CHAPTER 1

Molecular and Biochemical Toxicology: Definition and Scope

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1.1 INTRODUCTION

After the publication of the previous edition, toxicology saw a dramatic increase in the application of the principles and methods of molecular biology. Biochemical and molecular toxicology are concerned with the definition, at the molecular and cellular levels, of the cascade of events that is initiated by exposure to a toxicant and culminates in the expression of a toxic endpoint. Molecular techniques have provided a wealth of mechanistic information about the role of gene function in the interaction of xenobiotics and living organisms. The development of "knockout" mice with genes of interest deleted, along with the development of "humanized mice" with human genes inserted into their genome, has proven extremely valuable in investigations of toxicant metabolism and modes of toxic action. This edition, the fourth (retitled Molecular and Biochemical Toxicology), reflects this by the inclusion of chapters on molecular methods and the inclusion, in essentially every chapter, of molecular studies and approaches currently used in understanding the metabolic processing and mode of toxic action of xenobiotics.

Toxicology can be defined as the branch of science dealing with poisons. Having said that, attempts to define all of the various parameters lead to difficulties. The first difficulty is seen in the definition of a poison. Broadly speaking, a poison is any substance causing harmful effects in an organism to which it is administered, either deliberately or by accident. Clearly, this effect is dose-related inasmuch as any substance, at a low enough dose, is without effect, while many, if not most, substances have deleterious effects at some higher dose. Much of toxicology deals with compounds exogenous to the normal metabolism of the organism, with such compounds being referred to as xenobiotics. However, many endogenous compounds,

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including metabolic intermediates such as glutamate, or hormones such as thyroxine, are toxic when administered in unnaturally high doses. Similarly, trace nutrients such as selenium, which are essential in the diet at low concentrations, are frequently toxic at higher levels. Such effects are properly included in toxicology, while the endogenous generation of high levels of metabolic intermediates due to disease or metabolic defect is not, although the effects on the organism may be similar.

The expression of toxicity, hence the assessment of toxic effects, is another parameter of considerable complexity. *Acute toxicity*, usually measured as mortality and expressed as the lethal dose or concentration required to kill 50% of an exposed population under defined conditions (LD50 or LC50), is probably the simplest measure of toxicity. Nevertheless, it varies with age, gender, diet, the physiological condition of the animals, environmental conditions, and the method of administration. *Chronic toxicity* may be manifested in a variety of ways, including cancer, cataracts, peptic ulcers, and reproductive effects, to name only a few. Furthermore, chemicals may have different effects at different doses. For example, vinyl chloride is a potent hepatotoxicant at high doses and is a carcinogen with a very long latent period at low doses. Considerable variation also exists in the toxic effects of the same chemical administered to different animal species, or even to the same animal when administered via different routes. Malathion, for example, has relatively low toxicity to mammals, but is toxic enough to insects to be a widely used commercial insecticide.

1.2 TOXICOLOGY

Toxicology is clearly related to two of the applied biologies; medicine and agriculture. In medicine, clinical diagnosis and treatment of poisoning as well as the management of toxic side effects of clinical drugs are areas of significance. In agriculture, the development of selective biocides such as insecticides, herbicides, and fungicides is important, and their nontarget effects are of considerable public health significance. Toxicology may also be considered an area of fundamental science because the adaptation of organisms to toxic environments has important implications for ecology and evolution.

The tools of chemistry, biochemistry, and molecular biology are the primary tools of toxicology, and progress in toxicology is closely linked to the development of new methodology in these sciences. Those of chemistry provide analytical methods for toxicants and their metabolites, particularly for forensic toxicology, residue analysis, and toxicant metabolism; those of biochemistry provide methods for the investigation of metabolism and modes of toxic action; and those of molecular biology provide methods for investigations of the roles of genes and gene expression in toxicity.

1.3 BIOCHEMICAL TOXICOLOGY

Biochemical toxicology deals with processes that occur at the cellular and molecular levels when toxic chemicals interact with living organisms. Defining these interactions is fundamental to our understanding of toxic effects, both acute and chronic, and is essential for the development of new therapies, for the determination of toxic hazards, and for the development of new clinical drugs for medicine and biocides for agriculture.

The poisoning process may be thought of as a cascade of more or less distinct events. While biochemical and molecular toxicology are involved in all of these, their involvement in exposure analysis is restricted to the discovery and use of biomarkers of exposure (see Chapter 26). Following exposure, uptake involves the biochemistry of cell membranes and distribution, or transport processes within the body (Chapters 15 and 35). Metabolism, which may take place at portals of entry or, following distribution, in other organs, particularly the liver, may either detoxify toxicants or activate them to reactive metabolites more toxic than the parent chemical (see Chapters 9 through 14). Chemicals with intrinsic toxicity or reactive metabolites are involved in various modes of toxic action, usually initiated by interactions with macromolecules such as proteins and DNA. The study of modes of toxic action is a critically important area of toxicology (see Chapters 16 through 26). The final phase of detoxication, namely excretion (see Chapters 15 and 29), is studied at the cellular, organ, and intact organism levels.

Many of these aspects are studied at the organ level (discussed in Chapters 27 through 35), including portals of entry, respiratory toxicology, hepatotoxicology, nephrotoxicology, toxicology of the peripheral and central nervous systems, immunotoxicity, reproductive and developmental toxicity, and dermatotoxicity.

1.4 CELLULAR TOXICOLOGY

The culture of cells isolated from living organisms has been known since the early years of the twentieth century. By the 1950s the development of standardized culture media and the development of immortalized cell lines increased the utility of cultured cells in many areas of experimental biology, including toxicology. The use of cell culture in toxicological research is an established and useful approach for a number of reasons, including its use in investigating toxic effects on intact cellular systems in a situation less complex than that in the intact organism and its potential utility for routine toxicity testing systems for regulatory evaluations.

Some cells, such as hepatocytes, must be used in primary culture since they will not divide in culture and are relatively short-lived, while other cell lines are capable of division and can, in suitable media, be maintained indefinitely. In other cases, cells have been "immortalized" by fusion with tumor cells and thereafter retain the ability to divide in culture while, at the same time, maintaining many of the properties of the original nontumor cells. All of the various approaches to the use of cultured cells in biochemical and molecular toxicology are summarized in Chapter 8. The relatively recent union of the techniques of cell and molecular biology has been enormously productive for experimental toxicology since cells can be used for the expression of genetic constructs, reproduction of recombinant enzymes, and so on.

1.5 MOLECULAR TOXICOLOGY

The field of molecular biology is usually held to have begun with the description of the double helical structure of DNA by Watson and Crick in 1953, followed by the elucidation of the genetic code in the 1960s. In the subsequent half-century the techniques of molecular biology have expanded exponentially as has its importance in many, if not most, fields of biology. The success of the human genome project has given rise to an entire field devoted to the description of the complete genomes of organisms at all levels in the evolutionary tree. An overview of molecular techniques is presented in Chapter 2, and a review of toxicogenomics is presented in Chapter 3.

The techniques that have proven most valuable in toxicology include those of molecular cloning, the polymerase chain reaction, and the production of genetically modified mice. Microarrays, used to evaluate gene expression under various conditions, including exposure to toxicants, are becoming more important and, in concert with other molecular techniques, are being considered as potentially useful in such applied areas as hazard assessment and risk analysis.

Bioinformatics, which deals with the maintenance, analysis, and integration of genomic data, is discussed in Chapter 6.

1.6 PROTEOMICS AND METABOLOMICS

Since molecular biology is often held to be restricted to events involving nucleic acids, mention must be made of (a) proteomics, the analysis of all proteins in a sample of biological material, and (b) metabolomics, the analysis of all metabolites in a sample of biological material. These fields are discussed in Chapters 4 and 5.

1.7 CONCLUSIONS

The preceding brief description of the nature and scope of biochemical and molecular toxicology should make clear that the study of toxic action is a many-faceted subject, covering all aspects from the initial environmental contact with a toxicant to its toxic endpoints and to its ultimate excretion back into the environment. A considerable amount of material is summarized in the chapters following, but many essentials still remain to be discovered.

SUGGESTED READING

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