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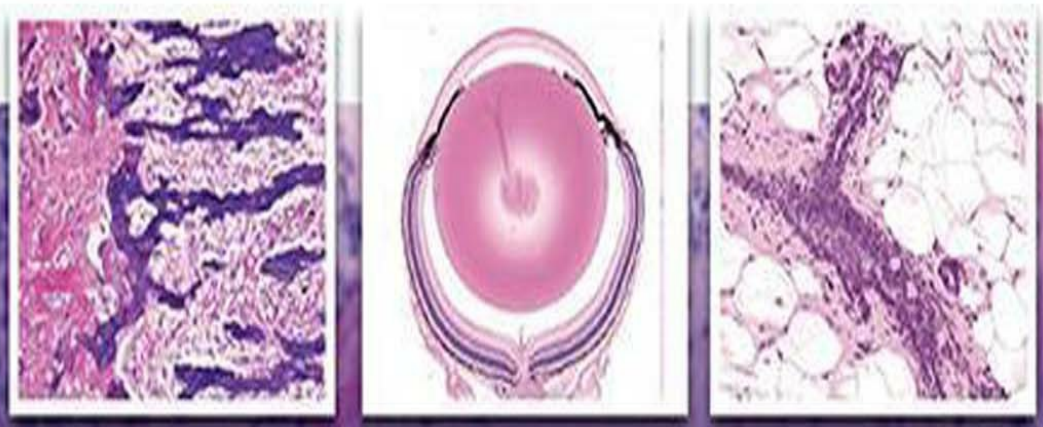
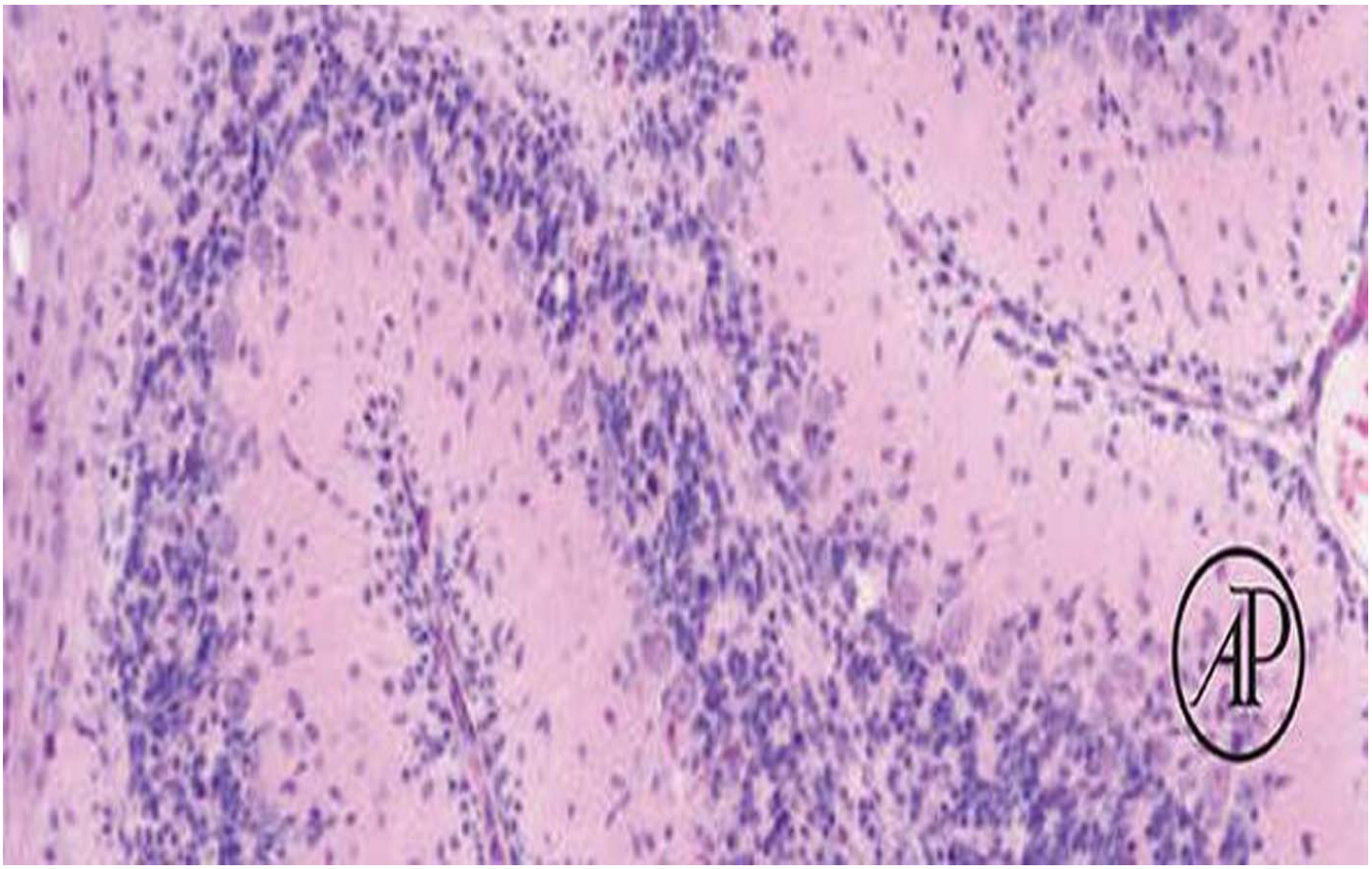


FUNDAMENTALS OF

TOXICOLOGIC PATHOLOGY

THIRD EDITION



Fundamentals of Toxicologic Pathology

THIRD EDITION

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“To teach is to learn…” Japanese Proverb

Dedication

We are profoundly grateful to those individuals responsible for our love of pathology and who mentored us during our careers as well as to our colleagues and students who inspired and supported us along this journey.

We also thank our partners, who encouraged and sustained us during our efforts to complete this revision.

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[†]In Memoriam

Preface

Matthew A. Wallig, Brad Bolon, Wanda M. Haschek, Colin G. Rousseaux and Beth W. Mahler

Fundamentals of Toxicologic Pathology, as stated in the preface of its first edition, "examines the interface between toxicology and pathology, providing an overview of structural alterations caused by toxicants and the mechanisms which result in those changes." It is designed as a "textbook" with easy and ready access to core information about toxicologic pathology. It is divided into two sections, Part I containing basic information pertaining to toxicology and pathology, and Part II in an organ systems format. The basis for this textbook is the *Handbook of Toxicologic Pathology*, a much larger reference work that comprehensively addresses virtually all aspects of toxicologic pathology and its multiple interfaces with numerous-related scientific disciplines. This edition updates and expands information presented in the second edition of *Fundamentals of Toxicologic Pathology*, not only to provide additional necessary core information regarding essential toxicologic principles, core mechanisms of injury, and basic tissue responses to toxic injury (Part I) but also to provide organ system-specific mechanisms of toxicity, responses to toxic injury, and basic methods of evaluating injury (Part II). The mammary gland and special senses have been added to the organ systems section.

As with the first and second editions, this third edition is focused toward entry-level professionals in the field of toxicologic pathology, mainly pathology/toxicology residents and graduate students, who require a brief but comprehensive overview that addresses the integration of structural and functional changes, which occur with toxic injury. In addition, this textbook is also a useful reference for more experienced pathologists, toxicologists, and other health professionals. Whether you are new to the field or already an accomplished practitioner, the editors and chapter authors hope that this volume serves you well.

The editors wish to acknowledge the efforts of the original authors of the chapters in the *Handbook of Toxicologic Pathology*, third edition, many of whom actively participated in updating and revising the chapters for this book.

CHAPTER 1

An Overview of Toxicologic Pathology

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OUTLINE

Introduction

Humans, animals, and/or the environment are exposed to dozens of xenobiotics (exogenous compounds or materials) each day, and thousands of xenobiotics over the course of time. These agents may be encountered as a single substance or in complex mixtures, in doses large or small, and for a limited period, intermittently or continuously over time. The impact of xenobiotics ranges from no detectable effect to toxicity of various severity to death. Toxicologic pathologists are instrumental in protecting the well-being of humans, animals, and the environment.

Even though toxicologic pathology was practiced for decades prior to the 1970s, widespread use of toxicologic pathology did not begin until the declaration of the "War on Cancer" in the United States by President Richard Nixon under the National Cancer Act in 1971. Over the past 50 years, this effort led to a major effort to identify carcinogens and toxicants that occur in the environment and workplace. At this time the National Cancer Institute (NCI), a subdivision of the US National Institutes of Health (NIH), initiated the first large-scale testing program, known as the bioassay program. In 1978 this program was transferred to the National Toxicology Program (NTP), located within the National Institute of Environmental Health Sciences (NIEHS), another subdivision of the NIH. To date, over 500 two-year rodent toxicity and carcinogenicity studies have been conducted by the NCI/NTP, resulting in identification of numerous environmental and workplace hazards.

Other federal agencies in the United States participating in this effort through the use of animal models, *in vitro* toxicology and molecular biology include the National Institute for Occupational Safety and Health (NIOSH) and the Environmental Protection Agency (EPA). In addition the US Food and Drug Administration (FDA) is responsible for ensuring the safety and efficacy of human and veterinary drugs, biological products, and medical devices as well as the safety of the food supply, cosmetics, products that emit radiation, and tobacco products. Similar programs to address concerns about the impact of environmental toxicants on human health also have been created in other countries, with well-known laboratories including the Fraunhofer Institute for Toxicology and Experimental Medicine in Hannover, Germany; the Institute for Applied Scientific Research (TNO) in the Netherlands; the Maltoni Institute in Bologna, Italy; and the British Industrial Biological Research Association (BIBRA). Together, these programs have substantially decreased human exposure to potentially harmful agents and thus have improved public health throughout the world.

Societies in developed countries have responded to concerns raised by focus groups and the general public by introducing legislation to address a number of issues regarding not only the effects of chemical dispersal on the environment but also the safety of products developed for industrial use. The increasing demands placed on industry to demonstrate that their products are "safe" have catalyzed an expanded interest in toxicologic pathology as a key means to develop better assessments of safety.

Industry, whether chemical or pharmaceutical, must provide safety assessment of their products to appropriate government agencies for approval prior to marketing. To address this requirement, an increasing number of pathologists have been recruited to evaluate anatomic pathology (morphologic) and clinical pathology (biochemical and hematologic) changes following exposure to xenobiotics of concern under controlled experimental conditions (i.e., safety testing). Since long-term exposure is anticipated with many environmental contaminants, a major focus of testing has been carcinogenesis using the 2-year rodent bioassay. Good Laboratory Practices (GLP) regulations, central to the industrial toxicologic pathologist's work, have been developed and implemented following exposure of fraud in laboratory testing of chemicals in the late 1970s. These regulations ensure the uniformity, reliability, and integrity of the data produced during safety assessment. Periodically, GLP regulations are revised to ensure that animal-derived data used to predict potential human responses are of the highest possible quality.

Because of the ability of toxicologic pathologists to integrate and interpret information from a broad range of disciplines, their efforts are considered critical to the identification, interpretation, and integration of functional and morphological changes from laboratory animals into safety assessment and risk management for agents to which humans might be exposed. The ability of the pathologist to work and

communicate effectively in a team setting with scientists in other disciplines (toxicologists, pharmacologists, and physicians, to name a few) is essential for a successful product development and risk assessment program—as well as for a fruitful career for the pathologist. In the process of acquiring a knowledge base concerning the safety and efficacy of agents for potential use by humans, or to which they may be exposed, communal experience over time has shown it to be necessary to describe the structural and functional effects caused by these compounds in a consistent manner, and to be able to predict the likelihood of these effects and whether or not they are harmful (or "adverse") under various conditions. Thus, dose–response characterizations and adversity decisions have become an integral and important part of the field of toxicologic pathology (Chapter 2: Biochemical and Molecular Basis of Toxicity). Because an understanding of the biology of diseases that are caused by xenobiotics is necessary at the molecular level to be able to predict low-dose effects of exposure, a considerable amount of research has gone into developing appropriate models that will envisage these adverse effects.

Toxicologic pathologists are involved in many functions that protect society. A principal role is safety assessment of many materials, including drugs, chemicals, biotechnology-derived products (e.g., biomolecules, cells, gene therapy vectors, and vaccines), medical devices (e.g., implantable devices, companion diagnostic kits), and nanoparticles. Toxicologic pathologists also participate in laboratory animal disease surveillance programs; drug discovery including identification and validation of therapeutic targets; phenotypic analysis of transgenic animals; characterization and validation of animal models; evaluation of product efficacy (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology), often using animal models; and the investigation of mechanisms of toxicity (Chapter 5: Morphologic Manifestations of Toxic Cell Injury). An underappreciated task for toxicologic pathologists is to accurately and clearly communicate the meaning of their work, to many audiences: other scientists, corporate managers, regulators, and even members of the general public.

Toxicologic pathologists have many career opportunities. The largest number of toxicologic pathologists is employed by industry, be it pharmaceutical, agrochemical, chemical, or contract research organizations (CROs). Toxicologic pathologists also work for regulatory agencies, academia, research organizations (private foundations and governments), and as consultants. Societies of toxicologic pathology (STPs) in many countries provide a "home" for those working in the discipline in regions such as North America, the United Kingdom, Europe, Japan, Latin America, China, and India. The key activities undertaken by these STPs are to provide their members with professional development opportunities (continuing education and networking) and to influence public policy by providing opinions ("best practice" and "points to consider" papers) designed to positively influence governmental policies that protect human, animal, and environmental health. Worldwide cooperation among STPs and an increasing emphasis on global harmonization promises job security to toxicologic pathologists for the foreseeable future.

What is Toxicologic Pathology?

Toxicologic pathology integrates the disciplines of pathology and toxicology most often in an experimental setting. Pathologists study the nature of disease (pathophysiology), evaluating changes produced in cells, tissues, organs, or body fluids in response to a "challenge," whether it is metabolic, infectious, neoplastic, immune-mediated, physical, or toxic in origin. Most diseases leave significant "footprints" in cells, fluids, and tissues (Chapter 5: Morphologic Manifestations of Toxic Cell Injury). Toxicologists, on the other hand, focus on the biochemical basis of the science of poisons (Chapter 2: Biochemical and Molecular Basis of Toxicity). The discipline of toxicologic pathology requires knowledge of both pathology and toxicology, as well as other related disciplines, such as statistics and experimental design, so that integration of anatomic pathology data, clinical pathology findings, and functional changes can be accomplished in a logical manner with respect to their biological significance. Contemporary toxicologic pathology also relies on an understanding of molecular biology, metabolomics, toxicogenomics, epigenetics, imaging, biomarkers, specialized techniques (such as immunohistochemistry), and quantitative approaches (e.g., morphometry, stereology, and digital image analysis) to pathology. The ability of modern biologists, including toxicologic pathologists, to relate these new platforms for gaining biological knowledge is driving the 21st century transformation from generalized to personalized medicine.

Pathologists are well versed in evaluating the manifestations of diseases, whether they occur in humans (medical) or in animals (veterinary). The toxicologic pathologist must have a mastery of both "experimental pathology" (i.e., disease investigations by which an appropriately designed study is undertaken to test a hypothesis) and "comparative pathology" (i.e., the relationship of anatomic, physiological, and pathological characteristics among various species) in the context of data interpretation and extrapolation from animals to the human population (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology). Another related but more expansive term is "environmental pathology," a branch of toxicologic pathology concerned with abiotic (unrelated to living organisms) environmental agents that influence human or animal health.

The perspective of a toxicologic pathologist differs from that of other pathologists. For example, a diagnostic pathologist interprets changes in tissues and body fluids from an individual or group to determine the cause of disease or death in that individual or wider population. However, the diagnostic pathologist must consider toxic agents as a possible cause of disease and may find evidence of contamination in the environment (polybrominated biphenyls in cattle, dichlorodiphenyltrichloroethane in raptors) or food (melamine in dogs and cats); in instances where both humans and animals may be exposed to the same toxic agent, findings by diagnostic pathologists may permit animals to serve as sentinels of disease for humans.

A forensic pathologist specializes in the investigation of death or disease that is "suspicious" in nature, with toxic agents as a possible cause of the condition. In contrast, the main role of the toxicologic pathologist, by contrast, is to determine the biological significance of alterations in form, function, or both, as manifested by altered structure of cells and tissues (lesions) or composition of body fluids (biomarkers), induced by a known chemical entity (often called the "test article" in the industrial setting). Toxicologic pathology is an essential part of hazard identification, dose–response data generation, and risk characterization, all of which are essential for risk analysis and assessment as well as risk management of human and animal exposure to potentially toxic agents (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology). Since these activities are largely confined to the industrial setting, toxicologic pathology is sometimes referred to as "industrial" pathology.

The Basis of Toxicologic Pathology

The foundation of pathology is the art and science of observation, with descriptions of altered morphology still an important basis for understanding diseased tissue. Pathology has its roots in common with other medical specialties dating back to antiquity. However, modern pathology began to develop during the 19th century, with Rudolf Virchow being considered the "father of pathology" due to his use of the microscope to view cellular changes in diseased tissues. The quest to understand the causes of disease resulted in efforts to associate lesions with their cause(s). As the discipline of pathology grew, associations were made between gross and microscopic lesions, alterations in body fluids (especially blood and urine), clinical alterations, and potential etiologies, resulting in the use of pathology as a routine diagnostic tool in medicine, initially for forensic purposes.

Observations of altered morphology were initially based on findings at autopsy or necropsy. With the development of the light

microscope and later the electron microscope, novel morphologic observations could be made at the cellular and subcellular levels (Chapter 4: Principles of Pharmacodynamics and Toxicodynamics). More recent techniques have been developed to detect changes at the tissue, cellular, molecular, and gene levels such as *in vivo* small animal imaging and toxicogenomics. Importantly, enhanced techniques for examination of altered components in blood and other body fluids from living animals have led to the development of biomarkers that can be used to predict the presence, severity, and progression of toxic injury.

Toxicologic pathology requires additional working knowledge of disciplines other than anatomic and clinical pathology, and toxicology. Disciplines that form a foundation for the toxicologic pathologist include cellular and molecular biology, biochemistry, physiology, microbiology, immunology, pharmacokinetics, pharmacodynamics, risk assessment, experimental design, and statistical evaluation. These disciplines are important in integration of clinical, biochemical, morphological, and functional changes to understand the biological significance and investigate mechanisms of action (MOAs).

A working knowledge of what the body does to a xenobiotic agent (Chapter 2: Biochemical and Molecular Basis of Toxicity; Chapter 3: Pharmacokinetics and Toxicokinetics) and what the agent can do to the body (Chapter 4: Principles of Pharmacodynamics and Toxicodynamics; Chapter 5: Morphologic Manifestations of Toxic Cell Injury) is essential. In both cases, there are many factors that affect these interactions. Examples include the route of exposure and the chemical form or composition of the toxic agent or test article, as well as the species, breed/strain (race), and individual variations in absorption, distribution, metabolism, and excretion.

Exposure to toxic substances may occur by a variety of routes. Dermal and inhalation exposures are the most common routes of unintentional exposure, while intentional exposure is frequently via the oral route. Fat-soluble substances such as phenolic compounds, vitamins D and K, and steroid hormones are readily absorbed through the skin; thus, it is important to recognize the chemical characteristics of the compounds in question (Chapter 23: Bone and Joints). Widespread inhalation exposure to potentially toxic gases and particles occurs in the workplace as well as in everyday life. In addition, exposures to gases are complicated by particles that may facilitate pulmonary exposure and add to the insult of the respiratory system as well as cardiovascular system (Chapter 14: Respiratory System). The oral (per os [PO]) route is a common route by which a toxic substance or drug may enter the body. Absorption of a substance across the gastrointestinal wall depends on its lipid solubility, pH, and ionization constant, and the nature of the mucosal lining at the site of absorption (Chapter 2: Biochemical and Molecular Basis of Toxicity; Chapter 15: Digestive System). Parenteral injections of toxins (noxious agents or biological origin, such as venoms) and therapeutic agents into dermal tissues (subcutaneous, SC); muscle bellies (intramuscular, IM); or blood vessels (intravascular, IV) represent other possible routes of exposure. The rates at which toxic agents rise and fall in blood and tissue as well as the persistence of agents within the body are affected by the route of exposure.

The route of exposure to be used in toxicity testing is determined by many parameters. Key factors include the chemical and physical properties of the compound, the route for its intended use or common exposure, and natural protective barriers, such as hair or skin. The route of administration may also be governed by special biological attributes of the test species and its environment, which is particularly important for fish and insects. Toxicity testing programs often incorporate bioassays using several routes of exposure with different pharmacokinetic profiles based on the peak test article accumulation (termed the "maximum concentration," or C_{max}) and the total accumulation over time (integrated as the "area under the curve," or AUC) (see Chapter 3: Pharmacokinetics and Toxicokinetics).

The Challenges Facing Toxicologic Pathology

As one contemplates the dimensions of the problem of safety assessment and evaluation of risk, one is reminded of Albert Einstein's remark, "No amount of experimentation could ever prove me right; a single experiment can prove me wrong." This comment epitomizes the dilemma of toxicologic pathology: the data may indicate that a substance is likely to be toxic or carcinogenic, but there is never the certainty to say that it is not.

Development of lesions that can be evaluated by the toxicologic pathologist is influenced by numerous genetic, epigenetic, microbial, environmental, and experimental factors. The importance of the microbiome in health and disease is only now starting to be appreciated and may be at least partly responsible for individual differences in response to toxic agents. As such, the toxicologic pathologist needs to understand how factors associated with the laboratory animal, the animal care and use program, the research facility environment, and the study conditions contribute to study findings so that the results of toxicity experiments can be properly interpreted. The issue of species variation in the handling of test substances makes extrapolation of results from rodents to humans difficult at best and at worst a tenuous process (Chapter 3: Pharmacokinetics and Toxicokinetics). It is doubtful whether a toxic response to a test substance administered at a high dose in a rat necessarily reflects the action of the same compound at a low dose in humans. At best, animal toxicity studies (high doses over short periods) represent a worst-case scenario for compound exposure in humans, and frequently is not an accurate representation of reality (low doses over long periods, and often multiple agents at a time). Such discrepancies may be exacerbated by the inherent genetic properties of inbred animal species versus typically outbred human populations; this situation often is handled in toxicity testing by using outbred rodent stocks (which are genetically heterogenous, like people) rather than highly inbred strains. Yet, given the state of our present knowledge and economic constraints, this method is the best we have on which to base social, political, legislative, and financial decisions.

Risk assessment characterizes potentially adverse findings (clinical, clinical pathology, and anatomic pathology) for a given species, usually humans. The risk assessment process has four main components: (1) hazard identification (does an agent cause toxicity?), (2) dose–response determination (at what doses is it toxic?), (3) exposure assessment (will individuals encounter the agent, and if so when and where), and (4) risk characterization (how likely is it that individuals will encounter the agent at a dose that may cause toxicity?). For most industrial toxicity studies, hazard identification and dose–response assessment for a test substance are central to the toxicologic pathologist's work. In contrast, exposure assessment and risk characterization are key tasks for environmental toxicologic pathologists.

Toxicologic pathologists and toxicologists participate in the risk assessment process on a daily basis. The toxicologic pathologist, together with the input from colleagues in toxicology, initially identifies the potential adverse health effects of test articles in laboratory animal species, defines the dose–response of the adverse effects, and then determines whether or not these effects are likely to express themselves in humans. An adverse health effect can only develop when exposure to a hazard occurs. However, it is important to keep in mind that not all test article–related effects are adverse, some effects are adaptive or actually desirable (i.e., pharmacological). Thus, the risk from test article exposure is the probability that a harmful response will manifest. The estimate regarding how likely it is that an adverse reaction will develop is termed risk characterization.

For many practicing toxicologic pathologists, the bioassay for determining the 2-year rodent carcinogenicity of compounds is central to their day-to-day work. The general approach to carcinogenicity testing involves treating large numbers of male and female rats and mice (50 animals per group to start, due to animal attrition over time) at several dose levels over a lifetime, followed by pathology evaluation. Unfortunately, the results are often equivocal or difficult to interpret, not least because some rodent carcinogens act by mechanisms that are not found in human cells. Given the high cost and questions regarding the relevance of the rodent cancer bioassay, a search for more predictive alternative assays continues. For example, genetically engineered mouse (GEM) models are starting to be used; these models have

been designed with built-in molecular defects that predispose the animals to develop cancer at an early age, thereby allowing for smaller numbers of animals and a shorter exposure period (6 months). Nonetheless, the GEM models still are not widely used for a variety of reasons. Accordingly, the search continues for alternatives that would limit or replace animal use. Toxicologic pathologists will be integral to the identification and validation of alternative animal models.

The validity of toxicity study data is only as good as the quality of that data. Therefore, it is important for the toxicologic pathologist to be familiar with and participate in development of the study design and methods to be utilized, including sample collection, and to understand the methodologies and interpretation of statistical evaluation. Furthermore, toxicologic pathologists who assess anatomic or clinical pathology endpoints for GLP studies must work closely with quality assurance (QA) specialists to ensure that the study data set is compiled correctly and documented according to established regulatory guidelines for multiple regions of the world.

Toxicologic pathology is an interpretive discipline, and anatomic pathology diagnoses in particular are subjective evaluations that may differ even among well-trained individuals. Furthermore, morphologic lesions seen in carcinogenicity studies can be confounded by a number of factors and often require negotiation among pathologists to obtain scientific consensus regarding their relevance. For this reason, it is now standard practice to use pathology peer-review and pathology working groups (PWGs) to validate the quality of the anatomic pathology data.

Due to the fact that anatomic pathology evaluation is an interpretive science that generates qualitative data and typically is not based on mechanical production of exact (quantitative) data, it is of paramount importance to utilize consistent nomenclature for lesions, such as that devised by the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND). The communication of the interpretation for the study findings requires careful consideration as well as succinct and accurate communication skills. Interpretation of clinical pathology data may be qualitative (e.g., morphologic findings for cytological preparations) or quantitative (e.g., clinical chemistry or hematology values measured on automated high-throughput analytical instruments), but often still requires cooperation among multiple toxicologic pathologists when deciding on its final significance in assessing risk.

Additional challenges in toxicologic pathology lie with laboratory animal issues, including the advancement of the 3Rs (Replacement, Reduction, and Refinement) to minimize the numbers of animals used in research and testing in toxicology, and changes in animal husbandry related to animal welfare. In conjunction with the 3Rs, emphasis now is being placed on the development of high-throughput screening assays such as in silico algorithms (computer modeling) and *in vitro* methods (e.g., cell cultures and tissue slices), and decreased use of nonrodent species (especially nonhuman primate models) in non-GLP studies. However, rodent use is increasing in toxicity testing due to the increased utilization of GEM models to assess basic biological mechanisms as well as test article efficacy and toxicity, and also the increasing sophistication in animal monitoring systems such as telemetric assessment of physiological parameters and noninvasive imaging methods, both of which can now be applied to rodents.

When the toxicity study is complete, the toxicologic pathologist must be able to synthesize the data into a comprehensive pathology report that communicates not only the main test article–related findings but also the pathologist's interpretation regarding their meaning (adverse vs nonadverse) to the test species under the conditions of the bioassay. This pathology report is combined with documents compiled by other scientists (biochemists, pharmacokineticists, etc.) to produce a final study report that describes the outcome of the entire study. Multiple study reports from toxicity tests undertaken in multiple species ultimately are considered together to produce an overview document that communicates the risk posed by exposure to a test article; such compilations are submitted to regulatory agencies as part of a product registration package.

The data in the pathology report also may be part of the grant documentation submitted to funding agencies, or may be adapted for publication in a scientific journal. The language used in the report needs to keep in mind the audience at which it is aimed. Will it need to be understood by other scientists who are not trained in pathology? Will it need to be understood by nonscientists? Excellent communication skills, both written and verbal, are important to the success of the toxicologic pathologist.

The toxic impact of a test article requires an accurate assessment of its risk, which entails a broad scientific knowledge concerning the nature of the harm and under what conditions its potential for harm may manifest. The main challenge today often is to compile biologically based mechanistic information for several species (ideally including human cells or tissues), the possession of which permits better estimations of how cells and organisms may respond to test articles. Mechanistic information helps to determine whether or not the hazard will be likely to develop in humans and may give quantitative information to suggest under what conditions a risk may actually occur. This information may also help identify subpopulations (based on genetic, geographic, or other differences) that are at greater or lesser risk. Thus, there is a critical need for a scientific understanding of the MOAs to reduce the extent of uncertainty associated with the assessment of risk.

Finally, the globalization of markets and corporations needs to be taken into consideration when considering modern challenges faced by toxicologic pathologists. As companies extend across multiple sites, often across multiple states and countries, and as outsourcing of discovery research and safety evaluation continues to increase, there is a need for harmonization of regulations and pathology nomenclature, as well as more uniform standards for professional training and practices. In the past, each country tended to have different regulations as well as training opportunities and requirements. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), a consortium of European, Japanese, and US regulatory agencies and pharmaceutical industries, has brought some uniformity to international regulations to ensure the quality, efficacy, and safety of pharmaceuticals. On a smaller scale, the STP, the European STP (ESTP), the British STP (BSTP), and the Japanese STP (JSTP) collaborate regularly to standardize toxicologic pathology nomenclature through the INHAND initiative. However, a unified global standard for training of toxicologic pathologists still needs to be defined. Globalization also has created a need for instant communication, with data- and image-sharing capabilities that often require toxicologic pathologists to become familiar with new technologies such as digital pathology. As the 21st century rolls on, toxicologic pathologists increasingly will need to adapt to new roles and technical innovations.

Resources required to address the various challenges faced by toxicologic pathologists are being developed by STP, based in North America, and other STPs around the world. The STP regularly circulates new information in *Toxicologic Pathology* and in the Society's website (www.toxpath.org). Key categories that are addressed in the STP outreach effort include continuing education for its members, affiliated societies, and regulators; up-to-date scientific reviews; position ("best practice" and "points to consider") papers for important issues facing the toxicologic pathology community, and opinion pieces on current regulatory issues. Similar publications are available from the BSTP, ESTP, and JSTP as well as in other related journals (*Experimental and Toxicologic Pathology* and the *Journal of Toxicologic Pathology*).

Training and Certification in Toxicologic Pathology

A toxicologic pathologist is a biomedical scientist with extensive clinical training, specialized training in comparative pathology, and subspecialization in toxicologic pathology. The majority of toxicologic pathologists worldwide are veterinarians with a veterinary medical degree (DVM or equivalent) and pathology training through a residency program, which generally culminates with board certification in

general pathology [e.g., diplomate status in the American or European College of Veterinary Pathology (ACVP or ECVP, respectively)]. In the United States, pathology training focuses on anatomic pathology and/or clinical pathology (depending on the training program), although there is some overlap both in training and the content of the ACVP board examination.

Very few training institutions provide training in toxicologic pathology per se, although trainees may be exposed to some toxicology courses and occasional diagnostic cases involving toxicant exposure. To a lesser extent, medically trained pathologists (MD or equivalent) or comparative/experimental pathologists (PhD or equivalent) are also involved in the practice of toxicologic pathology, especially in countries such as China and Japan; scientists with doctoral degrees alone (PhD) make up a very small group of current toxicologic pathologists. Research training may be obtained through an MS, PhD, or other postdoctoral training. Because of the differing scopes of training in diagnostic pathology (i.e., a medical-oriented degree and/or residency) versus experimental pathology (i.e., a research-oriented degree), individuals with both experiences generally enter the toxicologic pathology work force with more self-confidence and often require less time to gain proficiency.

Formal certification in toxicologic pathology is only available in Japan (diplomate, JSTP) and in some European countries. Many pathologists engaged in toxicologic pathology have chosen to demonstrate their expertise in toxicology by obtaining certification in toxicology [e.g., diplomate status provided through the American Board of Toxicology (ABT) in the United States or as a European Registered Toxicologist (ERT)]. For more experienced toxicologic pathologists, recognition as a Fellow by the International Academy of Toxicologic Pathology (IATP) is an option for showcasing their long-term expert practice in this profession.

Traditionally trained veterinary and medical pathologists encounter many unanticipated challenges in the transition from diagnostic pathologist to the experimental, regulatory-driven environment of the industrial toxicologic pathologist. During their years of training and diagnostic effort, pathologists typically provide both clinical and public health services in a diagnostic, hospital, or private laboratory setting. In such settings, they also serve the clinical community to support therapeutic approaches and prognoses. These laboratories generally function by internal work practices and procedures based on professionally recognized best practices with a degree of governmental oversight. However, the work practices of the diagnostic pathologist in these environments are not as severely constrained by the extensive GLP regulations mandating proper management and storage of data, QA review, peer review, animal welfare standards, organizational structure and personnel, and study design, except in the case of forensics. Adaptation to this enhanced degree of regulatory oversight represents one of the significant challenges faced by diagnostic pathologists who choose to make the transition to an industrial toxicologic pathology role.

The “Practitioner” of Toxicologic Pathology

A “practitioner” of toxicological pathology utilizes toxicologic pathology, on a daily basis regardless of the employment sector. As discussed earlier, the majority of toxicologic pathologists are employed by industry (including CROs), with smaller numbers in government, academia, and private consulting practices.

Regulatory (Industrial) Toxicologic Pathology

The majority of toxicologic pathologists in industry participate in regulatory-type, nonclinical studies performed in an experimental setting (applied research) in support of development of bio/pharmaceuticals and, to a lesser extent, agricultural and other chemicals, and medical devices. Anatomic pathology is usually a key part of these studies, whereby a standard tissue set (Table 1.1) is examined histologically for potential lesions. Toxicologic pathologists also participate in other aspects of drug development, particularly drug discovery (especially target discovery and validation), or can move up the corporate ladder into management (of pathology and/or toxicology departments, or sometimes of whole product development divisions). Optimal qualifications for entry-level industrial toxicologic pathologists in developed countries include a DVM (or equivalent) medical degree, board certification in veterinary pathology, and a degree (PhD) demonstrating research training. The PhD degree is valued because it fits its holders to face many of the challenges in toxicologic pathology stemming from new technologies (e.g., advanced molecular methods, innovative analytical instrumentation) and also provides some understanding of experimental design and statistical considerations.

Table 1.1

Society of Toxicologic Pathology Recommended Core List of Tissues to be Examined Histopathologically in Repeat-Dose Toxicity and Carcinogenicity Studies (for All Species Where Applicable)[a]

Adrenal gland	Pancreas
Aorta	Parathyroid gland
Bone with bone marrow[b]	Peripheral nerve
Brain	Pituitary
Cecum	Prostate
Colon	Salivary gland
Duodenum	Seminal vesicle
Epididymis	Skeletal muscle
Esophagus	Skin
Eye	Spinal cord
Gallbladder	Spleen
Harderian gland	Stomach
Heart	Testis
Ileum	Thymus
Jejunum	Thyroid gland
Kidney	Trachea
Liver	Urinary bladder
Lung	Uterus
Lymph node(s)	Vagina
Mammary gland[c]	Other organs or tissues with gross lesions
Ovary	Tissue masses

[a]This tissue list is intended to be a minimum core list that can be used for all types of repeat-dose toxicity and carcinogenicity studies, regardless of route of administration, species or strain of mammalian laboratory animal, duration of study, or class of drug to be tested. It is recommended that the route of administration be considered at the time of study design and that tissues relevant to the route of administration be added to this core list. For example, the addition of nasal cavity and turbinates, larynx, and tracheobronchial lymph nodes may be considered for inclusion in the tissue list for nasal inhalation studies. Likewise, depending upon the species or strain of laboratory animal, the addition of organs or tissues unique to or characteristic of that species or strain may be selected, as appropriate. It is also recommended that additional tissues that are known to be targets of the test article or those of its class be added to this core tissue list.

[b]For nonrodents, either rib or sternum. For rodents, femur including articular cartilage.

[c]Females only.

From Bregman, C.L., Alder, R.R., Morton, D.G., Regan, K.S., Yano, B.L., 2003. Recommended tissue list for histopathologic examination in repeat-dose toxicity and carcinogenicity studies: a proposal of the Society of Toxicologic *P*athology (STP). Toxicol. Pathol. 31 (2), 252–253.

Industrial toxicologic pathologists play a vital role in risk assessment. As new methods become validated and implemented, resulting in sophisticated visualization of altered morphology, new biomarkers, and gene-based technologies, toxicologic pathologists are expected to provide more than unambiguous diagnoses and a dose–response assessment via interpretation of routine hematoxylin and eosin–stained tissue sections and standard clinical pathology parameters. As toxicologic pathologists more frequently assume a greater role in product development, such as serving as Study Director for compounds under development as pharmaceutical agents, the impact of their observations, interpretations, and expert comments will become even greater. Therefore, issues such as consistency of terminology, study design (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology), statistical interpretation, integration of data into meaningful assessments of health risks, communication of risk, and risk management (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology) will need to be familiar to the toxicologic pathologist. The value of research skills is becoming of greater importance as more toxicologic pathologists in industry are becoming involved in the discovery arena, developing models for toxicity and efficacy testing and also investigating mechanisms of toxicity. The whole-animal focus of veterinary or medical training provides a critical viewpoint for product development teams that help in integrating data provided by other team members with more reductionist (molecule- or cell-oriented) perspectives.

Toxicologic Pathology Related to the Environment and Food Safety

Toxicologic pathologists involved in environmental pathology and food safety are generally employed by government agencies, such as the US EPA; US National Institute of Environmental Health Sciences (NIEHS, a division of the NIH); FDA; and US Department of Agriculture (USDA). However, some pathologists filling these roles are professionals in academia. There has been increasing recognition that the practice of toxicologic pathology can add valuable information for diagnosing environmental problems, detailing background disease prevalence, investigating mechanisms of toxicity, and developing alternate animal models for evaluating toxicity.

Human activities are the major source of environmental contaminants. Some contaminants, such as chlorinated or brominated organics and heavy metals as well as radioactive wastes, persist in the environment for indefinite periods and threaten human, wildlife, and domestic animal health. Environmental pathology deals with workplace exposures as well as air, water, and ground contaminants. Examples of workplace toxicants include asbestos, which causes asbestosis, lung cancer and mesothelioma, and diacetyl from popcorn butter flavoring, which can cause bronchiolitis obliterans (popcorn worker's lung). Air pollutants include sulfur dioxide, nitrogen oxides, ozone, and fine particulates, largely produced by industry and motor vehicles; these agents affect the respiratory and cardiovascular systems as well as possibly playing a role in metabolic syndrome and diabetes. Recently, lead from corroding water distribution pipes has been under the spotlight in Detroit and other cities, although lead exposure from chips of old paint is probably as important source in many communities. New environmental concerns include pharmaceuticals and antibiotics found in drinking water and industrial effluents, endocrine-disrupting chemicals, and the effect of "lifestyle" drugs (e.g., alcohol, marijuana, tobacco) on human populations (including those exposed by second-hand smoke).

The adverse effects of nutritional components and contaminants in food products are also important areas to which the toxicologic pathologist can add value. Nutritive and nonnutritive (typically preservative) chemicals are integral components of foods; indeed, foods may contain, intentionally or unintentionally, a wide range of chemicals from many sources that could be a potential health hazard. Toxicants (including toxins such as phycotoxins and mycotoxins) can enter the food chain at various levels of the food web, after which they can interact with other chemicals and compounds present in food, bio-accumulate, be metabolized into bio-products, or be modified during food processing and cooking. Knowledge of the chemical properties of substances and understanding their biological effects, MOAs, and pharmacokinetics (absorption, distribution, metabolism, bioaccumulation, and elimination) are important in assessing food safety.

Diagnostic Toxicologic Pathology

Diagnostic pathology identifies the cause of disease based on morphologic and/or clinical pathology findings, as well as history, clinical signs, and ancillary test results. It is important in all areas of pathology, both in spontaneous and in experimentally induced disease, including conditions associated with toxicant exposure. In experimental studies, it is important to separate out the effects of spontaneous disease and those induced by the experimental agent/test article. Diagnostic pathology is essential to investigate unexpected disease or death in laboratory animal colonies or prior to the termination of a study.

Diagnostic pathologists in academia and government also need to be familiar with the basics of toxicologic pathology since exposure to toxic agents is not uncommon in both companion and agricultural animals. Diagnostic pathologists working in human and veterinary medicine need to be familiar with tissue responses to drugs, harmful industrial and agricultural chemicals, environmental contaminants, and toxins produced by a wide variety of microbes, algae, fungi, and plants. Exposure to such agents may be accidental or intentional, and may occur in a single individual or in a group setting. Accidental exposure in humans may occur in an occupational setting or from environmental contamination, whereas intentional exposure or overdose can occur in malicious poisoning or suicide. Genetic susceptibility or underlying disease in exposed individuals or populations can increase risk posed by exposure to toxic agents.

In the veterinary diagnostic laboratory, toxicologic pathology will continue to be central to diagnosis and prevention of spontaneous, toxicant or toxin-induced disease. Since animals can serve as sentinels for human disease, the diagnostic laboratory is uniquely situated to identify sources of environmental contamination whether they are related to food, water, or other forms of exposure. Such contamination may affect the local animal population or may also affect human health. In addition, the utility of naturally occurring chemically induced diseases as models should not be underestimated, as it is often that diagnostic cases add crucial information to inform our understanding of the mechanisms of toxicity of these and similar chemical groups. One example of this paradigm was the identification of melamine and cyanuric acid (present in pet food) as the cause of acute nephrotoxicity in dogs and cats. Research based on such an outbreak in the United States led to the identification of melamine as the cause of nephrotoxicity in children in China due to contamination of milk formula (Chapter 11: Urinary System).

Investigative Toxicologic Pathology

Research in toxicologic pathology is essential for understanding the mechanisms of toxic injury (e.g., Chapter 6: Carcinogenesis: Manifestation and Mechanisms) and to fill data gaps that impede risk assessment. In addition, by obtaining an understanding of the pathogenesis of altered structure and function associated with toxicant exposure, normal physiological processes are often elucidated. For example, the use of teratogenic substances has aided our understanding of embryonic and fetal development (Chapter 25: Embryo, Fetus, and Placenta), and even helped to devise mechanism-based treatments to reduce or prevent certain classes of birth defects.

Toxicologic pathologists participate in research in many settings, including industry, academia, and government. Their unique skill set, which includes descriptive and comparative pathology, problem-solving, and broad-based education with a "whole-animal" orientation, brings an added dimension to many research areas.

Research in toxicologic pathology ranges from simple associative types of work including retrospective studies using archived tissues, cross-sectional surveys, and longitudinal studies of disease progression and remission to evaluation of the importance of toxicant-induced gene changes in initiating and sustaining various human diseases. In prospective investigations, the etiology is known, but the pathologist still uses morphological diagnoses to describe the disease process. Familiarity with experimental design and an understanding of statistical analysis are essential skills in investigative pathology. This output is considered in light of the key experimental variable(s), usually exposure to a xenobiotic at a particular dose. The output is a tested hypothesis as to the effect of the treatment by the experimental variable —xenobiotic "X"—generally by determining a difference between treated and untreated (negative control) animals as well as the relationship of xenobiotic exposure to the dose level. This relationship is essential in risk assessment.

The ongoing advent of new techniques and animal models will allow the field of investigative toxicologic pathology to progress and expand in scope and importance. Molecular biology and genomic techniques being used to probe the mechanisms of toxic injury. For example, activated oncogenes have been found in both human and animal tumors (Chapter 6: Carcinogenesis: Manifestation and Mechanisms), and so tumorigenesis associated with expression of constitutively activated oncogenes can be studied in transgenic mice, where the engineered proteins have been modified to always be functioning. Identification of an oncogene specifically activated by a given xenobiotic may aid in the extrapolation of data from bioassays conducted in rodents to predict human responses to that xenobiotic. In the case of genetically altered mice, the information obtained from studies using these animals has led to alternative models to study and test for carcinogenic potential. In fact, the p53 knockout mouse is presently being used to aid in identification of carcinogens.

Today's rapid pace of scientific advancement and the development of new technologies that can be exploited to address toxicological issues means that large strides will continue to be made in understanding mechanisms of xenobiotic-induced alterations and diseases. The combination of morphological techniques, which provide topographic specificity, with novel technologies that permit large-scale assessments of metabolic intermediates, proteins, and mRNA but generally lack topographic specificity, can be used to facilitate the study of mechanisms underlying xenobiotic-induced, microscopically detectable lesions. The combination of traditional lesion identification with laser capture microdissection, for example, will permit the direct molecular assessment of lesions by a variety of such new technologies.

Management Roles for Toxicologic Pathologists

As their careers progress, many scientists involved in product discovery and development find themselves moving toward more managerial roles, where they need to make decisions on compound development that necessitates the incorporation of techniques for risk management (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology). In fact, they may attain positions in upper management themselves. The toxicologic pathologist is well poised by both training and prior experience to fill such positions, as the management of scientific issues concerning product discovery and development is often best served by the integration of information arising from many interrelated disciplines (Figure 1.1).

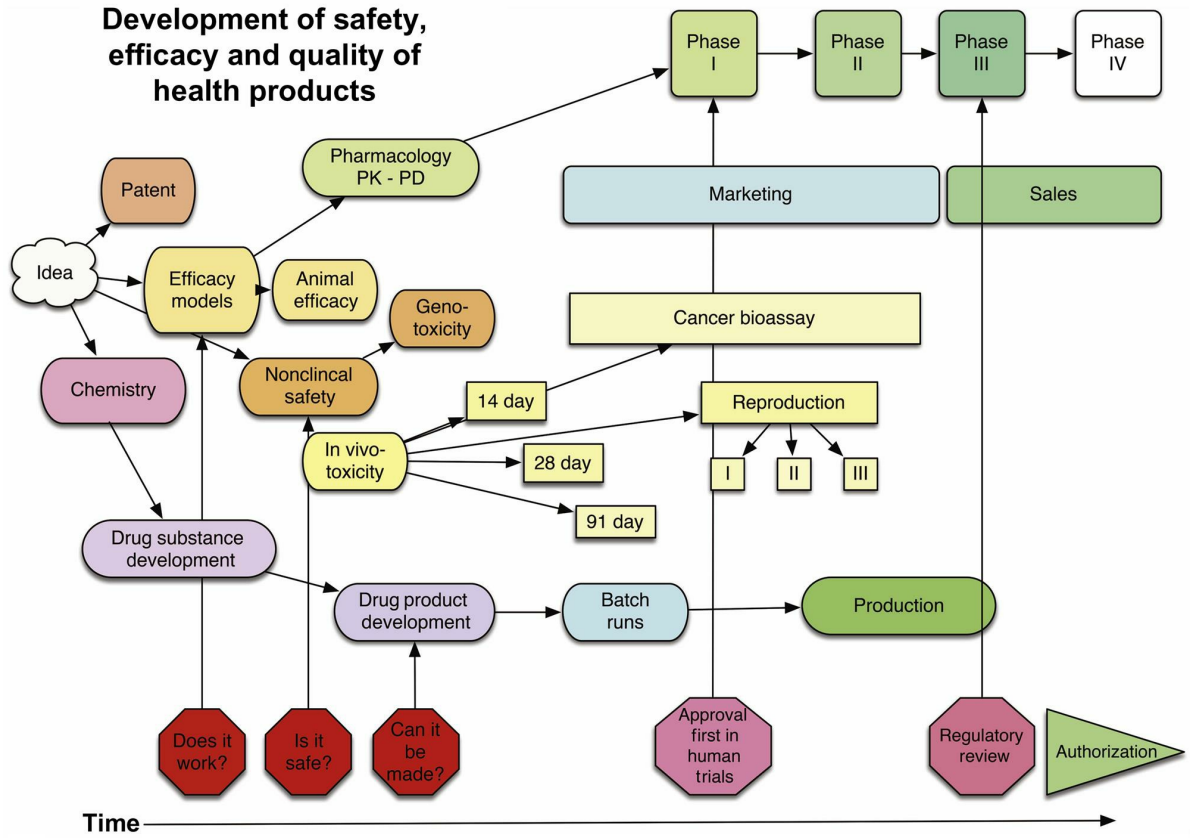

FIGURE 1.1 An example of the multidisciplinary understanding required for risk management in the development of health products. *Source*: From Haschek, WM., Rousseaux, C.G., Wallig, M.A. (Eds.), 2013. Handbook of Toxicologic Pathology, third ed. Academic Press, Figure 21.3, p. 653, with permission.

However, a manager requires more than just a solid toxicologic pathology background to succeed in such a role. Toxicologic pathologists in managerial positions also require strong oral and written communication skills to deal with upper-level managers (i.e., senior executives), regulators, corporate (including patent) lawyers, and external stakeholders, including prospective investors. In particular, genuine prowess in communication is a prerequisite for success in a managerial role, particularly when it comes to risk management (Chapter 7: Design of Studies and Risk Management in Toxicologic Pathology).

Summary

In summary, toxicologic pathologists are well suited to play many pivotal roles in decision-making within the framework of hazard identification and risk assessment/management. Their broad and thorough understanding of most biological processes, their perspective of biology as an integrative (i.e., whole animal) rather than reductionist (molecule- or cell-oriented) discipline, their ability to generate detailed data for decision-making, and their familiarity with the many limitations on the biological significance of such inherently subjective data make the toxicologic pathologist a critical member of the modern product discovery and development process. The growing number of new products being developed and produced worldwide as well as the many novel roles that will arise in this field during the next decades will offer toxicologic pathologists long and vibrant careers in serving to protect public health. For further information and details regarding specific aspects of toxicologic pathology (e.g., GLPs, quality assurance and quality control; a pathologist's role in drug discovery and development; peer review and PWGs), it is suggested that the reader refer to "*Haschek and Rousseaux's Handbook of Toxicologic Pathology*," Third Edition, Volumes I, II, and III, Elsevier, San Diego, CA (2013).

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PART I

Principles of Toxicologic Pathology

OUTLINE

CHAPTER 2

Biochemical and Molecular Basis of Toxicity

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Abstract

This chapter is focused on the fundamental principles of toxic mechanisms of injury. Toxicity is influenced by the amount of toxicant that reaches the target organ, the reactivity of the compound itself or metabolites that are formed by major biotransformation systems, and the potential for altered cellular function or gene expression. There are many mechanisms and pathways by which toxicity can develop. Fundamental mechanisms of toxicity are presented in this chapter, with examples provided to illustrate the changes as appropriate.

Keywords

Xenobiotic disposition; xenobiotic transporters; Phase I metabolism; Phase II metabolism; Phase III metabolism; cytochromes P450; glucuronidation; sulfation; glutathione; nuclear receptors reactive metabolites; mechanisms of cell death; oxidative stress; cell stress response; Nrf2; pathway; p53; apoptosis; necrosis; necroptosis; cell repair and proliferation

OUTLINE

Introduction

The cellular basis of toxicity encompasses the identification of a target organ of toxicity coupled with the features of how that organ responds to toxic stress. Since most organs are a composite of different cell types with a variety of functions, cellular targets of toxicity are determined by the type of insult and the mechanism of toxicity. The molecular basis of toxicity encompasses the breadth of changes from transcriptional, translational, and signal transduction pathways that are causally related to toxic responses. Although defined separately, molecular and cellular events are intertwined and contribute to sensitivity to toxicity, the nature of the toxic response, and the type of repair mechanisms that may ensue. An overarching concept in considering any mechanism of toxicity is that xenobiotic disposition plays a central role in the development of toxicity and is often a major determinant of the dose–response relationship for toxicity and a potential source for species differences in toxic responses.

In light of the breadth of target cells, target organs, and molecular pathways underlying toxic mechanisms, it is difficult to adequately address the multitude of mechanisms and pathways by which toxicants elicit adverse effects in cells or organs. This chapter focuses on the fundamental principles that contribute broadly to toxic or pathologic effects, starting with the characteristics that determine how a toxicant is delivered to its target, the major factors that determine toxic outcome and concluding with those that determine whether repair or regeneration occurs after toxic insult.

General Principles of Xenobiotic Disposition

The disposition of a xenobiotic is defined as the integrated action of its absorption, distribution, biotransformation, and elimination. The quantitative determination of these properties comprises the field of pharmacokinetics (or toxicokinetics), and collectively, disposition and kinetics ultimately determine the concentration of a compound at a target site for toxicity and dictate whether adverse effects will occur.

General Properties of Absorption

Biological Membranes

Cell membranes are comprised of a phospholipid bilayer wherein the polar head groups of the lipids are oriented toward the outer and inner surfaces of the membrane and the lipid tails are oriented inward forming a hydrophobic inner space. Cell membranes are typically 7–