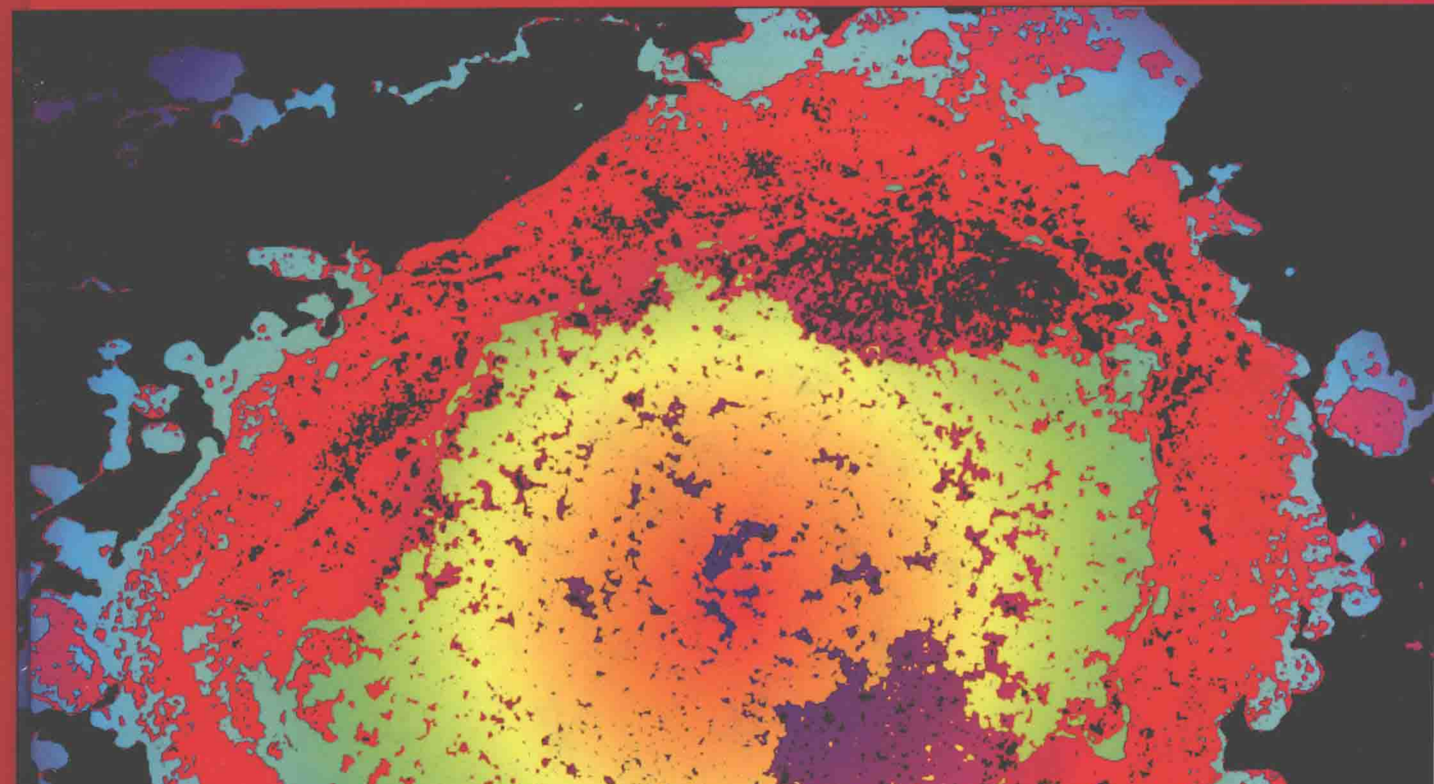
Principles of Biochemical Toxicology

John Timbrell



THIRD EDITION

TAYLOR & FRANCIS
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PRINCIPLES
of
BIOCHEMICAL TOXICOLOGY

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PRINCIPLES *of* BIOCHEMICAL TOXICOLOGY

For
Anna, Becky and Cathy

'. . . and she had never forgotten that, if you drink much from a bottle marked "poison", it is almost certain to disagree with you, sooner or later.'

From *Alice's Adventures in Wonderland, by Lewis Carroll*

Preface to the third edition

This third edition of *Principles of Biochemical Toxicology* has, like the previous edition, evolved against the background of my involvement with the teaching of toxicology to undergraduates reading for the degree in Toxicology and Pharmacology at the School of Pharmacy in London. I am saddened by the demise of that course, which I believe produced a significant number of well-rounded toxicological scientists. However, toxicology can and should be taught as part of a variety of courses and at different levels and I look forward to continuing that effort.

The objective of the book still remains that it should form a sound introduction to the basic principles of the subject from a biochemical and mechanistic viewpoint. It is a testament to the vitality and progression of toxicology that the increasing sophistication, complexity and expansion of the subject means that revision of at least parts of this book is essential every few years. However, a book this size cannot realistically cover all of the diverse aspects of toxicology in equal depth and detail and include all the new developments which are occurring, hence the extensive bibliography, which should be used to complement this text where more detail or other examples are wanted.

Happily the previous edition was written with the benefit of several months sabbatical time. Unfortunately this one has not and so is consequently a much less extensive revision. I hope that if and when a fourth edition is deemed necessary I shall have the luxury of an extended sabbatical period to complete what by then will be a major task! However, in this revision I have taken into account comments that have been made to me since the second edition was published and I hope I have improved both the text and diagrams.

The main addition to the book is the inclusion of summaries and questions at the end of each chapter. These are primarily for students to remind themselves of the key points covered in each chapter.

Again special thanks to Cathy, not only for her critical comments and the diagrams she has drawn or helped with, but also for her patience and support.

London, June 1998

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CHAPTER 1

Introduction

1.1 *Background*

Toxicology is the subject concerned with the study of the noxious effects of chemical substances on living systems. It is a multidisciplinary subject, as it embraces areas of pharmacology, biochemistry, chemistry, physiology and pathology, and although it has sometimes been considered as a subdivision of some of these other subjects, it is truly a scientific discipline in itself.

Toxicology may be regarded as the science of poisons; in this context it has been studied and practised since antiquity, and a large body of knowledge has been amassed. The ancient Greeks used hemlock and various other poisons, and **Dioscorides** attempted a classification of poisons. However, the scientific foundations of toxicology were laid by **Paracelsus** (1493–1541) and this approach was continued by **Orfila** (1787–1853). Nevertheless, development of toxicology as a separate science has been slow, particularly in comparison with subjects such as pharmacology and biochemistry, and toxicology has a much more limited academic base. This may in part reflect the nature of the subject, which has evolved as a practical art, and also the fact that many practitioners were mainly interested in descriptive studies for screening purposes or to satisfy legislation.

1.2 *Scope*

The interest in and scope of toxicology continues to grow rapidly and the subject is of profound importance to human and animal health. The increasing numbers (currently around 100 000) of foreign chemicals (xenobiotics) to which humans and other organisms in the environment are exposed underlies this growth. These include drugs, pesticides, environmental pollutants, industrial chemicals and food additives about which we need to know much, particularly concerning their safety. Of particular importance, therefore, is the ability to predict toxicity and this requires a sound mechanistic base to be successful. It is this mechanistic base that comes within the scope of biochemical toxicology, which forms the basis for almost all of the various branches of toxicology.

The development of toxicology has been hampered by the requirements of regulatory agencies which have encouraged the 'black box' approach of empiricism as discussed by Goldberg (see Bibliography). This routine gathering of data on toxicology, preferably of a negative nature, required by the various regulatory bodies of the industrial nations, has tended to constrain and regulate toxicology.

Furthermore, to paraphrase Zbinden, misuse of toxicological data and adverse regulatory action in this climate of opinion has discouraged innovative approaches to toxicological research and has become an obstacle to the application of basic concepts in toxicology. However, the emphasis on and content of basic science at recent toxicology congresses is testimony to the progress that has taken place in the period since Goldberg and Zbinden wrote their articles (see Bibliography and Reed, D. J., 1995).

Ideally, basic studies of a biochemical nature should be carried out if possible before, but at least simultaneously with, toxicity testing, and a bridge between the biochemical and morphological aspects of the toxicology of a compound should be built. It is apparent that there are many gaps in our knowledge concerning this connection between biochemical events and subsequent gross pathological changes. Without an understanding of these connections, which will require a much greater commitment to basic toxicological research, our ability to predict toxicity and assess risk from the measurement of various biological responses will remain inadequate.

Thus, any foreign compound which comes into contact with a biological system will cause certain perturbations in that system. These biological responses, such as the inhibition of enzymes, and interaction with receptors, macromolecules or organelles, may not be toxicologically relevant. This point is particularly important when assessing *in vitro* data, and involves the concept of a dose threshold, or the lack of such a threshold, in the 'one molecule, one hit' theory of toxicity.

1.3 *Biochemical aspects of toxicology*

Biochemical toxicology is concerned with the mechanisms underlying toxicity, particularly the events at the molecular level and the factors which determine and affect toxicity.

The interaction of a foreign compound with a biological system is two-fold: there is the effect of the organism on the compound and the effect of the compound on the organism. It is necessary to appreciate both for a mechanistic view of toxicology. The first of these includes the absorption, distribution, metabolism and excretion of xenobiotics, which are all factors of importance in the toxic process and which have a biochemical basis in many instances. The mode of action of toxic compounds in the interaction with cellular components, and at the molecular level with structural proteins and other macromolecules, enzymes and receptors, and the types of toxic response produced, are included in the second category of interaction. However, a biological system is a *dynamic* one and therefore a series of events may follow the initial response. For instance, a toxic compound may cause liver or kidney damage and thereby limits its own metabolism or excretion.

The anatomy and physiology of the organism affect all the types of interaction given

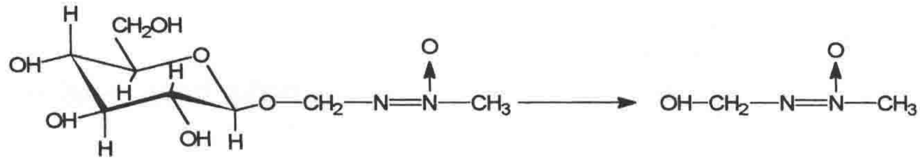

FIGURE 1.1 *Bacterial hydrolysis of cycasin.*

above, as may the site of exposure and entry of the foreign compound into the organism. Thus, the gut bacteria and conditions in the gastrointestinal tract convert the naturally occurring compound **cycasin**, methylazoxymethanol glycoside, into the potent carcinogen **methylazoxymethanol** (figure 1.1). Administered by other routes, cycasin is not carcinogenic.

The distribution of a foreign compound and its rate of entry determine the concentration at a particular site and the number and types of cells exposed. The plasma concentration depends on many factors, not least of which is the metabolic activity of the particular organism. This metabolism may be a major factor in determining toxicity, as the compound may be more or less toxic than its metabolites.

The excretion of a foreign substance may also be a major factor in its toxicity and a determinant of the plasma and tissue levels. All of these considerations are modified by species differences, genetic effects and other factors. The response of the organism to the toxic insult is influenced by similar factors. The route of administration of a foreign compound may determine the effect, whether systemic or local. For example, paraquat causes a local irritant effect on the skin after contact but a serious and often fatal lung fibrosis if it gains entry into the body and bloodstream. Normally only the tissues *exposed* to a toxic substance are affected unless there is an indirect effect involving a physiological mechanism such as an immune response. The distribution of a toxic compound may determine its target-organ specificity, as does the susceptibility of the particular tissue and its constituent cells. Therefore, the effect of a foreign compound on a biological system depends on numerous factors, and an understanding and appreciation of them is a necessary part of toxicology.

The concept of toxicity is an important one: it involves a damaging, noxious or deleterious effect on the whole or part of a living system which may or may not be reversible. The toxic response may be a transient biochemical or pharmacological change or a permanent pathological lesion. The effect of a toxic substance on an organism may be immediate, as with a pharmacodynamic response such as a hypotensive effect, or delayed, as in the development of a tumour.

It has been said that there are no harmless substances, only harmless ways of using them, which underscores the concept of toxicity as a relative phenomenon. It depends on the dose and type of substance, the frequency of exposure and the organism in question. There is no absolute value for toxicity, although it is clear that botulinum toxin has a very much greater relative toxicity or potency than DDT on a weight-for-weight basis (table 1.1). The derivation and meaning of the LD_{50} will be discussed in detail in Chapter 2. However, the LD_{50} is now seldom regarded as a useful parameter of toxicity except in particular circumstances such as the design of pesticides.

There are many different types of toxic compound producing the various types of toxicity detailed in Chapter 6. One compound

TABLE 1.1 *Acute LD_{50} values for a variety of chemical agents*

Agent	Species	LD_{50} (mg/kg body weight)
Ethanol	Mouse	10 000
Sodium chloride	Mouse	4000
Ferrous sulphate	Rat	1500
Morphine sulphate	Rat	900
Phenobarbital, sodium	Rat	150
DDT	Rat	100
Picrotoxin	Rat	5
Strychnine sulphate	Rat	2
Nicotine	Rat	1
d-Tubocurarine	Rat	0.5
Hemicholinium-3	Rat	0.2
Tetrodotoxin	Rat	0.1
Dioxin (TCDD)	Guinea-pig	0.001
Botulinum toxin	Rat	0.00001

Data from Loomis, T. A. (1974) *Essentials of Toxicology* (Philadelphia: Lea & Febiger).

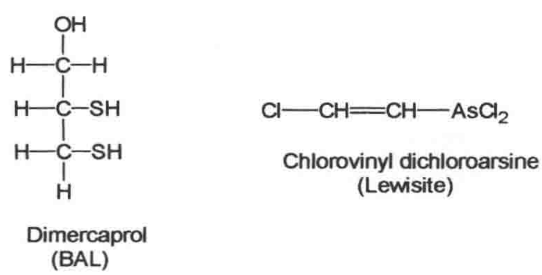

FIGURE 1.2 *Structures of Lewisite and dimercaprol or British Anti-Lewisite.*

may cause several toxic responses. For instance, vinyl chloride (figure 4.6) is carcinogenic after low doses with a long latent period for the appearance of tumours, but it is narcotic and hepatotoxic after single large exposures.

Investigation of the sites and modes of action of toxic agents and the factors affecting their toxicity as briefly summarized here is fundamental for an understanding of toxicity and also for its prediction and treatment.

For example, the elucidation of the mechanism of action of the war gas Lewisite (figure 1.2), which involves interaction with cellular sulphydryl groups, allowed the **antidote**, British Anti-Lewisite or **dimercaprol** (figure 1.2) to be devised. Without the basic studies performed by Sir Rudolph Peters and his colleagues, an antidote would almost certainly not have been available for the victims of chemical warfare.

Likewise, empirical studies with chemical carcinogens may have provided much interesting data but would have been unlikely to explain why such a diverse range of compounds cause cancer, until basic biochemical studies provided some of the answers.

1.4 *Summary*

Toxicology, also called the science of poisons, is a multi-disciplinary subject dealing with the noxious effects of chemicals on living systems. It has a long history in relation to the **art** of poisoning but has now become more **scientifically** based. The scientific foundations of toxicology were laid by Paracelsus. Toxicology is interrelated with the activities of regulatory authorities and its importance is a reflection of the large numbers of chemicals to which man and the environment are exposed. It relies on an understanding of the basic biochemistry and physiology of living systems and the relevant chemistry of toxic molecules. Thus the interaction of a chemical with a living system involves both an effect of the chemical on the biological system and of the biological system on the chemical. These interactions are affected by numerous factors.

The science of toxicology requires an appreciation of the fact that not all effects observed are toxicologically relevant. Toxicity is a damaging effect on whole or part of a living system.

An understanding of the mechanism of toxicity of a chemical is essential for a proper assesment of risk and can lead to the development of antidotes. There are no harmless chemicals, only harmless ways of using them.

1.5 *Review questions*

1 Which 16th century scientist was important in the development of toxicology and why?

2 Why is cycasin only carcinogenic when ingested by mouth?

3 How many times more toxic is botulinum toxin than nicotine in the rat?

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