Biochemical toxicology

a practical approach

Edited by
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Preface

Toxicology is a multi-disciplinary science dealing with the adverse effects of chemical and other agents on living systems and includes major contributions from biochemistry, pharmacology and pathology. Professional toxicologists are involved in establishing the safe limits of chemical substances intended for use as drugs, pesticides, food additives, cosmetics, and industrial chemicals. Research in toxicology is aimed at elucidating the nature and molecular mechanisms of toxic interactions. Such knowledge not only provides a basis for the practice of toxicology and allows the rational prediction of potential toxic hazards but also has been, and is, of great importance in elucidating the metabolic biochemistry of living organisms. Our primary aim in this book has been to provide detailed practical protocols and descriptions of methods which will allow biochemists to enter the fascinating area of toxicological research and will allow toxicologists to apply biochemical techniques and approaches to their studies. We believe that the book will prove indispensable to the novice in providing access to the all-important 'tricks of the trade' which are so often omitted from methods descriptions in research papers. However, we also believe that it will be valuable to experienced toxicologists in guiding them through the range of biochemical approaches which may be applied. The levels of biological complexity to which these methods are applied range from biochemical macromolecules, through subcellular preparations, to the whole animal. Of course, within the limitations of space it is not possible to cover every biochemical technique or biological preparation which can be used in toxicological research. However, we have aimed to include most of the more significant and fundamental practical approaches that are used in this area, and even some which have only recently been developed. The rationale for the choice of topics is provided in the Foreword and the only major topics which have been consciously omitted are *Mutagenicity Testing*, which is covered by another volume in this series edited by S.Venitt and J.M.Parry, and *Carcinogenicity Testing*, which is covered in a book edited by A.D.Dayan and R.W.Brimblecombe (MTP Press, Lancaster, 1978).

It is unfortunate that we have to introduce some notes of regret into this preface. However, it is with sadness that we have to record the untimely death of Professor Eric D.Wills, one of the first of our contributors to complete his chapter. We regret that he never saw the completed version of this book, nor indeed of his own textbook on the *Biochemical Basis of Medicine*. Both provide testimony to his clarity of expression and his erudition and will surely be fitting memorials. Our other note of regret is the long time span between the submissions of the first contributors and the last contributors to this book. The latter are in no way responsible for the publication delay since they responded at short notice to replace certain contributors who withdrew their commitment at a late stage. Although we accept full editorial responsibility for the result of this publication schedule, we do not believe that any contribution is diminished because of it. Indeed, we wish to express our thanks to all the authors for the quality of their contributions and for their forbearance. We are grateful to the staff at IRL Press and, in particular, to Eva Gooding for her unfailing patience and encouragement.

Finally, we wish to dedicate this book in honour of Professor Dennis Parke, Head of Department and Professor of Biochemistry at the University of Surrey, a post he

has held with distinction for the past twenty years. He is, of course, a world-renowned scientist in toxicology and biochemical pharmacology, but we wish to recognise here the significant and pioneering achievements he has made in promoting the science of toxicology in the United Kingdom through his research and his teaching.

Keith Snell and Barbara Mullock

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Abbreviations

BHT	2,6-ditert-butyl-p-methylphenol
BSP	bromosulphophthalein
CHO cells	Chinese hamster ovary cells
CIP	chloroform-isoamyl alcohol-phenol
CP	cyclophosphamide
DCPIP	2,4-dichloro-phenol-indophenol
DMSO	dimethylsulphoxide
EDTA	ethylenediamine tetraacetic acid
EGTA	ethyleneglycobis(β-aminoethyl)ether tetraacetic acid
g.l.c.	gas-liquid chromatography
GLDH	glutamate dehydrogenase
GOT	aspartate transaminase
GPT	alanine transaminase
HBSS	Hank's balanced salt solution
Hepes	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
h.p.l.c.	high-performance liquid chromatography
LAP	leucine aminopeptidase
LDH	lactate dehydrogenase
MEM	minimal essential medium
ODS	octadecyl silicate
PBS	phosphate-buffered saline
PDH	pyruvate dehydrogenase
PMSF	phenylmethylsulphonyl fluoride
PVP	polyvinyl pyrrollidone
RCR	respiratory control ratio
RSA	relative specific activity
S9	post-mitochondrial supernatant
SDH	sorbitol dehydrogenase
SDS	sodium dodecyl sulphate
TBA	thiobarbituric acid
TCA	trichloroacetic acid
t.l.c.	thin-layer chromatography
TMPD	tetramethyl-p-phenaline diamine
WME	William's medium E

Contents

Foreword

KEITH SNELL

The science of toxicology is not a 'pure' discipline; it is a unification of a number of scientific disciplines (e.g. biochemistry, pharmacology and pathology) orientated towards the common goal of the identification, quantification and mechanistic explanation of adverse interactions between a chemical substance and a living organism or biological system. It is concerned with investigations of toxicity; but toxicity is a subjective term since, for example, an anti-bacterial agent is clearly toxic to the organism it is directed against but hopefully not to the organism to which it is administered. Even within a single organism toxicity depends on dose; hence acetylsalicylic acid (aspirin) can have beneficial analgesic properties in humans at low doses, but can be a gastric irritant and ulcerogenic agent at high doses or after chronic administration. It is the goal of toxicity testing to define such parameters as dose-response characteristics and species selectivity and to refine these parameters (along with others) into a quantitative risk-benefit analysis that allows the value judgement of safety to be applied to a chemical substance which may have potential economic or therapeutic use for man. The approaches and methodologies of toxicity testing procedures are not dealt with in this book, but are covered elsewhere (1 – 4), and in general textbooks of toxicology (5 – 8). However, the development of appropriate toxicity testing procedures is critically dependent on fundamental studies on the molecular mechanisms of toxic effects which is the province of Biochemical Toxicology. Only with this basic knowledge is it possible to devise meaningful approaches to the detection of toxicity or indeed to make rational predictions about the nature of the toxic response which might determine the type of testing protocol to be employed. The present book focuses on the application of biochemical methods to investigations of mechanisms of toxicity. Such investigations aim to define the molecular targets of toxic interactions, so as to provide a biochemical explanation for the overt toxicity manifested in the whole organism as well as the basis for the selectivity of toxic actions.

Apart from the differential cellular sensitivity conferred by the presence of critical molecular targets, another major determinant of selectivity can be the generation of the ultimate toxicant chemical species at the susceptible site of toxic interaction. Investigations of this latter aspect involve considerations of pharmacokinetics and of xenobiotic metabolism. Thus the measurement of the parent chemical compound and its metabolic products in body fluids is an essential tool and the relevant techniques are considered in Chapter 1. Since the liver is the most biochemically active organ in the metabolism of xenobiotics, the activation or detoxification of chemical substances is frequently assessed using liver-derived systems. The most physiological of these is the intact perfused liver (Chapter 2). The use of liver cells, either freshly isolated or in primary culture (Chapter 3), has the advantage that a single cell type (hepatocyte) is being studied and that different experimental conditions can be employed with a preparation from a single animal. Even more defined is the liver microsomal subcellular fraction (Chapter 8), where the reactions and enzymes of many of the pathways of xenobiotic metabolism can be studied in isolation from many other intracellular biochemical pathways. A major attribute of this preparation is the cytochrome P-450

mixed function oxidase (monooxygenase) enzyme system which carries out the bioactivation of many toxic chemicals (9, 10). For this reason a crude microsomal preparation with supplementations is often included in biological preparations which otherwise have a limited capacity for the bioactivation of chemical toxicants (Chapters 3–5). Liver perfusion and cell culture techniques are also useful in defining and elucidating the cellular responses to toxic insult, free from the potential ambiguities of interpretation inherent in studies at the whole animal level (Chapters 2 and 3). Similarly, the post-implantation embryo culture system (Chapter 4) affords a useful tool for the study of teratological mechanisms without the ambiguities that might arise from maternal-conceptus interactions *in vivo*.

Ultimately, the sensitivity of a biological system to toxic insult is defined and characterised by the presence of critical molecular targets. Of these macromolecules, proteins possess highly specific functional characteristics and are difficult to consider in a generalised fashion; each must be studied individually. For other cellular macromolecules such as nucleic acids and lipids, it is feasible to study a more generalised interaction with the toxic chemical. In the case of nucleic acids, DNA is a critical toxicological target, through the covalent binding of reactive chemical toxicants, because of the known associations between chemical modification of DNA and mutagenicity and carcinogenicity (Chapter 5). For lipids, the most significant chemical damage comes from peroxidative attack (Chapter 6) and the consequent disturbances of structural integrity and functioning of biological membranes.

In many cases, the prime interest for the biochemist in linking the toxicant-target interaction to cellular damage, is the consequence for the normal functioning of the target molecule. A valuable approach in elucidating the mechanism of an agent at this level is to study the functional properties of the subcellular organelle in which the target macromolecule is located. More usually it is the cellular response to the toxicant which implicates a particular subcellular process, and then the demonstration of a direct effect on the isolated subcellular organelle will provide clues to the identity of the ultimate target molecule. These approaches are detailed in this book for subcellular fractions derived from the plasma membrane (Chapter 7), the endoplasmic reticulum (Chapter 8), mitochondria (Chapter 9), and lysosomes and peroxisomes (Chapter 10).

With the methodological details provided, it should be possible for biochemists to apply their skills to problems of toxicological interest. The principle aim of this book is to encourage such approaches and provide the practical means to follow them.

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CHAPTER 1

Methods for Studying Metabolism and Distribution *In Vivo* of Radiolabelled Drugs

L.GRAHAM DRING

1. INTRODUCTION

The methods used for studying drug metabolism and distribution have advanced greatly in the last 20 years, largely because of the increasing requirement for drug safety evaluation after the thalidomide disaster. That metabolic data would become part of the regulatory requirements for drug registration became apparent on the publication of the 'Goldenthal letter' (1) in the USA: 'Although at present, we are not insisting that metabolic data be submitted while the drug is under investigational exemption, we will expect to see information of this type in most New Drug Applications for new entities in the coming year'.

As a result, regulatory bodies have followed suit in many countries, thus in the UK the major requirements of the DHSS are set out in Notes on Applications for Clinical Trial Certificates (2). These notes recommend that metabolism studies include the following.

(i) *Plasma levels.* Single dose, peak drug levels and calculation of half-life in species used in toxicology. Chronic drug administration to identify any accumulation and to test for enzyme induction.

(ii) *Distribution.* To include plasma levels and autoradiography or quantitative studies, of major organs and the pregnant animal.

(iii) *Excretion.* Total urine and faeces collection. Times should allow reasonably complete recovery. Evidence for enterohepatic recycling.

(iv) *Metabolites.* Identification or separation conducted as far as is technically reasonable.

These are really the minimum requirements and most pharmaceutical companies when studying new chemical entities will have the basic pharmacology and biopharmaceutical information related to the drug and will be actively supporting the toxicity studies and pharmaceutical development. After judicious choice of formulation, route of administration and other biopharmaceutical factors, the animal experiments would comprise bioavailability/dose proportionality studies at the dose levels used in the toxicology studies. This can often give the toxicologist a valuable insight into the behaviour of the drug in the organism at increasing dose levels. It is also possible to generate much information using pregnant animals and the foetus which could help in the interpretation of the peri- and post-natal toxicology. Tissue distribution studies not only help the toxicologist, who may find that the accumulation of a drug in a particular organ goes far in explaining the toxicity to that organ, but also are of importance when assessing the feasibility of human studies with the radiolabelled drug.