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# Microsomes, Drug Oxidations, and Chemical Carcinogenesis

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# EXTRAMICROSOMAL EPOXIDE HYDRATION

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## I. INTRODUCTION

Epoxide hydrases are enzymes which add water to three-membered cyclic ethers (epoxides) to yield 1,2-diols. Microsomal epoxide hydrases, including those continuous with the nuclear membrane, have received a great deal of attention (see 1-3 and Oesch, this series, for a review), but for some substrates extramicrosomal epoxide hydration represents a major route of metabolism. This extramicrosomal hydration may be an important detoxification pathway since some, but not all, epoxides may be toxic, mutagenic or carcinogenic. Most of the work on extramicrosomal metabolism has dealt with a cytosolic or 100,000 g soluble enzyme(s), and the properties of the cytosolic and microsomal enzymes were recently compared (3). The purpose of this report is to summarize our current knowledge of extramicrosomal epoxide hydration largely concentrating on the activity in the cytosolic fraction.

## II. RESULTS AND DISCUSSION

### A. *Subcellular and Tissue Distribution of and Species, Age, and Sexual Differences in Epoxide Hydrase Activity*

Epoxide hydrase activity is either microsomal or nuclear when styrene oxide is used as a substrate; however, styrene oxide is one of the few substrates not hydrated by the cytosolic and mitochondrial subcellular fractions. Using methyl *cis*-epoxystearate as substrate, the presence of hydrase activity has been clearly established in the mitochondrial and cytosolic as well as the microsomal fractions (4). Cytosolic epoxide hydrase activity is found in all tissues of the rabbit and mouse examined, and it is consistently higher than that found in the microsomal fraction (3-6). Expoxide hydrase activity





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is in the cytosolic fraction of every vertebrate so far examined, but the activity is very low in the rat. Activity in the cytosol increases slowly with age in female Swiss-Webster mice, but there is a large increase in epoxide hydrase activity about the time of puberty in male mice (5-7).

*B. Substrate Selectivity of the Cytosolic Hydrase*

1. Terpenoid and Steroid Epoxides

Cytosolic epoxide hydration was first detected while investigating the mammalian metabolism of an epoxygeranyl phenyl ether pesticide (8,9), and the enzymatic nature of the epoxide hydrase activity in the cytosol acting on three terpenoid epoxides in mouse liver and kidney was then established (6). Squalene oxide and dioxide as well as lanosterol epoxide are hydrated by the cytosolic fraction (3). Sevanian and Hammock found that cholesterol-5α,6α-epoxide is metabolized slowly, if at all, by the cytosolic fraction of mouse liver, and its hydration is relatively slow in rat lung microsomes, with the rates still lower in the lung cytosolic fraction (10). Some hydration of this substrate seems to occur in the cytosolic fraction of the adrenal cortex (11). Further studies with terpenoid derivatives indicated that lipophilic mono and 1,2-disubstituted epoxides are more rapidly hydrated than tri and tetrasubstituted epoxides (12-14).

2. Other Aliphatic Epoxides

The cytosolic fraction hydrates a wide variety of aliphatic epoxides. The most rapidly hydrated compounds seem to have a lipophilic center on either side of the epoxide moiety such as epoxy fatty esters (3-5,7). Geometrical pairs of compounds such as *trans* and *cis*-stilbene oxides may prove very useful in evaluating the relative contribution of microsomal and cytosolic epoxide hydrases. For instance, *cis*-stilbene oxide is hydrated >30 times faster in the mouse liver microsomal than in the cytosolic fraction while *trans*-stilbene oxide is hydrated >700 times faster in the cytosolic fraction, which may explain the limitations of *trans*-stilbene oxide as an inducing agent in mammals other than the rat. Three closely related compounds illustrate the wide differences in the substrate selectivity of the cytosolic and microsomal enzymes. Styrene oxide is only hydrated by the microsomal fraction, *trans*-β-methylstyrene oxide is largely hydrated by the cytosolic fraction, while allylbenzene oxide is rapidly hydrated by both fractions. The cytosolic fraction will hydrate glycidyl ethers, but bicyclic epoxides appear to be poor substrates (3). The lack of hydration of styrene oxide by the cytosolic fraction, the neutral pH optimum of the enzyme and the low cytosolic epoxide hydrase ac-

tivity in tissues of the rat, in conjunction with the chronology of the experiments performed, illustrate how the epoxide hydrase activity in the cytosolic fraction was overlooked for a decade by many laboratories (3,7).

### C. *Reduction of Mutagenicity by Epoxide Hydrases*

The ability of the liver cytosolic and microsomal fractions from mouse, rat and guinea pig to reduce the mutagenicity of three aliphatic expoxides in the Ames' *Salmonella* assay could be largely explained by comparing the substrate selectivities of the microsomal and cytosolic epoxide hydrases for the three compounds and the relative enzyme levels in the three mammals (15). When a rat S9 preparation was used to activate benzo[a]pyrene and aflatoxin $B_1$, addition of the cytosolic fraction caused a statistically significant increase in the $HIS^+$ revertant colonies caused by both compounds.

### D. *Inhibition, Stimulation and Mechanism of Cytosolic Epoxide Hydrase Activity*

Epoxide hydrase in the cytosolic fraction is inhibited by divalent and monovalent copper ions as well as divalent zinc or iron (4), while trivalent iron stimulates epoxide hydrase activity. Trifluoromethyl ketones (potent "transition state mimics" for esterases acting on insect chemical mediators), also show promise as inhibitors of the cytosolic epoxide hydrase. All epoxides so far examined in the cytoplasmic fraction are hydrated stereospecifically in the *trans* manner (3-5,12,13). When an $^{18}O$ labeled trisubstituted epoxide was hydrated by the cytosolic fraction, attack by water occurred at the least hindered 2° carbon (16).

## III. CONCLUSION

As evidenced by the contributions to this volume, extramicrosomal epoxide hydration has received very little attention in comparison with the microsomal enzyme(s). Continued research is clearly needed to establish the relative contribution of microsomal and extramicrosomal hydrases and transferases to epoxide metabolism. Also, an appreciation is needed of the factors which may influence these metabolic pathways.

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