types. Covalent binding of hydroquinones to spindle-fiber proteins will inhibit cell replication. (2) Oxidative stress contributes to benzene toxicity. As the bone marrow is rich in peroxidase activity, phenolic metabolites of benzene can be activated there to reactive quinone derivatives, which can cause DNA damage, leading to cell mutation or apoptosis. Modulation of apoptosis may lead to aberrant hematopoiesis and neoplastic progression.

Toluene

Toluene is present in paints, lacquers, thinners, cleaning agents, glues, and many other products. It is also used in the production of other chemicals. Gasoline, which contains 5% to 7% toluene (w/w), is the largest source of atmospheric emissions and exposure of the general populace. Inhalation is the primary route of exposure, though skin contact occurs frequently. Toluene is a favorite of solvent abusers, who intentionally inhale high concentrations of the VOC.

Toluene is well absorbed from the lungs and GI tract. It rapidly accumulates in the brain, and subsequently, is deposited in other tissues according to their lipid content, with adipose tissue attaining the highest levels. Toluene is well metabolized, but a portion is exhaled unchanged.

T e CNS is the primary target organ of toluene and other alkylbenzenes. Manifestations of exposure range from slight dizziness and headache to unconsciousness, respiratory depression, and death. Occupational inhalation exposure guidelines are established to prevent significant decrements in psychomotor functions. Acute encephalopathic effects are rapidly reversible on cessation of exposure. Subtle neurologic effects have been reported in some groups of occupationally exposed individuals. Severe neurotoxicity is sometimes diagnosed in persons who have abused toluene for a prolonged period. Clinical signs include abnormal electroencephalographic (EEG) activity, tremors, and nystagmus, as well as impaired

hearing, vision, and speech. Magnetic resonance imaging has revealed permanent changes in brain structure, which correspond to the degree of brain dysfunction. T ese changes include ventricular enlargement, cerebral atrophy, and white matter hyperintensity, a characteristic profile termed toluene leukoencephalopathy.

Xylenes and Ethylbenzene

Large numbers of people are exposed to xylenes and ethylbenzene occupationally and environmentally. Xylenes and ethylbenzene, like benzene and toluene, are major components of gasoline and fuel oil. T e primary uses of xylenes industrially are as solvents and synthetic intermediates. Most of the aromatics released into the environment evaporate into the atmosphere.

Similar to toluene, xylenes and other aromatic solvents are well absorbed from the lungs and GI tract, distributed to tissues according to tissue blood flow and lipophilicity, exhaled to some extent, well metabolized by hepatic P450s, and largely excreted as urinary metabolites. Acute lethality of hydrocarbons (i.e., CNS depression) varies directly with lipophilicity. T ere is limited evidence that chronic occupational exposure to xylenes is associated with residual neurologic effects.

Xylenes and ethylbenzene have limited capacity to adversely affect organs other than the CNS. Mild, transient liver and/or kidney toxicity has been reported occasionally in humans exposed to high vapor concentrations of xylenes. T e majority of alkylbenzenes do not appear to be genotoxic or carcinogenic. Ethylbenzene and styrene are known animal carcinogens, but there are limited human data.

ALCOHOLS

Ethanol

Many humans experience greater exposure to ethanol (ethyl alcohol and alcohol) than to any other solvent. Ethyl alcohol is used as an additive in gasoline, as a solvent in industry, in many household products and pharmaceuticals including hand sanitizers, and in intoxicating beverages. Frank toxic effects are less important occupationally than injuries resulting from psychomotor impairment. Driving under the influence of alcohol is the major cause of fatal auto accidents. Blood alcohol level and the time necessary to achieve it are controlled largely by the rapidity and extent of ethanol consumption. Ethanol is distributed in body water and to some degree in adipose tissue. T e alcohol is eliminated by urinary excretion, exhalation, and metabolism. T e blood level in an average adult decreases by ~15 to 20 mg/dL per hour. T us, a person with a blood alcohol level of 120 mg/dL would require 6 to 8 h to reach negligible levels.

Ethanol is metabolized to acetaldehyde by three enzymes: (1) alcohol dehydrogenase (ADH) catalyzes oxidation of most of the ethanol to acetaldehyde, which is rapidly oxidized by acetaldehyde dehydrogenase (ALDH) to acetate; (2) catalase, utilizing H_2O_2 supplied by the actions of NADPH oxidase and xanthine oxidase, will normally account for more than 10% of ethanol metabolism; (3) CYP2E1, which is the principal isoform of the hepatic microsomal ethanol oxidizing system (MEOS).

ALDH activity is usually sufficiently high to metabolize large amounts of acetaldehyde to acetate. Caucasians, blacks, and Asians have varying percentages of different ALDH isozymes, which impact the efficiency of acetaldehyde metabolism. Some 50% of Asians have inactive ALDH, and these persons may experience flushing, headache, nausea, vomiting, tachycardia, and hyperventilation on ingestion of ethanol. Whereas this syndrome offers protection against developing alcoholism, it increases the risk of acetaldehyde-related cancers of the esophagus, stomach, colon, lung, head, and neck.

Gender differences in responses to ethanol are well recognized. Females exhibit slightly higher blood ethanol levels than men following ingestion of equivalent doses. T is phenomenon is due in part to more extensive ADH-catalyzed metabolism of ethanol by the gastric mucosa of males and to the smaller volume of distribution in women for relatively polar solvents such as alcohols. Also, women are more susceptible to alcohol-induced hepatitis and cirrhosis.

Fetal alcohol syndrome (FAS) is the most common preventable cause of mental retardation. Diagnostic criteria for FAS include (1) heavy maternal alcohol consumption during gestation; (2) pre and postnatal growth retardation; (3) craniofacial malformations including microcephaly; and (4) mental retardation. Less complete manifestations of gestational ethanol exposure are referred to as fetal alcohol spectrum disorder (FASD). Potential mechanisms causing FASD include (1) simple oxidative stress in fetal tissues, (2) alteration of neurotransmitter-gated ion channels such as the NMDA receptor, (3) alterations in the regulation of gene expression with reduced retinoic acid signaling or variant DNA methylation, (4) interference with mitogenic and growth factor responses involved in neural stem cell proliferation, (5) disturbances in molecules that mediate cell–cell interactions, and (6) derangements of glial proliferation, differentiation, and function. Overconsumption during all three trimesters of pregnancy can result in particular manifestations depending on the period of gestation during which insult occurs.

Human CYP2E1 is effective in production of reactive oxygen intermediates from ethanol that cause lipid peroxidation. Also, ethanol induces the release of endotoxin from gram-negative bacteria in the gut. T e endotoxin is taken up by Kupffer cells, causing the release of inflammatory mediators, which are cytotoxic to hepatocytes and chemoattractants for neutrophils.

Alcohol-induced tissue damage results from both nutritional disturbances and direct toxic effects. Malabsorption of thiamine, diminished enterohepatic circulation of folate, degradation of pyridoxal phosphate, and disturbances in the metabolism of vitamins A and D can occur. Prostaglandins released from endotoxin-activated Kupffer cells may be responsible for a hypermetabolic state in the liver. With the

increase in oxygen demand, the viability of centrilobular hepatocytes would be most compromised due to their relatively poor oxygen supply. Metabolism of ethanol via ADH and ALDH results in a shift in the redox state of the cell. T e resulting hyperlacticacidemia, hyperlipidemia, hyperuricemia, and hyperglycemia lead to increased steatosis and collagen synthesis.

Alcoholism can result in damage of extrahepatic tissues. Alcoholic cardiomyopathy is a complex process that may result from decreased synthesis of cardiac contractile proteins, attack of oxygen radicals, increases in endoplasmic reticulum Ca^{2+}-ATPase, and antibody response to acetaldehyde–protein adducts. Heavy drinking appears to deplete antioxidants and increases the risk of both hemorrhagic and ischemic strokes. T e brain and pancreas may be adversely affected in alcoholics.

T e associations between alcohol and cancers came primarily from epidemiologic case–control and cohort studies. Ethanol and smoking act synergistically to cause oral, pharyngeal, and laryngeal cancers. It is generally believed that alcohol induces liver cancer by causing cirrhosis or other liver damage and/or by enhancing the bioactivation of carcinogens.

Chronic ethanol consumption may promote carcinogenesis by (1) production of acetaldehyde, a weak mutagen and carcinogen; (2) induction of CYP2E1 with conversion of procarcinogens to carcinogens; (3) depletion of SAM and, consequently, global DNA hypomethylation; (4) increased production of inhibitory guanine nucleotide regulatory proteins and components of extracellular signal-regulated kinase-mitogen-activated protein kinase signaling; (5) accumulation of iron and associated oxidative stress; (6) inactivation of the tumor suppressor gene BRCA1 and increased estrogen responsiveness (primarily in the breast); and (7) impairment of retinoic acid metabolism.

Methanol

Methanol (methyl alcohol and wood alcohol) is found in a host of consumer products including windshield washer fluid, carburetor cleaners, and copy machine toner, and is used in the manufacture of formaldehyde and methyl tert-butyl ether. Serious methanol toxicity is most commonly associated with ingestion. Acute methanol poisoning in humans is characterized by an asymptomatic period of 12 to 24 h followed by formic acidemia, ocular toxicity, coma, and in extreme cases death. Visual disturbances develop between 18 and 48 h after ingestion and range from mild photophobia and blurred vision to markedly reduced visual acuity and complete blindness.

T e target of methanol within the eye is the retina, specifically the optic disk and optic nerve. Müller cells, rod, and cone cells are altered functionally and structurally, because cytochrome c oxidase activity in mitochondria is inhibited, resulting in a reduction in ATP.

T ough metabolized in liver, intraretinal conversion of methanol to formaldehyde and formate is critical. Metabolism of formate to CO_2 then occurs via a two-step, tetrahydrofolate (THF)-dependent pathway. Susceptibility to methanol toxicity is dependent on the relative rate of formate clearance. Conversion of formate to CO_2 is slower in primates than in rodents. In fact, formate acts as a direct ocular toxin and the acidotic state potentiates formate toxicity because the inhibition of cytochrome oxidase increases as pH decreases.

GLYCOLS

Ethylene Glycol

Ethylene glycol (EG) (1,2-dihydroxyethane) is a major constituent of antifreeze, deicers, hydraulic fluids, drying agents, and inks, and is used to make plastics and polyester fibers. T e most important routes of exposure are dermal and accidental or intentional ingestion. EG is rapidly degraded in environmental media.

T ree clinical stages of acute poisoning entail (1) a period of inebriation, the duration and degree depending on dose; (2) the cardiopulmonary stage 12 to 24 h after exposure, characterized by tachycardia and tachypnea, which may progress to cardiac failure and pulmonary edema; and (3) the renal toxicity stage 24 to 72 h postexposure. Metabolic acidosis can progress in severity during stages 2 and 3.

Absorption from the GI tract of rodents is very rapid and virtually complete. Dermal absorption in humans appears to be less extensive. EG is distributed throughout the total body water. As illustrated in Figure 24–2, EG is metabolized by NAD^+-dependent ADH to glycolaldehyde and on to glycolic acid. Glycolic acid is oxidized to glyoxylic acid by glycolic acid oxidase and lactic dehydrogenase. Glyoxylic acid may be converted to formate and CO_2, or oxidized by glyoxylic acid oxidase to oxalic acid. Metabolic acidosis in humans appears to be due to accumulation of glycolic acid. Hypocalcemia can result from calcium chelation by oxalic acid to form calcium oxalate crystals. Deposition of these crystals in tubules of the kidney and small blood vessels in the brain is associated with damage of these organs. Acute renal failure may follow. Additionally, hippuric acid crystals and direct cytotoxicity by other metabolites may act as damaging agents to the kidney in EG exposure. EG appears to have limited chronic toxicity potential, exhibits no evidence of carcinogenicity, and does not appear to be a reproductive toxicant.

Propylene Glycol

Propylene glycol (PG) is used as an intermediate in the synthesis of polyester fibers and resins, as a component of automotive antifreeze/coolants, and as a deicing fluid for aircraft. As PG is "generally recognized as safe" by the FDA, it is a constituent of many cosmetics and processed foods. Furthermore, it serves as a solvent/diluent for a substantial number of oral, dermal, and intravenous drug preparations. T e most important routes of exposure are ingesting and dermal contact. PG is readily metabolized by ADH to lactaldehyde, which is then oxidized by aldehyde dehydrogenase to lactate. Excessive lactate is

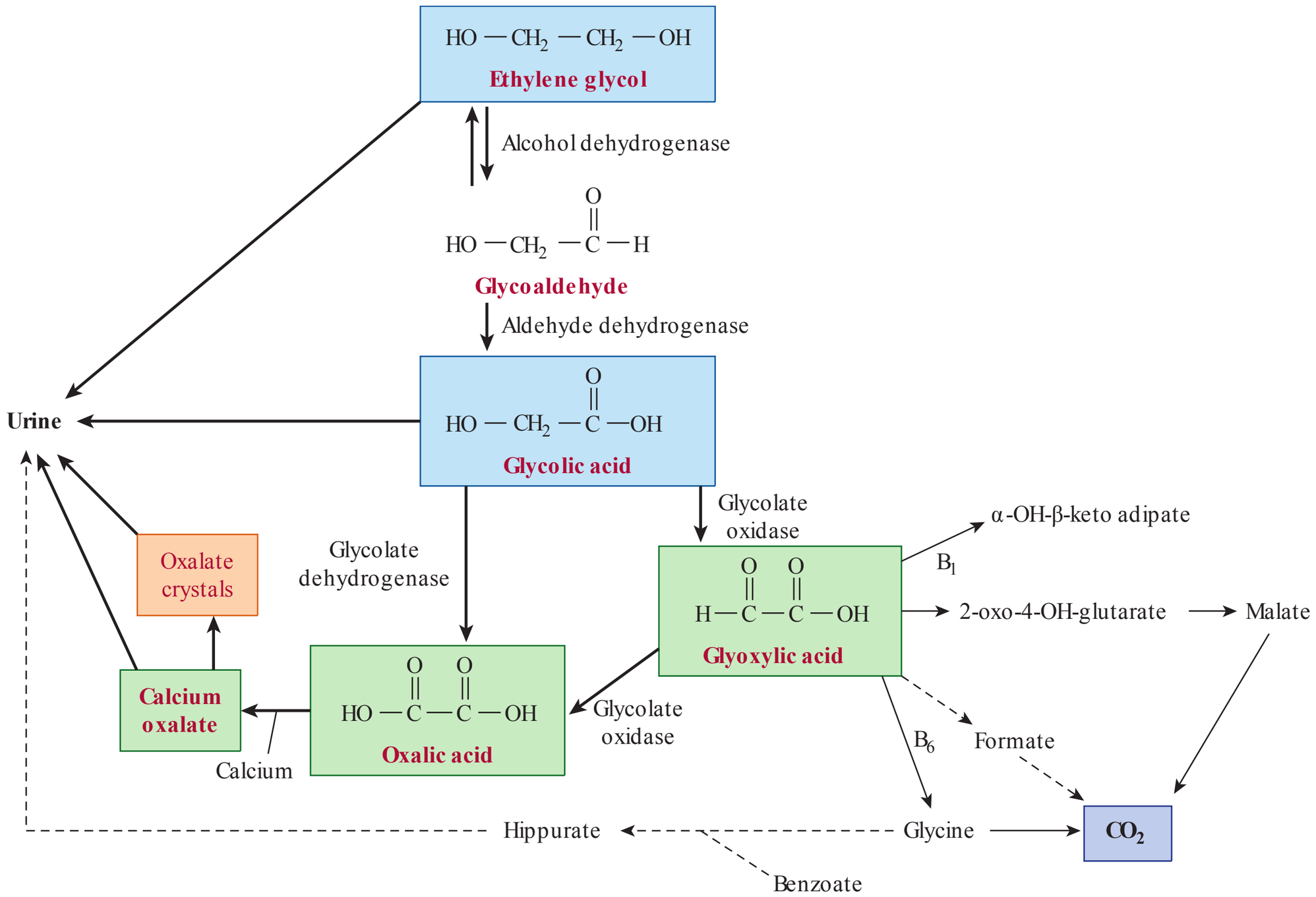

FIGURE 24–2 **Metabolic scheme for ethylene glycol in animals.** Key metabolites that have been observed in vivo are highlighted in boxes. Dashed lines are theoretical pathways that have not been verified in vivo or in vitro. (Adapted with permission from Corley RA, Bartels MJ, Carney EW, et al.: Development of a physiologically based pharmacokinetic model for ethylene glycol and its metabolite, glycolic acid, in rats and humans. Toxicol Sci, 2005 May;85(1):476–490.)

primarily responsible for the acidosis. PG has a very low order of acute and chronic toxicity.

GLYCOL ETHERS

T e glycol ethers include EG monomethyl ether, also called 2-methoxyethanol (2-ME; **CH3**—**O**—CH_2—CH_2—OH), EG dimethyl ether (**CH3**—**O**—CH_2—CH_2—**O**—**CH3**), 2-butoxyethanol (2-BE; **CH3**—**CH2**—**CH2**—**CH2**—**O**—CH_2—CH_2—OH), and 2-ME acetate (**CH3**—**CO**—**O**—CH_2—CH_2—O—CH_3). T ese solvents undergo rapid ester hydrolysis in vivo, and exhibit the same toxicity profile as unesterified glycols. T e glycol ethers are metabolized to alkoxyacetic acids, which are regarded as the ultimate toxicants. T eir acetaldehyde precursors have also been implicated.

Like glycol ether metabolism, glycol ether toxicity varies with chemical structure. With increasing alkyl chain length, reproductive and developmental toxicity decrease, whereas hematotoxicity increases.

Reproductive Toxicity

Epidemiologic studies have reported associations between glycol ether exposure and increased risk for spontaneous abortion, menstrual disturbances, and subfertility among women employed in the semiconductor industry. Reversible spermatotoxicity in males has been described for those exposed to glycol ethers. Typical responses include testicular and seminiferous tubule atrophy, abnormal sperm head morphology, necrotic spermatocytes, decreased sperm motility and count, and infertility.

Developmental Toxicity

Developmental toxicity in rodents includes a variety of minor skeletal variations, hydrocephalus, exencephaly, cardiovascular malformations, dilatation of the renal pelvis, craniofacial malformations, and digit malformations. T ere are significant associations for glycol ether exposure inducing cleft lip.

Hematotoxicity

Some glycol ethers are hemolytic to red blood cells. Typically, the osmotic balance of the cells is disrupted, they imbibe water and swell, their ATP concentration decreases, and hemolysis occurs. Humans are less susceptible than rodents to glycol ether–induced erythrocyte deformity and hemolysis.

AUTOMOTIVE GASOLINE AND ADDITIVES

Gasoline is a mixture of hundreds of hydrocarbons predominantly in the range of C_4 to C_{12}. Because its composition varies with the crude oil from which it is refined, the refining process, and the use of specific additives, generalizations regarding the toxicity of gasoline must be made carefully. Experiments conducted with fully vaporized gasoline may not be predictive of actual risk, because humans are exposed primarily to the more volatile components in the range of C_4 to C_5, which are generally less toxic than higher molecular-weight fractions.

The most extreme exposures occur to those intentionally sniffing gasoline for its euphoric effects. This dangerous habit can cause acute and chronic encephalopathies that are expressed as both motor and cognitive impairment. Ingestion of gasoline during siphoning events is typically followed by a burning sensation in the mouth and pharynx, as well as nausea, vomiting, and diarrhea resulting from GI irritation. Gasoline aspirated into the lungs may produce pulmonary epithelial damage, edema, and pneumonitis.

Oxygenated gasoline contains additives that boost its octane quality, enhance combustion, and reduce exhaust emissions. Benzene and 1,3-butadiene are classified as known or probable human carcinogens. The co-exposure of ethanol and gasoline shows additive and possibly synergistic toxic effects on growth, neurochemistry, and histopathology of the adrenal gland and respiratory tract. No significant epidemiologic association exists between methyl tertiary-butyl ether (MTBE) exposure and the acute symptoms commonly attributed to MTBE, including headache; eye, nose, and throat irritation; cough; nausea; dizziness; and disorientation. Because three MTBE animal cancer bioassays indicate kidney and testicular tumors in male rats and liver adenomas, leukemia, and lymphoma in female rats, MTBE is classified as a possible human carcinogen (group C).

CARBON DISULFIDE

The major uses of CS_2 are in the production of rayon fiber, cellophane, and CCl_4, and as a solubilizer for waxes and oils. Human exposure is predominantly occupational. Two distinct metabolic pathways for CS_2 exist: (1) the direct interaction of CS_2 with free amine and sulfhydryl groups of amino acids and polypeptides to form dithiocarbamates and trithiocarbonates; and (2) microsomal metabolism of CS_2 to reactive sulfur intermediates capable of covalently binding tissue macromolecules. The conjugation of CS_2 with sulfhydryls of cysteine or GSH results in the formation of 2-thiothiazolidine-4-carboxylic acid (TTCA), which is excreted in urine and has been frequently used as a biomarker of CS_2 exposure.

CS_2 is capable of targeting multiple organ systems including the cardiovascular system, CNS and PNS, male and female fertility, and eyes (retinal angiopathy and impairment of color vision). CS_2 toxicity requires frequent and prolonged exposures in occupational settings. The most common neurotoxic effect is a distal sensorimotor neuropathy that preferentially affects long axons in the PNS and CNS (particularly the ascending and descending tracks of the spinal cord and the visual pathways). Encephalopathy with motor and cognitive impairment has also been reported following chronic, low-level exposure to CS_2. The following clinical syndromes have been associated with CS_2: (1) acute and chronic encephalopathy (often with prominent psychiatric manifestations), (2) polyneuropathy (both peripheral and cranial), (3) Parkinsonism, and (4) asymptomatic CNS and PNS dysfunction. Pathological changes occur in both the CNS and PNS. CNS pathology consists of neuronal degeneration throughout the cerebral hemispheres, with maximal diffuse involvement in the frontal regions. Cell loss is also noted in the globus pallidus, putamen, and cerebellar cortex, with loss of Purkinje cells. Vascular abnormalities with endothelial proliferation of arterioles may be seen, sometimes associated with focal necrosis or demyelination. PNS changes consist primarily of myelin swelling and fragmentation and large focal axonal swellings, characteristic of distal axonopathy.

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QUESTIONS

1. Which of the following statements regarding solvents is FALSE?
   a. Solvents can be absorbed from the GI tract and through the skin.
   b. Equilibration of absorbed solvents/vapors occurs most quickly in the lungs.
   c. Solvents are small molecules that lack charge.
   d. Volatility of solvents increases with molecular weight.
   e. Most solvents are refined from petroleum.

2. What is the route in which most solvents enter the environment?
   a. chemical spills.
   b. contamination of drinking water.
   c. evaporation.
   d. improper waste disposal.
   e. wind.

3. All of the following statements are true EXCEPT:
   a. Most solvents can pass freely through membranes by diffusion.
   b. A solvent's lipophilicity is important in determining its rate of dermal absorption.
   c. Hydrophilic solvents have a relatively low blood:air partition coefficient.
   d. Biotransformation of a lipophilic solvent can result in the production of a mutagenic compound.
   e. Hepatic first-pass metabolism determines the amount of solvent absorbed in the GI tract.

4. Which of the following statements regarding age solvent toxicity is TRUE?
   a. GI absorption is greater in adults than it is in children.
   b. Polar solvents reach higher blood levels in the elderly than they do in children.
   c. Children are always more susceptible to solvent toxicity than are adults.
   d. Increased alveolar ventilation increases uptake of lipid-soluble solvents to a greater extent than water-soluble solvents.
   e. Increased body fat percentage increases clearance of solvent chemicals.

5. Huffing gasoline can result in which of the following serious health problems?
   a. renal failure.
   b. pneumothorax.
   c. Hodgkin's disease.
   d. encephalopathy.
   e. thrombocytopenia.

6. Which of the following statements regarding benzene is FALSE?
   a. High-level exposure to benzene could result in acute myelogenous leukemia (AML).
   b. Gasoline vapor emissions and auto exhaust are the two main contributors to benzene inhalation.
   c. Benzene is used as an ingredient in unleaded gasoline.
   d. Benzene metabolites covalently bind DNA, RNA, and proteins and interfere with their normal functioning within the cell.
   e. Reactive oxygen species can be derived from benzene.

7. Which of the following is NOT a criterion for fetal alcohol syndrome diagnosis?
   a. maternal alcohol consumption during gestation.
   b. pre and postnatal growth retardation.
   c. microcephaly.
   d. ocular toxicity.
   e. mental retardation.

8. Which of the following is NOT an important enzyme in ethanol metabolism?
   a. alcohol dehydrogenase.
   b. formaldehyde dehydrogenase.
   c. CYP2E1.
   d. catalase.
   e. acetaldehyde dehydrogenase.

9. Which of the following is NOT associated with glycol ether toxicity?
   a. irreversible spermatotoxicity.
   b. craniofacial malformations.
   c. hematotoxicity.
   d. seminiferous tubule atrophy.
   e. cleft lip.

10. Which of the following statements regarding chlorinated hydrocarbons is FALSE?
    a. Toxicities of trichloroethylene (TCE) are mediated mostly by reactive metabolites, not the parent compound.
    b. Glutathione conjugation is an important metabolic step of both trichloroethylene (TCE) and perchloroethylene (PERC).
    c. Many chlorinated hydrocarbons are used as degreasing agents.
    d. Chloroform interferes with intracellular calcium homeostasis.
    e. Carbon tetrachloride causes hepatocellular and kidney toxicity.

CHAPTER

25

Toxic Effects of Radiation and Radioactive Materials

David G. Hoel

INTRODUCTION

RADIATION BACKGROUND

- Types of Ionizing Radiation
- Relative Biologic Effectiveness and Quality Factors
- Units of Radiation Activity and Dose

RADIOBIOLOGY

- Nontargeted Radiation Effects
  - Bystander Effects
  - Genomic Instability
  - Adaptive Response
- Gene Expression
- Summary

CANCER EPIDEMIOLOGY

- Occupational Studies
- Nonoccupationally Exposed Groups
- Radionuclides
  - Radon
  - Radium
  - Plutonium
  - Radioiodine

NONCANCER EPIDEMIOLOGY

- Cardiovascular Disease
- Cataracts
- Mental Effects

DISCUSSION

KEY POINTS

- The four main types of radiation are due to alpha particles, electrons (negatively charged beta particles or positively charged positrons), gamma-rays, and X-rays.
- Alpha particles are helium nuclei (consisting of two protons and two neutrons), with a charge of +2, that are ejected from the nucleus of an atom.
- Beta particle decay occurs when a neutron in the nucleus of an element is effectively transformed into a proton and an electron, which is ejected.
- Gamma-ray emission occurs in combination with alpha, beta, or positron emission or electron capture. Whenever the ejected particle does not utilize all the available energy for decay, the excess energy is released by the nucleus as photon or gamma-ray emission coincident with the ejection of the particle.
- The Compton Effect occurs when a photon scatters at a small angle from its original path with reduced energy because part of the photon energy is transferred to an electron.
- Ionizing radiation loses energy when passing through matter by producing ion pairs (an electron and a positively charged atom residue).
- Radiation may deposit energy directly in DNA (direct effect) or may ionize other molecules closely associated with DNA, hydrogen, or oxygen, to form free radicals that can damage DNA (indirect effect).

INTRODUCTION

Ionizing radiations such as γ-rays and X-rays are radiations that have sufficient energy to displace electrons from molecules. T ese freed electrons then have the capability of damaging other molecules and, in particular, DNA. Atoms of the DNA target may be directly ionized or indirectly affected by the creation of a free radical that can interact with the DNA molecule. In particular, the hydroxyl radical is predominant in DNA damage. T us, the potential health effects of low levels of radiation are important to understand in order to be able to quantify their effects. Cancer has been the major adverse health effect of ionizing radiation. National Council on Radiation Protection (NCRP) Report 160 gives a summary breakdown of exposure sources in Figure 25–1.

RADIATION BACKGROUND

Types of Ionizing Radiation

When ionizing radiation passes through matter, it has the energy to ionize atoms so that one or more of its electrons can be dislodged and chemical bonds broken. Ionizing radiation is of two types: particulate and electromagnetic waves. Particulate radiation may either be electrically charged (α, β, proton) or have no charge (neutron). Ionizing electromagnetic radiation (photons) in the form of X-rays or γ-rays has considerably more energy than nonionizing radiation, such as ultraviolet and visible light. Radionuclides (i.e., radioactive atoms), being unstable, release both electromagnetic and particulate radiation during their radioactive decay. T e radionuclides decay into either stable elements or through a decay chain of successive radionuclides called decay daughters. T e types of radiation emitted, its rate of decay, and the energies of the released radiation are unique to each type of radionuclide. For example, the uranium decay series is illustrated in Figure 25–2, with specific details provided in Table 25–1.

T e rate of energy dissipation by a single event is referred to as linear energy transfer (LET). T e LET of a charged particle is the average energy lost due to interactions per unit length of its trajectory given as kiloelectron volts per micrometer (keV/μm). X-rays, γ-rays, and β particles of similar energies produce sparse ionization tracks and are classified as low-LET radiation. Particulate radiation (e.g., neutrons and α particles) causes interactions with large amounts of energy being dissipated within short distances. α-Particles (helium nucleus), which are released from the nucleus of some radionuclides, are slow-moving with a positive charge. Although they cannot penetrate a piece of paper or skin, they are of concern if ingested or inhaled. T e most recognized example is the lung cancer risk from the inhalation of radon (Rn 222) and its daughter products.

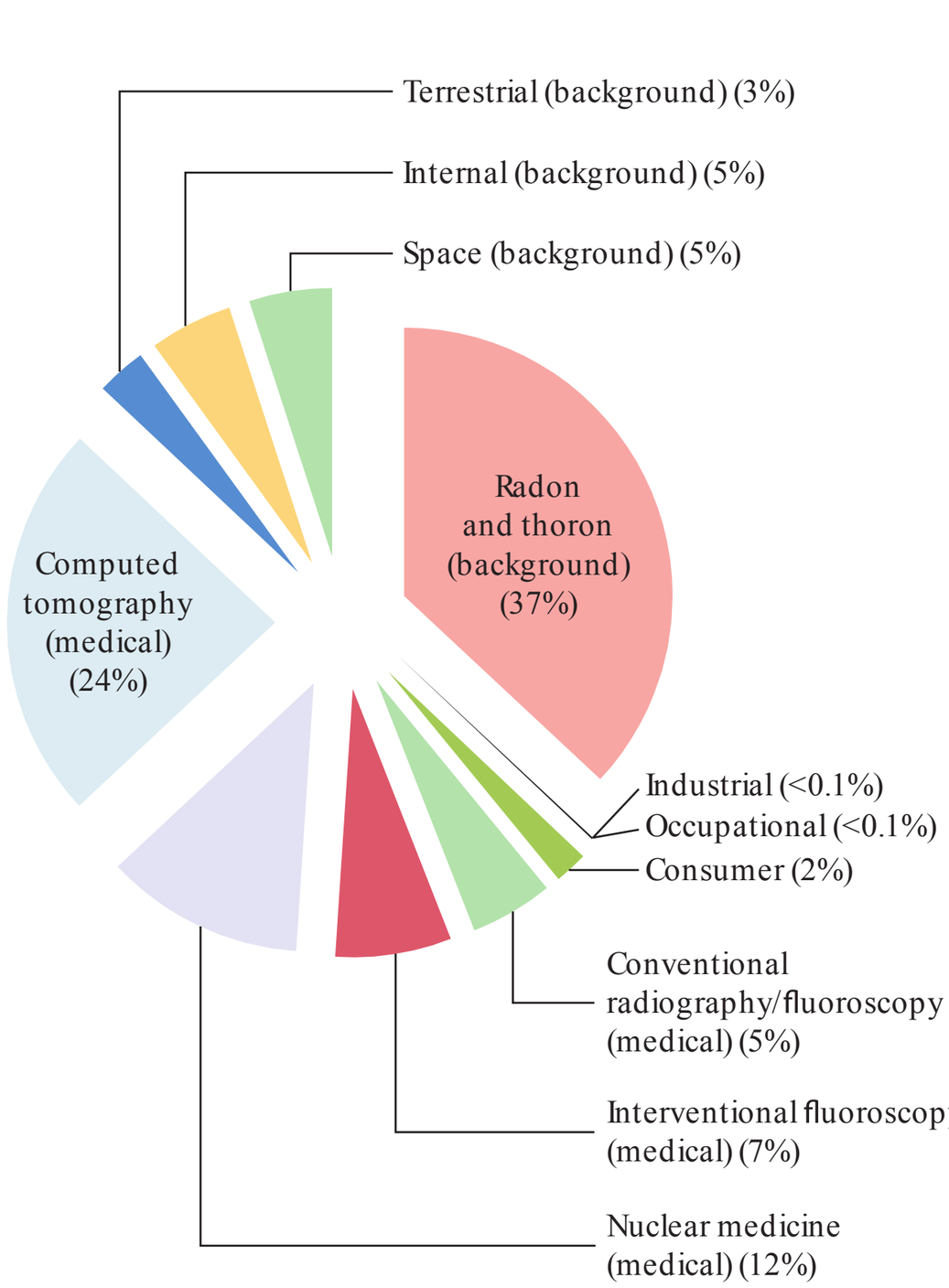

FIGURE 25–1 **Percent contribution of total effective dose to individuals** (Reproduced with permission from NCRP Report No. 160. Ionizing Radiation Exposure of the Population of the United States. Bethesda, MD: National Council on Radiation Protection and Measurements; 2009. http://NCRPpublications.org).

Relative Biologic Effectiveness and Quality Factors

T e various types of ionizing radiation have similar biologic effects that occur because of the ionization of molecules. However, without knowing the type of radiation, one cannot specify how much radiation is needed to produce a specific biologic effect. T is is because a given absorbed dose (energy per unit mass) of X-rays does not have the same biologic effect as an identical dose of neutrons. T e relative effectiveness of different types of radiation in producing biologic changes depends on deposition of energy. T e relative biologic effectiveness is numerically equal to the inverse of the ratio of absorbed doses of the two radiations required to produce equal biologic effects. T e difficulty is that the relative biologic effectiveness may differ depending on the biologic end point and it may also be dose-dependent.

Units of Radiation Activity and Dose

T e basic unit of radiation activity is the Becquerel (Bq), which is nuclear disintegrations per second. T e older unit of activity is the Curie (Ci), which corresponds to the number of disintegrations in 1 s from 1 g of radium 226 or 1 Ci = 3.7×10^{10} decays per second; thus, 1 Bq = 2.7×10^{-11} Ci. T e EPA continues to use the old unit of activity with regard to radon.

T e basic unit of dose is the Gray (Gy), which is the amount of energy released in a given mass of tissue. One Gray is defined as 1 joule of energy released in 1 kg of tissue. T e other common

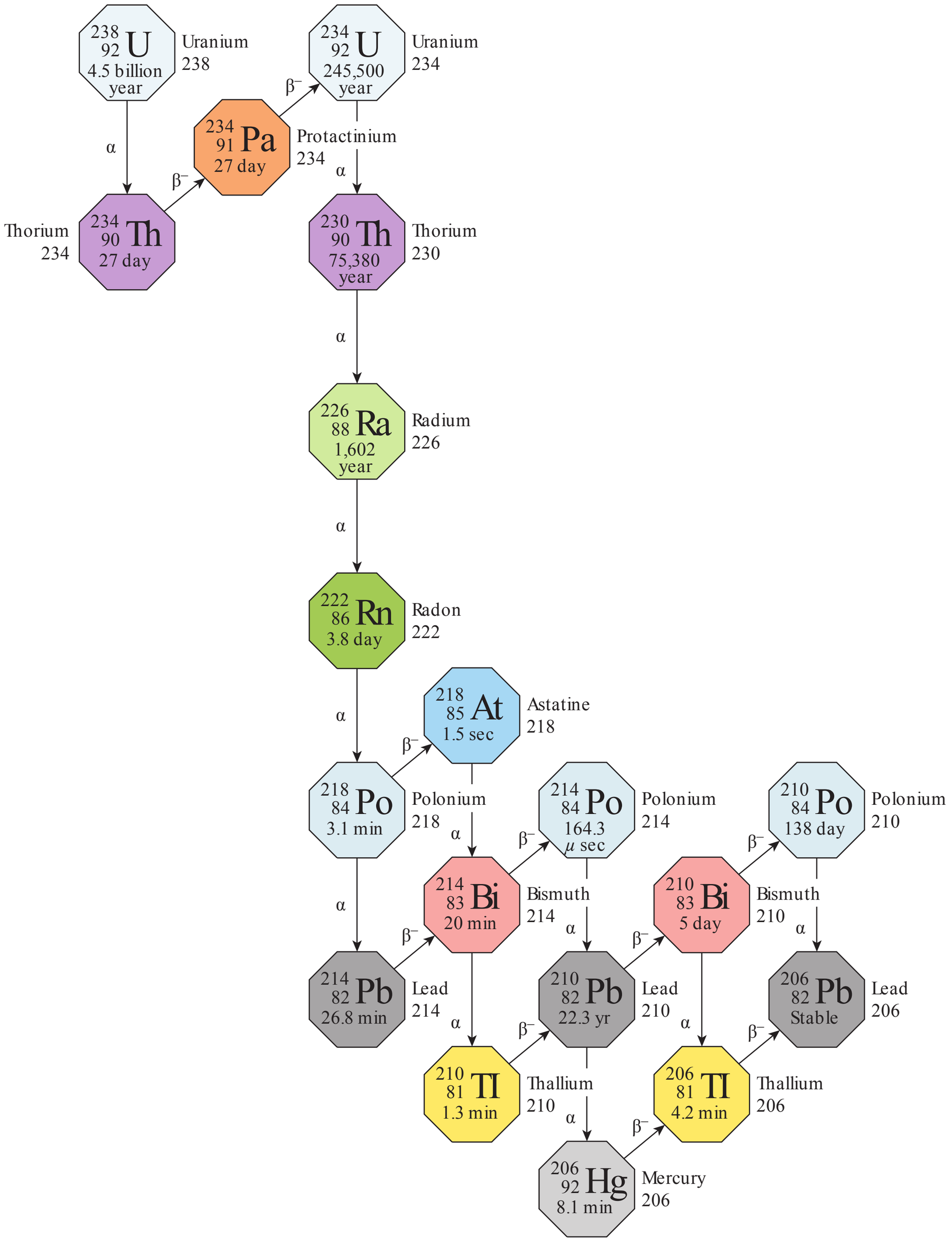

FIGURE 25–2 **Uranium decay chain.**

measure is the Sievert (Sv), which is a dose equivalent; that is, the dose in Gray multiplied by the appropriate quality factor.

RADIOBIOLOGY

Radiation biology has made significant progress in our understanding of radiation effects at low doses. Currently radiation cancer risk extrapolations make two assumptions: namely that the basic mode of action is linearly related to dose and that the individual cell is the unit of risk. However, effects occurring in nontargeted cells such as with induced genomic instability and bystander effects suggest that responses can occur nonuniformly over time at the tissue level. Following irradiation, various protective cellular processes occur that depend on the degree of damage and the tissue type. T ese mechanisms include DNA repair, intracellular metabolic oxidation/reduction reactions,

TABLE 25–1 Radioisotopes in the uranium decay series.

Nuclide	Decay Mode	Half-Life (a = Year)	Energy Released, MeV	Product of Decay
^{238}U	α	4.468×10^9 a	4.270	^{234}Th
^{234}Th	β^-	24.10 days	0.273	^{234m}Pa
^{234m}Pa	β− 99.84% IT 0.16%	1.16 min	2.271 0.074	^{234}U ^{234}Pa
^{234}Pa	β^-	6.70 h	2.197	^{234}U
^{234}U	α	245 500 a	4.859	^{230}Th
^{230}Th	α	75 380 a	4.770	^{226}Ra
^{226}Ra	α	1602 a	4.871	^{222}Rn
^{222}Rn	α	3.8235 days	5.590	^{218}Po
^{218}Po	α 99.98% β^- 0.02%	3.10 min	6.115 0.265	^{214}Pb ^{218}At
^{218}At	α 99.90% β^- 0.10%	1.5 s	6.874 2.883	^{214}Bi ^{218}Rn
^{218}Rn	α	35 ms	7.263	^{214}Po
^{214}Pb	β^-	26.8 min	1.024	^{214}Bi
^{214}Bi	β^- 99.98% α 0.02%	19.9 min	3.272 5.617	^{214}Po ^{210}Tl
^{214}Po	α	0.1643 ms	7.883	^{210}Pb
^{210}Tl	β^-	1.30 min	5.484	^{210}Pb
^{210}Pb	β^-	22.3 a	0.064	^{210}Bi
^{210}Bi	β^- 99.99987% α 0.00013%	5.013 days	1.426 5.982	^{210}Po ^{206}Tl
^{210}Po	α	138.376 days	5.407	^{206}Pb
^{206}Tl	β^-	4.199 min	1.533	^{206}Pb
^{206}Pb	–	Stable	–	–

cell cycle checkpoint controls, cellular signaling, senescence, and apoptosis.

A study reviewed in detail the effects of DNA damage after exposure to low doses of ionizing radiation. After an exposure to 5 mGy of low-LET radiation (average background per year), each cell nucleus is on average hit by one electron, resulting in 5 to 10 damaged bases, 2.5 to 5 single-strand breaks and 0.25 double-strand breaks.

Nontargeted Radiation Effects

Exposure to ionizing radiation can result in direct damage to the irradiated cells as well as producing effects in cells that were not irradiated (bystander effects). T ese nontargeted effects can occur in the nonirradiated neighbors of irradiated cells and at sites distant from the irradiated cells. Effects can also be observed in the progeny of an irradiated cell (genomic instability). Both targeted and nontargeted effects can result in DNA mutations, gene amplifications, chromosomal rearrangements, carcinogenesis, and cell death.

Bystander Ef ects—Radiation-induced bystander effects are those in which cells that have not been directly exposed to ionizing radiation react as though they have been exposed by receiving a biochemical signal from a radiation-exposed cell. T at is, they show chromosomal instability and other abnormalities, or die. For high-LET radiation a bystander effect has been shown for inducing cell lethality, chromosome aberrations, sister-chromatid exchanges, mutations, genomic instability, signal transduction pathways, and in vitro transformation. For low-LET radiation, the bystander effect has been limited to cell lethality and lethal mutations.

T ese bystander cells can be either adjacent or at some distance from the radiation-exposed cell. T e important issue from a risk assessment view is whether bystander effects are beneficial (e.g., adaptive response and apoptosis [removal of damaged cells]) or detrimental to the nonexposed cells, and what impact they may have on dose response at low doses. It should be noted that most observed effects are detrimental, but beneficial effects are more difficult to measure. However, bystander effects demonstrate that the organism and tissues communicate and are responding as an organized structure to radiation insult. Large DNA deletions are the major type of radiation-induced mutations. In bystander cells, however, the types of mutations are similar to those that occur spontaneously, with the majority being point mutations.

Genomic Instability—Genomic instability has been defined as the increase in rate of acquiring genetic change, and induced genomic instability can be observed in the progeny of irradiated cells and can persist for many generations. When a cell is saturated in repairing radiation damage it may change its gene-product profile without any specific genetic damage. T is has been suggested as a cause of genomic instability, which is an anti-inf ammatory response, and is a risk for malignancy.

Adaptive Response—In cells that are exposed to a low priming dose of radiation (e.g., 10 to 20 mGy) followed in a short time interval with a larger challenge dose (e.g., 1 Gy), the frequency of chromosomal aberrations induced by the challenge dose was found to be less than that from the challenge dose given alone. T is effect is referred to as "adaptive response." Studies have also shown that low doses of radiation may reduce the biologic background effect. T is has been shown for cell transformation and chromosomal damage. It has also been observed that the normal rate of cell transformation and chromosome damage can be decreased to below the normal background level after an initial low-dose radiation exposure. Adaptive responses have been observed both in vitro and in vivo for both cancer and genetic effects, which suggests that low doses may decrease radiation risk. T ese adaptive responses suggest that enhancing normal repair or protective processes make it possible to decrease the risk for low-dose radiation-induced cancer.

Gene Expression

It has been shown that dose, dose rate, radiation quality, and time since exposure result in variations in the response of genes, so that gene expression signatures may be markers of radiation exposure. Using gene expression methods, scientists have been able to distinguish a number of post-Chernobyl thyroid tumors and postradiotherapy thyroid tumors from their sporadic counterparts.

Summary

Cancer being the primary health concern from exposure to ionizing radiation, there is a focus on mechanisms and dose response as they relate to the induction of chromosomal aberrations and gene mutations because cancer is believed to be associated with these cellular responses. T e future of understanding low-dose radiation cancer risks will depend on the continued advancement of molecular biology, gene expression analysis, and computational biology.

CANCER EPIDEMIOLOGY

Epidemiologic studies have been extensive and provide the basis for our understanding of radiation-induced cancer effects. Radiation cancer studies are no different from other types of occupational and environmental cancer studies in that radiation-induced cancers are not distinguishable pathologically, and there are usual issues of exposure levels and durations, long latencies (e.g., 10 to 20 years for solid tumors), and study size. Generally for acute exposures only epidemiologic studies with exposures to relatively high doses of radiation (> 0.15 Sv) have shown such an excess of cancer. Because of these difficulties, the most informative studies are those that involve a large number of individuals with large radiation doses and follow-up of several decades. Table 25–2 lists radiation-linked human carcinogenicity.

Occupational Studies

T ere have been numerous studies over the years among nuclear workers, primarily at governmental facilities. In most of these studies, mortality rates were compared with those in the general population. In most cases, the cancer mortality rates were less than those for the general public, which may be due to the healthy worker effect. However, a series of analyses of workers at the Russian nuclear facility at Mayak have been published. T e Mayak workers generally experienced very high doses from both internal (plutonium, α particles) and external radiation exposures. High levels of body burdens of plutonium were found to have a relative risk of liver cancer and for bone cancer. Small nonsignificant increases were seen at low doses.

It is estimated that there are 2.3 million medical radiation workers worldwide. Epidemiologic studies of exposures of radiologists and radiologic technologists have been going on for many years. T ese workers were some of the earliest exposed to radiation with the first finding in 1902 that radiation can cause skin cancer. It was recognized in the 1940s that radiologists had increased rates of leukemia.

T e Chernobyl cleanup workers are of interest because of their higher exposures compared with other nuclear workers. T e excess relative risk for solid tumors was significant and the increase was observed in the highest dose interval with no increase in the lower-dose interval. Besides solid tumors, a significant increase in leukemia incidence was observed for those with increased exposures, compared with workers with lower exposures. Also, increases in the incidence of cardiovascular disease were observed among those at higher exposures.

TABLE 25–2 IARC: Tumor sites with sufficient evidence of human carcinogenicity.

Radiation Type	Major Study Populations	Tumor Sites (and Types) on Which Sufficient Evidence Is Based
α-particle and β-particle emitters		
Radon 222 and decay products	General population (residential exposure), underground miners	Lung
Radium 224 and decay products	Medical patients	Bone
Radium 226, radium 228, and decay products	Radium-dial painters	Bone, paranasal sinus, and mastoid process (radium 226 only)
Thorium 232 and decay products	Medical patients	Liver, extrahepatic bile ducts, gall bladder, leukemia (excluding CLL)
Plutonium	Plutonium-production workers	Lung, liver, bone
Phosphorus 32	Medical patients	Acute leukemia
Fission products, including strontium 90	General population, following nuclear reactor accident	Solid cancers, leukemia
Radioiodines, including iodine 131	Children and adolescents, following nuclear reactor accident	Thyroid
X-radiation or γ-radiation	Atomic-bomb survivors, medical patients; in utero exposure (of spring of pregnant medical patients and of atomic-bomb survivors)	Salivary gland, esophagus, stomach, colon, lung, bone, skin (BCC), female breast, urinary bladder, brain and CNS, leukemia (excluding CLL), thyroid, kidney (atomic-bomb survivors, medical patients); multiple sites (in utero exposure)
Solar radiation	General population	Skin (BCC, SCC, melanoma)
UV-emitting tanning devices	General population	Skin (melanoma), eye (melanoma, particularly choroid and ciliary body)

BCC, basal cell carcinoma; CLL, chronic lymphocytic leukemia; CNS, central nervous system; SCC, squamous cell carcinoma.

Nonoccupationally Exposed Groups

Studies of the population living in the high background radiation areas in Yangjiang, China evaluated dose reconstruction and noted a correlation between estimated radiation exposure and frequency of dicentric and ring chromosomes, which are recognized as a good biomarker of radiation exposure. They observed that for those in the high radiation background area, the incidence of these markers agrees with what has been observed in other studies of radiation exposures and chromosome aberrations. This result provides some evidence in support of the program's exposure estimates. In conclusion, the high-background Chinese studies have not shown an increase in cancer incidence at low-dose and dose-rate exposures.

A study of childhood cancers in relation to natural background radiation in Great Britain using the National Registry of Childhood Tumours involved radiation exposures estimated on the basis of the mother's residence at the time of birth. The study found a significant increase in leukemias in increased radiation exposure areas. Additionally, children who live within 5 km of a nuclear facility are also at an increased risk for the development of leukemia. The concept of population mixing, which basically is the idea that workers arriving in a typically rural area bring foreign infectious agents that in turn will affect local childhood leukemias, may explain increased leukemia effects in children when there is no evidence of radiation exposures from the nuclear plants.

The US National Research Council released recommendations on how a study could be carried out in the United States using cancer registry data. Previously the NCI analyzed cancer mortality rates in those counties with nuclear power reactors compared with control counties. Basically no differences were found; however, the use of counties as the analysis unit is likely inappropriate as a geographical area to detect any possible small effects.

Radionuclides

Radon—Radon is a natural radioactive gas produced by the decay of uranium and thorium. Originally, exposures to radon and its daughter radionuclides among uranium miners and some other groups of miners established that high exposures were a clear risk for lung cancer. The lung cancer risk was also significantly increased when the cases were restricted to exposures less than 200 Bq/m^3. The lung cancer effects were also consistent, with the risks projected downward from the higher exposed uranium miners.

Radium—There are 25 isotopes of radium of which four occur naturally (radium 223, 224, 226, and 228); the others are man-made or decay products of man-made radionuclides. Radium 226 with a half-life of 1601 years is by far the common natural form, followed by 228 with a half-life of 5.75 years. Radium 223 and 224 have half-lives of only a few days. Except for radium 228, which is a β emitter, the other three are all α emitters. The different isotopes have been used both occupationally as luminescent paint on watches and instruments (radium 226 and 228) and in medical applications (radium 223 and 224).

These uses, as well as radium found environmentally in drinking water, have provided material for many epidemiologic studies. Beginning in the 1920s, young women worked painting the dials of watches with paint containing radium 226 and 228. Many of them "pointed" the tips of their paintbrushes by mouth resulting in ingestion of relatively large amounts of radium for some of the women. Radium as a bone seeker resulted in increases in bone cancer as well as paranasal sinus cancers.

Bone sarcomas were also the major cancer effect among patients with tuberculosis and ankylosing spondylitis who were treated with high doses of radium 224 (mean bone surface dose of 30 Gy) in two cohort studies in Germany. There were increases in bone cancer in both studies, but there were also some increases in other cancer sites.

Plutonium—Plutonium is used for nuclear weapons production, and in the production of mixed oxide fuels. Most of the exposure to plutonium is to workers involved in the processing of plutonium in nuclear weapons (Pu 239) and in nuclear power generation (Pu 238). The major exposure to plutonium is by inhalation and is retained primarily in the lung, liver, and bone.

Radioiodine—Releases from nuclear facilities of fission-product radionuclides deposited in the environment as well as internal doses from the ingestion of foods containing fission products have been the result of the Chernobyl and Fukushima accidents. The major observable health effect has been childhood thyroid cancer resulting from the β emitter iodine 131. From studies of external radiation exposures in the A-bomb survivor studies as well as the children who were treated by radiation for tinea capitis (ring worm present on the scalp), it is clear that radiation is a risk for thyroid cancer for exposures to adolescents. The risk of radiation-related thyroid cancer was three times higher in iodine-deficient areas and the use of potassium iodide as a supplement reduced this risk of radiation-related thyroid cancer by a factor of 3.

NONCANCER EPIDEMIOLOGY

Cardiovascular Disease

What is of particular interest is cardiovascular disease (CV disease) mortality, because although it may have a lower relative risk from radiation exposure than solid tumors, it accounts for more total background deaths. Atherosclerosis is an inflammatory disease of the arteries, which can lead to ischemia of the heart. In a study of inflammatory biomarkers (TNF-α, IL-10, IgM, IgA, and IFN-γ) as well as erythrocyte sedimentation rates among A-bomb survivors, it was shown that these markers were associated with radiation exposure.

Cataracts

Cataracts were one of the earliest radiation-associated effects found after the discovery of X-rays. It has long been believed that it results from only high doses of radiation to the lens of the eye. There are basically three types of cataracts including nuclear or nuclear sclerosis, cortical, and posterior-subcapsular (PS) cataracts. Each of these clinical types has its known risk factors such as cigarette smoking for nuclear and possibly PS cataracts, while UV-B is a risk factor for cortical cataracts. Ionizing radiation is a risk factor for both cortical and PS cataracts but not nuclear sclerotic cataracts. Also there is limited evidence that those exposed at a younger age are at greater risk.

Mental Effects

In the A-bomb survivor analyses, significant effects on the developing brain were observed among those exposed during the period of the eighth week through the 25th week of gestation. During the most sensitive period of 8 to 15 weeks, there was an increased frequency of severe mental retardation, a diminution in IQ scores and school performances, as well as an increase in the occurrence of seizures. During this sensitive period there is a rapid increase in the number of neurons; they migrate to the cerebral cortex where they lose their capacity to divide, becoming perennial cells.

DISCUSSION

The major issue in radiation health effects is the causation of cancer. We now see noncancer effects such as CV disease at low-dose and also low dose-rate exposures that occur at environmental levels and in diagnostic medical screening. Currently the linear-no threshold (LNT) model is used to estimate these effects well below what can be observed in epidemiologic studies. The simple defense of the LNT model is that the physical energy deposition of ionizing radiation increases cancer risk linearly with increasing dose, and that the carcinogenic effectiveness is constant independent of dose.

It is recognized that a cell is not passively affected by the accumulation of lesions induced by ionizing radiation. The cell reacts through at least three main mechanisms: first by reacting against radiation-induced ROS; secondly by eliminating damaged cells by either apoptosis or through cell death during mitosis of unrepaired cells; and thirdly by immunosurveillance systems that eliminate clones of transformed cells.

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QUESTIONS

1. Which of the following is NOT a main type of radiation?
   a. alpha particles.
   b. microwaves.
   c. beta particles.
   d. gamma-rays.
   e. X-rays.

2. Which of the following statements regarding alpha particles is FALSE?
   a. Alpha particles are ejected from the nucleus of an atom.
   b. T e atomic number decreases by two after emission of an alpha particle.
   c. T e atomic weight decreases by two after emission of an alpha particle.
   d. Energies of most alpha particles range between 4 and 8 MeV.
   e. Alpha particles are helium nuclei.

3. Which of the following types of radiation is likely the MOST energetic?
   a. alpha particles.
   b. beta particles.
   c. positron emission.
   d. electron capture.
   e. photon emission.

4. Pair production and the Compton effect characterize which type of radiation's interaction with matter?
   a. alpha particles.
   b. beta particles.
   c. positron emission.
   d. electron capture.
   e. photon emission.

5. Which of the following statements regarding radiation DNA damage is FALSE?
   a. Ionizing radiation slows down by forming ion pairs.
   b. A main form of radiation DNA damage occurs by the production of free radicals.
   c. High-LET radiation causes more ionizations than does low-LET radiation.
   d. Most DNA damage caused by radiation happens directly.
   e. Direct and indirect ionization cause similar damage to DNA.

6. Low-LET radiation:
   a. causes large-scale ionizations throughout the cell.
   b. results from alpha particle emission.
   c. causes damage that is readily repaired by cellular enzymes.
   d. is also known as densely ionizing radiation.
   e. usually causes irreparable cell damage.

7. What is the most common type of DNA damage caused by low-LET radiation exposure?
   a. base damage.
   b. DNA protein cross-links.
   c. single-strand breaks.
   d. double-strand breaks.
   e. thymine-dimer formation.

8. Which of the following statements regarding radon exposure is FALSE?
   a. Miners are exposed to increased environmental radon levels.
   b. Radon exposure has been linked to the development of lung cancer.
   c. Smokers are at a higher risk from radon exposure.
   d. Radon levels are relatively higher in urban areas than in rural areas.
   e. T e use of open f ames indoors increases radon exposure.

9. T e largest dose of radiation is received from which of the following sources?
   a. inhalation.
   b. in body.
   c. cosmic.
   d. cosmogenic.
   e. terrestrial.

10. T e largest contributor to the effective dose of radiation in the U.S. population is which of the following?
    a. nuclear medicine.
    b. medical X-rays.
    c. terrestrial.
    d. internal.
    e. radon.

CHAPTER

26

Toxic Effects of Plants and Animals

John B. Watkins, III

INTRODUCTION

INTRODUCTION TO PLANT TOXICITIES

TOXIC EFFECTS BY ORGAN

- Skin
  - Irritant Contact Dermatitis
  - Allergic Contact Dermatitis
  - Photosensitivity
- Respiratory Tract
  - Allergic Rhinitis
  - Cough Reflex
- Gastrointestinal System
  - Direct Irritant Effects
  - Antimitotic Effects
  - Protein Synthesis Inhibition
- Cardiovascular System
  - Cardioactive Glycosides
  - Actions on Cardiac Nerves
  - Vasoactive Chemicals
- Liver
  - Hepatocyte Damage
  - Mushroom Toxins
  - Mycotoxins
- Kidney and Bladder
  - Carcinogens
  - Kidney Tubular Degeneration
- Blood and Bone Marrow
  - Anticoagulants
  - Bone Marrow Genotoxicity
  - Cyanogens
- Nervous System
  - Epileptiform Seizures
  - Excitatory Amino Acids
  - Motor Neuron Demyelination
  - Parasympathetic Stimulation
  - Parasympathetic Block
  - Sensory Neuron Block
- Skeletal Muscle and Neuromuscular Junction
  - Neuromuscular Junction
  - Skeletal Muscle Damage
- Bone and Tissue Calcification
  - Bone and Soft Tissue
- Reproduction and Teratogenesis
  - Abortifacients
  - Teratogens

CLINICAL STUDY OF PLANT POISONS

INTRODUCTION TO ANIMAL VENOMS

PROPERTIES OF ANIMAL TOXINS

ARTHROPODS

ARACHNIDA

- Scorpions
- Spiders
  - Agelenopsis Species (American Funnel Web Spiders)
  - Latrodectus Species (Widow Spiders)
  - Loxosceles Species (Brown or Violin Spiders)
  - Steatoda Species
  - Cheiracanthium Species (Running Spiders)
  - Theraphosidae Species (Tarantulas)
- Ticks

CHILOPODA (CENTIPEDES)

DIPLOPODA (MILLIPEDES)

INSECTA

- Heteroptera (True Bugs)
- Hymenoptera (Ants, Bees, Wasps, and Hornets)
  - Formicidae (Ants)
  - Apidae (Bees)
  - Vespidae (Wasps)
- Lepidoptera (Caterpillars, Moths, and Butterf ies)

MOLLUSCA (CONESNAILS)

REPTILES

- Lizards
- Snakes
  - General Information and Classification
  - Snake Venoms
  - Enzymes
  - Polypeptides
  - Toxicology
  - Snakebite Treatment

ANTIVENOM

POTENTIAL CLINICAL APPLICATION OF VENOMS

KEY POINTS

- Different portions of the plant (root, stem, leaves, seeds) often contain different concentrations of a toxic substance.
- T e age of a plant contributes to variability. Young plants may contain more or less of some constituents than mature plants.
- Climate and soil influence the synthesis of some toxins.
- Plants contain substances that may exert toxic effects on skin, lung, cardiovascular system, liver, kidney, bladder, blood, nervous system, bone, and the endocrine and reproductive systems.
- Contact dermatitis and photosensitivity are common skin reactions with many plants.
- Gastrointestinal effects range from local irritation to emesis and/or diarrhea.
- Cardiac glycosides in plants may cause nausea, vomiting, and cardiac arrhythmias in animals and humans.
- Venomous animals produce poison in a highly developed secretory gland or group of cells and can deliver their toxin during biting or stinging.
- Poisonous animals are those whose tissues, either in whole or in part, are toxic. Poisoning usually takes place through ingestion.
- T e bioavailability of a venom is determined by its composition, molecular size, amount or concentration gradient, solubility, degree of ionization, and the rate of blood flow into specific tissues.
- T e distribution of most venom fractions is rather unequal, being affected by protein binding, variations in pH, and membrane permeability, among other factors.
- A venom may be metabolized in several or many different tissues.
- Because of their protein composition, many toxins produce an antibody response; this response is essential in producing antisera.

INTRODUCTION

History is replete with stories of the earliest humans using plant extracts and animal venoms for hunting, war, assassination, and political intrigue for millennia. T e toxic properties of plants and animals often enhance their ability to survive. Some toxic compounds are used primarily to aid an animal in obtaining food while plants have developed toxic properties to specifically ward off being used as food. Toxins have been utilized as tools to study human biochemistry and physiology in order to pave the way for new pharmaceuticals. In fact, some components are in active development for clinical use.

INTRODUCTION TO PLANT TOXICITIES

T e plant kingdom contains potentially 300 000 species, and the toxic effects of plants serve primarily as defense mechanisms against natural predators. Toxic effects on humans can range from simple hay fever caused by exposure to plant pollen all the way to serious systemic reactions caused by ingestion of specific plants. Table 26–1 lists some of the poisoning syndromes plants can produce. Many variables that can affect the concentration of a plant's toxin and that can be a major factor in the severity of reaction one will experience on exposure include what part of the plant exposure is from, the age of the

TABLE 26–1 Poisoning syndromes caused by plants.

Syndrome	Genera	Mechanism(s)
Antimuscarinic	Atropa, Datura, Hyoscyanmus, Solanum	Blockade of muscarinic cholinoceptors
Cardiotoxic	Adenium, Digitalis, Convallaria, Nerium	Inhibition of cellular Na^+,K^+-ATPase increases contractility, enhanced vagal effect
Convulsants	Anemone, Conium, Labrunum, Nicotinia, Ranunculus	Blockade of gamma-aminobutyric acid (GABA) receptor on the neuronal chloride channel, alteration of acetylcholine homeostasis, mimic excitatory amino acids, sodium channel alteration, hypoglycemia
Cyanogenic	Eriobotrya, Hydrangea, Prunus	Gastric acid hydrolysis of cyanogenic glycosides releases cyanide
Dysrhythmia	Acotinum, Rhododendron, Veratrum	Sodium channel activation
Nicotinic	Conium, Laburnum, Lobelia, Nicotinia	Stimulation of nicotinic cholinoceptors
Pyrrolizidine	Crotalaria, Heliotropium, Senecia	Pyrroles injure endothelium of hepatic or pulmonary vasculature leading to veno-occlusive disease and hepatic necrosis
Toxalbumin	Abrus, Ricinus	Protein synthesis inhibitors leading to multiple organ system failure

plant, amount of sunlight and soil quality that the plant has grown in, and genetic differences within a species. Also, plant toxins fall under a number of different chemical structures, which is useful in understanding related toxins. Table 26–2 lists some of the common classifications.

TOXIC EFFECTS BY ORGAN

Skin

Irritant Contact Dermatitis—Plants that cause irritation of the skin on contact are rather common (Table 26–3). The trichomes, or barb-like hairs (Figure 26–1), found on stinging nettles (Urtica species, Urticaceae) puncture skin on contact and release an irritating sap containing a mixture of formic acid, histamine, acetylcholine, and serotonin. Mucuna pruriens (cowhage), which also deploys its toxin via barbed trichomes on contact, may cause pain, itching, erythema, and vesication. Mucinain, contained in the toxin, is the proteinase responsible for causing the pruritus.

Allergic Contact Dermatitis—Many people have experienced allergic dermatitis, most frequently from contact with poison ivy. Allergic dermatitis is an actual allergic reaction occurring within the skin as opposed to just a response to the presence of an irritant. Due to this immunological component, the severity of the reaction can range widely.

Philodendron scandens (Araceae, arum family) and the toxicodendron group of plants, which contain Rhus radicans (poison ivy, Figure 26–2), Rhus diversiloba (poison oak), and Rhus vernix (poison sumac), are all known to cause allergic dermatitis. In the Rhus species the allergen is a fat-soluble substance called urushiol that can penetrate the stratum corneum where it then binds to Langerhans cells in the epidermis. These haptenated cells then migrate to lymph nodes, where T cells are activated resulting in the allergic response.

TABLE 26–2 Chemical classification of plant toxins.

Chemical Category	Genera	Examples
Alkaloids	Atropa, Senecio, Nicotinia, Coffea, Papaver, Solanum, Acotinum	Tropines, pyrrolizidines, pyridines, purines, isoquinolines, steroids, diterpines
Glycosides	Digitalis, Aesculus	Steroids, coumarins
Proteinaceous compounds	Abrus, Amanitin, Lathyrus	Toxalbumins (abrin, ricin), polypeptides (amatoxins, phallotoxins, phalloidin), amines (aminopropionitrile)
Organic acids	Caladium, Dieffenbachia, Rheum	Oxalates
Alcohols	Cicuta, Eupatorium	Cicutoxin, tremetol
Resins and resinoids	Cannabis, Rhus	Tetrahydrocannabinol, urushiol

TABLE 26–3 Selective plants producing contact dermatitis.

Botanical Family	Genus Species	Common Name
Amaryllidaceae	Narcissus	Narcissus
Apocynaceae	Nerium oleander	Oleander
Bromeliaceae	Ananas comosus	Pineapple
Asteraceae	Ambrosia, Aster, Chrysanthemum, Rudbeckia hirta, Tagetes minuta	Ragweed, aster, chrysanthemum, Blackeyed Susan, Mexican marigold
Euphorbiaceae	Ricinus communis	Castor bean
Fumariaceae	Dicentra spectabilis	Bleeding heart
Ginkgoaceae	Ginkgo biloba	Ginkgo
Liliaceae	Allium cepa	Onion
Myrtaceae	Eucalyptus globulus	Eucalyptus
Pinaceae	Abies balsamea	Balsam fir
Saxifragaceae	Hydrangea	Hydrangea
Solanaceae	Lycopersicon esculentum, Solanum carolinense, S. turerosum	Tomato, horse nettle, potato
Umbelliferae	Daucus carota, Heracleum lanatum	Carrot, cow parsnip
Urticaceae	Urtica dioica, U. urens	Stinging nettle

Photosensitivity—Dermatitis does not necessarily have to be caused by skin contact. Consumption of Hypericum perforatum (St. John's wort) by animals can lead to serious dermatitis and even may be life threatening. The toxic agent is hypericin (a bianthraquinone) that, once ingested and dispersed systemically, causes photosensitization of the animal's skin. On exposure to sunlight, edematous lesions form on areas of skin that are not protected by hair such as the nose and ears.

FIGURE 26–1 **Stinging hairs of** Urtica ferox **(nettles).**

Respiratory Tract

Allergic Rhinitis—"Hay fever" or rhinitis from inhalation of plant pollens is a seasonal problem for many individuals. Trees, grasses, and weeds are all responsible to contributing to airborne pollen.

FIGURE 26–2 Toxicodendron radicans **(poison ivy).**

Grass species Poa and Festuca are major contributors along with pollen from several weed genera in the Asteraceae family (e.g., mugwort, Artemisia vulgaris, in Europe, and ragweed, Ambrosia sp., in North America).

Cough Reflex—Workers who process peppers have a significantly increased incidence of coughing when specifically handling Capsicum annuum (sweet pepper) and Capsicum *f*rutescens (red pepper). These two types of peppers produce the major irritants capsaicin and dihydrocapsaicin. Specific nerves in the airway have been found to be capsaicin-sensitive, which leads to the irritation and cough.

Gastrointestinal System

Direct Irritant Effects—Ingestion of a toxic plant can cause irritation of the gastrointestinal tract often resulting in nausea, vomiting, and diarrhea (Table 26–4). Toxic quinolizidine alkaloids are found in buffalo beans. Ingestion by children causes nausea, vomiting, dizziness, and abdominal discomfort. Also, consumption by livestock of the mature plant with seeds has been reported to be fatal.

Nuts from Aesculus hippocastanum (horse chestnut) and Aesculus glabra (Ohio buckeye) contain a glucoside called esculin. Ingestion by humans causes gastroenteritis, which increases in severity with the number of nuts consumed.

Antimitotic Effects—Podophyllotoxin is found in Podophyllum peltatum (May apple, Berberidaceae) especially in its foliage and roots. In low doses, mild purgation occurs; however, overdose results in nausea and severe paroxysmal vomiting. By binding microtubules, podophyllotoxin blocks mitosis from proceeding. This has made podophyllotoxin of interest for treatment of cancer.

Protein Synthesis Inhibition—The family Euphorbiaceae contains several genera that are known to be very toxic. The castor bean (Ricinus communis) is an ornamental plant that produces seeds that, if eaten by children or adults, causes no symptoms of poisoning for several days after ingestion. Gradually, gastroenteritis develops resulting in some loss of appetite, with nausea, vomiting, and diarrhea and can be deadly. The toxic agents are two lectins found in the beans: ricin I and ricin II of which ricin II is more toxic. Ricin II is made up of an A-chain and a B-chain. The B-chain is responsible for helping the A-chain get inside the cell. It binds to a terminal galactose residue on the cell membrane that then allows for the A-chain to be endocytosed. Once inside, the A-chain inactivates the 60s ribosomal subunit of cells

TABLE 26–4 Selective plants causing gastrointestinal irritation.

Common Name	Scientific Name	Toxic Part	Toxin
Amaryllis	Hippeastrum equestre	Bulb	Lycorine
Barberry	Berberis vulgaris	Root	Protoberberine and other isoquinoline alkaloids
Boxwood	Buxus sp.	Leaves, stems	Steroidal alkaloids
Buttercup	Ranunculus sp.	All parts	Ranunculin, protoanemonin
Crown of thorns	Euphorbia milii	All parts	Resiniferatoxin
Daffodil	Narcissus	All, especially bulb	Lycorine, narcissin, phenanthridine alkaloids
English Ivy	Hedera helix	All parts	Hederin from hederagenin
Euonymus	Euonymus sp.	All parts	Alkaloids
Hyacinth	Hyacinthus orientalis	Bulb	Calcium oxalate, lycorine
Iris	Iris	Bulb	Irritant resin
Mayapple	Podophyllum peltatum	Green fruit, roots	Podophyllotoxin
Mistletoe	Phoradendron flavescens	Berries, other parts	Phoratoxin
Pokeweed	Phytolacca americana	All parts	Phytolaccatoxin, related triterpines
Purging nut	Jatropha curcas	Seeds	Jatrophin (curcin) (toxalbumin)
Tung nut	Aleurites *f*ordii	Nut	Derivative of phorbol, saponins, toxalbumins
Wiseria	Wisteria sinensis	Pods	Wistarine (glycoside)

by catalytic depurination of an adenosine residue within the 28s rRNA, thereby blocking protein synthesis.

Cardiovascular System

Cardioactive Glycosides—Various plants that contain cardioactive glycosides include Digitalis purpurea (Figure 26–3), squill (Scilla maritima), which contains scillaren, lily of the valley, milkweeds, and other species listed in Table 26–5. The cardiac glycosides inhibit Na^+, K^+-ATPase.

Actions on Cardiac Nerves—Toxic alkaloids found in Veratrum viride (American hellebore, Liliaceae), Veratrum album (European hellebore), and Veratrum californicum cause nausea, emesis, hypotension, and bradycardia on ingestion. Veratrum album has been used for centuries to "slow and soften the pulse." The mixture of alkaloids includes protoveratrine, veratramine, and jervine that affects the heart by causing a repetitive response to a single stimulus resulting from prolongation of the sodium current. Aconitum species causes not only cardiac arrhythmias and hypotension, but ingestion causes gastrointestinal upset and neurological symptoms. This works by causing a prolonged sodium current with slowed repolarization of cardiac muscle and nerve fibers. Grayanotoxins bind to sodium channels in cardiac and muscle cells resulting in increased sodium conductance.

Vasoactive Chemicals—Mistletoe produces a toxin (phoratoxin and viscotoxin) that has marked effects on the cardiovascular system. It causes hypotension, vasoconstriction of the vessels in skin and skeletal muscle, and bradycardia resulting from negative inotropic actions on heart muscle.

Ingestion of the fungus Claviceps purpurea (ergot), which grows on grains that are used for food, causes vasoconstriction. In extreme cases, the vasoconstriction was severe enough that gangrene would develop in the extremities. Abortion in pregnant women is also common after ingestion of ergot-contaminated grains.

FIGURE 26–3 Digitalis purpurea **(common foxglove).**

Liver

Hepatocyte Damage—Ingestion of significant concentrations of pyrrolizidine alkaloids causes liver damage in the form of hepatic veno-occlusive disease associated with lipid peroxidation. Cattle that graze on grass contaminated with Senecio have been found to develop hepatitis that can progress to death

TABLE 26–5 Selective plants causing cardiotoxicity.

Common Name	Scientific Name	Toxic Part	Toxin
Azalea	Rhodendron sp.	All	Grayanotoxins
Death camus	Zigadenus	All	Zygadenine, veratrine
Foxglove	Digitalis sp.	Leaves, seeds	Digitalis glycosides
Larkspur	Delphenium ambiguum	All	Delphinine
Lily of the valley	Convallaria majalis	All	Convallarin, convallamarin
Milkweed	Asclepias sp.	Leaves, stem	(Hydroxycinnamoyl) desglucouzarin
Monkshood	Aconitum sp.	Leaves, roots, seeds	Aconitine, aconine
Oleander	Nerium oleander	All	Oleandrin, oleandrosine

if allowed to continue grazing. Human deaths have also been reported from consumption of contaminated wheat crops. The liver damage caused by ingestion clinically appears to be similar to cirrhosis and some hepatic tumors that can easily be mistaken to be the source of the disease.

Mushroom Toxins—Most nonedible mushrooms may cause mild discomfort and are not life threatening; however, repeated ingestion of the false morel, Gyromitra esculenta, has been found to cause hepatitis. Boiling generally inactivates the toxin gyromitrin. Most fatal poisonings related to wild mushrooms are from ingestion of different species within Amanita, Galerina, and Lepiota. Amanita phalloides (Figure 26–4) contains phalloidin and amatoxins. Phalloidin is capable of binding actin in muscle cells; however, it is not readily absorbed during digestion, which limits its harmful effects. The smaller α-, β-, and γ-amanitins are readily absorbed. Of the amatoxins, α-amanitin is the most toxic as it inhibits protein synthesis in hepatocytes by binding to RNA polymerase II. In addition to liver, intestinal mucosa and kidneys are also affected and serious clinical signs develop about three days after ingestion. In cases of severe poisoning, a liver transplant may be required. Amatoxin-α irreversibly inhibits acetylcholinesterase.

Mycotoxins—Fumonisin toxins are produced by the fungus Fusarium that is known to grow on corn. Ingestion in humans has been suggested to be associated with esophageal cancer.

Kidney and Bladder

Carcinogens—The bracken fern (P. aquilinum), which is extremely common worldwide, is the only higher plant known to be carcinogenic in animals under natural feeding conditions. The commonest bladder tumors in cattle are epithelial and mesenchymal neoplasms. Ptaquiloside, a norsesquiterpene glucoside, is the known carcinogen present in the fern and it has been found to alkylate adenines and guanines of DNA. Bovine consumption of bracken fern has been shown to significantly increase chromosomal aberrations.

FIGURE 26–4 Amanita phalloides **(death cap).**

Kidney Tubular Degeneration—Species of Xanthium (cocklebur, Asteraceae) have been found to contain the toxin carboxyatractyloside, which causes microvascular hemorrhages in multiple organs. The toxin causes tubular degeneration and necrosis in the kidney and centrilobular necrosis in the liver. Consumption of the mushroom species Cortinarius has been found to cause acute kidney injury but different species vary widely in toxicity and, therefore, edibility.

Blood and Bone Marrow

Anticoagulants—Fungal infections in sweet clover (Melilotus alba) have been found to produce dicumarol, a coumarin derivative that is a potent anticoagulant. Deaths in cattle have been reported and are caused by hemorrhages.

Bone Marrow Genotoxicity—Argemone (Papaveraceae), a species of poppy, produces sanguinarine, a benzophenanthridine alkaloid that is known to intercalate DNA and have carcinogenic potential.

Cyanogens—Cyanogens are found in a wide variety of plants including the kernels of apples, cherries, and peaches. Metabolism of amygdalin in peaches releases hydrocyanic acid that binds to the ferric ion in methemoglobin and cytochrome oxidase system, which, if severe enough, results in cyanide poisoning with death from asphyxiation.

Cassava produced from Manihot esculenta (Euphorbiaceae) is a major food source for some regions of Africa. The raw root contains a cyanogenic glucoside linamarin that must be removed during processing of the root for human consumption.

Nervous System

Epileptiform Seizures—The common and scientific names for selective plants that produce neurotoxins can be found in Table 26–6. Within the family Apiaceae, which contains carrots, the fleshy tubers of Cicuta maculata (water hemlock) produce neurotoxic cicutoxin (a C17-polyacetylene). Consumption of a single tuber can result in fatal poisoning, characterized by tonic–clonic convulsions, owing to the cicutoxin binding to GABA-gated chloride channels.

Members of the mint family (Labiatae) such as pennyroyal (Hedeoma), sage (Salvia), and hyssop (Hyssopus) are well known for their essential oils containing monoterpenes. Ingestion of these monoterpenes in concentrations much higher than those used for flavoring can cause tonic–clonic convulsions.

TABLE 26–6 Selective plants producing neurotoxicity.

Common Name	Scientific Name	Part	Toxin	Mechanism
Acacia tree	Acacia willardiana	Seeds	Willardiine	Glutamate receptor agonist
Alga	Digenea simplex Chondria armata	All	Kainic acid Domoic acid	Depolarization of glutamate-gated channels
Betel nut	Areca catechu	Nut	Guvacine Arecoline	GABA uptake inhibitor, anticonvulsant Stimulates muscarinic cholinoceptors; CNS stimulation
Buckthorn; Coyotillo	Karwinskia humboldtiana	Seeds, leaves	Tullidinol	Demyelination of motor neurons leading to paralysis
Chrysanthemum	Chrysanthemum cinerariifolium	Seeds	Pyrethrins	Stimulate sodium efflux from neurons in insects
Deadly nightshade	Atropa belladonna	Berries	Tropine alkaloid	Blockade of muscarinic cholinoceptors
Fly agaric mushroom	Amanita muscaria	All	Muscarine Muscimol	Stimulates muscarinic cholinoceptors; CNS stimulation GABA receptor agonist
Rhododendron	Rhododendron sp.	Leaves	Grayanotoxins	Stimulate sodium channel and membrane depolarization
Ryania	Ryania speciosa	Stems	Ryanodine	Stimulates calcium channels and muscle contraction
Poison nut tree	Strychnos nux vomica	All, especially seeds	Strychnine	Glycine receptor antagonist that produces convulsions
Tobacco	Nicotiana tabacum	Leaves	Nicotine	Nicotinic cholinoceptor agonist (low doses) or antagonist (high doses); CNS stimulation

Excitatory Amino Acids—Red algae (Digenia simplex) under certain conditions can proliferate rapidly leading to the notorious beach vacating "red tide" and producing kainic acid. Kainic acid may be ingested by humans who eat filter-feeding mussels that have eaten red algae. Acute symptoms are most notably gastrointestinal distress, headache, hemiparesis, confusion, and seizures. Severe exposure can result in severe memory deficits and sensorimotor neuropathy.

The fungi Amanita muscaria (fly agaric, Figure 26–5) and Amanita pantherian (panther agaric) produce the excitatory amino acid ibotenic acid and its derivative muscimol that is neurotoxic causing central nervous system depression, ataxia, hysteria, and hallucinations. Myoclonic twitching and seizures sometimes develop. Other genera of fungi have been marked for their hallucinogenic actions, notably Psilocybe, which contains psilocin and psilocybin.

Motor Neuron Demyelination—Karwinskia humboldtiana produces anthracenones in its seeds. Both human and livestock poisonings have been known to occur. Several days following ingestion, ascending flaccid paralysis develops with demyelination of large motor neurons in the legs and eventually leads to bulbar paralysis in fatal cases. In addition to neurotoxicity, the anthracenones in Karwinskia, especially peroxisomicine A_2, cause lung atelectasis, emphysema, and massive liver necrosis. Inhibition of catalase in peroxisomes has been proposed as the mechanism of cell toxicity.

Parasympathetic Stimulation—Certain mushrooms of the genera Inocybe, Clitocybe, and Omphalatus contain significant amounts of muscarine, the principal neurotransmitter in the

FIGURE 26–5 Amanita muscaria **(fly agaric).**

parasympathetic nervous system. Consumption of one of these species results in extreme parasympathetic activation resulting in urination, diarrhea, sweating, salivation, and lacrimation.

Parasympathetic Block—Atropine, l-hyoscyamine, and scopolamine are belladonna alkaloids that can be found in varying concentrations in several genera of Solanaceae, such as Datura stramonium (jimson weed), Hyoscyamus niger (henbane), Atropa belladonna (deadly nightshade, Figure 26–6), and Duboisia myoporoides (pituri). T ese alkaloids all effectively block the muscarinic receptor, essentially turning off the parasympathetic drive at the target organ. T is explains why tachycardia, dry mouth, dilated pupils, and decreased gastrointestinal motility all occur on ingestion of these toxins.

Sensory Neuron Block—Capsaicin found in C. annuum (sweet pepper) and C. *f*rutescens (red pepper) causes a burning sensation on vanilloid-type (VR1) sensory receptors. It also desensitizes the transient potential vanilloid 1 receptor (TRPV1) of sensory endings of C-fiber nociceptors to stimuli, a property that has therapeutic use in treating chronic pain. Capsaisin also can relax ileal smooth muscle.

Skeletal Muscle and Neuromuscular Junction

Neuromuscular Junction—Anabasine, an isomer of nicotine, is present in Nicotiana glauca (tree tobacco, Solanaceae) and produces prolonged depolarization of the junction after a period of excessive stimulation. Consumption of N. glauca leaves can result in flexor muscle spasm and gastrointestinal irritation, followed by severe, generalized weakness, and respiratory compromise. Curare, which is used as a poison placed on the tips of arrows, is a potent neuromuscular blocking agent that is obtained from Strychnos toxi*f*era and Chondrodendron tomentosum. Anabaena *fl*osaquae, a species of alga, can produce under the right conditions a neurotoxin anatoxin A that, when ingested by animals that drink pond water with the alga present, depolarizes and blocks the animal's nicotinic and muscarinic acetylcholine receptors, which can cause death from respiratory arrest within minutes to hours.

FIGURE 26–6 Atropa belladonna **(deadly nightshade).**

Skeletal Muscle Damage—Certain species of T ermopsis produce seeds that contain quinolizidine alkaloids. Livestock grazing on T ermopsis montana (false lupine, mountain goldenbanner) develop locomotor depression and recumbency due to areas of necrosis in skeletal muscle that have been found on autopsy.

Consumption of Cassia obtusi*f*olia (sicklepod, Leguminosae) seeds by livestock causes a degenerative myopathy in cardiac and skeletal muscle. Extracts of C. obtusi*f*olia have been found to inhibit NADH-oxidoreductase in bovine and swine mitochondria in vitro.

Bone and Tissue Calcification

Bone and So**f**t Tissue—Consumption of Solanum malacoxylon (Solanaceae) by sheep and cows can cause a marked decrease in bone calcium and calcification of the entire vascular system due to the presence of a water-soluble vitamin D–like substance. In severe cases other organs can also be affected such as the lungs, joint cartilage, and kidney.

Reproduction and Teratogenesis

Aborti**f**acients—Besides its actions on the nervous system, swainsonine, the active alkaloid in the legumes Astragalus and Oxytropus, also causes abortions in pregnant livestock that accidentally ingest these weeds. Foliage and seeds of Leucaena leucocephala, Leucaena glauca, and Mimosa pudica contain a toxic amino acid, mimosine, which on ingestion by cattle leads to uncoordinated gait, goiter, and reproductive disturbances including infertility and fetal death.

Teratogens—Ingestion of V. cali*f*ornicum (California false hellebore, Liliaceae) by pregnant sheep is known to cause malformations in its offspring that can include cyclopia, exencephaly, and microphthalmia. T e toxic alkaloid called jervine causes teratogenesis by blocking cholesterol synthesis that, among other things, prevents a proper response of fetal target tissue to the sonic hedgehog gene (Shh). Shh has an important role in proper developmental patterning of head and brain, and blocking cholesterol synthesis has been shown experimentally to cause a loss of midline facial structures.

CLINICAL STUDY OF PLANT POISONS

Old herbal remedies are a ripe field of study for many of their effects that can be beneficial yet toxic at high enough concentrations. A goal for new research is to elucidate the mechanism of action so that treatments can be tailored to the individual needs and toxic effects can be avoided or interactions with conventional drugs can be minimized. Recent research has shown that Uzara root extract reduced chloride secretion by the gut specifically by inhibiting Na^+, K^+-ATPase. T is effect was seen even in the presence of cholera toxin that causes potent diarrhea by increasing chloride secretion in the gut. Interestingly,

anemonin, which is the active skin irritant produced by species of Ranunculus (buttercup), has been found to show potent anti-inflammation effects under certain conditions. T e compound was found to reduce nitric oxide production that resulted in a lessened inflammatory response to inflammatory stimuli.

INTRODUCTION TO ANIMAL VENOMS

Venomous animals are capable of producing a poison in a highly developed exocrine gland or group of cells and can deliver their toxin during a biting or stinging act. Conversely, poisonous animals have no specific mechanism or structure for the delivery of their poisons, and poisoning usually takes place through ingestion. Animal venom may play a role in offense (as in the capture and digestion of food), in the animal's defense (as in protection against predators or aggressors), or in both functions. Venoms used in an offensive posture are generally associated with the oral pole, as in the snakes and spiders, while those used in a defensive function are usually associated with the aboral pole or with spines, as in the stingrays and scorpion fishes.

It is worth noting that animal and plant toxins tend to differ significantly in their chemical complexity, yet both are capable of causing massive harm. Plant toxins tend to be smaller compounds or proteins and often times a single offending substance can be pinpointed. Conversely, animal toxins must be studied in the context of the entire venom or poison that typically is very complex and contains many individual toxic compounds and very large proteins that essentially work together to cause their effects.

PROPERTIES OF ANIMAL TOXINS

Venoms are very complex, containing polypeptides, high- and low-molecular-weight proteins, amines, lipids, steroids, amino-polysaccharides, quinones, glucosides, and free amino acids, as well as serotonin, histamine, and other substances. Some venoms are known to consist of more than 25 proteins. T e venom is a source of peptides and proteins that act on myriad exogenous targets such as ion channels, receptors, and enzymes within cells and on cell membranes.

Novel instrument developments have permitted the greater application of mass spectrometry, coupled with various separation technologies, to tease out the complexity of natural venoms, thereby identifying the peptide and protein components of venoms. Figure 26–7 demonstrates the application of gel filtration and high-pressure liquid chromatography, as cone snail venom was fractionated into numerous peptides with varying activities. Similar fractionations have been performed

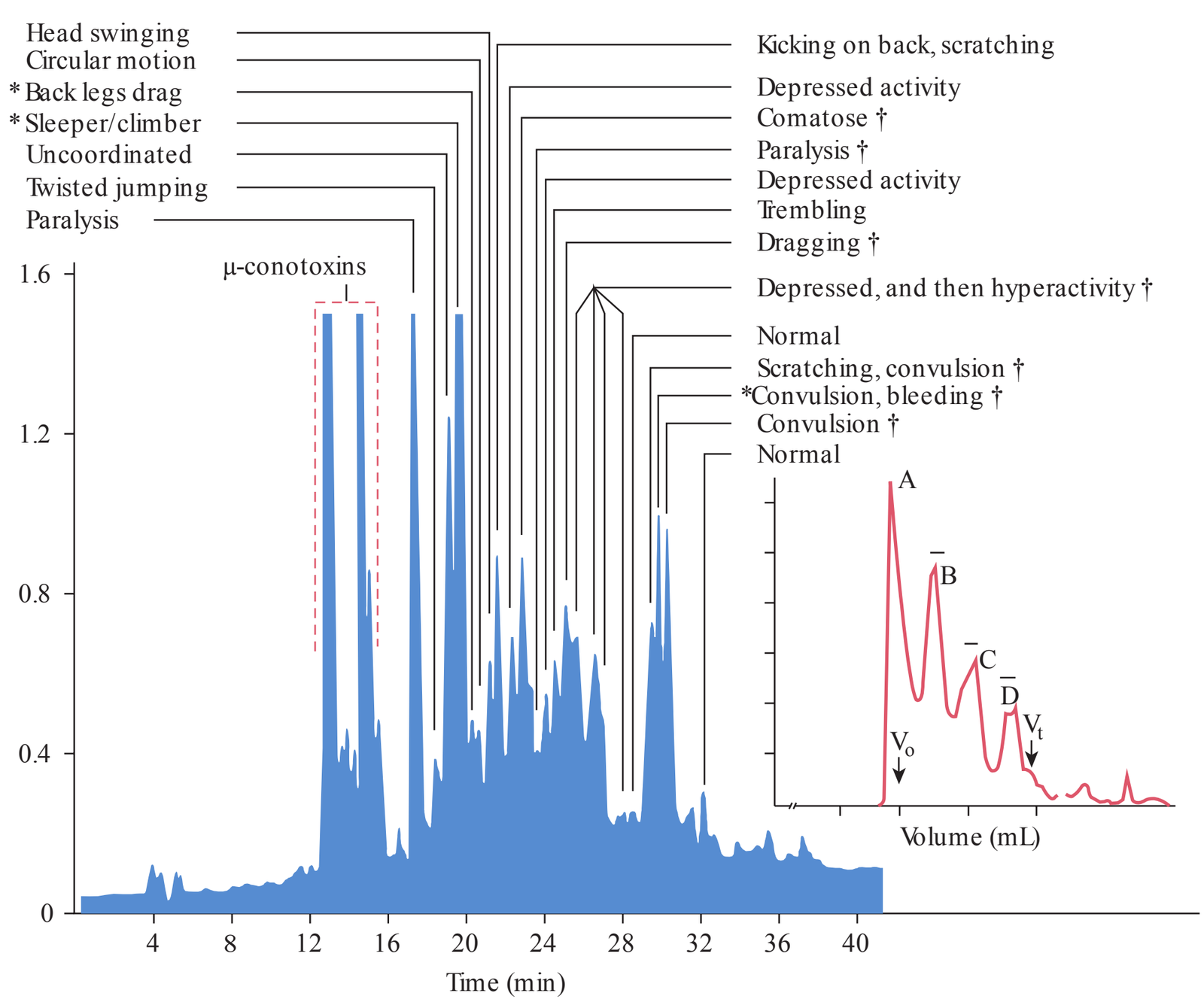

FIGURE 26–7 **Multiple biologically active components were obtained from** Conus geographus **venom by first subjecting the venom to gel filtration on Sephadex G-25 into four fractions and then separation of fraction B (which contains the α -conotoxins) by high-pressure liquid chromatography on a VYDAC C18 column using a trif uoroacetic acid–acetonitrile gradient.** Various peak fractions were then injected intracerebrally into mice and different responses were noted. (†) The fraction was lethal in at least one injected animal. (Reproduced with permission from Olivera BM, Rivier J, Clark C, et al.: Diversity of Conus Neuropeptides. Science, 1990 Jul 20;249(4966):257–263.)

on many other types of venom to identify the individual components. Unfortunately, studying the chemistry, pharmacology, and toxicology of venoms requires isolating and dismantling the venoms and losing the synergy among multiple components.

The bioavailability of a venom is determined by its composition, molecular size, amount or concentration gradient, solubility, degree of ionization, and the rate of blood flow into that tissue, as well as the properties of the engulfing surface itself. The venom can be absorbed by active or passive transport, facilitated diffusion, or pinocytosis, among other physiologic mechanisms. Besides the bloodstream, the lymph circulation not only carries surplus interstitial fluid produced by the venom but also transports larger molecular components and other particulates back to the bloodstream. The site of action and metabolism of venom is dependent on its diffusion and partitioning along the gradient between the plasma and the tissues where the components are deposited.

ARTHROPODS

Arthropods include the arachnids (scorpions, spiders, whip scorpions, solpugids, mites, and ticks), the myriapods (centipedes and millipedes), the insects (water bugs, assassin bugs, and wheel bugs), beetles (blister beetles), Lepidoptera (butterflies, moths, and caterpillars), and Hymenoptera (ants, bees, and wasps).

The number of deaths from arthropod stings and bites is unknown. Among the disease states that were confused with spider or arthropod bites or stings were erythema chronicum migrans, erythema nodosum, periarteritis nodosum, pyroderma gangrenosum, kerion cell-mediated response to a fungus, Stevens–Johnson syndrome, toxic epidermal necrolysis, herpes simplex, and purpura fulminans. Any arthropod may bite or sting and not eject venom.

ARACHNIDA

Scorpions

Of the more than 1000 species of scorpions, the stings of more than 75 can be considered of sufficient importance to warrant medical attention. Some of the more important scorpions are noted along with their location in Table 26–7.

Many scorpion venoms contain low-molecular-weight proteins, peptides, amino acids, nucleotides, and salts, among other components. Short-chain toxins appear to affect potassium or chloride channels, while the long-chain toxins affect mainly the sodium channels. The neurotoxic fractions are generally classified on the basis of their molecular size; the short-chain toxins are composed of 20 to 40 amino acid residues with 3 or 4 disulfide bonds and appear to affect potassium or chloride channels, while the long-chain toxins have 58 to 76 amino acid residues (6500–8500 Da) with four disulfide bonds and affect mainly the sodium channels. The toxins can selectively bind to a specific channel of excitable cells, thus impairing the initial depolarization of the action potential in the nerve and muscle that results in their neurotoxicity.

TABLE 26–7 Location of some medically important scorpions.

Genus	Distribution
Androctonus	North Africa, Middle East, Turkey
Buthus	France and Spain to Middle East and North Africa, Mongolia, China
Buthotus	Africa, Middle East, Central Asia
Centruroides	North, Central, South America
Heterometrus	Central and Southeast Asia
Leiurus	North Africa, Middle East, Turkey
Mesobuthus	Turkey, India
Parabuthus	Southern Africa
Tityus	Central and South America

The symptoms and signs of scorpion envenomation differ considerably depending on the species. Common offenders are members of the family Vejovidae and their sting gives rise to localized pain, swelling, tenderness, and mild paresthesia. Systemic reactions are rare, although weakness, fever, and muscle fasciculations have been reported.

Envenomations by some members of the genus Centruroides are clinically the most important. In children, their sting may produce initial pain, although some children do not complain of pain and are unaware of the injury. The child becomes tense and restless and shows abnormal and random head and neck movements. Often the child will display roving eye movements. Centruroides sculpturatus stings display visual signs, including nystagmus roving eye and oculogyric movements. Tachycardia, hypertension, and respiratory rates are increased. Fasciculations may be seen and the child may display ataxia. The respiratory distress may proceed to respiratory paralysis. As opposed to children, almost all adults complain of immediate pain after the sting. They become tense and anxious and develop tachycardia, hypertension, and increased respirations. Most adults become asymptomatic within 12 h.

Spiders

Of the 30 000 or so species, at least 200 have been implicated in significant bites on humans. Spiders are predaceous, polyphagous arachnids that generally feed on insects or other arthropods. Table 26–8 provides a short list of spiders with their associated toxins and the targets of their toxins.

All spiders except the Uloboridae family possess a venom apparatus that produces neurotoxins designed to paralyze or kill prey. Spider venoms are complex mixtures of low-molecular-weight components, including inorganic ions and salts, free acids, glucose-free amino acids, biogenic amines and neurotransmitters, and polypeptide toxins. The acylpolyamines are voltage-dependent open-channel blockers (sodium, calcium,

TABLE 26–8 Some significant spiders, their toxins, and the targets of the toxins.

Spider	Peptide	Target
Acanthoscurria gomesiana	Gomesin	PLM
Agelenopsis aperta	ω-AfaI-IVA μ-Afatoxin 1-6	Ca^{2+} Na^{+}
Grammostola spatula	HaTx1,2 GsMTx2,4 GSTxSIA	K^{+} MS Ca^{2+}
Hadronyche versuta	ω-ACTX-Hv1a ω-ACTX-Hv2a δ-ACTX-Hv1a	Ca^{2+} Ca^{2+} Na^{+}
Heteroscodra maculate	HmTx1,2	K^{+}
Ornithoctonus huwena	Huwentoxin I Huwentoxin IV	Ca^{2+} Na^{+}
Psalmopoeus cambridgei	PcTx1	ASIC
Phrixotrichus auratus	PaTx1,2	K^{+}
Thrixopelma pruriens	ProTxI,II	Na^{+}

Additional species, their toxins, and their targets may be obtained from the article by Corzo G, Escoubas P: Pharmacologically active spider peptide toxins. Cell Mol Life Sci 2003;60:2409-2426.
PLM, phospholipid membranes; Ca^{2+}, K^{+}, and Na^{+}, calcium, potassium, and sodium ion channels; MS, mechanosensitive ion channels; ASIC, acid-sensing ion channels.

and potassium channels) and/or blockers of the ion channel associated with glutamate receptors. They also act on nicotinic acetylcholine receptors.

Agelenopsis Species (American Funnel Web Spiders)—The American funnel web spider (Agelenopsis aperta) contains three classes of agatoxins that target ion channels. The α-agatoxins appear to be use-dependent, noncompetitive antagonists of the glutamate receptor channels. The μ-agatoxins cause increased spontaneous release of neurotransmitter from presynaptic terminals and repetitive action potentials in motor neurons. In addition, the μ-agatoxins are specific for insect sodium channels. The ω-amatoxins are a structurally diverse group of peptides that are selective for voltage-activated calcium channels. The four ω-amatoxins can be distinguished by sequence similarity and their spectrum of action against insect and vertebrate calcium channels. The action of the α-agatoxins is synergized by the μ-agatoxins causing channels to open at the normal resting potentials.

Latrodectus Species (Widow Spiders)—Found throughout the world in all continents with temperate or tropical climates, these spiders are commonly known as the black widow, brown widow, or red-legged spider (Figure 26–8). The latrotoxins, a family of high-molecular-weight proteins that are found in Latrodectus venoms, target different classes of animals including vertebrates, insects, and crustaceans. All latrotoxins

FIGURE 26–8 Latrodectus mactans **(female black widow spider).**

stimulate massive release of neurotransmitters after binding to specific neuronal receptors.

α-Latrotoxin is the most studied protein that is toxic only to vertebrates and not to insects or crustaceans. It is a presynaptic toxin that is said to exert its toxic effects on the vertebrate central nervous system depolarizing neurons by increasing intracellular [Ca^{2+}] and by stimulating exocytosis of neurotransmitters from nerve terminals. α-Latrotoxin and its mutants are versatile tools for the study of exocytosis. In particular, studies with this toxin have helped confirm the vesicular hypothesis of transmitter release, establish the requirement of calcium ion for endocytosis, characterize individual neurotransmitter sites in the central nervous system, and identify two families of important neuronal cell surface receptors.

Bites by the black widow are described as sharp and pinprick-like, followed by a dull, occasionally numbing pain in the affected extremity and by pain and cramps in one or several of the large muscle masses. Muscle fasciculations frequently can be seen, sweating is common, and lymphadenitis is frequently observed. Severe paroxysmal muscle cramps may occur and hypertension is a common finding, particularly in the elderly after moderate to severe envenomations. Blood studies are usually normal.

Loxosceles Species (Brown or Violin Spiders)—These primitive spiders are variously known in North America as the fiddle-back spider or the brown recluse (Figure 26–9). The venom of Loxosceles spiders appears to contain phospholipase, protease, esterase, collagenase, hyaluronidase, deoxyribonuclease, ribonuclease, dipeptides, dermonecrosis factors, and sphingomyelinase D. The venom has coagulation and vasoconstriction properties and it causes selective vascular endothelial damage. There are adhesions of neutrophils to the capillary wall with sequestration and activation of passing neutrophils by the perturbed endothelial cells.

FIGURE 26–9 Loxosceles reclusa **(male brown recluse spider) with the violin pattern on the dorsal cephalothorax.**

T e bite of this spider produces about the same degree of pain as does the sting of an ant, but sometimes the patient may be unaware of the bite. Pruritis over the area often occurs with reddening and elevated skin temperature at the lesion. With significant envenomations, hemorrhages may develop throughout the area, lymphadenopathy is common, and necrosis of the surrounding tissue may be visualized. Systemic symptoms and signs include fever, malaise, stomach cramps, nausea and vomiting, jaundice, spleen enlargement, hemolysis, hematuria, and thrombocytopenia. Fatal cases, while rare, usually are preceded by intravascular hemolysis, hemolytic anemia, thrombocytopenia, hemoglobinuria, and renal failure.

Steatoda Species—T ese spiders are variously known as the false black widow, combfooted, cobweb, or cupboard spiders. T e venom of Steatoda paykulliana stimulates the release of transmitter substances similar to Latrodectus. T e venom is said to form ionic channels that are permeable for bivalent and monovalent cations, and the duration of time in the open state depends on the membrane potential. S. paykulliana venom induces strong motor unrest, clonic cramps, exhaustion, ataxia, and then paralysis.

Cheiracanthium Species **(**Running Spiders**)**—Cheiracanthium punctorium, C. inclusum, C. mildei, C. diversum, and C. japonicum are often implicated in envenomations. Cheiracanthium tends to be tenacious and sometimes must be removed from the bite area. For that reason there is a high degree of identification following the bite of these spiders. T e most toxic venom fraction is said to be a protein of 60 kDa, and the venom is high in norepinephrine and serotonin.

T e patient usually describes the bite as sharp and painful. A reddened wheal with a hyperemic border develops. Small petechiae may appear near the center of the wheal. Skin temperature over the lesion is often elevated, but body temperature is usually normal. Lymphadenitis and lymphadenopathy may develop. C. japonicum produces more severe manifestations, including severe local pain, nausea and vomiting, headache, chest discomfort, severe pruritus, and shock.

Theraphosidae Species **(**Tarantulas**)**—True tarantulas are members of the family T eraphosidae. Tarantulas are predators and they feed on various vertebrate and invertebrate preys that are captured after envenomation with venoms that act rapidly and irreversibly on the central and peripheral nervous systems. In humans, reported bites elicit mild to severe local pain, strong itching, and tenderness that may last for several hours. Edema, erythema, joint stiffness, swollen limbs, burning feelings, and cramps are common.

T eraphosid spiders contain several toxins that are being evaluated for development as antiarrhythmic or as antinociceptive drugs. In particular, Grammostola mechanotoxin 4 from Grammostola spatulata has considerable promise as an antiarrhythmic. Protoxin I and II from T rixopelma pruriens have promise as analgesics because they inhibit the tetrodotoxin-resistant sodium channels.

Ticks

Tick paralysis is caused by the saliva of certain ticks of the families Ixodidae, Argasidae, and Nuttalliellidae. Ticks are known to transmit the organisms causing Lyme disease, Rocky Mountain spotted fever, babesiosis, leptospirosis, Q fever, ehrlichiosis, typhus, tick-borne encephalitis, and others.

Tick saliva contains a number of active constituents. For example, saliva from Ixodes scapularis contains apyrase (ATP-diphosphohydrolase), which hydrolyzes ADP that is released at the bite site thereby inhibiting ADP-induced platelet aggregation; kininase (ACE-like protein or angiotensin-converting enzyme-like protein), which hydrolyzes circulating kinins and reduces the host inflammatory response; glutathione peroxidase; serine protease inhibitors, which inhibit coagulation enzymes; an anticomplement protein that inhibits an enzyme in the alternative pathway for complement; an amine-binding protein that binds serotonin, histamine, and other biogenic amines.

As tick bites are often not felt, the first evidence of envenomation may not appear until several days later, when small macules 3 to 4 mm in diameter develop that are surrounded by erythema and swelling. T e patient often complains of dif culty with gait, followed by paresis and eventually locomotor paresis and paralysis. Problems in speech and respiration may ensue and lead to respiratory paralysis if the tick is not removed. T e saliva of Ixodes holocyclus has yielded a peptide holocyclotoxin-1 that may cause paralysis.

CHILOPODA (CENTIPEDES)

In the United States, the prevalent biting genus is a Scolopendra species. T e venom is concentrated within the intracellular granules, discharged into vacuoles of the cytoplasm of the secretory cells, and moved by exocytosis into the lumen of the gland; from thence ducts carry the venom to the jaws.

Centipede venoms contain high-molecular-weight proteins, proteinases, esterases, 5-hydroxytryptamine, histamine, lipids, and polysaccharides. The bite produces sharp pain, immediate bleeding, redness, and swelling. Localized tissue changes and necrosis have been reported, and severe envenomations may cause nausea and vomiting, changes in heart rate, vertigo, and headache.

DIPLOPODA (MILLIPEDES)

The repellent secretions expelled from the sides of their bodies contain a toxin of benzoquinone derivatives plus a variety of complex substances such as iodine and hydrocyanic acid, which the animal makes use of to produce hydrogen cyanide. Some species can spray these defensive secretions. The lesions produced by millipedes consist of a burning or prickling sensation and development of a yellowish or brown-purple lesion; subsequently, a blister containing serosanguinous fluid forms, which may rupture. Eye contact can cause acute conjunctivitis, periorbital edema, keratosis, and much pain; such an injury must be treated immediately.

INSECTA

Heteroptera (True Bugs)

The clinically most important of the true bugs are the Reduviidae (the reduviids): the kissing bug, assassin bug, wheel bug, or cone-nose bug of the genus *Triatoma*. The venom of these bugs appears to have apyrase activity and to lack 5-nucleotidase, inorganic pyrophosphatase, phosphatase, and adenylate kinase activities, but it is fairly rich in protease properties. It inhibits collagen-induced platelet aggregation. Three peptides isolated from the saliva are calcium channel inhibitors. The bites of *Triatoma* species are painful and give rise to erythema, pruritus, increased temperature in the bitten part, localized swelling, and—in those allergic to the saliva—systemic reactions such as nausea and vomiting and angioedema.

Hymenoptera (Ants, Bees, Wasps, and Hornets)

Formicidae (Ants)—Most ants have stings, but those that lack them can spray a defensive secretion from the tip of the gaster, which is often placed in the wound of the bite. Clinically important stinging ants are the harvesting ants (Pagonomyrmex), fire ants (Solenopsis), and little fire ants (Ochetomyrmex).

The venoms of the ants vary considerably. Formicinae ant venom contains about 60% formic acid. Fire ant venoms are rich in alkaloids. The sting of the fire ant gives rise to a painful burning sensation, after which a wheal and localized erythema develop, forming a vesicle that becomes purulent and turns into a pustle. The pustule may then break down, become a crust, or become a fibrotic nodule. In multiple stings there may be nausea, vomiting, vertigo, increased perspiration, respiratory difficulties, cyanosis, coma, and even death.

Apidae (Bees)—This family includes the bumble bees, honeybees, carpenter bees, and yellow jackets. The commonest stinging bees are Apis mellifera and the Africanized bee, Apis mellifera adansonii, and the incidence of Hymenoptera poisonings is increasing.

The venom contains biologically active peptides, such as melittin, apamine, mast cell–degranulating peptide, and others, as well as phospholipases A_2 and B, hyaluronidase, histamine, dopamine, monosaccharides, and lipids. Melittin tetramers cause a breakdown of the resting potential and rapid depolarization of nociceptors, which induces pain. Apamine is a blocker of calcium-dependent potassium channels and is thought to be the "lethal factor".

Bee stings typically produce immediate, sharp or burning pain, slight local erythema, and edema followed by itching. It is said that 50 stings can be serious and lead to respiratory dysfunction, intravascular hemolysis, hypertension, myocardial damage, hepatic changes, shock, and renal failure. With 100 or more stings, death can occur.

Vespidae (Wasps)—This family includes wasps and hornets. These venoms contain a high content of peptides, which include mastoparan in wasps and hornets and crabolin from hornet venom. These peptides release histamine from mast cells. Wasp kinins cause immediate pain, vasodilation, and increased vascular permeability leading to edema. These venoms also contain phospholipases and hyaluronidases, which contribute to the breakdown of membranes and connective tissue to facilitate diffusion of the venom.

Lepidoptera (Caterpillars, Moths, and Butterflies)

The urticating hairs, or setae, of caterpillars are effective defensive weapons that protect some species from predators. The toxic material found in the venom glands contains aristolochic acids, cardenolides, kallikrein, histamine and a fibrinolytic peptide. The spicules of Taumetopoea pityocampa contain a toxin that is a strong dermal irritant and highly allergenic peptide. In some parts of the world the stings of several species of Lepidoptera give rise to a bleeding diathesis, often severe and sometimes fatal.

MOLLUSCA (CONE SNAILS)

Human interest in this group of mollusks has been due to the beautiful patterns on their shells. Cone snails have a venom duct for synthesis and storage of venom and hollow harpoon-like teeth for injection of the venom. There are probably over 100 different venom components per species known as conotoxins. Molecular targets include G-protein-coupled receptors, neuromuscular transporters, and ligand- or voltage-gated ion channels. Some components have enzymatic activity. Figure 26–10 provides an overview of peptidic Conus venom components, indicating gene superfamilies, disulfide bond characteristics, and general targets.

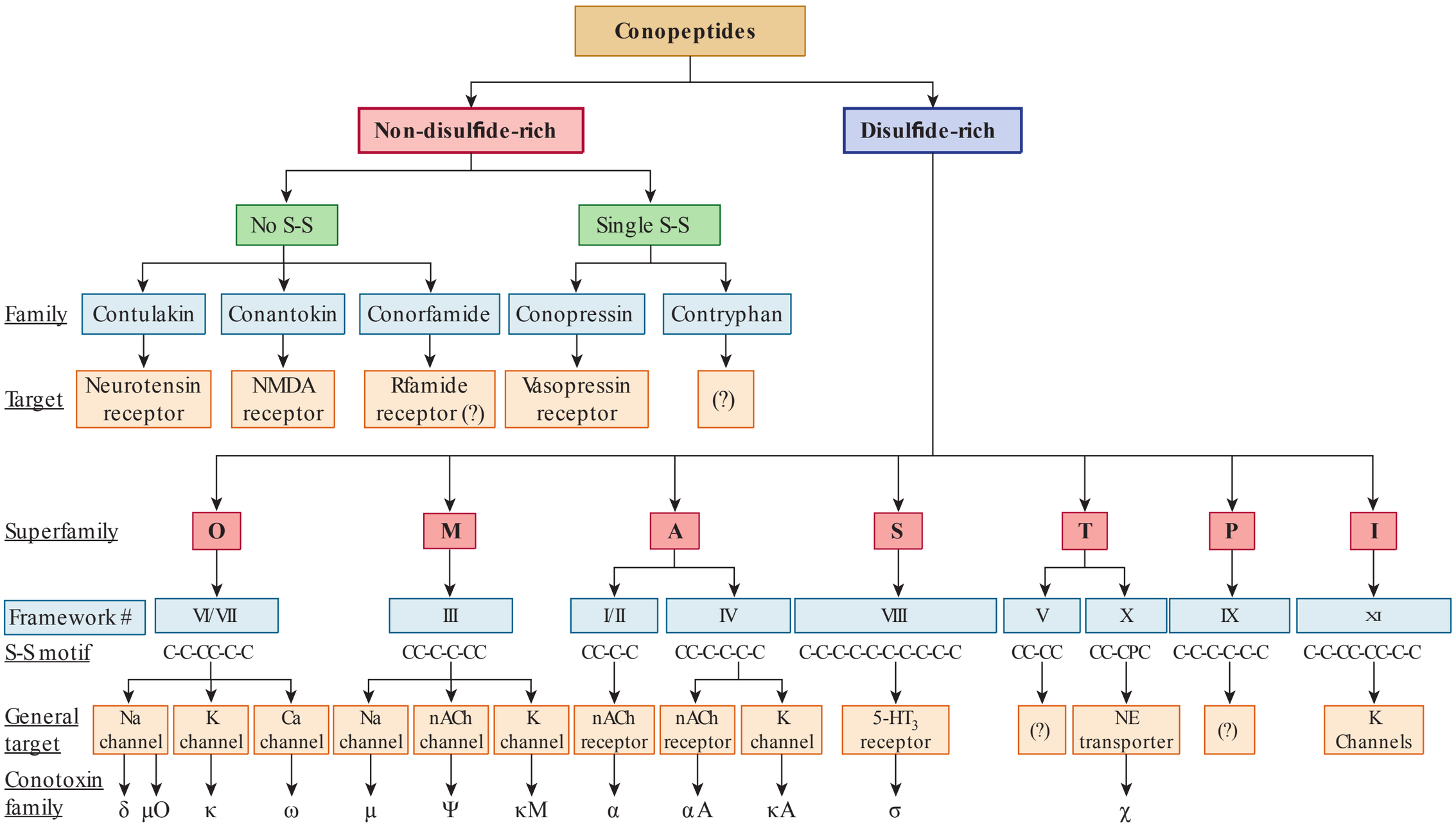

FIGURE 26–10 **Organizational diagram for** Conus **peptides, indicating gene superfamilies, disulfide patterns, and known pharmacologic targets.** Only the superfamilies of the disulfide-rich peptides are shown. (Reproduced with permission from Terlau H, Olivera BM: Conus venoms: A rich source of novel ion channel-targeted peptides. Physiol Rev, 2004 Jan;84(1):41–68.)

Cone snails could be called sophisticated practitioners of combination drug therapy. After injection, multiple conopeptides act synergistically to affect the targeted prey. The term toxin cabal has been applied to this coordinated action of the conopeptide mixture. The fish-hunting species Conus purpurascens apparently has two distinct cabals whose effects differ in time and space. The "lightning-strike cabal" causes immediate immobilization of the injected prey because various venom components inhibit voltage-gated sodium channel inactivation and block potassium channels, resulting in massive depolarization of axons in the vicinity of the injection site and a tetanic state. The second physiologic cabal, the "motor cabal," acts more slowly as conotoxins must be distributed throughout the body of the prey. The overall result is total inhibition of neuromuscular transmission. Various conopeptides inhibit presynaptic calcium channels that control neurotransmitter release, the postsynaptic neuromuscular nicotinic receptors, and the sodium channels involved in the muscle action potential.

REPTILES

Lizards

The Gila monster (Heloderma suspectum) and the beaded lizards (Heloderma horridum) are far less dangerous than is generally believed. Their venom is transferred from venom glands in the lower jaw through ducts that discharge their contents near the base of the larger teeth of the lower jaw. The venom is then drawn up along grooves in the teeth by capillary action. The venom of this lizard has serotonin, amine oxidase, phospholipase A, a bradykinin-releasing substance, helodermin, gilatoxin, and low-proteolytic as well as high-hyaluronidase activities. The clinical presentation of a helodermatid bite can include pain, edema, hypotension, nausea, vomiting, weakness, and diaphoresis. No antivenin is commercially available.

Snakes

General Information and Classification—Venomous snakes primarily belong to the following families: Viperidae (vipers), Elapidae, Atractaspididae, and Colubridae. Overall the Colubridae are considered the largest venomous family, and are composed of nearly 60% of all snakes.

Snake Venoms—These venoms are complex mixtures: proteins and peptides, consisting of both enzymatic and nonenzymatic compounds. Snake venoms also contain inorganic cations such as sodium, calcium, potassium, magnesium, and small amounts of zinc, iron, cobalt, manganese, and nickel. The metals in snake venoms are likely catalysts for metal-based enzymatic reactions. For example, in the case of some elapid venoms, zinc ions appear to be necessary for anticholinesterase activity, and calcium may play a role in the activation of phospholipase A and the direct lytic factor. Some proteases appear

to be metalloproteins. Some snake venoms also contain carbohydrates (glycoproteins), lipids, and biogenic amines, such as histamine, serotonin, and neurotransmitters (catecholamines and acetylcholine) in addition to positively charged metal ions. T e complexity of snake venom components is illustrated nicely in Figure 26–11.

Actions of snake venoms can be said to be broad ranging in several areas. A simplistic approach would group toxin components as neurotoxins, coagulants, hemorrhagins, hemolytics, myotoxins, cytotoxins, and nephrotoxins. Neurotoxins produce neuromuscular paralysis ranging from dizziness to ptosis; to ophthalmoplegia, flaccid facial muscle paralysis, and inability to swallow; to paralysis of larger muscle groups; and finally to paralysis of respiratory muscles and death by asphyxiation. Coagulants may have an initial procoagulant action that uses up clotting factors leading to bleeding. Coagulants may directly inhibit normal clotting at several places in the clotting cascade or via inhibition of platelet aggregation. In addition, some venom components may damage the endothelial lining of blood vessels leading to hemorrhage. Bite victims may show bleeding from nose or gums, from the bite site, and in saliva, urine, and stools. Myotoxins can directly impact muscle

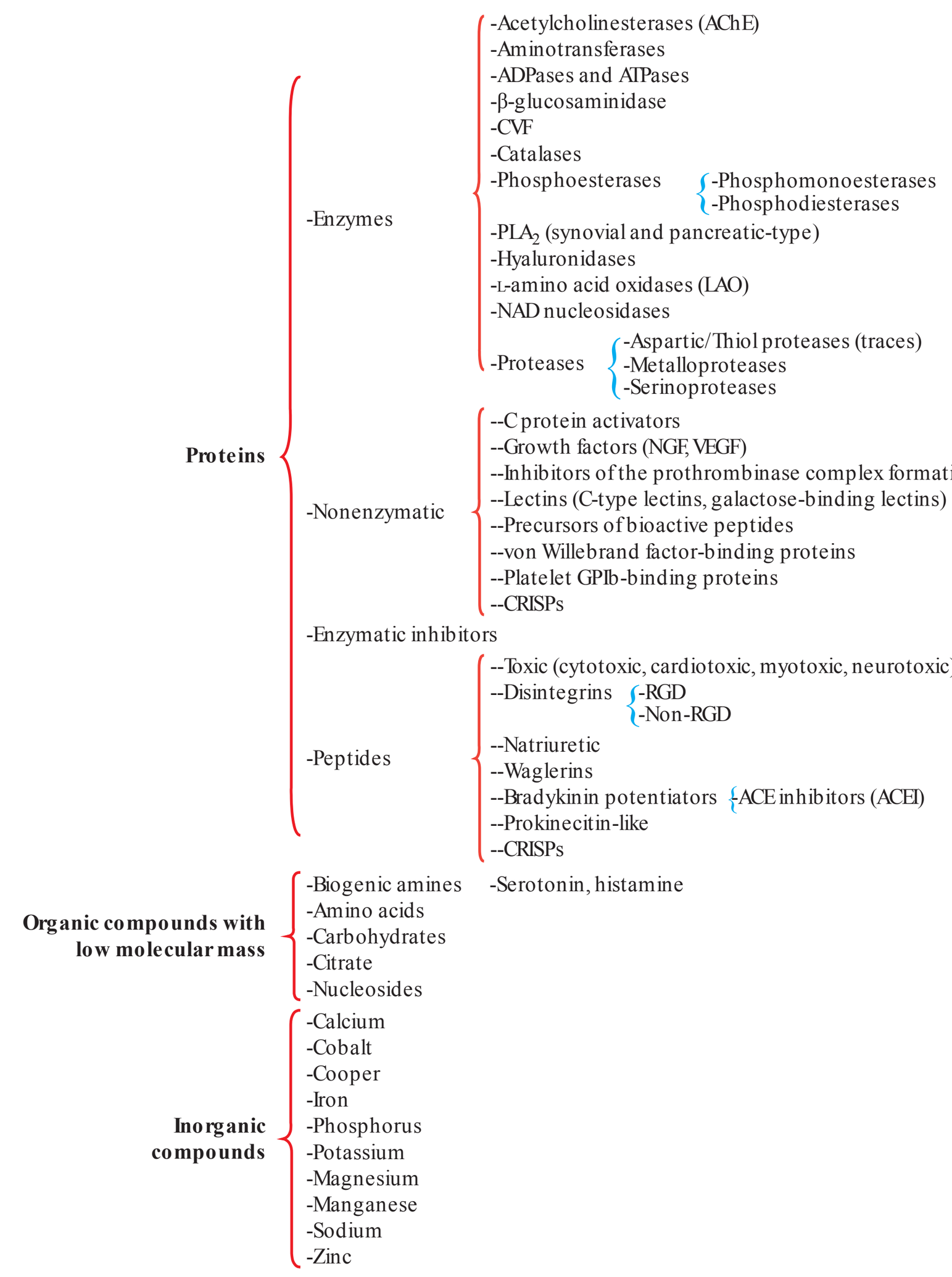

FIGURE 26–11 **Components of snake venoms.** ACE, angiotensin-converting enzyme; CRISP, cysteine-rich secretory protein; CVF, cobra venom factor–like proteins; LAO, l-amino acid oxidase; PLA_2, phospholipase A2; RGD, arginine–glycine–aspartate. (Reproduced with permission from Ramos OHP, Selistre-de-Araujo HS: Snake venom metalloproteases—structure and function of catalytic and disintegrin domains. Comp Biochem Physiol C Toxicol Pharmacol. 2006 Mar-Apr;142(3-4):328–346.)

contraction leading to paralysis or cause rhabdomyolysis or the breakdown of skeletal muscle. Myoglobinuria, or a dark brown urine, and hyperkalemia may be noted. Cytotoxic agents have proteolytic or necrotic properties leading to the breakdown of tissue. Typical signs include massive swelling, pain, discoloration, blistering, bruising, and wound weeping. Finally, nephrotoxins can cause direct damage to kidney structures leading to bleeding, damage to several parts of the nephron, tissue oxygen deprivation, and renal failure.

Enzymes—At least 26 different enzymes have been isolated from snake venoms. No single snake venom contains all 26 enzymes and some important snake venom enzymes are shown in Figure 26–11. Proteolytic enzymes that catalyze the breakdown of tissue proteins and peptides include peptide hydrolases, proteases, endopeptidases, peptidases, and proteinases. Collagenase is a specific kind of proteinase that digests collagen. T is activity has been demonstrated in the venoms of a number of species of crotalids and viperids. Hyaluronidase cleaves internal glycoside bonds in certain acid mucopolysaccharides resulting in a decrease in the viscosity of connective tissues. T e breakdown in the hyaluronic barrier allows other fractions of venom to penetrate the tissues, causing hyaluronidase to be called "spreading factor." Fibrin(ogen)olytic enzymes break down fibrin-rich clots and help to prevent further clot formation. An exciting development from the research on these enzymes is that one specific recombinant fibrinolytic enzyme derived from fibrolase called alfimeprase is progressing through clinical trials for the treatment of peripheral arterial occlusions. Phosphodiesterase has been found in the venoms of all families of poisonous snakes. It acts as an exonucleotidase, attacking DNA and RNA. Acetylcholinesterase, found in the cobra, catalyzes the hydrolysis of acetylcholine to choline and acetic acid thereby facilitating tetanic paralysis and capture of prey. Phospholipase A_2 is widely distributed in snake venoms, and this enzyme family interacts with other venom components often resulting in synergistic reactions.

T e snake venom metalloproteinases (SVMP) are enzymes that disrupt the hemostatic system that blocks the function of integrin receptors, a function that could alleviate a variety of pathological conditions such as inflammation, tumor angiogenesis and metastasis, and thrombosis. SVMPs degrade proteins such as laminin, fibronectin, type IV collagen, and proteoglycans from the endothelial basal membrane; degrade fibrinogen and von Willebrand factor enhancing the hemorrhagic action; and inhibit platelet aggregation and stimulate release of cytokines.

Polypeptides—Snake venom polypeptides are low-molecular-weight proteins that do not have enzymatic activity. More than 80 polypeptides with pharmacologic activity have been isolated from snake venoms. Most of the lethal activity of the poison of the sea snake Laticauda semi*f*asciata involves erabutoxins. Erabutoxin-a and α-cobratoxin are curamimetic at the mammalian neuromuscular junction. Disintegrins are a family of short cysteine-rich polypeptides that exhibit af nity for many ligand receptors. T e small basic polypeptide myotoxins are widely distributed in Crotalus snake venoms. T e specific agent crotamine from Crotalus durissus terri*f*icus venom induces skeletal muscle spasms and paralysis by changing the inactivation process of sodium channels leading to depolarization of the neuromuscular junction.

Toxicology—In general, the venoms of rattlesnakes and other New World crotalids produce alterations in the resistances and often in the integrity of blood vessels, changes in blood cells and blood coagulation mechanisms, direct or indirect changes in cardiac and pulmonary dynamics, and—with crotalids such as C. durrissus terri*f*icus and C. scutulatus—serious alterations in the nervous system and changes in respiration. In humans, the course of the poisoning is determined by the kind and amount of venom injected; the site where it is deposited; the general health, size, and age of the patient; the kind of treatment; and those pharmacodynamic principles noted earlier in this chapter. Death in humans may occur within less than 1 h or after several days, with most deaths occurring between 18 and 32 h. Hypotension or shock is the major therapeutic problem in North American crotalid bites.

Snakebite Treatment—T e treatment of bites by venomous snakes is now so highly specialized that almost every envenomation requires specific recommendations. However, three general principles for every bite should be kept in mind: (1) snake venom poisoning is a medical emergency requiring immediate attention and the exercise of considerable judgment; (2) the venom is a complex mixture of substances of which the proteins contribute the major deleterious properties, and the only adequate antidote is the use of specific or polyspecific antivenom; and (3) not every bite by a venomous snake ends in an envenomation. Venom may not be injected. In almost 1000 cases of crotalid bites, 24% did not end in a poisoning. T e incidence with the bites of cobras and perhaps other elapids is probably higher (see www.toxinology.com).

ANTIVENOM

Antivenoms have been produced against most medically important snake, spider, scorpion, and marine toxins. Antivenom consists of venom-specific antisera or antibodies concentrated from immune serum to the venom. Antisera contain neutralizing antibodies: one antigen (monospecific) or several antigens (polyspecific). Monovalent antivenoms have a high neutralization capacity, which is desirable against the venom of a specific animal. Neutralization capacity of antivenom is highly variable as there are no enforced international standards. Antivenom may cross-react with venoms from distantly related species and may not react with venom from the intended species. Nevertheless, in general, the antibodies bind to the venom molecules, rendering them ineffective.

All antivenom products may produce hypersensitivity reactions. Type I (immediate) hypersensitivity reactions are

caused by antigen cross-linking of endogenous IgE bound to mast cells and basophils. Binding of antigen by a mast cell may cause the release of histamine and other mediators, producing an anaphylactic reaction. Once initiated, anaphylaxis may continue despite discontinuation of antivenom administration. Type III hypersensitivity (serum sickness) may develop several days after antivenom administration. In these cases, antigen–antibody complexes are deposited in different areas of the body, often producing inflammatory responses in the skin, joints, kidneys, and other tissues. Fortunately, these reactions are rarely serious. T e risks of anaphylaxis should always be considered when one is deciding whether to administer antivenom.

POTENTIAL CLINICAL APPLICATION OF VENOMS

Toxin specificities for receptors and channels that facilitate the interface and coordination of neuromuscular activity are utilized and manipulated to study, model, diagnose, and sometimes treat acute and degenerative conditions. On closer examination of α-bungarotoxin and candoxin nicotinic acetylcholine receptor specificity, plans are under way to utilize the reversible and irreversible receptor binding in muscular and neuronal tissues, respectively, in Alzheimer patients. In addition to treating neurological diseases, specific α-toxins (longer chained) are also studied for their antiangiogenic capabilities in treating malignant tumor growth in patients suffering from small-cell lung carcinoma. In cases such as this, there is an inherent trade-off between promoting some degree of neurological deficit in light of combating tumor growth. Toxins such as the snake venom thrombin-like enzymes are valuable tools in both research and therapeutic applications. Fibrin(ogen)olytic enzymes that break down fibrin-rich clots preventing further clot formation may be useful as controls in blood clotting research or to treat heart attacks and strokes.

Animal venoms contain components that can reduce pain, can selectively kill specific cancers, may reduce the incidence of stroke via effects on blood coagulability, and function as antibiotics. Other venom components act as enzyme inhibitors. Finally, leeches, earthworms, helminths, snails, centipedes, spiders, and ticks all produce substances with potential clinical applications, such as osteoarthritis, deep vein thrombosis, antimicrobial action, inflammatory bowel disease, analgesia, and hyperlipidemia. Blood from mongoose, hedgehog, and opossum contains proteins that inhibit the hemorrhagins in snake venoms. T ese proteins may become valuable as agents of resistance to snakebites.

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QUESTIONS

1. All of the following statements regarding plant toxicity are true EXCEPT:
   a. Genetic variability plays a role in the toxicity of a plant.
   b. Plant toxins are most highly concentrated in the leaves.
   c. Young plants may have a higher toxin concentration than older plants.
   d. T e weather can influence the toxicity of plants.
   e. Soil composition can alter a plant's production of toxin.

2. Contact with which of the following plant species would be LEAST likely to cause an allergic dermatitis?
   a. Urtica.
   b. Philodendron.
   c. Rhus.
   d. Dendranthema.
   e. Hevea.

3. Which of the following statements regarding lectin toxicity is FALSE?
   a. Lectins have an af nity for N-acetylglucosamine on mammalian neurons.
   b. Consumption of lectins can cause severe gastrointestinal disturbances.
   c. T e fatality rate after ingestion of a fatal dose is very high.
   d. Some toxic lectins inhibit protein synthesis.
   e. A diet high in some lectins has been linked to reduced weight gain.

4. Colchicine, found in lily bulbs:
   a. causes severe dehydration.
   b. is sometimes used as a purgative.
   c. causes a severe contact dermatitis.
   d. inhibits sphingolipid synthesis.
   e. blocks microtubule formation.

5. Activation of a vanilloid receptor is characteristic of which of the following chemicals?
   a. acetylandromedol.
   b. capsaicin.
   c. colchicine.
   d. ergotamine.
   e. linamarin.

6. Which of the following plant species is known to cause cardiac arrhythmias on ingestion?
   a. Dief enbachia.
   b. Phytolacca americana.
   c. Digitalis purpurea.
   d. Pteridium aquilinum.
   e. Cicuta maculate.

7. Which of the following plant toxins does NOT affect the neuromuscular junction?
   a. nicotine.
   b. anabasine.
   c. curare.
   d. anatoxin A.
   e. muscimol.

8. Which of the following statements regarding animal toxins is FALSE?
   a. Animal venoms are strictly metabolized by the liver.
   b. T e kidneys are responsible for the excretion of metabolized venom.
   c. Venoms can be absorbed by facilitated diffusion.
   d. Most venom fractions distribute unequally throughout the body.
   e. Venom receptor sites exhibit highly variable degrees of sensitivity.

9. Scorpion venoms do NOT:
   a. affect potassium channels.
   b. affect sodium channels.
   c. affect chloride channels.
   d. affect calcium channels.
   e. affect initial depolarization of the action potential.

10. Which of the following statements regarding widow spiders is TRUE?
    a. Widow spiders are exclusively found in tropical regions.
    b. Both male and female widow spiders bite and envenomate humans.
    c. T e widow spider toxin decreases calcium concentration in the synaptic terminal.
    d. Alpha-latrotoxin stimulates increased exocytosis from nerve terminals.
    e. A severe alpha-latrotoxin envenomation can result in life-threatening hypotension.

11. Which of the following diseases is not commonly caused by tick envenomation?
    a. Rocky Mountain spotted fever.
    b. Lyme disease.
    c. Q fever.
    d. ehrlichiosis.
    e. cat scratch fever.

12. Which of the following is NOT characteristic Lepidoptera envenomation?
    a. increased prothrombin time.
    b. decreased fibrinogen levels.
    c. decreased partial thromboplastin time.
    d. increased risk of hemorrhaging.
    e. decreased plasminogen levels.

13. Which of the following animals has a venom containing histamine and mast cell–degranulating peptide that is known for causing hypersensitivity reactions?
    a. bees.
    b. ants.
    c. snakes.
    d. spiders.
    e. reduviids.

14. Which of the following enzymes is not typically found in snake venoms?
    a. hyaluronidase.
    b. lactate dehydrogenase.
    c. collagenase.
    d. phosphodiesterase.
    e. histaminase.

15. Which of the following statements regarding snakes is FALSE?
    a. Inorganic anions are often found in snake venoms.
    b. About 20% of snake species are venomous.
    c. Snake venoms often interfere with blood coagulation mechanisms.
    d. Proteolytic enzymes are common constituents of snake venoms.
    e. Snakebite treatment is often specific for each type of envenomation.

CHAPTER

27

Toxic Effects of Calories

Martin J. Ronis, Kartik Shankar, and Thomas M. Badger

BIOLOGY OF EATING AND DIGESTION

- Digestion of Foods
- Integrated Fuel Metabolism
- Set-Point Theory and Neural Control of Energy Balance

METHODS TO ASSESS ENERGY BALANCE

- Assessing Caloric Intake
- Assessing Caloric Content of Foods
- Assessing Energy Expenditure
- Assessing Body Composition
  - Anthropometric Analysis
  - Hydrodensitometry
  - Air Displacement Plesmography
  - Absorptiometry
  - Computerized Tomography
  - Nuclear Magnetic Resonance (NMR)
  - Electrical Impedance
  - Total Body Water
- Assessing Physical Activity

BIOLOGY OF OBESITY

- Obesity Risk: Genes and Fetal Environment

TOXICITY RELATED TO EXCESS CALORIC INTAKE/OBESITY

- Adaptation of Liver and Adipose Tissue to Excess Calories
- Ectopic Fat Deposition
- Metabolic Syndrome
- Therapeutic Options for Managing Metabolic Syndrome
- Nonalcoholic Steatohepatitis (NASH)
- Endocrine Dysfunction in Obesity, Metabolic Syndrome, and NAFLD
- Obesity and Cancer Risk

HEALTH BENEFITS AND LIFE EXTENSION ASSOCIATED WITH CALORIC RESTRICTION

TREATMENT OF OBESITY

- Lifestyle Modification: Dieting and Exercise
- Toxic Effects of Dieting
- Drug Therapy for Weight Loss

ECONOMIC, SOCIOLOGIC, AND LEGAL ASPECTS OF THE OBESITY EPIDEMIC

- Health Insurance and Obesity
- Changing the Environment: Family and Community Approaches to Healthy Eating and Physical Activity
- Food Labels
- Governmental and Corporate Issues

KEY POINTS

- Nutrients can broadly be defined as chemical substances found in food that are necessary for proper growth, development, reproduction, and repair.
- Energy in the body is derived from three main nutrient classes: carbohydrates, protein, and fat, which in turn are made up of sugars, amino acids, and free fatty acids, respectively.
- Hormonal messages generated by the pancreas, adipose tissue, and GI tract orchestrate multiple responses associated with caloric intake and utilization.
- T e "set-point" hypothesis proposes that food intake and energy expenditure are coordinately regulated in the central nervous system to maintain a relatively constant level of energy reserve and body weight.
- Dieting is defined as the use of a healthy, balanced diet that meets the daily nutritional needs of the body and that reduces caloric intake with increased moderate exercise.

BIOLOGY OF EATING AND DIGESTION

All biotic organisms derive energy from food to sustain life. T is energy "drives" various cellular functions, including digestion, metabolism, pumping blood, and muscle contractions. Nutrients can broadly be defined as chemical substances found in food that are necessary for proper growth and development, reproduction, and repair following injury.

Because most bacteria and higher organisms cannot carry out photosynthesis, they derive their energy by metabolism of preformed organic molecules, such as carbohydrates. In general, bacteria utilize simpler organic molecules and animals and humans require more complex macronutrients (proteins, fats, and carbohydrates) to meet their needs.

Digestion of Foods

T e process of digestion is a remarkable orchestration of many complex biochemical and physiologic events. Breakdown of food begins in the mouth via the actions of enzymes in saliva. In the stomach, food is acted upon by gastric juices, which contains high amounts of hydrochloric acid. Numerous enzymes supplied by the pancreas, liver, and gall bladder aid digestion in the small intestine.

T e latter parts of the small intestine, the jejunum and ileum, are primary sites of nutrient absorption. T e surface area of the intestinal mucosa available for absorption is greatly increased due to a combination of folds called valvulae conniventes (folds of Kerckring) and finger-like projections (villi) that are lined with enterocytes. Digestion of proteins begins in the stomach and continues in the lumen of the small intestine. T e jejunum is the site of absorption of amino acids, dipeptides, and tripeptides by amino acid and peptide carriers in the enterocyte brush border. Lipids are hydrolyzed by pancreatic and intestinal lipases. Bile salts, along with phospholipids, facilitate the absorption of lipids. Macronutrient molecules (proteins, sugars, and fatty acids) that end up in the circulation undergo metabolism in various tissues to be either oxidized to extract energy or stored for future utilization.

Integrated Fuel Metabolism

Energy in the body is derived from three main nutrient classes: carbohydrates, protein, and fat, which in turn are made up of sugars, amino acids, and free fatty acids, respectively. T e principal circulating fuels in the body, glucose and free fatty acids, are stored as glycogen and triglycerides, respectively. Triglycerides are stored in specialized cells (adipocytes) within large lipid droplets. Proteins are critical in maintaining structure and function and are catabolized for energy only under extreme conditions.

Maintaining a stable supply of substrate for utilization by the brain is required because the brain has little to no stored energy in the form of glycogen or triglycerides. Unlike the brain, the heart and to some degree the liver and skeletal muscles derive most of their energy needs through the oxidation of fatty acids.

Hormonal messages generated by the endocrine cells of the pancreas, adipose tissue (adipokines), and GI tract (gut neuropeptides) are critical to orchestrating the multiple processes associated with fuel flux and metabolism. Insulin is the principal hormone required to manage nutrient fuels in both fed and fasted states. A rise in glucagon and glucocorticoids (such as cortisol) promote lipolysis and breakdown of glycogen.

Set-Point Theory and Neural Control of Energy Balance

A number of redundant feedback mechanisms that maintain energy homeostasis in living systems regulate the balance between food intake and energy expenditure to maintain fuel reserves at preset levels. Under steady-state conditions, energy is normally utilized to maintain basal metabolic rate and thermogenesis, and to carry out cellular processes, organ-specific functions, and movement (muscle contractions). Excess fuels

are converted to triglycerides and stored in adipose tissues. Because adipose tissue is the major depot of preserving energy, signals derived from the periphery communicate with regions in the brain that coordinate energy balance. When total energy consumed equals the total energy required to meet basal metabolic needs, growth, thermogenesis, and physical activity, the individual is in energy balance, and maintaining this balance will result in relatively stable weight and healthy body composition.

One theory called the "set-point" hypothesis proposes that food intake and energy expenditure are coordinately regulated by defined regions in the central nervous system that signal to maintain a relatively constant level of energy reserve and body weight. Implicitly, the model requires the existence of four major components of an energy homeostasis system: (1) afferent signals relaying the levels of energy stores, (2) efferent processes regulating energy storage and expenditure, (3) efferent mechanisms controlling ingestive behavior, and (4) integrative centers in the brain to coordinate these processes. Studies have shown that the hypothalamus plays a central role in the control of energy balance, especially food intake. T e hormone leptin, which is secreted in proportion to body fat stores from the adipose tissue, was the first signal to be identified to be a homeostatic regulator of energy balance.

Two populations of neurons involved in appetite control in the brain are sensitive to the action of leptin and other neuropeptides, including orexigenic peptides neuropeptide Y and agouti-related peptide and the anorexigenic peptides proopiomelanocortin and cocaine and amphetamine-regulated transcript. Downstream projections from these neurons interact with the melanocortin receptor neurons and the neurons in the paraventricular nucleus of the hypothalamus. In addition to the hypothalamic control of appetite per se, reward and hedonic processes of "liking" and "wanting" food occur in the ventral striatum of the midbrain in conjunction with the mesolimbic dopamine system. In addition, the corticolimbic system of reward is controlled by areas in the prefrontal cortex, which integrates sensory, emotional, and cognitive information to coordinate behavioral responses. Hence, the homeostatic control of energy balance fits into the larger decision scheme of choice behavior via a complex neural system.

METHODS TO ASSESS ENERGY BALANCE

Assessing Caloric Intake

In animal studies, caloric intake can be quantitatively monitored by measuring the amount of food consumed by animals in metabolic cages. Caloric intake can be derived by multiplying the quantity (g/day) of diets consumed with the caloric density of the diet. A prospective method to collect information about current intake is maintenance of food records. T ese are usually carried out for a specific duration of time (three to seven days, generally including both week and weekend days) during which a written record of all food and beverages consumed is maintained. Details may include portion sizes, cooking methods, and patterns of eating.

Assessing Caloric Content of Foods

Accurate assessment of the caloric value of foods is essential for effective nutritional management in clinical and public policy arenas. T e general calorie factors of 4, 9, and 4 for the major sources of energy—carbohydrate, fat, and protein—have been widely used. T e heat released by combustion of a food in a bomb calorimeter is a measure of its gross energy. T e truly metabolizable energy can be derived by accounting for energy lost in urine (mainly from nitrogen) and on the body surface. Protein content is mainly determined via estimating nitrogen. Fat content can be assessed by measuring the sum of methanol–chloroform extractable total fatty acids that can be expressed as triglyceride equivalents. Carbohydrate content is generally measured by difference as the remaining energy after accounting for protein, fat, alcohol, and ash.

Assessing Energy Expenditure

T e total energy expenditure or metabolic cost for an average adult is primarily composed of three components: (1) basal energy expenditure, (2) thermic effect of food, and (3) energy expenditure associated with physical activity. Basal energy expenditure, also called as resting energy expenditure, is the energy expended when the individual is lying down and at complete rest, generally after sleep in the postabsorptive state. T e energy expenditure from physical activity consists of expenditure related to exercise and nonexercise activity thermogenesis.

Components of energy expenditure can be measured using either direct or indirect calorimetry. T e basic principle in direct calorimetry is to measure the actual heat produced by the organism in a highly controlled environment as an estimate of energy expenditure. Most commonly used methods to estimate energy expenditure involve indirect calorimetry. By using experimentally derived estimates for energy yields per mole of oxygen, heat production can be calculated based on the quantity of oxygen consumed.

Assessing Body Composition

Body composition assessments permit describing the overall mass of an individual organism in terms of water, fat mass, lean mass, protein, and minerals. In a simple two-compartment model of body composition assessment, total body mass is divided into fat mass (essential and nonessential fat) and fat-free mass (including lean mass and water). Lean mass in this scenario includes protein, carbohydrate, and minerals.

Anthropometric Analysis—Although individuals with greater body weight (mass) per height tend to have greater fat mass, total body weight may also be determined by increased muscle mass. T e simplest indirect measure of body fatness is

the relative proportion of body weight (in kilograms) to body height squared (meters^2), more commonly referred to as body mass index (BMI). BMI, however, is only an estimate: BMI does not always reflect fat mass, and care must be taken when using BMI as an index of body fat.

Hydrodensitometry—Using the density of the whole body and correcting for residual air in the lungs and GI tract, the relative body fat can be estimated using derived equations. T is procedure is also known as underwater weighing.

Air Displacement Plesmography—T is procedure employs the same principles as underwater weighing described above, except rather than the body displacing water, it displaces air. T is is probably the most accurate, precise, and cost-effective measure of total body fat, and is employed widely in clinical research in the United States.

Absorptiometry—In this technique imaging is performed throughout the entire body by a photon beam. T is allows imaging of both soft tissues and bone. Percentage of body fat, lean tissue, and bone mineral density can be computed for the whole body or specific sites based on the analysis of images.

Computerized Tomography—T e ability to generate three-dimensional cross-sectional images allows regional localization of adipose tissues, muscles, and organs (e.g., liver). Using the image data, percent body fat and lean mass can be calculated.

Nuclear Magnetic Resonance (NMR)—NMR works by interpreting radio-frequency signals of excited nuclei in an external magnetic field. T e physical characteristics of the hydrogen atom differ when the hydrogen is located on protein, fat, or water and this can be detected and quantitated to determine body composition.

Electrical Impedance—Bioelectrical impedance analysis and total body electrical conductivity measure total body composition based on measuring electrical impedance (the inverse of conductance) of an electric current passed through the body. Lean mass has more water and greater conductivity than fat mass and predictive equations are employed to derive fat and lean body mass.

Total Body Water—Body fat and lean mass can be calculated by estimating total body water using stable isotopes (either deuterium or O^{18}). Whereas body water occupies 73% of lean mass, fat-free mass can be estimated using appropriate assumptions.

Assessing Physical Activity

Devices such as accelerometers and pedometers can be utilized to empirically estimate activity. An important challenge in utilizing accelerometers is to convert the count data into energy expenditure, which is done using different regression models.

BIOLOGY OF OBESITY

Obesity Risk: Genes and Fetal Environment

Historically, human life was marked by unpredictable access to food. Fitness and survival of an individual were likely to be closely related to the ability to maximally seek, acquire, consume, and store energy (as fat) when food was available, and to select for mechanisms that reduce energy expenditure during times when food is scarce. T e advent of agrarian lifestyle and recent industrialization has meant that much of the developed and emerging world now has a drastically altered environment. Food is generally available for most people and our lifestyles require less physical activity and exertion. Hence, our genetic legacy in the context of caloric abundance acts as a powerful engine for weight gain, obesity, and its associated metabolic dysfunction. Natural variation and random mutation in genes controlling hypothalamic energy balance set-points occurred as human beings developed fire and social behaviors and were released from risk of predation. T e "drifty gene" hypothesis explains why even in societies where obesity is high, not everyone becomes obese. Obesity is a highly heritable trait and studies comparing monozygotic with dizygotic twins indicate that 40% to 75% of the interindividual difference in trait is accounted for by genetic variability. Several genes whose disruption causes severe monogenic forms of familial obesity have been described. Remarkably, most of these genes impair central control of food intake. However, the genetic basis of non-syndromic (common) obesity has remained elusive.

T e incidence of obesity continues to rise, including the prevalence among infants. As for many chronic diseases, it is now widely accepted that increased susceptibility to obesity can be programmed in utero and early postnatal life. Another important influence on risk of obesity in later life is maternal body composition (fat mass) at conception and gestational weight gain.

TOXICITY RELATED TO EXCESS CALORIC INTAKE/OBESITY

Many of the adaptive, physiologic responses to the positive energy balance produced as a result of overeating and inadequate physical activity result in toxicity over the long term. Short-term coordinated changes in metabolic pathways in white adipose tissue in response to overfeeding result in excess energy storage in the form of triglycerides, which leads to increased size of preexisting adipocytes (hypertrophy) and to formation of new adipocytes (hyperplasia). Under conditions of chronic excess ingested energy, the ef ciency of energy storage in adipose tissue is decreased and the body stores energy in ectopic sites. Triglycerides begin to accumulate in nonadipose tissues such as liver, skeletal muscle, and the pancreas as lipid droplets resulting in insulin resistance, inflammation, and tissue damage.

In addition, adipose tissue from obese individuals releases chemokines and cytokines, the so-called adipokines, which

contribute to a state of "metabolic inflammation." Non-esterified fatty acids and the other factors released from adipose tissue contribute to the development of metabolic syndrome in some overweight and obese individuals. T is is a cluster of components including insulin resistance, disruptions in lipid homeostasis (dyslipidemia), and elevated blood pressure, all of which substantially increase the risk for development of cardiovascular disease and type 2 diabetes.

Adaptation of Liver and Adipose Tissue to Excess Calories

Triglycerides and glycogen are used by the body to store excess caloric energy. Although obesity is often associated with overconsumption of high-fat diets, it can develop from excessive caloric intake of any food energy source, including carbohydrates and proteins. Dietary carbohydrates are converted to monosaccharides, mainly glucose and fructose, which are further metabolized in the liver and peripheral tissues. Excess glucose can be stored in the liver as the glucose polymer, glycogen. However, the majority of excess hepatic glucose is shunted into de novo fatty acid and triglyceride synthesis.

Recent DNA microarray analysis of gene expression in human adipose tissue biopsies suggests that coordinated upregulation of lipogenesis occurs in fat rapidly as a result of increased caloric intake. T e increase in triglyceride synthesis ultimately drives adipose tissue hypertrophy. In addition to adipocyte hypertrophy, excess calories also trigger proliferation and differentiation of pre-adipocytes in adipose tissue depots into new adipocytes, a process known as hyperplasia. It is clear that there is a limit to which adipose tissue can expand safely without damage to adipocytes and that when this limit is reached toxicity results.

When fat mass increases excessively, adipose tissue undergoes extensive structural remodeling. An extracellular matrix with high concentrations of collagen fibrils and fibronectin appears to be essential for maintenance of the structural integrity of adipocytes and for pre-adipocyte differentiation. At the point when adipocytes reach a certain size limit within a particular fat pad, the transcription factor, hypoxia-inducible factor 1α (HIF-1α), is expressed. HIF-1α regulates inappropriate remodeling of the extracellular matrix and development of fibrosis in adipose tissue in response to hypoxia and obesity. Fibrosis can be analyzed by collagen fibril staining and by col6a3 gene expression. Secreted protein acidic and rich in cysteine (SPARC), also known as osteonectin, is required for appropriate collagen synthesis during matrix remodeling. Both SPARC–/– mice and obesity-prone ob/ob mice where the collagen VI gene has been deleted display increased adipocyte and fat pad size, loose extracellular matrix structure, and reduced inflammation and metabolic disturbances after high-fat feeding. T e complex interactions between enlarging adipocytes and the fibrotic extracellular matrix trigger activation of MAP kinase pathways resulting in development of adipocyte insulin resistance, apoptosis, and necrosis, which in turn results in activation of resident macrophages in the fat and an inflammatory response (Figure 27–1).

Ectopic Fat Deposition

T e major sites for ectopic fat deposition (i.e., extra-adipocyte) are the liver and skeletal muscle. Correlation between central (visceral) adiposity, waist circumference, and ectopic fat deposition is better than that for BMI and is also highly correlated with progressive insulin resistance. In the liver, intrahepatocellular lipid accumulation, also known as fatty liver, or steatosis, is defined as an increase in hepatic lipid content above 5% by weight. To confirm that steatosis is actually present, histochemical staining of frozen sections for triglycerides using stains such as Oil Red O is required. Abnormal lipid accumulation in the liver in the absence of heavy alcohol usage is referred to as nonalcoholic fatty liver disease (NAFLD) and is associated with a wide spectrum of hepatic dysfunction. Simple steatosis is generally reversible with weight loss and/or lifestyle modification (e.g., diet and exercise). Hepatic lipid accumulation can occur as the result of one or more of the following: (1) increased fatty acid supply to the liver and increased fatty acid transporter expression, (2) increased de novo fatty acid and triglyceride synthesis, (3) decreased fatty acid oxidation, and (4) decreased synthesis and/or secretion of VLDL. Which of these processes predominates depends on the degree of obesity, total caloric intake, and diet composition. Reduced serum concentrations of the adipokine, adiponectin, that accompany development of obesity will result in increased hepatic fatty acid synthesis and reduced fatty acid degradation and thus contribute to development of steatosis.

T e other major site of ectopic fat deposition in obesity is skeletal muscle in the form of intramyocellular lipid (IMCL). IMCL has been shown to positively correlate with visceral adiposity.

Metabolic Syndrome

Overnutrition and a sedentary lifestyle lead to a clustering of metabolic and physiologic components called the metabolic syndrome. Central obesity (waist circumference), insulin resistance (increased fasting glucose above 100 mg/dL and increased fasting insulin, as a result of impaired glucose uptake into skeletal muscle/fat and increased glucose output by the liver, resulting from end-organ insensitivity to insulin), dyslipidemia (decreased serum HDL below 40 mg/dL in men and 50 mg/dL in women, and increased serum triglycerides above 150 mg/dL), and hypertension (blood pressure higher than 130/85) may be evident.

However, the relationship between obesity and whole-body insulin resistance appears to be mediated through increased circulating fatty acids and ectopic fat deposition in IMCL in skeletal muscle (Figure 27–2). Consumption of a very low calorie diet in obese subjects for as little as five days has been shown to produce marked decreases in IMCL and enhanced insulin sensitivity without significant changes in body fat mass. Reductions in glucose import into skeletal muscle with IMCL result from inhibition of translocation of the glucose transporter GLUT-4 from cytosolic- to membrane-associated compartments through the action of IMCL metabolites such as diacylglycerol (DAG),

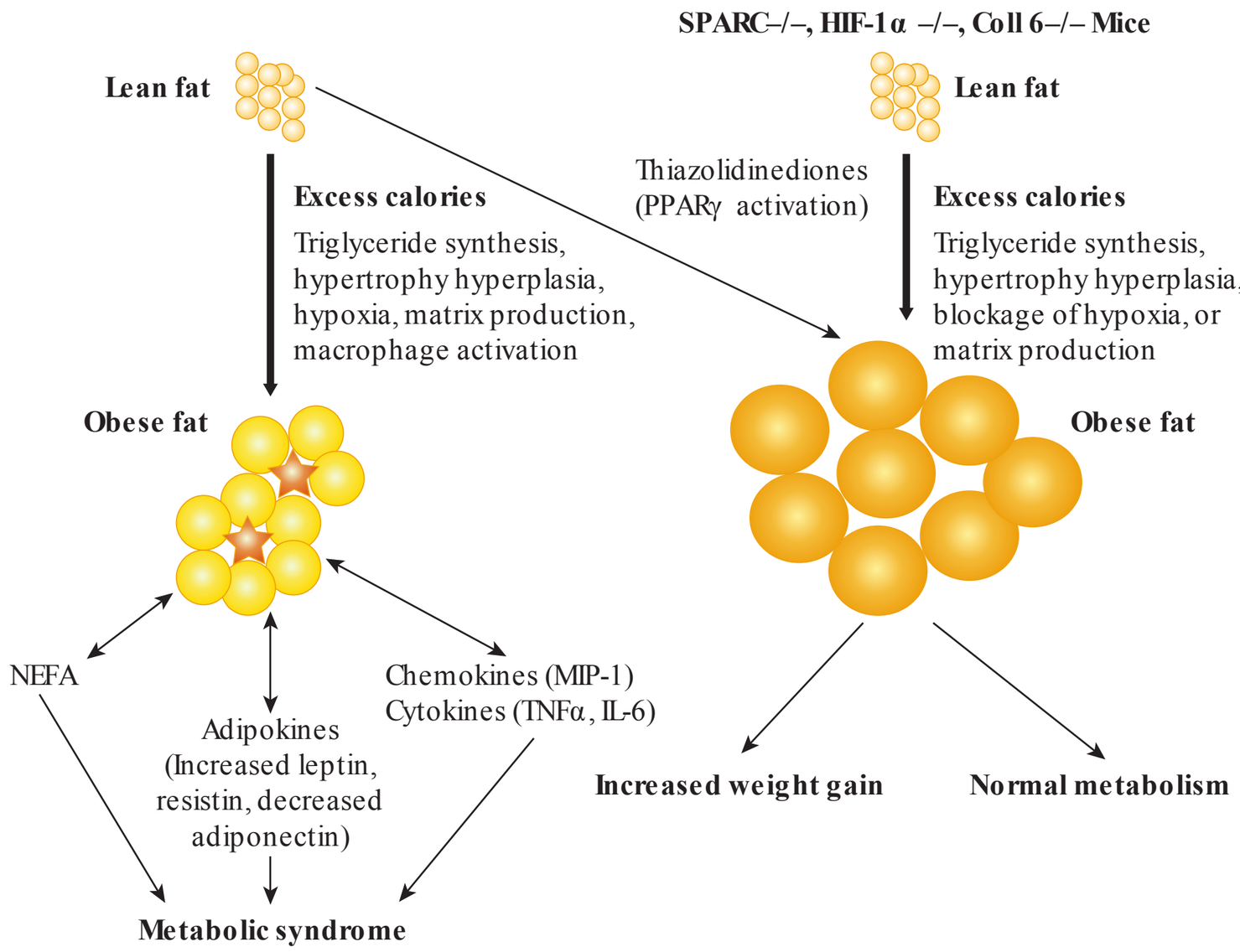

FIGURE 27–1 **Effects of excess calories (energy) on fat morphology under conditions leading to metabolic syndrome (left) or following stimulation of adipocyte differentiation and hyperplasia by thiazolidinedione treatment/in knockout mice incapable of normal responses to hypoxia (HIF-1α –/–)/in knockout mice incapable of normal extracellular matrix production (SPARC–/–, Coll 6–/–) (right).**

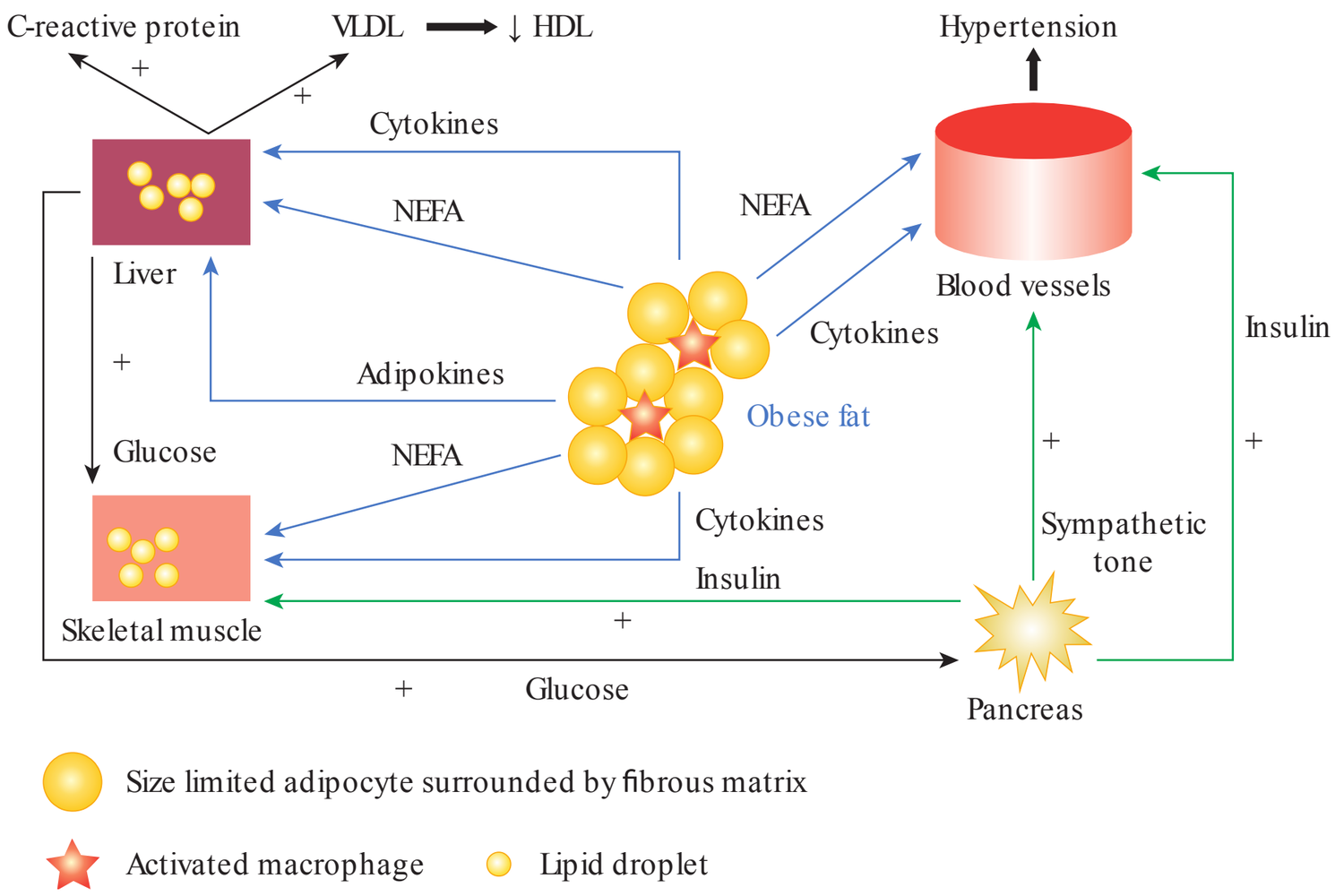

FIGURE 27–2 **Pathogenesis of metabolic syndrome.**

long-chain fatty acid CoAs, ceramides, and oxidized lipids. Reduced glucose transport also occurs in insulin-resistant adipose tissue itself and the negative effects of obesity are exacerbated because reduced insulin signaling in adipocytes also enhances expression of hormone-sensitive and adipose triglyceride lipases to further increase the release of nonesterified fatty acids. Insulin resistance in liver leads to excess glucose production as the result of a reduced ability of insulin to suppress the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK). T is contributes to systemic hyperglycemia and increased pancreatic

insulin production. Insulin resistance and steatosis are strongly correlated and interventions that lead to lower plasma insulin levels also decrease liver triglyceride content.

Therapeutic Options for Managing Metabolic Syndrome

Lifestyle modifications in the obese, including diets producing stable weight loss, long-term increased physical activity, or bariatric surgery (discussed below), are of benefit in treating all the components of metabolic syndrome, but suffer from limited compliance and significant risk of complications in the case of surgery. T erefore, routine clinical management has focused on pharmaceutical therapies for insulin resistance, hyperglycemia, dyslipidemia, and hypertension to reduce the risks of cardiovascular disease and type 2 diabetes.

Several classes of drugs are used to target insulin resistance. Dyslipidemia associated with metabolic syndrome is a major modifiable risk factor for cardiovascular disease that may be treated with the statins, which are inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG-CoA reductase).T ese compounds improve overall lipid profiles by decreasing LDL concentration, increasing HDL, and decreasing triglycerides.

Nonalcoholic Steatohepatitis (NASH)

Ectopic fat deposition in the liver is strongly correlated with obesity and insulin resistance. T e disease progression of nonalcoholic fatty liver disease (NAFLD) is first to NASH, which is characterized by cell death, inflammation, and fibrosis, then to cirrhosis in which liver function is significantly impaired, and ultimately to hepatocellular carcinoma (Figure 27–3).

Treatment with insulin sensitizers, bariatric surgery, and lifestyle modification (e.g., diet and exercise) resulting in weight loss and reduction of hepatic fat content improve NASH liver pathology.

Endocrine Dysfunction in Obesity, Metabolic Syndrome, and NAFLD

Hyperglycemia resulting from systemic insulin resistance provokes a compensatory increase in insulin secretion from the pancreas in obese individuals. Hyperinsulinemia then appears to have secondary effects on other endocrine systems. For example, growth hormone (GH) secretion is dramatically suppressed by obesity in both adults and children, but can be reversed by weight loss. Mechanisms appear to involve direct feedback effects of insulin on the pituitary and a reduction in secretion of the endogenous GH-releasing peptide ghrelin that is produced by the stomach and hypothalamic centers.

Increased serum concentrations of free androgens appear to explain, in part, why childhood obesity is associated with earlier pubertal development in girls. Hyperandrogenization is involved in the increased incidence of polycystic ovary syndrome in obese adolescent girls with metabolic syndrome, anovulatory cycles, and subfertility in obese women of childbearing age. Increased adipose tissue mass also results in increased estrogen production as a result of androgen aromatization in fat tissues. T is may also contribute to accelerated puberty in girls.

In obese boys, data suggest that puberty may be delayed owing to increased aromatization of androgens in adipose tissue. Negative feedback of estrogens at the level of the hypothalamic–pituitary axis may result in reduced luteinizing hormone secretion and reduced testosterone production.

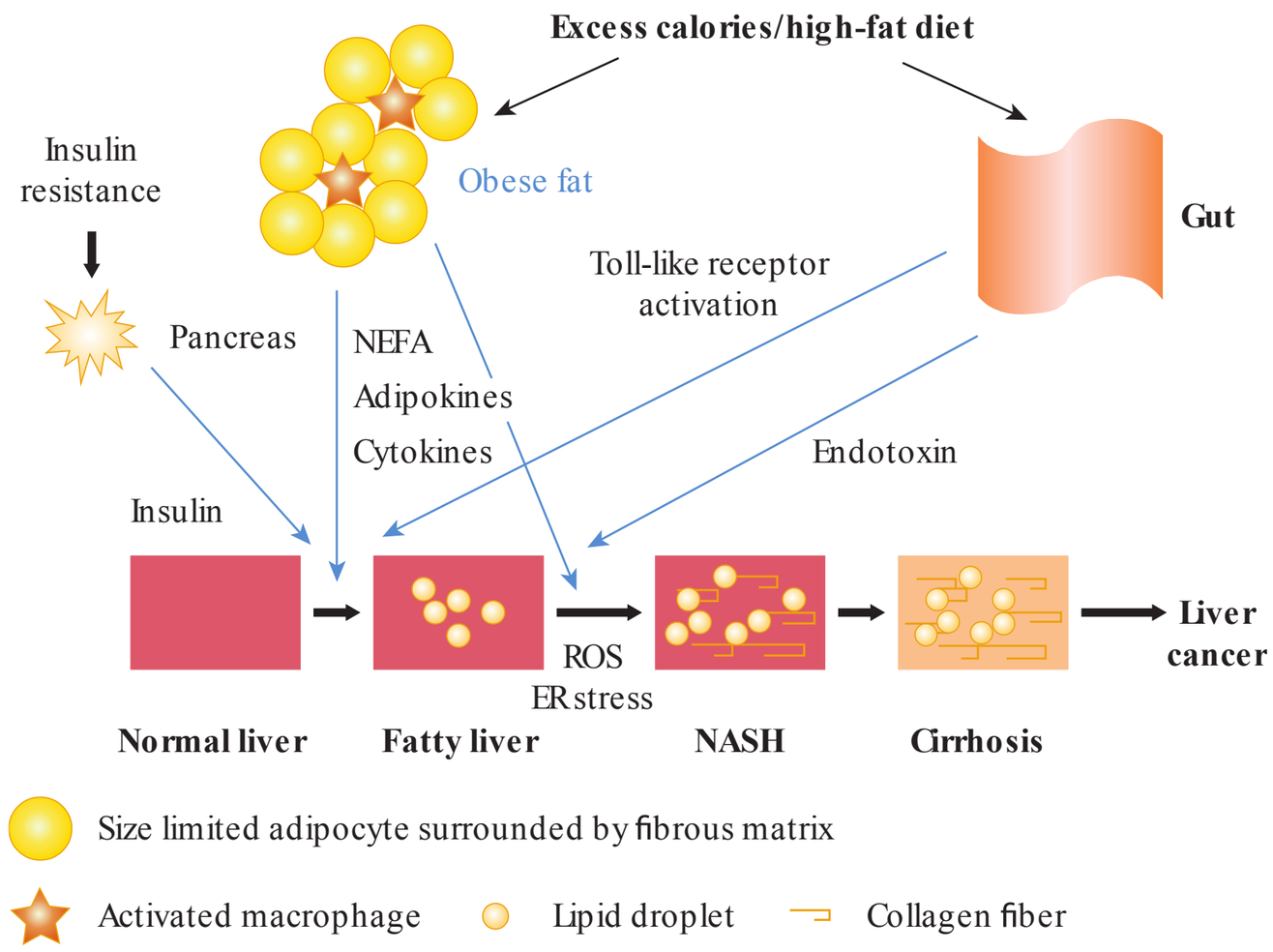

FIGURE 27–3 **Progression of nonalcoholic fatty liver disease (NAFLD).** ROS, reactive oxygen species; ER stress, endoplasmic reticulum stress.

TABLE 27–1 Estimated risk ratios* for cancer in relation to body mass index.

Cancer Type	Men	Women
Colon cancer	1.24	1.09
Gallbladder cancer	—	1.59
Leukemia	1.08	1.17
Malignant melanoma	1.17	—
Multiple myeloma	1.11	1.11
Esophageal adenocarcinoma	1.52	1.51
Renal cancer	1.24	1.34
Thyroid cancer	1.33	1.14
Prostate cancer	1.03	—
Postmenopausal breast cancer	—	1.12
Endometrial cancer†	—	1.73

*Per increase in body mass index by 5 kg/m^2 (Roberts DL, Dive C, Renehan AG: Biological mechanisms linking obesity and cancer risk: new perspectives. Annu Rev Med 61:301–316, 2010).
†For body mass indexes above 27 kg/m^2.

In addition to suppression in GH and gonadotropin secretion, hypothyroidism is common in individuals with metabolic syndrome. Reduced thyroid hormone concentrations may exacerbate NASH progression in the liver by increasing triglyceride synthesis, reducing fatty acid oxidation, and increasing hepatic cholesterol concentrations by reducing conversion to bile acids.

Obesity and Cancer Risk

Increased BMI is well known to be associated with significantly increased risk of a number of cancers. T ese include sex steroid–dependent endometrial, breast, and prostate cancer, GI tract cancers such as esophageal adenocarcinoma, and colon cancer and renal cancer (Table 27–1). Additional potential associations between obesity and cancer may involve depletion of cellular antioxidant systems as a result of the low-grade chronic systemic inflammation that accompanies morbid obesity and the possibility that mesenchymal stromal cells arising from expanding white adipose tissue may be recruited to tumors to promote angiogenesis and drive tumor progression.

HEALTH BENEFITS AND LIFE EXTENSION ASSOCIATED WITH CALORIC RESTRICTION

T e opposite of overfeeding is caloric restriction (CR; also known as dietary restriction). Over the past two decades, CR has been repeatedly shown to increase life span and reduce age-related disease in comparison with ad libitum feeding in a wide variety of organisms including yeast, nematodes, fruit flies, fish, many rodent species, and dogs. Preliminary data in humans suggest reproduction of many of the results from animal studies, including reduced fat and lean mass, reduced insulin, reduced energy expenditure, lower core body temperature, and improved lipid profiles. It has been suggested that the increased health and longevity associated with CR is related to reduced energy utilization, increased insulin sensitivity, and reduced inflammation.

TREATMENT OF OBESITY

Lifestyle Modif cation: Dieting and Exercise

As applied to body composition, dieting involves a plan or regimen to improve body composition. Trimming excess body fat generally requires reductions in total caloric intake, increases in the total energy expenditure (through exercise or physical activity), and modifications in diet composition. T is combined strategy is intended to reduce energy intake to a level low enough to drive the body to utilize stored fat as an energy source, thereby burning body fat.

Comprehensive lifestyle changes that promote a neutral energy balance include two major and closely linked components: (1) learning to consume only the amount of calories from high-quality foods necessary to support basal body energy needs plus energy needs to maintain physical activity and (2) selecting a reasonable physical activity plan that fits into the dieters' overall lifestyle patterns.

T e energy in food eaten can be "cost accounted" roughly as follows: (1) energy required to digest and absorb food, (2) energy utilized to support basal functions such as pumping blood and breathing, (3) energy for body functions other than basal functions such as walking and playing golf, and (4) nonutilized food calories such as food components not fully digested or absorbed.

Insulin has a major influence on carbohydrate, fat, and protein metabolism. Under conditions of adequate carbohydrate intake, insulin causes the sugar not utilized as fuel to be stored as fat and prevents utilization of fat as an energy source. T us, a high-carbohydrate diet tends to induce insulin secretion, which promotes carbohydrate energy storage as fat and tends to reduce the utilization of fat as an energy source.

Toxic Effects of Dieting

If the diet does not include all the required nutrients (i.e., an imbalanced diet), metabolism will suffer and, with time, this can result in health problems. Recalling the concept of hormesis, this is true whether in the case of deficiency of specific nutrients (deficiency disorders such as anemia or osteoporosis) or toxicity caused by excesses of a particular nutrient (such as thyroid impairment, vitamin deficiencies, mental confusion).

Some popular diet plans call for excess intake of a particular food and these can not only alter metabolism, but also interfere with medications.

Drug Therapy for Weight Loss

In addition to the diet plans described above, many overweight individuals turn to drug therapy to help lose body weight. Appetite suppressants, e.g., sympathomimetics such as diethylpropion, attempt to lessen the psychologic motivation for food, usually by acting on central nervous system appetite control centers, such as those in the hypothalamus. Although sympathomimetics can be used for long periods of time, their appetite-reducing effects tend to decrease after a few weeks in many people. T us, appetite suppressants are often used in the early stages of a weight loss program. People are likely to lose weight while taking sympathomimetics, but the weight loss is generally temporary without modifications in diet composition, eating behavior, and physical activity. Short-term use is usually accompanied by minor side effects such as thirst, irritability, constipation, stomach pain, dizziness, dryness of mouth, heightened sense of well-being, headache, irritability, nausea, nervousness or restlessness, trembling or shaking, and trouble sleeping. However, long-term use of appetite suppressants often times leads to more serious side effects: intracerebral hemorrhage, acute dystonia, myocardial injury, psychosis, cerebral arteritis, cardiac arrhythmias, heart valve damage, and even fatal pulmonary hypertension.

ECONOMIC, SOCIOLOGIC, AND LEGAL ASPECTS OF THE OBESITY EPIDEMIC

Health Insurance and Obesity

Obesity is not considered an illness for most insurance purposes. However, obesity can affect the cost of health insurance because as a group, obese people have a significantly greater risk of cardiovascular disease, hypertension, type 2 diabetes, and other health issues than lean people. Several health insurance companies use BMI as a measure of obesity and use BMI to compute disease risk and health insurance premiums. Obesity can result in high premiums and in the case of morbidly obese individuals, insurers may decline their application. Obesity is also regarded by insurance companies as a substantial risk for both life and disability policies. Clearly, costs increase proportionally with the degree of obesity.

Changing the Environment: Family and Community Approaches to Healthy Eating and Physical Activity

Basic practices that were common in past decades, when the general population was leaner, are lacking now when obesity is prevalent. Prior to 1970, the average BMI was 25.1 for men and 24.9 for women in the United States. Physical education (PE) classes were a regular feature of school curriculums; most meals were prepared using fresh produce, meats, and dairy products. It was common for children to walk or ride bicycles to school and to participate in games requiring physical activity during school recess and after school and on weekends. By 2002, there were fewer schools with PE classes, consuming electronic games and devices, fewer meals cooked from fresh components, more fast food, and a far more sedentary lifestyle than that prior to 1970. BMI values had risen to 27.8 in US men and 28.1 in US women. One approach to fighting the obesity issue is to bring back many of those practices used in the past. T ere are initiatives to establish community gardens, build community walking and riding trails, and teach people cooking and shopping skills that lead to healthier meal preparation. School systems are starting to return to PE classes on a regular basis and remove high-density caloric foods and drinks from vending machines.

Food Labels

T e Food and Drug Administration (FDA) is responsible for assuring that foods sold in the United States are properly labeled, regardless of origin (i.e., domestic or foreign). Food labels can be an important factor to help consumers in their food choices that can help prevent obesity and other diseases. Federal law requires that a minimal amount of information be listed on food packaging, including ingredients and nutrition data.

Governmental and Corporate Issues

T ere are increasing pressures from local, state, and federal governments in the United States to regulate various aspects of food production and marketing as a means of promoting health, reducing obesity, and its consequences. Food labeling is just one example of government intervention, whereby food processors and restaurants must provide a measure of nutrient and/or caloric content.

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QUESTIONS

1. Humans consume food to provide energy needed to
   a. drive cellular functions including digestion, metabolism, pumping blood, nerve activity, and muscle contractions.
   b. promote photosynthesis.
   c. synthesize oxygen in the lungs.
   d. prepare minerals for use in the body.
   e. produce carbon dioxide to fuel body functions.

2. Neural control of energy balance
   a. may be defined as the action of leptin on CNS function.
   b. may be defined as the action of hypothalamic cholinergic control of appetite and hedonic control.
   c. may involve a balance between food intake and energy expenditure.
   d. may involve a balance between leptin's action on orexigenic versus anorexigenic peptide expression.
   e. may involve adrenocortical control of hepatic function.

3. Body composition may be assessed by
   a. electrical impedance because lean mass has more water and greater conductivity than fat mass.
   b. anthropometric analysis of the body mass index.
   c. hydrodensitometry, which uses the density of the whole body and corrects for residual air in the lungs and GI tract to determine relative body fat.
   d. nuclear magnetic resonance.
   e. all of the above.

4. Ectopic fat deposition includes
   a. adipose tissue.
   b. skeletal muscle.
   c. lungs.
   d. heart.
   e. GI tract.

5. Excess calories may be
   a. stored as glucose in adipose tissue.
   b. stored as triglycerides in CNS tissue.
   c. stored as glycogen in CNS tissue.
   d. stored as glycogen in the liver.
   e. stored as triglycerides in the GI tract.

6. Metabolic syndrome is a constellation of actions including
   a. typically results from elevated fasting glucose, increased HDL, and hypertension.
   b. typically results from elevated fasting glucose, increased LDL, and hypertension.
   c. typically results from elevated fasting glucose, hypertriglyceridemia, and hypotension.
   d. typically results from elevated fasting glucose, hypotriglyceridemia, and truncal obesity.
   e. typically results from elevated fasting glucose, hypertriglyceridemia, and truncal obesity.

7. Excess caloric intake
   a. may lead to nonalcoholic steatohepatitis.
   b. is always correlated with obesity and insulin resistance.
   c. is characterized by elevations of serum ALT concentrations in all cases.
   d. leads to hepatic cirrhosis and liver cancer in almost all cases.
   e. is readily reversible by dieting.

8. Although dieting may effectively reduce body weight,
   a. toxicity may result from stimulation of adipokine release.
   b. toxicity may result from inhibition of drug metabolizing enzymes.
   c. toxicity may result from a loss of required nutrients.
   d. toxicity may result from extreme mental illness.
   e. toxicity may result from weight cycling.

9. Body mass index
   a. may be used as an indicator of suf cient caloric and essential nutrient intake.
   b. may be defined as body height divided by body weight squared.
   c. has risen insignificantly over the past 30 years in the United States.
   d. may not be used in the estimation of cancer risk in humans.
   e. may be defined as body weight divided by height squared.

10. Which of the following definitions is false?
    a. T e set-point hypothesis proposes that food intake and energy expenditure are coordinately regulated by defined regions in the brain that signal to maintain a relatively constant level of energy reserve and body weight.
    b. Hormonal messages generated by the endocrine cells of the pancreas, adipose tissue, and GI tract are involved in orchestrating multiple responses associated with caloric intake and caloric utilization.
    c. Caloric content of foods generally assumes factors of 4, 9, and 4 for carbohydrate, fat, and protein, respectively.
    d. T e body mass index (BMI) is an accurate method for assessing body composition.
    e. Liver, adipose, muscle, and other tissues adapt to excess caloric loads.

CHAPTER

28

Nanotoxicology

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NANOMATERIAL BASICS

Perspectives: Engineered Nanoparticles versus ambient particulate matter
Properties and Behaviors of ENPs versus Larger Particles
Classes of ENMs
Physicochemical Properties of Nanomaterials Relevant for Toxicity
Surface Area and Reactivity
Surface Charge
Surface Chemistry
Unique Quantum and Magnetic Properties
Geometry and Dimensions
Biopersistence

THE NANOMATERIAL BIOLOGIC INTERFACE

TOXICITY MECHANISMS

CAVEATS IN NANOTOXICOLOGIC ASSAYS

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ECOTOXICOLOGY OF ENMS

Environmental Uses and Exposures to Nanomaterials
Ecologic Risk Assessment of Manufactured Nanomaterials
Toxicity of Manufactured Nanomaterials
Complications of Assays
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Mechanisms of Toxicity

KEY POINTS

- Nanotechnology is the understanding and control of matter at nanoscale dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications.
- Nanotoxicology can be defined as the study of adverse effects of nanomaterials on living organisms and the environment.
- Surface properties are major determinants of biologic reactivity due to high surface area, surface charge, dissolution and release of metal ions, and redox activity leading to generation of ROS.
- T e respiratory tract is the major route for humans to exposure of nanomaterials.
- Surface properties are major determinants of biologic reactivity due to high surface area, surface charge, hydrophobicity and partitioning into lipid membranes, dissolution and release of metal ions, and redox activity.
- Dosemetric defines a dose in terms of an inherent property (physical, chemical, reactivity, etc.).

Nanotechnology has become a multibillion dollar industry worldwide, producing high volume, commercial nanomaterials including nanosilver, fullerenes, quantum dots, carbon nanotubes (CNTs), and metal oxide nanoparticles (NPs) (Figure 28–1). Nanotoxicology seeks to identify and characterize the hazards of engineered nanomaterials (ENMs) for purposes of risk assessment for humans and the environment, which requires a highly multidisciplinary team approach covering expertise in toxicology, biology, chemistry, physics, material science, geology, exposure assessment, physiologic-based pharmacokinetics (PBPK), and medicine. All these disciplines are necessary to develop testing strategies, establish toxicity ranking, determine "safe" exposure levels, and derive preventative exposure guidelines.

NANOMATERIAL BASICS

Perspectives: Engineered Nanoparticles versus Ambient Particulate Matter

Airborne ambient particulate matter (PM) can elicit adverse effects in the respiratory tract, in secondary organs and systemically (see Chapter 29). T e smallest fraction of PM, referred to as ultrafine particulates (UFPs, < 100 nm), has been associated with effects in the cardiovascular and central nervous system (CNS) as a consequence of their translocation to and distribution via blood circulation and neurons. Natural sources of ultrafine and nanoparticles include gas to particle conversions, forest fires, volcano eruptions, viruses, magnetotactic bacteria, mollusks, arthropods, fish and birds. Unintentional anthropogenic sources include internal combustion engines, power plants, metal fumes from smelting and welding and heated surfaces. Engineered nanoparticles would represent intentional sources. Epidemiologic studies have demonstrated that increased susceptibility to adverse effects from ambient particulate air pollution includes preexisting disease (asthma, diabetes), age (very young, elderly), or genetic background (polymorphism).

Properties and Behaviors of ENPs versus Larger Particles

Table 28–1 contrasts differences between NPs (< 100 nm) and larger particles (> 500 nm) in general characteristics, translocation propensity, and cellular effects assuming inhalational exposure. Biologic systems do not perceive a precise boundary at the 100 nm size threshold, but rather a gradual transition between nano- and larger-sized particles.

Efficient mucociliary and alveolar macrophage-mediated elimination following deposition in the respiratory tract is efficient for both nano-and larger particles once they are internalized by macrophages. NPs inhaled and deposited as singlets are too small to be efficiently recognized and phagocytized by alveolar macrophages—unless they aggregate or agglomerate to form larger particles—and thus overall alveolar macrophage-mediated clearance in the lung is poor. In contrast, uptake by epithelial cells and translocation into blood and lymphatic circulation occur regularly for NPs and only under heavy overload conditions for larger particles.

Classes of ENMs

Manufactured nanomaterials have an enormous range of composition, geometry, and complexity ranging from simple isometric forms (NPs), one-dimensional (1D) forms (fibers or tubes), and two-dimensional (2D) forms (plate-like or disk-like materials) as shown in Figure 28–2. A large fraction of the stable elements in the periodic table have now been cast into NPs. Nanomaterials may be applied to surfaces such as biomedical implants to enhance their function and biocompatibility, incorporated into nanostructured solids, or composites to improve strength, conductivity, and durability. As nanoscience progresses, there will be less emphasis on simple

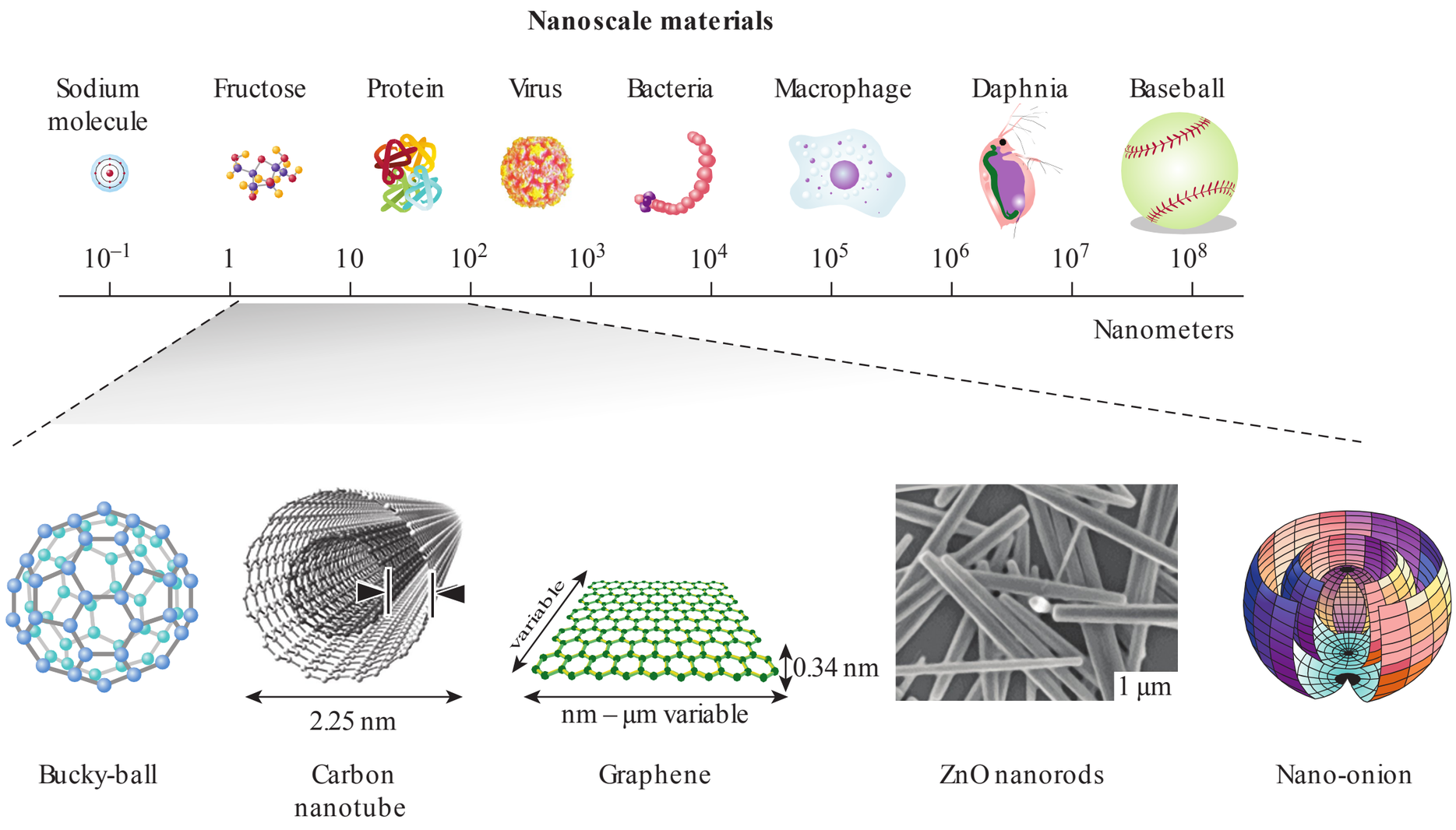


FIGURE 28–1 **Length scales for natural and synthetic structures (above) and some examples of engineered nanomaterials of varying size and shape (below).**

TABLE 28–1 What is different: nanoparticles versus larger particles (respiratory tract as portal of entry).

	Nanoparticles (< 100 nm)	Larger Particles (> 500 nm)
General characteristics		
Ratio: number or surface area/volume or mass	High	Low
Agglomeration in air, liquids	Likely (dependent on medium and surface)	Less likely
Deposition mechanism in respiratory tract	Diffusion; throughout resp. tract	Sedimentation, impaction, interception; throughout resp. tract
Protein/lipid adsorption in vitro	Very effective and important	Less effective
Protein/lipid adsorption in vivo	Yes	Some
Translocation to secondary target organs:	Yes	Generally not (to liver under "overload")
Clearance		
• Mucociliary	Probably yes	Efficient
• By alveolar macrophages	Poor	Efficient
• Into or across lung epithelium	Yes	Mainly under overload
• Lymphatic	Yes	Under overload
• Blood circulation	Yes	Under overload
• Sensory neurons (uptake + transport)	Yes	Not likely
Cell entry/uptake	Yes (caveolae; clathrin; lipid rafts; diffusion)	Yes (primarily phagocytic cells)
• Mitochondria	Yes	No
• Nucleus	Yes (< 40 nm)	No
Effects (caveat: dose!)**:**		
At secondary target organs	Yes	(No)
At portal of entry (resp. tract)	Yes	Yes
• Inflammation	Yes	Yes
• Oxidative stress	Yes	Yes
• Activation of signaling pathways	Yes	Yes
• Genotoxicity, carcinogenicity	Probably yes	Some

Data from Oberdörster G, Elder A, et al.: "Nanoparticles and the Brain: Cause for Concern?" Journal of Nanoscience and Nanotechnology, 2009;9:4996–5007.

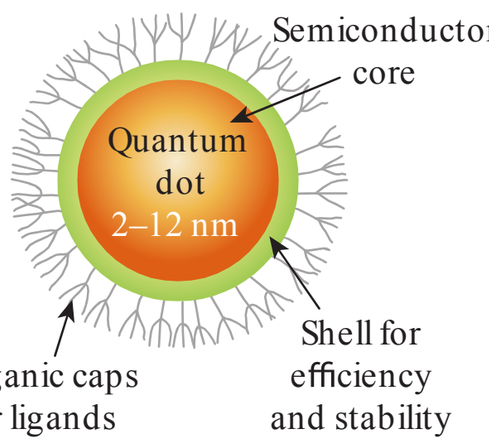
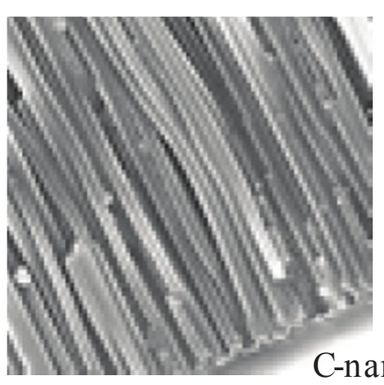
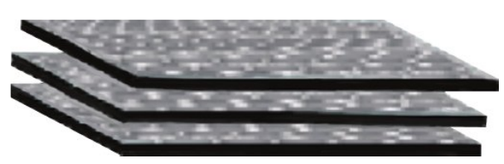
Chemistry \ Geometry	Isometric particles	1D; fibers/tubes	2D; plates, disks
Metals	Silver, gold nanoparticles Iron, cobalt, nickel magnetic NPs copper NP conducting inks	Gold or platinum nanowires	Silver nanoplates
Semiconductors	CdSe/ZnS quantum dots (see example)	Si, ZnO semiconducting nanowires, nanorods	Plate-like semiconductor nanocrystals
Ceramics	Zinc oxide, titanium dioxide pigments and sun screens, cerium oxide catalysts	Electrospun ceramic nanofibers for composite fillers	Nanoclays
Carbons	Fullerenes, carbon black, carbon nanohorns	Carbon nanotubes Carbon nanofibers	Graphene, graphene oxide few-layer graphene (example)
Polymers	Biodegradable polymer nanobeads for medical applications, branched dendrimers	Electrospun polymer nanofibers	
Examples:		 C-nanofiber array	 Few-layer graphene

FIGURE 28–2 **Classification of nanomaterials by geometry and chemistry.** The examples in this matrix illustrate the diversity in engineered nanomaterials, a diversity that continues to increase as new nanomaterials are synthesized.

geometries and chemistries, and more emphasis on complex material structures that combine nanoelements into active or smart structures.

Physicochemical Properties of Nanomaterials Relevant for Toxicity

Table 28–2 summarizes the nanomaterial properties thought to be relevant to biologic responses.

Surface Area and Reactivity—At the nanoscale, structures have a high surface-to-volume or surface-to mass ratio. T is high surface area of NPs is responsible for increased surface reactivity, increased adsorption of chemicals and strong catalytic activity. High surface reactivity is a desirable property for catalysis; however, the large number of exposed surface molecules or atoms exposes surface defects, vacant sites, and dangling chemical bonds that enhance chemical and redox reactivity.

Surface Charge—NPs have a strong tendency to form aggregates as a natural consequence of small size, which leads to strong intermolecular attractive forces. To obtain stable dispersions of unagglomerated nanomaterials, it is often necessary to add coatings that prevent particle–particle attachment or to impart an electrical charge that lead to particle–particle repulsion. T is is applicable to drug delivery whereby coating with biocompatible surfactants stabilizes NPs. Figure 28–3 depicts the characteristics of agglomerated and aggregated NPs.

TABLE 28–2 Physicochemical NP properties relevant to toxicology.

Size (aerodynamic, hydrodynamic) **Size distribution** **Shape** **Agglomeration/aggregation state** **Density** (material, bulk) **Chemical composition and phase** • Crystallinity • Dissolution and toxicant (ion) release • Coatings and bioavailable contaminants • Biopersistence **Surface properties** • Surface area (external, internal) • Electrical charge (zeta potential) • Redox activity • Hydrophobicity/hydrophilicity • Adsorptive capacity for biomolecules **Nanoscale quantum and magnetic properties (?)**	**Properties can change** • With method of production, preparation process, storage • When introduced into physiologic media, organism

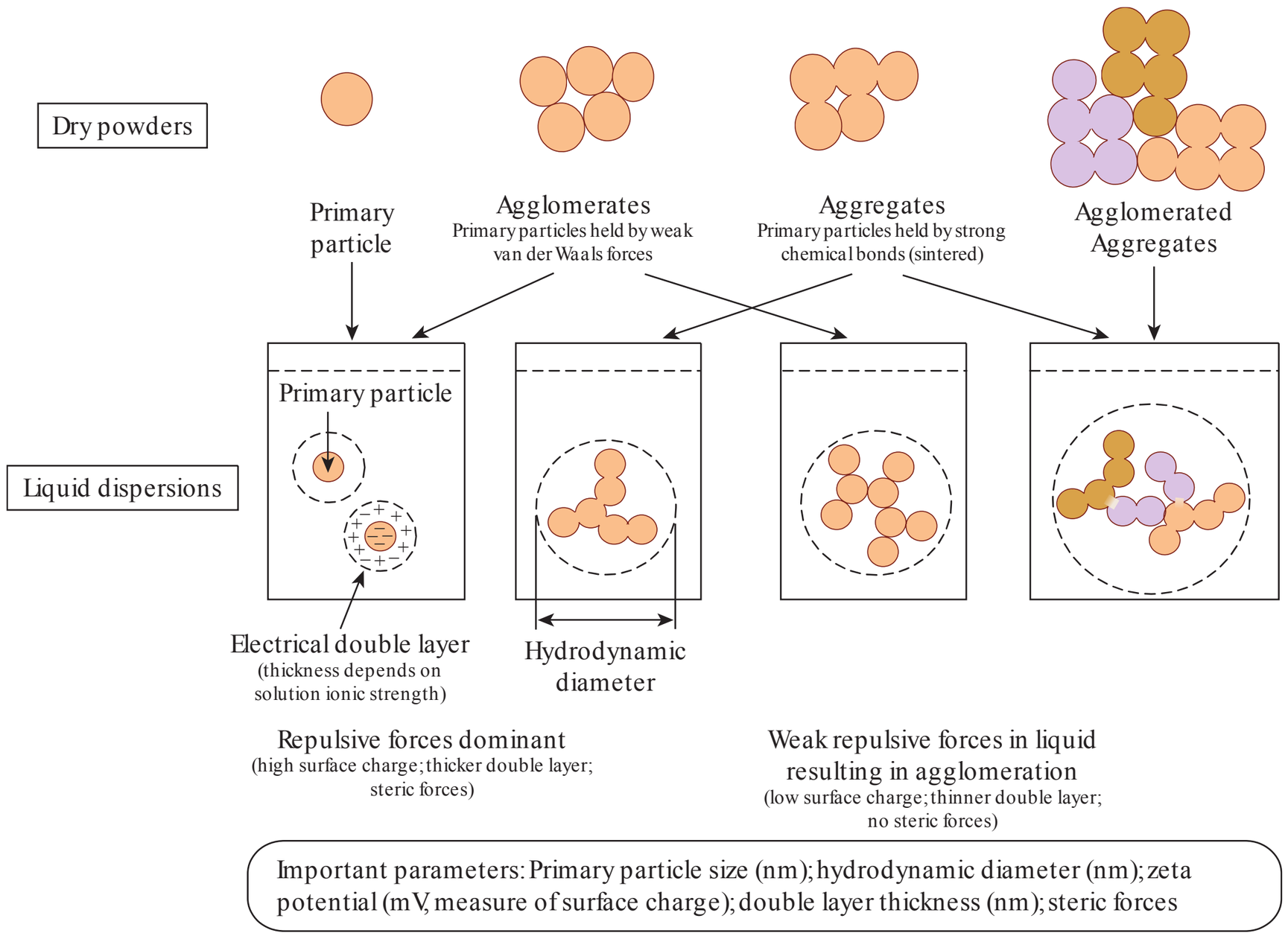


FIGURE 28–3 **Agglomeration and aggregation of nanoparticles in liquids and as dry powders.** (Modif ed with permission from Jiang JG, Oberdörster E, et al.: Characterization of size, surface charge, and agglomeration state of nanoparticle disperisons for toxicological studies, J Nanopart Res, 2009 Jan;11(1): 77–89.)

Surface Chemistry—High surface area and exposed surface atoms or molecules promote increased dissolution and release of ions from metallic or metal oxide NPs relative to bulk particles of the same chemical composition. Metal ions are toxic to bacteria and aquatic organisms by inhibition of enzymes and transport proteins. For example, ZnO NPs are incorporated into sunscreens where they absorb ultraviolet (UV) light; however, in water, Zn^{2+} ions are rapidly released and cause acute toxicity. Surface hydrophilicity of charged NPs increases their ability to be suspended in water, whereas surface hydrophobicity of fullerenes or grapheme repels water and enables these hydrophobic nanomaterials to partition into lipid membranes and enter target cells. In addition, surface defects expose electron active groups that donate an electron to molecular oxygen which generates superoxide anions which are reactive oxygen species (ROS).

CNTs are synthesized in the presence of metal catalysts that can undergo redox cycling and catalyze generation of highly reactive hydroxyl radical groups. Nanomaterials with high surface area can adsorb organic molecules such as polycyclic aromatic hydrocarbons that are potentially carcinogenic and quinones that also participate in generation of free radicals and redox cycling. Cationic NPs that have surface amide groups and cationic dendrimers are especially cytotoxic because they induce membrane damage, especially in lysosomes, that leads to accumulation of water and chloride ions and osmotic rupture.

Unique Quantum and Magnetic Properties—Ferromagnetic NPs less than 10nm in diameter respond to an external magnetic field. T is is exploited for contrast enhancement in diagnostic MRI and for hyperthermia induced by an external magnetic field to kill tumors targeted by magnetic NPs.

Geometry and Dimensions—T ese are important determinants in cellular uptake, systemic translocation, and potential toxicity. Nanomaterials can enter target cells by passive diffusion, direct physical penetration, or active, receptor-mediated uptake by endocytosis or phagocytosis depending on their size and extent of agglomeration. Small NPs appear to enter cells and organelles by passive diffusion. Single walled CNTs (SWCNTs) have been shown to directly puncture bacterial cells leading to osmotic lysis and death. SWCNTs have also been reported to translocate from the alveoli into the interstitium of the lung where they promote collagen deposition and interstitial fibrosis.

Biopersistence—Biopersistence of ENMs is an important factor in their environmental and biologic toxicity. Biopersistence is related to dissolution, which produces biologically active ionic species as well as has the ability to degrade the particle and clear it from biologic tissue or the environment. T e rate of dissolution of metal oxides is increased by natural organic matter in the aqueous environment; therefore, these NPs have low biodurability and it is predicted that they would not bioaccumulate in the environment.

Biopersistence in the lungs and pleural or peritoneal spaces is an important physicochemical characteristic of asbestos and man-made mineral fibers associated with carcinogenicity. Several recent studies have shown that carboxylated SWCNTs do not undergo oxidative degradation in the presence of stimulant fluid that mimics the lysosomal compartment of macrophages. However, oxidatively degraded SWCNTs did not induce lung inflammation or toxicity following pharyngeal aspiration in mice providing proof-of-principle for deliberate design of engineered CNTs that are biodegradable and less likely to induce disease following inhalation or injection for tumor imaging or drug delivery.

THE NANOMATERIAL BIOLOGIC INTERFACE

The high surface area of NPs provides a platform for adsorption of a variety of biologic molecules including proteins, lipids, and nucleic acids. A "protein corona" exists on the NP and governs its initial reaction with target cells. The interaction of nanomaterials with blood plasma proteins has been highly investigated due to its importance in drug delivery, circulation time, organ distribution, and clearance. The consequences of protein adsorption to NPs are not clear; although, depending on the NP surface, proteins may denature resulting in loss of normal structure and function, with altered enzyme activity or unfolding that exposes new antigenic determinants. An important potential pathologic consequence of serum protein adsorption to NPs is binding of fibrinogen leading to formation of blood clots.

NPs that are inhaled or ingested encounter a lipid mucus layer that provides a natural barrier to penetration of particulates and microorganisms. NPs may adhere to mucins causing enlargement of pore size with increased susceptibility to penetration of microorganisms. Smaller, charged NPs may be repelled by the hydrophilic domains and will not be able to penetrate the mucus layer. Aquatic organisms and bacterial biofilms are similarly surrounded.

ENPs also bind nucleic acids and have been proposed as gene delivery devices. Small grapheme oxide nanosheets can also intercalate into double-stranded DNA and induce DNA breaks in the presence of Cu^{2+} ions.

TOXICITY MECHANISMS

The mechanistic pathways associated with toxicity are predictable based on the physicochemical properties of ENMs. Oxidative stress due to direct generation of ROS at the surface of NPs or indirectly by target cells following internalization of NPs is a common mechanism responsible for toxicity of ENMs. The most vulnerable subcellular organelles and physiologic functions that can be perturbed by exposure to ENPs are summarized in Table 28–3.

The cell wall of bacteria and the plasma membrane of eukaryotic cells are the initial barriers to penetration of NPs. Carbon nanomaterials are proposed to act as "nanodarts" creating holes in the plasma membrane resulting in extracellular release of cytoplasmic contents as assessed by efflux of ribosomal RNA and decreased survival.

TABLE 28–3 In vitro mechanisms of nanoparticle toxicity.

1. Damage to cell wall and plasma membrane
2. Interference with electron transport and aerobic respiration
3. Induction of oxidant stress
4. Activation of cell signaling pathways
5. Perturbed ion homeostasis
6. Release of toxic metal ions from internalized nanoparticles
7. Disruption of lysosomal membrane integrity
8. Incomplete uptake or frustrated phagocytosis
9. Interference with cytoskeletal function
10. DNA and chromosomal damage

A wide variety of NPs have been designed to facilitate delivery of imaging agents, genes, proteins, and drugs into mammalian cells. NPs can also be designed to target specific cell surface receptors triggering internalization. In order to facilitate delivery, NPs can be engineered to escape from endosomes or lysosomes by coating with pH-sensitive polymers, viral caspids, cations, or biodegradable carriers.

NPs that are recognized by surface receptors may activate cell type–specific signaling pathways leading to cell proliferation or death by apoptosis, stress-related signaling, or calcium-mediated signal transduction events. Dysregulated intracellular calcium ion homeostasis may be the consequence of influx across a damaged plasma membrane permeability barrier or release of calcium ion from the major intracellular storage sites. Sustained elevation in intracellular calcium can cause cell death by necrosis.

Macrophages are the initial cells to phagocytize inhaled particulates deposited in the airways or alveoli. If they are longer than the diameter of macrophages, incomplete uptake occurs with prolonged generation of ROS by the respiratory burst mechanism of phagocytes and extracellular release of damaging lysosomal enzymes. In general, incomplete sequestration of NPs that are too large within lysosomes results in physical interference with cytoskeletal function that can cause impaired cell motility.

CAVEATS IN NANOTOXICOLOGIC ASSAYS

Due to their high surface area and hydrophobicity, NPs can adsorb vital dyes, cell culture micronutrients, or released cytokines.

SAFETY CONSIDERATIONS IN NANOMATERIAL DESIGN

In principle, it should be possible to engineer NPs with desirable surface properties for commercial or biomedical applications. Capping or coating of NPs using antioxidants may

decrease toxicity. Release of toxic metal ions from quantum dots and iron oxide NPs can be minimized using inorganic shells or biocompatible polymers. In addition, there is some evidence that CNTs are less pathogenic if they are shorter or entangled to hide their fibrous nature.

CASE STUDY: DESIGNING SAFER SUNSCREENS

As previously mentioned, due to its rapid dissolution in water and release of Zn_{2+} ions, ZnO NPs are considered as potential toxicants. TiO_2 NPs are used in sunscreens as well. T e potential of ZnO and TiO_2 NPs to induce photo toxicity and penetrate into the dermis has been a major concern for human safety of sunscreens. A series of skin penetration studies using both ex vivo and in vivo models showed that these NPs do not penetrate deeper than the outer most layer, stratum corneum, of intact skin. T us, it is suggested that the benefits of protection against carcinogenic UV light radiation provided by sunscreens formulated with ZnO or TiO_2 NPs outweigh the minimal risks associated with phototoxicity, DNA damage, and skin penetration.

MAMMALIAN TOXICOLOGY

Introduction

CNTs are a prime example of the two opposing faces of nanomaterials: Many highly desirable properties that are suitable for numerous beneficial applications contrast with reports of serious adverse effects in experimental animals. For example, the excitement of future use of CNTs for delivery of drugs, genes, and biosensors is dampened by reports of inflammatory fibrogenic and even mesothliogenic effects in laboratory rodents.

Concepts of Nanotoxicology

T e shape, size, and size distribution are important determinants for the deposition efficiency of inhaled materials throughout the respiratory tract. Uptake into cells is influenced by their surface charge, surface reactivity, the chemistry of surface coatings, and also surface defects to the material as synthesized or introduced during surface functionalization or processing.

Many ENPs are insoluble in the as-produced form and do not undergo simple dissolution but can undergo chemical oxidation in solution, tissue, or the environment to produce soluble species in a process that gradually degrades and eliminates the particle state. Such NPs can act via a "Trojan Horse" mechanism in that they are taken up into cells and subsequently dissolve, thereby creating a very high intracellular, ionic metal concentration that is cytotoxic.

Dosemetrics

Expressing dose–response relationships is most informative utilizing surface area of the NP. Figure 28–4 shows the pulmonary inflammatory dose–response relationship of two sizes of TiO_2 particles induced by intratracheal instillation in rates. A significantly greater influx of inflammatory neutrophils into the lung was induced by 25 nm TiO_2 per unit mass than by 250 nm TiO_2. T e result is a very steep dose–response for the nanosized TiO_2 and a flatter dose–response for the larger TiO_2. Likewise there was a clear separation of the dose–response when based on the number as dosemetric; however, when the same data was expressed based on particle surface area, a

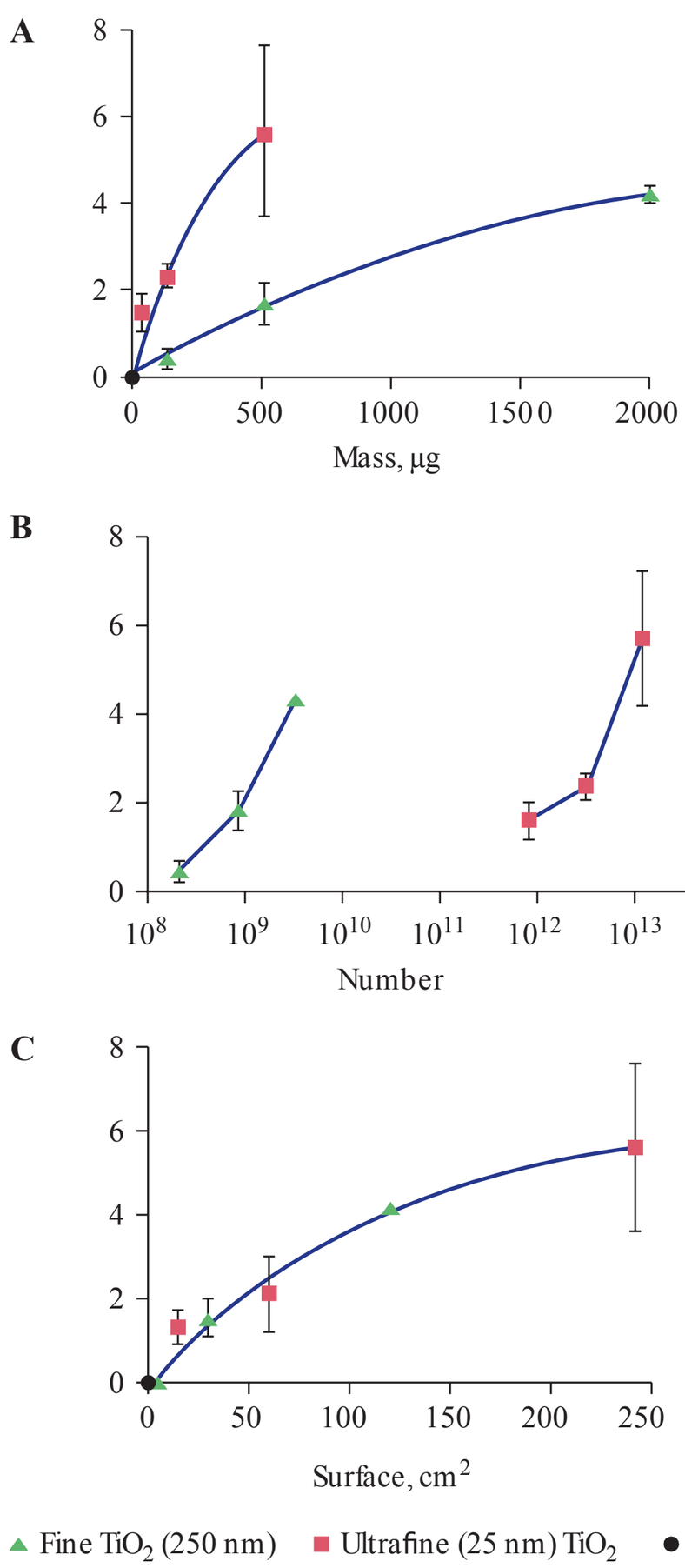


FIGURE 28–4 **Inf ammatory cell response (neutrophil number in lung lavage of rats 24 h after intratracheal instillation of two sizes of TiO_2 particles expressed by different dosemetrics.** Particle-mass **(A)**; -number **(B)**; -surface area **(C).** (Reproduced with permission from Oberdörster G, Oberdörster E, Oberdörster J: Concepts of Nanoparticle Dose metric and Response Metric, Environ Health Perspect, 2007 Jun;115(6):A290.)

common dose–response relationship emerged. More specifically, although the concept of particle surface area is plausible, the biologically available surface area is of greater value for defining a proper dosemetric.

Volume of NPs has been suggested as another dosemetric. T e "particle overload" hypothesis states as follows: When the volume of phagocytized particles in alveolar macrophages exceeds 6% of the normal macrophage volume, their physiologic clearance function becomes impaired; if the volume reaches 60%, clearance no longer functions. T is concept has been applied to estimate certain human occupational exposure limits. Dependent upon the situation, either surface area or volume dosemetric may be used. It is also important to remember that chemical properties of NPs are critical determinants of effects resulting from NP–cell interactions (Table 28–2).

Portals of Entry

T e respiratory tract, the gastrointestinal (GI) tract, and skin are the main organs of direct exposure of ENM. For medical application, injection will also be an important entry route. Intake via the respiratory tract is the most prevalent exposure route for occupational exposures. Additives of ENM to food and potential contamination of food result in exposure via GI tract. Based on available data, translocation of nanomaterial in vivo across GI-tract epithelial cells seems to be limited; however, DNA damage has been found in bone marrow of cells following very high gavage dosing of rats. Skin exposure via cosmetic and skin-care products occurs, although penetration of healthy skin by NP has not been demonstrated.

Dosing of the Respiratory Tract

Dosing of the respiratory tract of laboratory rodents involve the administration of materials as a bolus in a second or less. However, inhalation is the only physiologic method and should be considered the gold standard for exposure to airborne materials. Major differences between bolus-type and inhalation exposures relate to the dose rate, use of anesthesia, and the distribution of administered material within the respiratory tract.

Bolus delivery occurs within a fraction of a second, whereas inhalation at realistic concentration takes hours to months of exposure in order to deposit the same does in the lung. Treating a dose delivered by bolus to be the same that has accumulated in the lung over a lifelong exposure is not justifiable. Inundating cells abruptly with an extraordinarily high dose overwhelms the cell's defense mechanisms and leaves no time for developing adaptive responses. Consequently, mechanisms of effects induced but unrealistic high doses are different from those induced by relevant dose and dose rates.

Figure 28–5 illustrates a tremendous difference of inducing a pulmonary inflammation by either intratrachially instilling 200 µg of TiO_2 NP versus depositing the same dose by inhalation over a period of four hours for four days. T e difference

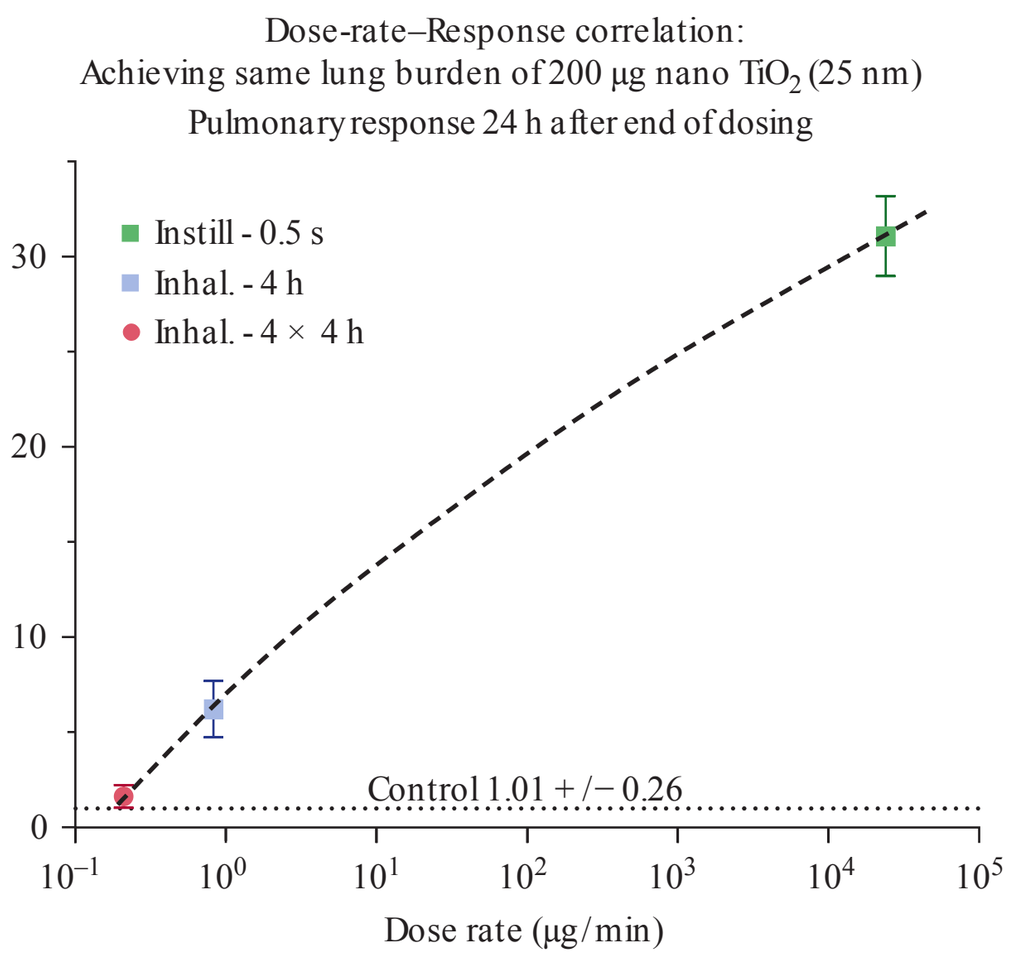


FIGURE 28–5 **Dose-rate–response correlation:** Deposition of 200 µg nano-TiO_2 in the lungs of rats either by instillation (high dose rate) or by inhalation (low dose rate) induces widely differing pulmonary inflammatory responses as determined by the appearance of inflammatory neutrophils in lung lavage. (Used with permission of G. Oberdörster).

in dose rate is significant with no response at the lowest dose rate of inhalation. T is supports that adaptive responses are an important physiologic protective mechanism, which need to be considered when interpreting results of nanomaterial toxicity testing. Despite the limitations of bolus-type delivery, they may be viewed as "proof of principle" with the findings to be confirmed by subsequent inhalation studies. T e concept of differential adsorption states that the physicochemical properties of nanoparticles such as size, surface properties, shape, dissolution, and others when in contact with media in the different body compartments, such as respiratory tract lining fluid, gastrointestinal secretions, etc., determine protein and lipid adsorption and thereby influence biodistribution across barriers and in target tissues and cells.

Respiratory Tract Deposition

Inhalation of ENMs results in significant deposition in the three compartments of the respiratory tract: the nasopharyngeal region from the nose/mouth to the larynx, the tracheobronchial region from the larynx to terminal bronchioles, and the alveolar region from the first generation of respiratory bronchioles to the last generation of alveolar ducts. T e deposition efficiency depends on particle characteristics, anatomical structure of the airways, and breathing parameters. Particle size, size distribution, density, and shape are the most important because they govern deposition in the respiratory tract by intertial impact, gravitational settling, and displacement by diffusion.

Studies have been performed involving nasal inhalation in rats and humans. Obvious differences between rats and humans are the maximum size of particles that are respirable, that is, will reach the alveolar region. In rats this is about 5 μm aerodynamic size, in humans about 15 μm. Although these sizes are outside the range of single NP, airborne NP occurs for the most part as agglomerates.

It should be noted in a reminder that realistic in vivo doses to cells of the respiratory tract are mostly orders of magnitude lower than doses that are typically applied in vitro to lung epithelial cell cultures. In the alveolar region where the airflow is very low, no deposition hotspots for NPs exist. T is nonhomogeneous deposition and formation of hotspots seem to correlate with predilection sites for bronchial carcinoma, which is further enhanced by less effective mucociliary clearance at carnal ridges.

Respiratory Tract Clearance and Disposition of NP: Nanomaterials

Once NPs are deposited in the respiratory tract they will encounter clearance mechanisms. However, there are several differences that separate NP from larger particles. Alveolar macrophages generally are attracted to deposited particles by chemotactic signals generated at the site of deposition. NPs may be too small to generate such signals leading to uptake into the pulmonary interstitium. Translocation into the interstitium and subsequently into blood and lymph circulation distinguishes NPs from microparticles. Figure 28–6 depicts the blood compartment as a plenum from which any tissue or organ can be reached by circulating NP. However, the amount of NP translocating from the lung to the blood circulation and accumulation in secondary organs is very low. Long-term retention studies with radioactive NPs have shown that clearance in extrapulmonary organs following the initial accumulation is very efficient, so after six months, with the exception of liver and spleen, only minor amounts were still present. Despite the low translocation rates, it has to be considered that continuous exposure may result in significant accumulation in some secondary organs.

Nanomaterials and the Brain

Organs with tight endothelial junctions, in particular the CNS, will not likely accumulate blood-borne NPs, unless the tight blood–brain barrier is damaged or NP surface has been specially modified. T e most efficient pathway of NP translocation to the CNS appears to be via olfactory sensory neurons from the nasal olfactory mucosa directly to the olfactory bulb. Results of epidemiologic studies of impaired cognitive function and of neurodegenerative brain pathology associated with exposure to traffic-related particles raised the question as to whether ambient UFPs as constituents of urban air pollution may be etiologically involved.

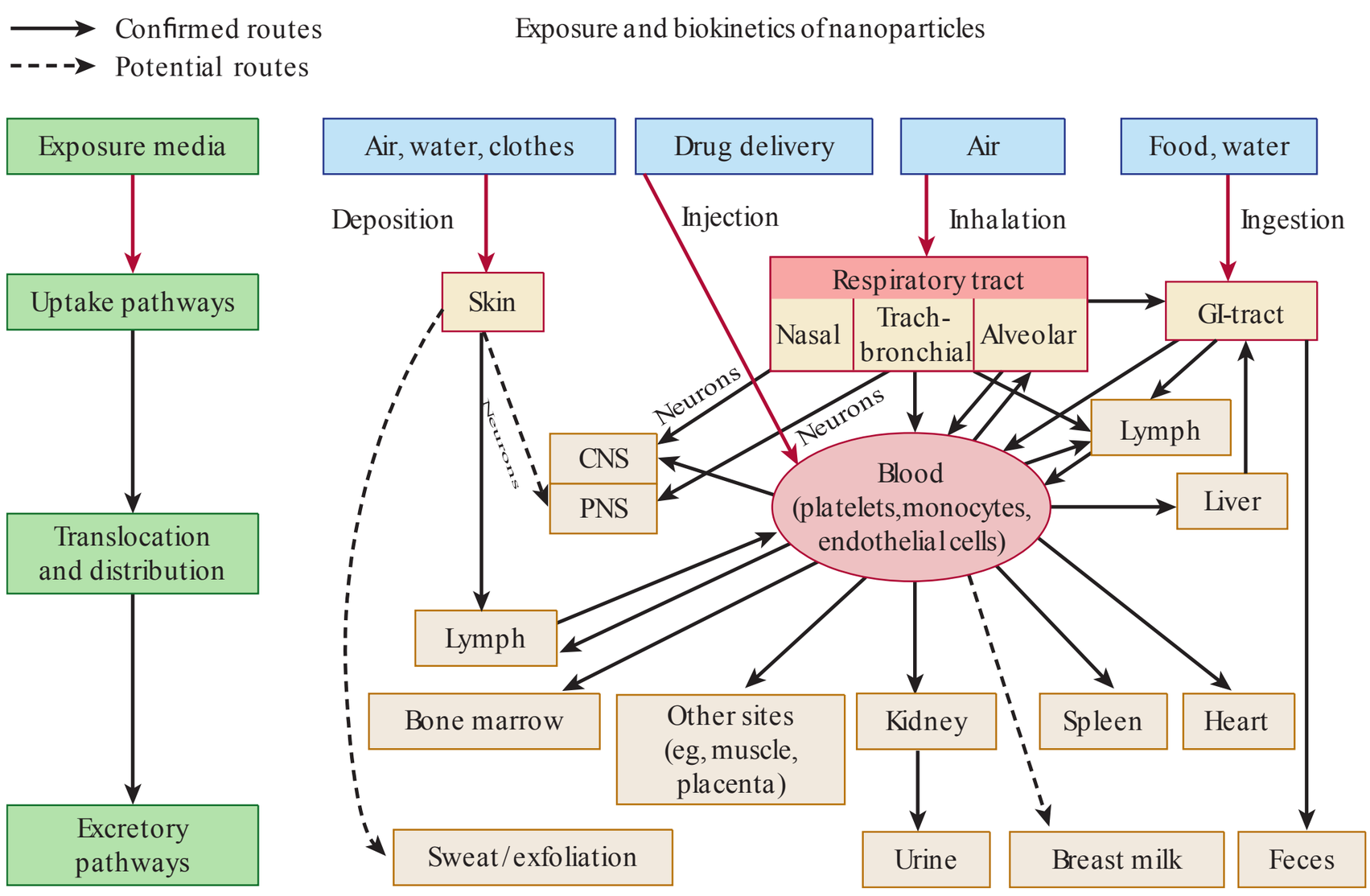


FIGURE 28–6 **Exposure and biokinetics of nanoparticle routes of exposure and biokinetics (uptake, distribution, elimination) of nanomaterials. Translocation rates in general are very low (see text).** (Reproduced with permission from Oberdörster G, Oberdörster E, Oberdörster J: Nanotoxicology: an emerging discipline evolving from studies of ultraf ne particles, Environ Health Perspect, 2005 Jul;113(7):823–839.)

Elimination of Nanomaterials

Elimination pathways for ENM from the body include mainly feces and urine. Urinary excretion is restricted to nanostructures < 5.5 nm in size for metal-based NP. Circulating fibrous structures of ENM, such as large MWCNTs, can collect in the urine of rats following intravenous application. T is phenomenon may be explained by a hydrodynamic lining of nanotubes so they will pass through glomerular pores. T e fecal excretory clearance pathway consists of several inputs: one is mucociliary clearance of deposited particles from the airways into the GI tract; another is the hepatobiliary clearance of blood-borne ENM via liver and bile into the small intestine. T is elimination pathway is also a well-known excretory path for heavy metals in the blood.

Another clearance pathway of deposited ENM in the lung involves translocation via interstitium or lymph to the pleura and subsequent elimination via lymphatic openings on the parietal pleura to mediastinal lymph nodes from where NP may enter the blood circulation via the thoracic ducts. T is pathway is of particular importance for fiber-shaped ENM because the size of the parietal stomata prevents efficient clearance of structures > 10 μm in length. As a consequence, the interaction of the retained fibers in the pleural cavity with mesothelial cells induce inflammatory and granulomatous responses and in long-term potentially mesothelioma.

CASE STUDY: MWCNTS

Bolus-type Exposures

Bolus-type delivery of CNTs to the respiratory tract of rats and mice revealed induction of dose-dependent significant inflammatory, granulomatous, and fibrogenic responses; they showed also that MWCNTs can reach subpleural and intrapleural sites. In addition, intraperitoneal injection studies clearly show the potential of CNTs, specifically MWCNTs, to induce severe adverse length-dependent effects at mesothelial sites once they reach the pleural cavity.

Inhalation Studies

Relatively few inhalation studies with CNTs in rodents have been reported. T e most meaningful and best justified for the risk assessment process would be a subchronic multiconcentration study with sufficient postexposure observation. However, short-term exposure to a relevant concentration is useful for dosemetric purposes when determining the biodistribution from deposition sites in the lung to secondary organs. Parameters varied, but within these variations outcomes ranged from no significant effects to severe pulmonary inflammation/oxidative stress responses.

Critical Appraisal of CNT In Vivo Studies

Given the importance of the physicochemical properties of CNTs for inducing adverse effects, it is of utmost importance to determine these properties, in particular as they appear in the airborne state at sites of human exposures, at occupational sites, or for the consumer. Adding dispersants for testing purposes will change surface properties; conceptually, inhalation studies in experimental animals for purposes of hazard identification should mimic human exposure conditions with regard to airborne size distribution. Of course, differences in respirability between humans and rodents must be considered and adjustments be made without use of surface altering dispersants.

Appropriately designed multiconcentration, subchronic inhalation studies, including a longer recovery period, are essential for deriving no observed adverse effect levels (NOAELs); results can be used as basis for deriving occupational exposure levels (OELs) by applying rodent/human dosemetric adjustments. Using results from bolus-type studies is difficult and raises questions, although national institute for occupational safety and health has combined results of fibrotic responses from diverse bolus-type and inhalation studies to derive a provisional recommended exposure level of 7 μg/m^3. T is REL is based on dose–response data from the available studies with bolus-type and short-term inhalation exposures and a subchronic inhalation study.

T ere has been no conclusive data regarding carcinogenic effects of realistic exposure to CNTs. T us, exposure should be avoided with appropriate measures (ventilation, filtration, personal protective equipment). T ere is an obvious and urgent need to perform additional long-term inhalation studies to assess carcinogenic potential.

Biologic Degradation of Carbon Nanomaterials—CNTs have been generally regarded as stable nondegradable materials, which has important implications for long-term health effects following inhalation into the lungs. Recently, however, SWCNT degradation has been observed in acellular assays that stimulate the phagolysosome of macrophages, but only if the tubes have been surface carboxylated, which introduces collateral defects in the side walls. Graphene oxide is also susceptible to oxidative attack by hydrogen peroxide and horseradish peroxidase. T ese observations may enable design of safer carbon materials that are potentially biodegradable in order to minimize adverse environmental and human health impacts. However, degradation of CNTs in vivo is still to be confirmed.

TOXICITY TESTING

In order to perform risk assessment, exposure and hazard data are required. To identify and characterize a hazard, in vitro and in vivo studies will be useful, and results should be derived via well-designed dose–response relationships. Key considerations include physicochemical characterization of the ENM to be tested, justification of the method(s) of dosing, selection of target cells, tissues, or animal species, and appropriate end points.

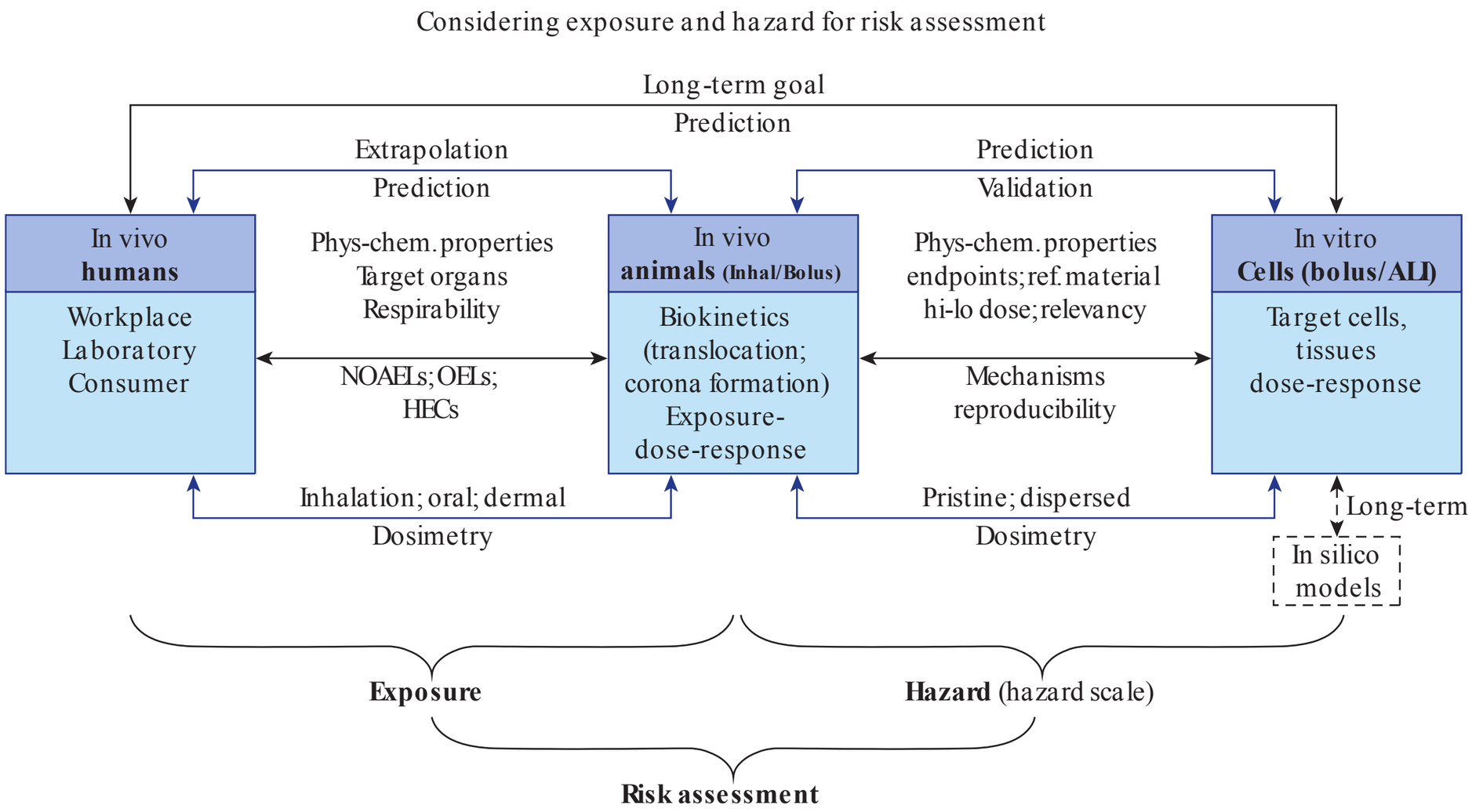


FIGURE 28–7 **Concepts and goals of nanomaterial toxicity testing (see text).**

In vitro studies should be stressed as far as uncovering underlying mechanisms of effects are concerned. T ey are also useful for toxicity ranking of nanomaterials for the purpose of hazard identification. In contrast, the design of in vivo studies allows the full evaluation of exposure dose–response relationships, which is necessary for the process of risk assessment (Figure 28–7).

For medicinal applications, injection is an important route of exposure requiring specific awareness with respect to assuring desired beneficial outcomes yet avoiding undesirable responses. For example, the desired pharmacologic target organelle for drug delivery by SWCNT are the lysosomes, whereas the mitochondria are the target organelles for SWCNT toxicity.

In Vitro Dosimetry

As most ENM toxicity studies are performed using in vitro assays, which are generally short-term, dosing-related questions are highly relevant. T e dose received by the cell is a function of colloidal dynamics in the culture medium governed by diffusion and settling phenomena, which in turn is governed by particle and media properties that include particle size, agglomeration state, shape, density, and charge. At equal mass concentrations in the medium, the magnitude of the cellular dose of ENM will differ significantly from those implied by the media concentration.

An in vitro sedimentation diffusion and dosimetry (ISDD) model may be used to predict the in vitro behavior and cell doses of particles. T e value of this model lies in the clear separation between exposure (concentration in the cell medium), the deposited dose on the cell surface, and the cellular dose. Knowledge about the time to deposit a certain dose allows consideration dose rate as a determinant for responses.

Predictive Toxicology

Critical elements for hazard identification based on toxicity testing of ENM are detailed information about their physicochemical properties prior to any experiments, the selection of appropriate target cells, validation of in vitro assays in terms of correlation and relevancy to in vivo results, the inclusion of biokinetics in the design of in vivo studies, and the inclusion of realistic doses in the design of dose–response in vitro and in vivo studies. Biokinetic information is crucial to identify potential secondary target organs based on significant accumulation of ENM. With real-world exposure scenarios relationships can be established to both characterize a hazard and assess a risk. An awareness of dosimetry-related aspects is of highest importance (Figure 28–7) for the risk assessment process, which often gets lost or is ignored because of a misconception of risk being analogous to hazard. Risk is a function of hazard and exposure, and neither aspect alone can determine risk.

Transition, Human-Eco-nanotoxicology

T e goals of nanotoxicology are to identify and characterize a hazard of ENMs for purposes or risk assessment for humans and the environment. Exposures throughout the life cycle of ENMs, from their source to their disposal, have to be considered for both. Dispersion and intermediate transformations in air, water, food, and soil are important modifiers of ENM–receptor interactions.

ECOTOXICOLOGY OF ENMS

Environmental Uses and Exposures to Nanomaterials

As with many chemicals in the marketplace, it is estimated that a portion of the nanomaterials used in industry and consumer products will enter the greater environment during some part of their life cycle, either through waste during production or through product use. T e exponential increase in use of ENMs in a multitude of industries and consumer products have been documented and shown exponential increase by the Woodrow Wilson Center's project on Emerging Technologies.

T e most common nanomaterials within cosmetics, clothing, personal care products, and sporting goods are silver and carbon. Other chemicals used in these products have been found to wash into the wastewater treatment system and end up in the aquatic environment. Despite removal potentials, it has been shown that NPs can be emitted through the wastewater process in the NP size range in significant quantities. Nanomaterials directly applied to a particular ecosystem, such as those for cleanup of environmental toxicants or as part of a pesticide formulation, may also lead to exposure.

Ecologic Risk Assessment of Manufactured Nanomaterials

For nanomaterials most of the ecologic risk assessment research has been conducted as the analysis of effects of a limited number of commercially available materials, using traditional acute single-organism mortality end points of a few select species and with little information regarding sublethal types of effects or other end points of concern at the community or ecosystem level. In addition, the concentration of exposures are much higher than what is considered to be a probably environmental level. Nanomaterials may also be transformed within the environment, and therefore the toxicity of the initial nanomaterial may not provide a complete idea of the toxicity over the lifetime of the material.

Toxicity of Manufactured Nanomaterials

Complications of Assays—Traditionally, ecotoxicology assays follow standard protocols and involve a group of species that has been selected to be representative of various organisms in the environment including bacteria, fish, birds, and insects. Some major issues in toxicology assays include delivery of nanomaterials in media and approximating environmental conditions, characterizing exposures, maintaining exposures throughout an assay, and determining the state of exposure throughout an assay. T e fact that many nanomaterials are not easily dispersible and aggregate substantially when introduced into common exposure media causes several issues. First, aggregated nanomaterials may no longer be in the nanosized range. Second, nanomaterials as they aggregate settle out of suspension, so depending on the organism involved the actual exposure may change over time as particles are effectively removed from suspension. Researchers have attempted to circumvent this issue by either changing the surface chemistry or by altering the exposure conditions. Unfortunately, changing the surface chemistry of a nanomaterial can also change its toxicity. Worse yet is that many of the coatings can cause toxicity on their own regardless of whether it is attached to the nanomaterial.

As part of determining the dose an organism actually encounters is determining how much of any nanomaterial actually reaches the organism and is taken up. T ere are several difficulties in measuring uptake including identifying the nanomaterial within the matrix of the organism versus inside the organism. Another complication is that of calculating the dose as a mass versus surface area. T e real adverse impacts of nanomaterials may not be due to the ambient environmental concentrations that arise but may be due to some subset of materials that are persistent and biomagnify in the environment.

Ecotoxicity of Nanomaterials

T e studies on the toxicity of ENMs to date conclude that toxicity varies with the type of nanomaterial and is not universal across materials. Most studies find some degree of toxicity but the concentrations of most nanomaterials that are needed to kill half the sample population are in the mg/L range, which is far above the estimates of potential exposures. Silver nanomaterials are some of the most widely used materials and appear to demonstrate the greatest toxicity of materials investigated in the literature. Silver in particular is toxic at μg/L doses to a variety of organisms. Rather than creating a free radical in media, the impacts of metal nanomaterials may be due to metal imbalance in cells after uptake and accumulation leading to apoptosis and cellular disregulation.

Nanosilver and possibly other nanomaterials based on soft metals may react with environmental sulfides to produce silver sulfide nanomaterials in which the silver bioavailability and toxicity is much reduced.

Mechanisms of Toxicity

As in mammalian toxicology studies, oxidative stress has been implicated as a major way in which nanomaterials exert toxicity either by generating free radicals within the suspension media or by changing the chemistry of the cells in which they come in contact. Metal oxide nanomaterials in particular have been found to generate oxidative stress with greater toxicity than their bulk counterparts. Metal nanomaterials have been found to cause a suite of effects, which in fish include negative impacts on respiration, oxidative stress, and development, in Caenorhabditis elegans increased mortality and decreased reproduction and inhibit algal growth.

T e toxicity of nanomaterials to aquatic organisms can be greatly dependent upon the interaction of nanomaterials with the media to which they are introduced. Nanomaterials may also impact the bioavailability and toxicity of other contaminants in the environment.

As the immune response is the first interaction of foreign substances with an organism, it has been shown that nanomaterials are stimulatory to the immune system of fish in particular and have an effect that is equal to the response to bacterial cell components, which may indicate an eventual cost to the organism. Nanomaterials also have the potential to be mutagenic and in fruit flies cause mutations that alter the phenotype significantly into the second generation.

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QUESTIONS

1. Which of the following is not a nanoparticle?
   a. carbon nanotubes.
   b. bucky-ball.
   c. graphene.
   d. zinc nanorods.
   e. bacteria.

2. Which of the following answers is not true regarding nanoparticles?
   a. NPs can originate from natural sources including forest fires, volcanoes, and viruses.
   b. NPs can originate from unintentional sources including internal combustion engines and electric motors.
   c. NPs can originate from unintentional sources including ferritin and magnetotactic bacteria.
   d. NPs can originate from intentional sources including carbon nanotubes and metal oxide nanoparticles.
   e. NPs can originate from natural, and intentional and unintentional anthropogenic sources.

3. In contrast to larger particles > 500 nm, nanoparticles
   a. are highly likely to enter the body by dermal absorption.
   b. are highly likely to enter the body through the respiratory tract.
   c. are unlikely to adsorb to protein or lipid.
   d. are efficiently removed from the lungs via mucociliary transport.
   e. are not likely to undergo uptake and transport in sensory neurons.

4. Which of the following statements is NOT true?
   a. Nanomaterials may be classified by geometry and chemistry.
   b. Engineered nanomaterials include quantum dots, C-nanofiber array, and few-layer grapheme.
   c. Agglomerates include primary particles held together by weak van der Waals forces.
   d. Aggregates include primary particles held by strong chemical bonds.
   e. Hydrodynamic diameter is unimportant in particle interactions.

5. Nanoparticles can exert toxicity by all of the following mechanisms except:
   a. damage to DNA and chromosomes.
   b. induction of oxidant stress.
   c. interference with biotransformation enzyme activities.
   d. activation of signaling pathways.
   e. release of toxic metal ions from internalized NPs.

6. Biodistribution of nanoparticles may be influenced by
   a. physicochemical properties such as plasma protein and respiratory tract mucus.
   b. physicochemical properties such as surface size and chemistry.
   c. physicochemical properties such as the gastrointestinal milieu.
   d. body compartment media including surface hydrophobicity.
   e. body compartment media including size.

7. Assays to determine the toxicity of manufactured nanoparticles suffer from all of the complications below except:
   a. the nanomaterial aggregate may no longer be in the nanosize range.
   b. aggregates of the nanoparticle may settle out of solution which may affect exposure dose.
   c. alterations in surface chemistry to stabilize suspension may evoke other issues in toxicity assessment.
   d. coatings of particles may have their own toxicity.
   e. uptake of the nanoparticle into an organism is easily determined.

8. T e goals of nanotoxicology are
   a. to identify and characterize hazards of engineered nanomaterials.
   b. to determine "safe" exposure levels.
   c. to determine biologic and biochemical actions.
   d. to determine manufacturing procedures and cost.
   e. to determine preventive exposure guidelines.

CHAPTER

29

Air Pollution*

Daniel L. Costa and Terry Gordon

AIR POLLUTION IN PERSPECTIVE

A Brief History of Air Pollution and Its Regulation

TOOLS TO ASSESS RISKS ASSOCIATED WITH AIR POLLUTION

Animal-to-Human Extrapolation: Issues and Mitigating Factors

OVERARCHING CONCEPTS

What Is an Adverse Health Effect?
Susceptibility

EXPOSURE

Air Pollution: Sources and Personal Exposure
Indoor versus Outdoor
Indoor Air in the Developing World
The Evolving Profile of Outdoor Air Pollution

EPIDEMIOLOGICAL EVIDENCE OF HEALTH EFFECTS

Outdoor Air Pollution
Acute and Episodic Exposures
Long-Term Exposures

POLLUTANTS OF OUTDOOR AMBIENT AIR

Classic Reducing-Type Air Pollution
Sulfur Dioxide
Sulfuric Acid and Related Sulfates
Particulate Matter
Metals
Gas–Particle Interactions
Ultrafine Carbonaceous Matter
Chronic Effects and Cancer
Photochemical Air Pollution
Chronic Exposures to Smog
Ozone
General Toxicology
Pulmonary Function Effects
Ozone Interactions with Copollutants
Nitrogen Dioxide
General Toxicology
Pulmonary Function Effects
Inflammation of the Lung and Host Defense
Other Oxidants
Aldehydes
Formaldehyde
Acrolein
Carbon Monoxide
Hazardous Air Pollutants

THE MULTIPOLLUTANT REALITY OF AIR POLLUTION

CONCLUSIONS

*This chapter has been reviewed by the US Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and the policies of the Agency.

KEY POINTS

- Reducing-type air pollution, characterized by SO_2 and smoke, is capable of producing deleterious human health ef ects.
- Photochemical air pollution arises from a series of complex reactions in the troposphere close to the earth's surface and comprises a mixture of ozone, nitric oxides, aldehydes, peroxyacetyl nitrates, and myriad reactive hydrocarbon radicals.
- Indoor air can be even more complex than outdoor air, and outdoor air can permeate the indoor environment in spite of the reduced air exchange in buildings.
- Sick-building syndrome may occur in new, poorly ventilated, or recently refurbished office buildings due to the outgasing of combustion products, volatile chemicals, biological materials and vapors, and emissions from furnishings.

AIR POLLUTION IN PERSPECTIVE

T e second half of the twentieth century was marked by remarkable changes in how the public viewed its relationship to the environment. From expansive urban factories with smokestacks belching opaque dark clouds of industrial effluent into a neutral blue sky, regulation and cost-efficient innovations by the private sector have reduced emissions. Decades to come will see change in our energy portfolio that is driven by cost and access, environmental impacts including climate change, and technological innovation. Nevertheless, so long as organically derived fuel is combusted to derive energy, its potential for impact on air quality and on public health and the environment will remain. As the developing world grows industrially, air pollution now is intercontinental with transport through the atmosphere via pathways close to the earth's surface as well as upper atmosphere. Air pollution now extends even into remote and wilderness areas, and significant damage to flora and crops can also occur.

Other issues facing many parts of the developing world tie closely to domestic culture and economy, as well as to the level of technological sophistication. Prime among these problems is exposure to carbon and soot from combustion of biomass in cooking and heating in domestic stoves. Approximately three billion people worldwide use biomass for home cooking in households with little ventilation. T e World Health Organization (WHO) estimates two million deaths per year as a result of these exposures, especially women who are exposed day in and day out over many years, often with their infant children by their sides. Understanding the intersection of technological as well as socioeconomic and political challenges will be at the core of any resolution to these issues.

A Brief History of Air Pollution and Its Regulation

For most of history, air pollution has been a problem of microenvironments and domestic congestion. T e smoky fires of early cave and hut dwellers choked the air inside their homes, and even when the emissions were vented outdoors, they simply combined with those of the neighbors to settle around the village on damp cold nights. With urbanization and a concomitant decrease in forest wood as a source of fuel to heat and cook, the need for energy led to the burning of easily accessible, dirty coal and the ambient release of sulfurous, sooty smoke. Industrialization brought kilns to make quicklime for construction and metal smelters needed for the development of progressive "modern" cities, only to push smoke and chemical emissions into the air. Unfortunately, the city dwellers who worked near these industries had to endure the bad air, while those of wealth frequently had country homes to which they could escape.

T e accidental release of 30 tons of methyl isocyanate vapor into the air of the shanty village of Bhopal, India, on December 3, 1984, killed an estimated 3 000 people within hours of the release, with several thousand delayed deaths and 200 000 injured or permanently impaired. T e tragedy shocked the world, and raised the issue of hazardous air pollutants (HAPs) to a new level of concern.

T e HAPs have since garnered more public and policy attention. T ere is concern for the acute ef ects of accidental releases of fugitive or secondary chemicals—such as phosgene, benzene, butadiene, and dioxin, into the air of populated industrial centers—and for potential chronic health ef ects, with cancer often being the focus of attention. While many of HAP chemicals are now better controlled than in the past, residual risk estimates are yet to be completed for many HAPs. T e database from which these assessments are made is called the Integrated Risk Information System (IRIS, www.epa.gov/iris/index.html) and currently contains 550 chemicals that have health data.

Internationally, the magnitude and control of air pollution sources vary considerably, especially among developing nations, which often forgo concerns for health and welfare because of cost and the desire to achieve prosperity. Figure 29–1 illustrates the international variation in air pollution–related mortality (outdoor and indoor) based on economic groupings. It is clear that there are wide dif erences reflecting economic imbalances—particularly prominent are the indoor particulate

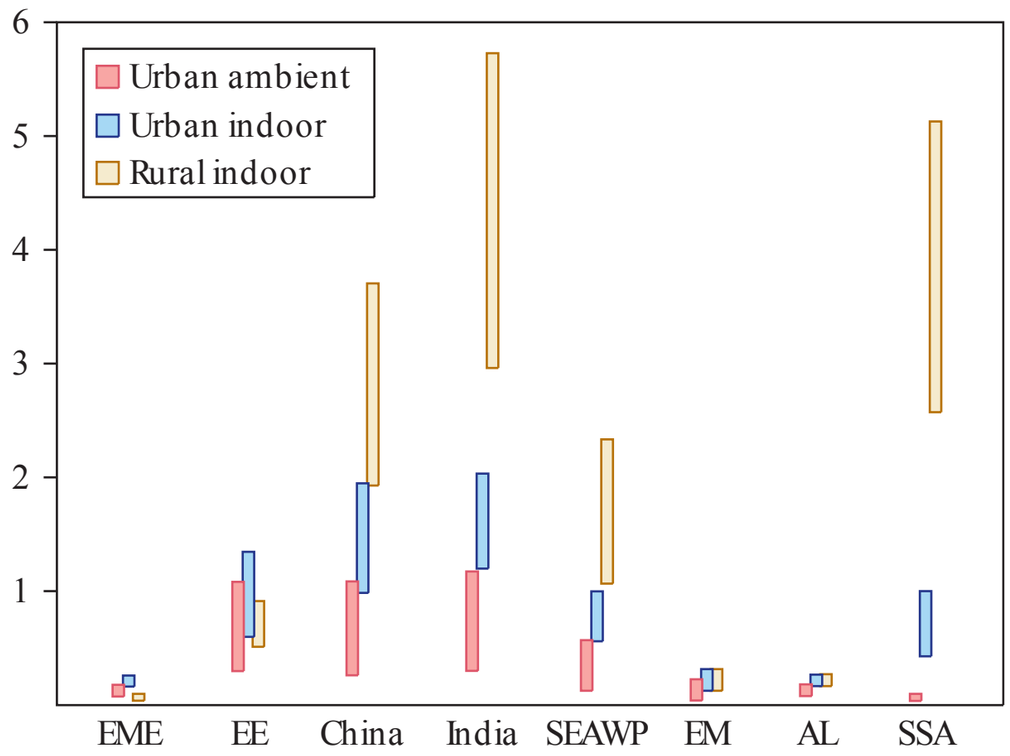

FIGURE 29–1 **Excess mortality due to outdoor and indoor particulate matter in various international economic groupings.** Bottom and top of each bar represent the lower and upper estimates of mortality, respectively, computed using the methodology of Schwela (2000): established market economies (EME), Eastern Europe (EE), China, India, Southeast Asia/Western Pacific (SEAWP), Eastern Mediterranean (EM), Latin America (AL), and Sub-Saharan Africa (SSA). (Modified with permission from Schwela D: Air Pollution in the Megacities of Asia—Seoul Workshop Report: Urban Air Pollution Management and Practice in Major and Megacities of Asia.)

levels in developing nations where biomass combustion is used for heating and cooking. These regions also contain many of the megacities of the world with major air pollution problems. In addition to local socioeconomic and political concerns, emissions of air pollutants have spawned problems of "international pollution." Long-range transport of polluted air masses from one country to another has been a global issue for several years. The air mass transport of acid sulfates from industrial centers of the Midwestern United States to southern Canada, and of NO_x and airborne mercury from China to the US are examples.

TOOLS TO ASSESS RISKS ASSOCIATED WITH AIR POLLUTION

Risk assessment is a formalized process whereby toxicity, exposure, and dose-dependent outcome data can be systematically integrated to estimate risk to a population. Figure 29–2 outlines a paradigm for incorporating all available data and risk assessments to providing evidence of "accountability" of applicable regulations on public health. The health database for any air pollutant may comprise data from animal toxicology, controlled human studies, and/or epidemiology. But, because each of these research approaches has inherent strengths and limitations, an appropriate assessment of an air pollutant requires the careful integration and interpretation of data from all three methodologies.

Epidemiological studies reveal associations between exposure to a pollutant(s) and the health effect(s) in the community or population of interest. Because data are garnered directly under real-world exposure conditions and often involve large numbers of people, the data are of direct utility to regulators assessing pollutant impacts. With proper design and analysis, studies can explore either acute or long-term exposures

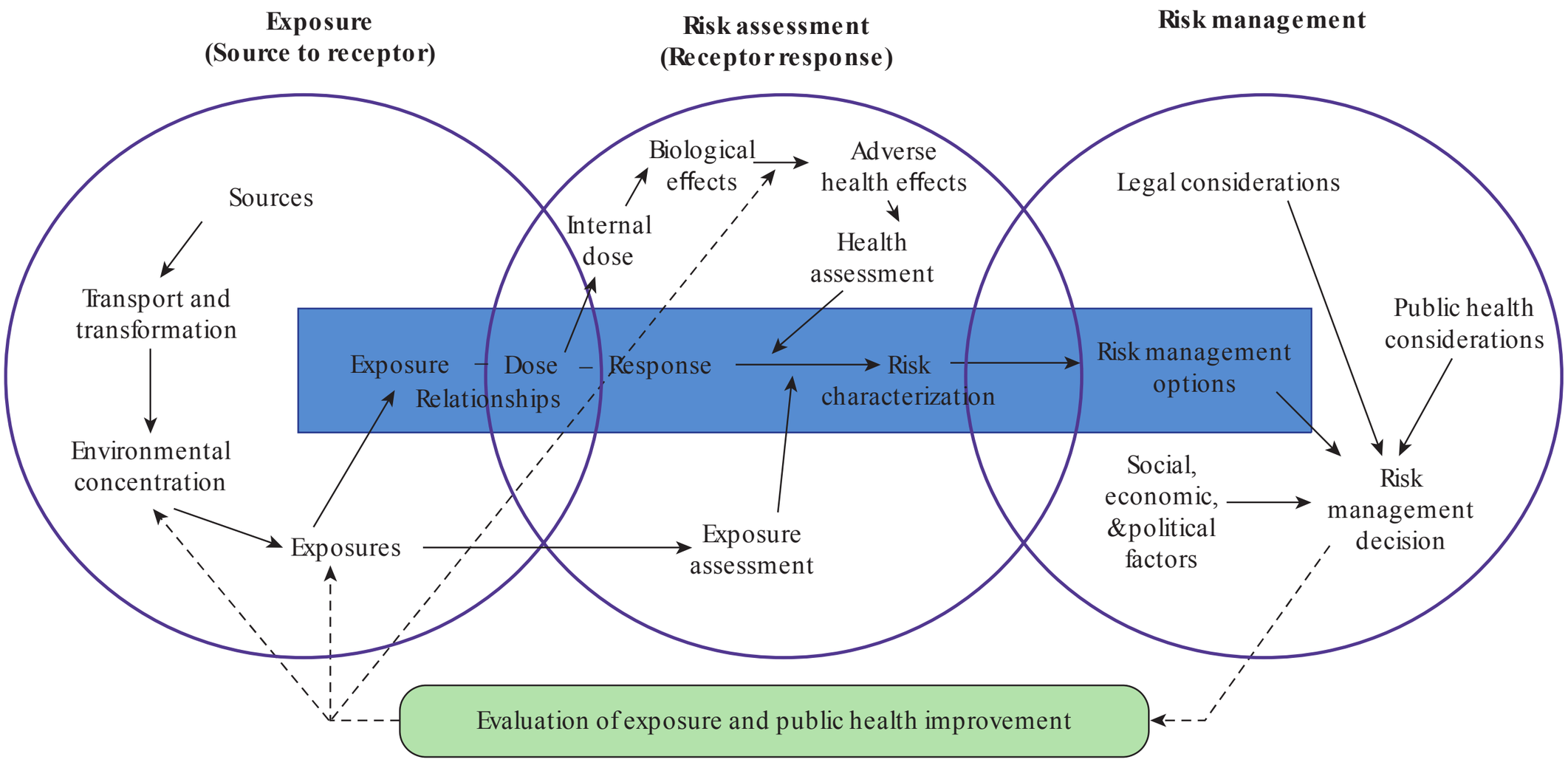

FIGURE 29–2 **NRC risk assessment paradigm.** Components of risk assessment within the left circle provide data to development of risk management as depicted in the right circle, modified to include an "accountability" component as a means to address air quality management impacts on the process risk reduction.

and theoretically can examine patterns in mortality and morbidity, both acute and chronic, especially if these responses appear disproportionately in population subsets (i.e., sensitive groups). However, it is difficult to control confounding personal variables in the population, such as genetic diversity and lifestyle dif erences among individuals, and population mobility are difficult to control. Perhaps most problematic is the lack of adequate exposure data—especially on a personal basis. Studies that involve controlled human exposures are very valuable in assessing potential human risk, since they are derived from the species of concern and are rooted in well-established clinical knowledge and experience.

Animal toxicology is used to predict or corroborate, through plausible mechanisms, suspected ef ects in humans. In the absence of human data, animal toxicology constitutes the essential first step of risk assessment: hazard identif cation. Animal toxicology is often required before any controlled human exposure can be conducted. It is particularly useful in elucidating pathogenic mechanisms involved in toxic injury or disease, providing basic knowledge that is critical to extrapolating databases across species, estimating uncertainties, and determining the relevance of information to humans. Knowledge of the toxic mechanism(s) provides the underpinnings to the "plausibility" of findings in the human context and, under carefully defined and highly controlled circumstances, may allow quantitative estimates of risk to human populations. Animal toxicology studies have been used to investigate all of the criteria air pollutants (ozone, sulfur dioxide, nitrogen dioxide, carbon monoxide, particulate matter and lead) and many of the HAPs (over 30 compounds listed) as well. T e strength of this discipline is that it can involve methods that are not practical in human studies and can provide more rapid turnaround of essential toxicity data under diverse exposure concentrations and durations. T e minimization of uncontrolled variables (e.g., genetic and environmental) may be the greatest strength of the animal bioassay.

Lastly, studies of botanical responses to air pollutants are now appreciated more than ever. Not only are commercial and native vegetation af ected by pollution but also some plant species are being exploited as sensitive "sentinels," warning of the impacts of pollution on both human and environmental receptors. Interestingly, some basic mechanisms (e.g., the involvement of antioxidants) between plants and animals have remarkable parallels.

Animal-to-Human Extrapolation: Issues and Mitigating Factors

T e value of animal toxicology in inhalation studies is highly dependent on the ability to extrapolate or relate empirical findings to real-world human scenarios. Several factors of study design play into the process of extrapolation (e.g., exposure concentration, duration, and patterns), but most important is the selection of the animal species that will serve as the toxicological model. Although cost and convenience are considered, whenever possible, ef ects that are homologous and involve the same mode of action between the study species and the human should guide the decision of the most appropriate test species. An essential, but often overlooked, part of response extrapolation from species to species is knowledge of the relative dosimetry of the pollutant along the respiratory tract. Significant advances in studies of the distribution of gaseous and particulate pollutants have been made through the use of empirical and mathematical models, the latter of which incorporate parameters of respiratory anatomy and physiology, fluid dynamics, and physical chemistry into predictions of deposition and retention. Empirical models combined with theoretical models aid in relating animal toxicity data to humans and help refine the study of injury mechanisms due to better estimates of the target dose.

OVERARCHING CONCEPTS

What Is an Adverse Health Effect?

When relating a health ef ect to an air pollutant, a response must be appreciated at two levels—that of the individual and that of the population. Clearly, an ef ect on an individual can be beyond an acceptable limit potentially putting that person's overall health in jeopardy, but this response may be lost in an index reflecting a population-based response. T e risk to a population reflects the averaging of individual responses or risks and may be measured as a shift in the normal distribution of some index of response for that population. Hence, on average, the entire population may be judged to be at some enhanced risk. T ese two forms of risk are clearly related, but most often in practice, the population risk is considered most appropriate from a public health perspective. It is also generally most credibly quantifiable.

Defining an air pollutant ef ect as "adverse" within the range of ef ects that may result from exposure is not always straightforward. Clearly, in humans, some ef ects would pass uncontested as adverse, e.g., death, acute life-threatening dysfunction or disease, irreversible impairments, and pain. In animal models, pathology has traditionally been the hallmark of an adverse ef ect. In either humans or animals, however, other ef ects that reflect minor and temporary dysfunctions or discomfort could be argued as not warranting significant or costly concern, especially if the ef ects are minor and transient with no long-term untoward outcomes. T is vein of thought would simply attribute these ef ects to be within normal physiologic ranges and are readily compensated within functional or biochemical reserve. T us, if one is to try to assess the impacts of air pollution on health, it is desirable that there exist some objective criteria to define what is indeed adverse based on the nature and the magnitude of the ef ect under evaluation. Moreover, distinguishing an air pollution ef ect from other adverse stimuli or disease processes can be complex and fraught with confounding factors, such as smoking and negative lifestyle factors.

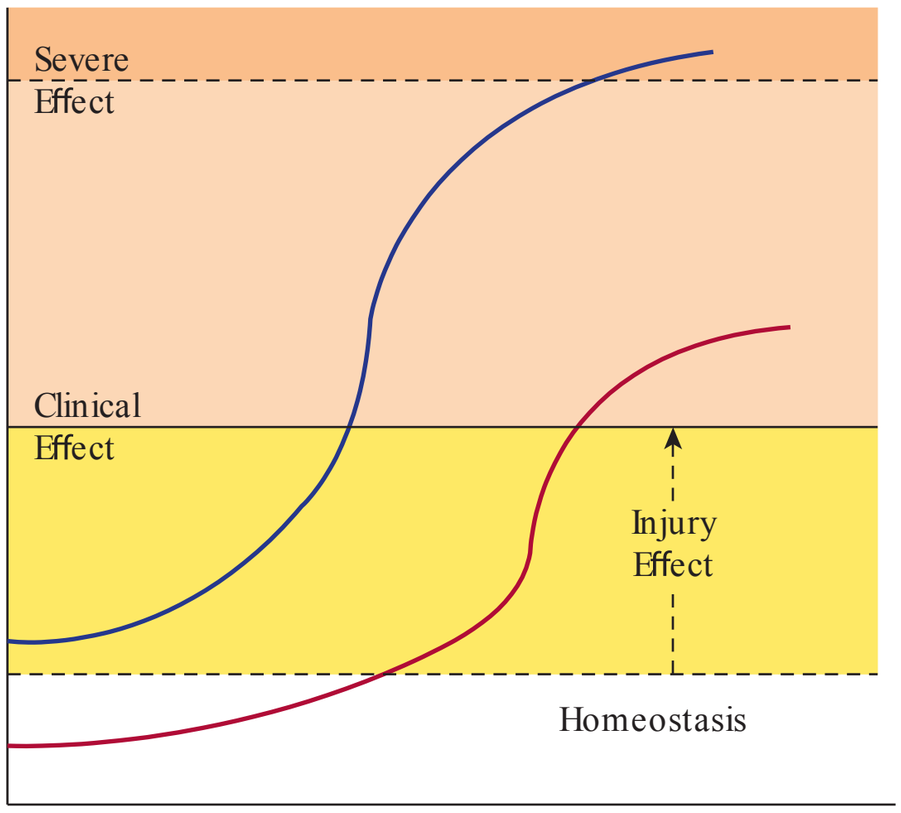

FIGURE 29–3 **Schematic illustration of the elements of the dose response to an air pollutant(s) of a susceptible versus a healthy individual.** The hypothetical susceptible individual may be more sensitive or may have a loss of reserve, either of which results in an inability to maintain homeostasis. The leftward shift or increased slope in the dose–response curve suggests an increase in responsiveness. Either situation may contribute to sensitivity and the likelihood of enhanced progression from subtle to severe outcomes.

Susceptibility

A common thread through these subject areas is the influential role of susceptibility, which can take the form of hyperresponsiveness or loss of reserve. What is a minor reversible effect in the majority of individuals may be a dysfunction that cannot be reversed or compensated in certain individuals (Figure 29–3). Obvious examples would be cardiopulmonary-compromised individuals who function with little or no reserve. As science continues to advance, especially in the realm of molecular biology where small signals can be detected that may forecast an adverse effect or otherwise may identify individuals or groups at risk, the definition of adverse will certainly need reexamination.

In actuality, there is no widely accepted definition for a "susceptible" individual and quite frequently the term is used interchangeably with "vulnerable." However, "vulnerability" refers to extrinsic nonbiological factors (e.g., an increased exposure to ambient air pollutants because one's school is located adjacent to a high-traffic-volume roadway), whereas "susceptibility" refers to intrinsic biological factors such as genetics, age, or preexisting disease. Factors that influence susceptibility and vulnerability are listed in Table 29–1.

Susceptible subpopulations that show exaggerated responsiveness to pollutants merit special mention. Some definable subgroups that are considered inherently more susceptible include children, the elderly, and those with a preexisting disease (e.g., asthma, cardiovascular disease, lung disease). The importance of susceptibility in air pollutant responses is gaining more and more attention as test subject responses that were once considered "outliers" in a controlled chamber study may well be evidence of unusual responsiveness.

TABLE 29–1 Factors that influence the response of individuals or subpopulations to ambient air pollutants.

Susceptibility Factors	Vulnerability/Exposure Factors
Preexisting cardiopulmonary disease	Proximity to point source
Genetic factors	Proximity to high-traffic-volume roadway
Age	Occupation
Gender	Activity level
Race/ethnicity	Use of air conditioning/building leakiness
Obesity	In utero exposure
Pregnancy	Geographic location (e.g., East versus West coast of the United States)
Diabetes	Lower social economic status

EXPOSURE

Air Pollution: Sources and Personal Exposure

Six major air pollutants (particulate matter (PM), O_3, NO_x, SO_2, CO, and Pb) are considered ubiquitous to industrialized communities and are thought to carry the greatest risk to human and environmental health. With the exception of O_3, these pollutants are emitted by anthropogenic combustion processes along with the myriad chemical compounds (mostly volatile organic compounds [VOCs]) considered under the category of HAPs. There are many natural sources of air pollutants as well (e.g., volcanoes, wildfires, windblown dust, natural biogenic vapors) but it is the anthropogenic sources that emit pollutants, which concentrate where people live, that raise concerns about potential health impacts. These factors do not dismiss the significance of potential risks posed by the natural emissions but put focus on the potential for human exposure and risk.

Assessing exposure to an air pollutant has long rested on observational measures of what is in the air. Exposure science is advancing rapidly and now utilizes approaches that range from novel statistical treatments of traditional exposure metrics to sophisticated models that systematically involve aerodynamic and microenvironmental characteristics to estimate or predict exposures to individuals or populations. Typically, inhalation studies of animals involve square-wave exposure patterns, although it is well appreciated that human exposures vary spatially and temporally.

Indoor versus Outdoor—People in the United States (and in most industrialized nations) spend in excess of 80% of their time indoors while at work, school, and home or in an automobile. Perhaps the most significant risk factor in indoor air for children is the presence of asthma. With nearly 10% of children with asthma, the risk of exposure to pollutants and allergens in concentrated form is particularly great.

As to the issue of total exposure, children and outdoor workers are thought to be more likely to encounter outdoor air pollution at its worst; in fact, the relatively high physical activity levels of these subgroups leads to larger doses of any given pollutant being delivered to the lungs. Defining personal exposure can be extremely difficult, as personal monitoring is tedious and expensive, and can sometimes be confounded by other contributions to the indicator being monitored. Hence, exposure measures are typically drawn from ambient measurements or derived from models developed from studies of groups of people carefully characterized across personal exposure modifiers—exercise, personal lifestyles, etc.

It is clear that indoor air can at times be more complex than outdoor air, a point often raised in challenging the legitimacy of studies that rely solely on outdoor monitoring data. Nevertheless, the national monitoring network for the criteria pollutants has been shown to reflect human exposure reasonably well for some pollutants, especially those that are nonreactive. Indeed, outdoor air permeates the indoor environment in spite of the reduced air exchange in most buildings. However, many variables determine how well components of the outdoor air infiltrate. T e complexity of the multiple sources underscores the importance of appreciating the total exposure scenario if we are to understand the nature of air pollution and its potential ef ects on human health (Figure 29–4).

T ere remain two broadly defined illnesses that are largely unique to the indoor building environment. T e first is "sick-building syndrome" (SBS), which is a collection of ailments defined by a set of persistent symptoms enduring for at least two weeks (Table 29–2) and appears to occur in at least 20% of those exposed. Frequently but not always, this syndrome occurs in new, poorly ventilated, or recently refurbished office buildings. T e suspected causes include combustion products, cleaning chemicals, biological emissions from mold, and vapor emissions from furnishings frequently exacerbated

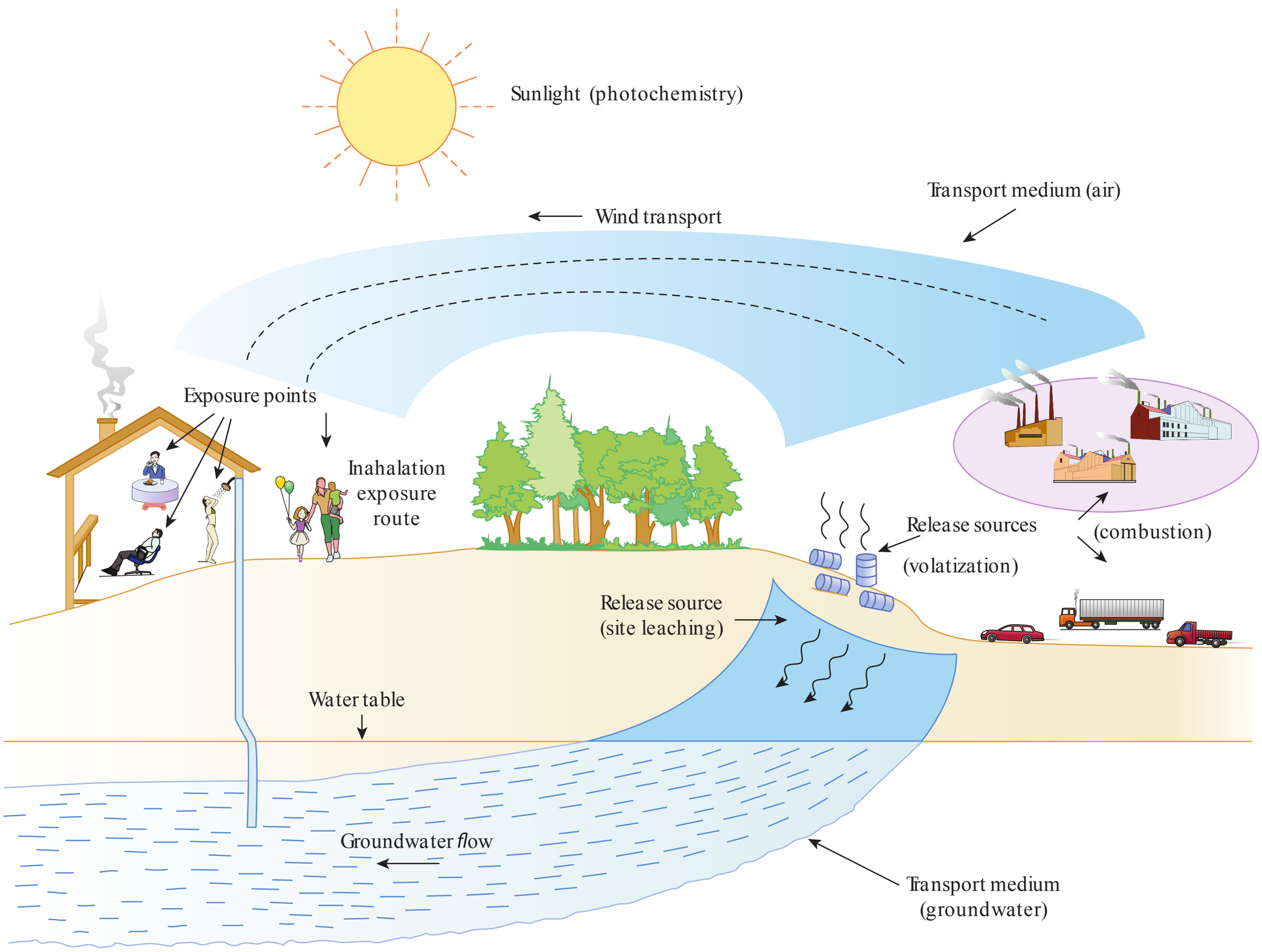

FIGURE 29–4 **Illustration of contributors to the total personal exposure paradigm showing how these indoor and outdoor factors interact.**

TABLE 29–2 Symptoms commonly associated with the sick building syndromes.

Eyes, nose, and throat irritation
Headaches
Fatigue
Reduced attention span
Irritability
Nasal congestion
Dif culty breathing
Nosebleeds
Dry skin
Nausea

by discomfort. T e perception of irritancy to the eyes, nose, and throat ranks among the predominant symptoms that can become intolerable with repeated exposures.

T e second syndrome (building-related illnesses) is a group of illnesses that consists of well-documented conditions with defined diagnostic criteria and generally recognizable etiology. T ese illnesses typically call for conventional medical treatment strategies, because simply exiting the building where the illness was contracted may not readily reverse the symptoms. Several biocontaminant-related illnesses include Legionnaires' disease, hypersensitivity pneumonitis, humidifier fever, as do allergies to animal dander, dust mites, and cockroaches. Medical treatment and mitigation of exposure (source elimination or personal protection) are generally needed to abate symptoms. Some typical outdoor pollutants can also be problematic indoors—CO from poorly vented heaters, NO_2, and many VOCs (passively emitted from new furniture or rugs, or from molds in the ventilation system) including trichloroethylene (a VOC common to the indoor air arising from chlorinated water or dry-cleaned clothes).

Indoor Air in the Developing World—Pollution of the indoor environment in the developing world is a major issue. Superimposed on the infiltration of ambient air pollutants are indoor emissions from cooking practices, the cultural use of incense, tobacco, and various other substances, such as perfumes. In less developed communities, unvented or poorly vented cookstoves that burn biomass are used much as they have been for centuries. Chronic lung diseases, such as bronchitis, emphysema, and cancer, are major killers of exposed women while children suf er from bronchitis and various other infectious lung diseases.

The Evolving Pro**fi**le o**f** Outdoor Air Pollution—Classically, ambient air pollution was distinguished on the basis of the chemical redox nature of its primary components SO_x and NO_x. T e classical types of air pollution were implicitly seasonal. Reducing-type air pollution occurred during winter periods of oil and coal combustion for heating and power coupled with meteorological inversions, while the oxidant atmospheres occurred during the warmer months of spring and summer, when sunlight is most intense and can catalyze reactions among the constituents of auto exhaust. Today the urban distinctions between reducing and oxidant smogs have become largely an academic exercise. As climate change progresses, there is expectation that the underlying chemistry will change further and alter these patterns.

Many megacities remain plagued by the classic reducing and oxidant forms of air pollution. Uncontrolled industrial and coal-fired power plant emissions surrounding cities such as Beijing and the northern sectors of Mexico City are dominated by sulfurous, particulate emissions, whereas southern Mexico City, Santiago, and Tokyo have substantially automobile-associated oxidant smogs. Impacts on health, visibility, and general welfare are clear and are bringing ever increasing public concern. Urban air pollution is a worldwide problem, where the estimate of people exposed to O_3 at potentially harmful levels exceeds 480 million.

EPIDEMIOLOGICAL EVIDENCE OF HEALTH EFFECTS

Outdoor Air Pollution

Acute and Episodic Exposures—A number of air pollution incidents have been documented where pollutant concentrations rose to levels that are clearly hazardous to human health. Where a single chemical has been accidentally released (e.g., methyl isocyanate in Bhopal, India), establishing the relationship between cause and ill ef ect is straightforward. However, most air pollution situations involve complex atmospheres, and establishing a specific cause other than the air pollution incident itself can be difficult.

Of the many air pollution studies over the last 25 years, none have had more impact on the perception of pollutant risk and the direction of research today than a series of epidemiological studies that showed an association between PM mass concentration and daily mortality. Studies utilizing novel time-series analyses that blunt the impact of weather, smoking, and other variables that might obscure patterns in health variables linked to the air monitoring data showed significant and consistent associations between health outcomes of ambient PM at levels previously thought to be safe. Time-series analyses are based on Poisson regression modeling to distinguish changes in daily death counts (or hospital admissions) associated with short-term changes in air pollution. T e statistical methodology applied in these time-series studies could detect short-term trends and minimized the ef ects of other pollutants and potential confounders with longer time constants.

PM stands as the preeminent air pollutant because of its health impact as well as the pollutant that opened the door to unsuspected targets of injury. T e major health outcome

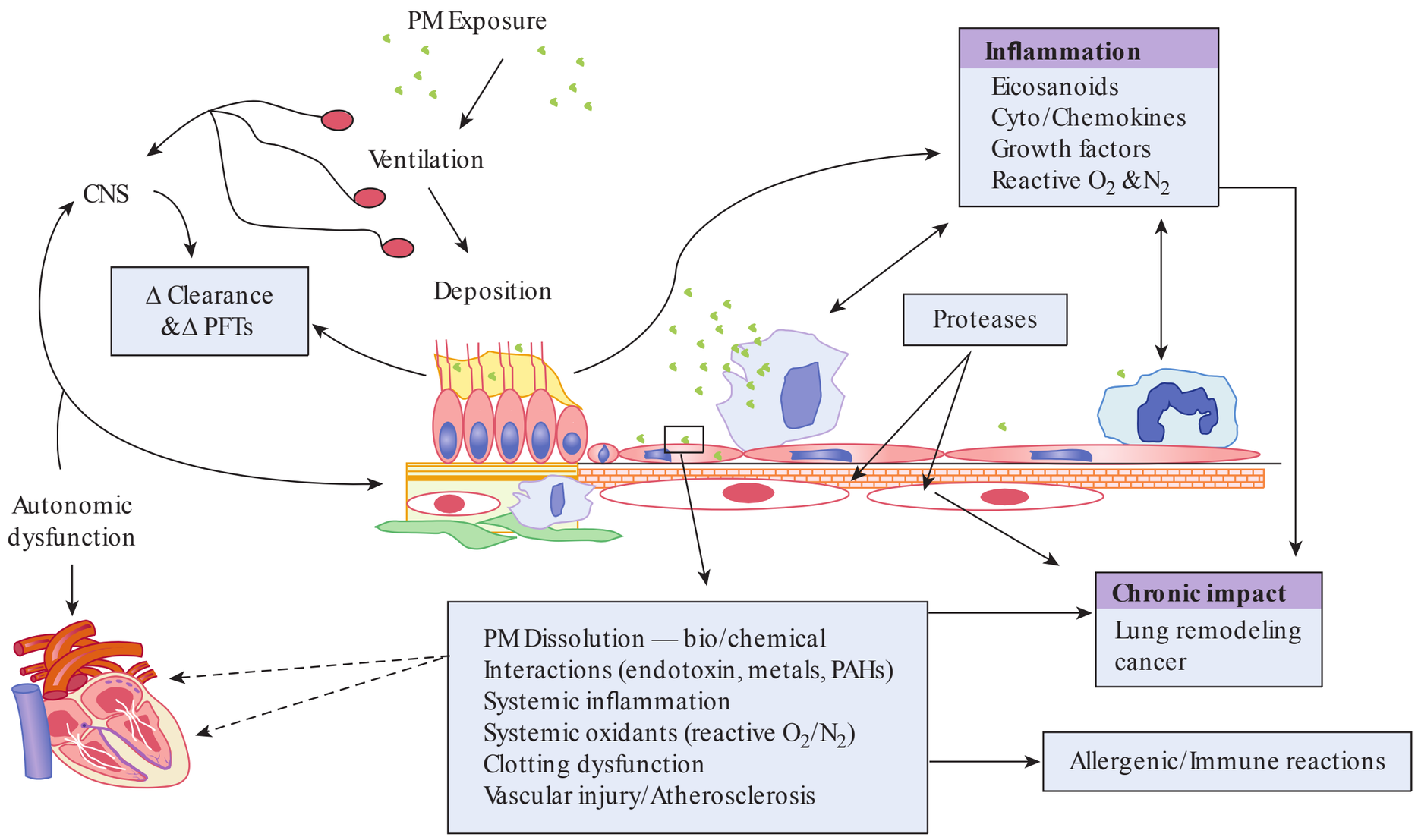

FIGURE 29–5 **Schematic of the multiple mechanisms thought to function in cardiopulmonary response(s) to air pollutants—derived from current hypothesized mechanisms for particulate matter.**

revealed in the study of PM has been the involvement of the cardiovascular system as a prime target for adverse impact. Both epidemiological and toxicological studies now point to major cardiac involvement in PM-associated mortality. Not surprisingly, effects are most apparent in subpopulations already compromised by cardiopulmonary and perhaps vascular diseases (e.g., diabetes). Several pathways have been proposed that attempt to link exposure and cardiac effects that may or may not include pulmonary mediation. These potential mechanisms are illustrated in Figure 29–5.

The association between PM and health outcomes apparently is linked to particle composition rather than mass alone. The actual "biochemical lesion" caused by PM is generally thought to involve oxidant mechanisms (generation of reactive oxygen and perhaps nitrogen species) by constituents or attributes (e.g., reactive surface area) of the particles at the cell or molecular level.

Long-Term Exposures—Epidemiological studies of the chronic effects of air pollution are difficult to conduct by the very nature of the goal: outcomes associated with long-term exposures. Looking back in time with retrospective, cross-sectional studies frequently were confounded with unknown variables and inadequate historical exposure data. A good example of the problem of confounding is cigarette smoking. Without extensive information on both active and passive smoking, the ability to discern the impact of an air pollution disease outcome such as chronic bronchitis and emphysema would be greatly impaired. Prospective studies, on the other hand, have the advantage of more precise control of confounding variables, such as the tracking of urinary cotinine as an index of tobacco smoke exposure, but they can be very expensive and require substantial time and dedication on the part of both the investigators and the study population. Depending on the study size and design, exposure assessments can be complex, and the loss of subjects due to dropout is sometimes unpredictable.

POLLUTANTS OF OUTDOOR AMBIENT AIR

Classic Reducing-Type Air Pollution

High concentrations of the reducing-type air pollution, characterized by SO_2 and smoke, are capable of producing dramatic human health effects. Empirical studies in human subjects and animals have long stressed the irritancy of SO_2 and its role in these incidents, while the full potential for interactions among the copollutants in the smoky, sulfurous mix has a mixed record of replication in the human exposure laboratory. It is an irritant gas that has a toxicology of its own and, through atmospheric reactions, can transform photochemically into sulfites or sulfates within a secondarily irritant particle.

Sulfur Dioxide

General Toxicology—Sulfur dioxide is a water-soluble irritant gas that is absorbed predominantly in the upper airways and that stimulates bronchoconstriction and mucus

secretion in a number of species, including humans. Early studies with relatively high exposure concentrations of SO_2 showed airway cellular injury and subsequent proliferation of mucus-secreting goblet cells. At concentrations < 1 ppm, such as might be encountered in the polluted ambient air of industrialized areas, long-term residents experience a higher incidence of bronchitis. Other factors (diet, access to health care, other pollutants) have been involved in this reversal reductions in ambient smoke and SO_2 are generally thought to be the most important.

T e penetration of SO_2 into the lungs is greater during mouth as opposed to nose breathing. An increase in the airflow during deep rapid breathing augments penetration of the gas into the deeper lung. As a result, persons exercising would inhale more SO_2 and, as noted with asthmatics, are likely to experience greater irritation. Once deposited along the airway, SO_2 dissolves into surface lining fluid as sulfite or bisulfite and is readily distributed throughout the body. It is thought that the sulfite interacts with sensory receptors in the airways to initiate local and centrally mediated bronchoconstriction.

Pulmonary Function Ef ects—T e basic pulmonary response to inhaled SO_2 is mild bronchoconstriction, which is reflected as a measurable increase in airflow resistance due to narrowing of the airways. Concentration-related increases in resistance have been observed in guinea pigs, dogs, and cats as well as humans. Airflow resistance increased more when the gas was introduced through a tracheal cannula than via the nose, since nasal scrubbing of the water-soluble gas was bypassed.

Sulfuric Acid and Related Sulfates—T e conversion of SO_2 to sulfate is favored in the environment with subsequent ammonia neutralization to ammonium sulfate [$(NH_4)_2SO_4$] or ammonium bisulfate [NH_4HSO_4]. During oil and coal combustion or the smelting of metal ores, sulfuric acid condenses downstream of the combustion processes with available metal ions and water vapor to form submicrometer sulfuric acid fume and sulfated fly ash. Photochemical reactions also promote acid sulfate formation via both metal-dependent and independent mechanisms, but most of the oxidation of SO_2 occurs within plumes as they disperse in the atmosphere. Stack emissions may undergo long-range transport to areas distant from the emission source, allowing considerable time for sunlight-driven chemistry.

General Toxicology—Sulfuric acid irritates by virtue of its ability to protonate (H^+) receptor ligands and other biomolecules. T is action can either directly damage membranes or activate sensory reflexes that initiate inflammation. Unlike other irritants, such as O_3 (see below), inhaled sulfuric acid does not appear to stimulate a classic neutrophilic lung inflammation. Rather, eicosanoid homeostasis appears to be disturbed resulting in macrophage dysfunction and altered host defense.

Pulmonary Function Ef ects—Sulfuric acid produces an increase in flow resistance in guinea pigs due to reflex airway narrowing, or bronchoconstriction, which impedes the flow of air into and out of the lungs. T is response might be thought of as a defensive measure to limit the inhalation of air containing noxious gases, but this explanation may be more teleological than fact. T e magnitude of the response is related to both acid concentration and particle size. T e thicker mucus blanket of the nose may also blunt (by dilution or neutralization by mucus buf ers) much of the irritancy of the deposited acid. In contrast, the less shielded distal airway tissues, with higher receptor density, would be expected to be more sensitive to the acid particles reaching that area.

Asthmatics appear to be somewhat more sensitive to the bronchoconstrictive ef ects of sulfuric acid than are healthy individuals, owing to hyperresponsive airways, so their tendency to constrict at low acid concentrations would be expected, just as asthmatic airways are sensitive to nonspecific airway smooth muscle agonists (e.g., carbachol, histamine, exercise). T e general correlation between airway responsiveness and inflammation that appears to be important in grading asthma severity and risk of negative clinical outcomes may also be predictive of responses to environmental stimuli.

Ef ects on Mucociliary Clearance and Macrophage Function—Sulfuric acid alters the clearance of particles from the lung. Mucus clearance appears to vary directly with the acidity ($[H^+]$) of the acid sulfate, with sulfuric acid having the greatest ef ect and ammonium sulfate the smallest. Collectively, there seems to be coherence in the data to rank sulfate irritancy: sulfuric acid > ammonium bisulfate > ammonium sulfate. Acidity $[H^+]$ appears to be the primary driver on most respiratory ef ects attributable to the acid sulfates even at the level of pulmonary macrophages.

Chronic Ef ects—Not surprisingly, sulfuric acid induces qualitatively similar ef ects in the airways as found at high concentrations of SO_2. As a fine aerosol, sulfuric acid deposits deeper along the respiratory tract, and its high specific acidity imparts greater injury on phagocytes and epithelial cells. T us, a primary concern with regard to chronic inhalation of acidic aerosols is the potential for bronchitis, since this has been a problem in occupational settings in which employees are exposed to sulfuric acid mists (e.g., battery plants).

Studies conducted with sulfuric acid have demonstrated that the airways of exposed animals become progressively more sensitive to challenge with acetylcholine, show a progressive decrease in diameter, and experience an increase in the number of secretory cells, especially in the smaller airways. T ese studies have expanded our knowledge of the biological response and its exposure-based relationship to sulfuric acid. It seems reasonable to postulate that chronic daily exposure of humans to ~100 $\mu g/m^3$ sulfuric acid may lead to impaired clearance and mild chronic bronchitis. T e possibility that chronic irritancy may elicit bronchitis-like disease in susceptible individuals (perhaps over a lifetime or in children because of dose dif erences) appears to be reasonable.

Particulate Matter

PM in the atmosphere can be solid, liquid, or a combination of both with a mélange of organic, inorganic, and biological compounds. The compositional matrix of PM can vary significantly depending on the emission source and secondary transformations, many of which involve gas to particle conversions. Long-range transport of emissions or transformation products can contribute significantly to the regional matrix of PM. Particles of larger size tend to have more local sources, the reason being that they are formed from dispersed dust and attrition of materials. Being of larger size, they tend to "fall out" or settle from the air due to gravity (although winds can in fact carry these particles great distances—e.g., Sahara desert particles have been found on the US East Coast). Particles in the range of 10 to 2.5 μm ($PM_{10-2.5}$—coarse PM) are highly inhalable by humans. In the urban setting there is considerable spatial and temporal heterogeneity of coarse PM while $PM_{2.5}$ appears more homogenous throughout a regional environment. The size designation of fine and coarse PM is based on their relative respirability—those in the range of PM_{10} are inhalable into the larger thoracic airways while the $PM_{2.5}$ is inhalable into the deeper reaches (gas exchange areas) of the lung (see Chapter 15).

Metals—There have been many standard acute and subchronic rodent inhalation studies with specific metal compounds, often as oxides, chlorides, or sulfates. These exposure studies relate most appropriately to occupational exposures. Metals may arise from natural as well as anthropogenic activities, and as a result metals are a common constituent in ambient PM. The metal profiles among regions differ appreciably in concentration and type and they also differ by the size mode of PM. Coarse PM (2.5–10 μm) arises largely from natural sources and thus has prominent earthen metals such as iron, sodium, silica, and magnesium—usually in oxide forms. Combustion-derived metals reflect the fuel source. For example, oil may have vanadium, nickel, and perhaps zinc and iron, while coal may have zinc and selenium. Their chemical forms vary from water-soluble salts to oxide and phosphate forms. Other metals are emitted from vehicles burning fuels to which metal compounds were added to alter functionality (e.g., lead, manganese, platinum) or as engine wear and catalyst by-products. Similarly, metals may also derive from brake (copper, iron), tire (zinc), and dispersed road (earthen silicates) wear. Metals have many biological properties, some essential to life while others being directly toxic to cells or act indirectly in a pro-oxidant toxic fashion. Thus, metals have garnered considerable interest regarding their role in PM toxicity.

Metal compounds can be separated nominally by physicochemical characteristics: those that are essentially water-insoluble (e.g., metal oxides and hydroxides such as those that might be released from high-temperature combustion sources or derived from the geocrustal matrix) and those that are soluble or somewhat soluble in water (often chlorides or sulfates such as those that might form under acidic conditions in a smoke plume or leach from acid-hydrated silicate particles in the atmosphere). Solubility appears to play a role in the toxicity of many inhaled metals by enhancing metal bioavailability (e.g., nickel from nickel chloride versus nickel oxide), but insolubility can also be a critical factor in determining toxicity by increasing pulmonary residence time within the lung (e.g., insoluble cadmium oxide versus soluble cadmium chloride). Moreover, some metals, either in their soluble forms or when partially coordinated on the surface of silicate or bioorganic materials, can promote electron transfer to form reactive oxidants.

Gas–Particle Interactions—As already noted, these gas–particle interactions can be extremely complex involving multiple components of the particles, gases/vapors, and sunlight and lead to toxicity of either the particle or the gas. Metal smelting or the combustion of coal can emit sulfuric acid that is physically associated with ultrafine metal oxide particles. Complex chemistry also occurs within the effluent of the combustion source. Similar interactions may result from gaseous pollutants that impair the clearance of particles from the lung or otherwise alter their metabolism. Studies focusing on irritancy and infectivity raise the prospect that realistic exposure scenarios of gaseous and particulate pollutants can interact through either chemical or physiologic mechanisms to enhance health risks of complex polluted atmospheres.

Ultrafine Carbonaceous Matter—Ultrafine carbon particles (often called black carbon) typically result from high-temperature pyrolysis or as the product of atmospheric transformation involving organic vapors and sunlight. The size of these particles allows them to slip between gas molecules moving primarily by diffusion and principles of Brownian motion. Agglomeration on surfaces or other particles in the air is their primary mode of dissipation. When concentrations exceed ~1 million/cm^3, they rapidly agglomerate with each other to form larger clumps or chains of ultrafine particles. As an air pollutant, therefore, elemental carbon particles generally do not exist as singlets except near their emission points—e.g., traffic or other high-temperature sources. Fine PM consists in part of agglomerates of carbonaceous organic material that if partially oxidized may be somewhat soluble in water. Some organic materials, which exist in the vapor form, condense on the ultrafine carbon (e.g., diesel PM). Estimates of the carbonaceous (including organic) content of ambient fine PM vary considerably but are nominally considered to be about 10% to 60% of the total mass depending on the urban or regional area.

Diesel particles vary widely in the ratio of organic and elemental carbonaceous materials, which in empirical studies has been shown to influence toxic outcomes, such as to their inflammatory and carcinogenic potential. Diesel exhaust that also contains significant amounts of gaseous pollutants: NO_x, CO, and SO_x as well as various VOCs and carbonyl irritants. Elemental carbon itself is generally considered to be of low toxicity, although long-term, high-concentration exposure conditions in rats can lead to lung "overload" where there is evidence of lung damage and carcinogenicity. In the environment, carbon

has the potential to act as a carrier of certain irritant gases. However, carbon in the ultrafine mode (< 0.1 μm) has been suggested to be more toxic than the fine mode (2.5 μm) form, perhaps due to enhanced surface reactivity or tissue penetration. Composition of the ultrafine particle also contributes to its effects and behavior. Ultrafine particles in the environment exist in extremely high numbers but contribute negligibly to mass. Recent commercial introduction of "engineered" nanoparticles brings many of the same concerns as ultrafines by virtue of their similar sizes. Additionally, being "engineered" particles, they may possess design features that "natural" combustion ultrafine (or nano) particles do not.

Chronic Effects and Cancer—The role of air pollution in human lung cancer is difficult to assess because the vast majority of respiratory cancers result from cigarette smoking. VOCs and nitrogen-containing and halogenated organics account for most of the compounds that are derived from combustion sources ranging from tobacco to power plants to incinerators to motor vehicles with potential carcinogenic effects. Human exposure to airborne toxicants is highly complex compositionally as well as in its temporal and spatial heterogeneity.

The lung cancer risk of any individual is some function of the carcinogenic nature of the substance, the amount of material deposited in the lungs, which is itself a function of the concentration in the ambient air, the physical and chemical properties of the inhalant that may determine deposition efficiency, and the cumulative volume of air inhaled. Of course, the innate susceptibility of the individual (including genotype and environmental factors such as diet, etc.) is also likely to be important. The majority of lung cancer risk from ambient air pollution lies within the PM fraction, including the polycyclic organic chemicals, along with the less volatile (semivolatile) nitroaromatics. These persistent organics associate with the PM matrix and thus could have a prolonged residence time at deposition sites within the respiratory tract. Genetic bioassays have revealed the potent mutagenicity, and presumably carcinogenicity, of various chemical fractions of ambient aerosols. Some of these compounds require metabolic transformation to activate their potency while others may be detoxified by their metabolism. Carcinogenic vapors such as benzene are inhaled but target the bone marrow producing leukemia.

The cells lining the respiratory tract turn over relatively quickly, since they interface with the ambient environment with every breath. Conceptually, their DNA would thus be vulnerable to carcinogenic or oxidant-induced replication errors that, when fixed as mutations, could give rise to tumors. Copollutants, such as irritant gases, that initiate inflammation may promote carcinogenic activity by damaging cells and further enhancing their turnover.

Photochemical Air Pollution

Photochemical air pollution (notably O_3) arises secondarily from a series of complex reactions in the troposphere activated by the ultraviolet (UV) spectrum of sunlight. In addition to O_3, it comprises a mixture of nitric oxides (NO_x), aldehydes, peroxyacetyl nitrates (PAN), and a myriad of aromatics and alkenes along with analog reactive radicals. If SO_2 is present, sulfates may also be formed and, collectively, they yield "summer haze." Likewise, the complex chemistry can generate organic PM, nitric acid vapor, and various condensates.

From the point of view of the toxicology of photochemical air pollutant gases, O_3 is by far the toxicant of greatest concern. It is highly reactive and more toxic than NO_x, and because its generation is fueled through cyclic hydrocarbon radicals, it reaches greater concentrations than the hydrocarbon radical intermediates. Although O_3 is of toxicological importance in the troposphere, in the stratosphere it plays a critical protective role. About 10 to 50 km above the earth's surface, UV light directly splits molecular O_2 into atomic $O^{\bullet}$, which then combines with O_2 to form O_3. The O_3 also dissociates back but much more slowly. The result is an accumulation of O_3 to several ppm within a relatively thin strip of the stratosphere forming an effective "permanent" barrier by absorbing the short-wavelength UV in the chemical process. This barrier had in recent years been threatened by various anthropogenic emissions (Cl_2 gas and certain chlorofluorocarbons) that enhance O_3 degradation (creation of an "O_3 hole"), but recent restrictions on the use of these degrading chemicals seem to have been effective in reversing this process. The benefits are believed to be a reduction of excess UV light infiltration to the earth's surface and reduced skin cancer risk.

This protective issue is quite different in the troposphere, where accumulation of O_3 serves no known purpose and poses a threat to the respiratory tract. Near the earth's surface, NO_2 arising from combustion processes efficiently absorbs longer-wavelength UV light, from which a free O atom is cleaved, initiating the following simplified series of reactions:

$$NO_2 + h\nu\,(\text{UV light}) \rightarrow O^{\bullet} + NO^{\bullet} \qquad (29\text{–}1)$$

$$O^{\bullet} + O_2 \rightarrow O_3 \qquad (29\text{–}2)$$

$$O_3 + NO^{\bullet} \rightarrow NO_2 \qquad (29\text{–}3)$$

This process is inherently cyclic, with NO_2 regenerated by the reaction of the $NO^{\bullet}$ and O_3. In the absence of unsaturated hydrocarbons (olefins and substituted aromatics) arising from fuel vaporization or combustion, as well as biogenic terpenes, this series of reactions would approach a steady state with little buildup of O_3. The free electrons of the double bonds of unsaturated hydrocarbons are attacked by free atomic $O^{\bullet}$, resulting in oxidized compounds and radicals that react further with $NO^{\bullet}$ to produce more NO_2. Thus, the balance of the reactions sequence shown in Eqs. (29–1) to (29–3) is tipped to the right, leading to buildup of O_3. This reaction is particularly favored when the sun's intensity is greatest at midday, utilizing the NO_2 provided by morning rush-hour traffic. Carbonyl compounds (especially short-chained aldehydes) are also by-products of these reactions. Formaldehyde and acrolein account for about 50% and 5%, respectively, of the total aldehyde content in urban atmospheres. Peroxyacetyl nitrate (CH_3COONO_2),

often referred to as PAN, and its homologs also arise in urban air, most likely from the reaction of the peroxyacyl radicals with NO_2.

Chronic Exposures to Smog

Epidemiological studies in human populations as well as empirical studies in laboratory animals have attempted to link degenerative lung disease with chronic exposure to photochemical air pollution. Cross-sectional and prospective field studies have suggested an accelerated loss of lung function in people living in areas of high pollution. However, as with many studies of this type, there were problems with confounding factors (meteorology, imprecise exposure assessment, and population variables). Studies have been conducted in children living in modern-day Mexico City, which has high oxidant and PM levels, noted severe epithelial damage and metaplasia as well as permanent remodeling of the nasal epithelium. When children migrated into Mexico City from cleaner, nonurban regions, even more severe damage was observed, suggesting that the tissue remodeling in the permanent residents imparted some degree of incomplete adaptation. Because the children were of middle-class origin, these observations were less likely confounded by socioeconomic variables. In fact, the epithelial cell damage in the nasal cavity of Mexico City children was inversely correlated with glutathione peroxidase, a marker of oxidative stress.

Ozone

General Toxicology—Ozone is the primary oxidant of concern in photochemical smog because of its inherent bioreactivity and its concentration relative to other reactive species. Current mitigation strategies for O_3 have been only largely unsuccessful owing to sustained population growth. With suburban sprawl and the downwind transport of air masses from populated areas to more rural environments, the geographic distribution of those exposed has also expanded, as has the temporal profile of individual exposure. In other words, ambient O_3 exposures are no longer stereotyped as brief 1 to 2 h peaks. Instead, there is more typically a prolonged period of exposure of 6 h or more at or near the NAAQS level.

Ozone induces a variety of effects in humans and experimental animals at concentrations that occur in many urban areas. These effects include morphologic, functional, immunologic, and biochemical alterations. Because of its low water solubility, a substantial portion of inhaled O_3 penetrates deep into the lung, but its reactivity is such that about 17% and 40% are scrubbed by the nasopharynx of resting rats and humans, respectively. Nevertheless, regardless of species, the region of the lung that is predicted to have the greatest O_3 deposition (dose per surface area) is the centriacinar region, from the terminal bronchioles to the alveolar ducts, also referred to as the proximal alveolar ductal region. Because O_3 penetration increases with increased tidal volume and flow rate, exercise increases the dose to the target area. Thus, it is important to consider the role of exercise-associated dosimetry in a study of O_3 or any inhalant before making cross-study comparisons, especially if that comparison is across species.

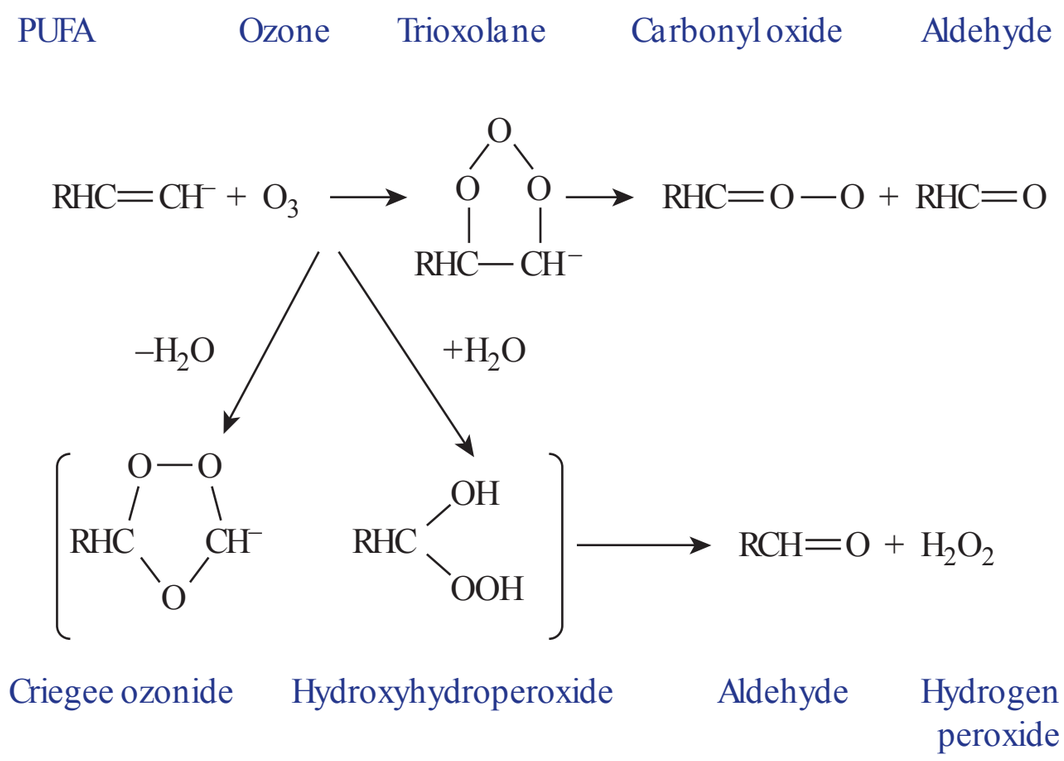

FIGURE 29–6 **Major reaction pathways of O_3 with lipids in lung lining fluid and cell membranes.** (Adapted with permission from the Air Quality Criteria Document for Ozone and Photochemical Oxidants, U.S. EPA, 1996.)

As a powerful oxidant, O_3 seeks to extract electrons from other molecules. The surface fluid lining the respiratory tract and the cell membranes that underlie the lining fluid contain a significant quantity of polyunsaturated fatty acids (PUFA), either free or as part of the lipoprotein structures of the cell. The double bonds within these fatty acids have a labile, unpaired electron that is easily attacked by O_3 to form ozonides that progress through a less stable zwitterion or trioxolane (depending on the presence of water); these ultimately recombine or decompose to lipohydroperoxides, aldehydes, and hydrogen peroxide. These pathways are thought to initiate propagation of lipid radicals and auto-oxidation of cell membranes and macromolecules (Figure 29–6).

Pulmonary Function Effects—Exercising human subjects exposed for 2 to 3 h to 0.12 to 0.4 ppm O_3 experience reversible concentration-related decrements in forced exhaled volumes (FVC and forced expiratory volume in one second [FEV_1]). It is not clear what mechanisms underlie the altered lung function (in terms of changes in FEV_1) produced by O_3. There is also evidence that the decrements in lung function are vagally mediated, and that the response can be abrogated by analgesics, such as ibuprofen and opiates, which also reduce pain and inflammation. Thus, pain reflexes involving C-fiber networks may be important in the reduction in forced expiratory volumes along with changes in vagal reflexes that alter airway reactivity and bronchoconstriction.

Airway responsiveness to specific (e.g., allergen) and nonspecific (e.g., cold air, inhaled methacholine) bronchoconstriction is another commonly used test of the pulmonary response to inhaled pollutants such as O_3. These types of tests are very important because airway hyperresponsiveness is a central feature of asthma and asthmatics are a sizeable subpopulation

(7% to 9% of the total population in the United States) that may be particularly sensitive to the adverse respiratory effects of inhaled pollutants.

Ozone Interactions with Copollutants—An approach simplifying the complexity of synthetic smog studies, yet addressing the issue of pollutant interactions, involves the exposure of laboratory animals or humans to binary or more complex synthetic mixtures of pollutants that occur together in ambient air. The most frequent combination involves interactions of O_3 and NO_2 or O_3 and PM (e.g., sulfuric acid or diesel particles). Not surprisingly, study design adds a level of complexity in interpretation such that evidence exists supporting either augmentation or antagonism of lung function impairments, lung pathology, and other indices of injury. This apparent conflict in the findings only emphasizes the need to carefully consider the myriad of factors that might affect studies involving multiple determinants and the nature of the exposure that is most relevant to reality.

As the number of interacting variables increases, so does the difficulty in interpretation. Studies of complex atmospheres involving acid-coated carbon combined with O_3 at near-ambient levels also show varied evidence of interaction on lung function and macrophage receptor activity. The statistical separation of the interacting variables and responses from the individual or combined components is difficult. However, it is indeed the complex mixture to which people are exposed that we wish to evaluate. Creative approaches to understanding mixture responses must be addressed in the future.

Nitrogen Dioxide

General Toxicology—Nitrogen dioxide, like O_3, is a deep lung irritant that can produce pulmonary edema if it is inhaled at high concentrations. Potential life-threatening exposure is a real-world problem for farmers, as near-lethal high levels of NO_2 can be liberated from fermenting fresh silage. Being heavier than air, the generated NO_2 and CO_2 displace air and oxygen at the base of silo and diffuse into closed spaces where workers can inadvertently get exposed to very high concentrations perhaps with depleted oxygen. Typically, shortness of breath rapidly ensues with exposures nearing 75 to 100 ppm NO_2, with delayed edema and symptoms of pulmonary damage. Not surprisingly, the symptoms are collectively termed "silo-filler's disease." Nitrogen dioxide is also an important indoor pollutant, especially in homes with unventilated gas stoves or kerosene heaters or in developing countries with the unvented burning of biomass fuels.

The distal lung lesions produced by acute NO_2 are similar among species. Theoretical dosimetry studies indicate that NO_2 is deposited along the length of the respiratory tree, with preferential deposition being in the distal airways. Damage is most apparent in the terminal bronchioles. At high concentrations, the alveolar ducts and alveoli are also affected, with type 1 cells again showing their sensitivity to oxidant challenge.

Pulmonary Function Effects—Exposure of normal human subjects to concentrations of ≤ 4 ppm NO_2 for up to 3 h produces no consistent effects on spirometry. A number of factors appear to be involved (e.g., exercise, inherent sensitivity of the asthmatic subject, exposure method).

Inflammation of the Lung and Host Defense—Unlike O_3, NO_2 does not induce significant neutrophilic inflammation in humans at exposure concentrations encountered in the ambient outdoor environment. There is some evidence for bronchial inflammation after 4 to 6 h at 2.0 ppm, which approximates the highest transient peak indoor levels of this oxidant. Exposures at 2.0 to 5.0 ppm have been shown to affect T lymphocytes, particularly $CD8^+$ cells and natural killer cells that function in host defenses against viruses. Although these concentrations may be high, epidemiological studies variably show effects of NO_2 on respiratory infection rates in children, especially in indoor environments.

Other Oxidants

PAN is thought to be responsible for much of the eye-stinging activity of smog. It is more soluble and reactive than O_3, and hence rapidly decomposes in mucous membranes. The cornea is a sensitive target and is prominent in the burning/stinging discomfort often associated with oxidant smogs.

Aldehydes

Carbonyl compounds, notably short-chained (2-4C) aldehydes, are common photo-oxidation products of unsaturated hydrocarbons. Two aldehydes are of major interest by virtue of their concentrations and irritancy: formaldehyde (HCHO) and acrolein ($H_2C{=}CHCHO$). They contribute to the odor as well as eye and sensory effects of smog. Formaldehyde accounts for about 50% of the estimated total aldehydes in polluted air, while acrolein, the more irritating of the two, accounts for about 5% of the total. Acetaldehyde (C_3HCHO) and many other longer-chain aldehydes make up the remainder, but they are not as intrinsically irritating, exist at low concentrations, and have less solubility in airway fluids.

Formaldehyde

Formaldehyde is a primary sensory irritant. Because it is very soluble in water, it is absorbed in mucous membranes in the nose, upper respiratory tract, and eyes. The dose–response curve for formaldehyde is steep: 0.5 to 1 ppm yields a detectable odor, 2 to 3 ppm produces mild irritation, and 4 to 5 ppm is intolerable to most people. Formaldehyde is thought to act via sensory C-fibers that signal locally as well as through the trigeminal nerve to reflexively induce bronchoconstriction through the vagus nerve.

Two aspects of formaldehyde toxicology have brought it from relative obscurity to the forefront of attention in recent years. One is its near ubiquitous presence in indoor

atmospheres as an of-gassed product of construction materials such as plywood, furniture, or improperly polymerized urea-formaldehyde foam insulation. In addition, the potential carcinogenicity of formaldehyde is a concern. Formaldehyde is a probable human carcinogen. There is epidemiological evidence that formaldehyde causes nasopharyngeal cancer, strong but not sufficient evidence of leukemia, and limited evidence of sinonasal cancer.

Acrolein

Because acrolein is an unsaturated aldehyde, it is more reactive than formaldehyde. It penetrates a bit deeper into the airways and may not have the same degree of sensory irritancy but it may cause more damage. Concentrations below 1 ppm cause irritation of the eyes and the mucous membranes of the respiratory tract. The mechanism of increased flow resistance appears to be mediated through both a local C-fiber and centrally mediated cholinergic reflexes. Ablation of the C-fiber network and atropine (muscarinic blocker) block this response.

Carbon Monoxide

Carbon monoxide is classed toxicologically as a chemical asphyxiant because its toxic action stems from its formation of carboxyhemoglobin, preventing oxygenation of the blood for systemic transport (see Chapter 11). Motor vehicles still account for two thirds of urban CO emissions. Other sources of CO include both main and sidestream tobacco smoke, and residential and commercial heating systems and mobile auxiliary heating units. No overt clinical human health effects have been demonstrated for COHb levels below 2%, while levels above 40% cause fatal asphyxiation.

Hazardous Air Pollutants

HAPs (so-called air toxics) represent an inclusive classification for air pollutants of anthropogenic origin that are generally of measurable quantity in the air, The HAPs include organic chemicals like acrolein, benzene, minerals like asbestos, polycyclic hydrocarbon such as benzo(a)pyrene, various metals and metal compounds like mercury and beryllium compounds, and pesticides such as carbaryl and parathion.

THE MULTIPOLLUTANT REALITY OF AIR POLLUTION

Pollutants in the atmosphere of any community vary considerably in space and time, and are charged by the varied output from a wide range of sources, only to be transformed stoichiometrically by a patterned intensity of sunlight. The reductionist approach examining one pollutant at a time has been successful in diminishing pollutants and improving public health. But there are likely chemical and physiologic interactions between and among pollutants that are of public health consequence that have not been appreciated. Parallel efforts within both the scientific and the regulatory/policy communities need to advance methods for evaluating and managing the effects of air pollution in a multipollutant manner.

CONCLUSIONS

The breadth and complexity of the problem of air pollution—from the development of credible databases to supporting regulatory action and decision making—have been the theme throughout. The classic and still most important air pollutants provide a foundation for understanding and appreciating the nuances of the issues and strategies for air pollution control and protection of public health. The key role of the toxicologist is to develop sensitive methods to assay responses to low pollutant concentrations, apply these methods to relevant exposure scenarios and test species, and develop paradigms to relate empirical toxicological data to real life through an understanding of mechanism. Last, the toxicologist must continually integrate laboratory data with those of epidemiology and clinical study to ensure their maximum utility.

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QUESTION

1. Which of the following compounds is NOT an oxidant-type air pollutant?
   a. NO_2.
   b. SO_2.
   c. O_3.
   d. radical hydrocarbons.
   e. aldehydes.

2. Which of the following pollutants contributes most to nontobacco-smoking lung cancer?
   a. asbestos.
   b. vinyl chloride.
   c. benzene.
   d. products of incomplete combustion.
   e. formaldehyde.

3. Inhalants, such as NO_2 and trichloroethylene, can increase proliferation of opportunistic pathogens in the lungs by:
   a. destroying goblet cells in the respiratory tract.
   b. damaging the alveolar septa.
   c. inactivating cilia in the respiratory tract.
   d. killing alveolar macrophages.
   e. dampening the immune system.

4. Which of the following is NOT a characteristic of SO_2 toxicology?
   a. SO_2 is a major reducing-type air pollutant.
   b. Increased airflow rate increases the amount of SO_2 inhaled.
   c. SO_2 inhalation causes vasoconstriction and increased blood pressure.
   d. SO_2 is predominately absorbed in the conducting airways.
   e. SO_2 inhalation increases mucus secretion in humans.

5. Which of the following would be MOST likely to occur on sulfuric acid exposure?
   a. vasoconstriction.
   b. decreased mucus secretion.
   c. an anti-inflammatory response.
   d. vasodilation.
   e. bronchoconstriction.

6. All of the following statements regarding particulate matter are true EXCEPT:
   a. Metals are most commonly released into the environment during coal and oil combustion.
   b. T e interaction of gases and particles in the atmosphere can create a more toxic product than the gas or particle alone.
   c. Solubility does not play a role in the bioavailability of a metal.
   d. T e earth's crust is an important source of atmospheric magnesium.
   e. Diesel exhaust contains reducing- and oxidant-type air pollutants.

7. Which of the following statements is NOT true?
   a. Ozone (O_3) combines with a nitric oxide radical to form NO_2.
   b. O_2 combines with an oxygen radical to form ozone.
   c. O_3 can cause damage to the respiratory tract.
   d. Accumulation of O_3 in the stratosphere is important for protection against UV radiation.
   e. Cl_2 gas is known to cause O_2 degradation.

8. Which of the following is NOT a likely symptom of NO_2 exposure?
   a. increased secretion by Clara cells.
   b. pulmonary edema.
   c. shortness of breath.
   d. loss of ciliated cells in bronchioles.
   e. decreased immune response.

9. Which of the following statements regarding aldehyde exposure is FALSE?
   a. T e major aldehyde pollutants are formaldehyde and acrolein.
   b. Formaldehyde is found in tobacco smoke, but acrolein is not.
   c. Acrolein causes increased pulmonary flow resistance.
   d. Formaldehyde exposure induces bronchoconstriction.
   e. T e water solubility of formaldehyde increases its nasopharyngeal absorption.

10. Carbon monoxide (CO) exerts its toxic ef ects via its interaction with which of the following?
    a. DNA polymerase.
    b. actin.
    c. kinesin.
    d. hemoglobin.
    e. microtubules.

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CHAPTER

30

Ecotoxicology

Richard T. Di Giulio and Michael C. Newman

INTRODUCTION

SOME DISTINCT ASPECTS OF EXPOSURE

TOXICANT EFFECTS

- Molecular and Biochemical Ef ects
- Gene Expression and Ecotoxicogenomics
  - Estrogen Receptor
  - Aryl Hydrocarbon Receptor
  - Genomics and Ecotoxicogenomics
  - Protein Damage
  - Oxidative Stress
  - DNA Damage
- Cellular, Tissue, and Organ Ef ects
  - Cells
  - Target Organs
- Organismal Ef ects
  - Mortality
  - Reproduction and Development
  - Disease Susceptibility
  - Behavior
  - Cancer
- Population
- Community
- Ecosystem to Biosphere

APPROACHES

- Toxicity Tests
- Biomarkers
- Population
- Community and Ecosystem
- Landscape to Biosphere

ECOLOGIC RISK ASSESSMENT

INTERCONNECTIONS BETWEEN ECOSYSTEM INTEGRITY AND HUMAN HEALTH

KEY POINTS

- Ecotoxicology is the study of the fate and effects of toxic substances on an ecosystem.
- Chemodynamics is, in essence, the study of chemical release, distribution, degradation, and fate in the environment.
- A chemical can enter any of the four matrices: the atmosphere by evaporation, the lithosphere by adsorption, the hydrosphere by dissolution, or the biosphere by absorption, inhalation, or ingestion (depending on the species). Once in a matrix, the toxicant can enter another matrix by these methods.
- T e biologic availability (or bioavailability) of a chemical is the portion of the total quantity of chemical present that is potentially available for uptake by organisms.
- Pollution may result in a cascade of events, beginning with effects on homeostasis in individuals and extending through populations, communities, ecosystems, and landscapes.
- Terrestrial toxicology is the science of the exposure to and effects of toxic compounds in terrestrial ecosystems.
- Aquatic toxicology is the study of effects of anthropogenic chemicals on organisms in the aquatic environment.

INTRODUCTION

Ecotoxicology is the study of contaminants in the biosphere and their effects on constituents of the biosphere. It has an overarching goal of explaining and predicting effect or exposure phenomena at several levels of biologic organization (Figure 30–1). Relevant effects to nonhuman targets range from biomolecular to global. As the need to predict major effects to populations, communities, ecosystems, and other higher level entities has become increasingly apparent, more cause–effect models relevant to these higher levels of biologic organization are added to the conventional set of toxicology models applied by pioneering ecotoxicologists. Contaminant chemical form, phase association, and movement among components of the biosphere are also central issues in ecotoxicology because they determine exposure, bioavailability, and realized dose.

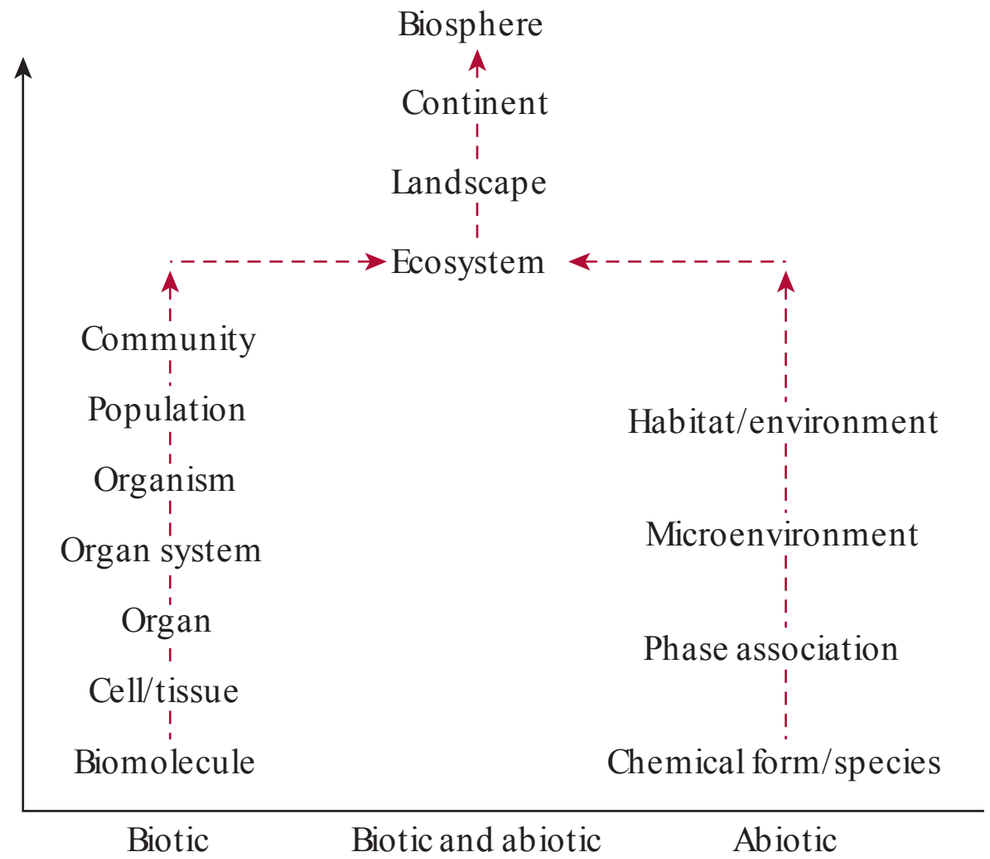

FIGURE 30–1 **Ecologic scales relevant to ecotoxicology.** Solely biologic scales relevant to ecotoxicology range from the molecular to the community levels: solely abiotic scales range from the chemical to the entire habitat. Biotic and abiotic components are usually combined at levels above the ecologic community and habitat. The ecologic community and physicochemical habitat combine to form the ecosystem. Ecologic systems can be considered at the landscape scale, that is, the combination of marine, freshwater, and terrestrial systems at a river's mouth. Recently, the continental and biospheric scales have become relevant as in the cases of ozone depletion, acid precipitation, and global warming.

SOME DISTINCT ASPECTS OF EXPOSURE

Ecotoxicology commonly uses sparse information for a few species to predict effects to many species and their interactions. Relevant exposure routes are the conventional ingestion, inhalation, and dermal absorption. But, unique features of exposure pathways must be accommodated for species that ingest a wide range of materials using distinct feeding mechanisms, breathe gaseous or liquid media using different structures, and come into dermal contact with a variety of gaseous, liquid, and solid media.

Prediction of oral exposure can be limited because species feed on different materials; however, conventional principles about oral bioavailability remain relevant. Many techniques applied to determining human oral bioavailability are available to the ecotoxicologist. As an example, some birds are uniquely at high risk of lead poisoning because they ingest and then use lead shot as grit. T e birds grind shot in their gizzards under acidic conditions, releasing significant amounts of dissolved lead.

Estimation of chemical speciation is central to predicting bioavailability of water-associated contaminants. Speciation can determine the bioavailability of dissolved metals. Movements of nonionic and ionizable organic compounds across the gut or gills are strongly influenced by lipid solubility and pH partitioning, respectively. Consequently, determination of a compound's lipophilicity or calculation of pH- and pK_a-dependent ionization facilitates some predictive capability for bioavailability.

T e free ion activity model (FIAM) states that uptake and toxicity of cationic trace metals are best predicted from their free ion activity or concentration, although exceptions exist.

Bioavailability, bioaccumulation, or exposure concentrations for sediment-associated toxicants are also approached by considering chemical speciation and phase partitioning. Metals in sediments are either incorporated into one of the many solid phases or dissolved in the interstitial waters surrounding the sediment particles. Bioavailable metals have been estimated by normalizing sediment metal concentrations to easily extracted iron and manganese concentrations because solid iron and manganese oxides sequester metals in poorly bioavailable solid forms.

Another issue of importance to the ecotoxicologist is the possibility of biomagnification, the increase in contaminant concentration as it moves through a food web. Biomagnification can result in harmful exposures to species situated high in the food web such as birds of prey.

TOXICANT EFFECTS

One approach to this complex topic of ecotoxicologic effects is to organize effects according to biologic levels of organization. One may consider effects, in ascending order, at the subcellular (molecular and biochemical), cellular, organismal, population, community, and ecosystem levels of organization. Ecotoxicology deals with, theoretically at least, all species, and in line with other aspects of natural resource management, the primary concern is one of sustainability. T e policies and regulations surrounding chemical effects in natural ecosystems are designed to protect ecologic features such as population dynamics, community structures, and ecosystem functions.

Molecular and Biochemical Ef ects

T is lowest level of organization includes fundamental processes associated with the regulation of gene transcription and translation, biotransformation of xenobiotics, and the deleterious biochemical effects of xenobiotics on cellular constituents including proteins, lipids, and DNA.

Gene Expression and Ecotoxicogenomics

Xenobiotics can affect gene transcription through interactions with transcription factors and/or the promoter regions of genes. In the context of environmental toxicology, perhaps the most studied xenobiotic effects involve ligand-activated transcription factors. T ese intracellular receptor proteins recognize and bind specific compounds, thus forming a complex that binds to specific promoter regions of genes, thereby activating transcription of mRNAs, and ultimately translation of the associated protein.

Estrogen Receptor—T e dominant natural ligand for this nuclear receptor is estradiol (E2). Binding of E2 with estrogen receptor (ER) produces a complex that can then bind to estrogen response elements (ERE) of specific genes that contain one or more EREs, thereby causing gene transcription. Genes regulated in this manner by E2–ER play various important roles in sexual organ development, behavior, fertility, and bone integrity.

A number of chemicals can serve as ligands for ER; in most cases these "xenoestrogens" activate gene transcription acting as receptor agonists. Some of these xenoestrogens include diethylstilbestrol (DES), DDT, methoxychlor, endosulfan, surfactants (nonyl-phenol), some PCBs, bisphenol A, and ethinyl E2, a synthetic estrogen observed in municipal effluents and surface waters. Environmental exposures to these chemicals are sufficient to perturb reproduction or development. Moreover, endocrine disruption by environmental xenoestrogens appears to be stronger for wildlife than for humans, likely due to instances of elevated exposures that are less prone to confounding factors than is typically the case for human exposures. Egg-laying vertebrates provide a biomarker of estrogen exposure—vitellogenin production, which is produced in the liver and transferred to the ovary to become a key component of yolk protein. Increased vitellogenin production in males is useful biomarker of estrogenic chemical exposures.

Aryl Hydrocarbon Receptor—T e aryl hydrocarbon receptor (AHR) is a member of the basic helix–loop–helix Per ARNT Sim (bHLH-PAS) family of receptors/transcription factors that is involved in development, as sensors of the internal and external environment in order to maintain homeostasis, and in establishment and maintenance of circadian clocks. Characterized genes that are upregulated by the AHR system code for enzymes involved in the metabolism of lipophilic chemicals, including organic xenobiotics and some endogenous substrates such as steroid hormones. T ese enzymes include mammalian CYP1A1, 1A2, and 1B1 and their counterparts in other vertebrates, glutathione transferase, glucuronosyltransferase, alcohol dehydrogenase, and quinone oxidoreductase.

Some ubiquitous pollutants that act as AHR ligands and markedly upregulate gene transcription via the AHR–ARNT signaling pathway include the polycyclic aromatic hydrocarbons (PAHs) and the polyhalogenated aromatic hydrocarbons (pHAHs). In general, pHAH-type AHR ligands are more potent AHR ligands and enzyme inducers than PAHs.

Ethoxyresorufin O-deethylase (EROD) activity is of en used as a biomarker for AHR-related changes. Elevated activities of hepatic EROD have been associated with exposures to PCBs, dioxins, PAHs, and complex mixtures of these associated with harbor sediments, municipal effluents, paper mill effluents, refinery effluents, and oil spills.

Genomics and Ecotoxicogenomics—Ecotoxicogenomics has great potential for elucidating impacts of chemicals of ecologic concern and ultimately for playing an important role in ecologic risk assessments (ERAs) and regulatory ecotoxicology. Genome sequencing of many species has set the stage for genome-wide analysis of gene expression (transcriptomics), changes in protein production (proteomics), and metabolite

profiles (metabolomics). Appropriate bioinformatic analysis can help reveal biologically meaningful patterns of gene expression af er exposures to various toxicants. Specific areas to which these emerging fields can contribute include prioritization of chemicals investigated in ERAs, identification of modes of action of pollutants, identification of particularly sensitive species, and effect prediction at higher levels of organization.

Protein Damage—Acetylcholinesterase (AChE) degrades the neurotransmitter acetylcholine, and controls nerve transmission in cholinergic nerve tracts. T e widely used organophosphate and carbamate classes of insecticides kill by inhibiting AChE, and this mechanism is operative for "nontarget" organisms including invertebrates, wildlife, and humans. Of particular ecologic concern has been the ingestion of AChE-inhibiting insecticides with food items or granular formulations (mistaken as seed or grit) by birds and aquatic animal exposures from agricultural run-off. Another example is the inhibition of delta-aminolevulinic acid dehydratase af er lead exposure. Studies of this enzyme have been exploited as a biomarker for lead exposure in humans and wildlife. In addition to enzyme inhibition, chemicals can damage proteins in other ways, including oxidative damage and the formation of stable adducts similar to those formed with DNA.

Oxidative Stress—Oxidative stress has been defined as the point at which production of ROS exceeds the capacity of antioxidants to prevent damage. Numerous environmental contaminants act as prooxidants and enhance production of ROS. T e resulting oxidative damage can account wholly or partially for toxicity. Mechanisms by which chemicals enhance ROS production include redox cycling, interactions with electron transport chains (notably in mitochondria, microsomes, or chloroplasts), and photosensitization. Redox cycling chemicals include diphenols and quinones, nitroaromatics and azo compounds, aromatic hydroxylamines, paraquat, and certain metal chelates, particularly of copper and iron.

Photosensitization is an important mechanism in aquatic systems. Ultraviolet (UV) radiation (specifically UV-B and UV-A) can penetrate surface waters to varying depths, depending on the wavelength of the radiation and the clarity of the water. T e UV radiation generates ROS and other free radicals via excitation of photosensitizing chemicals, including common pollutants of aquatic systems.

ROS can drive redox status to a more oxidized state, potentially reducing cell viability. T ese ROS-mediated impacts and others have been associated with several human diseases including atherosclerosis, arthritis, cancer, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. With the exception of cancer, the role of ROS in specific diseases in wildlife has received little attention. It is reasonable to assume that oxidative stress accounts in part for the toxicity of diverse pollutants to free-living organisms.

DNA Damage—T e importance of DNA as a molecular target was discussed in Chapter 9, and the most important human health issue is associated with cancer. Cancer is an important health outcome associated with chemical exposures in wildlife, particularly for bottom-dwelling fishes. In the context of ecotoxicology, the most widely studied form of damage has been the formation of stable DNA adducts, DNA strand breaks, and oxidized DNA bases. Adduct formation is particularly common with exposure to PAHs. PAHs must be activated to reactive metabolites to form these adducts.

Cellular, Tissue, and Organ Ef ects

Ce lls—Most free-living organisms routinely experience energy deficits. For example, food resources are of en scarce during the winter for many animals, which adapt by conserving energy (by hibernating or lowering metabolism) or by storing energy beforehand (as is the case for many migratory birds). T us, the effects of pollutants on mitochondrial energy metabolism can be of particular importance to wildlife.

Lysosomes, which are involved in the degradation of damaged organelles and proteins, sequester many environmental contaminants, including metals, PAHs, and nanoparticles. T e accumulation of xenobiotics by lysosomes can elicit membrane damage, which warns of pathologic effects in both invertebrates and vertebrates.

Chemical effects on nuclei have been examined in ecologic contexts. Micronuclei are chromosomal fragments that are not incorporated into the nucleus at cell division, and chemical exposures can markedly increase their frequency. Elevated micronuclei numbers have been observed in erythrocytes in fish and in hemocytes in clams from a PCB-polluted harbor.

Target Organs—An important target organ in ecotoxicology of nonmammalian aquatic vertebrates and many invertebrates is the gill, which is the major site of gas exchange, ionic regulation, acid–base balance, and nitrogenous waste excretion. Gills are immersed in a major exposure medium for these animals (surface water), so metabolically active epithelial cells are in direct contact with this medium. T ey also receive blood supply directly from the heart. Common structural lesions in gills include cell death (via necrosis and apoptosis), rupture of the epithelium, hyperplasia, and hypotrophy of various cell populations that can lead to lamellar fusion, epithelial swelling, and lif ing of the respiratory epithelium from the underlying tissue. Chloride cells have a major role in ionic homeostasis, and they can be compromised af er exposure to metals, such as cadmium, copper, lead, silver and zinc. In some cases, this may be due to inhibition of ATPases and/or increased membrane permeability.

Organismal Ef ects

Mortality—Chemical pollution of the environment does not generally attain levels sufficient to outrightly kill wildlife. T e ecotoxicologic concerns are the long-term, chronic impacts of chemicals on organismal variables such as reproduction and development, behavior, and disease susceptibility, and how such impacts parlay into effects at the population and higher

levels of organization. However, mortality is an end point in exposure studies.

Reproduction and Development—Contaminant effects on development are of en difficult to discern in field studies, due to the small size of embryos and the fact that developmental impacts are generally either lethal or greatly reduce survival. Because early life stages of most organisms are generally more sensitive to xenobiotics than other life stages, developmental impacts merit careful attention by ecotoxicologists.

Chlorinated hydrocarbons continue to generate concerns although many (DDT and other insecticides, and PCBs) have had their production and use sharply curtailed. T e dioxins (TCDD) and coplanar PCBs compromise cardiac development, among other effects in vertebrates, and these developmental perturbations are largely receptor-mediated and dependent on binding of the chemical (such as TCDD) with the AHR.

Hydrocarbons, in large part PAHs, associated with oil spills, contaminated sediments, paper mill effluents, and creosote used for wood treatment have profound developmental effects in fish embryos. In many cases, the effects observed visually appear similar to those observed in fish embryos exposed to dioxins and coplanar PCBs, and include malformed hearts ("tube hearts"), craniofacial deformities, hemorrhaging, and edema of the pericardium and yolk sac, the latter resulting in a distended, faintly blue yolk sac that gives this syndrome the name "blue sac disease."

Disease Susceptibility—T e potential impacts of environmental contaminants on immune systems that render organisms more susceptible to disease are of great concern. Numerous laboratory studies have demonstrated chemical impacts on immune systems in animals of ecologic relevance. T ese include pesticides in amphibians, PCBs in channel catfish, heavy metals in rainbow trout, PAHs in bivalves, and flame retardants (polybrominated diphenyl ethers) in American kestrels. T e potential effects of chemicals on immune function and disease susceptibility in wildlife is an important problem in ecotoxicology and future work with powerful genomic tools will help make significant advances in our understanding.

Behavior—Relatively subtle effects on behaviors associated with mating and reproduction, foraging, predator–prey interactions, preference/avoidance of contaminated areas, and migration have potentially important ramifications for population dynamics. In some cases, the biochemical mechanisms underlying behavioral effects have been elucidated, which may assist our understanding of these issues and provide useful biomarkers for behavioral toxicants in field studies.

Chemicals causing behavioral effects in wildlife are known to be neurotoxicants. Behavioral effects of insecticides have been observed in fish. For example, impacts of the organophosphate diazinon on olfactory-mediated behaviors such as the alarm response and homing in the Chinook salmon have been observed, as well as similar thresholds for the effects of another organophosphate (chlorpyrifos) on swimming and feeding behaviors and on AChE inhibition in coho salmon (Oncorhynchus kisutch). Mercury, particularly as methylmercury, comprises another potent neurotoxin that has been shown to perturb behavior in wildlife.

Environmental contaminants not generally thought of as neurotoxicants have also been shown to perturb behavior. For example, cadmium and copper have been shown to impact olfactory neurons and associated behaviors (preference/avoidance to chemicals, including pheromones) in several fish species. Copper exposure in zebrafish also led to loss of neurons in the peripheral mechanosensory system ("lateral line"), which could lead to altered behaviors associated with schooling, predator avoidance, and rheotaxis (physical alignment of fish in a current). Clearly, numerous mechanisms of chemical toxicity can result in behavioral impacts, including direct toxicity to neurons, alterations in hormones that modulate behaviors, and impaired energy metabolism. Also, impaired behavior may comprise a sublethal impact with substantive ecologic consequence.

Cancer—Beginning in the 1960s, numerous cases of cancer epizootics in wildlife that are associated with chemical pollution, particularly in specific fish populations, have been reported in North America and northern Europe. As in humans, cancer in these animals occurs largely in relatively older age classes and therefore is of entimes considered a disease unlikely to directly impact population dynamics or other ecologic parameters. However, this may not always be the case, particularly in species that require many years to attain sexual maturity and/or have low reproductive rates.

Lifestyle is a major contributor to differential cancer susceptibility; benthic (bottom-dwelling) species such as brown bullhead (Ameiurus nebulosus) and white sucker (Catostomus commersoni) in freshwater systems, and English sole (Parophrys vetulus) and winter flounder (Pseudopleuronectes americanus) in marine systems generally exhibit the highest cancer rates in polluted systems. T e bulk of chemicals in these systems associated with cancer epizootics, such as PAHs, PCBs, and other halogenated compounds, reside in sediments; benthic fish live in contact with these sediments and prey in large measure on other benthic organisms. T e molecular and biochemical pathways underlying chemical carcinogenesis, such as PAH metabolism, DNA damage, and effects on oncogenes are qualitatively similar between most fish and mammalian species examined.

It is noteworthy that many reports of elevated cancer rates in free-living animals occur in fish, with few reports of potentially chemically related cancers to our knowledge in other vertebrates. It is likely that elevated exposures play an important role in the relatively high frequency of reports of cancers in benthic fish; relative inherent sensitivities among mammals, birds, reptiles, and amphibians, and fish are unclear.

Population

A population is a collection of individuals of the same species that occupy the same space and within which genetic information can be exchanged. Population ecotoxicology covers a wide

TABLE 30–1 A summary of one popular set of rules of thumb for assessing plausibility of a causal association in an ecologic epidemiology.

Rule	Description
1. Strength of association	How strong the association is between the possible cause and the effect, e.g., a very large relative risk
2. Consistency of association	How consistently is there an association between the possible cause and the effect, e.g., consistent among several studies with different circumstances
3. Predictive performance	How good is the prediction of effect made from the presence/level of the possible cause
4. Monotonic trend	How consistent is the association between possible cause and effect to a monotonic trend (i.e., either a consistent increase or decrease in effect level/prevalence with an increase in exposure)
5. Inconsistent temporal sequence	The effect, or elevated level of effect, occurs before exposure to the hypothesized cause
6. Factual implausibility	The hypothesized association is implausible given existing knowledge
7. Inconsistency with replication	Very poor reproducibility of association during repeated field assessments encompassing different circumstances or repeated formal laboratory testing

range of topics with core research themes being (1) epidemiology of chemical-related diseases, (2) effects on general population qualities including demographics and persistence, and (3) population genetics.

The level of belief warranted for possible contaminant-related effects in nonhuman populations is assessed by applying routine epidemiologic methods. Rules of thumb for gauging the level of belief warranted by evidence that emerged from human epidemiology are also applied in population ecotoxicology (Table 30–1).

Defining and predicting alterations in population size, dynamics, and demographic composition owing to toxicant exposure are important. Some species populations fluctuate within a range of densities. These fluctuations are characteristic of the species' strategy for maintaining itself in various types of habitats and toxicant exposure could potentially change this range. Combined with decreases in population densities driven by external forces such as weather events, these toxicant-induced modifications of the average population densities and dynamics can increase the risk of a population's density falling so low that local extinction occurs. Toxicants can change a species population's vital rates, such as age- and sex-dependent death, birth, maturation, and migration rates. Combined, these changes determine the population density and distribution of individuals among ages and sexes during exposure.

Individuals of the same species often are grouped into subpopulations within a habitat and all of these subpopulations together comprise a metapopulation (Figure 30–2). Subpopulations in the metapopulation have different levels of exchange and different vital rates that depend on the nature of their habitat. Spatial distances and obstacles or corridors for migration influence migration among patches; habitat quality determines vital rates.

The genetics of exposed populations are studied to understand changes in tolerance to toxicants and to document toxicant influence on field populations. Some populations have the capacity to become more tolerant of toxicants via selection. Genetic qualities are also used to infer past toxicant influence in an exposed population. Another piece of evidence demonstrating past toxicant influence on populations can be a change in genetic diversity. A drop in genetic diversity in populations is thought to be an adverse effect because genetic diversity is required in populations to evolutionarily adapt to environmental changes. Toxicants can influence genetic diversity by purely stochastic means.

Community

An ecologic community is an assemblage of populations occupying a defined habitat at a particular time. Populations

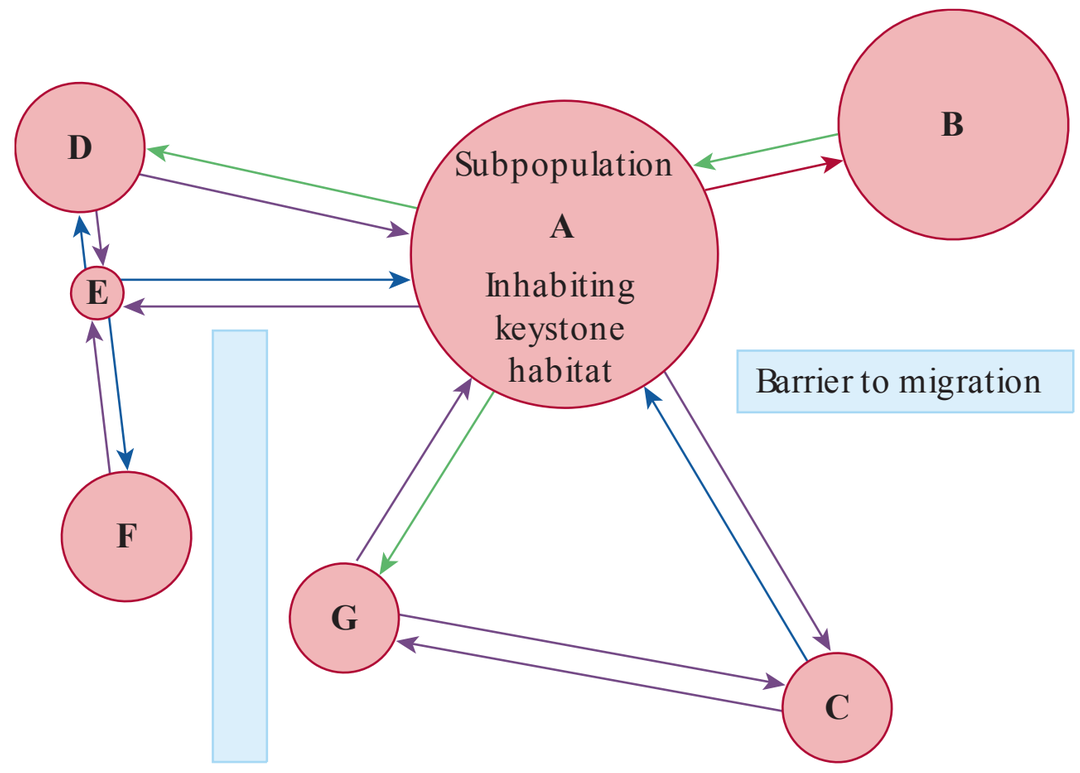

FIGURE 30–2 **Metapopulations are composed of subpopulations that differ in their vital rates and tendency to exchange individuals.** In this illustration, subpopulation A occupies a keystone habitat. The loss of subpopulation A would devastate the metapopulation. Also, loss of the migration corridor between subpopulations A, B, and D would devastate the metapopulation. In contrast, the loss of subpopulation F would not influence the metapopulation to the same degree.

in a community interact in many ways and, because these many interactions are complex, a community has properties that are not predictable from those of its component populations. Some species play a crucial role (keystone species) or numerical dominance (dominants) and these are essential to maintaining community structure, which refers to the number of species present and the numbers of individuals present in each of these species. Community may also refer to the distribution of species among different functional groups such as decomposers, detritivores, primary producers, primary consumers (herbivores), and secondary consumers (carnivores that consume herbivores).

Communities take on characteristic structures as predicted by the law of frequencies: the number of individual organisms in a community is related by some function to the number of species in the community. Ecotoxicants can alter the resulting community structure in predictable ways by either directly impacting the fitness of individuals in populations that make up the community or by altering population interactions.

Recently, structural and functional qualities in communities have been combined to generate multimetric indices such as the Index of Biotic Integrity (IBI). Ecologic insight is used to select and then numerically combine community qualities such as species richness, health of individual animals in a sample, and the number of individuals in a sample belonging to a particular functional group, such as number of piscivorous fish. T e IBI score for a study site is calculated and compared with that expected for an unimpacted site in order to estimate its biologic integrity.

Another central theme in community ecotoxicology is toxicant transfer during trophic interactions. Toxicant concentrations can decrease (biodiminution), remain constant, or increase (biomagnification) with each trophic transfer within a food web. Metals that biomagnify are mercury and the alkali metals, cesium, and rubidium. Zinc, an essential metal that is actively regulated in individuals, can exhibit biomagnification or biominification depending on whether ambient levels are below or above those required by the organism to function properly.

Most individuals in a community can feed on different species depending on their life stage, seasons, and relative abundances of prey species. T ese trophic interactions are best described as occurring in a trophic web, not a trophic chain.

Ecosystem to Biosphere

Ecosystems, the functional unit of ecology, are composed of the ecologic community and its abiotic habitat. T e ecotoxicologist is interested in understanding how toxicants diminish an ecosystem's capacity to perform essential functions and to understand toxicant movement within different ecosystem components enough to assess exposure.

Conventional ecosystem studies involve descriptions of contaminant concentrations and movements in easily defined ecosystems such as lakes, forests, or fields. Some toxicants, especially those subject to wide dispersal by air or water, cannot be completely understood in this framework, so a landscape scale might be chosen instead. As an example, acid precipitation might be examined in the context of an entire watershed, mountain range, or even a continental region. Still other ecotoxicants require a global context in order to fully understand their movements and accumulation. As an example, hexachlorobenzene concentration in tree bark collected worldwide showed a clear latitudinal gradient.

APPROACHES

Toxicity Tests

Toxicity testing encompassing representative animals and plants at different levels of organization offers a practical approach to characterize chemical effects on biologic systems. Toxicity tests address the potential direct effects of toxic substances on individual ecosystem components in a controlled and reproducible manner. Ecotoxicology tests feature a wide variety of aquatic (including algae, invertebrates, tadpoles, bivalves, shrimp, and fish), avian (quail and duck), and terrestrial (soil microorganisms, crops, honey bees, earthworms, and wild mammals) species. Species are selected based on their traditional use as laboratory animals, but also on ecologic relevance, which further complicates global harmonization of ecologic testing. In addition, testing of aquatic species requires monitoring of water quality, investigation of the solubility and stability of the test substance under the conditions of testing, and determination of nominal versus measured concentrations. Testing can be conducted in aqueous systems without renewal of test substance (static), renewal at predetermined time intervals (static renewal), or continuous flow of test substance through the test compartment (flow-through).

In acute toxicity testing, single species are exposed to various concentrations of the test agent. T e most common end point in acute tests is death. Abnormal behavior and other gross observations are commonly noted, and nonlethal end points occasionally apply. Data from different test concentrations are used to derive concentration–response curves. T e LC_{50} represents the concentration of test substance killing 50% of the tested animals and EC_{50} the concentration of test substance affecting 50% of the test population during a specified period of time, such as growth; the IC_{50} is the concentration causing a 50% reduction in a nonquantal measurement (such as movement) for the test population. Other quantitative values are the lowest observed effect concentration (LOEC), that is, the lowest concentration where an effect is observed, and the no observed effect concentration (NOEC), the highest concentration resulting in no adverse effects.

Short-term laboratory studies conducted with single species are useful for rapid screening, provide information on thresholds for effects, and selective and comparative toxicity, and can be used as range finders to guide subsequent, of en more involved, studies. Long-term and reproductive studies evaluate the effects of substances on organisms over extended periods

of time and/or sequential generations (chronic toxicity, life cycle, and reproduction).

Unique to ecotoxicology are the more elaborate microcosm, mesocosm, and field studies. Microcosms are representative aquatic or terrestrial ecosystems created under laboratory conditions that include a number of relevant species (such as protozoa, plankton, algae, plants, and invertebrates). Simulated field studies or mesocosms can be created in the laboratory or in the field (e.g., artificial streams and ponds) or consist of enclosures of existing habitats, containing representative soil, water, and biota. Lastly, full-scale field studies (aquatic organisms, terrestrial wildlife, and pollinators) evaluate the effects of a substance on wildlife under real-life scenarios of actual use conditions of a product (e.g., pesticide field usage rate), and thus are more complicated, subject to considerable variability, and require extensive knowledge of the local population and community dynamics.

As a final point, plant studies are a significant component of ecologic toxicity testing, particularly for pesticide registration, and involve tiered testing of both target area and nontarget terrestrial and aquatic plants. End points of phytotoxicity include seedling emergence and growth, vegetative vigor, etc. Central to the toxicity testing with plants are the substrate and environmental conditions, which greatly influence plant health.

Biomarkers

T e term "biomarker" is most of en employed to refer to molecular, physiologic, and organismal responses to contaminant exposure that can be quantified in organisms inhabiting or captured from natural systems. Biomarkers do not directly provide information concerning impacts on the higher levels of organization that ecotoxicology ultimately endeavors to discern. Nevertheless, biomarkers of en provide important ancillary tools for discerning contaminant exposures and potential impacts of ecologic importance. Biomarkers can provide sensitive early warning signals of incipient ecologic damage.

Chemical specificity among biomarkers is also highly variable and is imbued with trade-offs. Nonspecific biomarkers may be preferred if complex mixtures are being studied. T e larger the number of biomarkers, the more expensive and time-intensive the study. Effects of environmental variables such as temperature, time of day or year, salinity, and dissolved oxygen and physiologic variables including sex, age, reproductive status, and nutritional status needed to be accounted for and controlled. Many biomarkers are invasive and require sacrifice of the organism in order to obtain needed tissues. T is can be problematic, particularly in cases involving rare species or charismatic species such as marine mammals. In such cases, and in others where feasible, the use of noninvasive biomarkers is either preferred or required. Biomarkers can provide powerful tools as early warning signals of ecologic damage, to assist in assessments of environmental contamination, and in determining the effectiveness of various environmental management decisions such as cleanups. However, careful case-specific thought is required for the selection of biomarkers.

Population

Demographic surveys or experiments can be conducted for exposed populations. Some studies explore age-specific vital rates but others are designed to explore vital rates for different ages such as nestling, fledgling, juvenile, and adult. Most result in data sets that can be analyzed profitably using either a simple life table or more involved matrix analysis. T e matrix method allows one to describe the population state and also to understand the sensitivity of the population to effects on vital rates for various ages or stages. T e value of such studies lies in the ability to integrate effects on several factors into a projection of population consequences. Demographic studies are becoming more common in ecotoxicology, especially with species amenable to laboratory manipulation.

Conventional studies of increased tolerance af er generations of exposure and molecular genetic surveys of exposed populations are the primary approaches by which genetic consequences are assessed. Increased tolerance is usually detected by subjecting individuals from the chronically exposed population and a naive population to toxicant challenge and formally testing for tolerance differences. Alternatively, a change associated with a tolerance mechanism might be examined in chronically exposed and naive populations. Close examinations of population genetics associated with contaminated habitats are also used to infer consequences of multigenerational exposure.

Community and Ecosystem

Most studies of community and ecosystem effects use modified methods developed in community and systems ecology. T e approach affording the most control and ability to replicate treatments involves laboratory microcosms. A microcosm is a simplified system that is thought to possess the community or ecosystem qualities of interest. T e experimental control and reproducibility associated with microcosms come at the cost of losing ecologic realism. Gaining back some realism by giving up some degree of tractability, outdoor mesocosms are also applied to community and ecosystem ecotoxicology. Mesocosms are larger experimental systems, usually constructed outdoors that also attempt to simulate some aspect of an ecosystem such as community species composition. Terrestrial ecotoxicologists apply the term enclosure instead of mesocosm for such experimental units. Terrestrial mesocosms can be pens, enclosures, or large soil plots depending on the effects being quantified. Field studies are the third means of exploring effects at the community or ecosystem level. T e high realism of associated findings from field studies is balanced against the difficulty of achieving true replication and sufficient control of other factors influencing the system's response. Field studies can involve manipulations such as introducing toxicant into replicate water bodies; however, the majority of field studies involve biomonitoring of an existing, notionally impacted,

community or ecosystem. Because mesocosm and field studies involve data generation in the presence of many uncontrolled variables and poor replication or pseudoreplication, appropriate multivariate statistical techniques for recognizing patterns among locations or through time are required.

Landscape to Biosphere

Technologies for acquiring, processing, and analyzing large amounts of information have been essential. Archived and new imagery from satellites and high-altitude platforms are now integrated with off-the-shelf geographic information systems (GIS) sof ware with affordable computers. Much of this imagery is gathered with remote sensing technology, and arrays of sensors are being assembled to enable real-time input. Remote sensing data from satellites or aircraf provide information for wide spatial areas and the rapidly emerging, ground- or water-based observing system networks have begun to produce extremely rich data streams.

ECOLOGIC RISK ASSESSMENT

ERA applies ecotoxicologic knowledge to support environmental decision making (Figure 30–3). A widely dispersed ecotoxicant such as acid precipitation or widely used product such as a herbicide might require assessment of risk at a landscape or subcontinental scale. Ecotoxicants requiring a global ERA might include greenhouse gases contributing to global warming, hydrofluorocarbons depleting the ozone layer, and persistent organic pollutants that accumulate to harmful concentrations in polar regions far from their release point at industrialized latitudes.

Adaptations are based on the context of an ERA. Some ERAs address existing situations. Considerable field information might be available for such a retroactive ERA and epidemiologic methods might be applied advantageously. In contrast, predictive ERAs assess possible risk associated with a future or proposed toxicant exposure.

Exposure characterization describes or predicts contact between the toxicant and the assessment end point. Depending on the ERA context, this could involve a simple calculation of average exposure, or a temporally and spatially explicit description of amounts present in relevant media. Toxicant sources, transport pathways, kinds of contact, and potential costressors are also defined.

Ecologic effect characterization describes the qualities of any potential effects of concern, the connection between the potential effects and the assessment end point, and how changes in the level of exposure might influence the effects manifesting in the assessment end point. Normally, a statement about the strength of evidence associated with the descriptions and uncertainties is presented in the ecologic effect characterization.

Risk characterization uses the analysis of exposure and ecologic effects to address questions of risk. Although it is desirable to have an explicit statement of risk, that is, the probability of a specified intensity of an adverse effect occurring to the assessment end point, generally only a qualitative likelihood is expressed. Nevertheless, the risk characterization must provide details surrounding the statement, including important uncertainties.

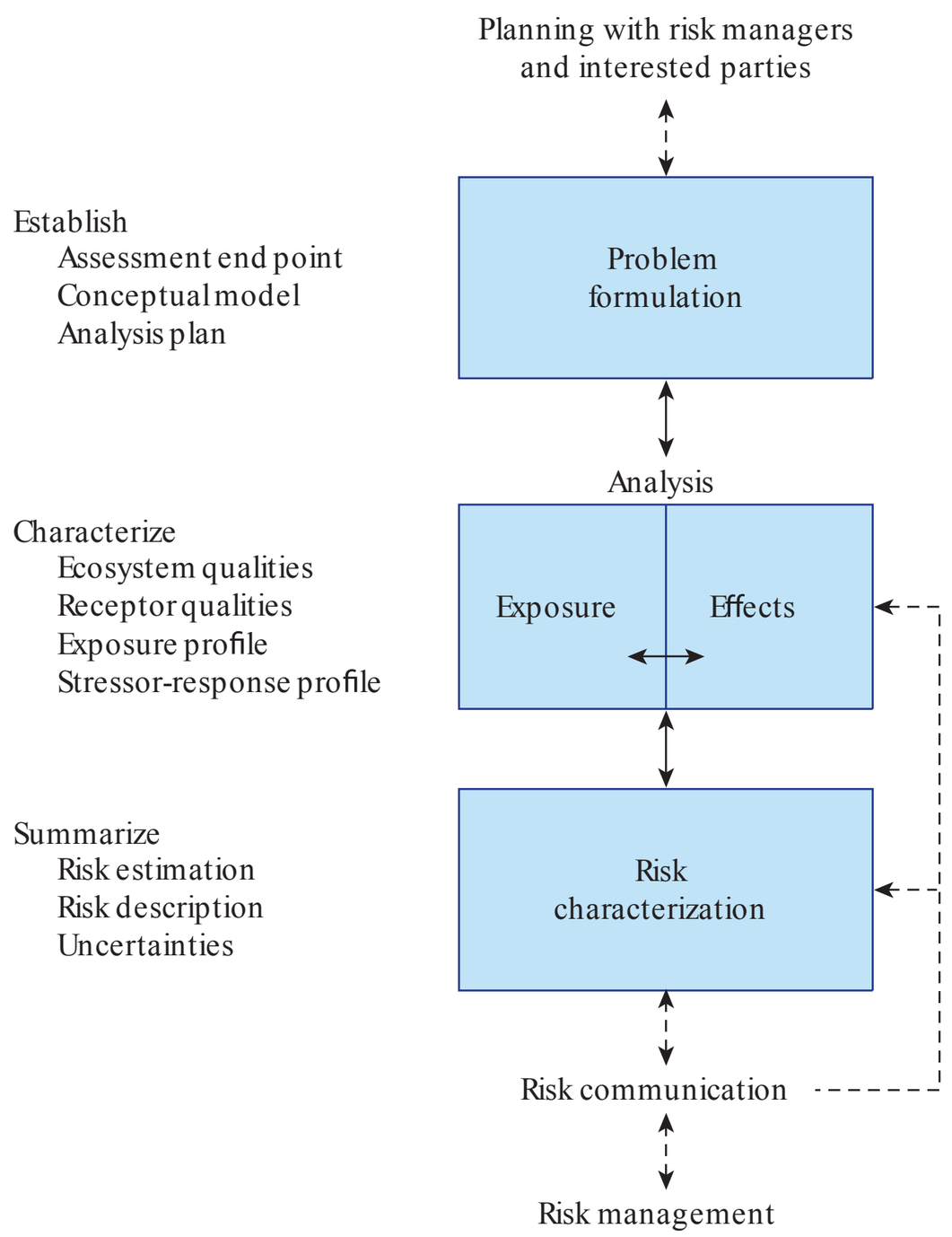

FIGURE 30–3 **The general form of an ecologic risk assessment including problem formulation, analysis, and risk characterization stages.** Problem formulation is done in dialog with risk managers and stakeholders, and involves a clear statement of the ecologic entity to be assessed, a conceptual model for the process, and a plan for conducting the assessment. The analysis stage involves exposure and effect characterizations. Using the context developed during problem formulation and information organized together in the analysis stage, a statement of risk and associated uncertainties are made in the risk characterization stage.

INTERCONNECTIONS BETWEEN ECOSYSTEM INTEGRITY AND HUMAN HEALTH

It is important to consider interconnections between human health and ecologic integrity, or health. By determining how chemicals and other anthropogenic stressors degrade ecosystems and impact human health and well-being, and vice versa, a holistical understanding of the results of environmental contamination is obtained. For example, a conceptual model attempts to elucidate the interconnections linking natural and

social systems in a circular manner with continuous feedbacks. T e natural system produces both positive outputs (such as natural resources and raw materials) and negative outputs (such as hurricanes and disease vectors) to the social system. T e culture and institution of the social system in turn transform the natural system outputs in various ways and subsequently deliver various positive outputs (consumer goods and conservation efforts) and negative outputs (pollution and deforestation) to the natural system. T ese outputs influence the quantity and quality of life (human and nonhuman) of the natural system, and the circular flow of resources continually creates conditions that influence the well-being of individuals, societies, and ecosystems.

T is rather abstract model formalizes the interconnections between human and ecologic health that most of us intuitively sense. Some of these connections are obvious. Chemical contamination of seafoods valued by humans is one example. Others are less clear but potentially very significant, such as human impacts on aquatic systems that foster the propagation of human disease vectors, or human impacts on global climate that may concomitantly impact humans and ecosystems in varied and complex ways. Collaboration among biomedical, environmental, and social scientists and policymakers will catalyze the integrated protection of human and ecosystem health.

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Q U E S T I O N S

1. What is the mode by which a chemical enters the lithosphere?
   a. evaporation.
   b. adsorption.
   c. dissolution.
   d. absorption.
   e. diffusion.

2. T e bioavailability of contaminants in the hydrosphere is directly related to:
   a. chemical concentration.
   b. amount of chemical.
   c. water solubility of chemical.
   d. toxicity of chemical.
   e. molecular size of chemical.

3. All of the following regarding biomarkers are true EXCEPT:
   a. Dermal absorption is considered an external dose.
   b. Biomarkers of susceptibility are useful in extrapolating wildlife disease to human diseases.
   c. Induction of certain enzymes is an important biomarker.
   d. T e biologically effective dose is the amount of internal dose needed to elicit a certain response.
   e. T e effects of chemical exposure can be different across species.

4. Which of the following processes is LEAST likely to be affected by endocrine-disrupting agents?
   a. enzyme activity.
   b. transcription.
   c. hormone secretion.
   d. signal transduction.
   e. DNA replication.

5. Estrogen exposure has been shown to cause all of the following in wildlife species EXCEPT:
   a. sexual imprinting.
   b. altered sex hormone levels.
   c. immune suppression.
   d. gonadal malformations.
   e. sex reversal.

6. Which of the following is FALSE regarding terrestrial ecotoxicology?
   a. Terrestrial organisms are generally exposed to contaminants via ingestion.
   b. Predation is an important confounder of measurements in terrestrial toxicology field studies.
   c. Reproductive tests are not important in measuring end points in toxicity tests.
   d. Enclosure studies are better able to control for environmental factors in field studies.
   e. Toxicity tests usually test the effects of an oral chemical dose.

7. An important type(s) of compound that is far more toxic in water than in air is/are:
   a. organic compounds.
   b. photochemicals.
   c. vapors.
   d. lipid-soluble xenobiotics.
   e. metals.

8. Which of the following are used to record end point toxicity of aquatic toxicity tests?
   a. LD_{50} and ED_{50}.
   b. LC_{50} and EC_{50}.
   c. reproductive tests.
   d. LD_{50} and LC_{50}.
   e. LD_{50} and EC_{50}.

9. Biologic availability is:
   a. the total amount of chemical within an organism.
   b. the concentration of chemical in an environmental reservoir.
   c. the threshold concentration of a chemical needed for toxic effect.
   d. the concentration of chemical within an organism.
   e. the proportion of chemical potentially available for uptake.

10. Chemodynamics does NOT study:
    a. the fate of chemicals in the environment.
    b. the rate at which chemicals are metabolized.
    c. the distribution of chemicals in the environment.
    d. the effects of toxic substances on the environment.
    e. the release of chemicals into the environment.

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CHAPTER

31

Food Toxicology

Frank N. Kotsonis and George A. Burdock

UNIQUENESS OF FOOD TOXICOLOGY

Nature and Complexity of Food
Importance of the Gastrointestinal Tract

SAFETY STANDARDS FOR FOODS, FOOD INGREDIENTS, AND CONTAMINANTS

The Food, Drug, and Cosmetic Act
Methods Used to Evaluate the Safety of Foods, Ingredients, and Contaminants
Safety Evaluation of Direct Food and Color Additives
Exposure: The Estimated Daily Intake
Assignment of Concern Level (CL) and Required Testing
Safety Determination of Indirect Food Additives
Safety Requirements for GRAS Substances
Establishing Safe Conditions of Use for New Foods, Macroingredients and New Technologies
Transgenic Plant (and New Plant Varieties) Policy
Nanotechnology
Safety Requirements for Dietary Supplements
Assessment of Carcinogens
Carcinogenicity as a Special Problem

ADVERSE REACTIONS TO FOOD OR FOOD INGREDIENTS

TOXIC SUBSTANCES IN FOOD

Metals, Hydrocarbons, N-Nitroso Substances and Mycotoxins
Toxins in Fish, Shellfish, and Turtles
Microbiologic Agents
Bovine Spongiform Encephalopathy

CONCLUSION

KEY POINTS

- Food is an exceedingly complex mixture of nutrient and nonnutrient substances.
- A substance listed as Generally Recognized as Safe (GRAS) achieves this determination on the adequacy of safety, as shown through scientific procedures or through experience based on common use.
- An estimated daily intake (EDI) is based on two factors: the daily intake of the food in which the substance will be used and the concentration of the substance in that food.
- Food hypersensitivity (allergy) refers to a reaction involving an immune-mediated response, including cutaneous reactions, systemic effects, and even anaphylaxis.
- The vast majority of food-borne illnesses in developed countries are attributable to microbiologic contamination of food.

UNIQUENESS OF FOOD TOXICOLOGY

The nature of food is responsible for the uniqueness of food toxicology. Food contains hundreds of thousands of substances that have not been fully characterized or tested. Food cannot be commercially produced in a definable environment under strict quality controls and thus cannot meet the rigorous standards of chemical identity, purity, and good manufacturing practice met by most consumer products. The fact that food is harvested from the soil, the sea, or inland waters or is derived from land animals subject to the unpredictable forces of nature makes the constancy of raw food unreliable. Food is

more complex and variable in composition than all other substances to which humans are exposed, and humans are exposed more to food than to any other chemicals!

Nature and Complexity of Food

Food is an exceedingly complex mixture whether it is consumed in the "natural" (unprocessed) form or as a highly processed "Meal Ready to Eat" (MRE). Nonnutrient substances (substances other than carbohydrates, proteins, fats, or vitamins/minerals) may be contributed by food processing, but nature provides the vast majority of nonnutrient constituents. Table 31–1 indicates that natural, or minimally processed, foods contain far more nonnutrient than nutrient constituents.

Nonnutrient substances include plant hormones and naturally occurring pesticides, antinutrients such as lectins, saponins, trypsin, and/or chymotrypsin inhibitors in soybeans, phytates that may bind minerals, antithiamines, and frankly toxic constituents such as tomatine or cycasin. Some 7800 volatile chemicals have been identified in food. Moreover, nonnutrient substances may be classified as food additives. Approximately 200 flavoring ingredients, most already in food naturally, may be added to food, often in concentrations similar to that which is found naturally.

Importance of the Gastrointestinal Tract

T e gut is a large, complex, and dynamic organ with vast absorptive surface. T e GI transit time provides for adequate exposure of ingesta to a variety of processing conditions including, but not limited to, variable pH, digestive acids and enzymes (trypsin, chymotrypsin, etc., from the pancreas and carbohydrases, lipases, and proteases from the enterocytes), saponification agents (in bile), and a luxuriant bacterial flora (estimated to be 100 trillion organisms in adults). In addition, enterocytes possess an extensive capacity for the metabolism of xenobiotics that may be second only to the liver, with a full complement of phase (type) I and phase (type) II reactions present.

TABLE 31–1 Nonnutrient substances in food.

Food	Number of Identified Nonnutrient Chemicals
Cheddar cheese	160
Orange juice	250
Banana	325
Tomato	350
Wine	475
Coffee	625
Beef (cooked)	625

Reproduced with permission from Smith RL: Does one man's meat become another man's poison? Trans Med Soc Lond 1991-1992;108:6–17.

TABLE 31–2 Systems transporting enteric constituents.

System	Enteric Constituent
Passive diffusion	Water, chloride, fats (as micelles), short- and medium-chain fatty acids
Facilitated diffusion	Fructose, D-xylose, 6-deoxy-1,5-anhydro-D-glucitol, glutamic acid, aspartic acid, short-chain fatty acids, glucose, galactose, xenobiotics with carboxy groups, sulfates, glucuronide esters, lead, cadmium, zinc
Active transport	Cations, anions, sugars, vitamins, nucleosides (pyrimidines, uracil, and thymine, which may be in competition with 5-fluorouracil and 5-bromouracil), cobalt, manganese (which competes for the iron transportation system)
Pinocytosis	Long-chain lipids, vitamin B_{12} complex, azo dyes, maternal antibodies, botulinum toxin, hemagglutinins, phalloidins, E coli endotoxins, virus particles

T e constituents of food and other ingesta (e.g., drugs, contaminants, and inhaled pollutants dissolved in saliva and swallowed) are physicochemically heterogeneous, and the primary mechanisms for intestinal absorption are passive or simple diffusion, active transport, facilitated diffusion, and pinocytosis. Each mechanism characteristically transfers a defined group of constituents from the lumen into the body (Table 31–2).

Apart from its duties of absorption and metabolism, the GI tract is also the largest immunologic organ in the body and is constantly exposed to a large number of antigens in food (approximately 88 kg of protein annually) and commensal and ingested bacteria. One cell layer away from these antigens is the lamina propria of the GI tract, which contains the mucosal-associated lymphoid tissue, composed of lymphocytes and antigen-presenting cells, as well as unique dendritic cells, which interact with dietary antigens and ultimately determine whether an antigen is tolerated or an immune response is launched.

SAFETY STANDARDS FOR FOODS, FOOD INGREDIENTS, AND CONTAMINANTS

The Food, Drug, and Cosmetic Act

T e Food, Drug, and Cosmetic (FD&C) Act presumes that traditionally consumed foods are safe if they are free of contaminants. To ban such foods, the FDA must have clear evidence that death or illness can be traced to the consumption of a particular food. T e FD&C Act permits the addition of substances to food to accomplish a specific technical effect if the substance is determined to be Generally Recognized as Safe (GRAS). T e act requires that scientific experts base a GRAS determination on the adequacy of safety, as shown through

scientific procedures or through experience based on common use. If a food contains an unavoidable contaminant even with the use of current good manufacturing practices (CGMP), it may be declared unfit as food if the contaminant may render the food injurious to health. Foods containing unavoidable contaminants are not automatically banned, but the FDA has regulatory tolerance levels or more informal action levels on the tolerable quantity of unavoidable contaminants.

T e primary factors that must be considered in the evaluation of animal drugs are (1) consumption and absorption by the target animal, (2) metabolism of the drug by the target food animal, (3) excretion and tissue distribution of the drug and its metabolites in food animal products and tissues, (4) consumption of food animal products and tissues by humans, (5) potential absorption of the drug and its metabolites by humans, (6) potential metabolism of the drug and its metabolites by humans, and (7) potential excretion and tissue distribution in humans of the drug, its metabolites, and the secondary human metabolites derived from the drug and its metabolites. T us, the pharmacokinetic and biotransformation characteristics of both the animal and the human must be considered in an assessment of the potential human health hazard of an animal drug.

In addition to allowing GRAS substances to be added to food, the act provides for a class of substances that are regulated food additives, which must be approved and regulated for their intended use by the FDA. Two distinct types of color additives have been approved for food use: those requiring certification by FDA chemists and those exempt from certification. Most certified colors approved for food use bear the prefix FD&C (such as FD&C Blue No. 1). Orange B and Citrus Red No.2 are the two certified colors lacking the FD&C designation. Such color additives consist of structures that cannot be synthesized without a variety of impurities, and so must be carefully monitored and certified as safe before use in food products. Food colors that are exempt from certification are derived primarily from natural sources.

T e importance of using safety warning labeling is demonstrated by the effort to protect particularly susceptible consumers who have food allergies or food intolerance. Although accidental exposure is common, avoidance of the offending foods is the only successful noninterventional approach. Food allergy is the leading cause of anaphylaxis, often requiring hospitalization. It is estimated that 3% to 4% of adults and about 6% of young children in the United States suffer from food allergies, and food allergies account for 35% to 50% of all cases of anaphylaxis.

Methods Used to Evaluate the Safety of Foods, Ingredients, and Contaminants

Safety Evaluation of Direct Food and Color Additives—T e safety of any substance added to food must be established on the basis of specific intended conditions of use or uses in food. Factors that need to be considered include (1) the purpose for use of the substance, (2) the food to which the substance is added, (3) the concentration level used in the proposed foods, and (4) the population expected to consume the substance.

Exposure: The Estimated Daily Intake—Exposure is most often referred to as an estimated daily intake (EDI) and is based on two factors: the daily intake (I) of the food in which the substance will be used and the concentration (C) of the substance in that food. In estimates of consumption and/or exposure, one must also consider other sources of consumption for the proposed intended use of the additive if it already is used in other foods for another purpose, occurs naturally in foods, or is used in nonfood sources.

Before approval, regulatory agencies require evidence that a food additive is safe for its intended use(s) and that the EDI is less than its acceptable daily intake (ADI). T e ADI is generally based on results from animal toxicology studies.

Assignment of Concern Level (CL) and Required Testing—Structures of functional groups in food additives are assigned to categories (A, B, and C) based on their relative harmful nature (category A is least harmful and category C is most harmful). Based on structure assignment and calculated exposure, a CL for a certain additive can be assigned (Table 31–3). An additive with a higher CL (CLIII) is more likely to be dangerous than one with a lower CL (CLI). Once the CL is established, a specific test battery is prescribed, as shown in Table 31–4.

Safety Determination of Indirect Food Additives—Indirect food additives are substances that are not added directly to food but enter food by migrating from surfaces that contact food. T ese surfaces may be from packaging material (cans, paper, and plastic) or surfaces used in processing,

TABLE 31–3 Assignment of concern level.

Structure Category A	Structure Category B	Structure Category C	Concern Level
< 0.05 ppm in the total diet (< 0.0012 mg/kg per day)	< 0.025 ppm in the total diet (< 0.00063 mg/kg per day)	< 0.0125 ppm in the total diet (< 0.00031 mg/kg per day)	I
≥ 0.05 ppm in the total diet (≥ 0.0012 mg/kg per day)	≥ 0.025 ppm in the total diet (≥ 0.00063 mg/kg per day)	≥ 0.0125 ppm in the total diet (≥ 0.00031 mg/kg per day)	II
≥ 1 ppm in the total diet (≥ 0.025 mg/kg per day)	≥ 0.5 ppm in the total diet (≥ 0.0125 mg/kg per day)	≥ 0.25 ppm in the total diet (≥ 0.0063 mg/kg per day)	III

TABLE 31–4 Summary of the toxicity tests recommended for different levels of concern.*

Toxicity Studies†	Concern Levels I	II	III
Short-term tests for genetic toxicity	X	X	X
Metabolism and pharmacokinetic studies		X	X
Short-term (28-day) toxicity studies with rodents	X‡		
Subchronic (90-day) toxicity studies with nonrodents	X‡		
Reproduction studies with teratology phase	X‡	X‡	
One-year toxicity studies with nonrodents		X	
Carcinogenicity studies with rodents		X§	
Chronic toxicity/carcinogenicity studies with rodents		X§,¶	

*http://www.fda.gov/food/guidanceregulation/guidancedocumentsregulatoryinformation/ingredientsadditivesgraspackaging/ucm2006826.htm
†Not including dose range-finding studies, if appropriate.
‡Including neurotoxicity and immunotoxicity screens.
§An in utero phase is recommended for one of the two recommended carcinogenicity studies with rodents, preferably the study with rats.
¶Combined study may be performed as separate studies.

holding, or transporting food. T e level of overall consumption of these materials determines the testing required by the FDA to allow certain foods to be packaged in certain ways.

Safety Requirements for GRAS Substances—T e FD&C Act regards foods as GRAS when they are added to other food, such as green beans in vegetable soup. It also regards a number of food ingredients as GRAS. A list of examples of substances regarded as GRAS is given in Table 31–5. It is important to reemphasize that GRAS substances, though used like food additives, are not food additives; this allows GRAS substances to be exempt from the premarket clearance restrictions applied to food additives.

Establishing Safe Conditions of Use for New Foods, Macroingredients and New Technologies

Transgenic Plant (and New Plant Varieties) Policy—New and novel foods or ingredients and new technologies present new challenges and may require innovative methods for determining safety. Scientists have employed biotechnology to add one or more specific genes into crops like soybeans, corn, cotton, and canola, to improve pest and disease management, resulting in agronomic, economic, environmental, health, and social benefits for farmers. Irrespective of the breeding method used to produce a new plant variety, tests must be done to ensure that the levels of nutrients or toxins in the plants have not changed and that the food is still safe to consume. In particular, tests on new plant varieties must demonstrate that any new proteins produced in the plant by genetic engineering are nontoxic and nonallergenic.

Nanotechnology—Nanotechnology offers some distinct advantages in delivery systems using micelles and liposomes and other technologic advantages such as nanoemulsions (emulsion stability), biopolymeric nanoparticles (encapsulation technology), and cubosomes (solubilized hydrophobic, hydrophilic, and amphiphilic molecules, among other uses). Nanotechnology allows new and more ef cient uses of old products by enhancing solubility, facilitating controlled release, improving bioavailability, and protecting labile substances (including micronutrients and bioactive substances) during processing, storage, and distribution.

TABLE 31–5 Examples of GRAS substances and their functionality.

CFR Number	Substance	Functionality
Substances Generally Recognized as Safe 21 CFR 182		
182.2122	Aluminum calcium silicate	Anticaking agent
182.8985	Zinc chloride	Nutrient supplement
Direct food substances af rmed as Generally Recognized as Safe 21 CFR 184		
184.1005	Acetic acid	Several
184.1355	Helium	Processing aid
Indirect food substances af rmed as Generally Recognized as Safe 21 CFR 186		
186.1025	Caprylic acid	Antimicrobial
186.1374	Iron oxides	Ingredient of paper and paperboard
	Notified GRAS substances with "No Objection"	
GRN 305	Carnobacterium maltaromaticum strain CB1 (viable and heat-treated)	(Antimicrobial) inhibitor of Listeria monocytogenes
GRN 211	Xanthan gum (with reduced pyruvate)	Stabilizer, emulsifier, thickener, suspending and bodying agent, and foam enhancer

GRAS, Generally Recognized as Safe.

FDA has not yet promulgated specific guidelines for testing. However, particle behavior and characteristics at the nanoscale enable applications that can affect safety, effectiveness, performance, quality, and, where applicable, public health impact of FDA-regulated products. Nanoparticle properties may be due to altered chemical, biologic, or magnetic properties, altered electrical or optical activity, increased structural integrity, or other unique characteristics of nanoscale materials not normally observed in their larger counterparts. Conversion of an approved product (GRAS or food additive) to the nanoscale may well render the material unsafe.

Safety Requirements for Dietary Supplements

Dietary supplements have a special status within the law and the regulations: supplements are regarded as foods or food constituents and not food additives or drugs. The standard of safety uses the concept of reasonable expectation *of* no harm. This is a lesser safety standard than the reasonable certainty standard for substances added to *f*oods. The basis for this rationale is that consumption of a dietary supplement is by choice, not involuntary as for a food (i.e., food must have a presumption of safety). Thus, there is a higher standard of safety for food. Also, because (1) there is a deliberate choice involved in consuming a dietary supplement and (2) the daily recommended intake is clearly stated on the label, there is an implied assumption of some risk on the part of the consumer.

Assessment of Carcinogens

Carcinogenicity as a Special Problem—The Delaney clause of the FD&C Act prohibits the approval of regulated food additives "found to induce cancer when ingested by man or animals." It must be emphasized that the Delaney prohibition applies only to the approval of food additives, color additives, and animal drugs; it does not apply to unavoidable contaminants, GRAS substances, or ingredients sanctioned by the FDA or USDA before 1958. To be a carcinogen under the Delaney clause, a food or color additive must be demonstrated to directly induce cancer when ingested by humans or animals. This is interpreted to mean that the findings of cancer must be clearly reproducible and that the cancers found are not secondary to nutritional, hormonal, or physiologic imbalances. This position allows the agency to argue that changing the level of protein or fat in the diet does not induce cancer but simply modulates tumor incidence.

ADVERSE REACTIONS TO FOOD OR FOOD INGREDIENTS

Food hypersensitivity (allergy) refers to a reaction involving an immune-mediated response. An allergic reaction may be manifested by one or more of the symptoms listed in Table 31–6. Cutaneous reactions and anaphylaxis are the most common symptoms associated with food allergy. Any protein in food may act as an allergen; some of the allergenic components of common food allergens are listed in Table 31–7.

TABLE 31–6 Symptoms of IgE-mediated food allergies.

Cutaneous	Urticaria (hives), eczema, dermatitis, pruritus, rash
Gastrointestinal	Nausea, vomiting, diarrhea, abdominal cramps
Respiratory	Asthma, wheezing, rhinitis, bronchospasm
Other	Anaphylactic shock, hypotension, palatal itching, swelling including that of tongue and larynx, methemoglobinemia*

*An unusual manifestation of allergy reported to occur in response to soy or cow milk protein intolerance in infants.

Data from Murray KF, Christie, DL: Dietary protein intolerance in infants with transient methemoglobinemia and diarrhea. J Pediatr 122:90, 1993. Elsevier; Taylor SL, Scanlan RA (eds.): Food Toxicology: A Perspective on the Relative Risks. New York: Marcel Dekker, 1989.

Food idiosyncrasies are generally defined as quantitatively abnormal responses to a food substance or additive. They may resemble hypersensitivity, but do not involve immune mechanisms. Examples of such reactions and the foods that are probably responsible are given in Table 31–8.

TABLE 31–7 Known allergenic food proteins.

Food	Allergic Proteins
Cow's milk	Casein, β-lactoglobulin, α-lactalbumin
Egg whites	Ovomucoid, ovalbumin
Egg yolks	Livetin
Peanuts	Ara h 2, peanut I
Soybeans	β-Conglycinin (7S fraction), glycinin (11S fraction), Gly mIA, Gly mIB, Kunitz trypsin inhibitor
Codfish	Gad cI
Shrimp	Antigen II
Green peas	Albumin fraction
Rice	Glutelin fraction, globulin fraction
Cottonseed	Glycoprotein fraction
Peach, guava, banana, mandarin, strawberry	30 kDa protein
Tomato	Several glycoproteins
Wheat	Gluten, gliadin, globulin, albumin
Okra	Fraction I

Data from Taylor SL, Scanlan RA (eds.): Food Toxicology: A Perspective on the Relative Risks. New York: Marcel Dekker, 1989.

TABLE 31–8 Idiosyncratic reactions to foods.

Food	Reaction	Mechanism
Fava beans	Hemolysis, sometimes accompanied by jaundice and hemoglobinuria; also, pallor, fatigue, nausea, dyspnea, fever and chills, abdominal and dorsal pain	Pyramidene aglycones in fava bean cause irreversible oxidation of GSH in G-6-PD-deficient erythrocytes by blocking NADPH supply, resulting in oxidative stress of the erythrocyte and eventual hemolysis
Chocolate	Migraine headache	Phenylethylamine-related
Beets	Beetanuria: passage of red urine (often mistaken for hematuria)	Excretion of beetanin in urine after consumption of beets
Asparagus	Odorous, sulfurous-smelling urine	Autosomal dominant inability to metabolize methanthiol of asparagus and consequent passage of methanthiol in urine
Red wine	Sneezing, flush, headache, diarrhea, skin itch, shortness of breath	Diminished histamine degradation: deficiency of diamine oxidase (?), histamines present in wine
Choline- and carnitine-containing foods	Fish odor syndrome: foul odor of body secretions	Choline and carnitine metabolized to trimethylamine in gut by bacteria, followed by absorption but inability to metabolize to odorless trimethylamine N-oxide
Milk	Abdominal pain, bloating, diarrhea	Lactase deficiency
Fructose-containing foods	Abdominal pain, vomiting, diarrhea, hypoglycemia	Reduced activity of hepatic aldolase Btoward fructose-1-phosphate

TABLE 31–9 Anaphylactoid reactions to food.

Food	Reaction	Mechanism
Western Australian salmon (Arripis truttaceus)	Erythema and urticaria of the skin, facial flushing and sweating, palpitations, hot flashes of the body, headache, nausea, vomiting, and dizziness	Scombroid poisoning; high histamine levels demonstrated in the fish
Fish (spiked with histamine)	Facial flushing, headache	Histamine poisoning; histamine concentration in plasma correlated closely with dose ingested
Cape yellow tail (fish) (Seriola lalandii)	Skin rash, diarrhea, palpitations, headache, nausea and abdominal cramps, paresthesia, unusual taste sensation, and breathing dif culties	Scombroid poisoning; treated with antihistamines
Sulfite sensitivity	Bronchospasm, asthma	Sulfite oxidase deficiency to metabisulfites in foods and wine
Tuna, albacore, mackerel, bonito, mahimahi, and bluefish	Reaction resembling an acute allergic reaction	Scombroid poisoning; treated with antihistamines and cimetidine
Cheese	Symptoms resembling acute allergic reaction	Responds to antihistamines; histamine poisoning

Anaphylactoid reactions are historically thought of as reactions mimicking anaphylaxis (and other "allergic-type" responses) through direct application of histamine. Ingestion of some types of fish that have been acted upon by certain microorganisms to produce histamine may result in an anaphylactoid reaction also called "scombrotoxicosis" (Table 31–9). Sulfite-induced bronchospasm was first noticed as an acute sensitivity to metabisulfites sprayed on restaurant salads and in wine.

Also referred to as false food allergies, pharmacologic food reactions are characterized by exaggerated responses to pharmacologic agents in food and possibly due to receptor sensitization. In contrast, metabolic food reactions differ from other categories of adverse reactions in that the foods are more or less commonly eaten and demonstrate toxic effects only when eaten in excess or improperly processed. Specific examples are provided in Table 31–10. T e susceptible population exists as a result of its own behavior, that is, the "voluntary" consumption of food as a result of a limited food supply or an abnormal craving for a specific food.

TOXIC SUBSTANCES IN FOOD

Metals, Hydrocarbons, N-Nitroso Substances and Mycotoxins

Certain substances are unavoidable in food because of their widespread use; presence in the earth's crust, which has resulted in their becoming a persistent and/or ubiquitous contaminant

TABLE 31–10 Metabolic food reactions.

Food	Reaction	Mechanism
Lima beans, cassava roots, millet (sorghum) sprouts, bitter almonds, apricot, and peach pits	Cyanosis	Cyanogenic glycosides releasing hydrogen cyanide on contact with stomach acid
Cabbage family, turnips, soybeans, radishes, rapeseed, and mustard	Goiter (enlarged thyroid)	Isothiocyanates, goitrin, or S-5-vinyl-thiooxazolidone interferes with utilization of iodine
Unripe fruit of the tropical tree Blighia sapida, common in Caribbean and Nigeria	Severe vomiting, coma, and acute hypoglycemia sometimes resulting in death, especially among the malnourished	Hypoglycin A, isolated from the fruit, may interfere with oxidation of fatty acids, so that glycogen stores have to be metabolized for energy, with depletion of carbohydrates, resulting in hypoglycemia
Leguminosae, Cruciferae	Lathyritic symptoms: neurologic symptoms of weakness, leg paralysis, and sometimes death	L-2,4-Diaminobutyric acid inhibition of ornithine transcarbamylase of the urea cycle, inducing ammonia toxicity
Licorice (glycyrrhizic acid)	Hypertension, cardiac enlargement, sodium retention	Glycyrrhizic acid mimicking mineralocorticoids
Polar bear and chicken liver	Irritability, vomiting, increased intracranial pressure, death	Vitamin A toxicity
Cycads (cycad flour)	Amyotrophic lateral sclerosis (humans), hepatocarcinogenicity (rats and nonhuman primates)	Cycasin (methylazoxymethanol); primary action is methylation, resulting in a broad range of effects from membrane destruction to inactivation of enzyme systems

in the environment; or presence as a product of normal food processing.

Among the natural elements, approximately 22 are known to be essential nutrients of the mammalian body. These elements are referred to as micronutrients and include iron, zinc, copper, manganese, molybdenum, selenium, iodine, cobalt, and even aluminum and arsenic. However, lead, cadmium, and mercury are familiar as contaminants (or at least have more specifications setting their limits in food ingredients). The prevalence of these elements as contaminants is not due so much to their ubiquity in nature but rather to their use by humans (see Chapter 23 for the toxicology of these metals).

Polychlorinated hydrocarbons have extensive use as pesticides, solvents, and heat-transfer agents. As a result of their facile nature, their resulting wide-range uses, and resistance to degradation (and ease of detection), chlorinated hydrocarbons have been found in a wide variety of foods. Polybrominated biphenyls and polybrominated biphenyl ethers are used in electrical equipment, paint, and plastics.

Nitrogenous compounds such as amines, amides, guanidines, and ureas can react with oxides of nitrogen (NO_x) to form N-nitroso compounds (NOCs). These compounds originate from two sources: environmental formation and endogenous formation. Environmental sources have declined over the last several years but still include foods (e.g., nitrate-cured meats) and beverages (e.g., malt beverages), cosmetics, occupational exposure, and rubber products.

Food-borne mycotoxins (toxins elaborated by fungi), such as the carcinogenic and hepatotoxic aflatoxins, and the hyperestrogenic mycotoxin zearalenone have considerable potential to contribute to human disease. Details of mycotoxins effects are listed in Table 31–11.

Toxins in Fish, Shellfish, and Turtles

Seafood toxins under FDA policy have a zero tolerance, with any detectable level considered cause for regulatory action. Ciguatera, scaritoxin, and maitotoxin are neurotoxins (anticholinesterase) found in 11 orders, 57 families, and over 400 species of fish as well as in oysters and clams. Ciguatera is originally made by dinoflagellates and biotransformed into the active form by fish; after consumption, humans experience gastrointestinal disorders, neurologic symptoms, or death. Palytoxin is produced by the zoanthid soft coral of the genus Palythoa, and fish, crabs, and polychaete worms, living in close association with or eating this mass, may become contaminated with palytoxin. The toxin has been reported in mackerel, parrotfish, and several species of crabs. Victims report a bitter, metallic taste from the meat (most often muscle, liver, ovary, and digestive tract), followed immediately by nausea, vomiting, and diarrhea. Within several hours, symptoms include myoglobinuria, a burning sensation around the mouth and extremities, muscle spasms, dyspnea, and dysphonia. Death may result from myocardial injury. Brevetoxins, produced by dinoflagellates (Gymnodinium breve) and concentrated in filter-feeding organisms, bind to voltage-dependent sodium channels. Symptoms after human consumption include nausea, tingling, and numbness of the oral area, loss of motor control, and severe muscular ache, all of which resolve in a few days. Saxitoxin is found in shellfish feeding on dinoflagellates; blockade of ion

TABLE 31–11 Selected mycotoxins produced by various molds: some of their effects and the commodities that are potentially contaminated.

Mycotoxin	Source	Effect	Commodities Contaminated
Aflatoxins B_1, B_2, G_1, G_2	Aspergillus flavus, A. parasiticus	Acute aflatoxicosis, carcinogenesis	Corn, peanuts, and others
Aflatoxin M_1	Metabolite of AFB_1	Hepatotoxicity	Milk
Fumonisins B_1, B_2, B_3, B_4, A_1, A_2	Fusarium verticillioides	Renal and liver carcinogenesis	Corn
Trichothecenes (e.g., T-2, deoxynivalenol, diacetoxyscirpenol)	Fusarium and Myrothecium	Hematopoietic toxicity, meningeal hemorrhage of brain, "nervous" disorder, necrosis of skin, hemorrhage in mucosal epithelia of stomach and intestine, emesis, feed refusal, immune suppression	Cereal grains, corn
Zearalenones	Fusarium	Estrogenic effect	Corn, grain
Cyclopiazonic acid	Aspergillus, Penicillium	Muscle, liver, and splenic toxicity	Cheese, grains, peanuts
Kojic acid	Aspergillus	Hepatotoxic?	Grain, animal feed
3-Nitropropionic acid	Arthrinium sacchari, A. saccharicola, A. phaeospermum	Central nervous system impairment	Sugarcane
Citreoviridin	Penicillium citreoviride, P. toxicarium	Cardiac beriberi	Rice
Cytochalasins E, B, F, H	Aspergillus and Penicillium	Cytotoxicity	Corn, cereal grain
Sterigmatocystin	Aspergillus versiolar	Carcinogenesis	Corn
Penicillinic acid	Penicillium cyclopium	Nephrotoxicity, abortifacient	Corn, dried beans, grains
Rubratoxins A, B	Penicillium rubrum	Hepatotoxicity, teratogenic	Corn
Patulin	Penicillium patulatum	Carcinogenesis, liver damage	Apple and apple products
Ochratoxin	Aspergillus ochraceus, A. carbonarius, Penicillium verrucosum	Endemic nephropathy, carcinogenesis	Grains, peanuts, grapes, green coffee
Citrinin	Aspergillus and Penicillium	Nephrotoxicity	Cereal grains
Penitrem(s)	Aspergillus, Claviceps, and Penicillium	Tremors, incoordination, bloody diarrhea, death	Moldy cream cheese, English walnuts, hamburger bun, beer
Ergot alkaloids	Claviceps purpurea	Ergotism	Grains

channels in neural transmission at neuromuscular junctions leads to paresthesia and muscular weakness. Domoic acid is also found in shellfish; an analog of the neurotransmitter glutamine, it leads to damage to the hippocampus and other brain areas, causing various neurologic symptoms. Tetrodotoxin is consumed by humans by eating improperly prepared pufferfish. It causes paralysis of the central nervous system and peripheral nerves by blocking the movement of all monovalent cations, leading to muscular paralysis, respiratory distress, and sometimes death. Chelonitoxin is found in sea turtles and causes necrosis of the myocardium and pulmonary edema.

There are naturally occurring toxins that are innate to a particular marine species, but do not involve marine algae or other environmental influences. Escolar (Lepidocybium *flavobrunneum*) and Oilfish or Cocco (Ruvettus pretiosus) contain a strong purgative oil, which when consumed can cause diarrhea known as gempylid fish poisoning, gempylotoxism, or keriorrhea. The toxin consists of wax esters (C_{32}, C_{34}, C_{36}, and C_{38} fatty acid esters), the primary component of which is $C_{34}H_{66}O_2$. Another innate toxin is tetramine. It is found in the salivary glands of Buccinum, Busycon, or Neptunia spp., a type of whelk or sea snail that is distributed in temperate and tropic waters and has long been a food source for humans. This is heat-stable neurotoxin, tetramine, which upon ingestion by humans causes, among other symptoms, eyeball pain, headache, dizziness, abdominal pain, ataxia, tingling in the fingers, nausea, and diarrhea. Finally, the meat of the Greenland shark (Somniosus microcephalus) and the pacific sleeper shark (Somniosus pacificus) contains trimethylamine oxide, which breaks down to trimethylamine in the gut, probably by enteric bacteria. The neurotoxic trimethylamine produces ataxia in both humans and dogs.

Microbiologic Agents

Most U.S. food-related illness results from microbial contamination. Botulism is due to toxin produced by C. botulinum and C. butyricum in improperly canned foods. The toxin interferes with acetylcholine at peripheral nerve endings, leading to respiratory distress and respiratory paralysis. C. perfringens food poisoning occurs when meat has been contaminated with intestinal contents at slaughter, and then roasted and inadequately stored, allowing C. perfringens to grow and elaborate its toxin. The toxin causes death of enterocytes and severe fluid loss as diarrhea. Bacillus cereus makes two toxins; one causes vomiting and is elaborated in improperly prepared rice, whereas the other causes vomiting and can be present in various foods. Staphylococcus aureus, which is normal flora of human skin and nasal discharge, produces a wide variety of endo- and exotoxins. Foods are usually contaminated after cooking by persons handling them and then keeping the foods at room temperature for several hours. Cattle are natural reservoirs of Escherichia coli; outbreaks of E. coli are associated with improperly prepared beef as well as unpasteurized juices and raw vegetables from plants fertilized with manure.

Bovine Spongiform Encephalopathy

Bovine spongiform encephalopathy (BSE, or mad cow disease) is transmitted by an infectious protein called a prion. Present in diseased cows, prions are transmitted to humans in meat that is improperly handled. BSE manifests clinically as neurologic deterioration leading to death.

CONCLUSION

Food consists of myriad chemical substances in addition to the macro- and micronutrients that are essential to life. There are two principal means by which a food can be toxic and still be considered a food: (1) an ordinarily nontoxic food has become toxic (through some act of man or nature), if even for a small subpopulation (e.g., allergy, intolerance); or (2) overconsumption of a food not ordinarily considered toxic at historic levels of use. The vast majority of food-borne illnesses are attributable to microbiologic contamination of food. Thus, the overwhelming concern for food safety must be directed toward preserving the microbiologic integrity of food.

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QUESTIONS

1. Which of the following statements regarding food complexity is FALSE?
   a. Many flavor additives are nonnutrient substances.
   b. Foods are subjected to environmental forces that alter their chemical composition.
   c. T ere are more nonnutrient chemicals in food than nutrient chemicals.
   d. A majority of nonnutrient chemicals are added to food by humans.
   e. Food is more variable and complex than most other substances to which humans are exposed.

2. Which of the following foods contains the most nonnutrient chemicals?
   a. beef.
   b. banana.
   c. tomato.
   d. orange juice.
   e. Cheddar cheese.

3. Which of the following is considered an indirect food additive?
   a. nitrites.
   b. plastic.
   c. food coloring.
   d. EDTA.
   e. citric acid.

4. Estimated daily intake (EDI) is based on which of the following?
   a. metabolic rate.
   b. daily intake.
   c. substance concentration in a food item.
   d. body mass index.
   e. concentration of substance in a food item and daily intake.

5. Which of the following is NOT characteristic of IgE-mediated food allergies?
   a. urticaria.
   b. wheezing.
   c. hypertension.
   d. nausea.
   e. shock.

6. Which of the following wheat proteins is famous for being allergenic?
   a. casein.
   b. ovalbumin.
   c. livetin.
   d. gluten.
   e. glycinin.

7. Which of the following foods contains a chemical that causes hypertension by acting as a noradrenergic stimulant?
   a. cheese.
   b. peanuts.
   c. shrimp.
   d. chocolate.
   e. beets.

8. What is the mechanism of saxitoxin, found in shellfish?
   a. interference with ion channels.
   b. direct neurotoxicity.
   c. interference with DNA replication.
   d. binding to hemoglobin.
   e. interference with a stimulatory G protein.

9. Which of the following foods can cause a reaction that mimics iodine deficiency?
   a. chocolate.
   b. shellfish.
   c. peanuts.
   d. fava beans.
   e. cabbage.

10. Improperly canned foods can be contaminated with which of the following bacteria, causing respiratory paralysis?
    a. C. per*f*ringens.
    b. R. ricketsii.
    c. S. aureus.
    d. C. botulinum.
    e. E. coli.

CHAPTER

32

Analytical and Forensic Toxicology

Bruce A. Goldberger and Diana G. Wilkins*

ANALYTICAL TOXICOLOGY

ROLE IN GENERAL TOXICOLOGY

ROLE IN FORENSIC TOXICOLOGY

TOXICOLOGIC INVESTIGATION OF A POISON DEATH

- Case History and Specimens
- Toxicologic Analysis
- Interpretation of Analytical Results

CRIMINAL POISONING OF THE LIVING

FORENSIC URINE DRUG TESTING

HUMAN PERFORMANCE TESTING

COURTROOM TESTIMONY

ROLE IN CLINICAL TOXICOLOGY

ROLE IN THERAPEUTIC MONITORING

SUMMARY

KEY POINTS

- Analytic toxicology involves the application of the tools of analytic chemistry to the qualitative and/or quantitative estimation of chemicals that may exert adverse effects on living organisms.
- Forensic toxicology involves the use of toxicology for the purposes of the law; by far the most common application is to identify any chemical that may serve as a causative agent in inflicting death or injury on humans or in causing damage to property.
- The toxicologic investigation of a poison death involves (1) obtaining the case history in as much detail as possible and gathering suitable specimens, (2) conducting suitable toxicologic analyses based on the available specimens, and (3) the interpretation of the analytic findings.
- The toxicologist as an expert witness may provide two objectives: testimony and opinion. Objective testimony usually involves a description of analytic methods and findings. When a toxicologist testifies as to the interpretation of analytic results, that toxicologist is offering an "opinion."

With its roots in forensic applications, analytical toxicology involves the application of the tools of analytical chemistry to the qualitative and/or quantitative estimation of chemicals that may exert effects on living organisms. Forensic toxicology involves the use of toxicology for the purposes of the law. The most common application is to identify any chemical that may serve as a causative agent in inflicting death or injury on humans, or in causing damage to property. There is no substitute for the unequivocal identification of a specific chemical substance that is demonstrated to be present in tissues from the victim at a sufficient concentration to explain the injury with a reasonable degree of scientific probability or certainty. For this reason, forensic toxicology and analytical toxicology have long shared a mutually supportive partnership.

*Drs Goldberger and Wilkins acknowledge the contribution of Alphonse Poklis, PhD, who authored this chapter in previous editions of Casarett & Doull's Toxicology.

ANALYTICAL TOXICOLOGY

Forensic toxicologists learned long ago that when the nature of a suspected poison is unknown, a systematic, standardized approach must be used to identify the presence of most common toxic substances. An approach that was first suggested by Chapuis in 1873 in Elements de Toxicologie is based on the origin or nature of the toxic agent. Such a system can be characterized as follows:

1. Gases—Gases are most simply measured by means of gas chromatography.
2. Volatile substances—T ese are generally liquids of various chemical types that vaporize at ambient temperatures. Gas chromatography is the simplest approach for separation and quantitation.
3. Corrosive agents—T ese include mineral acids and bases. Many corrosives consist of ions that are normal tissue constituents. Chemical techniques can be applied to detect these ions when they are in great excess over normal concentrations.
4. Metals—Metals are encountered frequently as occupational and environmental hazards. Separation involves destruction of the organic matrix by chemical or thermal oxidation.
5. Anions and nonmetals—T ese present an analytical challenge as they are rarely encountered in an uncombined form.
6. Nonvolatile organic substances—T ese constitute the largest group of substances that must be considered by analytical toxicologists. T is group includes drugs, pesticides, natural products, pollutants, and industrial compounds. T ese substances are solids or liquids with high boiling points. A scheme for analysis of these chemicals is illustrated in Figure 32–1. Separation procedures rely on differential extractions of biologic tissues and fluids

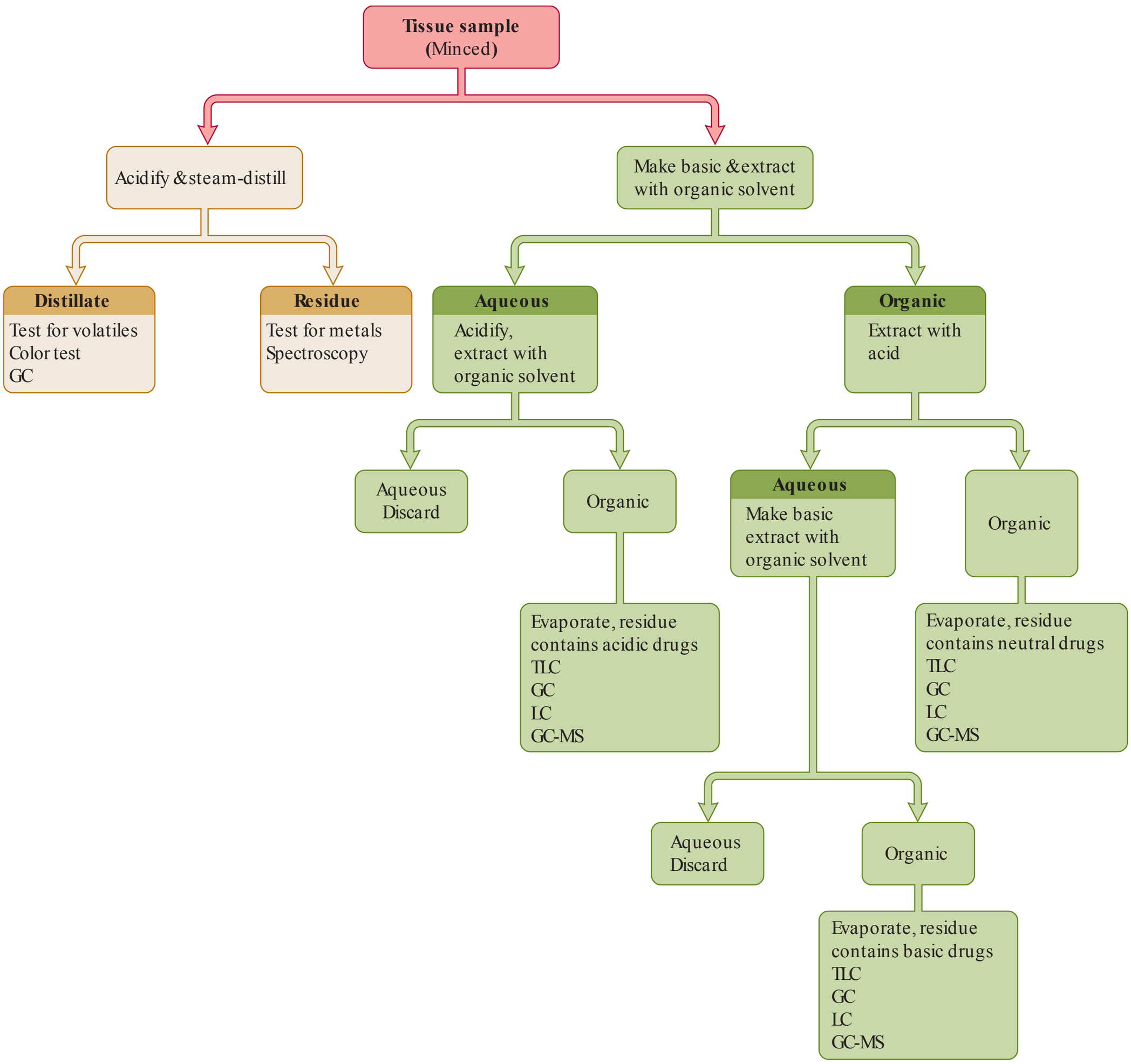

FIGURE 32–1 **A scheme of separation for poisons from tissues.**

and this process is of en tedious and inefficient, with poor recovery of the analyte. Immunoassay may permit avoidance of extractions and facilitate quantification.

7. Miscellaneous—T is category covers the large number of compounds that cannot be detected by routine application. Venoms and other toxic mixtures of proteins or uncharacterized constituents fall into this class.

ROLE IN GENERAL TOXICOLOGY

It is universally acknowledged that the chemical under study must be either pure or the nature of any contaminant well-characterized to enable interpretation of the experimental results with validity. Chemicals may degrade when in contact with air, by exposure to ultraviolet or other radiation, by interaction with constituents of the vehicle or dosing solution, and by other means. Developing an analytical procedure by which these changes can be recognized and corrected is essential in achieving consistent and reliable results over the course of a study.

Finally, analytical methods are necessary to determine the bioavailability of a compound that is under study. Some substances with low water solubility are difficult to introduce into an animal, and a variety of vehicles may be investigated. However, a comparison of the blood concentrations for the compound under study provides a simple means of comparing the effectiveness of vehicles.

ROLE IN FORENSIC TOXICOLOGY

T e duties of a forensic toxicologist in postmortem investigations include the qualitative and quantitative analysis of drugs or poisons in biologic specimens collected at autopsy and the interpretation of the analytical findings with respect to the physiologic and behavioral effects of the detected chemicals on the deceased at the time of injury and/or death. T e cause of death in cases of poisoning cannot be proved beyond contention without toxicologic analysis that confirms the presence of the toxicant in either body fluids or tissues of the deceased.

Additionally, the results of postmortem toxicologic testing provide valuable epidemiologic and statistical data. Forensic toxicologists are of en among the first to alert the medical community to new epidemics of substance abuse and the dangers of abusing over-the-counter drugs. Similarly, they of en determine the chemical identity and toxicity of novel analogs of psychoactive agents that are subject to abuse, including "designer drugs" such as "china white" (methylfentanyl), "ecstasy" (methylenedioxymethamphetamine), and GHB (gamma-hydroxybutyric acid).

TOXICOLOGIC INVESTIGATION OF A POISON DEATH

T e toxicologic investigation of a poison death may be divided into three steps: (1) obtaining the case history and suitable specimens, (2) the toxicologic analyses, and (3) the interpretation of the analytical findings.

Case History and Specimens

Today, thousands of compounds are readily available that are lethal if ingested, injected, or inhaled. Usually, a limited amount of specimen is available on which to perform analyses; therefore it is imperative that before the analyses are initiated, as much information as possible concerning the facts of the case be collected. T e age, sex, weight, medical history, and occupation of the decedent as well as any treatment administered before death, the gross autopsy findings, the drugs available to the decedent, and the interval between the onset of symptoms and death should be noted. In a typical year, a postmortem toxicology laboratory will perform analyses for such diverse poisons as over-the-counter medications (e.g., analgesics, antihistamines), prescription drugs (e.g., benzodiazepines, opioids), drugs of abuse (e.g., cocaine, marijuana, methamphetamine), and gases (e.g., inhalants, carbon monoxide).

Specimens of many different body fluids and organs are necessary, as drugs and poisons display varying affinities for body tissues. It is paramount that the handling of all specimens be authenticated and documented. Fluids and tissues should be collected before embalming, as this process will dilute or chemically alter the poisons present, rendering their detection difficult or impossible. Although forensic toxicology laboratories typically receive blood, urine, liver tissue, and/or stomach contents for identification of xenobiotics, they have been increasingly called upon to meet the analytical challenges of many alternative types of samples. Nontraditional matrices, such as bone marrow, hair, vitreous humor, and nails, among others, may be submitted to the laboratory. For example, on occasion, toxicologic analysis is requested for cases of burned, exhumed, putrefied, or skeletal remains. Finally, in severely decomposed bodies, the absence of blood and/or the scarcity of solid tissues suitable for analysis have led to the collection and testing of maggots (fly larvae) feeding on the body.

Toxicologic Analysis

Before the analysis begins, several factors must be considered, including the amount of specimen available, the nature of the poison sought, and the possible biotransformation of the poison. In cases involving oral administration of the poison, the gastrointestinal (GI) contents are analyzed first because large amounts of residual unabsorbed poison may be present. T e urine may be analyzed next, as the kidney is the major organ of excretion for most poisons and high concentrations of toxicants and/or their metabolites of en are present in urine. Af er absorption from the GI tract, drugs or poisons are carried to the liver before entering the general systemic circulation; therefore, the first analysis of an internal organ is conducted on the liver.

A thorough knowledge of drug biotransformation is of en essential before an analysis is performed. T e parent compound and any major pharmacologically active metabolites should be isolated and identified. Many screening tests, such as immunoassays, are specifically designed to detect not the parent drug but its major urinary metabolite.

T e analysis may be complicated by the normal chemical changes that occur during the decomposition of a cadaver. T e autopsy and toxicologic analysis should be started as soon af er death as possible. However, many poisons—such as arsenic, barbiturates, mercury, and strychnine—are extremely stable and may be detectable many years af er death.

Forensic toxicology laboratories analyze specimens by using a variety of analytical procedures. Initially, nonspecific tests designed to determine the presence or absence of a class or group of analytes may be performed directly on the specimens. Examples of tests used to rapidly screen urine are the FPN (ferric chloride, perchloric, and nitric acid) color test for phenothiazine drugs and immunoassays for the detection of amphetamines, benzodiazepines, and opiate derivatives, among others. Today, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS) are the most widely applied methodology in toxicology and are generally accepted as unequivocal identification for all drugs.

Interpretation of Analytical Results

Once the analysis of the specimens is complete, the toxicologist must interpret his or her findings with regard to the physiologic or behavioral effects of the toxicants on the decedent at the concentrations found. Specific questions may be answered, such as the route of administration, the dose administered, and whether the concentration of the toxicant present was sufficient to cause death or alter the decedent's actions enough to cause his or her death. Assessing the physiologic or behavioral meanings of analytical results is of en the most challenging aspect confronted by the forensic toxicologist.

In determining the route of administration, the toxicologist notes the results of the analysis of the various specimens. As a general rule, the highest concentrations of a poison are found at the site of administration. T erefore, the presence of large amounts of drugs and/or poisons in the GI tract and liver indicates oral ingestion, while higher concentrations in the lungs than in other visceral organs can indicate inhalation or intravenous injection.

T e physiologic effects of most drugs and poisons are generally correlated with their concentrations in blood or blood fractions such as plasma and serum. T e survival time between the administration of a poison and death may be sufficiently long to permit biotransformation and excretion of the agent. Blood values may appear to be nontoxic or consistent with therapeutic administration. Death from hepatic failure af er an acetaminophen overdose usually occurs at least three to four days af er ingestion. Postmortem acetaminophen concentrations in blood may be consistent with the ingestion of therapeutic doses. T erefore, fatal acetaminophen overdose is determined by case history, central lobular necrosis of the liver, and, if available, analysis of serum specimens collected from the decedent when he or she was admitted to the emergency department.

A new extension of forensic toxicology is the analysis of impurities of illicit drug synthesis in biologic specimens. Many drugs of abuse, particularly methamphetamine, are illicitly manufactured in clandestine laboratories. T ere are several popular methods of methamphetamine synthesis; when these are applied in clandestine laboratories, side reactions or incomplete conversion of the reactants yield an impure mixture of methamphetamine and synthetic impurities. T ese impurities can be characteristic of a particular synthetic method and suggest the synthetic method that was used to produce the drug; point to a possible common source of illicit production; and provide a link between manufacturers, dealers, and users.

CRIMINAL POISONING OF THE LIVING

Over the past few decades, forensic toxicologists have become more involved in the analysis of specimens obtained from living victims of criminal poisonings. Generally, this increase in testing is a result of two types of cases: (1) administration of drugs to incapacitate victims of kidnapping, robbery, or sexual assault and (2) poisoning as a form of child abuse.

While alcohol is still of en a primary factor in cases of alleged sexual assault, common drugs of abuse or other psychoactive drugs are of en involved (Table 32–1). Of particular concern are the many potent inductive agents medically administered prior to general anesthesia. Many of these drugs, such as benzodiazepines and phenothiazines, are available today through illicit sources or legal purchase in foreign

TABLE 32–1 Distribution of drugs of abuse encountered in urine specimens in 1179 cases of alleged sexual assault.*

Rank	Drug/Drug Group	Incidence
1	No drugs found	468
2	Ethanol	451
3	Cannabinoids	218
4	Benzoylecgonine (cocaine metabolite)	97
5	Benzodiazepines	97
6	Amphetamines	51
7	Gamma-hydroxybutyrate (GHB)	48
8	Opiates	25
9	Propoxyphene	17
10	Barbiturates	12

*Thirty-five percent of the drug-positive specimens were positive for more than one drug.
Data from ElSohly MA, Salamone SJ: Prevalence of drugs used in cases of alleged sexual assault. J Anal Toxicol, 1999;23(3):141–146.

countries. When administered surreptitiously, they cause sedation and incapacitate the victim while also producing amnesia in the victim as to the events while drugged, without causing severe central nervous system depression. T ese cases of en present a difficult analytical challenge to the toxicologist. Usually, the victim does not bring forth an allegation of assault until 24 h to several days af er the attack. T us, the intoxicating drug may have been largely eliminated or extensively metabolized such that extremely low concentrations of drug or metabolites are present in the victim's blood, urine, and/or hair specimens.

Poisoning as a form of child abuse involves the deliberate administration of toxic or injurious substances to a child, usually by a parent or other caregiver. Common agents used to intentionally poison children have included syrup of ipecac, table salt, laxatives, diuretics, antidepressants, sedative-hypnotics, and narcotics. As in the case of sexual assault, sophisticated MS testing methods may be required to detect such agents as emetine and cephaeline, the emetic alkaloids in syrup of ipecac.

FORENSIC URINE DRUG TESTING

Concerns regarding the potentially adverse consequences of substance abuse for the individual, the workplace, and society have led to widespread urine analysis for controlled or illicit drugs. Currently, such testing is conducted routinely by the military services, regulated transportation and nuclear industries, many federal and state agencies, public utilities, federal and state criminal justice systems, and numerous private businesses and industries. Significant ethical and legal ramifications are associated with such testing. T ose having positive test results may not receive employment, be dismissed from a job, be court-martialed, or suffer a damaged reputation.

Forensic urine drug testing (FUDT) differs from other areas of forensic toxicology in which urine is the only specimen analyzed and testing is performed for a limited number of drugs and metabolites. Under the federal certification program, analyses are performed for a limited number of classes or drugs of abuse. Initial testing is performed by immunoassays on rapid, high-throughput chemistry analyzers. A confirmation analysis in FUDT-certified laboratories is performed by GC-MS and LC-MS/MS. FUDT results are reported only as positive or negative for the drugs sought.

Many individuals who are subject to regulated urine testing have devised techniques to mask their drug use either by physiologic means such as the ingestion of diuretics or by attempting to adulterate the specimen directly with bleach, vinegar, or other products that interfere with the initial immunoassay tests. T us, specimens are routinely tested for adulteration by checking urinary pH, creatinine, and specific gravity and noting any unusual color or smell. Recently a mini-industry has developed to sell various products that are alleged to "beat the drug test" by interfering with the initial or confirmatory drug test. T us, FUDT laboratories now routinely test not only for drugs of abuse, but also for a wide variety of chemical adulterants. In most instances, a positive test result for adulteration has as serious a consequence as a positive drug test.

HUMAN PERFORMANCE TESTING

Forensic toxicology activities also include the determination of the presence of ethanol and other drugs and chemicals in blood, breath, or other specimens and the evaluation of their role in modifying human performance and behavior. T e most common application of human performance testing is to determine impairment while driving under the influence of ethanol or drugs. Several studies have demonstrated a relatively high occurrence of drugs in impaired or fatally injured drivers. T ese studies tend to report that the highest drug-use accident rates are associated with the use of such illicit or controlled drugs as cocaine, benzodiazepines, marijuana, and phencyclidine. Before driving under the influence of drugs is as readily accepted by the courts as ethanol testing, legal and scientific problems regarding drug concentrations and driving impairment must be resolved.

COURTROOM TESTIMONY

T e forensic toxicologist of en is called upon to testify in legal proceedings as an "expert witness." An expert witness may provide two types of testimony: objective testimony and "opinion." Objective testimony by a toxicologist usually involves a description of his or her analytical methods and findings. When a toxicologist testifies as to the interpretation of his or her analytical results or those of others, that toxicologist is offering an "opinion." Whether a toxicologist appears in criminal or civil court, workers' compensation, or parole hearings, the procedure for testifying is the same: direct examination, cross-examination, and redirect examination. Regardless of which side has called for the expert witness, the toxicologist should testify with scientific objectivity. An expert witness is called to provide informed assistance to the jury, not to judge the case.

ROLE IN CLINICAL TOXICOLOGY

Analytical toxicology in a clinical setting plays a role very similar to its role in forensic toxicology. As an aid in the diagnosis and treatment of toxic incidents, as well as in monitoring the effectiveness of treatment regimens, it is useful to clearly identify the nature of the toxic exposure and measure the amount of the toxic substance that has been absorbed. Frequently, this information, together with the clinical state of the patient, permits a clinician to relate the signs and symptoms observed to the anticipated effects of the toxic

agent. T is may permit a clinical judgment as to whether the treatment must be vigorous and aggressive or whether simple observation and symptomatic treatment of the patient are sufficient.

A cardinal rule in the treatment of poisoning cases is to support vital cardiopulmonary function and to remove any unabsorbed material, limit the absorption of additional poison, and hasten its elimination. Although the instrumentation and the methodology used in a clinical toxicology laboratory are similar to those utilized by a forensic toxicologist, a major difference between these two applications is responsiveness. In emergency toxicology testing, results must be communicated to the clinician within hours to be meaningful for therapy. Primary examples of the usefulness of emergency toxicology testing are the rapid quantitative determination of acetaminophen, salicylate, alcohols, and glycol serum concentrations in instances of suspected overdose.

Ethanol is the most common chemical encountered in emergency toxicology. Although relatively few fatal intoxications occur with ethanol alone, serum values are important in the assessment of behavioral, physiologic, and neurologic function, particularly in trauma cases where the patient is unable to communicate and surgery with the administration of anesthetic or analgesic drugs is indicated. Intoxications from accidental or deliberate ingestion of other alcohols or glycols—such as methanol from windshield deicer or paint thinner, isopropanol from rubbing alcohol, and ethylene glycol from antifreeze—are of en encountered in emergency departments. Following ingestion of methanol or ethylene glycol, patients of en present with similar neurologic symptoms and severe metabolic acidosis due to the formation of toxic aldehyde and acid metabolites. A rapid quantitative serum determination for these intoxicants will indicate the severity of intoxication and the possible need for dialysis or therapy with an alcohol dehydrogenase inhibitor (fomepizole).

ROLE IN THERAPEUTIC MONITORING

Historically, the administration of drugs for long-term therapy was based largely on experience. A dosage amount was selected and administered at appropriate intervals based on what the clinician had learned was generally tolerated by most patients. If the drug seemed ineffective, the dose was increased; if toxicity developed, the dose was decreased or the frequency of dosing was altered. At times, a different dosage form might be substituted. Establishing an effective dosage regimen was particularly difficult in children and the elderly.

T e factors responsible for individual variability in responses to drug therapy include the rate and extent of drug absorption, distribution, and binding in body tissues and fluids, rate of metabolism and excretion, pathologic conditions, and interaction with other drugs. Monitoring of the plasma or serum concentration at regular intervals will detect deviations from the average serum concentration, which, in turn, may suggest that one or more of these variables need to be identified and corrected. Drugs that are commonly monitored during therapy are presented in Table 32–2.

TABLE 32–2 Drugs commonly indicated for therapeutic monitoring.

Antiarrythmics
Digoxin
Digitoxin
Lidocaine
Procainamide and N-acetylprocainamide
Quinidine
Antibiotics
Amikacin
Chloramphenicol
Gentamicin
Tobramycin
Vancomycin
Anticancer
Methotrexate
Anticonvulsants
Carbamazepine
Gabapentin
Lamotrigine
Phenobarbital
Phenytoin
Primidone
Topiramate
Valproic acid
Zonisamide
Antidepressants
Amitriptyline/nortriptyline
Desipramine/imipramine
Doxepin/nordoxepin
Antipsychotics
Clozapine
Pimozide
Bronchodilators
Caf eine
Theophylline
Immunosuppressants
Azathioprine
Cyclosporine
Mycophenolic acid
Sirolimus
Tacrolimus
Mood stabilizing
Lithium

SUMMARY

T e analytical techniques employed by forensic toxicologists have continued to expand in complexity and improve in reliability and sensitivity. Many new analytical tools have been applied

to toxicologic problems in almost all areas of the field, and the technology continues to open new areas of research. Forensic toxicologists continue to be concerned about conducting unequivocal identification of toxic substances in such a manner that the results can withstand a legal challenge. T e issues of substance abuse, designer drugs, increased potency of therapeutic agents, and widespread concern about pollution, and the safety and health of workers present challenges to the analyst's knowledge, skills, and abilities. As these challenges are met, analytical toxicologists will continue to play a substantial role in the expansion of the discipline of toxicology.

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QUESTIONS

1. Which of the following is most commonly used as a drug of sexual assault?
   a. narcotics.
   b. amphetamines.
   c. benzodiazepines.
   d. ethanol.
   e. antidepressants.

2. All of the following statements regarding analytic/forensic toxicology are true EXCEPT:
   a. Analytic toxicology uses analytic chemistry to characterize a chemical's adverse effect on an organism.
   b. Medical examiners and coroners are most important in determining cause of death.
   c. Tissues and body fluids are vital in forensic toxicology.
   d. Forensic toxicology is used for purposes of the law.
   e. Chapuis first characterized a system for classifying toxic agents.

3. Which of the following criteria is NOT routinely used to check for adulteration of drug urine analysis?
   a. urea.
   b. pH.
   c. color.
   d. specific gravity.
   e. creatinine.

4. Which blood alcohol concentration (BAC) is most commonly used as the statutory definition of DUI?
   a. 0.04.
   b. 0.06.
   c. 0.08.
   d. 0.12.
   e. 0.16.

5. Which of the following drugs is NOT properly matched with its most common analytic method?
   a. benzodiazepines—GC/MS.
   b. ibuprofen—TLC/HPLC.
   c. amphetamines—immunoassays.
   d. barbiturates—GC/immunoassays.
   e. ethanol—immunoassays.

6. For which of the following drugs is serum NOT used during toxicology testing?
   a. ethanol.
   b. cocaine.
   c. aspirin.
   d. barbiturates.
   e. ibuprofen.

7. Which of the following is LEAST important in determining variability in response to drug therapy?
   a. drug interactions.
   b. distribution in body tissue.
   c. body mass index.
   d. pathologic conditions.
   e. rate of metabolism.

8. Which of the following statements is FALSE regarding steady state?
   a. Steady-state concentrations are proportional to the dose/dosage interval.
   b. Steady state is attained af er approximately four half-lives.
   c. T e steady-state concentrations are proportional to F/Cl.
   d. Monitoring of steady-state drug concentration assumes that an effective concentration is present.
   e. Fluctuations in concentration are increased by slow drug absorption.

9. Which of the following is an indirect method of measuring a chemical or its metabolite?
   a. blood test.
   b. hair sample.
   c. urinalysis.
   d. hemoglobin adduct detection.
   e. breath analysis.

10. Which of the following statements regarding analytic/forensic toxicology is TRUE?
    a. Antidepressants are commonly used to incapacitate victims.
    b. It is easy to test for and prove that marijuana is a factor in an automobile accident.
    c. Heroin is the drug most commonly encountered in emergency toxicology.
    d. Toxicologists can play an important role in courtroom testimonies.
    e. Ethanol intoxication of en results in death.

CHAPTER

33

Clinical Toxicology

Louis R. Cantilena Jr.

HISTORY OF CLINICAL TOXICOLOGY

INTRODUCTION OF THE POISON CONTROL CENTER

CLINICAL STRATEGY FOR TREATMENT OF THE POISONED PATIENT

- Clinical Stabilization
- Clinical History in the Poisoned Patient
- Physical Examination
- Laboratory Evaluation
- Radiographic Examination
- Prevention of Further Poison Absorption
- Enhancement of Poison Elimination
- Use of Antidotes in Poisoning
- Supportive Care of the Poisoned Patient

CASE EXAMPLES OF SPECIFIC POISONINGS

- Acetaminophen
- Ethylene Glycol
- Valproic Acid

CONCLUSION

KEY POINTS

- Clinical toxicology encompasses the expertise in the specialties of medical toxicology, applied toxicology, and clinical poison information.
- Important components of the initial clinical encounter with a poisoned patient include stabilization of the patient, clinical evaluation (history, physical, laboratory, and radiology), prevention of further toxin absorption, enhancement of toxin elimination, administration of antidote, and supportive care with clinical follow-up.

HISTORY OF CLINICAL TOXICOLOGY

T e history of poisoning and poisoners goes back to ancient times. Formulas for creating poisonous and noxious vapors have been found in Chinese writings dating back to 1000 BC. Documentation regarding the use of antidotes can be found in Homer's Odyssey and Shastras from 600 BC. Additional history is found in Chapter 1.

INTRODUCTION OF THE POISON CONTROL CENTER

In the United States, poison control centers are staffed by a medical director (medical toxicologist), administrator, specialists in poison information, and educators for poison prevention programs. Personnel provide direct information to patients with expert recommendations for medical treatment, critical diagnostic and treatment information for health care professionals, education for health care professionals, and poison prevention activities through public education. Poison control centers serve as a potential early-warning system for a potential chemical or biologic terrorist attack.

CLINICAL STRATEGY FOR TREATMENT OF THE POISONED PATIENT

T e following general steps represent important components of the initial clinical encounter with a poisoned patient:

1. Stabilization of the patient
2. Clinical evaluation (history, physical, laboratory, and radiology)
3. Prevention of further toxin absorption

TABLE 33–1 Clinical features of toxic syndromes.

	Blood pressure	Pulse	Temperature	Pupils	Lungs	Abdomen	Neurologic
Sympathomimetic	Increase	Increase	Slight increase	Mydriasis	NC	NC	Hyperalert, increased reflexes
Anticholinergic	Slight increase or NC	Increase	Increase	Mydriasis	NC	Decreased bowel sounds	Altered mental status
Cholinergic	Slight decrease or NC	Decrease	NC	Miosis	Increased bronchial sounds	Increased bowel sounds	Altered mental status
Opioid	Decrease	Decrease	Decrease	Miosis	NC or rales (late)	Decreased bowel sounds	Decreased level of consciousness

4. Enhancement of toxin elimination
5. Administration of antidote (if available)
6. Supportive care and clinical follow-up

Clinical Stabilization

The first priority in the treatment of the poisoned patient is stabilization. Initial assessment of airway, respiration, and circulation is crucial. Some toxins or drugs can cause seizures early in the course of presentation. The steps and clinical procedures incorporated to stabilize a critically ill, poisoned patient are numerous and include, if appropriate, support of ventilation, circulation, and oxygenation. In critically ill patients, sometimes treatment interventions must be initiated before a patient is truly stable.

Clinical History in the Poisoned Patient

The primary goal of taking a medical history in poisoned patients is to determine, if possible, the substance ingested or the substance to which the patient has been exposed as well as the extent and time of exposure. In the setting of a suicide attempt, patients may not provide any history or may give incorrect information so as to increase the possibility that they will successfully bring harm to themselves. Information sources commonly employed in this setting include family members, emergency medical technicians who were at the scene, a pharmacist who can sometimes provide a listing of prescriptions recently filled, or an employer who can disclose what chemicals are available in the work environment.

In estimating the level of exposure to the poison, one generally should maximize the possible dose received. That is, one should assume that the entire prescription bottle contents were ingested, that the entire bottle of liquid was consumed, or that the highest possible concentration of airborne contaminant was present in the case of a patient poisoned by inhalation.

With an estimate of dose, the toxicologist can refer to various information sources to determine what the range of expected clinical effects might be from the exposure. The estimation of expected toxicity greatly assists with the triage of poisoned patients. Estimating the timing of the exposure to the poison is frequently the most difficult aspect of the clinical history in the setting of treatment of the poisoned patient.

Taking an accurate history in the poisoned patient can be challenging and in some cases unsuccessful. When the history is unobtainable, the clinical toxicologist is left without a clear picture of the exposure history. In this setting, the treatment proceeds empirically as an "unknown ingestion" poisoning.

Physical Examination

A thorough physical examination is required to assess the patient's condition, determine the patient's mental status, and, if altered, determine possible additional causes such as trauma or central nervous system infection. Whenever possible, the patient's physical examination parameters are categorized into broad classes referred to as toxic syndromes (toxidromes), constellations of clinical signs that, taken together, are likely associated with exposure from certain classes of toxicologic agents. Categorization of the patient's presentation into toxic syndromes allows for the initiation of rational treatment based on the most likely category of toxin responsible, even if the exact nature of the toxin is unknown. Table 33–1 lists clinical features of the major toxic syndromes. Occasionally a characteristic odor detected on the poisoned patient's breath or clothing may point toward exposure or poisoning by a specific agent (Table 33–2).

TABLE 33–2 Characteristic odors associated with poisonings.

Odor	Potential Poison
Bitter almonds	Cyanide
Eggs	Hydrogen sulfide, mercaptans
Garlic	As, organophosphates, DMSO, thallium
Mothballs	Naphthalene, camphor
Vinyl	Ethchlorvynol
Wintergreen	Methylsalicylate

DMSO, dimethyl sulfoxide.

Laboratory Evaluation

Table 33–3 lists drugs or other chemicals that are typically available for immediate measurement in a hospital facility. As one can see, the number of agents for which detection is possible in the rapid-turnaround clinical setting is extremely limited compared with the number of possible agents that can poison patients. T is further emphasizes the importance of recognizing clinical syndromes for poisoning and for the clinical toxicologist to initiate general treatment and supportive care for the patient with poisoning from an unknown substance.

For the substances that can be measured on a rapid-turnaround basis in an emergency department setting, the quantitative measurement can often provide both prognostic and therapeutic guidance.

Predictive relationships of drug plasma concentration and clinical outcome and/or suggested concentrations that require therapeutic interventions are available for several agents including salicylates, lithium, digoxin, iron, phenobarbital, and theophylline. Some authors have identified "action levels" or toxic threshold values for the measured plasma concentrations of various drugs or chemicals. Generally, these values represent mean concentrations of the respective substance that have been retrospectively shown to produce a significant harmful effect.

Because of the limited clinical availability of "diagnostic" laboratory tests for poisons, toxicologists utilize specific, routinely obtained clinical laboratory data—especially the anion gap and the osmol gap—to determine what poisons may have been ingested. An abnormal anion or osmol gap suggests a differential diagnosis for significant exposure. Both calculations are used as diagnostic tools when the clinical history suggests poisoning and the patient's condition is consistent with exposure to agents known to cause elevations of these parameters (i.e., metabolic acidosis, altered mental status, etc.).

T e anion gap is calculated as the difference between the serum Na ion concentration and the sum of the serum Cl and HCO_3 ion concentrations. A normal anion gap is < 12. When there is laboratory evidence of metabolic acidosis, the finding of an elevated anion gap would suggest systemic toxicity from a relatively limited number of agents (Table 33–4).

TABLE 33–3 List of tests that are commonly measured in a hospital setting on a stat basis.

Acetaminophen	Osmolality
Acetone	Phenobarbital
Carbamazepine	Phenytoin
Carboxyhemoglobin	Procainamide/NAPA
Digoxin	Quinidine
Ethanol	Salicylates
Gentamicin	Theophylline
Iron	Tobramycin
Lithium	Valproic Acid
Methemoglobin	

NAPA, N-acetylprocainamide.

TABLE 33–4 Dif erential diagnosis of metabolic acidosis with elevated anion gap: "AT MUD PILES".

A	Alcohol (ethanol ketoacidosis)
T	Toluene
M	Methanol
U	Uremia
D	Diabetic ketoacidosis
P	Paraldehyde
I	Iron, isoniazid
L	Lactic acid
E	Ethylene glycol
S	Salicylate

T e second calculated parameter from clinical chemistry values is the osmol gap. T e osmol gap is calculated as the numerical difference between the measured serum osmolality and the serum osmolarity calculated from the clinical chemistry measurements of the serum sodium ion, glucose, and blood urea nitrogen (BUN) concentrations. T e normal osmol gap is < 10 mOsm. An elevated osmol gap suggests the presence of an osmotically active substance (methanol, ethanol, ethylene glycol, and isopropanol) in the plasma that is not accounted for by the sodium ion, glucose, or BUN concentrations.

Although calculation of both the AG and the osmol gap can provide very useful information from readily available clinical chemistry measurements, these determinations must be interpreted cautiously in certain clinical settings. For example, even though a patient may have ingested a large, significantly toxic amount of methanol, if measured late in the clinical course of the exposure, the osmol gap may not be significantly elevated as most of the osmotically active methanol has left the plasma and has been biotransformed or cleared but is still producing serious clinical effects.

Radiographic Examination

T e use of clinical radiographs to visualize drug overdose or poison ingestions is relatively limited due to lack of radiopacity. Generally, plain radiographs can detect a significant amount of ingested oral medication containing ferrous or potassium salts. In addition, certain formulations that have an enteric coating or certain types of sustained release products are radiopaque as well.

T e most useful radiographs ordered in a case of overdose or poisoning include the chest and abdominal radiographs

and the computed tomography (CT) study of the head. T e abdominal radiograph has been used to detect recent lead paint ingestion in children, and ingestion of halogenated hydrocarbons, such as carbon tetrachloride or chloroform, that may be visualized as a radiopaque liquid in the gut lumen. Abdominal plain radiographs have been helpful in the setting where foreign bodies are detected in the gastrointestinal tract, such as would be seen in a "body packer," or one who smuggles illegal substances by swallowing latex or plastic storage vesicles filled with cocaine or some other substance. Occasionally these storage devices rupture and the drug is released into the gastrointestinal tract, with serious and sometimes fatal results.

Plain radiography and other types of diagnostic imaging in clinical toxicology can also be extremely valuable for the diagnosis of toxin-induced pathology. For example, the detection of drug-induced noncardiac pulmonary edema is associated with serious intoxication with salicylates and opioid agonists. Another example of the use of radiologic imaging in clinical toxicology is with CT of the brain. Significant exposure to carbon monoxide (CO) has been associated with CT lesions of the brain consisting of low-density areas in the cerebral white matter and in the basal ganglia, especially the globus pallidus.

Prevention of Further Poison Absorption

During the early phases of poison treatment or intervention for a toxic exposure via the oral, inhalational, or topical route, a significant opportunity exists to prevent further absorption of the poison by minimizing the total amount that reaches the systemic circulation. For toxins presented by the inhalational route, the main intervention used to prevent further absorption involves removing the patient from the environment where the toxin is found and providing adequate ventilation and oxygenation for the patient. For topical exposures, clothing containing the toxin must be removed and the skin washed with water and mild soap taking care not to cause cutaneous abrasions that may enhance dermal absorption.

T e four primary methods to prevent continued absorption of an oral poison are induction of emesis with syrup of ipecac, gastric lavage, oral administration of activated charcoal, and whole bowel irrigation. Although potentially indicated for individuals who are hours away from a medical facility, syrup of ipecac use for induction of emesis in the treatment of a potentially toxic ingestion has declined. Risk of cardio- and neurotoxicity and lower effectiveness at removing the toxicant than desired limit its use. Likewise, gastric lavage, which involves placing an orogastric tube into the stomach and aspirating fluid, and then cyclically instilling fluid and aspirating until the ef uent is clear, is limited by the risk of aspiration during the lavage procedure and evidence of limited effectiveness.

For many years, orally administered activated charcoal has been routinely incorporated into the initial treatment of a patient poisoned by the oral route. T e term activated means that the charcoal has been specially processed to be more efficient at adsorbing toxins.

T e usefulness of whole bowel irrigation for a poisoned patient is very limited. Considerable absorption of the toxicant can occur before the procedure "washes" the lumen of the GI tract clear of unabsorbed material. T e best evidence for efficacy of this procedure in the setting of poisoning is for removal of ingested packets of illegal drugs swallowed by people smuggling the material and hoping to avoid detection by concealing the agents in their intestines.

Enhancement of Poison Elimination

T ere are several methods available to enhance the elimination of specific poisons or drugs once they have been absorbed into the systemic circulation. T e primary methods employed for this use today include alkalinization of the urine, hemodialysis, hemoperfusion, hemofiltration, plasma exchange or exchange transfusion, and serial oral activated charcoal.

T e use of urinary alkalinization results in enhancement of the renal clearance of weak acids. T e basic principle is to increase the pH of urinary filtrate to a level sufficient to ionize the weak acid and prevent renal tubule reabsorption of the molecule (ion trapping). Although there are potentially similar advantages to be gained from acidification of the urine in order to enhance the clearance of weak bases, this method is not used because acute renal failure and acid–base and electrolyte disturbances are associated with acidification.

T e dialysis technique, either peritoneal dialysis or hemodialysis, relies on passage of the toxic agent through a semipermeable dialysis membrane so that it can subsequently be removed. Hemodialysis incorporates a blood pump to pass blood next to a dialysis membrane, which allows agents permeable to the membrane to pass through and reach equilibrium. Some drugs are bound to plasma proteins and so cannot pass through the dialysis membrane; others are distributed mainly to the tissues and so are not concentrated in the blood, making dialysis impractical. Hemodialysis has been shown to be clinically effective in the treatment of poisoning by the drugs and toxins shown in Table 33–5.

T e technique of hemoperfusion is similar to hemodialysis except there is no dialysis membrane or dialysate involved in the procedure. T e patient's blood is pumped through a perfusion cartridge, where it is in direct contact with adsorptive material (usually activated charcoal). Protein binding does

TABLE 33–5 Dif erential diagnosis of elevated osmol gap.

Methanol
Ethanol
Ethylene glycol
Isopropanol

not significantly interfere with removal by hemoperfusion. Because of the more direct contact of the patient's blood with the adsorptive material, the medical risks of this procedure include thrombocytopenia, hypocalcemia, and leukopenia.

The technique of hemofiltration is relatively new in clinical toxicology applications. As in the case of hemodialysis, the patient's blood is delivered through hollow fiber tubes and an ultrafiltrate of plasma is removed by hydrostatic pressure from the blood side of the membrane. The perfusion pressure for the technique is generated either by the patient's blood pressure (for arteriovenous hemofiltration) or by a blood pump (for venovenous hemofiltration). Needed fluid and electrolytes removed in the ultrafiltrate are replaced intravenously with sterile solutions.

The use of either plasma exchange or exchange transfusions has been relatively limited in the field of clinical toxicology. Although the techniques afford the potential advantage of being able to remove high-molecular-weight and/or plasma protein-bound toxins, their clinical utility in poison treatment has been limited. Plasma exchange, or pheresis, involves removal of plasma and replacement with frozen donor plasma, albumin, or both with intravenous fluid. The risks and complications of this technique include allergic-type reactions, infectious complications, and hypotension. Exchange transfusion involves replacement of a patient's blood volume with donor blood. The use of this technique in poison treatment is uncommon and mostly confined to inadvertent drug overdose in a neonate or premature infant.

Serial oral administration of activated charcoal, also referred to as multiple-dose activated charcoal (MDAC), has been shown to increase the systemic clearance of various drug substances. The mechanism for the observed augmentation of nonrenal clearance caused by repeated doses of oral charcoal is thought to be transluminal efflux of the drug from the blood to the charcoal passing through the gastrointestinal tract. The activated charcoal in the gut lumen serves as a "sink" for the toxin. A concentration gradient is maintained and the toxin passes continuously into the gut lumen, where it is adsorbed to charcoal. In addition, MDAC is thought to produce its beneficial effect by interrupting the enteroenteric–enterohepatic circulation of drugs. The technique involves continuing oral administration of activated charcoal beyond the initial dosage every 2 to 4 h. An alternative technique is to give a loading dose of activated charcoal via an orogastric tube or nasogastric tube, followed by a continuous infusion intragastrically. A list of agents for which MDAC has been shown to be an effective means of enhanced body clearance is given in Table 33–6.

TABLE 33–6 Chemicals for which hemodialysis has been shown effective as a treatment modality for poisoning.

Alcohols	Meprobamate
Antibiotics	Metformin
Boric acid	Paraldehyde
Bromide	Phenobarbital
Calcium	Potassium
Chloral hydrate	Salicylates
Fluorides	Strychnine
Iodides	Theophylline
Isoniazid	Thiocynates
Lithium	Valproic acid

Use of Antidotes in Poisoning

A relatively small number of specific antidotes are available for clinical use in the treatment of poisoning. The U.S. Food and Drug Administration (FDA) has placed incentives for sponsors to develop drugs for rare diseases or conditions through the Orphan Drug Act.

The mechanism of action of various antidotes is quite different. For example, a chelating agent or Fab fragments specific to digoxin will work by physically binding the toxin, preventing the toxin from exerting a deleterious effect in vivo, and, in some cases, facilitating body clearance of the toxin. Other antidotes pharmacologically antagonize the effects of the toxin. Atropine, an antimuscarinic, anticholinergic agent, is used to pharmacologically antagonize at the receptor level the effects of organophosphate insecticides that produce lethal cholinergic, muscarinic effects. Certain agents exert their antidote effects by chemically reacting with biologic systems to increase detoxifying capacity for the toxin. For example, sodium nitrite is given to patients poisoned with cyanide to cause formation of methemoglobin, which serves as an alternative binding site for the cyanide ion, thereby making it less toxic to the body.

Supportive Care of the Poisoned Patient

The supportive care phase of poison treatment is very important. Not only are there certain poisonings that have delayed toxicity, but there are also toxins that exhibit multiple phases of toxicity. Close clinical monitoring can detect these later-phase poisoning complications and allow for prompt medical intervention.

Another important component of the supportive care phase of poison treatment is the psychiatric assessment. Generally, a patient who has attempted suicide should be constantly monitored until he or she has been evaluated by the psychiatric consultant and judged to be at low risk for being without constant surveillance. In many cases, it is not possible to perform a psychiatric interview of the patient during the early phases of treatment and evaluation. Once the patient has been stabilized and is able to communicate, a psychiatric evaluation should be obtained.

CASE EXAMPLES OF SPECIFIC POISONINGS

Acetaminophen

A 16-year-old female patient arrives in the ED by ambulance after being found by a parent in what appeared to be an intoxicated state with empty pill bottles scattered about her room. T e parent reports the patient was despondent recently after breaking up with her boyfriend. T e patient is tearful and reports abdominal pain and admits to drinking alcohol and taking over-the-counter (OTC) pills in an apparent suicide attempt. T e estimated time of ingestion is 6 h prior to arrival in the ED. T e patient does not use prescription, OTC medications, or dietary supplements and is not known to have a history of regular consumption of alcoholic beverages or use illicit drugs.

On physical examination the blood pressure was 118/80 mm Hg, pulse 88/min and regular, respiratory rate 18/min, and temperature 37.0°C. She was awake and oriented, responded to questions appropriately with slightly slurred speech. Other pertinent findings included normal bowel sounds with mild epigastric tenderness. T e neurologic examination was only significant for slightly slurred speech.

T e patient was given 1.5 g/kg oral activated charcoal as a slurry in a sorbitol cathartic. Forty minutes later, the laboratory results showed a mildly increased white blood cell count, liver transaminase values elevated to approximately three times the upper limit of normal, and an acetaminophen concentration was 308 μg/mL. Based on the Rumack–Matthew nomogram, which plots acetaminophen plasma concentration versus hours of after ingestion, one can discern whether hepatic toxicity is probable. For example, a plasma acetaminophen concentration of 308 μg/mL at approximately 6 h after ingestion was well within the "probable hepatic toxicity" range, and treatment with N-acetylcysteine NAC was initiated.

T e patient received the first dose of IV NAC in the ED and was admitted to the medical ward to complete the treatment course of IV NAC. Transient increases of hepatic transaminases were measured over the ensuing two days of the hospitalization. T e psychiatry consultation service determined she was not actively suicidal; she was discharged from the hospital two days after admission with scheduled psychiatric and medical follow-up appointments.

T e clinical presentation of patients poisoned with acetaminophen is sufficiently confusing in some cases; it is difficult to estimate the time of ingestion. Due to the paucity of clinical symptoms with acute overdose, most clinicians will request an acetaminophen concentration be measured for any patient suspected of having a toxic exposure to any substance. T e paucity of signs and symptoms associated with an acetaminophen overdose makes inadvertent missing of a potentially fatal overdose until the window for maximum antidote effectiveness has passed.

Acetaminophen in normal individuals is inactivated by sulfation and glucuronide conjugation, with about 4% biotransformed by CYP2E1 to a toxic metabolite that is normally detoxified by conjugation with glutathione and excreted as the mercapturate. Patients who are concurrently using, or have recently used, agents that induce CYP2E1 may produce more than 4% of the toxic metabolite. When there is evidence (medical history) of concurrent chemicals that induce CYP2E1, the treatment nomogram from acetaminophen should be modified to a lower threshold for treatment with NAC.

Follow-up liver biopsy studies of patients who have recovered three months to a year after hepatotoxicity have demonstrated no long-term sequelae or chronic toxicity. A very small percentage (0.25%) of patients in the national multiclinic study conducted in Denver may progress to hepatic encephalopathy with subsequent death. T e clinical nature of the overdose is one of a sharp peak of serum glutamic-oxaloacetic transaminase (SGOT) by day 3, with recovery to less than 100 IU/L by day seven or eight. Patients with SGOT levels as high as 20 000 IU/L have shown complete recovery and no sequelae one week after ingestion.

Laboratory evaluation of a potentially poisoned patient is crucial in terms of both hepatic measures of toxicity and plasma levels of acetaminophen. Accurate estimation of acetaminophen in the plasma should be done on samples drawn at least 4 h after ingestion, when peak plasma levels can be expected.

Once an accurate plasma level has been obtained, it should be plotted on the Rumack–Matthew nomogram to determine if NAC therapy is indicated. T is nomogram is based on a series of patients with and without hepatotoxicity and their corresponding measured plasma acetaminophen concentrations.

Ethylene Glycol

A 37-year-old female was brought to the ED after being found unresponsive in her home. At the scene, emergency medical personnel administered oxygen and naloxone and performed a finger stick for glucose (standard procedure for a person with altered mental status and suspected toxic ingestion), which showed a normal value of 95 mg/dL. T e patient's spouse reported that she had been depressed and despondent with the recent loss of her job. No empty pill bottles or liquid containers were found with her at home.

Upon arrival to the hospital, she remained comatose. Her vital signs were: blood pressure 105/65 mm Hg, pulse 78/min, respiratory rate elevated at 32/min, and her body temperature was normal. T e remainder of the physical examination was significant as her pupils were 3 mm and sluggishly reactive to light; the lung and heart examinations were normal; the abdominal examination revealed diminished but present bowel sounds, and no tenderness, organomegaly, or masses were detected. T e rectal examination was normal; the stool was without detectable gross or occult blood. Neuro examination was nonfocal with a diminished gag reflex.

T e patient was placed on a cardiac monitor, an IV line was started, clinical laboratory specimens were obtained, and she was placed on oxygen, given naloxone, thiamine, and dextrose (50%) intravenously. Chest and abdominal radiography was without abnormality. A 12-lead ECG was also normal. Faced with the uncertainty of oral ingestion versus topical and inhalation exposure, a decision was made to proceed with gastric decontamination. T e patient was endotracheally intubated to protect her airway before an orogastric tube was placed. Gastric lavage was performed and no blood was found. T e fluid withdrawn from the stomach was bright yellow in appearance and slightly viscous. When a Wood's lamp illuminated this fluid in a darkened room, fluorescence was observed. T is finding suggests the presence of automotive antifreeze that contains ethylene glycol. Activated charcoal (2.0 g/kg) was placed via the orogastric tube into the stomach with a cathartic even though the efficacy for binding ethylene glycol is limited; the use of activated charcoal here was for other, potentially unknown coingestants. Clinical laboratory results returned showing the following:

Na = 140 mEq/L	K = 3.1 mEq/L
Cl = 94 mEq/L	HCO_3 = 8 mEq/L
BUN = 12 mg/dl	Glucose = 100 mg/dl

Arterial blood gas:
pH = 7.20; pCO_2 = 20 mm Hg; pO_2 = 98 mm Hg

T e complete blood count was normal, the urine analysis was normal, measured serum osmolarity was 330 mOsm/kg, and acetaminophen and salicylate levels were below the limits of detection, and the urine toxicology screen was negative.

T e laboratory results were interpreted as follows: a metabolic acidosis with elevated AG (AG = 38) and an elevated osmol gap (40 mOsm). T ese findings are consistent with either methanol or ethylene glycol poisoning (Tables 33–4 and 33–5). T e patient was treated with IV fomepizole (4-methylperazole), sodium bicarbonate was given intravenously for the profound metabolic acidosis, and the patient underwent hemodialysis. After 4 h of hemodialysis, the acid–base and electrolyte abnormalities were corrected but the patient remained comatose. T e patient underwent a second 4-h course of hemodialysis 8 h later to again correct her metabolic acidosis with the appearance of minor renal injury (serum creatinine increased to 1.8 mg/dL). She regained normal consciousness within 18 h and her renal function recovered completely within three days. Subsequently, the patient admitted that she intentionally drank more than half a container of antifreeze with the intent of harming herself. She was evaluated by the psychiatry consultation service and transferred to their service for further care.

Ethylene glycol exerts primary toxicity after undergoing biotransformation by alcohol dehydrogenase to glycolic acid and then to glycolic and oxalic acid by the action of aldehyde dehydrogenase. T e latter two acid metabolites are thought to be responsible for both the renal and the acid–base toxicity observed during poisoning by ethylene glycol. If untreated or treated too late, ethylene glycol poisoning can result in fatal cerebral edema with seizures as well as irreversible renal damage.

Valproic Acid

A 33-year-old male was brought to the ED after being found unresponsive with two empty prescription pill bottles of extended release valproic acid at his side. He was last seen 8 h prior to being found unresponsive and was then in normal health. The pharmacy confirmed that monthly prescriptions, each containing 30, 250 mg extended release valproic acid tablets, had been dispensed within the preceding three months.

T e patient was unresponsive to verbal or tactile stimulation. Vital signs were blood pressure 85/55 mm Hg, pulse 94/min, respiratory rate 20/min, and temperature 33.2°C. Naloxone was administered without effect. A cardiac monitor showed sinus rhythm. T e physical examination showed the patient to be without obvious signs of trauma; the skin was cool and without track marks; the pupils were 2 mm and poorly reactive to light; bowel sounds were diminished. T e rectal examination was negative for occult blood. T e neurologic examination revealed coma without focal motor abnormalities and an absent gag reflex.

Initial laboratories showed mild metabolic acidosis with elevated serum lactate, an increased anion, slightly increased serum ammonia, normal glucose, liver function tests, and renal function tests. T e chest and abdominal radiographs were normal. T e 12-lead ECG showed a prolonged QT interval without arrhythmia. T e patient was endotracheally intubated to protect his airway prior to gastric lavage that yielded some pill fragments only. T e patient was placed on a ventilator to support his respiration. Activated charcoal (1.5 g/kg) was administered via the orogastric tube immediately following the lavage procedure. T e blood pressure continued to remain low despite IV fluid administration. A STAT valproic acid serum measurement showed the concentration was 572 μg/mL.

Blood pressure responded to low-dose vasopressors (IV dopamine) with continued IV fluid administration. A repeat serum valproic acid concentration was 890 μg/mL at 2 h postadmission. Serial oral activated charcoal (every 4 h) was initiated via the orogastric tube and hemodialysis was started 3 h after admission. IV l-carnitine was given when a repeat serum ammonia concentration was further elevated at 94 mg/dL. Subsequent measured plasma concentrations of valproic acid gradually declined to < 100 μg/mL over the next 48 h after one additional hemodialysis session was conducted. T e patient regained consciousness 24 h after admission and made a full recovery by the fourth hospital day. T e psychiatry consultation service accepted the patient in transfer to

their inpatient service after he was medically cleared by the toxicology service.

T e increasing plasma concentrations of the toxic substance despite gastric decontamination procedures can occur after ingestion of an extended release formulation, which is pharmaceutically designed to slowly dissolve in the gastrointestinal tract and provide for ongoing sustained release of the active drug product as opposed to immediate release of the agent. Drug substances that demonstrate the "slow release" profile without having been formulated in a sustained release dosage form include salicylates and barbiturates as well as formulations of iron supplements. T e presence of a drug bezoar or concretion can be dangerous because the treating team could erroneously stratify a patient based on the initial measured plasma concentration and be unprepared for severe toxicity or a prolonged toxicity time course.

Moderate to severe valproic acid intoxication leads to depletion of l-carnitine, which may cause the observed hyperammonemia. T e FDA has recently approved the use of IV l-carnitine for the treatment of valproic acid poisoning in the setting of hepatotoxicity, hyperammonemia, large overdoses of valproate by history, or measured serum concentrations of valproic acid exceeding 450 μg/mL.

CONCLUSION

Clinical toxicology encompasses the expertise in the specialties of medical toxicology, applied toxicology, and clinical poison information specialists. T e clinical science has significantly evolved to the present state of the discipline over the past 50 years or more. T e incorporation of evidence-based, outcome-driven practice recommendations has significantly improved the critical evaluation of treatment modalities and methods for poison treatment. A careful diagnostic approach to a poisoned patient is essential, as important medical history is often absent or unreliable. Skillful use of antidotes is an important component of the practice of medical toxicology. Continued research will increase the repertoire of effective treatments for poisoning and ultimately improve clinical practice.

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QUESTIONS

1. What is the primary goal in taking a history in a poisoned patient?
   a. determining drug allergies.
   b. determining susceptibility to drug overdose.
   c. determining likelihood of an attempted suicide.
   d. determining the ingested substance.
   e. determining the motive behind the poisoning.

2. Who is most likely to give incorrect information while taking a history of a poisoned patient?
   a. patient.
   b. EMT.
   c. employer.
   d. pharmacist.
   e. family members.

3. Which of the following sets of clinical features characterizes an anticholinergic toxic syndrome?
   a. increased blood pressure, decreased heart rate, decreased temperature.
   b. decreased blood pressure, increased heart rate, decreased temperature.
   c. increased blood pressure, increased heart rate, increased temperature.
   d. decreased blood pressure, decreased heart rate, decreased temperature.
   e. increased blood pressure, decreased heart rate, increased temperature.

4. Which of the following sets of clinical features characterizes a sympathomimetic toxic syndrome?
   a. miosis, decreased bowel sounds, decreased alertness.
   b. decreased heart rate, increased temperature, mydriasis.
   c. hyperalertness, decreased blood pressure, miosis.
   d. increased temperature, increased heart rate, miosis.
   e. mydriasis, increased blood pressure, hyperalertness.

5. Which of the following drugs CANNOT be tested for in a hospital on a stat basis?
   a. ethanol.
   b. cocaine.
   c. aspirin.
   d. phenytoin.
   e. digoxin.

6. Which is NOT included in the differential diagnosis of an elevated anion gap?
   a. ethanol.
   b. methanol.
   c. diabetes.
   d. ethylene glycol.
   e. diarrhea.

7. An elevated osmol gap might suggest which of the following?
   a. methanol poisoning.
   b. chronic vomiting.
   c. lactic acidosis.
   d. diabetic ketoacidosis.
   e. chronic diarrhea.

8. Which of the following is LEAST likely to prevent further poison absorption?
   a. induction of emesis.
   b. activated charcoal.
   c. gastric lavage.
   d. syrup of ipecac.
   e. parasympathetic agonist.

9. Which of the following would NOT be used to enhance poison elimination?
   a. oral activated charcoal.
   b. hemoperfusion.
   c. acidification of urine.
   d. hemodialysis.
   e. plasma exchange.

10. Which of the following might be used as an antidote for patients with cyanide poisoning?
    a. syrup of ipecac.
    b. atropine.
    c. chelating agents.
    d. sodium nitrite.
    e. quinine.

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CHAPTER 34

Occupational Toxicology

Peter S. T orne

INTRODUCTION

WORKPLACES, EXPOSURES, AND STANDARDS

- Determinants of Dose
- Occupational Exposure Limits

OCCUPATIONAL DISEASES

- Routes of Exposure
- Agents Associated with Diseases
- Occupational Respiratory Diseases
- Other Occupational Diseases

TOXICOLOGIC EVALUATION OF OCCUPATIONAL AGENTS

- Evaluation of Occupational Risks
- Establishing Causality
- Animal Toxicology Testing for Establishing Acceptable Levels of Exposure
- Worker Health Surveillance
- Linkage of Animal Studies and Epidemiologic Studies

EXPOSURE MONITORING

- Environmental Monitoring for Exposure Assessment
- Biologic Monitoring for Exposure Assessment

CONCLUSION

KEY POINTS

- Occupational toxicology is the application of the principles and methodology of toxicology toward chemical and biologic hazards encountered at work.
- In occupational environments, exposure is often used as a surrogate for dose.
- Occupational exposure limits do not correspond to the level of exposure below which the probability of impairing the health of the exposed workers is acceptable.
- Diseases arising in occupational environments involve exposure primarily through inhalation, ingestion, or dermal absorption.

INTRODUCTION

T e work environment with its chemical and biologic hazards plays a role in the occurrence of adverse human health effects. Occupational toxicology is the application of the principles and methodology of toxicology toward chemical and biologic hazards encountered at work. T e objective of the occupational toxicologist is to prevent adverse health effects in workers that result from their work environment. Because the work environment often presents exposures to complex mixtures, the occupational toxicologist must also recognize exposure combinations that are particularly hazardous.

It is often difficult to establish a causal link between a worker's illness and job. First, the clinical expressions of occupationally induced diseases are often indistinguishable from those arising from nonoccupational causes. Second, there may be a long interval between exposure and the expression of disease. T ird, diseases of occupational origin may be multifactorial

with personal or other environmental factors contributing to the disease process. Ongoing assessments of occupational risk must occur as new hazards arise with the emergence of new technologies.

WORKPLACES, EXPOSURES, AND STANDARDS

Approximately 40% of the global work force works in agricultural production. T e demographics of laborers in industrial nations has shifted away from jobs in heavy industry toward jobs in the service sector and high-technology industries.

Determinants of Dose

Dose is defined as the amount of toxicant that reaches the target tissue over a defined time span. In occupational environments, exposure is often used as a surrogate for dose. T e response to a toxic agent is dependent on both host factors and dose. Figure 34–1 illustrates the pathway from exposure to subclinical disease or adverse health effect and suggests that there are important modifying factors: contemporaneous exposures, genetic susceptibility, age, gender, nutritional status, and behavioral factors. T ese modifying factors can inf uence whether a worker remains healthy, develops subclinical disease that is repaired, or progresses to illness. As illustrated in Figure 34–1, the dose is a function of exposure concentration, exposure duration, and exposure frequency. Individual and environmental characteristics also can affect dose. Table 34–1 indicates determinants of dose for exposure via the inhalation and dermal routes. Personal protective equipment must be used properly to maximize effectiveness.

TABLE 34–1 Determinants of toxicant dose.

Inhalation exposure
• Airborne concentration
• Particle size distribution
• Respiratory rate
• Tidal volume
• Other host factors
• Duration of exposure
• Chemical, physical, or biologic properties of the hazardous agent
• Effectiveness of personal protective devices
Dermal exposure
• Concentration in air, droplets, or solutions
• Degree and duration of wetness
• Integrity of skin
• Percutaneous absorption rate
• Region of skin exposed
• Surface area exposed
• Preexisting skin disease
• Temperature in the workplace
• Vehicle for the toxicant
• Presence of other chemicals on skin

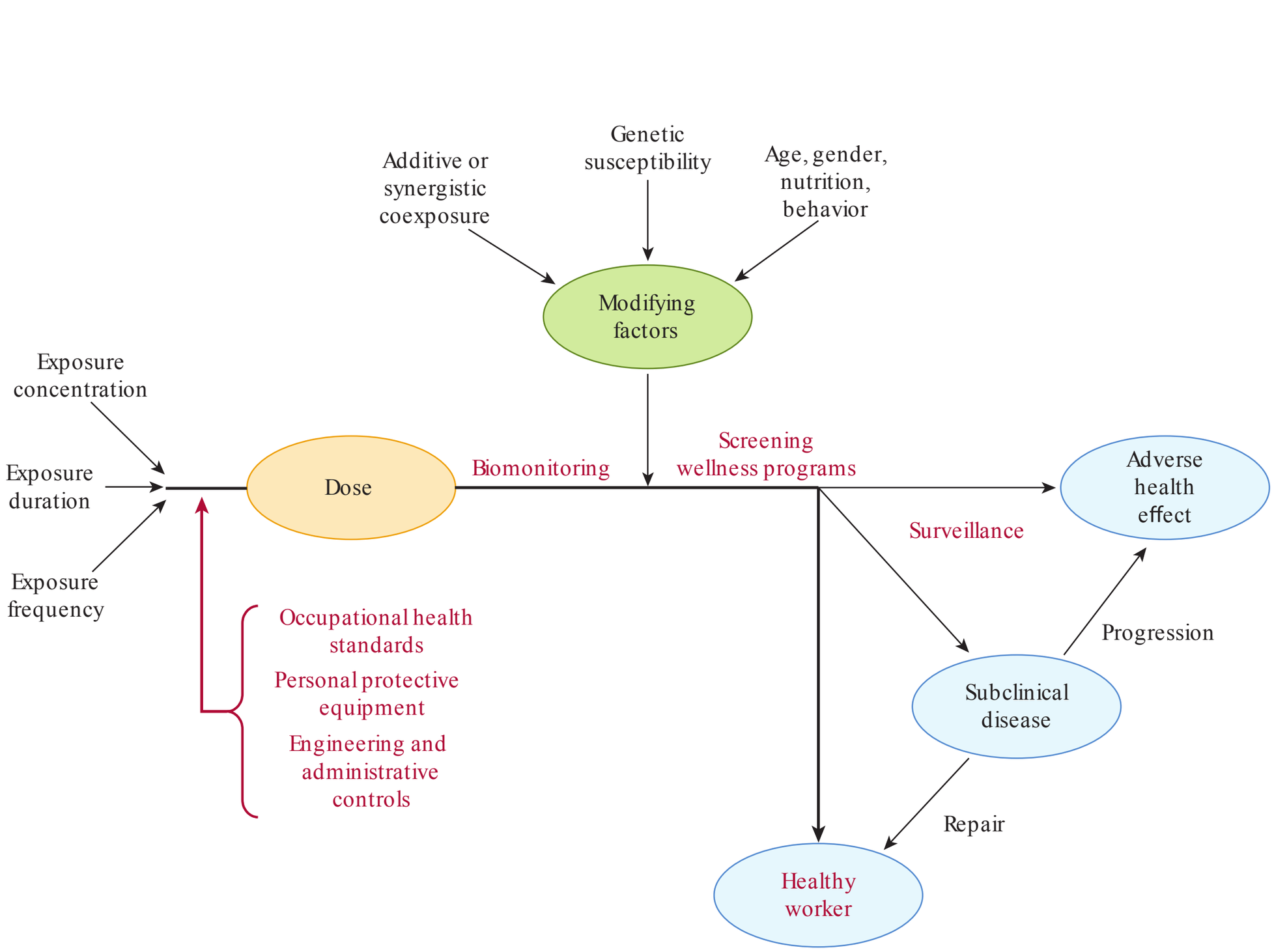

FIGURE 34–1 **Pathway from exposure to disease, showing modifying factors and opportunities for intervention.**

Occupational Exposure Limits

Workplace exposure limits exist for chemical, biological, and physical agents in order to promote worker health and safety. For chemical and biological agents, exposure limits are expressed as acceptable ambient concentration levels (occupational exposure limits [OELs]) or as concentrations of a toxicant, its metabolites, or a specific marker of its effects (biologic exposure indices [BEIs]).

OELs are established as standards by regulatory agencies or as guidelines by research groups or trade organizations. In the United States, the Occupational Safety and Health Administration under the Department of Labor promulgates legally enforceable standards known as permissible exposure limits (PELs). T e National Institute for Occupational Safety and Health (NIOSH), under the Centers for Disease Control and Prevention, publishes recommended exposure limits that are frequently updated and are generally more stringent than PELs.

T e European Commission has established legally enforceable binding occupational exposure limit values (BOELVs) and biologic limit values for the protection of worker health and safety.

T e American Conference of Governmental Industrial Hygienists is a trade organization that annually publishes OELs for chemicals and for physical agents. T ese take the form of threshold limit values (TLVs) and BEIs. T ey are developed as guidelines and are not enforceable standards.

OELs correspond to the level of exposure below which the probability of impairing the health of the exposed workers is acceptable. To determine that the risks from an occupational hazard are acceptable, it is necessary to characterize the hazard, identify the potential diseases or adverse outcomes, and establish the relationship between exposure intensity or dose and the adverse health effects.

OCCUPATIONAL DISEASES

Routes of Exposure

Diseases arising in occupational environments involve exposure primarily through inhalation, ingestion, or dermal absorption. Exposures leading to occupational infections may arise through inhalation or ingestion of microorganisms, from needle sticks in health care workers, or from insect bites among those who work outdoors. Additionally, poisonings from toxic plants or venomous animals can occur through skin inoculation (e.g., zookeepers, horticulturists, or commercial skin divers).

Agents Associated with Diseases

Table 34–2 outlines some major occupational diseases and examples of toxicants that cause them. Table 34–3 lists known human carcinogens (group 1), for which there are extensive occupational exposure.

Occupational Respiratory Diseases

Occupational lung diseases (such as coal workers' pneumoconiosis, asbestosis, and occupational asthma) are largely responsible for the creation of the occupational regulatory framework. Although death rates are fairly low, many exposures result in debilitating illnesses. Many of the diseases listed in Table 34–2 are known by other names that refer to a particular occupation or agent. One example is hypersensitivity pneumonitis, an allergic lung disease marked by interstitial lymphocytic pneumonitis and granulomatous lesions. Hypersensitivity pneumonitis is also known as extrinsic allergic alveolitis, farmer's lung disease, bagassosis (sugar cane), humidifier fever, Japanese summer house fever, pigeon breeder's lung, and maple bark stripper's lung, depending on the occupational setting in which it arises. Although we often think of these as the same disease, it is important to recognize that the exposures and physiologic responses they induce are complex and may differ in the manifestation of the disease.

Toxic gas injuries are often characterized by leakage of both f uid and osmotically active proteins from the vascular tissue into the interstitium and airways. T e vapors of anhydrous ammonia combine with water in the tissues of the eyes, sinuses, and upper airways and form ammonium hydroxide, quickly producing liquefaction necrosis. Chemicals with lower solubility, such as nitrogen dioxide, act more on the distal airways and alveoli and take longer to induce tissue damage.

Occupational asthma occurs when airways restrict in response to some stimulus present in the workplace. In chemical-based industries, plastic and rubber polymer precursors, diisocyanates, reactive dyes, and acid anhydrides are recognized low-molecular-weight sensitizing compounds. Biocides and fungicides used in metal fabrication and machining, custodial services, lawn and turf growing, and agriculture are also chemicals associated with occupational asthma. A number of metals can induce sensitization and asthma, including chromium, cobalt, nickel, platinum, and zinc. Enzymes include α-amylase among bakery workers and subtilisin, a protease used in laundry detergents.

Animal handlers, processors, and laboratory technicians who work with animals can become immunologically sensitized to urine or salivary proteins in many vertebrates; proteins in bat guano and bird droppings; animal dander; serum proteins in blood products; dust from horns, antlers, and tusks; or the shells of crustaceans. Very high rates of sensitization can occur in shellfish processors. Arthropods such as insect larvae, cockroaches, mites, or weevils are recognized inducers of work-related asthma. Plants and plant products (e.g., soy f our, spices, and coffee beans) can also cause asthma among workers. Exposure to fungi, especially of the genera Aspergillus, Penicillium, Rhizopus, Mucor, and Paecilomyces, are associated with allergic rhinitis and asthma. T ese are especially present in sawmills, woodchip handling, and composting facilities.

An emerging area of concern is adverse effects of respiratory exposures to manufactured nanomaterials. Occupational exposures occur in the manufacture of the nanomaterials and in their use in fabricating materials and consumer products. Exposures can also occur when nanomaterials are cut or shaped and when product waste is discarded. Engineered

TABLE 34–2 Examples of occupational diseases and the toxicants that cause them.

Organ System or Disease Group	Disease	Causative Agent
Lung and airways	Acute pulmonary edema, bronchiolitis obliterans	Nitrogen oxides, phosgene, diacetyl
	Allergic rhinitis	Pollens, fungal spores
	Asphyxiation	Carbon monoxide, hydrogen cyanide, inert gas dilution
	Asthma	Toluene diisocyanate, α-amylase, animal urine proteins
	Asthma-like syndrome	Swine barn environments, cotton dust, bioaerosols
	Bronchitis, pneumonitis	Arsenic, chlorine
	Chronic bronchitis	Cotton dust, grain dust, welding fumes
	Emphysema	Coal dust, cigarette smoke
	Fibrotic lung disease	Silica, asbestos
	Hypersensitivity pneumonitis	Thermophilic bacteria, avian proteins, pyrethrum, Penicillium, Aspergillus
	Metal fume fever	Zinc, copper, magnesium
	Mucous membrane irritation	Hydrogen chloride, swine barn environments
	Organic dust toxic syndrome	"Moldy" silage, endotoxin
	Upper respiratory tract inflammation	Endotoxin, peptidoglycan, glucans, viruses
Cancer	Acute myelogenous leukemia	Benzene, ethylene oxide
	Bladder cancer	Benzidine, 2-naphthylamine, 4-biphenylamine
	Gastrointestinal cancers	Asbestos
	Hepatic hemangiosarcoma	Vinyl chloride
	Hepatocellular carcinoma	Aflatoxin, hepatitis B virus
	Mesothelioma, lung carcinoma	Asbestos, arsenic, radon, bis-chloro methyl ether
	Skin cancer	Polycyclic aromatic hydrocarbons, ultraviolet irradiation
Skin	Allergic contact dermatitis	Natural rubber latex, isothiazolins, poison ivy, nickel
	Chemical burns	Sodium hydroxide, hydrogen fluoride
	Chloracne	TCDD, polychlorinated biphenyls
	Irritant dermatitis	Sodium dodecyl sulfate
Nervous system	Cholinesterase inhibition	Organophosphate insecticides
	Neuronopathy	Methyl mercury
	Parkinsonism	Carbon monoxide, carbon disulfide
	Peripheral neuropathy	N-Hexane, trichloroethylene, acrylamide
Immune system	Autoimmune disease	Vinyl chloride, silica
	Hypersensitivity	See entries for allergic rhinitis, asthma, hypersensitivity pneumonitis, allergic contact dermatitis
	Immunosuppression	TCDD, lead, mercury, pesticides
Renal disease	Indirect renal failure	Arsine, phosphine, trinitrophenol
	Nephropathy	Paraquat, 1,4-dichlorobenzene, mercuric chloride
Cardiovascular disease	Arrhythmias	Acetone, toluene, methylene chloride, trichloroethylene
	Atherosclerosis	Dinitrotoluene, carbon monoxide
	Coronary artery disease	Carbon disulfide
	Cor pulmonale	Beryllium
	Systemic hypotension	Nitroglycerine, ethylene glycol dinitrate
Liver disease	Fatty liver (steatosis)	Carbon tetrachloride, toluene
	Cirrhosis	Arsenic, trichloroethylene
	Hepatocellular death	Dimethylformamide, TCDD
Reproductive system	Male	Chlordecone (Kepone), dibromochloropropane, hexane
	Female	Aniline, styrene
	Both sexes	Carbon disulfide, lead, vinyl chloride
Infectious diseases	Arboviral encephalitides	Alphavirus, Bunyavirus, Flavivirus
	Aspergillosis	Aspergillus niger, A. fumigatus, A. flavus
	Cryptosporidiosis	Cryptosporidium parvum
	Hepatitis B	Hepatitis B virus
	Histoplasmosis	Histoplasma capsulatum
	Legionellosis	Legionella pneumophila
	Lyme disease	Borrelia burgdorferi
	Psittacosis	Chlamydia psittaci
	Tuberculosis	Mycobacterium tuberculosis hominis

TCDD, 2,3,7,8-tetrachlorodibenzo-para-dioxin.

TABLE 34–3 Occupational exposure agents classified by IARC as group 1 definite human carcinogens.

Agent	Industries and Occupations Where Some Workers May be Exposed
Particulate matter	
Asbestos	Miners, abatement workers, construction workers, sheet metal workers, steam fitters, shipyard workers
Crystalline silica (quartz or cristobalite)	Stone and ceramics industry, foundries, construction, abrasives manufacturing
Erionite	Waste treatment workers, building materials manufacturing
Hematite	Underground mining
Talc containing asbestiform fibers	Ceramics industry
Wood dust	Wood and wood-products industries, pulp and paper industry, wood working trades
Metals	
Arsenic and arsenic compounds	Miners, nonferrous metal smelting, arsenical pesticide manufacturers and applicators
Beryllium	Specialty metallurgy workers, avionics, electronics, nuclear industry
Cadmium and cadmium compounds	Cadmium smelting, battery production, dyes and pigment making, electroplating
Gallium arsenide	Microelectronics manufacturing
Hexavalent chromium compounds	Chromate production plants, dye and pigment making, welders, tanners
Nickel compounds*	Nickel smelting, welding
Organic chemicals	
Aflatoxin	Animal feed industry, grain handling and processing
4-Aminobiphenyl	Chemical industry, dyes and pigment manufacturing
Benzene	Refineries, shoe industry, chemical, pharmaceutical and rubber industry, printing industry
Benzidine	Chemical industry, dyes and pigment manufacturing
Benzo(a)pyrene	Coke oven emissions, coal tar pitch volatiles, diesel exhaust, environmental tobacco smoke
Bis(chloromethyl) ether and chloromethyl ether (technical grade)	Chemical industry, laboratory reagent, plastic manufacturing
1,3-Butadiene	Chemical industry, petrochemical plants, styrene–butadiene rubber manufacturing
Coal tars and pitches	Coke production, coal gasification, refineries, foundries, road paving, hot tar roofing
Ethylene oxide	Chemical industry, dry vegetable fumigation, hospital sterilizing
Formaldehyde	Textiles, composite wood industry, chemical industry, medical laboratories
4,4′-Methylenebis(2-chloroaniline)	Epoxy resin manufacturing, polyurethane product fabrication
Mineral oils, untreated and mildly treated	Metal machining and honing, roll steel production, printing
2-Naphthylamine	Chemical industry, dyestuffs, and pigment manufacturing
2,3,4,7,8-Pentachlorodibenzofuran	Hazardous waste processing, chlorophenoxy herbicide production and use, pulp and paper industry
3,4,5,3′,4′-Pentachlorobiphenyl	Hazardous waste processing, waterway dredging, transformer handling, pulp and paper industry
Shale oils or shale-derived lubricants	Mining and processing, cotton textile industry
Soots	Chimney sweeps, heating and ventilation contractors, firefighters, metallurgical workers
2,3,7,8-Tetrachlorobibenzo-para-dioxin (TCDD)	Hazardous waste processing, chlorophenoxy herbicide production and use, pulp and paper industry
Vinyl chloride	Plastics industry, production of polyvinyl chloride products and copolymers
Other agents with occupational exposure	
Environmental tobacco smoke	Restaurant, bar and entertainment industry; other smoke-exposed workers
Occupational exposures as a painter	Commercial painting
Leather dust	Garment industry, auto seat fabrication, saddle and tack manufacturing
Magenta dye (rosaniline, pararosaniline)	Dye manufacture, textile dying, commercial art and printing
Mustard gas	Production, soldiers, some research laboratories
Exposures in the rubber industry	Work in rubber manufacturing industries
Strong inorganic acid mists containing sulfuric acid	Steel industry, petrochemical industry, fertilizer industry, pickling industry
Physical Agents	
Ionizing radiation†	Radiology and nuclear medicine staff, nuclear workers, miners, hazardous waste workers
Solar radiation	Farmers, gardeners and landscapers, lifeguards, construction workers

*Certain combinations of nickel oxides and sulfides.
†Includes X-rays, γ-rays, neutrons, radon gas, and α and β particle–emitting substances internally deposited.

nanomaterials may be carbon-based, metal-based, or biologic in nature. Inhaled nanomaterials may induce pulmonary toxicity or they can cause adverse effects in other tissues through adsorption and transport, generation of toxic substances by their dissolution or degradation, or by crossing key physiologic barriers, or cell and nuclear membranes.

Other Occupational Diseases

Occupational toxicants may induce diseases in a variety of sites distant from the lung or skin. These include tumors arising in the liver, bladder, gastrointestinal tract, or hematopoietic system and are attributable to a variety of chemical classes.

Nervous system damage can be central, peripheral, or both. It may be acute, as with some organophosphate exposures, or chronic, as with organomercury poisoning or acrylamide-induced neuropathy. Immune system injury may arise from the immunosuppressive effects of chemicals or from hypersensitivity leading to respiratory or dermal allergy or systemic hypersensitivity reactions. Autoimmune syndromes have been associated with occupational exposures to crystalline silica and vinyl chloride.

Occupational diseases of the cardiovascular system include atherosclerosis, various arrhythmias, impaired coronary blood supply, systemic hypotension, and right ventricular hypertrophy usually due to pulmonary hypertension. Liver diseases include carbon tetrachloride–induced fatty liver. Occupational diseases of the reproductive system can be gender- and organ-specific, or may affect both sexes. Disease due to exposures to infectious agents occurs in such occupations as veterinarians, health care workers, biomedical researchers, and farmers.

Both industrial and nonindustrial indoor environments may pose occupational hazards due to the presence of chemical or biologic agents. Problems with ventilation and use of synthetic building materials have led to a rise in complaints associated with occupancy in buildings. Volatile and semivolatile chemicals are released from process materials in manufacturing, building materials, f oor coverings, furniture, cleaning products, biocides, and microorganisms. In some cases, the occupied space of a building may be clean and dry, but local amplification sites for molds, such as damp closets or sub-f oors, may develop. Airborne viruses, bacteria, and fungi are responsible for a variety of building-related illnesses.

TOXICOLOGIC EVALUATION OF OCCUPATIONAL AGENTS

Evaluation of Occupational Risks

To recommend an acceptable exposure level to an industrial chemical, one must attempt to define the risks associated with adverse effects in the most sensitive exposed populations. It then remains to decide what proportion of exposed subjects may still develop an adverse effect at the proposed acceptable exposure level.

Establishing Causality—In complex occupational environments, it may be difficult to establish a causal relationship between a toxic substance and a disease. A matrix was developed to evaluate the weight of evidence for a causal association between a toxicant and an occupational disease (Figure 34–2). Evidence from well-conducted in vitro studies, animal studies, human challenge studies (intentional clinical exposure to humans), case reports, and epidemiologic investigations are evaluated. T is evaluation is guided by seven criteria. If a chemical were thoroughly studied in animals, humans, and in vitro studies and produced clear and convincing evidence of an exposure–response relationship in controlled studies that used appropriate models and relevant endpoints, then that would constitute compelling evidence of a causal relationship between that chemical and that disease.

To evaluate with some degree of confidence, the level of exposure at which the risk of health impairment is acceptable, a body of toxicologic information is required. Five

	Assessment of exposure to specific agents	Consideration or control of confounders	Evidence of a dose – response relationship	Consistent results from different studies	Objective clinical data	Endpoints related to human pathology	Appropriate subjects or models
In vitro studies							
Animal studies							
Human challenge studies							
Case studies							
Epidemiology studies							

For each type of study listed in the first column weight the quality of data from existing studies based on the criteria listed in the column headings as follows:

0 No evidence or condition is not met
1 Equivocal evidence or condition is partially met
2 Some evidence or condition is mostly met
3 Clear evidence or condition is convincingly met

FIGURE 34–2 **Matrix for assessing the strength of an association between a toxicant and an occupational disease.**

sources of data may be available to inform the occupational risk-assessment process. T ese sources include in vitro assays, animal toxicology studies, human challenge studies, case reports, and epidemiology studies.

Animal Toxicology Testing for Establishing Acceptable Levels of Exposure

Animal studies provide valuable data from which to estimate the level of exposure at which the risk of health impairment is acceptable. Comparison of the animal studies with epidemiology testing is provided in Table 34–4. T e duration of tests necessary to establish an acceptable level for occupational exposure is primarily a function of the type of toxic action suspected. It is generally recognized that for systemically acting chemicals, subacute and short-term toxicity studies are usually insufficient for proposing OELs. Subacute and short-term toxicity tests are usually performed to find out whether the compound exhibits immunotoxic properties and cumulative characteristics. T ey also aid in selection of the doses for long-term exposures. Studies designed to evaluate reproductive effects and teratogenicity should also be considered.

Information derived from exposure routes similar to those sustained by workers is clearly most relevant. T e choice of what studies to perform using which routes of administration must be evaluated scientifically for each toxicant. Important considerations include its target sites and mechanism of action, metabolism, the nature of its adverse effects, and how workers are exposed to the toxicant. Investigations that can make use of specific physiologic or biochemical tests, based on knowledge of the principal target organ or function, produce highly valuable information and increase confidence in the OEL derived from them.

Worker Health Surveillance

T e primary objective of occupational toxicology is to provide both periodic screening of general health and wellness and health exposure monitoring tailored to recognized hazards of the workplace. Monitoring of exposures to toxicants in the workplace may be important in detecting excessive exposures before the occurrence of significant biologic disturbances and health impairment. When a new chemical is being used on a large scale, careful clinical surveillance of workers and monitoring of workplaces should be instituted. Evaluation of the validity of the proposed OEL derived from animal experiments through workplace surveillance is the major goal.

Epidemiologic studies designed to assess exposure–response relationships will have more validity if both the target dose and the critical biologic changes are monitored in exposure–response studies. Knowledge of the fate of the chemical in the organism and its mechanism of action are required. Because early biomarkers of effect are subtle and individual variations

TABLE 34–4 Comparison of epidemiologic studies and experimental exposure studies.

	Observational Epidemiologic Studies	Experimental Animal Exposure Studies
Toxicant exposure	Reflects true exposure among population at risk	Controlled to represent major toxicant of interest
Character	Complex and variable in space and time May include nonoccupational exposures to toxicant or related compounds	Usually one or two test compounds May not reflect complexity of human exposures
Frequency and duration	Work day, work week, and years in that job May be task specif c	Acute, subacute, subchronic, chronic
Exposure route	Inhalation, ingestion, percutaneous, or a combination	Injection, inhalation, oral, or dermal. Rarely a combination by design
Appropriateness of dose	Reflect the actual range of exposure	Often doses studied are far higher than human exposures
Assessment	Environmental sampling, or measurement of biomarkers May be retrospective and based on employer records, group-based approaches, or questionnaires	Measurement of administered dose with or without measurement of biomarkers Sampling of exposure chamber air for inhalation studies
Species considerations	Humans—cohorts or cases and controls Must protect the safety and conf dentiality of subjects	Laboratory animals, usually inbred strains of mice or rats Must ensure proper care and use of animals
Representativeness	May exist a selection bias such that the study population may not represent the occupational work force	Experimental animal species may not represent humans
Relevance to human health	Directly relevant if appropriate outcomes are studied	Relevant if species differences are known Of limited relevance if species or strain effects on absorption, distribution, metabolism, and disease are unknown
Analytical challenges	Selection bias, misclassif cation, and confounding in characterization of outcomes Within- and between-subject variance may be high	Control of genetics, feeding, and housing between exposed and control groups Low variance in outcomes

exist in the response to a chemical insult, results generally require a statistical comparison between a group of exposed workers and a similar group of workers without the exposure of interest. If exposure induces an adverse effect, it is expected that these studies may permit establishment of the relationship between integrated exposure (intensity × time) and frequency of abnormal results and, consequently, a redefinition of the OEL.

In cases where a surveillance program was not instituted before the introduction of a new chemical, it is more difficult to establish the efficacy of the exposure limit. In this situation, evaluation depends on retrospective cohort studies or case-control studies or on cross-sectional studies on workers who have already sustained exposure. In fact, case reports of isolated overexposures resulting from specific incidents such as containment breaches, chemical spills, or vessel or pipe ruptures can provide useful information. Such observations may indicate whether human symptomatology is similar to that found in animals and may suggest functional or biologic tests that might prove useful for routine monitoring of exposed workers.

Linkage of Animal Studies and Epidemiologic Studies

In the field of occupational toxicology, close cooperation between those conducting animal studies and those conducting studies of workers is essential for examining risks associated with overexposure to chemicals and other toxicants. Several occupational carcinogens have been identified clearly through combined epidemiologic and experimental approaches. For example, the carcinogenicity of vinyl chloride was first demonstrated in rats, and a few years later, epidemiologic studies confirmed the same carcinogenic risk for humans. T is observation stimulated several investigations on the metabolism of vinyl chloride in animals and on its mutagenic activity in in vitro systems, leading to a better understanding of its mechanism of carcinogenicity.

Studies of the metabolic handling of occupational toxicants in animals are instrumental in the characterization of reactive intermediates and may suggest unsuspected risks or indicate new methods of biologic monitoring. Conversely, clinical observations on workers may stimulate studies of the metabolism or the mechanism of toxicity of a toxicant in animals, thereby revealing the health significance of a biologic disturbance.

Arsenic is one of the very few compounds for which there are limited data of predictive value from animal studies to human health effects. Inorganic arsenic has been shown conclusively to cause human cancers of many organs, but not to cause cancer in animals. T is demonstrates that the occupational toxicologist cannot rely solely on animal or epidemiologic studies. A combined approach is necessary in order to identify, elucidate, and prioritize risks and to develop interventions and techniques for worker health surveillance.

EXPOSURE MONITORING

Environmental Monitoring for Exposure Assessment

A critical element of establishing OELs is the accurate and uniform assessment of exposure. Methodology for exposure assessment must be specifically tailored to the agent under study and the environment in which it appears. To assess airborne exposures, personal samples taken in the breathing zone are generally used. Repeated random sampling is usually the best approach to developing unbiased measures of exposure. Recent studies have demonstrated that group-based approaches, assessing exposures to groups rather than to individuals, are more efficient in terms of measurement effort for obtaining a desired level of accuracy.

Although one cannot assess dose directly through exposure monitoring, it has distinct advantages over biomonitoring, which cannot provide route-specific exposure data. Exposure monitoring allows one to quantify workplace exposure by route through selective air monitoring in the breathing zone of the worker and dermal dosimetry using absorptive material affixed to the workers' skin or clothing. Environmental monitoring techniques are generally less expensive and less invasive than techniques involving the collection and analysis of biologic samples such as blood or urine. Spatial, temporal, and work practice associations can be established by air monitoring and can suggest better interventions and engineering controls. Exposure monitoring allows one to quantify workplace exposure by route through selective air monitoring in the breathing zone of the worker and dermal dosimetry using absorptive material affixed to the workers' skin or clothing.

A fully validated sampling and analysis method requires specification of the sampling methods; sample duration, handling, and storage procedures; the analytic method and measurement technique; the range, precision, accuracy, bias, and limits of detection; quality assurance issues; and known interferences. It is also important to document intralaboratory and interlaboratory variability. Once a standard method is established, it must be closely followed in every detail in order to assure consistency of results.

Biologic Monitoring for Exposure Assessment

Biomonitoring consists of the measurement of toxicants, their metabolites, or molecular signatures of effect in specimens from humans or animals, including urine, blood, feces, exhaled breath, hair, fingernails or toenails, bronchial lavage, breast milk, and adipose tissue. T ese may serve as biomarkers of exposure, biologic effect, or susceptibility. Emerging technologies will allow measurement and monitoring of chemicals in the body and transmission of the data from indwelling biosensors. Biomonitoring data provide a measurement of exposure based on internalized dose, or the amount

of chemical stored in one or in several body compartments or in the whole body, and, thus, account for all exposures by all routes for the assessed analyte.

T e term internalized dose may have different meanings. T e measured biomarker may ref ect the amount of chemical absorbed shortly before sample collection, as with the concentration of a solvent in exhaled air or in a blood sample obtained during the work shift. It may ref ect exposure during the preceding day, as with the measurement of a metabolite in blood or urine collected after the end of exposure. For toxicants with a long biologic half-life, the measured parameter may ref ect exposure accumulated over a period of weeks or months, as with arsenic in toenails. Internal dose may refer to the amount of chemical stored in one or in several body compartments or in the whole body (the body burden).

T e greatest advantage of using biologic measurements is that the biologic parameter of exposure is more directly related to the adverse health effects than environmental measurements. It may offer a better estimate of the risk than can be determined from ambient monitoring. Biologic monitoring accounts for uptake by all exposure routes.

Several factors can inf uence uptake. Personal hygiene habits vary from one person to another, and there is some degree of individual variation in the absorption rate of a chemical through the lungs, skin, or gastrointestinal tract. Because of its ability to encompass and evaluate the overall exposure (whatever the route of entry), biologic monitoring also can be used to test the overall efficacy of personal protective equipment such as respirators, gloves, or barrier creams. Another consideration with biologic monitoring is the fact that the nonoccupational exposures (hobbies, residential exposures, dietary habits, smoking, and second jobs) also may be expressed in the biologic sample.

Relationships between air monitoring and biologic monitoring may be modified by factors that inf uence the fate of an occupational toxicant in vivo. Metabolic interactions can occur when workers are exposed simultaneously to chemicals that are biotransformed through identical pathways or that modify the activity of the biotransformation enzymes. Furthermore, metabolic interferences may occur between occupational toxicants and alcohol, tobacco, food additives, prescription drugs, natural product remedies, or recreational drugs. Changes in any of several biologic variables (weight, body mass, pregnancy, diseases, immune status, etc.) may modify the metabolism of an occupational chemical. T ese factors have to be taken into consideration when the results of biomonitoring are interpreted. Whatever the parameter measured, whether it is the substance itself, its metabolite, or an early biomarker of effect, the test must be sufficiently sensitive and specific to provide meaningful data in the range of workplace exposures.

CONCLUSION

In summary, environmental and biologic monitoring should be regarded as complementary elements in an occupational health and safety program. T e working environment will always present the risk of overexposure of workers to various toxicants. Recognition of these risks should not wait until epidemiologic studies have defined hazardous levels. A combined experimental, clinical, and epidemiologic approach is most effective for evaluating the potential risks, promulgating scientifically based occupational health standards, and implementing workplace controls to ensure adherence to the standards.

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QUESTIONS

1. Which of the following is NOT a modifying factor that can influence the likelihood of disease?
   a. age.
   b. dose.
   c. nutritional status.
   d. gender.
   e. genetic susceptibility.

2. Which of the following is LEAST likely to increase occupational inhalation of a chemical?
   a. increased airborne concentration.
   b. increased respiratory rate.
   c. increased tidal volume.
   d. increased particle size.
   e. increased length of exposure.

3. Which would increase the likelihood of toxic dosage through dermal exposure?
   a. no preexisting skin disease.
   b. toxic exposure to thick skin.
   c. increased percutaneous absorption rate.
   d. low surface area of exposure.
   e. high epidermal intercellular junction integrity.

4. Prolonged arsenic exposure could cause:
   a. infertility.
   b. cirrhosis.
   c. cor pulmonale.
   d. skin cancer.
   e. nephropathy.

5. Which of the following lung diseases has the highest occupational death rate?
   a. asbestosis.
   b. coal workers' pneumoconiosis.
   c. byssinosis.
   d. hypersensitivity pneumonitis.
   e. silicosis.

6. Lyme disease is caused by which of the following?
   a. B. burgdorferi.
   b. H. capsulatum.
   c. M. tuberculosis.
   d. L. pneumophila.
   e. C. psittaci.

7. Asbestos exposure is unlikely to cause:
   a. lung cancer.
   b. GI cancer.
   c. emphysema.
   d. pulmonary fibrosis.
   e. mesothelioma.

8. Exposure to which of the following can cause autoimmune disease?
   a. mercury.
   b. nitrogen dioxide.
   c. vinyl chloride.
   d. lead.
   e. flavivirus.

9. Which of the following might be linked to parkinsonism?
   a. nitrogen dioxide.
   b. zinc.
   c. copper.
   d. magnesium.
   e. carbon monoxide.

10. Which of the following infectious agents can cause hepatocellular carcinoma?
    a. flavivirus.
    b. bunyavirus.
    c. alphavirus.
    d. hepatitis C virus.
    e. hepatitis B virus.

Answers to Chapter Questions

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1. b.
2. a.
3. a.
4. d.
5. b.

Chapter 2

1. b.
2. c.
3. b.
4. d.
5. e.
6. e.
7. b.
8. c.
9. d.
10. a.

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7. c.
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5. e.
6. c.
7. b.
8. d.
9. b.
10. c.

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8. c.
9. c.
10. d.

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9. e.
10. e.

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5. a.
6. c.
7. d.
8. c.
9. e.
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5. c.
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