Induction of Microsomal Enzymes by Foreign Chemicals and Carcinogenesis by Polycyclic Aromatic Hydrocarbons: G. H. A. Clowes Memorial Lecture

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I feel very honored indeed, and also very humble, to be selected as the 21st G. H. A. Clowes Memorial Lecturer. The research that I have done would not have been possible without the help, encouragement, and dedication of my many associates and collaborators during the past 25 years, and all of these individuals share with me in today's honor. I am also extremely grateful to the Burroughs Wellcome Co. and to Hoffmann-La Roche Inc. for their support of my research in Tuckahoe from 1960 to 1970 and in Nutley from 1970 to the present. Looking back at the names of earlier recipients of the Clowes Award, I realize that I am the 6th Clowes Lecturer who, at one time or another in his career, has been associated with the McArdle Laboratory for Cancer Research at the University of Wisconsin. I believe that the large number of awardees associated with McArdle is telling us something about the genius of Dr. Harold Rusch who established McArdle with a handful of outstanding young investigators. Dr. Rusch helped to develop in his associates an intense devotion to fundamental cancer research, and he provided his colleagues with an atmosphere that encouraged them to have critical but constructive interactions with each other in ways that stimulated excellence in research by all of the members of the group. In 1952, I had the good fortune of coming to McArdle as a graduate student of Drs. James and Elizabeth Miller. Although I failed in my first research assignment, which was to synthesize pure 2-amino-1-naphthol, the Millers had great patience with me, and they tried very hard to instill their high standards into my research. Whatever small accomplishments I may have made to biomedical research were in large part the result of the excellent training and encouragement that I received from Jim and Betty Miller.

An important problem that has been of great interest to me for many years is individuality in the response of human beings and other living organisms to foreign chemicals. Why does a drug or an environmental pollutant cause toxicity in one person and not in another person? Why do some cigarette smokers develop lung cancer whereas other cigarette smokers do not? One of the causes of variability in the response of human beings to a foreign chemical is individuality in the rate of metabolism of the chemical. By the mid-1960s, it was recognized by clinical pharmacologists that the plasma half-lives and steady state plasma concentrations of drugs varied by as much as 10- to 20-fold among different individuals (42, 115, 204, 318). Because it seemed likely that there were also large differences in the metabolism of chemical carcinogens in different individuals, we investigated the metabolism of BP by surgical biopsy samples of human liver obtained for medical reasons (61, 176), by placentas obtained after normal childbirth (323, 324), and by cultured human foreskin obtained from normal neonates after circumcision (6, 188). In all of these studies, we found variability in the rate of metabolism of BP to fluorescent phenolic metabolites by tissue samples that were obtained from different individuals. More recent studies on differences in the ability of liver samples from different individuals to metabolize some chemical carcinogens are illustrated in Chart 1. A 6- to 9-fold difference in the metabolism of BP to fluorescent phenols and in the metabolism of BP 7,8-dihydrodiol and aflatoxin B1 to mutagens was observed among the 10 liver samples studied (60, 81) (Chart 1).

Genetic and environmental factors each contribute to varia-

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1 Presented on April 27, 1981, at the 72nd Annual Meeting of the American Association for Cancer Research, Washington, D. C. Some additional material has been added to provide a more complete manuscript.

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over the years, and I would like to focus a major portion of my presentation on this topic. The ability in the metabolism of foreign chemicals in different individuals (2, 59, 64, 81, 82, 307–309, 323) and to variability in the biotransformation of foreign chemicals in the same individual who is examined on several occasions (5, 81, 82). Variability in the in vivo metabolism of a prototype drug that occurs when it is given to an individual on several occasions is an approach that we have used for assessing the influence of environment and life style on the metabolism of foreign chemicals in human beings. In these studies, we found that the amount of day-to-day variation in the in vivo metabolism of 3 prototype drugs by several normal volunteers depended on both the drug and the subject studied (5, 81, 82). Since genes and environment interact at so many different levels, it is not possible to quantify accurately the relative contributions of each to the variability that occurs in the metabolism of foreign chemicals in human beings. Factors that influence the metabolism of foreign chemicals in humans include age, disease states, dietary and nutritional factors, hormonal changes in the body, and the ingestion of foreign chemicals. The ability of foreign chemicals to induce the synthesis of microsomal enzymes\(^1\) that metabolize the compound administered as well as other foreign chemicals and endogenous substrates has been of particular interest to me over the years, and I would like to focus a major portion of my presentation on this topic.

**Induction of Microsomal Enzymes by Foreign Chemicals**

In 1952, Richardson et al. (260) reported that the hepatocarcinogenic activity of 3' -methyl-4-dimethylaminoazobenzene was greatly reduced when this aminoazo dye was fed to rats with MC, a carcinogenic polycyclic aromatic hydrocarbon. Additional research by the Millers and their associates demonstrated that several polycyclic aromatic hydrocarbons inhibited the carcinogenicity of 3'-methyl-4-dimethylaminoazobenzene and 2-acetylanilinofluorene (225). An explanation for the unexpected antagonism of aminoazo dye carcinogenesis by polycyclic hydrocarbons came from studies by Raymond Brown and myself while we were graduate students with the Millers. In these studies, we found that administration of MC and other polycyclic aromatic hydrocarbons to rats increased the levels of liver enzymes that metabolize aminoazo dyes by \(N\)-demethylation and azo link reduction to noncarcinogenic products (35, 78, 226). The marked stimulatory effect of a single injection of MC on the metabolism of aminoazo dyes by rat liver is shown in Chart 2. Additional studies revealed that polycyclic aromatic hydrocarbons could stimulate their own metabolism (79). The rapid induction of hepatic BP hydroxylase activity that occurs after a single injection of BP is shown in Chart 3. During the course of these studies, we demonstrated that the \(N\)-demethylation of 3-methyl-4-monomethylaminoazobenzene and the hydroxylation of BP occurred in liver microsomes and required NADPH and oxygen (61, 79), and we also identified several hydroxylated products and quinones as metabolites of the in vitro biotransformation of BP (79). In these early studies, we found that ethionine blocked the induction of hepatic aminoazo dye and BP metabolism, and this

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\(^1\) Microsomal enzymes derived from endoplasmic reticulum catalyze the metabolism of foreign chemicals and endogenous substrates by oxidation, reduction, hydration, and conjugation.
block was prevented by methionine (78, 79). These observations, coupled with the lack of an in vitro effect of the hydrocarbon inducers on monooxygenase activity, suggested that the hydrocarbons induced the de novo synthesis of liver microsomal enzymes that metabolize aminoazo dyes and polycyclic aromatic hydrocarbons.

In 1957, I went to Dr. Bernard B. Brodie's laboratory at the NIH to explore the possibility that microsomal enzyme induction was a broad phenomenon that was caused by many foreign chemicals. While at the NIH, I worked with Dr. John J. Burns who asked me to identify metabolites of 2 centrally acting muscle relaxant drugs, zoxazolamine and chlorzoxazone. These studies resulted in the isolation and identification of hydroxylated metabolites of zoxazolamine and chlorzoxazone from human urine as well as the demonstration of the NADPH-dependent hydroxylation of zoxazolamine and chlorzoxazone by rat liver microsomes (63, 67, 84). The structures of zoxazolamine, chlorzoxazone, and their metabolites are shown in Chart 4. During the course of these studies, we found that treatment of rats for 4 days with several structurally unrelated chemicals stimulated the metabolism of zoxazolamine (65), and this drug became a useful prototype substrate for studies on the induction of microsomal monooxygenases. While at the NIH, Dr. Burns and I studied the possible induction of microsomal monooxygenases by barbiturates and several structurally unrelated drugs that were known to stimulate the synthesis of ascobic acid in the rat. We found that barbiturates and other previously known stimulators of ascobic acid synthesis were potent inducers of hepatic aminoazo dye N-demethylase activity and that polycyclic aromatic hydrocarbon enzyme inducers were potent stimulators of ascobic acid synthesis (62). During these studies, we demonstrated that treatment of rats with several structurally unrelated drugs increased the levels of liver microsomal enzymes that metabolized the compound administered as well as other substrates, and evidence was also obtained for induced enzyme synthesis and for enhanced synthesis of total microsomal protein (65). While we were studying the effects of drugs and polycyclic aromatic hydrocarbons as inducers of microsomal monooxygenases at the NIH, Drs. Herbert Remmer and Ryuichi Kato working independently also demonstrated a stimulatory effect of barbiturates and other drugs on drug metabolism (161, 163, 256, 257). Although the enhanced metabolism of foreign chemicals that occurs after exposure of an organism to a chemical is often an adaptive protective response that enhances the detoxification and elimination of the compound from the organism, some chemicals are metabolized to toxic products, and the induction of microsomal enzymes may increase the toxicity of these chemicals.

The induction of monooxygenases by foreign chemicals is widespread in nature and has been observed to occur in mammals, fish, insects, plants, bacteria, and many other organisms. Several hundred synthetic and naturally occurring compounds with diverse structures are now known to increase the levels of microsomal enzymes when administered to experimental animals. Examples of inducers of microsomal monooxygenases include drugs, steroids, industrial chemicals, pesticides, herbicides, food preservatives, certain dyes used as coloring agents, polycyclic aromatic hydrocarbons, and normally occurring constituents in our diet.

**Effect of Enzyme Induction on the in Vivo Metabolism of Chemicals**

The magnitude of the change in the metabolism and action of a chemical that may occur after enzyme induction in animals is illustrated by data obtained from rats given the muscle relaxant drug zoxazolamine, which is metabolized by liver microsomal enzymes to the pharmacologically inactive compound, 6-hydroxyzoxazolamine (Chart 4). Administration of BP to rats rapidly stimulated hepatic zoxazolamine hydroxylase activity, and maximum enzyme levels were observed within 24 hr (65). Although some increase in zoxazolamine hydroxylase activity was found 24 hr after starting phenobarbital administration, maximum stimulation was not obtained unless the animals were treated for 3 to 4 days (65). The increased amount of zoxazolamine hydroxylase activity in liver microsomes of rats treated with phenobarbital or BP was associated with an accelerated rate of metabolism of zoxazolamine in vivo. The half-life of zoxazolamine was 9 hr in control rats, 48 min in phenobarbital-pretreated rats, and only 10 min in rats pretreated with BP (Chart 5). These dramatic changes in the half-life of zoxazolamine were paralleled by changes in the duration of action of the drug. Control rats given a high dose of zoxazolamine were paralyzed for 730 min, rats pretreated for 4 days...
days with phenobarbital were paralyzed for 102 min, and rats pretreated for 1 day with BP were paralyzed for only 17 min (65).

Several studies demonstrated that microsomal enzyme induction enhances the in vivo metabolism of polycyclic aromatic hydrocarbons (187, 269, 270, 321). Treatment of rats with MC 24 hr prior to an injection of 7,12-dimethylbenz(a)anthracene decreased the tissue concentrations of 7,12-dimethylbenz(a)anthracene and its 7-hydroxymethyl and 12-hydroxymethyl metabolites (187). During the course of these studies, we found that treatment of rats with MC increased the levels of liver microsomal enzymes that metabolized 7,12-dimethylbenz(a)anthracene, and we also found that treatment with the enzyme inducer stimulated the further metabolism of the hydroxymethyl metabolites of 7,12-dimethylbenz(a)anthracene (187). It was of considerable interest that the further metabolism of primary metabolites was inhibited by high concentrations of 7,12-dimethylbenz(a)anthracene in the incubation mixture so that the profile of metabolites observed depended on the concentration of substrate (187). The accumulation of primary metabolites under conditions of high substrate concentration and their further metabolism under conditions of low substrate concentration are important general concepts that have also been pointed out in studies with BP (132). The induction of increased amounts of BP hydroxylase activity in rat liver microsomes is reflected in vivo by an enhanced rate of biliary excretion of BP metabolites and by decreased blood and tissue concentrations of BP (269, 270, 321). The stimulatory effect of BP on its own metabolism is also illustrated by a decreased tissue concentration of this compound when it is administered chronically. At 24 hr after a single p.o. dose of 1 mg of [3H]BP to adult rats, the concentration of this hydrocarbon in dorsal fat was 249 ng/g, whereas at 24 hr after 7 daily doses of the tritiated hydrocarbon, the concentration of BP in dorsal fat was only 24 ng/g (270).

Occurrence of Multiple Cytochrome P-450s and Specificity in their Induction

During the course of studies at the NIH, we found that polycyclic aromatic hydrocarbons have marked specificity as inducers of microsomal monoxygenases in rat liver, and we suggested the presence in rat liver of multiple monoxygenases that are under separate regulatory control (67). In these studies, it was found that treatment of rats with BP increased the levels of liver microsomal enzymes that hydroxylate BP and 3-hydroxysteroids and that N-demethylate 3-methyl-4-monomethylnaphtalene, but little or no induction occurred for the hydroxylation of chlorozoxazone or the N-demethylation of Benadryl (67). Although treatment of rats with BP caused a dramatic decrease in the pharmacological action of zoxazolamine by stimulating its in vivo metabolism, treatment with BP did not stimulate the metabolism of hexobarbital or decrease its pharmacological action (65). Studies by Axelrod et al. (12) and by Weisburger et al. (320) suggested that different animal species have monoxygenases with different catalytic properties, and additional studies indicated that inhibitors of monoxygenase activity exert specificity with regard to the reactions inhibited (13). Our observations and those by Axelrod and Weisburger suggested the presence of multiple monoxygenases in the liver, and studies during the next several years demonstrated that multiple cytochrome P-450s were terminal oxygenases for the oxidative metabolism of drugs, carcinogens, and many other foreign chemicals.

Initial reports that carbon monoxide inhibited monooxygenase activity in liver (61) and placenta (267) were followed by a series of experiments by Klingenberg (170), Garfinkel (108), and Omura and Sato (241) that led to the identification of a carbon monoxide-binding cytochrome in liver microsomes. This hemoprotein was called cytochrome P-450 by Omura and Sato (241). The role of cytochrome P-450 in the hydroxylation of drugs was elucidated in a series of elegant experiments by Cooper et al. (92), and the induction of increased levels of hepatic cytochrome P-450 by phenobarbital was demonstrated by Orrenius and Ernster (242) and by Remmer and Merker (258). Subsequent studies showed that treatment of rats with polycyclic aromatic hydrocarbons caused the appearance of a microsomal hemoprotein with spectral properties that were different from those of cytochrome P-450 in control rats and from those of cytochrome P-450 in rats treated with phenobarbital (7, 175, 277). The hemoprotein in liver microsomes from rats treated with MC was called cytochrome P-450 by Cooper et al. (92). Studies by Alvares et al. (8) demonstrated that induction of the spectral changes by MC was prevented by inhibitors of protein and RNA synthesis suggesting that MC had stimulated the de novo synthesis of a new cytochrome P-450.

Testosterone has been used extensively as a prototype substrate that undergoes multiple hydroxylation reactions. During the course of studies on the hydroxylation of testosterone in the 6β-, 7α-, and 16α-positions by rat liver microsomes, we found that the 3 hydroxylation reactions were under separate regulatory control and that one hydroxylation reaction could be selectively stimulated or inhibited without influencing the others (74). For instance, chlortion markedly inhibited the 16α-hydroxylation reaction but had little or no effect on the 6β- or 7α-hydroxylation reaction. In addition, different patterns of development with age and specific effects of enzyme inducers on the 3 hydroxylation reactions were observed. We found that carbon monoxide inhibited the microsomal 6β-, 7α-, and 16α-hydroxylation of testosterone to different extents. The CO/O2 ratios needed for 50% inhibition of the 16α-, 6β-, and 7α-hydroxylation of testosterone were 0.93, 1.54, and 2.36, respectively (72). The photochemical action spectrum for the 3 hydroxylation reactions revealed that light at about 450 nm was more effective than light at other wave lengths in reversing the CO inhibition. These results suggested the presence in liver microsomes of several cytochrome P-450s that function in the hydroxylation of testosterone at the various positions and that the cytochrome responsible for the 16α-hydroxylation reaction was more sensitive to CO than the cytochromes which function for the 6β- and 7α-hydroxylation reactions.

Although studies with liver microsomes provided strong evidence that phenobarbital and MC induced the de novo synthesis of different monoxygenases, a definitive demonstration of the induction of different monoxygenases required the solubilization and purification of these membrane-bound enzymes from the tissues of animals treated with phenobarbital and MC. In 1957, we reported on the use of detergent (deoxycholate) for the solubilization of liver microsomal monoxygenase activity (61), and subsequent studies have used various detergents for the solubilization and purification of the monoxygenase
systems. In 1968 and 1969, Drs. Lu and Coon reported on the resolution and reconstitution of a monooxygenase system from rabbit liver microsomes (206, 207), and they found that cytochrome P-450, lipid, cytochrome P-450 reductase, and NADPH were all required for optimal catalytic activity. Following these studies, Dr. Lu joined my laboratory and together with Dr. Kuntzman we initiated a program to purify and reconstitute the cytochrome P-450s from control rats and from rats treated with phenobarbital and MC. We found that the cytochrome P-450s that were partially purified from liver microsomes of control, phenobarbital-treated, and MC-treated rats had markedly different substrate specificities when they were reconstituted with excess NADPH-cytochrome P-450 reductase and lipid (77, 206, 209). In addition, the different partially purified cytochrome P-450s had different regioselectivities for the metabolism of testosterone in the 6β-, 7α-, and 16α-positions (77, 209). These results provided the first direct demonstration for the presence in liver microsomes of multiple cytochrome P-450s with different catalytic activities. Additional studies by numerous investigators have resulted in the isolation of many highly purified forms of cytochrome P-450 from the liver and extrahepatic tissues of animals (89, 184, 210). In much of the research on the identification and purification of the multiple cytochrome P-450s, microsomal enzyme inducers have been used to increase the concentrations of specific forms of cytochrome P-450, thereby facilitating the identification and purification of these hemoproteins.

Levin and his associates have purified to apparent homogeneity 4 different cytochromes P-450 (P-450a, P-450b, P-450c, and P-450d) from liver microsomes of rats treated with various inducers (264, 265), and they found remarkably different NH2- and COOH-terminal amino acid sequences for these hemoproteins (24, 25). The large differences in the NH2- and COOH-terminal amino acid sequences for these 4 forms of cytochrome P-450 in rat liver microsomes indicate that each of the hemoproteins is a separate gene product. Cytochrome P-450b is the major P-450 in liver microsomes from phenobarbital-treated rats, cytochrome P-450c (also called cytochrome P-448 or P-450) is the major P-450 in liver microsomes from rats treated with MC, and cytochrome P-450d is the major P-450 in liver microsomes from rats treated with isosafrole. The availability of several pure cytochrome P-450s led to the preparation of highly specific antibodies for these hemoproteins and to the use of these antibodies for the quantification of specific cytochrome P-450 isozymes in tissues (299, 300). It was found that treatment of rats with phenobarbital for 4 days caused a 50-fold increase in the concentration of cytochrome P-450b in liver microsomes, a 3-fold increase in the concentration of cytochrome P-450a, and no change in the amount of cytochrome P-450c. Treatment of rats with MC caused a 50-fold increase in the concentration of cytochrome P-450c in liver microsomes, a 4-fold increase in cytochrome P-450a, and no increase in cytochrome P-450b. Treatment of rats with Aroclor 1254 caused a 45- to 60-fold increase in the concentrations of both cytochromes P-450b and P-450c in liver microsomes as well as a 5-fold increase in the concentration of cytochrome P-450a. The ability of Aroclor 1254 to increase greatly the levels of several cytochromes P-450s in rat liver microsomes may explain why the 9000 × g liver supernatant fraction obtained from rats treated with Aroclor 1254 is so effective in metabolizing a diverse group of chemicals to mutagens in the Ames system. It should be pointed out that, although the antibodies to cytochromes P-450a, P-450b, and P-450c are highly specific, the possibility of reaction of an antibody (even a monoclonal antibody) with more than one cytochrome P-450 is an important problem. The antibody for cytochrome P-450b described above does cross-react with a newly isolated hemoprotein (cytochrome P-450e) that is present in small amounts in liver microsomes from rats treated with phenobarbital or polychlorinated biphenyls (266). Additional studies revealed that cytochrome P-450e is structurally very similar to cytochrome P-450b (266), but the 2 hemoproteins are encoded by different mRNAs (314). The recent use of monoclonal antibodies for the characterization of the cytochrome P-450 isozymes in cells opens up a new dimension for future studies on the structure, function, and regulation of the multiple cytochrome P-450s.

Each of the highly purified cytochrome P-450s has its own characteristic but often overlapping substrate specificity (89, 184, 210), suggesting that the rates of detoxification and metabolic activation of foreign chemicals will depend on the relative amounts of the various cytochrome P-450s that are present in an organism. The catalytic activities of cytochromes P-450a, P-450b, and P-450c toward 6 substrates are described in Table 1. Cytochrome P-450b has good catalytic activity for the oxidative N-demethylation of benzphetamine and for the 16α-hydroxylation of testosterone, whereas the other cytochrome P-450s that were studied have poor catalytic activity for these reactions. Cytochrome P-450c has good catalytic activity for the hydroxylation of BP and zoxazolamine, and this hemoprotein also has high catalytic activity for the biotransformation of BP and other polycyclic aromatic hydrocarbons to mutagens and to their proximate and ultimate carcinogenic metabolites (198, 264, 292, 354).

Wood et al. (354) have utilized the purified cytochrome P-450s, purified epoxide hydrolase, and short incubation periods under carefully defined conditions for studies on the metabolic activation of chemicals to mutagens. Marked differences in the abilities of cytochromes P-450b and P-450c to metabolize BP to mutagens during a 5-min incubation in liquid medium are shown in Chart 6. Using purified cytochrome P-450c and short incubation conditions, we found that purified epoxide hydrolase partially inhibits the accumulation of mutagenic metabolites of BP (354), and similar results were reported by Bentley et al. (18) in studies with liver microsomes, purified epoxide hydrolase, and the usual incubation conditions of Ames. In additional studies, we found that epoxide hydrolase and cytochrome P-

<table>
<thead>
<tr>
<th>Substrate</th>
<th>P-450a</th>
<th>P-450b</th>
<th>P-450c</th>
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<tbody>
<tr>
<td>Aniline</td>
<td>5.1</td>
<td>6.7</td>
<td>9.3</td>
</tr>
<tr>
<td>Benzphetamine</td>
<td>3.2</td>
<td>176.0</td>
<td>5.1</td>
</tr>
<tr>
<td>BP</td>
<td>0.1</td>
<td>0.5</td>
<td>19.2</td>
</tr>
<tr>
<td>Zoxazolamine</td>
<td>0.9</td>
<td>2.4</td>
<td>27.9</td>
</tr>
<tr>
<td>7-Ethoxycoumarin</td>
<td>0.6</td>
<td>12.1</td>
<td>48.5</td>
</tr>
<tr>
<td>Testosterone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6β-Hydroxy-</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>7α-Hydroxy-</td>
<td>4.4</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>16α-Hydroxy-</td>
<td>&lt;0.1</td>
<td>2.4</td>
<td>&lt;0.1</td>
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Table 1: Catalytic activities of purified cytochromes P-450a, P-450b, and P-450c from rat liver microsomes (184, 264)
forms of cytochrome P-450 that are induced and also on the 
or unimportant for an organism is complex and depends on the 
the cytochrome P-450 enzymes. The answer to whether the 
induction of the microsomal cytochrome P-450s is good, bad,
amounts of potentially toxic chemicals that are metabolized by 
cytochrome P-450s on the toxicity of chemicals in an organism.

living in an environment where we are exposed to various
Effects of Enzyme Induction on the Toxicity of Chemicals

Examples of chemicals that induce different forms or mix-
tures of forms of cytochrome P-450 in rat liver microsomes
include MC, phenobarbital, isosafrole, pregnenolone 16α-car-
bonitrile, ethanol, cholestryamine, clofibrate, and Aroclor 1254
research is needed to determine the number of cytochrome P-
450s in liver and in extrahepatic tissues and to determine the
factors that regulate the synthesis and activity of these en-
zymes. Studies on the mechanism of microsomal enzyme
induction revealed the presence of a cytoplasmic receptor that
bids 2,3,7,8-tetrachlorodibenzo-p-dioxin and polycyclic aro-
matic hydrocarbons, and this receptor is important for the
induction of microsomal monooxygenases by these com-

450c were both required for the metabolism of BP 7,8-oxide
to mutagenic metabolites (354), and this observation provided a
direct demonstration that epoxide hydrolase can play a role in
the metabolism of chemicals to toxic products. It seems likely
that studies with the purified cytochromes P-450 and epoxide
hydrolase will have increasingly greater importance for the
elicitation of the pathways of metabolic activation and detox-
ification of chemical carcinogens.

Examples of chemicals that induce different forms or mix-
tures of forms of cytochrome P-450 in rat liver microsomes

Effects of Enzyme Induction on the Toxicity of Chemicals

I would like to consider the effects of high levels of the
cytochrome P-450s on the toxicity of chemicals in an organism.
This is a particularly important question because all of us are
living in an environment where we are exposed to various
amounts of potentially toxic chemicals that are metabolized by
the cytochrome P-450 enzymes. The answer to whether the
induction of the microsomal cytochrome P-450s is good, bad,
or unimportant for an organism is complex and depends on the
forms of cytochrome P-450 that are induced and also on the
chemical environment of the organism. The effect of induction
by barbiturates or polycyclic aromatic hydrocarbons on the
acute toxicity of several chemicals is shown in Table 2. Al-
though pretreatment of rats with barbiturates protects animals
from the lethal effects of warfarin, meprobamate, and strych-
nine by stimulating the metabolism of these compounds to
nontoxic products (66, 138, 162), this same treatment in-
creases the toxicity of Schradan, carbon tetrachloride, bro-
mobenzene, and acetaminophen by stimulating the metabolism
of these compounds to toxic products (109, 162, 229, 366).
Although induction of the cytochrome P-450s with phenobar-
bital is associated with an increased toxicity of bromobenzene,
the induction of a different profile of cytochrome P-450s with
MC is associated with protection of rats from the hepatotoxicity
of bromobenzene (366). The later induction regimen also pro-
tects rats from the lethal effect of a high dose of zoxazolamine
but increases the amount of liver necrosis caused by acet-
aminophen (66, 229).

Suitable inducers of microsomal enzymes inhibit the carci-
ngenicity of aminoazo dyes (225, 260), 2-acetylaminofluo-
rene (225), BP (317), 7,12-dimethylbenz[a]anthracene (136),
aflatoxin B, (221), 4-dimethylaminostilbene (288), urethan
(361), diethylstilbestrolamine (178), and bracken fern (243),
previously by stimulating the metabolism of the carcinogen via
detoxification pathways to a greater extent than via toxification
pathways. Butylated hydroxyanisole and butylated hydroxytol-
Uene are examples of compounds that inhibit the carcinogenic-
ity of several chemicals in mice (318), and recent studies have
shown that butylated hydroxyanisole is an effective inducer of
aniline hydroxylase, epoxide hydrolase, and glucuronyl trans-
ferase activities in liver microsomes (48, 49). In addition, treat-
ment of mice with butylated hydroxyanisole increases the levels
of glutathione S-transferase, glucose-6-phosphate dehydro-
genase, and UDP-glucose dehydrogenase in the liver cytosol
(17, 48). Butylated hydroxyanisole also increases the concen-
tration of free sulphydryl groups in tissues (16), and the in-
creased levels of sulphydryl groups may protect animals by
reacting with and inactivating ultimate carcinogens. The induc-
tion profile of butylated hydroxyanisole is very different from
that which has been reported for phenobarbital, polycyclic
hydrocarbons, and several other inducers of microsomal en-
zymes.

In most instances studied, pretreatment of animals with a
microsomal enzyme inducer or the combined administration
of the inducer and a carcinogen inhibits the tumorigenic effect
of the chemical administered. However, this is not always the
case. The combined administration of phenobarbital and saf-

Table 2

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Effect</th>
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| Phenobarbital  | 1. Protection from lethal effects of warfarin, meprobamate,
| or thiopental |   and strychnine (66, 138, 162)                            |
|               | 2. Increased lethal effects of Schradan and carbon tetra-
|               |   chloride (109, 162)                                     |
|               | 3. Increased liver necrosis caused by bromobenzene and    |
|               |   acetaminophen (229, 366)                                |
| MC            | 1. Protection from lethal effect of zoxazolamine (66)    |
|               | 2. Decreased liver necrosis caused by bromobenzene (366)  |
|               | 3. Increased liver necrosis caused by acetaminophen (229) |


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role causes more hepatic tumors than safrole alone (337). It is of considerable interest that several inducers of liver microsomal enzymes are promotors of liver tumors (252). For example, phenobarbital inhibits the carcinogenicity of 2-acetylaminofluorene when the 2 compounds are fed together, but phenobarbital causes the appearance of additional liver tumors when the barbiturate is administered chronically after the cessation of a low initiating dose of 2-acetylaminofluorene (252, 253). Although several microsomal enzyme inducers are promotors of liver tumors, it is not known whether the induction of microsomal enzymes and tumor promotion are causally related.

**Stimulatory Effect of Drugs and Environmental Chemicals on Drug Metabolism in Humans**

The induction of microsomal enzymes observed in animals has significance in humans, and there are many examples of drugs that stimulate their own metabolism or the metabolism of other chemicals in humans (59, 64). An early example of a stimulatory effect of one drug on the metabolism of a second drug in humans is the stimulatory effect of barbiturates on the metabolism of coumarin anticoagulants. We found that treatment of rats with phenobarbital for several days increased the activity of liver microsomal enzymes that metabolize the anticoagulant drugs bishydroxycoumarin and warfarin (94, 138), and we also found that administration of 65 mg of phenobarbital per day lowered the plasma levels and decreased the anticoagulant activity of bishydroxycoumarin in humans (94). The potential hazards of this interaction were investigated in a canine model (322), and the results of a representative study are shown in Chart 7. In this study, a dog was given 1 mg of bishydroxycoumarin per kg of body weight every 48 hr for several weeks until a constant concentration of drug in the plasma and a constant anticoagulant effect (prolonged prothrombin time) were achieved. When phenobarbital was administered to the dog without changing the bishydroxycoumarin regimen, the concentration of bishydroxycoumarin in the plasma decreased, and the anticoagulant effect of the drug was abolished. Even when there was a 5-fold increase in the dose of bishydroxycoumarin, adequate anticoagulant therapy and an adequate concentration of bishydroxycoumarin in the plasma were not achieved while phenobarbital treatment was continued. When phenobarbital administration was discontinued, the concentration of bishydroxycoumarin in the plasma and the anticoagulant effect of this drug increased during the next few days to such an extent that the dog bled severely and required vitamin K to save its life. These results indicate that combined therapy with a coumarin anticoagulant and a stimulator of drug metabolism can be hazardous if the enzyme inducer is withdrawn and therapy with the anticoagulant is continued without an appropriate decrease in the dose. The interaction between phenobarbital and bishydroxycoumarin is an example of a drug interaction that has led to serious consequences and to some deaths in humans. Since patients are often treated with combinations of drugs, the possibility of drug interactions is an important consideration during human drug therapy.

Chemicals in the workplace can stimulate the oxidative metabolism of drugs in humans. People exposed to large amounts of DDT (255), polychlorinated biphenyls (4, 173), Kepone (114), or combinations of DDT and lindane (171) in chemical factories have an enhanced rate of oxidative drug metabolism. Similarly, anesthesiologists who are exposed to large amounts of volatile anesthetic chemicals in the operating room (102, 118) and gasoline station attendants who are exposed to high levels of petroleum (117) also have an enhanced rate of drug metabolism. These results indicate that people who are occupationally exposed to enzyme-inducing chemicals in their environment or who are treated with enzyme-inducing drugs will have an enhanced rate of metabolism of some drugs, and it is likely that these individuals will also have an enhanced rate of metabolism of some environmental chemicals that are metabolized by the same enzymes that metabolize drugs.

**Stimulatory Effect of Cigarette Smoking on the Metabolism of Chemicals**

Since BP and many other polycyclic aromatic hydrocarbons are liver microsomal enzyme inducers that are present in cigarette smoke, Dr. Richard Welch and I studied the effects of cigarette smoke on monooxygenase activity in both rats and humans. We found that exposure of pregnant rats to cigarette smoke stimulated the hydroxylation of BP in the placenta and in the maternal liver, lung, and intestine (326). Increased activity was also found in fetal liver, indicating that enzyme inducers in cigarette smoke pass through the placenta and reach the fetus. Additional studies revealed that several polycyclic aromatic hydrocarbons present in cigarette smoke are potent inducers of BP hydroxylase activity in rat placenta (321, 324). Examples of these inducers include BP, DBA, BA, chrysene, 3,4-benzofluorene, anthracene, pyrene, and fluoranthene (324). Since cigarette smoke and hydrocarbons in cigarette smoke induced monooxygenase activity in rat placenta, we collaborated with Drs. Paul Poppers and Michael Finster at Columbia University to investigate the effects of cigarette smoking on the oxidative metabolism of several drugs and carcinogens by human placentas that were obtained after normal childbirth. We found that placentas from cigarette smokers had markedly higher levels of BP hydroxylase (323, 324), aminozadodo N-demethylase (324), and zoxazolamine hydroxylase (154) activities than did placentas from nonsmokers (Table 3). The average BP hydroxylase activity in the placentas of smokers was about 60-fold higher than in the placentas of nonsmokers. Similar studies indicated that the average zoxazolamine hydroxylase activity in the placentas from smokers was about 15-fold higher than in the placentas from nonsmokers.
Cigarette smoking caused a much smaller stimulatory effect on the O-dealkylation of 7-ethoxycoumarin (141) and did not change the oxidative aromatization of Δ4-androstene-3,17-dione to estradiol and estrone (85). Our results suggested the presence in human placenta of several monooxygenases that are under separate regulatory control.

Large interindividual differences occurred for the amount of BP hydroxylase activity in placentas that were obtained from smokers and non-smokers (85, 141, 154, 323, 324). BP hydroxylase activity varied about 6-fold among the placentas from non-smokers and about 80-fold among the placentas from smokers. The highest BP hydroxylase activity in the placentas from the smoking group was at least 400-fold higher than the lowest BP hydroxylase activity in the placentas from the non-smoking group. It was also of interest that placental BP hydroxylase activity varied about 6-fold among the placentas from smokers and non-smokers (85, 141, 154, 323, 324). BP hydroxylase activity varied over a 70-fold range among subjects who smoked about the same number of cigarettes (Table 4), and we suggested that variability among different cigarette smokers in the induction of enzymes that metabolize chemical carcinogens in tobacco smoke may help explain why some people develop cancer when exposed to cigarette smoke and others do not (323, 324). A few years later, we suggested the use of tissue culture systems for the determination of basal activity and inducibility of carcinogen-metabolizing enzymes in human tissues as an approach for detecting differences in the abilities of different individuals to metabolize carcinogens and other toxic chemicals. Our initial studies to determine the feasibility of this approach were encouraging and revealed that BA caused the induction of BP hydroxylase activity in cultured human foreskin (6, 188). A 3-fold variation in basal BP hydroxylase activity in foreskins from different individuals was observed as well as marked individuality in the degree of induction in the different foreskins studied. The amount of induction varied from about 70 to 500% in the 13 foreskins examined (6, 188), and similar results were obtained by others in studies with cultured human lymphocytes and monocytes (15, 44, 164). In more recent studies, Dr. C. Harris and his associates have demonstrated marked interindividual differences in the metabolic activation of several carcinogens to DNA-bound adducts by several human tissue culture systems (119). It is possible that appropriate metabolic studies on the bioactivation and detoxification of environmental carcinogens by cultured human cells from different individuals may help in the identification of individuals who have a high cancer risk.

Since the metabolism of phenacetin to its major metabolite, N-acetyl-p-aminophenol (Chart 8), occurs by a polycyclic hydrocarbon-inducible enzyme system in rat liver (83), we utilized phenacetin as a prototype drug to determine whether or not cigarette smoking stimulates the in vivo metabolism of some chemicals in humans. Collaborative studies with Dr. Eugene Pantuck at Columbia University and with Dr. Ronald Kuntzman revealed that cigarette smoking lowered the plasma levels of phenacetin (Chart 9) administered p.o. without changing its plasma half-life or the plasma levels of total N-acetyl-p-aminophenol (247, 248). The ratio of the concentration of total N-acetyl-p-aminophenol to that of phenacetin in plasma was markedly increased in the cigarette smokers. These results suggested that cigarette smoking stimulated the in vivo metabolism of phenacetin in the gastrointestinal tract and/or during absorption.

Table 3
Effect of cigarette smoking on the metabolism of chemicals by human placenta

<table>
<thead>
<tr>
<th>Reaction measured</th>
<th>Nonsmokers</th>
<th>Cigarette smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP hydroxylation (85, 141, 154, 323, 324)</td>
<td>0.5 ± 0.1</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>3-Methyl-4-monooethylaminonoazobenzene N-demethylation (324)</td>
<td>&lt;4</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>Zoxazolamine hydroxylation (154)</td>
<td>0.4 ± 0.1</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>7-Ethoxyccoumarin O-dealkylation (141)</td>
<td>29 ± 3</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>Estrogen biosynthesis (85)</td>
<td>44 ± 9</td>
<td>47 ± 5</td>
</tr>
</tbody>
</table>

* Average ± S.E.
* nmol fluorescent phenolic metabolites equivalent to 3-hydroxy-BP formed per g placenta per hr.
* nmol 3-methyl-4-aminooazobenzene formed per g placenta per hr.
* nmol tritiated water formed per g placenta per hr.
* nmol 7-ethoxycoumarin formed per g placenta per hr.
* nmol estradiol and estrone formed per g placenta per hr from Δ4-androstene-3,17-dione.

Table 4
Interindividual differences in BP hydroxylase activity in placentas from cigarette smokers (85, 324)

<table>
<thead>
<tr>
<th>Subject</th>
<th>3-Hydroxy-BP formed (nmol/g placenta/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.9</td>
</tr>
<tr>
<td>B</td>
<td>2.2</td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
</tr>
<tr>
<td>D</td>
<td>2.6</td>
</tr>
<tr>
<td>E</td>
<td>5.0</td>
</tr>
<tr>
<td>F</td>
<td>5.2</td>
</tr>
<tr>
<td>G</td>
<td>7.4</td>
</tr>
<tr>
<td>H</td>
<td>17.0</td>
</tr>
<tr>
<td>I</td>
<td>17.4</td>
</tr>
<tr>
<td>J</td>
<td>20.9</td>
</tr>
<tr>
<td>K</td>
<td>60.2</td>
</tr>
<tr>
<td>L</td>
<td>65.6</td>
</tr>
<tr>
<td>M</td>
<td>67.8</td>
</tr>
</tbody>
</table>

Chart 8. Metabolism of phenacetin to N-acetyl-p-aminophenol.

Chart 9. Plasma levels of phenacetin in cigarette smokers and nonsmokers at various times after the p.o. administration of 900 mg of phenacetin (247, 248). Each value represents the mean ± S.E. from 9 subjects.

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its first pass through the liver. Subsequent studies revealed that cigarette smokers have shorter plasma half-lives of antipyrene (121), theophylline (144, 152), and caffeine (251) than do nonsmokers, but cigarette smokers do not have an enhanced metabolism of phenytoin, meperidine, or nortriptyline (219, 238, 282).

Effect of Diet on the Metabolism of Chemicals

In 1950, Drs. G. C. Mueller and J. A. Miller reported that riboflavin adenine dinucleotide was required for the enzymatic reductive cleavage of a carcinogenic aminoazo dye to noncarcinogenic metabolites in the liver (232). This observation provided an explanation for the protective effect of dietary riboflavin on aminoazo dye hepatocarcinogenesis (166, 228) and drugs in humans. An important feature of this clinical research, on the effects of dietary factors on the metabolism of prototype chemicals in our diet is receiving increasingly more attention (93, 227, 306). Since human beings receive a large part of their exposure to exogeneous chemicals through the diet, it is critical to determine the effects of different diets on the metabolism and action of foreign chemicals. These studies have been extended by many investigators, and the effects of dietary factors on the metabolism of foreign chemicals in experimental animals have been reviewed recently (45, 123). Substances in food that may influence the metabolism of drugs include macronutrients (carbohydrate, protein, fat), trace substances important to human health (vitamins, trace metals, etc.), and numerous nonnutrient, lipop-soluble foreign chemicals. Although the latter diverse group of substances has not been well characterized, the presence of these chemicals in our diet is receiving increasingly more attention (93, 227, 306). Since human beings receive a large part of their exposure to exogeneous chemicals through the diet, it is important to determine the effects of different diets on the metabolism and action of drugs, environmental carcinogens, and other environmental chemicals. Because of these considerations, Dr. Attallah Kappas and I initiated a research program on the effects of dietary factors on the metabolism of prototype drugs in humans. An important feature of this clinical research, which was done in collaboration with Dr. Eugene Pantuck at Columbia University and with Drs. Alvito Alvares and Karl Anderson at the Rockefeller University, was the use of normal volunteers as their own controls. In these studies, we examined the metabolism of prototype drugs before, during, and after a specific dietary change.

Stimulatory Effect of Dietary Charcoal-broiled Beef on Human Drug Metabolism. Charcoal-broiled beef, a food which contains relatively high concentrations of polycyclic aromatic hydrocarbons, is eaten by large numbers of people. Because of the possible influence of this food on the metabolism of foreign chemicals, we studied the effect of feeding charcoal-broiled beef on the metabolism of foreign chemicals in rats and in humans. The in vitro metabolism of phenacetin by intestine (245) and the metabolism of BP by liver and placenta (120) were stimulated severalfold when rats were fed a diet which contained charcoal-broiled beef. Additional studies in humans revealed that feeding charcoal-broiled beef (14 oz/day) for 4 days markedly lowered the plasma concentrations of subsequently administered phenacetin (Chart 10) without changing its plasma half-life or the plasma levels of total N-acetyl-p-aminophenol (80, 244). Thus, the ratio of the concentration of total N-acetyl-p-aminophenol to that of phenacetin in plasma was markedly higher after the subjects were fed charcoal-broiled beef, and the ratio decreased when the subjects returned to the control diet. Our results indicated that feeding charcoal-broiled beef greatly increased the metabolism of phenacetin during its first pass through the liver and/or in the gastrointestinal tract. Additional studies revealed that feeding charcoal-broiled beef for 4 to 5 days increased the metabolic clearance rates of antipyrene and theophylline by 38 and 30%, respectively (159). It was of interest that an occasional individual failed to respond to the effects of charcoal-broiled beef feeding. The reason(s) for individuality in responsiveness to charcoal-broiled beef feeding is not known.

Stimulatory Effect of Dietary Cabbage and Brussels Sprouts on Human Drug Metabolism. Dr. L. Wattenberg and his associates have shown that intestinal BP hydroxylase activity in rodents is very sensitive to changes in the content of cruciferous vegetables in the diet (315). Feeding rats a diet containing brussels sprouts or cabbage markedly stimulated the intestinal metabolism of BP, 7-ethoxycoumarin, and phenacetin (246, 315). Indole-3-acetinol, indole-3-carbonil, and 3,3'-diindolylmethane have been identified as examples of strong inducers of monoxygenase activity that are present in brussels sprouts and cabbage (205).

The effects of feeding high levels of cabbage and brussels sprouts for several days on the metabolism of antipyrene and phenacetin were studied in 10 normal volunteers (249). Feeding the cabbage- and brussels sprouts-containing diet for 3 days decreased the mean plasma half-life of antipyrene by 13% and increased its metabolic clearance rate by 11%. Returning the subjects to the control diet for 6 days resulted in a 17% increase in the plasma half-life of antipyrene and a 13% decrease in its metabolic clearance rate. Although these effects of feeding cabbage and brussels sprouts are small, they are statistically significant. Feeding the cabbage- and brussels sprouts-containing diet for 6 days resulted in mean plasma concentrations of phenacetin in humans (80, 244). Nine subjects were given a p.o. dose of 900 mg of phenacetin after 7 days on control diet, 4 days on a charcoal-broiled beef diet, and 7 days on the control diet a second time. Each value represents the mean ± S.E.
concentrations of phenacetin that were decreased 34 to 67% at the various times after the p.o. administration of this drug (Table 5). The mean ratio of the plasma concentration of total N-acetyl-p-aminophenol to the plasma concentration of phenacetin was increased when the subjects were fed cabbage and Brussels sprouts, and the ratio returned towards control values when the subjects were again fed the control diet for several days. These results suggest that feeding a diet that contains cabbage and Brussels sprouts stimulates the metabolism of phenacetin in the gastrointestinal tract and/or during its first pass through the liver. In addition to the above observations, the ratio of the plasma concentration of conjugated N-acetyl-p-aminophenol to the concentration of unconjugated N-acetyl-p-aminophenol was increased 40 to 50% when the subjects were fed the cabbage- and Brussels sprouts-containing diet, suggesting that this diet enhances the conjugation of N-acetyl-p-aminophenol (249).

Regulation of Human Drug Metabolism by Dietary Carbohydrate and Protein. Changes in the protein and carbohydrate content of the diet markedly influence human drug metabolism (Table 6) (3, 160). For these studies, 6 normal volunteers were fed their customary home diet for 2 weeks, a high-protein-low-carbohydrate diet for 2 weeks, and their customary home diet again for 2 weeks. The plasma half-life of antipyrine was determined on Day 10 of each of the 4 study periods, and the plasma half-life of theophylline was determined on the last day of each of the 4 study periods. The average half-lives of antipyrine and theophylline increased 63 and 46%, respectively, when the 6 subjects were shifted from the high-protein-low-carbohydrate diet to the isocaloric high-carbohydrate-low-protein diet, and it was of interest that each individual had his own characteristic response to this alteration in diet. The increase in antipyrine half-lives among the 6 subjects ranged from no change in one subject to 111% in another subject. The increase in theophylline half-lives among the 6 subjects when they were shifted from the high-protein-low-carbohydrate diet to the high-carbohydrate-low-protein diet ranged from 14 to 71%. The above dietary changes also caused significant changes in the metabolic clearance rates of antipyrine and theophylline, and a similar stimulation of antipyrine and theophylline metabolism was observed when the amount of dietary protein was increased at the expense of fat (10). An increase in the amount of either saturated or unsaturated fat in the diet at the expense of carbohydrate, however, had little or no effect on the metabolism of antipyrine or theophylline (10). Recent studies have shown that increasing the ratio of protein to carbohydrate in the diet can have clinical importance since this dietary change was found to decrease the effectiveness of theophylline in children with asthma (104). The results of studies with charcoal-broiled beef, with cabbage and Brussels sprouts, and with changes in the ratio of protein to carbohydrate in the diet indicate that dietary changes can significantly influence the oxidative metabolism of prototype drugs in humans, and it is likely that dietary factors can also influence the metabolism and activity of chemical carcinogens in humans.

Activation of Monoxygenases by Flavonoids. Several years ago, Wiebel and Gelboin showed that the addition of 7,8-benzo flavone to liver microsomes from MC-treated rats markedly inhibited BP hydroxylase activity (328, 329). These investigators also found that 7,8-benzo flavone caused an age-dependent stimulation of BP hydroxylase activity when the flavone was added to liver microsomes from untreated rats (328, 329). Dr. J. Kapitulnik and I studied the possible use of 7,8-benzo flavone as a probe for the properties of monoxygenases in human liver, and we found that the addition of 7,8-benzoflavone to homogenates or microsomes from human liver caused a manyfold stimulation of BP hydroxylation (156). The marked activating effect (sometimes greater than 10-fold) of 7,8-benzo flavone on BP hydroxylation that we observed with human liver prompted us to study the effects of several flavonoids on monoxygenase activity in this tissue. The structures of some

Table 5
Effect of a diet containing Brussels sprouts and cabbage on the plasma concentration of phenacetin in humans (249)
Ten subjects were given a dose of phenacetin (900 mg p.o.) after they had ingested a control diet for 9 days, a Brussels sprouts- and cabbage-containing diet for 6 days, and the control diet for 9 additional days. Each value represents the mean ± S.E.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Plasma concentration of phenacetin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 hr</td>
</tr>
<tr>
<td>Control (1st time)</td>
<td>2316 ± 1150</td>
</tr>
<tr>
<td>Brussels sprouts and cabbage</td>
<td>982 ± 402</td>
</tr>
<tr>
<td>Control (2nd time)</td>
<td>1570 ± 946</td>
</tr>
</tbody>
</table>

Table 6
Effect of carbohydrate and protein content of the diet on human drug metabolism (3, 160)
Antipyrine (18 mg/kg) was administered p.o. on Day 10 and theophylline (5 mg/kg) was administered p.o. on Day 14 of each 14-day dietary regimen. Each value represents the mean ± S.E. for 6 subjects.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Antipyrine Half-life (hr)</th>
<th>Antipyrine Metabolic clearance rate (ml/min)</th>
<th>Theophylline Half-life (hr)</th>
<th>Theophylline Metabolic clearance rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home diet</td>
<td>16.2 ± 1.6</td>
<td>37 ± 1</td>
<td>8.1 ± 1.0</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>44% Protein, 35% carbohydrate, 21% fat</td>
<td>9.6 ± 0.4</td>
<td>58 ± 4</td>
<td>5.2 ± 0.4</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>10% Protein, 70% carbohydrate, 20% fat</td>
<td>15.6 ± 1.7</td>
<td>39 ± 4</td>
<td>7.6 ± 0.7</td>
<td>52 ± 4</td>
</tr>
<tr>
<td>Home diet</td>
<td>14.2 ± 0.6</td>
<td>40 ± 3</td>
<td>7.5 ± 0.7</td>
<td>52 ± 2</td>
</tr>
</tbody>
</table>
of the flavonoids that were studied are shown in Chart 11. Except for the synthetic flavonoid, 7,8-benzoflavone, all of the compounds shown in this chart are normal constituents of herbaceous plants commonly found in man’s environment, and many of these flavonoids are present in our diet. The results of our studies demonstrated that the addition of quercetin, kaempferol, morin, or chrysin to human liver microsomes inhibited BP hydroxylation, but the addition of 7,8-benzoflavone, flavone, tangeretin, or nobiletin to human liver microsomes caused a severalfold activation in the hydroxylation of BP (36). The activating effect of the latter 4 flavonoids is shown in Chart 12. Similar studies demonstrated that the activator flavonoids also increased the metabolism of aflatoxin B1 to mutagens and to aflatoxin B2,3-dihydrodiol (36, 37). In other studies, we found that the in vitro addition of 7,8-benzoflavone to human liver microsomes stimulated the metabolism of BP 7,8-dihydrodiol, B(E)P 9,10-dihydrodiol, antipyrine, and zoxazolamine, but the oxidative metabolism of hexobarbital, coumarin, and 7-ethoxycoumarin was not appreciably influenced (36, 81, 156, 289). Specificity in the activating effect of 7,8-benzoflavone on the metabolism of some substrates but not on the metabolism of other substrates suggested the presence of multiple monooxyn acids in human liver microsomes.

The effects of flavone and 7,8-benzoflavone on the metabolism of BP by 5 highly purified cytochrome P-450 isozymes obtained from rabbit liver microsomes were examined, and we found that the responses to flavone and 7,8-benzoflavone were dependent on the cytochrome P-450 isozyme studied (134). BP metabolism was stimulated more than 5-fold by the addition of flavone to a reconstituted monoxygenase system consisting of NADPH, cytochrome P-450 reductase, dilauroylphosphatidylcholine, and cytochrome P-450LM3c or cytochrome P-450LM2 (Chart 13). In contrast to these observations, an inhibitory effect of flavone on BP metabolism was observed when cytochrome P-450LM2, cytochrome P-450LM3b, or cytochrome P-450LM5 was used as the source of hemoprotein in the reconstituted monoxygenase system (Chart 13). Studies on the mechanism of the activating effects of flavone and 7,8-benzoflavone on BP hydroxylation in cholate-solubilized liver microsomes from rabbits and humans suggested that these flavonoids stimulated BP metabolism at least in part by enhancing the interaction between cytochrome P-450 and cytochrome P-450 reductase, thereby facilitating the flow of electrons to cytochrome P-450 (133).

Recent studies indicate that the activation of microsomal monoxygenases may have significance in vivo. The addition of flavone to liver microsomes obtained from neonatal rats caused a stimulation in the metabolism of [4,6-3H]zoxazolamine to 6-hydroxyzoxazolamine as measured by the formation of tritiated water, and we investigated whether flavone could stimulate the hydroxylation of zoxazolamine in vivo (181). In this study, 740 nmol of [4,6-3H]zoxazolamine were injected i.p. either alone or together with 5 μmol of flavone into neonatal rats. Total-body homogenates were made at various times after...
the injection, and tritiated water was measured and expressed as 6-hydroxyzoxazolamine. As shown in Chart 14, the i.p. injection of flavone caused an immediate stimulation in the in vivo metabolism of zoxazolamine. Animals treated with flavone formed severalfold more 6-hydroxyzoxazolamine than did control animals during the first 15 to 120 min after the injection. The ability of an in vitro activator of microsomal monooxygenases to immediately stimulate the oxidative metabolism of a chemical in vivo is a new finding, and further studies are needed to determine the pharmacological and toxicological implications of this observation.

**Effect of Enzyme Induction on the Metabolism of Steroids and Other Endogenous Substrates**

Factors that influence the hydroxylation of foreign chemicals by liver microsomes also influence the liver microsomal hydroxylation of steroid hormones (59, 70, 174). This research pointed out that steroid hormones are normal body substrates for the microsomal monooxygenases and that phenobarbital and other microsomal enzyme inducers can stimulate the metabolism of steroid hormones. Treatment of rats with phenobarbital for 4 days increased the hydroxylation of androgens, estrogens, progestational steroids, and adrenocortical steroids by enzymes in liver microsomes (Chart 15), and this effect was paralleled by a decreased action and an enhanced in vivo metabolism of exogenously administered steroids (73). Pretreatment of rats with phenobarbital and other inducers of liver microsomal enzymes decreased the estrogenic action of estradiol and estrone (192, 194, 325), decreased the androgenic action of testosterone (195, 196), and decreased the anesthetic action of progesterone and deoxycorticosterone (68, 177). The marked inhibitory effect of treating rats with phenobarbital or the insecticide chlordane on the uterotrophic action of estrone is shown in Table 7. Pretreatment of rats with phenobarbital also decreased the uterotrophic action of synthetic estrogens and progestational steroids used in oral contraceptives and stimulated the liver microsomal metabolism of some of these compounds (189, 193). Phenobarbital and other enzyme-inducing drugs have been shown to stimulate the 6β-hydroxylation of cortisol in guinea pig liver microsomes (69), to enhance the urinary excretion of 6β-hydroxycortisol in humans (43, 73, 250, 261), to increase the urinary excretion of polar metabolites of testosterone in humans (286), to decrease the effectiveness of a synthetic glucocorticoid in steroid-dependent asthmatics (33), and to stimulate the metabolism of synthetic estrogens and progestational steroids contained in oral contraceptives (28). Anticonvulsants and rifampicin are examples of drugs that stimulate the metabolism of the oral contraceptive, ethynyl estradiol, in humans (22, 27, 28), and unwanted pregnancies have occurred in women taking oral contraceptives simultaneously with anticonvulsants or rifampicin (cf. Ref. 189). Some examples of the stimulatory effect of enzyme-inducing

![Graph](chart14.png)

**Chart 14.** In vivo activation of zoxazolamine metabolism by flavone (181). Neonatal rats were given i.p. injections of 740 nmol of [4,6-3H]zoxazolamine alone or together with 5 μmol of flavone. Total body homogenates were made at various times after the injection, and 3H2O was measured and expressed as 6-hydroxyzoxazolamine formed. Points, mean; bars, S.E.

![Graph](chart15.png)

**Chart 15.** Stimulatory effect of phenobarbital treatment on steroid hydroxylation by rat liver microsomes (68, 70, 194). Immature rats were given i.p. injections of 37 mg of sodium phenobarbital per kg of body weight twice daily for 4 days. The animals were killed the following day and liver microsomes were prepared and incubated with 14C-labeled steroid in the presence of NADPH. Formation of polar metabolites with the chromatographic mobility of hydroxylated substrate was measured.

**Table 7**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>Estrone (μg)</th>
<th>Uterine wet wt (mg)</th>
<th>Inhibition of response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>19.5 ± 0.6</td>
<td>30.8 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phenobarbital</td>
<td>18.7 ± 0.2</td>
<td>19.7 ± 0.5</td>
<td>91</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>27.3 ± 0.9</td>
<td>40.2 ± 1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chlordane</td>
<td>27.4 ± 1.1</td>
<td>28.3 ± 1.5</td>
<td>93</td>
</tr>
</tbody>
</table>
drugs on the urinary excretion of 6β-hydroxycortisol in humans are shown in Chart 16. In this study, treatment of humans with N-phenylbarbital or phenylbutazone for several weeks stimulated the urinary excretion of 6β-hydroxycortisol. Additional studies pointed out that people who are exposed to high levels of DDT in a pesticide factory have an enhanced urinary excretion of 6β-hydroxycortisol (255). Microsomal enzyme inducers stimulate the metabolism of many endogenous substrates such as steroid hormones, sterols, bile acids, thyroid hormone, prostaglandins, fatty acids, lipid-soluble vitamins, and bilirubin (71, 179), but the physiological importance of these effects is still not very well understood. Newborn infants from women treated with phenobarbital before delivery have a lower serum concentration of unconjugated bilirubin (301), an altered profile of steroid metabolites (described above), an enhanced turnover of thyroid hormone (180, 239), and a lower plasma concentration of 25-hydroxycholecalciferol (137, 287) which is associated with a high incidence of osteomalacia in this population.

### Carcinogenesis by Polycyclic Aromatic Hydrocarbons

At this point in my lecture, I would like to change topics and talk about our studies on carcinogenesis by polycyclic aromatic hydrocarbons. It was in about 1972 that I developed a strong interest in doing more detailed studies on the metabolism of polycyclic aromatic hydrocarbons and in identifying proximate and ultimate carcinogenic metabolites of these compounds. It was apparent that research of this type would require a major chemical synthetic program, and I started asking chemists about the possibility of synthesizing arene oxides of BP so that we could study the metabolism and biological activity of these compounds. All the chemists that I asked, except for one, said this would be terribly difficult if not impossible. When I asked Dr. Donald Jerina at the NIH if it might be possible to synthesize some of the arene oxides of BP and about the possibility of a collaboration, Dr. Jerina replied that he was interested in a collaboration and that he could easily provide 4 of the arene oxides of BP, and, "by the way," he said, "I can also give you several dihydrodiols and quinones and all twelve of the isomeric phenols of BP." This was the start of an intensive collaboration with Dr. Jerina's laboratory which is still continuing today. The magnitude of the synthetic program initiated by Dr. Jerina has been enormous. During the course of this research, Dr. Jerina and his associates have synthesized more than 200 potential metabolites and reference compounds derived from the polycyclic aromatic hydrocarbon class of carcinogens. Many of the compounds that were synthesized are highly reactive, and some are optically active enantiomers. Almost all of the compounds have been of the type that are extremely difficult to make. I am fortunate to be collaborating with Dr. Jerina who is not only an outstanding synthetic chemist but also an outstanding biochemist and biologist. Dr. Jerina and the members of his laboratory share equally with the members of my laboratory in whatever accomplishments we may have made to the identification of proximate and ultimate carcinogenic metabolites of the polycyclic aromatic hydrocarbons. Drs. Haruhiko Yagi, Dhiren Thakker, and Roland Lehr (now at the University of Oklahoma) from Dr. Jerina's laboratory and Wayne Levin, Dr. Alexander Wood, and Richard Chang from my laboratory have been the principal contributors to this program.

Evidence for the metabolic activation of a polycyclic aromatic hydrocarbon to reactive intermediates was first reported in 1951 by E. C. Miller. She applied BP to mouse skin and found covalent binding of metabolites of this hydrocarbon to skin protein (224). This study was followed by many other investigations indicating that application of carcinogenic polycyclic hydrocarbons to mouse skin resulted in the covalent binding of metabolites to DNA, RNA, and protein. Dr. Charles Heidelberger, who was an earlier Clowes Lecturer, pioneered in much of this research and in research on the ability of polycyclic hydrocarbons to cause malignant transformations in cultured cells (129). The importance of DNA binding for the carcinogenicity of polycyclic hydrocarbons on mouse skin was suggested in 1964 by Brookes and Lawley (31, 32), who found a correlation between the carcinogenicity of several polycyclic hydrocarbons on mouse skin and the covalent binding of these hydrocarbons to mouse skin DNA. In 1957, the metabolism of BP to several hydroxylated products and to quinones by a NADPH-dependent monoxygenase in liver microsomes was described (79), and several years later the liver microsomal metabolism of BP to DNA-bound metabolites was demonstrated (110, 112). Although the possibility that epoxides (arene oxides) were reactive metabolites of polycyclic aromatic hydrocarbons was suggested by Boyland in 1950 (26), it was 1968 before the metabolism of a polycyclic hydrocarbon to an arene oxide was first reported (147, 148). In this study, Jerina and his associates demonstrated the metabolism of naphthalene to naphthalene 1,2-oxide by liver microsomes. Arene oxides are now recognized as the immediate metabolic precursors of trans-dihydrodiols (by hydration with epoxide hydrolase) and as intermediates in the formation of phenols by isomerization (95, 145).

During a remarkable 5-year interval from 1973 to 1978, research in the laboratories of Borgen, Brookes, Gelboin, Grover and Sims, Harvey, Slaga, and Weinstein and by our laboratories in Nutley and Bethesda led to the demonstration that one of the diastereomeric BP 7,8-diol-9,10-epoxides (see Chart 17 for structures) is an ultimate carcinogenic metabolite of BP. The metabolism of BP to BP 7,8-oxide, to BP 7,8-dihydrodiol, and to the diastereomeric BP 7,8-diol-9,10-epox-
Monooxygenase system from MC-treated rats metabolized BP to phenols and quinones, but little or no metabolism to dihydriodols occurred in the absence of epoxide hydrolase (Chart 18). The addition of purified epoxide hydrolase to the monooxygenase system resulted in the appearance of chromatographic peaks corresponding to the 4.5-, 7.8-, and 9.10-trans-dihydriodols of BP (see Chart 17 for structures), and there was a concomitant decrease in phenolic metabolites (Chart 18). BP 4.5-oxide that was formed during the incubation of BP with the purified cytochrome P-450-dependent monooxygenase system was sufficiently stable to appear as a peak on the chromatogram, and the accumulation of BP 4.5-oxide during the incubation was completely prevented by the addition of epoxide hydrolase to the monooxygenase system (Chart 18). The change in profile of metabolites of BP that was observed when the ratio of epoxide hydrolase to cytochrome P-450 was varied provided strong evidence for the cytochrome P-450-dependent metabolism of BP to its 4.5-, 7.8-, and 9.10-oxides which could either be metabolized to trans-dihydriodols by epoxide hydrolase or undergo isomerization to phenols. The use of purified cytochrome P-450 and epoxide hydrolase as tools for the elucidation of metabolic pathways and as an approach for demonstrating the metabolic activation and detoxification of polycyclic hydrocarbons is described in more detail elsewhere (131, 190, 236, 237, 290, 291, 293, 352, 354).

Mutagenic Activity of BP Metabolites

We chose to study the mutagenic activity of known and potential BP metabolites as an initial test of their biological activity because the mutagenic activity of chemicals provides a simple method for evaluating their ability to interact with genetic material in a way that results in an altered and heritable phenotype, and we thought that studies on the mutagenicity of BP derivatives would help in the determination of how BP is activated to its ultimately reactive forms. The inherent mutagenic activities of potential BP metabolites and other BP derivatives were examined in Salmonella typhimurium strains TA 98, TA 100, and TA 1538 and in Chinese hamster V-79 cells (76, 296, 338, 349, 359). Both the bacterial and mammalian cells selected for testing the mutagenic activity of BP deriva-

Chart 18. Profile of metabolites formed during the incubation of BP with a purified cytochrome P-450c-dependent monooxygenase system in the presence and absence of purified epoxide hydrolase (131). Diol Fractions 1, 2, and 3 correspond to BP 9.10-dihydriodol, BP 4.5-dihydriodol, and BP 7.8-dihydriodol, respectively. Phenol Fractions 1 and 2 each correspond to mixtures of phenolic metabolites. Quinone Fraction 1 has the chromatographic mobility of BP 1,6-, 3,6-, and 4,5-quinones, whereas Quinone Fraction 2 has the chromatographic mobility of BP 4.5-oxide and the 6,12- and 11,12-quinones of BP. Quinone Fraction 2 contains primarily BP 4,5-oxide.

Metabolism of BP to Arene Oxides and Dihydriodols by Purified Cytochrome P-450 and Epoxide Hydrolase

In 1974, we reported on the use of high-pressure liquid chromatography for the separation of BP metabolites (131), and similar studies were reported independently by Selkirk et al. (271). We also reported on the use of purified hepatic cytochrome P-450 and purified hepatic epoxide hydrolase for the elucidation of pathways of BP metabolism (131). In this study, a reconstituted purified cytochrome P-450c-dependent

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Note: The diagram includes the following key points:
- **BP 4,5-oxide**
- **BP 9,10-oxide**
- **BP 7,8-oxide**
- **BP 7,8-diol-9,10-epoxide-1**
- **BP 7,8-diol-9,10-epoxide-2**

The chart also illustrates the metabolic pathways involving epoxide hydrolase and cytochrome P-450. The metabolic reactions are depicted with arrows indicating the conversion of one compound to another.
tives lack detectable levels of polycyclic hydrocarbon-metabolizing enzymes and thus the parent hydrocarbon, BP, is inactive in these test systems.

In 1975, we reported that BP 4,5-oxide (K-region oxide) was a potent mutagen in several strains of S. typhimurium and in Chinese hamster V-79 cells, and we indicated that BP 7,8-oxide and BP 9,10-oxide were weakly mutagenic (349). In the following year, the diastereomeric BP 7,8-diol-9,10-epoxides were reported to have high mutagenic activity (135, 235, 339, 359). The results of our research with more than 30 derivatives of BP indicated that the diastereomeric BP 7,8-diol-9,10-epoxides and BP H₂-9,10-epoxide (see Table 16 for structure) were the most mutagenic compounds tested in S. typhimurium strains TA 98 and TA 100 and in Chinese hamster V-79 cells (76, 359). It was of considerable interest that the relative mutagenic activities of the various compounds depended on the test system used. In strain TA 1538 of S. typhimurium, BP 4,5-oxide had about the same mutagenic activity as did BP 7,8-diol-9,10-epoxide 1, but BP 4,5-oxide was a more potent mutagen in this strain of bacteria than was BP 7,8-diol-9,10-epoxide 2 (76, 86, 338, 339, 359). In all 3 bacterial strains, BP 7,8-diol-9,10-epoxide 1 was a more potent mutagen than was BP 7,8-diol-9,10-epoxide 2, while the reverse was true in Chinese hamster V-79 cells (359). We found that BP 7,8-diol-9,10-epoxides 1 and 2 were, respectively, about 40- and 100-fold more mutagenic toward Chinese hamster V-79 cells than was BP 4,5-oxide (76, 339, 359). The high mutagenic activities of the BP 7,8-diol-9,10-epoxides and 9,10-epoxy-7,8,9,10-tetrahydro-BP are undoubtedly an underestimate of intrinsic biological activity because these epoxides are unstable in aqueous solution and they undergo spontaneous solvolysis and hydronium ion-catalyzed solvolysis to biologically inactive tetraols (327, 359). During the course of our studies, we found that BP 4,5-oxide and BP H₂-9,10-epoxide were rapidly metabolized to nonmutagenic products by purified epoxide hydrolase (343, 354, 359), and we also found that the BP 7,8-diol-9,10-epoxides and other diol-epoxides were refractory to attack by epoxide hydrolase (343, 345, 359).

Since the chemically synthesized BP 4,5-oxide and the BP 7,8-diol-9,10-epoxides 1 and 2 used for the mutagenicity studies described above are racemates, the optically pure (+)- and (−)-enantiomers of each compound were prepared (54, 360), and we determined their mutagenic activities (54, 348). Substantial differences were observed for the mutagenic activities of the (+)- and (−)-enantiomers of the BP 7,8-diol-9,10-epoxides 1 and 2 in bacterial and mammalian cells (Table 8). In strains TA 98 and TA 100 of S. typhimurium, the (−)-enantiomer of BP 7,8-diol-9,10-epoxide 1 was 1.3 to 9.5 times more mutagenic than were the other 3 optically active stereoisomers. In Chinese hamster V-79 cells, however, the (+)-enantiomer of BP 7,8-diol-9,10-epoxide 2 was 6 to 18 times more mutagenic than were the other 3 isomers. This study suggests the presence of highly stereoselective receptors, detoxification mechanisms and/or transport systems. The data also demonstrate that the relative mutagenic activities of the four BP 7,8-diol-9,10-epoxides depend on the test system used. It will be shown later that the high mutagenic activity of the (+)-BP 7,8-diol-9,10-epoxide 2 in Chinese hamster V-79 cells accurately predicted the high tumorigenicity of this isomer in mice (Table 15; Chart 21).

Studies on the mutagenicity of the (+)-[4S,5R]- and (−)-[4R,5S]-enantiomers of BP 4,5-oxide revealed that the (−)-enantiomer was more mutagenic than the (+)-enantiomer in S. typhimurium strains TA 98 and TA 100 and in Chinese hamster V-79 cells (54). Whenmixtures of the enantiomers were studied in Chinese hamster V-79 cells, synergistic cytotoxic and mutagenic responses were observed. The greatest cytotoxic and mutagenic responses occurred with a 3:1 mixture of the (−)- and (+)-enantiomers of BP 4,5-oxide, respectively (Table 9). Further research is needed to determine the mechanism of this synergism.

### Specificity in the Metabolism of BP to Optically Active Enantiomers

Since optically active enantiomers have different biological activities, the stereochemical course of BP metabolism by the cytochrome P-450-dependent monoxygenase system and by epoxide hydrolase may play a critical role in the expression of the biological activity of BP. Accordingly, we investigated the biotransformation of BP to its optically active metabolites (151, 185, 282, 296, 297, 298), and similar studies on the stereochemical course of BP metabolism were done independently by Gelboin and his associates (135, 362–365). Liver microsomal enzymes from rats treated with MC metabolized BP to the (−)-enantiomers of BP 4,5-dihydrodiol, BP 7,8-dihydrodiol, and BP 9,10-dihydrodiol to a >10-fold excess relative to the (+)-enantiomers (295, 297, 298, 362, 365). The metabolism

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**Table 8**

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>S. typhimurium</th>
<th>Chinese hamster V-79 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain TA 98</td>
<td>Strain TA 100</td>
</tr>
<tr>
<td>(−)-BP 7,8-diol-9,10-epoxide 1</td>
<td>5200</td>
<td>9500</td>
</tr>
<tr>
<td>(−)-BP 7,8-diol-9,10-epoxide 2</td>
<td>1800</td>
<td>1000</td>
</tr>
<tr>
<td>(+)-BP 7,8-diol-9,10-epoxide 1</td>
<td>3900</td>
<td>5200</td>
</tr>
<tr>
<td>(+)-BP 7,8-diol-9,10-epoxide 2</td>
<td>2500</td>
<td>6000</td>
</tr>
</tbody>
</table>

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Mutagenic activity is expressed as revertants/nmol/plate for the S. typhimurium strains and as 8-azaguanine-resistant colonies/nmol/10⁵ surviving cells for the Chinese hamster V-79 cells.
of BP to the (+)-{S,S} and (-)-{R,R}-enantiomers of BP 7,8-dihydrodiol by liver microsomes from control rats and from rats treated with phenobarbital or MC is shown in Table 10. In all cases, the (-)-{R,R}-enantiomer was the major metabolite formed. Studies with other hydrocarbons revealed that BA, DBA, chrysene, and phenanthrene were also metabolized by rat liver microsomes predominantly to the {R,R}-enantiomer of several dihydrodiols (292). Interestingly, the degree of enantiomeric purity of the metabolites formed depended on the source of microsomes (kinds of cytochrome P-450s), the hydrocarbon substrate studied, and the positional dihydrodiol isomer measured (292).

Marked stereoselectivity occurred for the metabolism of the optically pure (+)-{S,S} and (-)-{R,R}-enantiomers of BP 7,8-dihydrodiol to the optical enantiomers of the BP 7,8-diol-9,10-epoxides 1 and 2 by rat liver microsomes (295, 298). Incubation of the (-)-{R,R}-enantiomer of BP 7,8-dihydrodiol (the metabolically predominant enantiomer) with liver microsomes from rats pretreated with MC resulted in the stereoselective metabolism of this dihydrodiol to the (+)-BP 7,8-diol-9,10-epoxide 2 to a 6-fold greater extent than to the (-)-BP 7,8-diol-9,10-epoxide 1 (Table 11). When the (+)-{S,S}-enantiomer of BP 7,8-dihydrodiol was used as the substrate, highly stereoselective metabolism to (+)-BP 7,8-diol-9,10-epoxide 1 was observed. Interestingly, liver microsomes from MC-treated rats metabolized both the (+)- and (-)-enantiomers of BP 7,8-dihydrodiol with greater stereoselectivity than did liver microsomes from control rats or from rats treated with phenobarbital (Table 11). These differences in stereoselectivity in the metabolism of the BP 7,8-dihydrodiols to specific diol-epoxide isomers probably reflect differences in the proportion of the various cytochrome P-450s present in liver microsomes from control rats and from rats treated with phenobarbital and MC. Gelboin and Coon have recently shown that several purified cytochrome P-450 isozymes from rabbit liver microsomes have

### Table 9

**Mutagenic and cytotoxic activity of mixtures of optically pure enantiomers of BP 4,5-oxide in Chinese hamster V-79 cells (54)**

<table>
<thead>
<tr>
<th>% of total dose</th>
<th>Mutation frequency (8-azaguanine-resistant colonies/10⁵ surviving cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-BP 4,5-oxide</td>
<td>(-)-BP 4,5-oxide</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>10</td>
<td>90</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 10

**Optical purity of BP 7,8-dihydrodiol formed during the incubation of BP with liver microsomes from control and induced rats (151, 295)**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>(+)-{S,S}</th>
<th>(-){R,R}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>MC</td>
<td>4</td>
<td>96</td>
</tr>
</tbody>
</table>

### Table 11

**Metabolism of optically pure enantiomers of BP 7,8-dihydrodiol to the BP 7,8-diol-9,10-epoxides by rat liver microsomes (295)**

Rats were given i.p. injections of sodium phenobarbital (75 mg/kg) or MC (25 mg/kg) once daily for 4 days, and the animals were killed the following day. Liver microsomes were prepared and incubated with BP and NADPH. Tetrals and triols derived from the BP 7,8-diol-9,10-epoxides 1 and 2 were quantified.

<table>
<thead>
<tr>
<th>Substrate Products formed</th>
<th>Control microsomes</th>
<th>Phenobarbital microsomes</th>
<th>MC microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-BP 7,8-dihydrodiol</td>
<td>53</td>
<td>51</td>
<td>83</td>
</tr>
<tr>
<td>(-)-Diol-epoxide 1</td>
<td>24</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>(+)-Diol-epoxide 2</td>
<td>53</td>
<td>54</td>
<td>90</td>
</tr>
<tr>
<td>(+)-BP 7,8-dihydrodiol</td>
<td>10</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>(-)-Diol-epoxide 2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
markedly different stereoselectivities in their metabolism of the 
(−){[R,R]} enantiomer of BP 7,8-dihydriodiol to BP 7,8-diol-
9,10-epoxides 1 and 2 (97, 98). Since only one of the 4
possible optically active BP 7,8-diol-9,10-epoxides has high
tumorigenic activity (Table 15; Chart 21), our results and those
of Gelboin and Coon indicate that factors influencing the rela-
tive amounts of nucleic acid adducts derived from BP 7,8-diol-
group of guanine in nucleic acids, was the major nucleic acid
tumorigenic activity (Table 15; Chart 21), our results and those
of Gelboin and Coon indicate that factors influencing the rela-
tive amounts of nucleic acid adducts derived from BP 7,8-diol-
9,10-epoxide 1 and 2, covalently bound to the 2-amino
group of guanine in nucleic acids, was the major nucleic acid
adduct observed when BP was metabolized by bovine bronchi-
al explants (142, 233, 319), by 10T½ mouse embryo fibro-
blasts (34), and by explants or cultured cells from human
bronchus, lung, and colon (11, 143, 272). In some of these
studies, evidence was also presented for the formation of small
amounts of nucleic acid adducts derived from BP 7,8-diol-
9,10-epoxide 1. Studies with cultured BHK 21/C13 cells (167,
259), cultured hamster embryo cells (14, 140), secondary
mouse embryo fibroblast cells (259), baby hamster kidney cells
(273), and human alveolar tumor cells (47) provided additional
evidence for the binding of both BP 7,8-diol-9,10-epoxides 1
and 2 to DNA after exposure of the cells to BP. It is of
considerable interest that the profile of DNA adducts depends
on the test system studied. In hamster embryo cell cultures
exposed to BP, there is a higher amount of the guanine adduct
of BP 7,8-diol-9,10-epoxide 1 and an adenine adduct than in
10T½ cells (14, 140).

In our studies with a target tissue susceptible to BP carci-
nogenesis, both (+)-BP 7,8-diol-9,10-epoxide 2 and (−)-BP
7,8-diol-9,10-epoxide 1 were found covalently bound to DNA,
RNA, and protein of skin after topical application of BP to the
backs of mice (172, 230). Interestingly, the relative amounts of
the 2 diol-epoxides bound depended on whether binding to
DNA, RNA, or protein was measured, but in all cases the amount of (+)-BP 7,8-diol-9,10-epoxide 2 that was bound was
greater than the amount of bound (−)-BP 7,8-diol-9,10-epox-
ide 1. Our studies and those using tissue culture systems
demonstrated that most of the binding of the BP diol-epoxides
to DNA and RNA occurred at the exocyclic 2-amino group of
guanine. The metabolism of BP to diol-epoxides and the struc-
tures of the guanine adducts of the BP diol-epoxides that were
isolated from mouse skin DNA and RNA after application of BP
are shown in Chart 19. More recently, evidence was presented
for the covalent binding of 9-hydroxy-BP 4,5-oxide to DNA (as
a minor adduct) after application of BP to mouse skin (312). It
is unlikely that this adduct has important role in the initiation
of skin tumors since BP 4,5-oxide and 9-hydroxy-BP have little
or no tumorigenic activity on mouse skin (155, 202).
mice that were treated (202, 331). When a lower dose of hydrocarbon was studied, BP 7,8-oxide was significantly less carcinogenic than was BP (Chart 20). Histological examination revealed that most of the tumors in the treated animals were squamous cell carcinomas.

BP 7,8-dihydrodiol was considerably more tumorigenic than was BP 7,8-oxide when tested as a complete carcinogen on mouse skin. The application of 0.15 μmol of BP or BP 7,8-dihydrodiol to mouse skin once every 2 weeks for 60 weeks resulted in a tumor incidence of 100%, whereas only 20% of the mice treated with an equimolar amount of BP 7,8-oxide had skin tumors (Chart 20) (203). BP 7,8-dihydrodiol was at least equipotent to BP as a complete carcinogen at several doses (201, 203), and other studies have shown that BP 7,8-dihydrodiol is a potent tumor initiator on mouse skin (55, 285). In contrast to these observations, BP 4,5-dihydrodiol and BP 9,10-dihydrodiol have little or no tumorigenic activity (280, 296). It was of considerable interest that BP H4-7,8-epoxide and BP H4-7,8-diol, compounds related to BP 7,8-oxide and BP 7,8-dihydrodiol but with the double bond saturated with hydrogen at the 9,10-position of the molecule, were inactive in eliciting tumors on mouse skin (201, 203) (Chart 20). The strong carcinogenic activities of BP 7,8-oxide and BP 7,8-dihydrodiol combined with the lack of carcinogenicity of these compounds when the 9,10-double bond was removed strongly suggested that the carcinogenicity of BP 7,8-oxide and BP 7,8-dihydrodiol on mouse skin was due to the metabolic conversion of these compounds to one or more of the BP 7,8-diol-9,10-epoxides.

Although BP 7,8-diol-9,10-epoxide 1 was inactive as a complete carcinogen on mouse skin, BP 7,8-diol-9,10-epoxide 2 did have very weak tumorigenic activity when compared to BP (201). In these studies, 100% of the mice had skin tumors when they were treated with 0.4 μmol of BP once every 2 weeks for 60 weeks, and 13% of the animals had skin tumors when they were treated with an equimolar dose of BP 7,8-diol-9,10-epoxide 2. Additional studies by Slaga et al. (281, 285) indicated that BP 7,8-diol-9,10-epoxide 2 had considerably weaker activity than did BP as a tumor initiator on mouse skin. Although BP 7,8-diol-9,10-epoxide 2 was only weakly carcinogenic on mouse skin and BP 7,8-diol-9,10-epoxide 1 was inactive, we found that both compounds were highly active in causing hypertrophy and hyperplasia when they were applied topically (30).

High Tumorigenicity of Racemic BP 7,8-dihydrodiol and BP 7,8-diol-9,10-epoxide 2 in Newborn Mice. Since the newborn mouse is highly susceptible to the effects of many chemical carcinogens (96, 304, 305), we tested potential proximate and ultimate carcinogenic metabolites of BP and other polycyclic aromatic hydrocarbons in a newborn mouse tumor model. In these studies, the hydrocarbons were injected i.p. into Swiss-Webster mice on the first, 8th, and 15th days of life, and the animals were killed several months later. The predominant tumors observed were pulmonary tumors (mostly adenomas with some adenocarcinomas), malignant lymphomas, and liver tumors. The kinds of tumors observed depended on the hydrocarbon studied, the dose administered, and the duration of the study. The newborn mouse tumor model has been a particularly useful test system for identifying proximate and ultimate carcinogenic metabolites of polycyclic aromatic hydrocarbons. We found that BP 7,8-oxide was moderately active in
tetraols derived from BP 7,8-diol-9,10-epoxide 2 had little or no tumorigenic activity, whereas BP 7,8-diol-9,10-epoxide 2 and BP 7,8-dihydrodiol were highly tumorigenic at the 28-nmol dose tested. After correcting for the small number of lung tumors in control mice, the data in Experiment 2 indicate that BP 7,8-dihydrodiol and BP 7,8-diol-9,10-epoxide 2 were, respectively, about 15- and 40-fold more active than BP in causing pulmonary tumors (157). The studies described in Table 12 indicate that BP 7,8-dihydrodiol is a proximate carcinogenic metabolite and that BP 7,8-diol-9,10-epoxide 2 is an ultimate carcinogenic metabolite of BP in the newborn mouse. These data provided the first demonstration of proximate and ultimate carcinogenic metabolites for the polycyclic aromatic hydrocarbon class of carcinogens. Although the reason(s) for the high tumorigenic activity of racemic BP 7,8-diol-9,10-epoxide 2 in newborn mice and the much weaker activity of this compound on mouse skin is not known, our results illustrate the importance of studies with several animal models during the evaluation of the carcinogenicity of chemicals.

**High Tumorigenic Activity of 2-Hydroxy-BP.** A comparison of the carcinogenic activity of BP with that of its 12 possible isomeric phenols revealed that only 2- and 11-hydroxy-BP were complete carcinogens on mouse skin (155, 331). 2-Hydroxy-BP was almost equipotent to BP in causing skin tumors; 11-hydroxy-BP was weakly active; and 1-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, and 12-hydroxy-BP were inactive. 2-Hydroxy-BP was about 4-fold more active than an equimolar dose of BP in causing pulmonary tumors in the newborn mouse tumor model, and 6-hydroxy-BP was inactive (53). Additional studies with the 12 isomeric BP phenols indicated that only 2-hydroxy-BP had strong tumor-initiating activity in an initiation-promotion model on mouse skin (278). 2-Hydroxy-BP is the only known example of a phenolic polycyclic aromatic hydrocarbon with strong carcinogenic activity, and additional studies are needed to determine how 2-hydroxy-BP exerts its carcinogenic effect and to determine whether or not 2-hydroxy-BP is a metabolite of BP.

**Tumorigenicity of Optically Active Metabolites of BP.** An examination of the tumorigenic activity of the (+)-[7S,8S]- and (-)-[7S,8S]-enantiomers of BP 7,8-oxide revealed that the (+)-enantiomer, which is the major enantiomer formed metabolically from BP, is several-fold more active than the (-)-enantiomer as an initiator of tumors on mouse skin and in causing pulmonary tumors in newborn mice (185) (Table 13). Interestingly, in both tumor models, we found that racemic BP 7,8-oxide produced more tumors than either optical enantiomer alone (Table 13). Without any enantiomeric interaction, the tumorigenic activity of racemic BP 7,8-oxide should have been intermediate to the activity of the (+)- and (-)-enantiomers. This is not the case, and our data demonstrate a synergistic interaction for the carcinogenicity of the optical enantiomers. Further studies are needed to determine the mechanism of this synergistic interaction.

An examination of the tumorigenic activities of the (+)-[7S,8S]- and (-)-[7R,8R]-enantiomers of BP 7,8-dihydrodiol (158, 199) revealed that (+)-BP 7,8-dihydrodiol, which is the major enantiomer formed metabolically from BP, was several-fold more active than (+)-BP 7,8-dihydrodiol as a tumor initiator on mouse skin (Chart 21) and in causing pulmonary tumors and malignant lymphomas in newborn mice (Table 14). When the 4 optically active BP 7,8-diol-9,10-epoxides were tested for tumorigenicity on mouse skin and in newborn mice (41, 279), significant tumorigenicity in both tumor models was found only for (+)-BP 7,8-diol-9,10-epoxide 2 (Table 15; Chart 21), which is the major diol-epoxide isomer formed metabolically from BP. Although (+)-BP 7,8-diol-9,10-epoxide 2 was
less active than BP as a tumor initiator on mouse skin (Chart 21), this diol-epoxide isomer was 50 to 100 times more active than BP in causing pulmonary tumors when injected into newborn mice (Tables 12 and 15). The injection of only 7 or 14 nmol of ( + )-BP 7,8-diol-9,10-epoxide 2 into newborn mice during the first 15 days of life caused a high incidence of pulmonary tumors, but little or no tumorigenicity was observed after an equimolar dose of BP or the other 3 diol-epoxide isomers (Table 15). The data in Table 14 indicating that a high dose of ( + )-BP [7S,8S]dihydridiol has tumorigenic activity suggest that ( + )-BP-7,8-diol-9,10-epoxide 1, the major diol-epoxide metabolite of ( + )-BP [7S,8S]dihydridiol (Table 11), may have tumorigenic activity if a sufficiently high dose could be given. It is of interest that the highly tumorigenic ( + )-BP 7,8-diol-9,10-epoxide 2 was the most mutagenic of the 4 diol-epoxides of BP that were tested in Chinese hamster V-79 cells whereas the relative mutagenic activities of the 4 diol-epoxides in S. typhimurium strain TA 98 or TA 100 did not predict the tumorigenic activities of these compounds (Table 8).

The stereochemical course for the metabolism of BP to its proximate and ultimate carcinogenic metabolites and the absolute stereochemistry of each metabolite are summarized in Chart 22. Major pathways for the metabolism of BP to its diol-epoxides by liver microsomal enzymes are given by the heavy arrows, and minor pathways are given by the thin arrows. The metabolism and carcinogenicity studies described here have demonstrated that BP is metabolized by cytochrome P-450 to the proximate carcinogen ( + )-BP 7,8-oxide [7R,8S] to a much greater extent than to the (—)-enantiomer of this arene oxide. ( + )-BP 7,8-oxide is then stereospecifically metabolized by epoxide hydrolase to the proximate carcinogen ( + )-BP 7,8-dihydridiol [7R,8R] which in turn is stereoselectively metabolized by cytochrome P-450 to the ultimate carcinogen ( + )-BP 7,8-diol-9,10-epoxide 2 [7R,8S,9S,10R]. It is of interest to note that liver enzymes metabolize BP predominantly to the only BP 7,8-diol-9,10-epoxide isomer with high tumorigenic activity.

**Bay Region Theory of Polycyclic Aromatic Hydrocarbon Carcinogenesis**

Several observations made during the course of our research with Dr. Jerina led to a new theory (the bay region theory) that has successfully predicted proximate and ultimate carcinogenic metabolites of more than a dozen polycyclic aromatic hydrocarbons. The prototype of a bay region in a polycyclic hydrocarbon is the sterically hindered region between C-4 and C-5 of phenanthrene. Examples of bay regions and bay-region diol-epoxides of polycyclic aromatic hydrocarbons are given in Chart 23. The bay region theory suggested that bay-region diol-epoxides of polycyclic aromatic hydrocarbons are prime candidates for ultimate carcinogens and that the amount of metabolism of the parent hydrocarbon to bay-region diol-epoxides should be important for the expression of the carcinogenicity of the parent hydrocarbon. The findings that led to the bay region theory, which was first proposed by Jerina and Daly (146), included (a) high chemical reactivity and mutagenic
activity of bay-region epoxides of BP relative to non-bay-region epoxides (149,359), (b) observations from a survey of the literature indicating that substituents which could block the formation of bay-region diol-epoxides on the angular benzo ring of BA derivatives inhibited the carcinogenicity of these compounds (146), and (c) the results of Dewar perturbational molecular orbital calculations predicting a greater ease of carbocation formation at the benzylic carbon atom for bay-region epoxides and diol-epoxides than for their corresponding non-bay-region epoxides and diol-epoxides (149).

Examination of the properties of the BP 7,8-diol-9,10-epoxides that account for their high biological activity revealed that 9,10-epoxy-7,8,9,10-tetrahydro-BP (BP H4-9,10-epoxide; bay-region epoxide) is an extremely potent mutagen whereas 7,8-epoxy-7,8,9,10-tetrahydro-BP (BP H4-7,8-epoxide; non-bay-region epoxide) is a considerably less potent mutagen (359) (Table 16). In addition, the bay-region BP 7,8-diol-9,10-epoxides 1 and 2 are much more mutagenic than are BP 7,10-diol-8,9-epoxide, the BP 9,10-diol-7,8-epoxides 1 and 2, or the BP 7,8- and 9,10-oxides (75, 296, 349, 359) (Table 16). These results indicate that a saturated benzo ring and a bay-region 9,10-epoxide group in BP are required for high mutagenic activity, but hydroxyl groups on the benzo ring are not required.

The mutagenicities of bay-region and non-bay-region tetrahydroepoxides of BP, BA, chrysene, and phenanthrene were compared to the $\Delta E_{\text{bene}}/\beta$ values which are calculated indices for the ease of carbocation formation at the benzylic carbon atom. It can be seen in Table 17 that, for each of the 4 pairs of tetrahydroepoxides that we tested, the bay-region tetrahydro-
Table 16
Mutagenicity of benzo-ring epoxides and arene oxides of BP (75, 296, 349, 359)

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. typhimurium</th>
<th>Chinese hamster</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain TA 98</td>
<td>Strain TA 100</td>
</tr>
<tr>
<td>BP 7,8-diol-9,10-epoxide 1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>BP 7,8-diol-9,10-epoxide 2</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>BP 7,10-diol-8,9-epoxide</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>BP 9,10-diol-7,8-epoxide 1</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>BP 9,10-diol-7,8-epoxide 2</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>BP H-9,10-epoxide</td>
<td>95</td>
<td>90</td>
</tr>
<tr>
<td>BP H-7,8-epoxide</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>BP 9,10-oxide</td>
<td>1</td>
<td>0.6</td>
</tr>
<tr>
<td>BP 7,8-oxide</td>
<td>1</td>
<td>0.6</td>
</tr>
</tbody>
</table>

epoxide has higher mutagenicity and a higher $\Delta E_{\text{homo}}/\beta$ value than does the corresponding non-bay-region tetrahydroepoxide (343, 346, 352, 359). Recent studies have also indicated similar results for the heterocyclic hydrocarbon benz(acycl)acridine where the bay-region tetrahydro-1,2-epoxide is more mutagenic than the non-bay-region tetrahydro-3,4-epoxide (342).

Proximate and Ultimate Carcinogenic Metabolites of BA

Studies with BA were initiated as an early test of the bay region theory. Even though BA is only a weak carcinogen, perturbational molecular orbital calculations indicated an unusual ease of carbocation formation at the bay-region 1-position for the BA 3,4-diol-1,2-epoxides when compared with other BA diol-epoxides (149). Because of these considerations, Dr. Jerina and our laboratory predicted that BA 3,4-dihydrodiol would be a prime candidate for a proximate carcinogenic metabolite of BA and that the diastereomeric BA 3,4-diol-1,2-epoxides 1 and 2 (epoxide oxygen cis and trans to the benzylic hydroxyl group, respectively) would be prime candidates for ultimate carcinogenic metabolites of BA. The expected pathway for the metabolism of BA to its bay-region diol-epoxides is shown in Chart 24. An examination of the metabolism of BA by rat liver microsomes or by a combination of a purified cytochrome P-450-dependent reconstituted monoxygenase system and purified epoxide hydrolase revealed that BA was indeed metabolized to BA 3,4-dihydrodiol, but the amount of this dihydrodiol formed relative to the 5,6- and 8,9-dihydrodiols was very low (293, 302). The predominant enantiomer of BA 3,4-dihydrodiol that was formed metabolically had $\left(-\right)[3R,4R]$ absolute stereochemistry (294), and studies on the further metabolism of the $\left(-\right)[3R,4R]$-enantiomer of BA 3,4-dihydrodiol by rat liver microsomes revealed the stereoselective for-
Table 17

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative mutagenic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. typhimurium</td>
</tr>
<tr>
<td></td>
<td>Strain TA98</td>
</tr>
<tr>
<td>BP H4-7,8-epoxide</td>
<td>0.488</td>
</tr>
<tr>
<td>BP H4-9,10-epoxide</td>
<td>0.794</td>
</tr>
<tr>
<td>BA H4-3,4-epoxide</td>
<td>0.628</td>
</tr>
<tr>
<td>BA H4-1,2-epoxide</td>
<td>0.766</td>
</tr>
<tr>
<td>Chrysene H4-1,2-epoxide</td>
<td>0.526</td>
</tr>
<tr>
<td>Chrysene H4-3,4-epoxide</td>
<td>0.640</td>
</tr>
<tr>
<td>Phenanthrene H4-1,2-epoxide</td>
<td>0.540</td>
</tr>
<tr>
<td>Phenanthrene H4-3,4-epoxide</td>
<td>0.658</td>
</tr>
</tbody>
</table>

In accord with observations on the mutagenic activity of the BA derivatives, we found that BA 3,4-dihydrodiol was at least 10 times more tumorigenic than BA or the 1,2-, 5,6-, 8,9- and 10,11-dihydrodiols of BA when tested as a tumor initiator on mouse skin (Table 19) (351). In addition, the injection of 2800 nmol of BA 3,4-dihydrodiol into newborn mice during the first 15 days of life caused at least 30 times more pulmonary tumors than did an equimolar dose of BA or the other metabolically possible BA dihydrodiols, and BA 3,4-dihydrodiol also had exceptionally high activity in causing malignant lymphomas (Table 19) (335). Examination of the tumorigenicity of the bay-region BA 3,4-diol-1,2-epoxides as initiators of skin tumors in mice (191) revealed that BA 3,4-diol-1,2-epoxide 1 and BA 3,4-diol-1,2-epoxide 2 were many times more tumorigenic than BA and that BA 3,4-diol-1,2-epoxide 2 was somewhat more tumorigenic than BA 3,4-dihydrodiol (Table 20). Injection of 280 nmol of these compounds into newborn mice during their first 15 days of life indicated that BA 3,4-diol-1,2-epoxide 2
Chart 24. Metabolism of BA to a BA 3,4-diol-1,2-epoxide. Although BA 3,4-diol-1,2-epoxide 2 has been identified as a metabolite of BA 3,4-dihydrodiol, the metabolic formation of BA 3,4-diol-1,2-epoxide 1 has not yet been demonstrated.

**Chart 25. Metabolism of BA and its dihydrodiols to mutagens by a purified cytochrome P-450c-dependent monooxygenase system from rat liver microsomes (353).**

was at least 30-fold more active than BA 3,4-dihydrodiol and at least 60-fold more active than BA in causing lung tumors whereas BA 3,4-diol-1,2-epoxide 1 was less than one-tenth as active as its diastereomer (Table 20) (330). High tumor-initiating activity of BA 3,4-dihydrodiol and BA 3,4-diol-1,2-epoxide 2 on mouse skin was also reported by Slaga and his associates, and these investigators found that BA 1,2-diol-3,4-epoxide 2, BA 10,11-diol-8,9-epoxide 2, and the BA 8,9-diol-10,11-epoxides 1 and 2 had very low or no tumorigenic activity (284). It is of interest that, although evidence has been presented that BA 8,9-diol-10,11-epoxide 2 and BA 3,4-diol-1,2-epoxide 2 are both covalently bound to DNA after application of BA to mouse skin (90), only the latter diol-epoxide is highly tumorigenic. The results of the tumorigenicity studies described here, coupled with the observation that small amounts of BA 3,4-dihydrodiol and BA 3,4-diol-1,2-epoxide 2 are formed metabolically from BA by liver microsomes (292–294), indicate that BA 3,4-dihydrodiol is a proximate carcinogenic metabolite of BA and that

**Table 18**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chinese hamster</td>
</tr>
<tr>
<td></td>
<td>S. typhimurium</td>
</tr>
<tr>
<td></td>
<td>strain TA 100</td>
</tr>
<tr>
<td></td>
<td>histidine revert-</td>
</tr>
<tr>
<td></td>
<td>ants/nmol/plate</td>
</tr>
<tr>
<td></td>
<td>(hisidine revert-</td>
</tr>
<tr>
<td></td>
<td>ants/nmol/plate)</td>
</tr>
<tr>
<td></td>
<td>(hisidine revert-</td>
</tr>
<tr>
<td></td>
<td>ants/nmol/plate)</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 1</td>
<td>1650</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 2</td>
<td>850</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 2</td>
<td>16</td>
</tr>
<tr>
<td>BA 8,9-diol-10,11-epoxide 1</td>
<td>50</td>
</tr>
<tr>
<td>BA 8,9-diol-10,11-epoxide 2</td>
<td>50</td>
</tr>
<tr>
<td>BA 10,11-diol-8,9-epoxide 1</td>
<td>6</td>
</tr>
<tr>
<td>BA 10,11-diol-8,9-epoxide 2</td>
<td>50</td>
</tr>
</tbody>
</table>

**Table 24. Metabolism of BA to a BA 3,4-diol-1,2-epoxide.** Although BA 3,4-diol-1,2-epoxide 2 has been identified as a metabolite of BA 3,4-dihydrodiol, the metabolic formation of BA 3,4-diol-1,2-epoxide 1 has not yet been demonstrated.

**Table 25. Metabolism of BA and its dihydrodiols to mutagens by a purified cytochrome P-450c-dependent monooxygenase system from rat liver microsomes (353).**

**Table 18. Mutagenicity of BA diol-epoxides (76, 343)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chinese hamster</td>
</tr>
<tr>
<td></td>
<td>S. typhimurium</td>
</tr>
<tr>
<td></td>
<td>strain TA 100</td>
</tr>
<tr>
<td></td>
<td>histidine revert-</td>
</tr>
<tr>
<td></td>
<td>ants/nmol/plate</td>
</tr>
<tr>
<td></td>
<td>(hisidine revert-</td>
</tr>
<tr>
<td></td>
<td>ants/nmol/plate)</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 1</td>
<td>1650</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 2</td>
<td>850</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 2</td>
<td>16</td>
</tr>
<tr>
<td>BA 8,9-diol-10,11-epoxide 1</td>
<td>50</td>
</tr>
<tr>
<td>BA 8,9-diol-10,11-epoxide 2</td>
<td>50</td>
</tr>
<tr>
<td>BA 10,11-diol-8,9-epoxide 1</td>
<td>6</td>
</tr>
<tr>
<td>BA 10,11-diol-8,9-epoxide 2</td>
<td>50</td>
</tr>
</tbody>
</table>

was at least 30-fold more active than BA 3,4-dihydrodiol and at least 60-fold more active than BA in causing lung tumors whereas BA 3,4-diol-1,2-epoxide 1 was less than one-tenth as active as its diastereomer (Table 20) (330). High tumor-initiating activity of BA 3,4-dihydrodiol and BA 3,4-diol-1,2-epoxide 2 on mouse skin was also reported by Slaga and his associates, and these investigators found that BA 1,2-diol-3,4-epoxide 2, BA 10,11-diol-8,9-epoxide 2, and the BA 8,9-diol-10,11-epoxides 1 and 2 had very low or no tumorigenic activity (284). It is of interest that, although evidence has been presented that BA 8,9-diol-10,11-epoxide 2 and BA 3,4-diol-1,2-epoxide 2 are both covalently bound to DNA after application of BA to mouse skin (90), only the latter diol-epoxide is highly tumorigenic. The results of the tumorigenicity studies described here, coupled with the observation that small amounts of BA 3,4-dihydrodiol and BA 3,4-diol-1,2-epoxide 2 are formed metabolically from BA by liver microsomes (292–294), indicate that BA 3,4-dihydrodiol is a proximate carcinogenic metabolite of BA and that
Table 19

Tumorigenicity of BA dihydrodiols on mouse skin and in newborn mice (335, 351)

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Skin study*</th>
<th>Newborn study$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of mice</td>
<td>% of mice</td>
</tr>
<tr>
<td></td>
<td>with skin</td>
<td>tumors/mouse</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23</td>
<td>0.30</td>
</tr>
<tr>
<td>BA 1,2-dihydrodiol</td>
<td>80</td>
<td>4.80</td>
</tr>
<tr>
<td>BA 5,6-dihydrodiol</td>
<td>20</td>
<td>0.20</td>
</tr>
<tr>
<td>BA 8,9-dihydrodiol</td>
<td>17</td>
<td>0.21</td>
</tr>
<tr>
<td>BA 10,11-dihydrodiol</td>
<td>14</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* Mice received a single topical application of 2 μmol of compound followed 18 days later by twice-weekly applications of 16 nmol of 12-O-tetradecanoylphorbol-13-acetate for 20 weeks.

$^b$ Mice were given i.p. injections of 0.4, 0.8, and 1.6 μmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 22 weeks of age.

BA 3,4-diol-1,2-epoxide 2 is an ultimate carcinogenic metabolite of BA. It is of interest that BA 3,4-diol-1,2-epoxide 1 is also more tumorigenic than BA, but this diol-epoxide has not yet been identified as a metabolite of BA. Metabolism studies suggest that the low carcinogenic activity of BA is caused at least in part from a low rate of conversion of BA to BA 3,4-dihydrodiol relative to the formation of other dihydrodiols of BA. Only 1 to 4% of the total metabolites of BA formed by rat liver microsomes can be accounted for as its 3,4-dihydrodiol with a bay-region double bond (293). Studies on the tumorigenicity of the (+)- and (-)-enantiomers of BA 3,4-dihydrodiol revealed that the (-)-[3R,4R]-enantiomer, which is the metabolically predominant enantiomer (292), is much more tumorigenic than the (+)-[3S,4S]-enantiomer on mouse skin and in

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A. H. Conney

Table 20

Tumorigenicity of bay-region diol-epoxides of BA on mouse skin and in the newborn mouse (191, 330)

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Skin study*</th>
<th>Newborn study*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of mice with skin tumors</td>
<td>Skin tumors/mouse</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>0.03</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 2</td>
<td>43</td>
<td>1.30</td>
</tr>
<tr>
<td>BA 3,4-diol-1,2-epoxide 1</td>
<td>70</td>
<td>1.90</td>
</tr>
</tbody>
</table>

* Mice received a single topical application of 0.4 μmol of compound followed 7 days later by twice-weekly applications of 16 nmol of 12-O-tetradecanoylphorbol-13-acetate for 20 weeks.

*Mice were given i.p. injections of 40, 80, and 160 nmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 26 weeks of age.

newborn mice (191, 330). Studies are now in progress to determine the tumorigenicity of the 4 optically active isomers derived from the diastereomeric BA 3,4-diol-1,2-epoxides.

Proximate and Ultimate Carcinogenic Metabolites of Chrysene

Chrysene is a weak carcinogen with 2 equivalent bay regions (Table 21). The bay region theory predicts that a chrysene 1,2-diol-3,4-epoxide is an ultimate carcinogenic metabolite of chrysene and that chrysene 1,2-dihydrodiol is a proximate carcinogenic metabolite. Chrysene is metabolized predominantly to its 1,2- and 3,4-dihydrodiols (213, 237). Liver microsomes from MC-treated rats produced the (−)[1R,2R]-enantiomer of chrysene 1,2-dihydrodiol (bay-region diol-epoxide precursor) with 80% enantiomeric purity (237). However, when liver microsomes from phenobarbital-treated rats were used, the chrysene 1,2-dihydrodiol that was formed was only 10% enantioomerically pure (237). Metabolism of the metabolically formed (−)[1R,2R]-enantiomer of chrysene 1,2-dihydrodiol by liver microsomes from MC-treated rats resulted in the predominant formation of a bay-region 1,2-diol-3,4-epoxide in which the oxirane ring and the hydroxyl group are trans (Isomer 2) (237).

Metabolic activation of chrysene 1,2-dihydrodiol by liver microsomes or a purified cytochrome P-450-dependent monoxygenase system resulted in the formation of metabolites that were at least 20 times more mutagenic to strain TA 100 of S. typhimurium than were the metabolites formed from chrysene or chrysene 3,4- or 5,6-dihydrodiol (355). When the double bond at the bay-region 3,4-position of chrysene 1,2-dihydrodiol was saturated, the resulting tetrahydrodiol (chrysene H₄-1,2-diol) could not be metabolized to mutagens, suggesting that a bay-region chrysene 1,2-diol-3,4-epoxide was the active mutagenic metabolite formed from chrysene 1,2-dihydrodiol (355). Additional studies revealed that the 1,2-diol-3,4-epoxides of chrysene which have a bay-region epoxide either cis (Isomer 1) or trans (Isomer 2) to the benzylic hydroxyl group have high mutagenic activity in bacterial and mammalian cells (346).

Studies on the tumorigenicity of chrysene and its metabolically possible trans-dihydrodiols on mouse skin revealed that chrysene 1,2-dihydrodiol has higher tumor-initiating activity than chrysene (200, 283) whereas the 3,4- and 5,6-dihydrodiols of chrysene have no appreciable tumorigenic activity (Table 21) (200). trans-1,2-Dihydroxy-1,2,3,4-tetrahydrochrysene, which has a saturated bay-region 3,4-bond, has less than 25% of the tumorigenic activity of chrysene 1,2-dihydrodiol (200). Tumorigenicity studies in the newborn mouse indicated that chrysene 1,2-dihydrodiol and chrysene 1,2-diol-3,4-epoxide 2 are at least 10- and 50-fold more active, respectively, than chrysene in causing pulmonary tumors (Table 21), and chrysene 1,2-diol-3,4-epoxide 1 has little or no tumorigenic activity in this test system (38). Recent studies on the tumorigenic activities of optically active metabolites of chrysene have shown that the metabolically formed (−)-chrysene 1,2-dihydrodiol and (+)-chrysene 1,2-diol-3,4-epoxide 2 are more tumorigenic on mouse skin and in the newborn mouse than their mirror image enantiomers (52). The results of these studies
## Table 21
Tumorigenicity of chrysene and its derivatives on mouse skin and in newborn mice (38, 200, 346)

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Skin study*</th>
<th>Newborn study*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of mice with skin tumors</td>
<td>% of mice with lung tumors</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>Chrysene</td>
<td>52</td>
<td>1.45</td>
</tr>
<tr>
<td>Chrysene 1,2-dihydrodiol</td>
<td>79</td>
<td>3.38</td>
</tr>
<tr>
<td>Chrysene 3,4-dihydrodiol</td>
<td>10</td>
<td>0.10</td>
</tr>
<tr>
<td>Chrysene 5,6-dihydrodiol</td>
<td>7</td>
<td>0.07</td>
</tr>
<tr>
<td>Chrysene H4-3,4-epoxide</td>
<td>77</td>
<td>1.50</td>
</tr>
<tr>
<td>Chrysene 1,2-diol-3,4-epoxide 1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chrysene 1,2-diol-3,4-epoxide 2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Mice received a single topical application of 4 µmol of compound except that animals treated with chrysene H4-3,4-epoxide received 2 µmol of compound in a separate experiment. Seven days later, the mice were treated with 16 nmol of 12-O-tetradecanoylphorbol-13-acetate twice weekly for 25 weeks. Mice were given i.p. injections of 0.2, 0.4, and 0.8 µmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 38 to 42 weeks of age.

Indicate that the (-)-(1R,2R)-enantiomer of chrysene 1,2-dihydrodiol and the (+)-enantiomer of chrysene 1,2-diol-3,4-epoxide 2 are proximate and ultimate carcinogenic metabolites of chrysene.

### Proximate and Ultimate Carcinogenic Metabolites of DBA

DBA is a strong carcinogen with 2 equivalent bay regions (Table 22). In contrast to the weak carcinogen BA which is metabolized only slightly to its 3,4-dihydrodiol, DBA is extensively metabolized to its DBA 3,4-dihydrodiol with a double bond in the bay-region 1,2-position, and significant amounts of the 1,2- and 5,6-dihydrodiols are also formed (236, 275). The bay region theory predicts that DBA 3,4-dihydrodiol is a proximate carcinogenic metabolite of DBA and that one or more of the DBA 3,4-diol-1,2-epoxides are ultimate carcinogenic metabolites of DBA. Metabolic activation of DBA and the 1,2-, 3,4- and 5,6-dihydrodiols of DBA by liver microsomes or by a highly purified cytochrome P-450-dependent monoxygenase system revealed that DBA 3,4-dihydrodiol was activated to products that were more mutagenic to strain TA 100 of S. typhimurium than were the metabolites of DBA or the metabolites of the 1,2- or 5,6-dihydrodiols of DBA (357). Saturation of the bay-region double bond at the 1,2-position of DBA 3,4-dihydrodiol produced a tetrahydrodiol (DBA H2-3,4-diol) which was poorly activated by microsomes or the purified monoxygenase system (357). The high mutagenic activity of DBA 3,4-dihydrodiol after metabolism and the importance of the double bond at the bay-region 1,2-position of the dihydrodiol strongly suggest that a bay-region 3,4-diol-1,2-epoxide is an ultimate mutagenic metabolite of DBA.

Examination of the tumorigenicity of the 1,2-, 3,4-, and 5,6-dihydrodiols of DBA revealed that only the 3,4-dihydrodiol of DBA had significant tumorigenic activity on mouse skin and in newborn mice. The mice received a single topical application of 4 µmol of compound except that animals treated with chrysene H4-3,4-epoxide received 2 µmol of compound in a separate experiment. Seven days later, the mice were treated with 16 nmol of 12-O-tetradecanoylphorbol-13-acetate twice weekly for 25 weeks. Mice were given i.p. injections of 0.2, 0.4, and 0.8 µmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 38 to 42 weeks of age.
Table 22

Table: Tumorigenicity of DBA dihydrodiols on mouse skin and in newborn mice (40)

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Dose (nmol)</th>
<th>% of mice with skin tumors</th>
<th>Skin tumors/mouse</th>
<th>Dose (nmol)</th>
<th>% of mice with lung tumors</th>
<th>Lung tumors/mouse</th>
<th>% of male mice with liver tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>160</td>
<td>0.10</td>
<td>10</td>
<td>160</td>
<td>0.14</td>
<td>0</td>
</tr>
<tr>
<td>DBA 1,2-dihydrodiol</td>
<td>10</td>
<td>14</td>
<td>0.14</td>
<td>70</td>
<td>18</td>
<td>0.18</td>
<td>0</td>
</tr>
<tr>
<td>DBA 3,4-dihydrodiol</td>
<td>160</td>
<td>57</td>
<td>1.52</td>
<td>420</td>
<td>18</td>
<td>0.21</td>
<td>0</td>
</tr>
<tr>
<td>DBA 5,6-dihydrodiol</td>
<td>160</td>
<td>3</td>
<td>0.03</td>
<td>420</td>
<td>7</td>
<td>0.07</td>
<td>0</td>
</tr>
<tr>
<td>DBA H₄-3,4-diol</td>
<td>160</td>
<td>29</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mice received a single topical application of compound followed 7 days later by twice-weekly applications of 16 nmol of 12-O-tetradecanoylphorbol-13-acetate for 25 weeks.

** Mice were given i.p. injections of 1/7, 2/7, and 4/7 of the total dose of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 25 to 29 weeks of age.

the newborn mouse (40) (Table 22). Although DBA 3,4-dihydrodiol was somewhat less active than DBA in causing skin and pulmonary tumors, this dihydrodiol was uniquely active in causing liver tumors (Table 22). DBA H₄-3,4-diol, with the saturated bay-region double bond, was considerably less tumorigenic than DBA 3,4-dihydrodiol on mouse skin. The high tumor-initiating activity of DBA 3,4-dihydrodiol on mouse skin was also observed by Slaga and his associates, but these investigators indicated that DBA 3,4-diol-1,2-epoxide 2 had little or no tumorigenicity in this animal model (283). Although DBA 3,4-diol-1,2-epoxide 2 was reported to be inactive as a tumor initiator on mouse skin, the overall results of studies with DBA and its derivatives suggest that DBA 3,4-dihydrodiol and one or more of the bay-region DBA 3,4-diol-1,2-epoxides are proximate and ultimate mutagenic and carcinogenic metabolites of DBA.

Proximate and Ultimate Carcinogenic Metabolites of B(c)Ph

B(c)Ph is a weak carcinogen with a bay region between 2 angular benzo rings (Table 23). The bay region theory predicts that a B(c)Ph 3,4-diol-1,2-epoxide, if formed metabolically, is a prime candidate as an ultimate carcinogenic metabolite of the parent hydrocarbon. We found that B(c)Ph 3,4-dihydrodiol, the precursor of the bay-region diol-epoxides of B(c)Ph, was metabolized to mutagens by liver microsomes or a purified cytochrome P-450-dependent monooxygenase system several-fold more effectively than was B(c)Ph or its 1,2- and 5,6-dihydrodiols (344). When the bay-region double bond at the 1,2-position of B(c)Ph 3,4-dihydrodiol was saturated with hydrogen, the resulting tetrahydrodiol could not be metabolically activated to mutagens. These data suggest that one or more of the bay-region B(c)Ph 3,4-dihydrodiol was saturated with hydrogen, the resulting tetrahydrodiol could not be metabolically activated to mutagens. These data suggest that one or more of the bay-region B(c)Ph 3,4-diol-1,2-epoxides is an ultimate mutagenic metabolite of B(c)Ph. In accord with these observations, we found that B(c)Ph 3,4-diol-1,2-epoxide 1, in which the bay-region epoxide oxygen and the benzylic hydroxyl group are cis and B(c)Ph 3,4-diol-1,2-epoxide 2, in which the bay-region epoxide oxygen and the benzylic hydroxyl group are trans, have strong mutagenic activity in bacterial and mammalian cells (344). Studies on the tumor-initiating activity of B(c)Ph and its
metabolically possible dihydrodiols and bay-region diol-epoxides on mouse skin revealed that B(c)Ph 3,4-dihydrodiol was more tumorogenic than B(c)Ph whereas the 1,2- and the 5,6-dihydrodiols of B(c)Ph were inactive (197) (Table 23). When the analog of B(c)Ph 3,4-dihydrodiol with a saturated 1,2-bond in the bay region [B(c)Ph H₄,3,4-diol] was tested, it was inactive (Table 23), suggesting that B(c)Ph 3,4-dihydrodiol required metabolism at the bay-region 1,2-position for tumorigenicity (197). The data in Table 23 also demonstrate that the diastereomeric bay-region 3,4-diol-1,2-epoxides of B(c)Ph are at least 50- to 100-fold more active than is B(c)Ph as tumor initiators on mouse skin (150, 197). An interesting aspect of this study is the extraordinarily high tumorigenic activity on mouse skin displayed by both diastereomers. The B(c)Ph 3,4-diol-1,2-epoxides are much more tumorogenic than BA 3,4-diol-1,2-epoxide 2 (Table 20), which had previously been the most active diol-epoxide tested on mouse skin (191, 284). It is of considerable interest that the highly tumorigenic B(c)Ph bay-region diol-epoxides have relatively low chemical reactivity as measured by either their spontaneous or their acid-catalyzed solvolysis in water (150). Further studies are needed to provide an explanation for the greater than expected tumorigenicty of the bay-region diol-epoxides of B(c)Ph.

**Proximate and Ultimate Carcinogenic Metabolites of MC**

MC is a strong carcinogen, and the structure of this compound is shown in Table 24. Studies on the metabolism of MC by purified cytochrome P-450 in the presence and absence of purified epoxide hydrolase failed to provide evidence for the formation of significant amounts of the 9,10-dihydrodiol with a bay-region 7,8-double bond (290, 291). Since 2 major metabolites of MC are 1- and 2-hydroxy-MC, we studied the metabolism of these alcohols to determine if they were converted to the 9,10-dihydrodiols (290, 291). As the 1-hydroxy-MC was metabolized by rat liver microsomes and by a purified cytochrome P-450-dependent monooxygenase system in the presence of epoxide hydrolase to a pair of diastereomeric trans-9,10-di-hydrodiols in which the 10-hydroxy group is either cis or trans to the 1-hydroxyl group (290, 291). Upon metabolic activation by monoxygenases, we found that one of these 9,10-dihydrodiols was among the most mutagenic MC derivatives tested toward both bacterial and mammalian cells (347).

Studies on the tumorigenic activity of the two 9,10-dihydrodiols of 1-hydroxy-MC revealed that one of these compounds had about the same activity as did MC and was considerably more active than was 1-hydroxy-MC in initiating tumors on mouse skin (186). The metabolically predominant isomer of 1-hydroxy-MC 9,10-dihydrodiol was more active than MC or 1-hydroxy-MC in causing pulmonary and liver tumors in the newborn mouse (186) (Table 24). In these studies, 1-hydroxy-MC 9,10-dihydrodiol was about 10-fold more active than MC in causing liver tumors in male mice, and 1-hydroxy-MC was inactive (Table 24). These results indicate that 1-hydroxy-MC 9,10-dihydrodiol with an adjacent bay-region double bond is a proximate carcinogenic metabolite of MC. In our studies (186), as well as those by others (46, 274), 2-hydroxy-MC and 2-keto-MC have strong tumorigenic activity. It would be of interest to determine the tumorigenic activity of the 9,10-dihydrodiols of 2-hydroxy-MC and 2-keto-MC.

Studies by Malaveille et al. (214) have shown that MC 9,10-dihydrodiol is metabolically activated to a potent mutagen, and studies by Chouroulinek et al. (57) have shown that the 9,10-dihydrodiol of MC was more active as a tumor initiator than were the 4,5-, 7,8-, or 11,12-dihydrodiols of MC. In this later investigation, MC 9,10-dihydrodiol was somewhat less tumorigenic than MC. Additional evidence that MC is activated via the formation of bay-region diol-epoxides was obtained from studies on the nature of the DNA-bound adducts of MC (91, 168, 169, 311). These studies indicated that several of the covalently bound adducts were saturated in positions 7, 8, 9, and 10 of the hydrocarbon which is consistent with the formation and covalent binding of 9,10-diol-7,8-epoxides of MC. In further studies, it was concluded that one of the DNA-bound adducts is a 1- or 2-hydroxy derivative of MC saturated in positions 9, 10, 11, and 12 of the hydrocarbon which is consistent with the formation of bay-region diol-epoxides obtained from studies on the nature of the DNA-bound adducts of MC (91, 168, 169, 311). These studies indicated that several of the covalently bound adducts were saturated in positions 7, 8, 9, and 10 of the hydrocarbon which is consistent with the formation and covalent binding of 9,10-diol-7,8-epoxides of MC. In further studies, it was concluded that one of the DNA-bound adducts is a 1- or 2-hydroxy derivative of MC saturated in positions 7, 8, 9, and 10 of the molecule (169). The available data indicate that one or more of the MC 9,10-diol-7,8-epoxides and/or their 1- and 2-hydroxy derivatives are ultimate carcinogenic metabolites of MC.

---

**Table 23**

Tumor-initiating activity of B(c)Ph and its derivatives on mouse skin (150, 197)

Mice received a single topical application of compound followed 7 days later by twice weekly applications of 16 nmol of 12-O-tetradecanoylphorbol-13-acetate for 20 weeks.

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Dose (μmol)</th>
<th>% of mice with tumors</th>
<th>Tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4</td>
<td>0.025</td>
<td>7.13</td>
</tr>
<tr>
<td>Bay region</td>
<td>0.4</td>
<td>0.03</td>
<td>1.63</td>
</tr>
<tr>
<td>B(c)Ph</td>
<td>0.4</td>
<td>7.13</td>
<td>1.63</td>
</tr>
<tr>
<td>B(c)Ph 1,2-dihydrodiol</td>
<td>0.4</td>
<td>28.0</td>
<td>0.17</td>
</tr>
<tr>
<td>B(c)Ph 3,4-dihydrodiol</td>
<td>0.4</td>
<td>3.03</td>
<td>1.07</td>
</tr>
<tr>
<td>B(c)Ph 5,6-dihydrodiol</td>
<td>0.4</td>
<td>3.03</td>
<td>1.07</td>
</tr>
<tr>
<td>B(c)Ph H₄,3,4-diol</td>
<td>0.4</td>
<td>3.03</td>
<td>1.07</td>
</tr>
<tr>
<td>B(c)Ph 3,4-diol-1,2-epoxide 1</td>
<td>0.025</td>
<td>53.0</td>
<td>6.47</td>
</tr>
<tr>
<td>B(c)Ph 3,4-diol-1,2-epoxide 2</td>
<td>0.025</td>
<td>57.0</td>
<td>7.13</td>
</tr>
</tbody>
</table>
A. H. Conney

Table 24
Tumorigenicity of MC and its derivatives in newborn mice (186)
Mice were given i.p. injections of 7, 14, and 28 nmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 36 to 39 weeks of age.

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Pulmonary tumors</th>
<th>Hepatic tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of mice with tumors</td>
<td>Tumors/mouse</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>0.11</td>
</tr>
<tr>
<td>MC</td>
<td>82</td>
<td>1.77</td>
</tr>
<tr>
<td>1-Keto-MC</td>
<td>8</td>
<td>0.08</td>
</tr>
<tr>
<td>1-Hydroxy-MC</td>
<td>26</td>
<td>0.29</td>
</tr>
<tr>
<td>1-Hydroxy-MC 9,10-dihydrodiol</td>
<td>94</td>
<td>4.47</td>
</tr>
<tr>
<td>2-Keto-MC</td>
<td>67</td>
<td>1.03</td>
</tr>
<tr>
<td>2-Hydroxy-MC</td>
<td>14</td>
<td>0.20</td>
</tr>
<tr>
<td>MC 11,12-oxide</td>
<td>13</td>
<td>0.13</td>
</tr>
<tr>
<td>MC 11,12-dihydrodiol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Proximate and Ultimate Carcinogenic Metabolites of DB(a,h)P and DB(a,i)P

DB(a,h)P and DB(a,i)P are hexacyclic aromatic hydrocarbons that have high tumorigenic activity in mice, and both of these hydrocarbons have 2 bay regions. The structures of these compounds and some of their derivatives are given in Table 25. Quantum mechanical calculations designed to predict the ease of triol carbocation formation from diol-epoxides indicated that the bay-region diol-epoxides of DB(a,h)P and DB(a,i)P should have very high chemical reactivity (149), and studies of their solvolysis have confirmed these predictions (345). Recent studies by Hecht et al. (125) have resulted in the identification of DB(a,i)P 3,4-dihydrodiol as a metabolite of DB(a,i)P that is formed by rat liver.

Mutagenicity studies with several benzo-ring derivatives of
Table 25

Tumorigenicity of DB(a,h)P, DB(a,i)P, and their derivatives on mouse skin and in the newborn mouse (51)

<table>
<thead>
<tr>
<th>Compound administered</th>
<th>Skin study</th>
<th></th>
<th>Newborn study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of mice</td>
<td>Pulmonary tumors</td>
<td>% of male mice</td>
</tr>
<tr>
<td></td>
<td>with tumors</td>
<td>Tumors/mouse</td>
<td>with tumors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tumors/male mouse</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>0.10</td>
<td>27</td>
</tr>
<tr>
<td>DB(a,h)P</td>
<td>72</td>
<td>5.52</td>
<td>97</td>
</tr>
<tr>
<td>DB(a,h)P 1,2-dihydrodiol</td>
<td>80</td>
<td>4.40</td>
<td>98</td>
</tr>
<tr>
<td>DB(a,i)P H4-1,2-diol</td>
<td>67</td>
<td>3.60</td>
<td>82</td>
</tr>
<tr>
<td>DB(a,h)P 1,2-diol-3,4-epoxide 2</td>
<td>79</td>
<td>5.25</td>
<td>97</td>
</tr>
<tr>
<td>DB(a,i)P</td>
<td>81</td>
<td>5.00</td>
<td>100</td>
</tr>
<tr>
<td>DB(a,i)P 3,4-dihydrodiol</td>
<td>61</td>
<td>1.64</td>
<td>95</td>
</tr>
<tr>
<td>DB(a,i)P H3-3,4-diol</td>
<td>67</td>
<td>2.03</td>
<td>86</td>
</tr>
<tr>
<td>DB(a,i)P 3,4-diol-1,2-epoxide 2</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
</tbody>
</table>

**a** Mice received a single topical application of 600 nmol of compound followed 7 days later by twice-weekly applications of 16 nmol of 12-O-tetradecanoylphorbol-13-acetate for 16 weeks.

**b** Mice were given i.p. injections of 12.5, 25, and 50 nmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 49 to 54 weeks of age.

DB(a,h)P and DB(a,i)P showed that DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol, the expected dihydrodiol precursors of the bay-region diol-epoxides, were metabolized to mutagenic products by a cytochrome P-450-dependent mono-oxygenase system to a greater extent than their respective parent hydrocarbons, and the tetrahydrodiol derivatives with no adjacent bay-region double bond were only poorly activated to mutagenic metabolites (345). Additional studies have shown that synthetic bay-region diol-epoxides of DB(a,h)P and DB(a,i)P, in which the benzylic hydroxyl group and the oxirane ring are trans (Isomer 2), have high inherent mutagenic activity in bacterial and mammalian cells (345).
The results of tumorigenicity studies with DB(a,h)P, DB(a,i)P, and several of their derivatives are summarized in Table 25 (51). DB(a,h)P 1,2-dihydrodiol and DB(a,i)P 3,4-dihydrodiol, the immediate metabolic precursors of the bay-region diol-epoxides, had tumor-initiating activity on mouse skin about equal to their respective parent hydrocarbons (Table 25). In newborn mice, these dihydrodiols were the most tumorigenic dibenzopyrene derivatives tested, and they had 3- to 8-fold greater activity than their parent hydrocarbons in producing pulmonary tumors. The bay-region diol-epoxides DB(a,h)P 1,2-diol-3,4-epoxide 2 and DB(a,i)P 3,4-diol-1,2 epoxide 2 had less tumorigenic activity than did their dihydrodiol precursors, both on mouse skin and in the newborn mouse. These bay-region diol-epoxides were also less active than their parent hydrocarbons on mouse skin, and they had tumorigenic activity equal to or somewhat less than their parent hydrocarbons in the newborn mouse. DB(a,h)P H₂-1,2-diol and DB(a,i)P H₂-3,4-diol differ from their corresponding dihydrodiols in that the adjacent bay-region double bonds are saturated with hydrogen. As expected, these tetrahydrodiols had only 10 to 12% of the tumorigenic activity of the dihydrodiols for the lung of newborn mice, but both tetrahydrodiols had appreciable tumor-initiating activity on mouse skin, and DB(a,i)P H₂-3,4-diol had significant tumorigenic activity for the liver in newborn mice. Although these results suggest that non-bay-region metabolites may be responsible for some of the tumorigenic effects of DB(a,h)P and DB(a,i)P, these hydrocarbons are symmetrical molecules with 2 equivalent bay regions, and the unexpectedly high tumorigenic activity of the tetrahydrodiols on mouse skin and for the liver may be due to the formation of a diol-epoxide in the other bay region of the molecule. Evidence in support of this concept comes from the complete lack of activity of 2,10-difluoro-DB(a,i)P as a tumor initiator on mouse skin and in causing pulmonary and liver tumors in the newborn mouse (Table 25). Low tumorigenicity of 2,10-difluoro-DB(a,i)P was also observed by Hecht et al. (125) and by Boger et al. (21). The difluoro derivative, which has fluorine in both benzo rings, undergoes little or no metabolism to dihydrodiols that are precursors of bay-region diol-epoxides (125). The available data suggest that bay-region diol-epoxides are ultimate mutagenic and carcinogenic metabolites of DB(a,h)P and DB(a,i)P.

**Mutagenic and Carcinogenic Activities of Bay-Region Epoxides and Diol-Epoxides of Phenanthrene and B(e)P**

Phenanthrene, the simplest polycyclic aromatic hydrocarbon with a bay region, is generally considered to have little or no carcinogenic activity. The bay-region 3,4-epoxy-1,2,3,4-tetrahydropyrene (phenanthrene H₄-3,4-epoxide) is a moderately potent mutagen in strains TA 98 and TA 100 of S. typhimurium and in Chinese hamster V-79 cells, but the diastereomeric bay-region 1,2-diol-3,4-epoxides 1 and 2 of phenanthrene are considerably less mutagenic (346). The structures of these compounds are shown in Table 26. Examination of the tumorigenic activity of several phenanthrene derivatives revealed that the metabolically possible 1,2-, 3,4-, and 9,10-dihydrodiols of phenanthrene have little or no activity as tumor initiators on mouse skin (346). Interestingly, phenanthrene H₄-3,4-epoxide (bay-region tetrahydro-epoxide) is weakly active as a tumor initiator on mouse skin (346) and in causing pulmonary and hepatic tumors when injected into newborn mice (Table 26) (38). Studies on the tumorigenicity of the diastereomeric phenanthrene-1,2-diol-3,4-epoxides in the newborn mouse tumor model revealed that both of these bay-region diol-epoxides are inactive in causing pulmonary tumors (38). The greater tumorigenic activity of phenanthrene H₄-3,4-epoxide relative to the bay-region diol-epoxides is in accord with the relative mutagenic activities of these compounds in bacteria and in mammalian cells (346). B(e)P, a symmetrical hydrocarbon with 2 identical bay regions, has little or no tumorigenic activity in the mouse. The structures of B(e)P and some of its possible proximate and ultimate carcinogenic metabolites are shown in Table 27. Recent studies have shown that very little B(e)P is metabolized to B(e)P 9,10-dihydrodiol (expected precursor of the bay-region diol-epoxides) by cultured hamster embryo cells (211) and rat liver microsomes (212). In addition, little or no B(e)P 9,10-dihydrodiol is converted to the bay-region diol-epoxides by rat liver microsomes (356). Studies on the tumorigenicity of B(e)P and B(e)P 9,10-dihydrodiol revealed that these compounds have no significant tumor-initiating activity on mouse skin and fail to induce lung tumors in newborn mice (39). However, B(e)P 9,10-dihydrodiol did produce a significant incidence of hepatic tumors when injected into newborn mice, suggesting that some bay-region diol-epoxide may have been formed (Table 27). Studies on the intrinsic mutagenicity of the bay-region diol-epoxides of B(e)P indicate that these com-
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Table 27
Tumorigenicity of B(e)P and its derivatives in newborn mice (39, 50)

In Experiment 1, the mice were given i.p. injections of 0.4, 0.8, and 1.6 μmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 62 to 66 weeks of age. In Experiment 2, the mice were given i.p. injections of 0.1, 0.2, and 0.4 μmol of compound on the first, 8th, and 15th days of life, respectively. The animals were killed at 39 to 43 weeks of age.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Compound administered</th>
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<th>Hepatic tumors</th>
</tr>
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<tr>
<td>1</td>
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<td>48</td>
<td>11</td>
</tr>
<tr>
<td></td>
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<td>41</td>
<td>21</td>
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<tr>
<td></td>
<td>B(e)P 4,5-dihydrodiol</td>
<td>51</td>
<td>17</td>
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<tr>
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<td>B(e)P 9,10-dihydrodiol</td>
<td>35</td>
<td>61</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B(e)P</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>B(e)P 9,10-diol-11,12-epoxide 1</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>B(e)P 9,10-diol-11,12-epoxide 2</td>
<td>25</td>
<td>21</td>
</tr>
</tbody>
</table>

Other Hydrocarbons That Fit the Predictions of the Bay-Region Theory

Our collaborative research with Dr. Jerina's laboratory that is described above has demonstrated proximate and ultimate carcinogens and mutagens for 10 different polycyclic aromatic hydrocarbons. For each hydrocarbon, the proximate and ultimate carcinogens and mutagens that we identified were in agreement with the bay region theory. Further support for the bay region theory has come from studies with additional hydrocarbons in several other laboratories.

Examination of the fluorescent properties of DNA-bound adducts derived from 7-methylbenz(e)anthracene (303, 310) and 7,12-dimethylbenz(a)anthracene (20, 100, 139, 231, 311)
provided evidence for the covalent binding of the angular benzo
ing of these hydrocarbons to DNA. Studies with the 3,4-
dihydrodiols of 7-methylbenz(a)anthracene and 7,12-dimethyl-
benz(a)anthracene (expected precursors of the bay-region
diol-epoxides) indicated that both of these dihydrodiols un-
dergo metabolic activation to mutagens and cause malignant
transformations to a greater extent than do their parent hy-
drocarbons (215–218). Tumorigenicity studies on mouse skin
revealed that the 3,4-dihydrodiols of 7-methylbenz(a)-
anthracene, 8-methylbenz(a)anthracene, and 7,12-dimethyl-
benz(a)anthracene were more active tumor initiators than
their parent hydrocarbons (56, 262, 332, 333). In addition, the
3,4-dihydrodiols of 7,12-dimethylbenz(a)anthracene and 7-hy-
droxymethyl-12-methylbenz(a)anthracene caused more lung
and liver tumors than did 7,12-dimethylbenz(a)anthracene
when injected into newborn mice (334). Hydrogenation of the
bay-region 1,2-double bond of 7,12-dimethylbenz(a)anthra-
cene resulted in 1,2,3,4-tetrahydro-7,12-dimethylbenz(a)an-
thrace which had only one-tenth of the tumor-initiating activity
of the parent hydrocarbon on mouse skin (99). Although
1,2,3,4-tetrahydro-7,12-dimethylbenz(a)anthracene was a
much less effective tumor initiator than the parent hydrocarbon,
the tetrahydro derivative still possessed strong tumor-initiating
activity, possibly by metabolic activation at non-bay-region
positions.

Hecht and his associates have provided evidence for the metabolic activation of 5-methylchrysene to bay-region 1,2-
diol-3,4-epoxides (124, 126–128, 223). These studies indic-
ated that the major DNA adduct observed after application of
5-methylchrysene to mouse skin was derived from a bay-region
1,2-diol-3,4-epoxide. In addition, 5-methylchrysene-1,2-dihy-
drodiol (precursor of bay-region diol-epoxides) was metabo-
lically activated to mutagens to a greater extent than was 5-
methylchrysene, and 5-methylchrysene-1,2-dihydrodiol was
more tumorigenic on mouse skin than was 5-methylchrysene
or its other metabolically possible dihydrodiols. Substitution
with fluorne at positions 1, 3, or 12 of 5-methylchrysene
inhibited the formation of the 1,2-dihydrodiol and inhibited
carcinogenicity.

Evidence has been obtained for the metabolism of the strong
carcinogen 15,16-dihydro-11-methylcyclopenta(a)phenan-
thren-17-one to a trans-3,4-diol-1,2-epoxide (bay-region diol-
epoxide) that is bound covalently to DNA (88). In addition, the
trans-3,4-dihydrodiol of 15,16-dihydro-11-methylcyclopenta-
a(a)phenanthren-17-one (bay-region diol-epoxide precursor)
is metabolized to mutagens to a greater extent than is the
parent compound (88), and this dihydrodiol is also more active
than the parent compound as a tumor initiator on mouse skin
(87).

Studies with the carcinogetic 1,4-dimethylphenanthrene
have provided evidence that its 7,8-dihydrodiol (precursor of the
bay-region diol-epoxide) is a major metabolite and that this
dihydrodiol is a proximate mutagenic metabolite of 1,4-dimeth-
lyphenanthrene (183).

Recent studies with several benzofluoranthene derivatives
are in accord with the bay region theory (9, 182). Benzo(b)fluoranthene 9,10-dihydrodiol, which is the immediate
precursor of a bay-region diol-epoxide, was as active as
benzo(b)fluoranthene in initiating tumors on mouse skin.
Benzo(j)fluoranthene 9,10-dihydrodiol, which is the expected
metabolic precursor of a diol-epoxide in a 4-sided pseudo-bay
region, had appreciable tumor-initiating activity but was less
active than benzo(j)fluoranthene. Benzo(k)fluoranthene 8,9-
dihydrodiol cannot form a bay-region diol-epoxide, and this
compound was inactive as a tumor initiator on mouse skin.

Summary and Concluding Remarks

Multiple cytochrome P-450s with characteristic but overlap-
ping substrate specificities metabolize polycyclic aromatic hy-
drocarbons and many other classes of precarcinogens to their
proximate and ultimate carcinogenic metabolites, and the same
enzymes also participate in the detoxification of these com-
pounds. The selective regulation of the cytochrome P-450
enzymes by genetic and environmental factors is an important
area of cancer research since changes in the activity or amount
of specific forms of cytochrome P-450 will alter both the extent
of metabolism of a substrate and the relative yields of its
various metabolites. I have given several examples of specificity
in the induction and activation of cytochrome P-450 isozyms,
and I have indicated how this can alter the action of drugs,
chemical carcinogens, and other environmental pollutants as
well as the metabolism and action of endogenous substrates
such as steroid hormones, lipid-soluble vitamins, and prosta-
glandins. The ability of microsomal enzyme inducers to inhibit
the action of some chemical carcinogens by enhancing their
detoxification was pointed out as well as certain problems with
this approach to cancer prevention. The toxicological signifi-
cance of the induction of the cytochrome P-450 system in
mammals and in other living organisms depends on the cyto-
chrome P-450 isozyms that are induced and on the identity of
the chemicals in the environment of the organism. Marked
interindividual differences occur for the metabolism of chemi-
cals in human beings, and these differences are caused by
complex interactions between genes and environment. The
ingestion of drugs, the smoking of cigarettes, exposure to
halogenated hydrocarbon insecticides or other environmental
pollutants, and changes in the diet can all influence the metab-
olism of chemicals in humans. It is likely that these same factors
influence the metabolism and action of environmental carcin-
o gens in humans.

Bay-region diol-epoxides have been identified as important
ultimate carcinogenic metabolites for the polycyclic aromatic
hydrocarbon class of carcinogens. The marked selectivity of
cytochrome P-450 and epoxide hydrolase for the metabolism
of polycyclic aromatic hydrocarbons to specific isomers of diol-
epoxides and the critical importance of stereochemical factors
for the expression of biological activity have been particularly
interesting aspects of our research. Studies on the biotrans-
formation of BP and chrysene by liver microsomes from MC-
treated rats or by purified cytochrome P-450c and purified
epoxide hydrolase have demonstrated the stereoselective me-
tabolism of these hydrocarbons to [R,R]-enantiomers of trans-
dihydrodiols that are precursors of bay-region diol-epoxides.
In each case, the predominant [R,R]-dihydrodiol that is formed
metabolically is considerably more carcinogenic than its mirror
image enantiomer. These [R,R]-dihydrodiols undergo stereo-
selective metabolism predominantly to highly tumorigenic bay-
region diol-epoxides with the epoxide oxygen trans to the
benzylic hydroxyl group. Examination of the carcinogeticity
of the 4 optically active bay-region diol-epoxides of BP and the
4 optically active bay-region diol-epoxides of chrysene demon-
strated that for each series of compounds only the metabolically predominant (+)-diol-epoxide 2 isomer with the epoxide (-)-enantiomers of BP 4,5-oxide have a synergistic mutagenic effect in Chinese hamster V-79 cells (Table 9).

The identification of bay-region diol-epoxides as ultimate carcinogenic metabolites of some polycyclic aromatic hydrocarbons should help in the search for naturally occurring and synthetic chemicals that inactivate these ultimate carcinogens. Recent studies demonstrated that riboflavin 5'-phosphate functions as an acid catalyst (350, 358). Studies on the mechanisms of these effects indicate that riboflavin 5'-phosphate functions as an acid catalyst and enhances the conversion of BP 7,8-diol-9,10-epoxide 2 to covalent adducts with the diol-epoxide (268). The identification and use of naturally occurring and synthetic chemicals that antagonize ultimate carcinogens are potentially important approaches to cancer prevention.

Acknowledgments
I dedicate this manuscript to my wife, Diana, and to my past and present colleagues. I am very fortunate in having a wonderful wife and a wonderful group of associates with a deep commitment to biomedical research.

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References
A. H. Conney


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Induction of Microsomal Enzymes by Foreign Chemicals and Carcinogenesis by Polycyclic Aromatic Hydrocarbons: G. H. A. Clowes Memorial Lecture

Allan H. Conney


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