Ann. Rev. Pharmacol. Toxicol. 1980. 20:513–31

MOLECULAR PROPERTIES AND BIOLOGICAL FUNCTIONS OF MICROSOMAL EPOXIDE HYDRASE

❖6779

Anthony Y. H. Lu and Gerald T. Miwa

Department of Animal Drug Metabolism and Radiochemistry,
Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

INTRODUCTION

Many drugs, mutagens, and environmental carcinogens are aromatic or olefinic compounds and are metabolized in mammals by enzymes localized in the endoplasmic reticulum of various tissues (1, 2). Biologically active arene oxides which are formed by the microsomal cytochrome P-450–containing monooxygenase system are the initial products from many of these compounds, polycyclic aromatic hydrocarbons being one class that has been extensively investigated (3–7). These reactive epoxides and arene oxides can be hydrated enzymatically via microsomal epoxide hydrase (epoxide hydratase, EC 4.2.1.63) to chemically less reactive *trans*-dihydrodiols. In addition, arene oxides can undergo nonenzymatic isomerization to phenols, be conjugated with glutathione via the enzyme glutathione-S-epoxide transferase, and also react with DNA, RNA, and proteins to form covalently bound products. Since certain of these arene oxides have toxic, mutagenic, and carcinogenic properties, the ability of epoxide hydrase to convert epoxides to dihydrodiols has been recognized as an important metabolic step in the detoxification of these compounds. However, most recent investigations (8–16) have also shown that the cytochrome P-450 system and epoxide hydrase convert many polycyclic aromatic hydrocarbons to more reactive bay region diol epoxide derivatives. Thus, epoxide hydrase plays a central role in both the inactivation of mutagenic and carcinogenic metabolites of polycyclic aromatic hydrocar-

0362-1642/80/0415-0513$01.00

bons as well as in the activation of these metabolites to more toxic compounds.

In order to examine the role of metabolism in the cytotoxicity and carcinogenicity of these foreign chemicals, a basic understanding of the properties and mechanism of action of the enzymes involved in these activation and inactivation processes is necessary. In this article, we describe recent progress on the purification and characterization of microsomal epoxide hydrase, its mechanism of action, and its role in mutagenesis and carcinogenesis. For additional information on this enzyme, readers are referred to several other review articles (6, 17, 18).

MOLECULAR PROPERTIES OF EPOXIDE HYDRASE

Location and Distribution

The pioneering studies of Oesch, Jerina & Daly (19, 20) established that rat epoxide hydrase is located primarily in the endoplasmic reticulum in close association with the cytochrome P-450 system, that it is present in various animal species and organs, and that its activity increases during maturation. In addition, liver microsomal epoxide hydrase activity can be induced in vivo by compounds such as phenobarbital and 3-methylcholanthrene, but the induction of epoxide hydrase and the cytochrome P-450–containing monooxygenase system is apparently under separate genetic control (21, 22). The biological half-life of epoxide hydrase in a primary fetal rat liver cell culture has been estimated to be 72 hr (23).

With the development of more sensitive enzyme assays the ubiquity of epoxide hydrase in a wide variety of mammalian and nonmammalian vertebrate species has been established (24, 25). In addition, enzyme activity is present essentially in all organs and tissues of rats and mice (26). Recently, the presence of a cytosolic epoxide hydrase in mouse liver has been reported (27). This soluble enzyme appears to be involved in the metabolism of a variety of lipophilic substrates including insect juvenile hormone.

Topology

Epoxide hydrase activity in liver microsomes can be activated 30% to 60% by various neutral and anionic detergents (28). This activation by detergent and studies with proteases and the nonpenetrating chemical agent diazobenzene sulfonate suggest that epoxide hydrase may be buried deeply in the hydrophobic lipid constituting the microsomal membrane (29). On the other hand, the antigenic site of the enzyme appears to be exposed on the cytoplasmic side of the membrane since antibody produced against purified epoxide hydrase can interact with microsomes in the presence or absence of detergent and produces immunoprecipitates (30, 31). However, it is

uncertain from these studies whether the catalytic site of the enzyme is exposed or buried because the antibody-antigen complex retains catalytic activity.

Biochemical Properties of Purified Epoxide Hydrase

Liver microsomal epoxide hydrase was first partially purified by Oesch & Daly (20). More recently, the enzyme has been purified to apparent homogeneity as judged by sodium dodecylsulfate gel electrophoresis by several laboratories (32–35). The purified enzyme contains a single polypeptide with a molecular weight of approximately 50,000 in the presence of sodium dodecylsulfate, but exists as high molecular weight aggregates ($\sim$ 600,000) in the absence of dodecylsulfate.

The absolute absorption spectrum of the purified epoxide hydrase has a single absorption maximum at 280 nm and a shoulder around 290 nm indicative of a high tryptophan content. The absence of an absorption peak or shoulder in the 320 to 560 nm region indicates that the protein does not contain a prosthetic group such as heme or flavin. Metal analysis of the purified enzyme showed the presence of insignificant amounts of Fe, Zn, Cu and the absence of Mn and Mo (32). Thus, it appears that the catalytic function of epoxide hydrase does not require the presence of any prosthetic groups.

Amino acid analysis indicates that the purified epoxide hydrase has a relatively high content of tryptophan and tyrosine, and a high percentage of nonpolar residues (32, 36). Bentley et al (36) have reported that in their purified epoxide hydrase preparation, the N-terminal amino acid is blocked while the C-terminal amino acid is either asparagine or glutamine. On the other hand, DuBois and co-workers (37) have identified methionine and leucine as the N- and C-terminal amino acids, respectively, for their purified epoxide hydrase preparation. The reason for this discrepancy is presently unexplained.

Substrate Specificity of Purified Epoxide Hydrase

The substrate specificity of the purified rat epoxide hydrase, like the membrane-bound enzyme, is very broad. Thus, the purified rat enzyme can hydrate a variety of alkene oxides (such as styrene 7,8-oxide and octene 1,2-oxide) as well as the K-region arene oxides of phenanthrene, 7-methylbenzo(a)anthracene, benzo(a)anthracene, benzo(a)pyrene, 3-methylcholanthrene and dibenzo(a,h)anthracene, and the non-K-region arene oxides of naphthalene and benzo(a)pyrene (35, 38, 39). Among the oxides tested, phenanthrene 9,10-oxide is the best substrate whereas dibenzo(a,h)anthracene 5,6-oxide is the poorest. Of the four arene oxides of benzo(a)pyrene studied, the hydration rates of benzo(a)pyrene 4,5-, 7,8-, and 9,10-oxides are

similar but the hydration rate for benzo(a)pyrene 11,12-oxide is considerably lower. Studies with these four benzo(a)pyrene oxides also show that the 4,5-oxide has the lowest apparent K_m whereas the 11,12-oxide has the highest apparent K_m (38). The rates of metabolism of the arene oxide of polycyclic aromatic hydrocarbons cannot be predicted based only on the location of the oxide ring which can be at the K-region, non-K-region, or a sterically hindered region (40). However, it is clear that the highly mutagenic or carcinogenic bay region diol epoxides of several polycyclic aromatic hydrocarbons are either not substrates or are rather poor substrates of epoxide hydrase (40–43).

Role of Lipid in Catalysis

Since epoxide hydrase is anchored in the microsomal membrane and many of its substrates are highly lipophilic, its lipid environment would be expected to influence the catalytic properties of the enzyme or the local concentration of substrates near the active site of the enzyme. Indeed, a survey of the literature indicates that the apparent K_m of some substrates is dependent on the amount of microsomes used in the assay mixture (38, 44). These results indicate that the classical Michaelis-Menten equation which was derived for a single phase system may not adequately describe catalysis by epoxide hydrase since the membrane-bound enzyme in microsomal suspension consists of a lipid bilayer phase and an aqueous phase.

The effect of lipid on the kinetics of hydration by the purified, lipid-depleted epoxide hydrase is dependent on the particular substrate being studied and most likely related to the lipid/water partition coefficient of the compound (38). The removal of 90% of the total lipid from liver microsomes does not affect hydration of styrene 7,8-oxide nor does addition of microsomal lipid to lipid-depleted microsomes or to purified epoxide hydrase (32). The addition of dilauroyl phosphatidylcholine or heated liver microsomes has little or no effect on the hydration rate of octene 1,2-oxide or on its apparent K_m value. However, with more lipophilic compounds such as benzo(a)pyrene 4,5-, and 11,12-oxides, the presence of phospholipid or heat-inactivated microsomes extends the time linearity of the reaction and increases the apparent K_m under initial rate conditions. In the case of hydration of benzo(a)anthracene 5,6-oxide, phosphatidylcholine eliminates substrate inhibition.

The kinetic parameters of the hydration of benzo(a)pyrene 11,12-oxide have been examined in detail (38). When microsomes are used as the source of enzyme, the apparent K_m values for benzo(a)pyrene 11,12-oxide are estimated to be 120, 1200, and 5500 μM in reaction mixtures containing 100 μg, 500 μg, and 1000 μg microsomal protein, respectively. When

purified epoxide hydrase is used as the enzyme source, the apparent K_m for benzo(a)pyrene 11,12-oxide is independent of enzyme concentration but dependent on added lipid concentration. Thus, in the absence of added dilauroyl phosphatidylcholine or in the presence of this lipid at a concentration below its critical micelle concentration (0.4 to 1.0 X 10^{-4} M), the observed K_m for benzo(a)pyrene 11,12-oxide (18 μM) remains constant and can be considered to be the true K_m for this substrate. However, when the lipid concentration is greater than the critical micelle concentration, the apparent K_m value increases linearly with lipid concentration. In fact, the addition of lipid above the critical micelle concentration gives kinetic patterns that mimic competitive inhibition. This apparent inhibition is due to partition of the substrate between the aqueous phase and the lipid micelles. As a result, the concentration of substrate in the aqueous phase available to the enzyme is lower than it would be in the absence of lipid micelles. The above results are consistent with a model (38) in which lipid-soluble substrates partition between lipid micelles and the aqueous medium.

MULTIPLE FORMS OF MICROSOMAL EPOXIDE HYDRASE

Induction Studies

Pretreatment of animals with various inducers results in differential effects on the metabolism of a variety of drugs, carcinogens, and endogenous compounds and provides strong supporting evidence for the presence of multiple forms of cytochrome P-450 in liver microsomes (2, 45, 46). A similar approach has not answered the question as to whether multiple forms of epoxide hydrase exist in liver microsomes prepared from a single species. Jerina et al (47) reported that prior treatment of rats with phenobarbital increased the rate of hydration for 11 substrates from 100% to 160%, whereas 3-methylcholanthrene treatment increased these same activities by only 40% to 80%. Bresnick and co-workers (48) also found that phenobarbital administration elevated the microsomal hydration of four substrates by 130% to 170%. However, when 3-methylcholanthrene was used as the inducer, differential effects were observed. Thus, the hydration of octene 1,2-oxide was increased at a dose of 20 mg/kg by 29% after 2 days and at a dose of 40 mg/kg by 40% after 3 days. In contrast, the rates of hydration of styrene 7,8-oxide, naphthalene 1,2-oxide, and 3-methylcholanthrene 11,12-oxide were unaltered after 3-methylcholanthrene treatment. Since the extent of induction by 3-methylcholanthrene was weak and also variable, these results should be interpreted with caution. Thus, the apparent lack of a differential effect by inducers on the metabolism of a variety of alkene and arene oxides could mean either there is only one species of epoxide hydrase

in rat liver microsomes or there are several different enzymes but under similar genetic control.

Purification Studies

In 1971, Oesch et al (49) reported that specific activity of several structurally unrelated alkene and arene oxides determined by a partially purified epoxide hydrase preparation from guinea pig liver microsomes was increased approximately 26 to 30 fold over the starting material whereas the hydration of benzene oxide was only increased 4-fold by the same preparation. In addition, the hydration of styrene 7,8-oxide was not inhibited by benzene oxide but was inhibited by other alkene and arene oxides. Subsequent study by Dansette and co-workers (50), using partially purified epoxide hydrase preparation from rat liver microsomes, confirmed that benzene oxide did not inhibit the hydration of styrene 7,8-oxide and naphthalene 1,2-oxide. Based on these results, it has been suggested that these partially purified preparations contain at least two different epoxide hydrases. However, this interpretation has been brought into question by the results of more recent studies (51) which indicate that benzene oxide has a relatively high K_m compared to styrene oxide and that the hydration of benzene oxide is extremely sensitive to detergent inhibition. Thus, the low purification factor for hydration of benzene oxide could be attributed to the presence of small amounts of detergent in the enzyme preparation, and the low affinity of benzene oxide for the enzyme could explain the lack of benzene oxide inhibition on the hydration of other substrates.

Although rat liver microsomal epoxide hydrase has been purified to homogeneity by several laboratories (32–35), one cannot completely rule out the possible presence of more than one form of electrophoretically similar epoxide hydrase in these preparations. Indeed, several interesting observations were noted when the properties of these purified enzyme preparations were examined. For example, the pH optimum and the purification factor for different substrates are not the same (38, 39). In addition, modifiers such as metyrapone and cyclohexene oxide exert differential effects on the hydration of different substrates using either the purified enzymes or liver microsomes (31, 40). Thus, metyrapone greatly stimulated the hydration of styrene 7,8-oxide, naphthalene 1,2-oxide, and octene 1,2-oxide, but strongly inhibited the hydration of benzo(a)pyrene 11,12-oxide and dibenzo(a,h)-anthracene 5,6-oxide. It somewhat inhibited the hydration of benzo(a)pyrene 7,8- and 9,10-oxides and slightly stimulated the hydration of phenanthrene 9,10-oxide, benzo(a)anthracene 5,6-oxide, and benzo(a)pyrene 4,5-oxide. Differential inhibition of the hydration of these substrates by cyclohexene oxide was also observed (31).

Although these results suggest the presence of more than one enzyme in the purified rat epoxide hydrase preparations, they are also subject to other interpretations. For example, because of the lipophilicity and other complications, the assay conditions for some of the substrates may not be optimal. In addition, the purified enzyme preparations undoubtedly still contain small amounts of detergent which could differentially affect hydration rates of the various substrates. Thus, the different purification factors (ranging from 10 to 60 fold) for different substrates might simply reflect the complexity of the assays. An alternate interpretation for the differential effects of metyrapone and cyclohexene oxide could be that epoxide hydrase possesses multiple catalytic and effector sites. The binding of an effector to the enzyme could cause the enzyme to undergo a conformational change which would affect these catalytic sites resulting in the differential effects for different substrates. In agreement with this hypothesis is the report by DuBois et al (37) that the purified liver microsomal epoxide hydrase from phenobarbital-treated rats contains only a single N-terminal and C-terminal amino acid and that the sequencing of the first 20 amino acids adjacent to the N-terminal gives no indication of the presence of more than a single polypeptide in the preparation.

On the other hand, Guengerich and co-workers (35, 52) have recently provided evidence for the presence of multiple forms of liver microsomal epoxide hydrase in individual rat and human tissues. Different forms have been physically separated by column chromatography and purified to apparent homogeneity. The purified enzymes have different amino acid compositions and can also be distinguished by their immunochemical properties. Although each form possesses very broad substrate specificity, their activities toward different substrates are significantly different. Interestingly, these different forms have similar molecular weights as determined by gel electrophoresis in the presence of sodium dodecylsulfate, and modifiers such as metyrapone and cyclohexene oxide still exert differential effects with different substrates. Furthermore, the level of each of these forms can be altered by in vivo pretreatment with various inducers.

Epoxide Hydrase from Different Species and Tissues

Microsomal preparations derived from various animal species and different tissues exhibit different activities toward different substrates, suggesting the presence of a distinct epoxide hydrase in different animal species and different tissues (25, 39). Immunochemical studies using antibodies produced against purified rat liver epoxide hydrase also support this idea (31, 40, 44, 53). Thus, antibodies prepared against rat liver epoxide hydrase do not cross-react with rabbit or human liver microsomal epoxide hydrase and

cross-react poorly with guinea pig and hamster liver microsomal epoxide hydrase, indicating significant species differences in liver microsomal epoxide hydrase.

An immunological approach has also been used to establish that liver microsomal epoxide hydrase prepared from rats, B6 mice, and D2 mice are immunologically identical (31, 40). The antibody prepared against rat liver epoxide hydrase reacts with rat lung and kidney microsomal epoxide hydrase forming a single immunoprecipitin band with a line of identity with the purified rat liver enzyme. Furthermore, Burchell and co-workers (54) have shown that human and guinea pig liver epoxide hydrases are immunologically indistinguishable.

HUMAN LIVER EPOXIDE HYDRASE

Human liver microsomes contain relatively high levels of epoxide hydrase activity as compared to either untreated or phenobarbital-treated rats or other animal species (19, 55, 56). Kapitulnik et al (56) used microsomes from human autopsied livers to study the hydration of a variety of alkene and arene oxides and have found that the substrate specificities of the human and rat enzymes are very similar. In addition, human epoxide hydrase has been purified and found by polyacrylamide gel eletrophoresis in the presence of sodium dodecylsulfate to have a minimum molecular weight of 49,000 (35, 57), indistinguishable from the rat enzyme. Despite these similarities, the human and rat enzymes are distinct proteins since antibody produced against rat epoxide hydrase does not cross-react with the purified human enzyme (52, 57). Immunochemical data (52) also indicate that there is more than one form of human liver epoxide hydrase and that the level of individual forms can either be induced or repressed by various foreign chemicals resulting in individual variations in the composition of epoxide hydrase present in the human population.

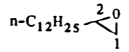
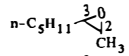
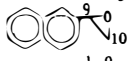
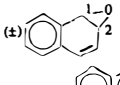
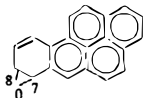
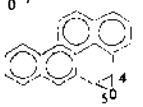
MECHANISM OF ACTION

Epoxide hydrase catalyzes the hydration of epoxides to *trans*-1,2-dihydrodiols. The enzyme is highly regiospecific since monosubstituted 1,1- and *cis*-1,2-disubstituted epoxides containing at least one large lipophilic group are readily hydrated in contrast to *trans*-1,2-, tri-, and tetra-substituted epoxides (17, 58). Using *cis*-^{18}O-labeled epoxides or $H_2{}^{18}O$, Hanzlik et al (59) have shown that hydration occurs at the sterically least hindered epoxide carbon atom. As can be seen in Table 1, hydration of *cis*-1,2-disubstituted epoxides occur exclusively at the least hindered epoxide carbon atom. Indeed, regiospecific discrimination is not abolished until almost complete symmetry exists around the epoxide ring as is the case with

benzo(a)pyrene 4,5-oxide (Table 1, VIII) whose enzymatic hydration has not been found to be regiospecific (63).

The enzymic hydration of arene oxides and *cis*-1,2-disubstituted epoxides occurs with almost complete inversion of configuration at the epoxide carbon atom undergoing hydration and results in the formation of *trans*-1,2-dihydrodiol products. This selective *trans*-addition of water to the epoxide in combination with a high degree of stereoselectivity can result in unique isomeric products with high optical purity. For example, stereoselective hydration of racemic benzo(a)pyrene 4,5-oxide results in almost exclusive formation of (–)benzo(a)pyrene 4,5-dihydrodiol (Table 1, VIII). Furthermore, the enzymic hydration of a series of *para*-substituted stilbene-1,2-oxides of known stereochemistry results in highly selective attack (> 80%) at the epoxide carbon atom with the S-absolute stereoconfiguration (64). Epoxide hydrase is not always so highly stereospecific, however, since racemic naphthalene-1,2-oxide is hydrated to a mixture of enantiomeric products containing only a small excess (28–35%) of the (–)-*trans*-1,2-dihydrodiol product (Table 1, VI) (62). The stereoselectivity of hydration of racemic benzo(a)pyrene 7,8-oxide (VII) has also been studied but the results are equivocal since the optical composition of the dihydrodiol prod-

Table 1 Regiospecificity and stereospecificity of epoxide hydrase

	Substrate	Position of attack	Percentage of attack	Percentage of optical enantiomers formed	Reference
I		8	90%		60
II		8	89%		61
III		1	>98%		59
IV		2	85%		61
V		10	97%		59
VI		2	~90%	(–) in small excess	62
VII		8	>98%	(–) 54%, (+) 46% (–) 84%, (+) not reported	63 16
VIII		4 5	~60% ~40%	(–) 89%, (+) 11%	63

ucts have been reported to be both highly enriched in the (–)-enantiomer (86%) (16) or not significantly enriched (54%) (63).

Epoxide hydration occurs chemically under both acid- and base-catalyzed conditions. Consequently, both acid-catalyzed (65) and nucleophilic mechanism (59, 66) have been proposed for the epoxide hydrase–catalyzed reaction. The acid-catalyzed reaction mechanism appears unlikely, however, since for many substrates (I, II, and V in Table 1), the direction of epoxide opening is incompatible with resonance or inductive stabilization of the carbonium ion that would result from protonation of the epoxide oxygen. Dansette et al (64) have systematically examined the influence of inductive effects on epoxide hydrase–catalyzed reactions with a series of *para*-substituted stilbene oxides. A comparison of Hammett σ constants for these substituents relative to hydration activity indicates that a carbonium ion is not formed in the rate-limiting step. In addition, the complete inversion of configuration observed at the hydrated epoxide carbon is inconsistent with a carbonium ion intermediate but does suggest a back-sided attack by an incoming nucleophile.

In chemical model systems, the nucleophilic hydration of epoxides closely mimics the enzyme-catalyzed reaction in regiospecificity (59) and solvent isotope effects (64). This base-catalyzed hydration reaction occurs at the least hindered epoxide carbon atom with a solvent isotope effect similar to that observed for the enzyme-catalyzed reaction. Furthermore, a variety of other nucleophiles attack the sterically least hindered epoxide carbon atom and yield *trans*-addition products (66).

General base activation of water to a nucleophilic species is known to occur enzymatically by metal coordination to water (67) or by histidine imidazole deprotonation of water. The enzyme carbonic anhydrase has been shown to activate water through metal coordination (67) but this mechanism does not appear to be operating in the epoxide hydrase–catalyzed reaction since metal chelators fail to inhibit epoxide hydrase (20, 32, 59) and the enzyme does not contain significant amounts of iron, zinc, copper, manganese, or molybdenum (32).

On the other hand, DuBois et al (68) have demonstrated that a histidine imidazole residue is involved in the general base-catalyzed activation of water by epoxide hydrase. Employing the active-site directed inhibitor 2-bromo-4'-nitroacetophenone, these investigators were able to specifically alkylate one of the 15 histidine residues which resulted in the complete inactivation of the purified enzyme. Furthermore, the rate of inactivation was controlled by an amino acid residue with an apparent pK_a of 7.6, similar to histidine. Thus, the available evidence indicates that epoxide hydrase–catalyzed hydration occurs by nucleophilic attack at the least hindered epoxide carbon atom by an incoming hydroxyl ion resulting in

ring opening away from this ion and the formation of *trans*-dihydrodiol products. Water appears to be activated to a nucleophilic species through a general base mechanism involving a single histidine imidazole residue.

ROLE OF EPOXIDE HYDRASE IN BENZO(A)PYRENE-INDUCED MUTAGENESIS AND CARCINOGENESIS

In recent years, significant progress has been made in our understanding of the roles played by the cytochrome P-450 system and epoxide hydrase in the metabolic activation of polycyclic aromatic hydrocarbons to ultimate mutagens and carcinogens. The extensive studies (8–16, 69–71) on the metabolism, mutagenicity, and carcinogenicity of benzo(a)pyrene led to the formulation of the bay-region theory (72), which predicts that dihydrodiol epoxides situated on a saturated, angular benzo-ring would be most likely responsible for the mutagenicity and carcinogenicity of polycyclic aromatic hydrocarbons. Thus, in order that benzo(a)pyrene be mutagenic and carcinogenic it must be epoxidated initially at the 7,8-position to form 7,8-oxide, followed by hydration to form 7,8-dihydrodiol and further epoxidated to form the highly reactive benzo(a)pyrene 7,8-dihydrodiol-9,10-epoxide. Epoxidation at the 4,5-position is also an important pathway leading to the highly mutagenic but not carcinogenic 4,5-oxide of benzo(a)-pyrene.

Using purified rat liver microsomal epoxide hydrase in combination with the purified cytochrome P-450 and P-448 systems, Wood and co-workers (13, 41–43) have examined the role of epoxide hydrase in the biotransformation of benzo(a)-pyrene and its derivatives to mutagenic metabolites as measured in the Ames' bacterial mutation test (73–75). From these studies, it is clear that the role of epoxide hydrase in the biotransformation of benzo(a)pyrene can be either beneficial or harmful depending on the particular metabolic pathways involved and whether the resulting oxide intermediates are substrates for the enzyme. For example, the presence of epoxide hydrase effectively eliminates the mutagenicity of benzo(a)pyrene 4,5-oxide by catalyzing its transformation to a relatively nonmutagenic product *trans*-4,5-dihydroxy-4,5-dihydrobenzo(a)-pyrene [benzo(a)pyrene 4,5-dihydrodiol]. In this case, the role of epoxide hydrase is to inactivate a harmful product. On the other hand, the hydration of benzo(a)pyrene 7,8-oxide by the enzyme results in the formation of *trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene [benzo(a)pyrene 7,8-dihydrodiol] which can be further epoxidated by the cytochrome P-448 system to form the bay-region diol-epoxide. Although benzo(a)pyrene 7,8-oxide and benzo(a)pyrene 7,8-dihydrodiol are only weak mutagens (76), the resulting bay-region diol-epoxide is highly

mutagenic and carcinogenic (11, 12, 14, 41, 69–72). The role of epoxide hydrase in this case is one of activation to a potentially harmful product by producing a precursor for the formation of an ultimate carcinogen. In addition, the studies of Wood et al (41–43) have shown that the bay-region diol epoxide of several polycyclic aromatic hydrocarbons is either a poor substrate or not a substrate for the enzyme since addition of epoxide hydrase either has no effect on the mutagenicity of these compounds or only decreases the activity by 20% to 30%. Thus, depending on the particular metabolic pathway, epoxide hydrase can either play the role of an activator or an inactivator, or has no effect on the mutagenicity of polycyclic aromatic hydrocarbon derivatives.

Bentley and co-workers (77) have employed a different approach in evaluating the relative importance of different metabolic pathways on the mutagenic activity of benzo(a)pyrene. In their studies, mouse liver microsomes were used as the source of activating enzyme, and purified rat liver microsomal epoxide hydrase was added as a way of evaluating the effect of increasing amounts of epoxide hydrase on benzo(a)pyrene-mediated mutagenesis. When liver microsomes from untreated or phenobarbital-treated C3H mice were used, addition of purified epoxide hydrase decreased benzo(a)pyrene-mediated mutagenic activity to low levels in a dose-dependent fashion indicating that the mutagenic metabolites were almost completely inactivated by epoxide hydrase. When liver microsomes from 3-methylcholanthrene–treated mice were used, only about 50% of the mutagenic activity could be eliminated by the addition of epoxide hydrase. These results can be explained in the following way. Since liver microsomes from untreated and phenobarbital-treated animals preferentially epoxidate the benzo(a)pyrene molecule at the 4,5-position (78–80) yielding the highly potent mutagen benzo(a)pyrene 4,5-oxide which can be readily inactivated by epoxide hydrase (13), it can be concluded that the 4,5-pathway is primarily responsible for the mutagenic activity of benzo(a)pyrene with these two activating systems. When liver microsomes from 3-methylcholanthrene–treated mice are used, the situation is more complex. Since cytochrome P-448 in 3-methylcholanthrene–treated animals oxidized benzo(a)pyrene preferentially at the 7,8-position, the addition of more epoxide hydrase enhances the conversion of benzo(a)pyrene 7,8-oxide to 7,8-dihydrodiol, the precursor of the highly mutagenic diol epoxides which are not significantly inactivated by epoxide hydrase. Thus, the partial inhibition of mutagenic activity of benzo(a)pyrene in 3-methylcholanthrene–treated mice by added epoxide hydrase indicates that both the 4,5- and 7,8-metabolic pathways of benzo(a)pyrene are responsible for the mutagenic activity of benzo(a)pyrene.

One can conclude from these studies that one way of minimizing the mutagenicity of benzo(a)pyrene would be to direct more metabolism

through the 4,5-pathway with the cytochrome P-450 system and at the same time increase the level of epoxide hydrase so as to inactivate the resulting mutagenic benzo(a)pyrene 4,5-oxide. Oesch and co-workers (81, 82) have recently found a compound that produces these effects. These investigators found that *trans*-stilbene oxide is a potent inducer of rat liver epoxide hydrase but it does not significantly affect the level of microsomal cytochrome P-450 or the overall rate of benzo(a)pyrene metabolism. When rats are treated with *trans*-stilbene oxide, there is a shift in the profile of the metabolism of benzo(a)pyrene by their liver microsomes toward the 4,5-position. In addition, the mutagenic activity of benzo(a)pyrene is greatly decreased compared to that observed with microsomes from untreated rats.

IN VITRO ACTIVATION AND INHIBITION

Because of the vital role of microsomal epoxide hydrase in chemical mutagenesis and carcinogenesis, various attempts have been made to modulate epoxide hydrase activity. One technique has been to find compounds that affect hydrase activity in vitro, and a large number of compounds have been tested as potential activators and inhibitors of microsomal epoxide hydrase (21, 58). For example, 1,1,1-trichloropropene 2,3-oxide and cyclohexene oxide are potent inhibitors of styrene 7,8-oxide hydration. Both compounds have been used to demonstrate the detoxification role of epoxide hydrase since in many cases the addition of these compounds causes the increase in toxicity of the testing compounds (18, 21). However, interpretation of these results should be cautious since 1,1,1-trichloropropane 2,3-oxide at high concentrations also inhibits the cytochrome P-450 system and lowers the GSH level.

The activity of microsomal and purified epoxide hydrase can be stimulated by the in vitro addition of compounds such as metyrapone, 1-(2-isopropylphenyl)imidazole, and 1-(2-cyanophenyl)imidazole (21, 31, 83). Ganu & Alworth (84) carried out a structure-activity study and found that chalcone oxide and 9-fluorenone are more active than metyrapone as stimulators of microsomal hydration of styrene 7,8-oxide. Based on the study of 12 structurally related compounds, it is concluded that compounds containing an aryl carbonyl substituted with an additional hydrophobic group are the most effective in vitro stimulators of epoxide hydrase as judged by styrene 7,8-oxide hydration. Subsequently, Alworth et al (85) reported that a series of flavones are also potent stimulators of microsomal hydration of styrene 7,8-oxide. Flavone is the most active, followed by 7,8-benzoflavone. Kinetic analysis suggests that these stimulators bind at a site which is distinct from the binding site of styrene oxide (31, 58, 84).

Despite the identification of in vitro stimulators and inhibitors of epoxide hydrase, one should be cautious in trying to extrapolate this information to

the in vivo situation in which one would like to modulate epoxide hydrase's role in mutagenesis and carcinogenesis. As shown in the study of Levin et al (31, 40), the effect of activators such as metyrapone and inhibitors such as cyclohexene oxide on the catalytic activity of epoxide hydrase is dependent on the substrates being hydrated. Thus, while metyrapone stimulates the hydration of styrene 7,8-oxide, octene 1,2-oxide, and naphthalene 1,2-oxide, this compound actually inhibits the hydration of benzo(a)pyrene 11,12-oxide, 3-methylcholanthrene 11,12-oxide, and dibenzo(a,h)anthracene 5,6-oxide. It either slightly stimulates or slightly inhibits the hydration of phenanthracene 9,10-oxide, benzo(a)anthracene 5,6-oxide, benzo(a)pyrene 4,5- 7,8-, and 9,10-oxides. For this reason, the in vivo use of an epoxide hydrase stimulator may be beneficial in controlling the mutagenic and carcinogenic activity of some chemicals but could also be extremely harmful in other cases depending on the particular metabolic pathway and the particular alkene or arene oxide intermediate involved.

EPOXIDE HYDRASE AS PRENEOPLASTIC ANTIGEN

Farber and co-workers (86, 87) have described a preneoplastic antigen (PN antigen) induced in rat liver hyperplastic nodules by 2-acetylaminofluorene, ethionine, safrole, aflatoxin B_1, nitrosamines, and aminoazo dyes. This antigen is located primarily in the endoplasmic reticulum and can be detected immunologically in hyperplastic nodules and hepatomas. It is also present in normal rat liver microsomes although at much lower levels (88).

Recently, the PN antigen has been purified from microsomes of 2-acetylaminofluorene–induced hyperplastic nodules in rats (88). Furthermore, Levin et al (89) have identified the PN antigen as the liver microsomal epoxide hydrase based on the following observations: 1. Purified PN antigen from hyperplastic nodules and purified epoxide hydrase from phenobarbital-treated rats have identical minimum molecular weights (49,000) determined by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate. The concentration of this 49,000 molecular weight protein species in liver microsomes from hyperplastic nodules is much higher than that found in liver microsomes of untreated rats. 2. PN antigen and purified epoxide hydrase are immunochemically identical on the basis of Ouchterlony double-diffusion analysis. 3. Both PN antigen and epoxide hydrase catalyze the hydration of a number of arene oxides to dihydrodiols. However, purified PN antigen is only about 10% as active as the purified microsomal epoxide hydrase. The low activity of purified PN antigen is most likely due to inactivation by preparative gel electrophoresis during purification. 4. Hyperplastic nodule microsomes from 2-acetylaminofluo-

rene–treated rats catalyze the hydration of a variety of alkene and arene oxides at rates 6 to 10 fold greater than the liver microsomes of untreated rats. In addition, the induction of microsomal epoxide hydrase by 2-acetylaminofluorene measured by hydration parallels the appearance of PN antigen detected by immunological techniques.

The elevated epoxide hydrase activity induced in hyperplastic nodules and in hepatomas by hepatocarcinogens is a very interesting observation but the physiological and biochemical importance of this phenomenon is unknown. Nevertheless, this finding could lead to further investigation of a possible role for microsomal epoxide hydrase in liver tumorigenesis and other physiological functions.

ACKNOWLEDGMENTS

We wish to thank Dr. W. L. Alworth, Dr. B. Burchell, Dr. R. P. Hanzlik, and Dr. F. Oesch for sending us preprints and unpublished observations and Dr. P. Wislocki and Mrs. S. West for reading the manuscript and making critical suggestions. Our thanks are also due to Mrs. G. Mancinelli for her help in the preparation of the manuscript.

Literature Cited

1. Gillette, J. R. 1967. Biochemistry of drug oxidation and reduction by enzymes in hepatic endoplasmic reticulum. *Adv. Pharmacol.* 4:219–61
2. Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19:317–66
3. Miller, J. A. 1970. Carcinogenesis by chemicals: An overview. *Cancer Res.* 30:559–76
4. Daly, J. W., Jerina, D. M., Witkop, B. 1972. Arene oxides and the NIH shift: The metabolism, toxicity and carcinogenicity of aromatic compounds. *Experientia* 28:1129–49
5. Sims, P., Grover, P. L. 1974. Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. *Adv. Cancer Res.* 20:165–274
6. Jerina, D. M., Daly, J. W. 1974. Arene oxides: A new aspect of drug metabolism. *Science* 185:573–82
7. Heidelberger, C. 1975. Chemical carcinogenesis. *Ann. Rev. Biochem.* 44:79–121
8. Borgen, A., Darvey, H., Castagnoli, N., Crocker, T. T., Rasmussen, R. E., Wang, I. Y. 1973. Metabolic conversion of benzo(a)pyrene by Syrian hamster liver microsomes and binding of metabolites to DNA. *J. Med. Chem.* 16:502–6
9. Sims, P., Grover, P. L., Swaisland, A., Pal, K., Hewer, A. 1974. Metabolic activation of benzo(a)pyrene proceeds by a diol-epoxide. *Nature* 252:326–28
10. King, H. W. S., Osborne, M. R., Beland, F. A., Harvey, R. 1976. (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an intermediate in the metabolism and binding to DNA of benzo(a)pyrene. *Proc. Natl. Acad. Sci. USA* 73:2679–81
11. Newbold, R. F., Brooks, P. 1976. Exceptional mutagenicity of benzo(a)pyrene diol epoxide in cultured mammalian cells. *Nature* 261:52–54
12. Thakker, D. R., Yagi, H., Lu, A. Y. H., Levin, W., Conney, A. H., Jerina, D. M. 1976. Metabolism of benzo(a)pyrene: Conversion of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene to highly mutagenic 7,8-diol-9,10-epoxides. *Proc. Natl. Acad. Sci. USA* 73:3381–85
13. Wood, A. W., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M., Conney, A. H. 1976. Metabolism of benzo(a)pyrene derivatives to mutagenic products by highly purified hepatic microsomal enzyme. *J. Biol. Chem.* 251: 4882–90
14. Huberman, E., Sachs, L., Yang, S. K., Gelboin, H. V. 1976. Identification of mutagenic metabolites of benzo(a)py-

rene in mammalian cells. *Proc. Natl. Acad. Sci. USA* 73:607–11
15. Weinstein, I. B., Jeffrey, A. M., Jennette, K. W., Blobstein, S. H., Harvey, R. G., Harris, C., Autrup, H., Kasai, H., Nakanishi, K. 1976. Benzo(a)pyrene diol epoxides as intermediates in nucleic acid binding *in vitro* and *in vivo*. *Science* 193:592–95
16. Yang, S. K., McCourt, D. W., Leutz, J. C., Gelboin, H. V. 1977. Benzo(a)pyrene diol epoxides: Mechanism of enzymatic formation and optically active intermediates. *Science* 196:1199–1201
17. Oesch, F. 1973. Mammalian epoxide hydrases: Inducible enzymes catalyzing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. *Xenobiotica* 3:305–40
18. Oesch, F. 1978. Epoxide hydrase. In *Progress in Drug Metabolism,* Vol. 3, ed. J. W. Bridges, L. F. Chasseaud, pp. 253–301. London: Wiley
19. Oesch, F., Jerina, D. M., Daly, J. 1970. A radiometric assay for hepatic epoxide hydrase activity with 7-^{3}H-styrene oxide. *Biochim. Biophys. Acta* 227:685–91
20. Oesch, F., Daly, J. 1971. Solubilization, purification and properties of a hepatic epoxide hydrase. *Biochim. Biophys. Acta* 227:692–97
21. Oesch, F., Jerina, D. M., Daly, J. W., Rice, J. M. 1973. Induction, activation, and inhibition of epoxide hydrase: An anomalous prevention of chlorobenzene-induced hepatotoxicity by an inhibitor of epoxide hydrase. *Chem.-Biol. Interact.* 6:189–202
22. Oesch, F., Morris, N., Daly, J. W., Gielen, J. E., Nebert, D. W. 1973. Genetic expression of the induction of epoxide hydrase and aryl hydrocarbon hydroxylase activities in the mouse by phenobarbital or 3-methylcholanthrene. *Mol. Pharmacol.* 9:692–96
23. Goujon, F. M., van Cantfort, J., Gielen, J. E. 1980. Comparison of aryl hydrocarbon hydroxylase and epoxide hydratase induction in primary fetal rat liver cell culture. *Biochem. Pharmacol.* In press
24. Walker, C. H., Bentley, P., Oesch, F. 1978. Phylogenetic distribution of epoxide hydratase in different vertebrate species, strains and tissues measured using three substrates. *Biochem. Biophys. Acta* 539:427–34
25. Oesch, F., Schmassmann, H., Bentley, P. 1978. Specificity of human, rat and mouse skin epoxide hydratase towards K-region epoxides of polycyclic hydrocarbons. *Biochem. Pharmacol.* 27: 17–20
26. Oesch, F., Glatt, H., Schmassmann, H. 1977. The apparent ubiquity of epoxide hydratase in rat organs. *Biochem. Pharmacol.* 26:603–7
27. Gill, S. S., Hammock, B. D., Casida, J. E. 1974. Mammalian metabolism and environmental degradation of the juvenoid 1-(4'-ethylphenoxy)-3,7-dimethyl-6,7-epoxy-*trans*-2-octene and related compounds. *J. Agric. Food Chem.* 22:386–95
28. Burchell, B., Bentley, P., Oesch, F. 1976. Latency of epoxide hydratase and its relationship to that of UDP-glucuronyl transferase. *Biochim. Biophys. Acta* 444:531–38
29. Seidegard, J., Moron, M. S., Eriksson, L. C., DePierre, J. W. 1978. The topology of epoxide hydratase and benzpyrene monooxygenase in the endoplasmic reticulum of rat liver. *Biochim. Biophys. Acta* 543:29–40
30. Oesch, F., Bentley, P. 1976. Antibodies against homogeneous epoxide hydratase provide evidence for a single enzyme hydrating styrene oxide and benzo(a)pyrene 4,5-oxide. *Nature* 259:53–55
31. Levin., W., Thomas, P. E., Korzeniowski, D., Seifried, H., Jerina, D. M., Lu, A. Y. H. 1978. Liver microsomal epoxide hydratase: Activation, inhibition, and immunological properties of the purified enzyme. *Mol. Pharmacol.* 14: 1107–20
32. Lu, A. Y. H., Ryan, D., Jerina, D. M., Daly, J. W., and Levin, W. 1975. Liver microsomal epoxide hydrase: Solubilization, purification and characterization. *J. Biol. Chem.* 250:8283–88
33. Bentley, P., Oesch, F., 1975. Purification of rat liver epoxide hydratase to apparent homogeneity. *FEBS Lett.* 59:291–95
34. Knowles, R. G., Burchell, B. 1977. A simple method for purification of epoxide hydratase from rat liver. *Biochem. J.* 163:381–83
35. Guengerich, F. P., Wang, P., Mitchell, M. B., Mason, P. S. 1979. Rat and human liver microsomal epoxide hydrase: Purification and evidence for the existence of multiple forms. *J. Biol. Chem.* 254:12248–54
36. Bentley, P., Oesch, F., Tsugita, A. 1975. Properties and amino acid composition of pure epoxide hydratase. *FEBS Lett.* 59:296–99
37. DuBois, G. C., Appella, E., Armstrong, R., Levin, W., Lu, A. Y. H., Jerina, D.

M. 1979. Hepatic microsomal epoxide hydrase: Chemical evidence for a single polypeptide chain. *J. Biol. Chem.* 254:6240–43

38. Lu, A. Y. H., Jerina, D. M., Levin, W. 1977. Liver microsomal epoxide hydrase: Hydration of alkene and arene oxides by membrane-bound and purified enzymes. *J. Biol. Chem.* 252: 3715–23
39. Bentley, P., Schmassmann, H., Sims, P., Oesch, F. 1976. Epoxides derived from various polycyclic hydrocarbons as substrates of homogeneous and microsome-bound epoxide hydratase: A general assay and kinetic properties. *Eur. J. Biochem.* 69:97–103
40. Levin, W., Jerina, D. M., Thomas, P. E., Lu, A. Y. H. 1978. Physical and catalytic properties of purified rat liver epoxide hydrase. In *Conjugation Reactions in Drug Biotransformation,* ed. A. Aitio, pp. 337–46. Amsterdam: Elsevier/North-Holland Biochem. Press
41. Wood, A. W., Wislocki, P. G., Chang, R. L., Levin, W., Lu, A. Y. H., Yagi, H., Hernandez, O., Jerina, D. M., Conney, A. H. 1976. Mutagenicity and cytotoxicity of benzo(a)pyrene benzoring epoxides. *Cancer Res.* 36:3358–66
42. Wood, A. W., Levin, W., Ryan, D., Thomas, P. E., Yagi, H., Mah, H. D., Thakker, D. R., Jerina, D. M., Conney, A. H. 1977. High mutagenicity of metabolically activated chrysene 1,2-dihydrodiol: Evidence for bay region activation of chrysene. *Biochem. Biophys. Res. Commun.* 78:847–54
43. Wood, A. W., Chang, R. L., Levin, W., Lehr, R. E., Schaefer-Ridder, M., Karle, J. M., Jerina, D. M., Conney, A. H. 1977. Mutagenicity and cytotoxicity of benz(a)anthracene diol epoxides and tetrahydroepoxides: Exceptional activity of the bay region 1,2-epoxides. *Proc. Natl. Acad. Sci. USA* 74:2746–50
44. Lu, A. Y. H., Levin, W., Thomas, P. E., Jerina, D. M., Conney, A. H. 1978. Enzymological properties of purified liver microsomal cytochrome P-450 system and epoxide hydrase. In *Carcinogenesis,* Vol. 3, *Poly-nuclear Aromatic Hydrocarbons,* ed. P. W. Jones, R. I. Freudenthal, pp. 243–52. New York: Raven
45. Mannering, G. J. 1971. Properties of cytochrome P-450 as affected by environmental factors. Qualitative changes due to administration of polycyclic hydrocarbons. *Metabolism.* 20:228–45
46. Conney, A. H., Lu, A. Y. H., Levin, W., Somogyi, A., West, A., Jacobson, M., Ryan, D., Kuntzman, R. 1973. Effect of enzyme inducers on substrate specificity of the cytochrome P-450's. *Drug Metab. Dispos.* 1:199–210
47. Jerina, D. M., Dansette, P. M., Lu, A. Y. H., Levin, W. 1977. Hepatic microsomal epoxide hydrase: A sensitive radiometric assay for hydration of arene oxides of carcinogenic aromatic hydrocarbons. *Mol. Pharmacol.* 13:342–51
48. Bresnick, E., Mukhtar, H., Stoming, T. A., Dansette, P. M., Jerina, D. M. 1977. Effect of phenobarbital and 3-methylcholanthrene administration on epoxide hydrase levels in liver microsomes. *Biochem. Pharmacol.* 26:891–92
49. Oesch, F., Jerina, D. M., Daly, J. W. 1971. Substrate specificity of hepatic epoxide hydrase in microsomes and in a purified preparation: Evidence for homologous enzymes. *Arch. Biochem. Biophys.* 144:253–61
50. Dansette, P. M., Yagi, H., Jerina, D. M., Daly, J. W., Levin, W., Lu, A. Y. H., Kuntzman, R., Conney, A. H. 1974. Assay and partial purification of epoxide hydrase from rat liver microsomes. *Arch. Biochem. Biophys.* 164:511–17.
51. Oesch, F., Bentley, P., Platt, K. L., Golan, M. D. 1980. Enzymic hydration of benzene oxide: Assay and properties. *Arch. Biochem. Biophys.* In press
52. Guengerich, F. P., Wang, P., Mason, P. S., Mitchell, M. B. 1979. Rat and human microsomal epoxide hydrase: Immunological characterization of various forms of the enzyme. *J. Biol. Chem.* 254:12255–59
53. Knowles, R. G., Burchell, B. 1977. Mouse liver epoxide hydratase: Purification and identity with the rat liver enzyme. *Biochem. Soc. Trans.* 5:731–32
54. Burchell, B. 1979. Personal communication.
55. Oesch, F., Thoenen, H., Fahrlaender, H. 1974. Epoxide hydrase in human liver biopsy specimens: Assay and properties. *Biochem. Pharmacol.* 23: 1307–17
56. Kapitulnik, J., Levin, W., Lu, A. Y. H., Morecki, R., Dansette, P. M., Jerina, D. M., Conney, A. H. 1977. Hydration of arene and alkene oxides by epoxide hydrase in human liver microsomes. *Clin. Pharmacol. Ther.* 21:158–65
57. Lu, A. Y. H., Thomas, P. E., Ryan, D., Jerina, D. M., Levin, W. 1979. Purification of human liver microsomal epoxide hydrase: Differences in the properties of the human and rat enzymes. *J. Biol. Chem.* 254:5878–81
58. Oesch, F., Kaubisch. N., Jerina, D. M., Daly, J. W. 1971. Hepatic epoxide hy-

drase: Structure-activity relationships for substrates and inhibitors. *Biochemistry* 10:4858–66
59. Hanzlik, R. P., Edelman, M., Michaely, W. J., Scott, G. 1976. Enzymatic hydration of ^{18}O epoxides. Role of nucleophilic mechanisms. *J. Am. Chem. Soc.* 98:1952–55
60. Jerina, D. M., Ziffer, H., Daly, J. W. 1970. The role of the arene oxide-oxepin system in the metabolism of aromatic substrates. IV. Stereochemical consideration of dihydrodiol formation and dehydrogenation. *J. Am. Chem. Soc.* 92:1056–61
61. Hanzlik, R. P., Heideman, S., Smith, D. 1978. Regioselectivity in enzymatic hydration of *cis*-1,2-disubstituted ^{18}O-epoxides. *Biochem. Biophys. Res. Commun.* 82:310–15
62. Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman-Nirenberg, P., Udenfriend, S. 1970. 1,2-Naphthalene oxide as an intermediate in the microsomal hydroxylation of naphthalene. *Biochemistry* 9:147–56
63. Thakker, D. R., Yagi, H., Levin, W., Lu, A. Y. H., Conney, A. H., Jerina, D. M. 1977. Stereospecificity of microsomal and purified epoxide hydrase from rat liver. *J. Biol. Chem.* 252:6328–34
64. Dansette, P. M., Makedonska, V. B., Jerina, D. M. 1978. Mechanism of catalysis for the hydration of substituted styrene oxides by hepatic epoxide hydrase. *Arch. Biochem. Biophys.* 187:290–98
65. Watabe, T., Akamatsu, K., Kiyonaga, K. 1971. Stereoselective hydrolysis of *cis*- and *trans*-stilbene oxides by hepatic microsomal epoxide hydrolase. *Biochem. Biophys. Res. Commun.* 44:199–204
66. Jeffrey, A. M., Yeh, H. J. C., Jerina, D. M., DeMarinis, R. M., Foster, C. H., Piccolo, D. E., Berchtold, G. A. 1974. Stereochemical course in reactions between nucleophiles and arene oxides. *J. Am. Chem. Soc.* 96:6929–37
67. Coleman, J. E. 1971. Metal ions in enzymic catalysis. *Prog. Bioorg. Chem.* 1:159–344
68. DuBois, G. C., Appella, E., Levin, W., Lu, A. Y. H., Jerina, D. M. 1978. Hepatic microsomal epoxide hydrase: Involvement of a histidine at the active site suggests a nucleophilic mechanism. *J. Biol. Chem.* 253:2932–39
69. Malaveille, C., Bartsch, H., Grover, P. L., Sims, P. 1975. Mutagenicity of non-K-region diols and diol-epoxides of benz(a)-anthracene and benzo(a)pyrene in S. typhimurium TA 100. *Biochem. Biophys. Res. Commun.* 66:693–700
70. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Jerina, D. M., Conney, A. H. 1976. High mutagenicity and toxicity of a diol epoxide derived from benzo(a)pyrene. *Biochem. Biophys. Res. Commun.* 68: 1006–12
71. Kapitulnik, J., Wislocki, P. G., Levin, W., Yagi, H., Jerina, D. M., Conney, A. H. 1978. Tumorigenicity studies with diol-epoxides of benzo(a)pyrene which indicate that (±)-*trans*-7β, 8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene is an ultimate carcinogen in newborn mice. *Can. Res.* 38:354–58
72. Jerina, D. M., Lehr, R., Schaefer-Ridder, M., Yagi, H., Karle, J. M., Thakker, D. R., Wood, A. W., Lu, A. Y. H., Ryan, D., West, S., Levin, W., Conney, A. H. 1977. Bay-region epoxides of dihydrodiols: A concept explaining the mutagenic and carcinogenic activity of benzo(a)pyrene and benzo(a)anthracene. In *Origins of Human Cancer*, ed. H. H. Hiatt, J. D. Watson, J. A. Winstein, pp. 639–58. Cold Spring Harbor Lab.
73. Ames, B. N., Durston, W. E., Yamasaki, E., Lee, F. D. 1973. Carcinogens are mutagens: A simple system combining liver homogenates for activation and bacteria for detection. *Proc. Natl. Acad. Sci. USA* 70:2281–85
74. Ames, B. N., Lee, F. D., Durston, W. E. 1973. An improved bacterial test system for the detection and classification of mutagens and carcinogens. *Proc. Natl. Acad. Sci. USA* 70:782–86
75. McCann, J., Spingarn, N. E., Kobori, J., Ames, B. N. 1975. Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. *Proc. Natl. Acad. Sci. USA* 72:979–83
76. Wislocki, P. G., Wood, A. W., Chang, R. L., Levin, W., Yagi, H., Hernandez, O., Dansette, P. M., Jerina, D. M., Conney, A. H. 1976. Mutagenicity and cytotoxicity of benzo(a)pyrene arene oxides, phenols, quinones, and dihydrodiols in bacterial and mammalian cells. *Cancer Res.* 36:3350–57
77. Bentley, P., Oesch, F., Glatt, H. 1977. Dual role of epoxide hydratase in both activation and inactivation of benzo(a)-pyrene. *Arch. Toxicol.* 39:65–75
78. Rasmussen, R. E., Wang, I. Y. 1974. Dependence of specific metabolism of benzo(a)pyrene on the inducer of hy-

droxylase activity. *Cancer Res.* 34: 2290–95
79. Holder, G., Yagi, H., Dansette, P., Jerina, D. M., Levin, W., Lu, A. Y. H., Conney, A. H. 1974. Effects of inducers and epoxide hydrase on the metabolism of benzo(a)pyrene by liver microsomes and a reconstituted system: Analysis by high pressure liquid chromatography. *Proc. Natl. Acad. Sci. USA.* 71:4356–60
80. Yang, S. K., Selkirk, J. K., Plotkin, E. V., Gelboin, H. V. 1975. Kinetic analysis of the metabolism of benzo(a)pyrene to phenols, dihydrodiols, and quinones by high-pressure chromatography compared to analysis by aryl hydrocarbon hydroxylase assay, and the effect of enzyme induction. *Cancer Res.* 35: 3642–50
81. Schmassmann, H., Oesch, F. 1978. *Trans*-stilbene oxide: A selective inducer of rat liver epoxide hydratase. *Mol. Pharmacol.* 14:834–47
82. Bucker, M., Golan, M., Schmassmann, H. U., Glatt, H. R., Stasiecki, P., Oesch, F. 1979. The epoxide hydratase inducer *trans*-stilbene oxide shifts the metabolic epoxidation of benzo(a)pyrene from the bay- to the K-region and reduces its mutagenicity. *Mol. Pharmacol.* 16:656–66
83. Liebman, K. C., Ortiz, E. 1973. New potent modifiers of liver microsomal drug metabolism: 1-Arylimidazoles. *Drug Metab. Dispos.* 1:775–79
84. Ganu, V. S., Alworth, W. L. 1978. Epoxide hydrase: Structure activity relationships of *in vitro* stimulators of the microsomal activity. *Biochemistry* 17: 2876–81
85. Alworth, W. L., Dang, C. C., Ching, L. M., Viswanathan, T. 1980. Stimulation of mammalian epoxide hydrase activity by flavones. *Xenobiotica.* In press
86. Epstein, S. M., Ito, M., Merkow, L., Farber, E. 1967. Cellular analysis of liver carcinogenesis: The induction of large hyperplastic nodules in the liver with 2-fluorenyldiacetamide or ethionine and some aspects of their morphology and glycogen metabolism. *Cancer Res.* 27:1702–11
87. Farber, E. 1973. Hyper lastic liver nodules. *Methods Cancer Res.* 7:345–75
88. Griffin, M. J., Kizer, D. E. 1978. Purification and quantitation of preneoplastic antigen from hyperplastic nodules and normal liver. *Cancer Res.* 38:1136–41
89. Levin, W., Lu, A. Y. H., Thomas, P. E., Ryan, D., Kizer, D. E., Griffin, M. J. 1978. Identification of epoxide hydrase as the preneoplastic antigen in rat liver hyperplastic nodules. *Proc. Natl. Acad. Sci. USA* 75:3240–43