BIOCHEMICAL PHARMACOLOGY AND TOXICOLOGY

Volume I
Methodological Aspects of Drug Metabolizing Enzymes

Edited by
DAVID ZAKIM

and
DONALD A. VESSEY

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SERIES PREFACE

The total number of known chemical compounds exceeds 6 million and is increasing at the rate of 25,000 per month. The number of chemical compounds to which humans are commonly exposed is estimated to be in excess of 60,000 and is increasing in number and diversity. Given that many new chemical compounds have been discovered to have unanticipated toxicities, the increasing chemical diversity in our environment presents a substantial health threat to us and to other living things. Greater predictability is needed and will come only through a basic understanding of the target systems and the nature of their interaction with the xenobiotic.

We believe that the need to understand this increasing chemical challenge to biological systems will stimulate research in this area. This research will involve the application of basic biochemistry to the study of important pharmacological and toxicological problems. Because investigators in these fields are led to the study of biochemical systems that are new to them, there is a need for a treatise to introduce investigators in a practical way to the biochemistry of those systems.

It is the aim of this series to address the basic biochemistry of a number of systems of interest to pharmacologists and toxicologists. We intend to provide a fundamental understanding of the areas presented and to develop a working knowledge by providing methodological details. Further, it is hoped that these presentations will encourage research into these areas by focusing attention on significant unresolved issues.

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April 1985

PREFACE

Biological systems have evolved enzymes that detoxify naturally occurring toxins as well as a variety of compounds added to the environment by our industrial society. Alternatively, these enzymes occasionally catalyze reactions that lead to a metabolic activation of compounds to toxins. In both situations the xenobiotic metabolizing enzymes are affecting the health and survival of the organism.

The lack of knowledge of the detailed properties of enzymes that modify the biological activity of endogenous and exogenous chemicals underlies many problems in toxicology and therapeutics. The administration of a drug or toxin in amounts that exceed an animal's metabolic capacity can lead to a serious, if not fatal, disease. A more significant and frequent clinical problem is the unpredictable and untoward reaction to a therapeutic dose of a drug, especially in geriatric patients or those on polypharmacy regimens. These kinds of reactions, which may account for as much as 10% of all hospital admissions in the United States, can often be traced with certainty to competition between drugs for a common detoxification pathway. It is likely that most of these

reactions will eventually be understood in this context. In addition, the need to detoxify small amounts of environmental pollutants could be affected by therapeutic agents and vice versa.

It is clear, therefore, that understanding the kinetic properties of the xenobiotic metabolizing enzymes, the manner in which they are regulated, their muliplicity and the chemical basis for their substrate specificities, will have a significant, positive effect on the value of medical intervention. It is also recognized that understanding the induction of xenobiotic metabolizing enzymes may have practical medical significance. It is certain that a great deal of new knowledge relevant to basic biology and biochemistry will come from studies of xenobiotic metabolizing enzymes.

The number of investigators interested in studying xenobiotic metabolizing enzymes continues to increase. However, most of these enzymes have a complexity that often serves to deter investigators who might otherwise wish to work in this area or, at least, limits the depth of their enquiry. These complexities exist because such enzymes differ from simple soluble fraction enzymes in that generally they are tightly bound to membranes, they have very broad substrate specificities, there are multiple molecular forms of each enzyme, their substrates are relatively insoluble in water, and their kinetic properties are often complex. In this volume we have focused our attention on those drug metabolizing enzymes that possess these complexities. The enzymes addressed are: the epoxide hydratases, the sulfotransferases, the UDP-glucuronyl-transferases, the monoamine oxidases, the cytochromes P450, and the glutathione S-transferases.

In writing about each of these enzymes, the authors have endeavored to provide sufficient basic information to allow the reader to approach the current research literature with understanding. They have also included lengthy discussions of the methods currently used to characterize the enzymes and the limitations and pitfalls associated with these methods. The aim has been to provide a fundamental treatise that will not only introduce people to the varied aspects of the study of these enzymes but also provide the necessary details for actually undertaking such investigations. We feel fortunate having found authors who feel

the same need for such a text and who possess the high level of scholarship necessary to undertake this endeavor. We thank John Wiley & Sons for providing us with this opportunity.

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MEMBRANE-BOUND AND SOLUBLE-FRACTION EPOXIDE HYDROLASES

Methodological Aspects

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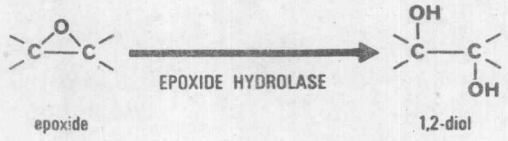
1. INTRODUCTION

Epoxides are highly strained three-membered cyclic ethers which vary considerably in reactivity (Figure 1). The toxicity of these compounds often is related directly to their reactivity, and many of the electrophilically reactive epoxides are mutagenic, carcinogenic, or cytotoxic.[1]

The occurrence of epoxides is widespread. They are formed *in vivo* in several important biosynthetic pathways and as metabolites of xenobiotics. Leukotriene A_4 (2)[2] and squalene 2,3-oxide (1j)[3,4] are two examples of epoxidized biosynthetic intermediates. Numerous olefinic and aromatic xenobiotics, including many drugs used clinically, are metabolized to epoxides by microsomal cytochrome P-450-dependent monooxygenases,[5] and epoxides may also be formed by peroxide rearrangement. Two well-known examples of xenobiotics metabolized to epoxides are aflatoxin B_1 (3)[1] and benzo (a)pyrene (see **1h**). Many natural products contain epoxides, including mycotoxins such as the 12,13-epoxytrichothecenes which are important contaminants of animal and human foodstuffs.[6] Epoxides are also used extensively in industry as intermediates and stabilizers. Manson has provided an extensive review of the vast number of epoxides to which humans are exposed.[7]



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Epoxide hydrolases (EC 3.3.2.3)—formerly named epoxide hydrases or epoxide hydratases (EC 4.2.1.63)—catalyze the conversion of epoxides to 1,2 diols. The diols resulting from such hydration are more polar, more readily excretable, and generally represent a detoxification process. However, the diols of certain polycyclic aromatic hydrocarbons can be metabolized further by

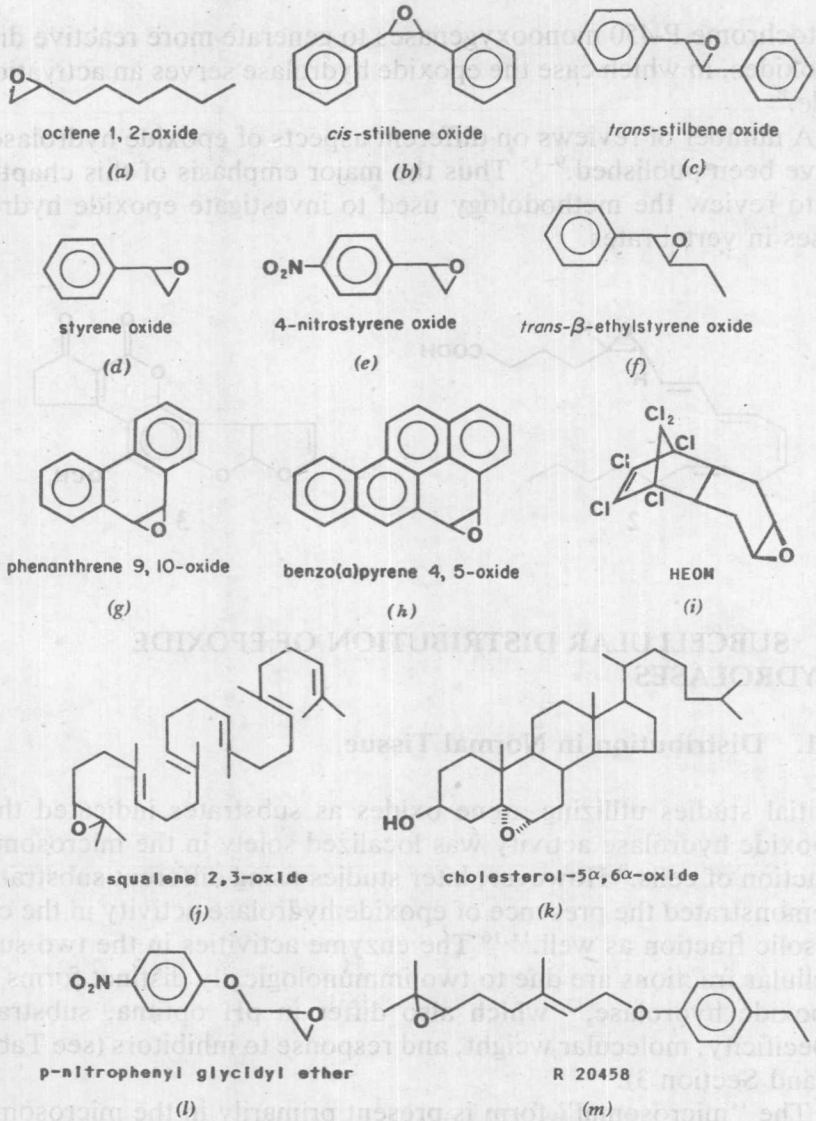

Figure 1. Commonly used substrates of epoxide hydrolases. HEOM = 1,2,3,4,9,9-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-methanonapthalene. R 20458 = 1-(4′-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-2*E*-octene. *Note*: Epoxides may be named as either the oxide of an alkane (e.g., octane 1,2-oxide) or trivially as the oxide of an alkene (e.g., octene oxide). Both methods of nomenclature have been used extensively in the literature.