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# The Highly Tumorigenic 3,4-Dihydrodiol Is a Principal Metabolite Formed from Dibenzo[a,h]anthracene by Liver Enzymes

M. NORDQVIST*, D. R. THAKKER*, W. LEVIN†, H. YAGI*, D. E. RYAN†, P. E. THOMAS†, A. H. CONNEY†, AND D. M. JERINA*

*Laboratory of Bioorganic Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, MD 20014 and †Department of Biochemistry and Drug Metabolism, Hoffmann-La Roche Inc., Nutley, NJ 07110

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SUMMARY

NORDQVIST, M., D. R. THAKKER, W. LEVIN, H. YAGI, D. E. RYAN, P. E. THOMAS, A. H. CONNEY, AND D. M. JERINA. The highly tumorigenic 3,4-dihydrodiol is a principal metabolite formed from dibenzo[a,h]anthracene by liver enzymes. *Mol. Pharmacol.*, **16**: 643–655 (1979).

Pretreatment of rats with 3-methylcholanthrene or Aroclor-1254 caused a 2- to 3-fold enhancement in the rate of metabolism of [$^{14}$C]-dibenzo[a,h]anthracene (DBA) by liver microsomes and altered the relative amounts of some of the metabolites formed compared to metabolism by microsomes from untreated rats. These changes consisted mainly of a decrease in phenols and an increase in unidentified minor metabolites. Pretreatment of rats with phenobarbital caused less marked changes in the profile of metabolites formed from DBA, and the rate of metabolism of DBA was significantly reduced per nmol of cytochrome P-450 compared to microsomes from untreated rats. Under all incubation conditions, DBA 3,4-dihydrodiol was the major dihydrodiol metabolite which accounted for 24–28% of the total metabolites. Less than half this amount of the 1,2-dihydrodiol was formed, and only trace amounts of the K-region 5,6-dihydrodiol could be detected. Two major phenolic metabolite fractions were detected. After 3-methylcholanthrene- or Aroclor-pretreatment, metabolites more polar than the dihydrodiols were also formed. In the absence of epoxide hydrase, DBA was converted primarily into phenols by a purified and reconstituted cytochrome P-448 monooxygenase system. Addition of highly purified epoxide hydrase resulted in the formation of DBA 1,2- and 3,4-dihydrodiols as well as more polar metabolites with a concomitant decrease in the amounts of phenolic metabolites. The predominance of DBA 3,4-dihydrodiol as a metabolite on incubation of DBA either with rat liver microsomes or with the highly purified, reconstituted monooxygenase system in the presence of epoxide hydrase is of considerable importance since this is the most potent carcinogenic metabolite of DBA presently known. The 3,4-dihydrodiol formed from DBA by liver microsomes from 3-methylcholanthrene-treated rats was found to be 60% optically pure. Evidence is presented which indicates that bay region diol epoxides are formed from racemic DBA 3,4-dihydrodiol.

## INTRODUCTION

Dibenzo[a,h]anthracene was the first chemically pure substance reported to induce neoplasia (1). It is now generally accepted that DBA and other polycyclic aromatic hydrocarbons require metabolic transformation to highly reactive metabolites (ultimate carcinogens) before they co-



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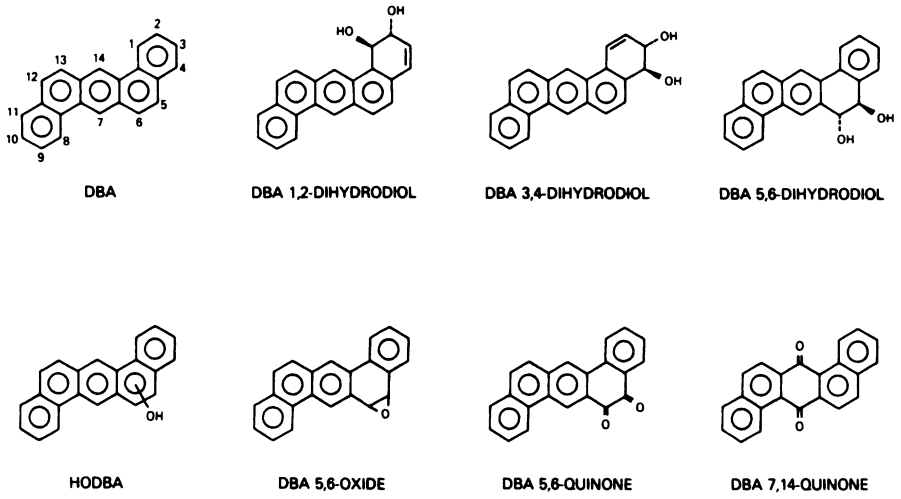


FIG. 1. *Structures of DBA and potential metabolites of DBA used as chromatographic standards for HPLC; stereochemistry is relative*

valently bind to cellular macromolecules and cause cancer (2–5). In an attempt to explain and predict the carcinogenicity of polycyclic aromatic hydrocarbons, a hypothesis termed the "bay-region" theory has been formulated (6–9). The theory predicts that the most tumorigenic dihydrodiol (proximate carcinogen) of a given hydrocarbon will be on an angular benzo-ring and will have a bay-region double bond. Metabolic epoxidation of this double bond produces a bay-region diol epoxide, the proposed ultimate carcinogenic metabolite.

Recent studies on the metabolic activation of DBA[1] and its dihydrodiols to bacterial mutagens (10) established that DBA 3,4-dihydrodiol, which has a bay-region 1,2-double bond (Fig. 1), caused a higher mutagenic response in *S. typhimurium* than did DBA or its 1,2- and 5,6-dihydrodiols. Since DBA $H_4$-3,4-diol, a compound in which the bay-region 1,2-double bond of the 3,4-dihydrodiol is saturated, was poorly activated, a bay-region 3,4-diol-1,2-epoxide is the probable metabolite responsible for the high mutagenicity on activation of the 3,4-dihydrodiol. For reasons which are presently unclear, the K-region 5,6-dihydrodiol can also be metabolically activated to products which are highly mutagenic but only when high enzyme or high substrate concentrations are used in the mutagenesis assay.

Metabolism of DBA has been studied previously in whole animals (11–13), liver homogenates (14, 15) and microsomal preparations (15–17) by thin-layer chromatography and uv analysis. Although evidence has been presented for all three possible dihydrodiols as metabolites of DBA (15), only the 5,6-dihydrodiol was available as a synthetic standard. Arene oxides are thought to be the precursors of all these dihydrodiols (3, 16–18).

The principal aim of the present investigation has been to establish the extent to which DBA is converted to the 3,4-dihydrodiol by liver microsomes from control or induced rats as well as by a highly purified and reconstituted monooxygenase system. It is important to determine the amount of this dihydrodiol formed since the bay-region theory predicts that the 3,4-diol-1,2-epoxide(s) of the weak carcinogen benzo[a]anthracene (BA) should be more reac-

[1] Abbreviations used: DBA, dibenzo[a,h]anthracene; DBA 1,2-dihydrodiol, *trans*-1,2-dihydroxy-1,2-dihydrodibenzo[a,h]anthracene; DBA 3,4- and 5,6-dihydrodiols, other *trans* dihydrodiols of DBA; DBA $H_4$-3,4-diol, *trans*-3,4-dihydroxy-1,2,3,4-tetrahydrodibenzo[a,h]anthracene; 4-hydroxy-$H_4$-DBA, 4-hydroxy-1,2,3,4-tetrahydrodibenzo[a,h]anthracene; HODBA, phenols of DBA; DBA 5,6-oxide, dibenzo[a,h]anthracene 5,6-oxide; DBA 5,6-quinone, dibenzo[a,h]anthracene 5,6-quinone; DBA 7,14-quinone, dibenzo[a,h]anthracene 7,14-quinone; BA, benzo[a]anthracene; BP, benzo[a]pyrene; HPLC, high pressure liquid chromatography.

tive than those of DBA (6–9). Yet, DBA is known to be a much more potent carcinogen than BA (19). Since the theory makes no attempt to take into account the extent of metabolism at a particular position of a hydrocarbon, a possible explanation for the inversion in predicted carcinogenic activity is that BA is very poorly converted into a bay region 3,4-diol-1,2-epoxide(s) while this is a major route of metabolism for DBA. Recent studies have shown that the 3,4-dihydrodiol of BA is indeed a very minor metabolite of this hydrocarbon (20), while earlier results suggested that the 3,4-dihydrodiol of DBA might be a major metabolite (15).

## MATERIALS AND METHODS

*Chemicals.* [$^{14}$C]-DBA (Fig. 1) was obtained from Amersham-Searle. When analyzed by HPLC, the material had <0.6% radiochemical impurity in the metabolite region and was free of uv-detectable impurities. The compound was used at a specific activity of 11.6 mCi/mmol. DBA 1,2- and 3,4-dihydrodiols (Fig. 1) were prepared as previously described (21). DBA 5,6-dihydrodiol and DBA 5,6-oxide were synthesized by modifications of the methods outlined for K-region arene oxides (22). DBA 5,6- and 7,14-quinone were prepared by established procedures (23, 24). A presumed mixture of 5- and/or 6-HODBA was obtained by acid catalyzed dehydration of the K-region 5,6-dihydrodiol (25). Aroclor-1254 (lot KC-12-638) was obtained from Monsanto Chemical Co., St. Louis, MO.

*Incubations.* Incubation mixtures with liver microsomes contained 0.25 to 2.00 mg of microsomal protein, 200 μmol of potassium phosphate buffer (pH 7.4), 6 μmol of $MgCl_2$, 2 μmol of NADPH, and 100 nmol of [$^{14}$C]-DBA (in 100 μl of acetone) in a total volume of 2.00 ml. For incubations with the reconstituted monooxygenase system (*cf.* 26, 27) with and without addition of purified epoxide hydrase (42 to 334 units)[2], microsomes were replaced by 0.10 to 0.20 nmol of cytochrome P-448, 1500 units of purified cytochrome c reductase[2] and 60 μg of dilauroyl phosphatidylcholine, and the pH of the potassium phosphate buffer was 7.0. All incubations with DBA were at 37° for 10 min except for one experiment, in which the incubation time was 30 min. Incubations of unlabelled DBA 3,4-dihydrodiol (3.2 μmol) were done at 37° for 15 min with 20 mg microsomal protein from 3-methylcholanthrene-treated rats, 5.0 mmol of potassium phosphate buffer (pH 7.4), 0.15 mmol of $MgCl_2$ and 0.05 mmol of NADPH in a total volume of 50 ml. Unconverted substrates and metabolites were extracted into ethyl acetate:acetone (2:1) after incubation. The organic phase was dried over anhydrous sodium sulphate and evaporated under nitrogen. Residues were dissolved in 150 μl of methanol (DBA metabolites) or 250 μl of tetrahydrofuran (DBA 3,4-dihydrodiol metabolites) and analyzed by HPLC.

Liver microsomes were prepared from immature (50–60 g) male rats of the Long-Evans strain (28). The cytochrome P-450 content of the microsomes from control-, phenobarbital-, 3-methylcholanthrene- and Aroclor-pretreated animals was 0.87, 2.10, 1.69 and 3.50 nmol/mg of protein, respectively, determined as described by Omura and Sato (29). Highly purified cytochrome P-448 (26), NADPH-cytochrome c reductase (30) and homogeneous epoxide hydrase (31) were prepared by established procedures.

*Analysis of metabolites.* Synthetic reference compounds and metabolites of DBA were chromatographed on a DuPont Zorbax ODS (octadecylsilane) column (6.2 mm × 25 cm). A linear gradient (1.2 ml/min) from 70% methanol in water to 100% methanol was used at a rate of gradient change of 1%/min after an initial delay of 1 min (Spectra Physics, model 3500B). The effluent was monitored at 280 nm. Fractions were collected every 0.3 min throughout the metabolite profile except for the substrate peak, which was collected as a single fraction. Radioactivity of the fractions was determined by scintillation spectrometry (Aquasol, Packard Tricarb Model B2450). Results obtained from the microsomal and the reconstituted monooxygenase system were corrected for zero-time experiments,

[2] One unit of epoxide hydrase activity is defined as 1 nmol of styrene glycol formed per min. One unit of cytochrome c reductase is defined as 1 nmol of cytochrome c reduced per min at 22°.

analyzed as described above. For preparative isolation of DBA 3,4-dihydrodiol metabolites, a linear gradient (1.2 ml/min) of 50% methanol in water to 100% methanol was used at a rate of change of 1%/min.

Both retention times on HPLC and fluorescence spectra were used to establish the identities of the metabolites of DBA. Metabolite fractions were collected from the HPLC column and diluted with methanol prior to spectroscopic analysis. The 1,2- and 3,4-dihydrodiol fractions had fluorescence emission and excitation spectra identical to those of the authentic synthetic standards (21); emission maxima at 410 nm for the 1,2-dihydrodiol and at 407 and 430 nm for the 3,4-dihydrodiol (excitation at 277 nm). The amount of radioactive metabolite which was cochromatographic with synthetic 5,6-dihydrodiol (emission maxima at 370 and 390 nm) was generally too small to allow confirmation of its presence by fluorescence. Two radioactive metabolite fractions were detected in the phenol region of the chromatograms. These metabolite fractions (phenol-1 and -2 fractions) were established to be phenolic by the marked spectral changes which occurred on addition of sodium methoxide. The original spectra returned upon acidification with ammonium chloride. If DBA 3,4-dihydrodiol is metabolized to 3,4-diol-1,2-epoxides, benzo-ring tetraols, triols, and a keto diol could be detected as break-down products since such diol epoxides are predicted to be highly reactive (6–9). For this reason, the fluorescence spectra of the metabolites of DBA and of DBA 3,4-dihydrodiol were compared to that of 4-hydroxy-$H_4$-DBA (21), which also has an 8,9-dialkyl substituted BA chromophore (see RESULTS). Fluorescence spectra were measured with a Perkin-Elmer Spectrofluorimeter, model MPF-3L.

*Enantiomeric purity of metabolically formed DBA 3,4-dihydrodiol.* Racemic DBA 3,4-dihydrodiol was allowed to react with the acid chloride of (−)-α-methoxy-α-trifluoromethylphenylacetic acid in dry pyridine. The resulting pair of diastereomeric bis esters (32, 36) were separated by chromatography on the ODS column with a gradient of 80% to 100% methanol in water at a rate of change of 1%/min and a flow rate of 1.2 ml/min; retention times 20.0 min and 20.7 min. The two diastereomer peaks were of equal size and had uv maxima nearly identical to authentic dihydrodiol. Radioactive 3,4-dihydrodiol formed from [$^{14}$C]-DBA by liver microsomes from 3-methylcholanthrene-treated rats was diluted with racemic DBA 3,4-dihydrodiol, converted to the diastereomeric bis esters and chromatographed as above to determine enantiomeric purity radiochemically.

## RESULTS

*Chromatographic properties of synthetic derivatives of DBA.* The three metabolically probable *trans* dihydrodiols of DBA were well separated from each other, as well as from phenols and quinones of DBA, by HPLC on a reverse phase ODS column (Fig. 2A). The bay-region 1,2-dihydrodiol emerged first from the ODS column, followed by the K-region 5,6-dihydrodiol and finally by the 3,4-dihydrodiol. The order of elution of these dihydrodiols is predictable based on considerations of conformation and polarity (32). The K-region phenols (5/6-HODBA), the 5,6-oxide and 5,6-quinone emerged as one peak designated as phenol fraction-1. Ultraviolet spectra before and after chromatography established that DBA 5,6-oxide survived the HPLC column intact. The DBA 1,2- and 3,4-dihydrodiols were dehydrated with a 1:1 mixture of 6 N HCl:methanol at room temperature for 0.5 hr to produce two phenolic products in each case. Based on absorbance at 280 nm, about 80% of the phenol produced from the 1,2-dihydrodiol and 40% of the phenol produced from the 3,4-dihydrodiol chromatographed in phenol fraction-1 (*cf.* Fig. 2B). The major dehydration product from the 3,4-dihydrodiol (60%) eluted in phenol fraction-2. Mass spectra of these three major dehydration products ($M^+$ = 294) confirmed that they were phenols. A minor dehydration product (~20%) of the 1,2-dihydrodiol chromatographed between the phenol-1 and -2 fractions. By analogy to isomeric phenols of other hydrocarbons (32), 2- and 3-HODBA might be expected to chromatograph in the phenol-1 fraction while 4- and possibly 1-HODBA should

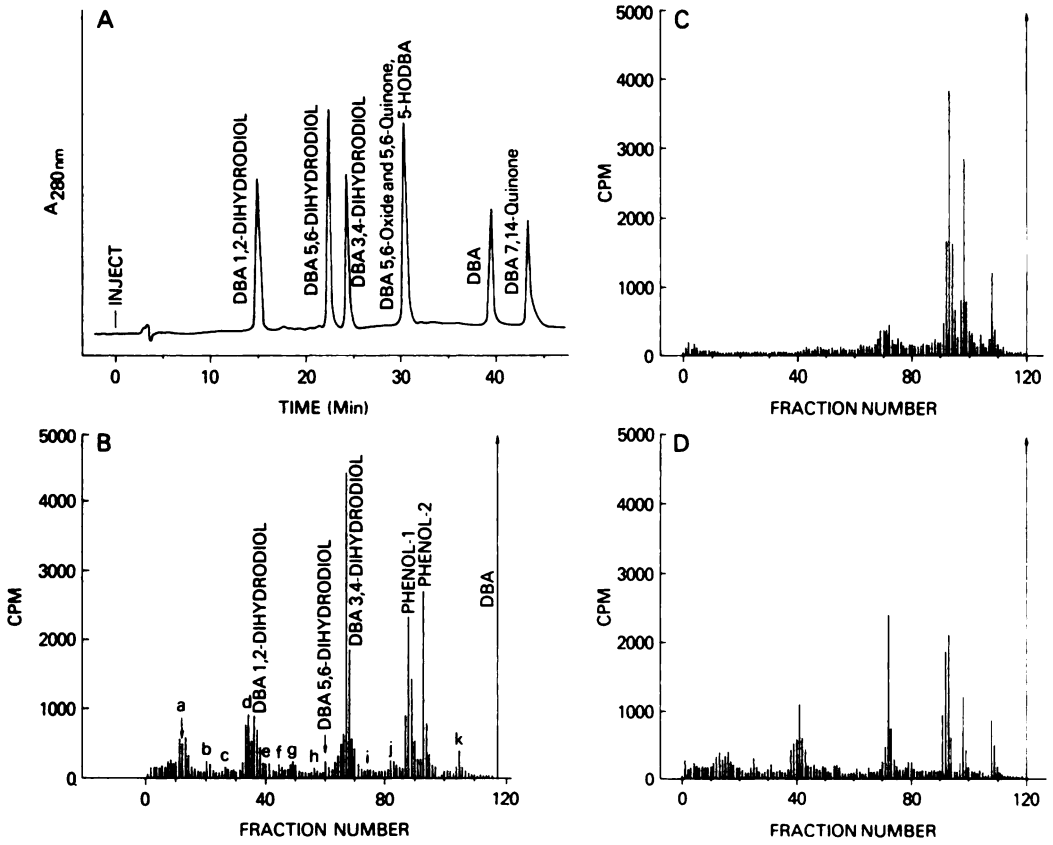


FIG. 2. *Chromatographic separation of DBA metabolites on HPLC*

Synthetic derivatives (A) and radioactive metabolites formed by microsomes from Aroclor-pretreated rats (B) or by the purified cytochrome P-448 system in the absence (C) and presence (D) of epoxide hydrase. Incubation and chromatographic conditions are described in MATERIALS AND METHODS. The gradient used was 70% to 100% methanol in water at a rate of change of 1% per min.

chromatograph with the phenol-2 fraction. DBA 7,14-quinone eluted after the parent hydrocarbon. The low polarity of this quinone may partly be explained by the high symmetry of the molecule in that both of the quinone oxygen atoms form part of a hindered bay region. Although no evidence was found for the formation of the 7,14-quinone, its close proximity to the substrate made detection of this potential metabolite difficult.

*Effect of inducers on liver microsomal metabolism of DBA.* Results obtained from incubations of [$^{14}$C]-DBA (50 nmol/ml) with various microsomal preparations under conditions which gave constant turnover (nmol products/nmol hemeprotein/min) with respect to the protein concentrations used are given in Table 1. Liver microsomes from 3-methylcholanthrene-pretreated rats metabolized DBA at a 2.5- to 3-fold higher rate than microsomes from untreated rats. Aroclor-pretreatment similarly resulted in a 2-fold higher rate of conversion. Phenobarbital-pretreatment caused a significant decrease in the rate of metabolism of DBA compared with control microsomes when the data were expressed per nmol cytochrome P-450. Comparison of the rate of metabolism of DBA with that of BA and BP by liver microsomes from control, phenobarbital-treated, or 3-methylcholanthrene-treated animals shows that DBA is metabolized at only ⅓ to ⅙ the rate of the other two hydrocarbons by these enzyme preparations (Table 2).

The profile of radioactive metabolites formed by liver microsomes from Aroclor-

pre-treated rats is shown in Fig. 2B. Microsomes from phenobarbital-treated, 3-methylcholanthrene-treated and untreated rats produced qualitatively similar metabolite profiles. DBA 3,4-dihydrodiol was found to be a major metabolite (24–28% of the total metabolites) formed by all microsomal preparations (see Table 1). The 1,2-dihydrodiol was formed to less than half this extent (8–13%) by the microsomal enzyme preparations. The amount of radioactive metabolites which cochromatographed with the 5,6-dihydrodiol was quite small (2–5% of the total metabolites), with the maximum amount being formed by microsomes from phenobarbital-pretreated rats. In the case of metabolism by microsomes from phenobarbital-pretreated rats, fluorescence spectroscopy confirmed that at least some of the radioactivity which cochromatographed with the 5,6-dihydrodiol was indeed associated with this dihydrodiol. The (peaks a–k, Table 1 and Fig. 2B) in varying amounts which total as much as 32% of the overall metabolism. These are presumed to be mainly secondary oxidative metabolites. They are most extensively formed in incubations which result in a high percent conversion of substrate to its metabolites. The sum of the polar peaks a–c (*cf*, Fig. 2B), for example, account for 8–10% of the metabolites formed by microsomes from Aroclor-

TABLE 1

*Metabolites of DBA obtained with rat liver microsomes and a purified and reconstituted system containing cytochrome P-448*

| Protein[b] | Metabolites[a] | | | | | | Total conversion | Recovery |
|---|---|---|---|---|---|---|---|---|
| | 1,2-Diol | 5,6-Diol | 3,4-Diol | Phenol-1 | Phenol-2 | Total unknown metabolite peaks[e] | | |
| Microsomes (control) | 8.3 | 1.9 | 26 | 33 | 14 | 17 | 4.8 | 87 |
| | (0.04) | (0.01) | (0.13) | (0.16) | (0.07) | (0.08) | (0.55) | |
| Microsomes (phenobarbital) | 11 | 4.5 | 28 | 30 | 11 | 15 | 4.4 | 87 |
| | (0.02) | (0.01) | (0.05) | (0.06) | (0.02) | (0.03) | (0.21) | |
| Microsomes (3-methyl-cholanthrene) | 13 | 1.7 | 24 | 18 | 15 | 28 | 24.8 | 80 |
| | (0.15) | (0.02) | (0.28) | (0.21) | (0.18) | (0.33) | (1.47) | |
| Microsomes (Aroclor) | 8.8 | 1.6 | 25 | 19 | 14 | 32 | 16.7 | 82 |
| | (0.07) | (0.01) | (0.20) | (0.15) | (0.11) | (0.25) | (0.95) | |
| Cytochrome P-448 + epoxide hydrase[c] | 11 | 1.6 | 17 | 22 | 8.0 | 40 | 12.2 | 78 |
| | (0.54) | (0.08) | (0.80) | (1.07) | (0.38) | (2.07) | (6.1) | |
| Cytochrome P-448[d] | 1.7 | 1.3 | 4.9 | 41 | 23 | 28 | 10.3 | 73 |
| | (0.06) | (0.05) | (0.19) | (1.55) | (0.89) | (1.05) | (5.2) | |

[a] The upper row of numbers represents the percent of each metabolite (as a % of total metabolites) formed, and the lower row represents nmols of product formed per nmol of hemeprotein per min. Total conversion was calculated from the total radioactivity above blank which emerged from the column prior to DBA. Recovery is the percentage of radioactivity emerging before DBA in discrete metabolite peaks.

[b] The incubations with microsomes from control, phenobarbital- and 3-methylcholanthrene-treated rats contained 0.5 mg protein/ml, whereas the incubation with Aroclor-induced microsomes contained 0.25 mg protein/ml. The concentration of the cytochrome P-448 in the reconstituted system was 0.1 nmol/ml. Hemeprotein contents of the microsomal preparations are given in the MATERIALS AND METHODS.

[c] The incubation medium contained 42 units of epoxide hydrase.

[d] Since the reconstituted monooxygenase system is free of epoxide hydrase as measured both by SDS-gel electrophoresis and catalytic activity with several arene oxides as substrates, the radioactivity in the three dihydrodiol regions must either arise by spontaneous hydrolysis of their arene oxide precursors or be minor secondary oxidative metabolites which chromatograph in these regions. As yet, spontaneous hydrolysis has not been detected for non-K-region arene oxides.

[e] This entry comprises the total of the minor metabolite peaks a–k (cf. Fig. 2B) which were formed (generally >1% each) by all enzyme preparations. Generally peaks a and k individually accounted for 20–40% of these minor metabolites with peaks d and i often being the next largest.

TABLE 2

*Comparison of the rate of metabolism (nmol products/nmol hemeprotein/min) of DBA, BA, and BP by liver microsomes from control and pretreated rats*

| Substrate (50 nmol/ml) | Microsomes | | | % active dihydrodiol[a] |
|---|---|---|---|---|
| | Control | Phenobarbital | 3-Methylcholanthrene | |
| DBA | 0.5 | 0.2 | 1.5 | 24–28% |
| BA[b] | ~2 | 1.4 | 7 | 1.5–4% |
| BP[c] | 1.4 | 1.1 | 5 | 5–12% |

[a] This entry refers to the biologically most active of the metabolically possible dihydrodiols from each hydrocarbon, the DBA 3,4-, BA 3,4-, and BP 7,8-dihydrodiols.

[b] Data taken from reference 20.

[c] Data taken from reference 27.

and 3-methylcholanthrene-pretreated rats (17–25% conversion of substrate, Table 1).

The effects of extent of metabolism of DBA by liver microsomes from 3-methylcholanthrene-pretreated rats on the profile of metabolites are shown in Fig. 3. Several marked changes were observed when the extent of conversion was varied from 16% to 69% by increasing both the concentration of protein and the incubation time (see legend, Fig. 3). The minor metabolites a–c (*cf*, Fig. 2B) increased 3.5-fold to 20% of the total metabolites, and the phenol-1 fraction was decreased by 70%. Notably, the phenol-1 fraction constitutes 27% of the total metabolites at low conversion of substrate, but only 8% at high conversion. DBA 3,4-dihydrodiol is also a major metabolite (25%) at low conversion, but is not significantly reduced at high conversion (22% of the total metabolites). Thus, metabolites in the phenol-1 fraction appear to be the best substrates for further metabolism.

*Metabolism of DBA 3,4-dihydrodiol.* Since DBA 3,4-dihydrodiol is the most mutagenic (10) and tumorigenic (33) metabolite known for DBA, further metabolism of the synthetic dihydrodiol was examined (Fig. 4). Five metabolite peaks (arrows, Fig. 4) were detected which had fluorescence spectra that were virtually identical to that of 4-hydroxy-$H_4$-DBA, indicating a substituted BA chromophore (Fig. 5). Since the pair of diastereomeric 3,4-diol-1,2-epoxides could form four tetraols, two triols, and a keto diol (34–37), as many as seven such peaks are possible. Metabolites such as phenolic dihydrodiols (35, 36) could account for some of the other products shown in Fig. 4. The number and amounts of metabolites of the 3,4-dihydrodiol which have the substituted BA chromophore clearly indicate that extensive conversion to 3,4-diol-1,2-epoxides had occurred. When the metabolites in peaks 1–5 (Fig. 4) were isolated and rechromatographed on the gradient profile used to separate metabolites of DBA (Fig. 2), peaks 1 and 2 chromatographed in the 1,2-dihydrodiol region, peak 3 with peak f, peak 4 with peak g, and peak 5 with the 5,6-

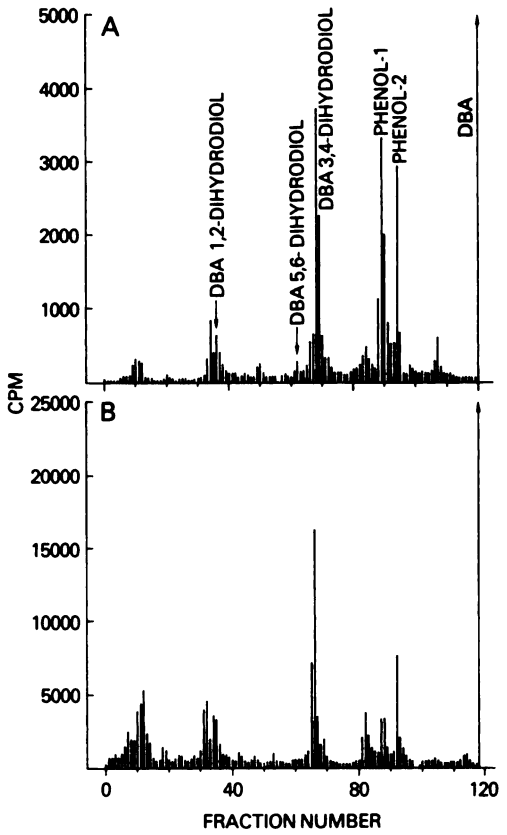


FIG. 3. *Effect of extent of metabolism on the HPLC profile of metabolites of 50 μM DBA by liver microsomes from 3-methylcholanthrene-pretreated rats*

(A) 16% conversion on 10 min incubation with 0.25 mg protein/ml, (B) 69% conversion on 30 min incubation with 1.0 mg protein/ml.

dihydrodiol. Fluorescence spectra of DBA metabolites c, d, f, and g indicated that these peaks were largely composed of compounds which exhibited a 1,2,3,4-$H_4$-DBA chromophore identical to that of 4-hydroxy-$H_4$-DBA. These results indicate that at least some DBA 3,4-diol-1,2-epoxide is formed from DBA under our incubation conditions.

For both BP and BA, the more tumorigenic (−)-[R,R]-enantiomers of their 7,8- and 3,4-dihydrodiols, respectively, are the predominant enantiomers formed from the parent hydrocarbons[3]. In each case, the diester of the (−)-dihydrodiol with (−)-α-methoxy-α-trifluorophenylacetic acid elutes first from ODS columns. Although absolute stereochemistry has yet to be assigned to the (+)- and (−)-enantiomers of DBA 3,4-dihydrodiol, the early eluting diastereomeric bis ester was found to be four times as radioactive as the late eluting bis ester. Thus DBA 3,4-dihydrodiol formed from [$^{14}$C]-DBA by liver microsomes from 3-methylcholanthrene-treated rats is about 60% optically pure.

*Effects of epoxide hydrase on the metabolism of DBA by a highly purified cytochrome P-448-containing system.* The HPLC profiles of the metabolites of [$^{14}$C]-DBA (50 nmol/ml) obtained after incubation with purified cytochrome P-448 (0.1 nmol/ml) in the absence and presence of epoxide hydrase are shown in Fig. 2. In the absence of epoxide hydrase, mainly three radioactive fractions were obtained; phenol-1, phenol-2 and peak *k* (Fig. 2C, Table 1). Analysis of phenol-1 and phenol-2 by base-induced shifts of fluorescence bands in neutral and basic media verified their phenolic nature. In the presence of epoxide hydrase (42 units) dihydrodiol peaks appeared and the phenol fractions became less prominent (Fig. 2D). Phenol-1 was still the major radioactive peak (Table 1) but the proportions of DBA 1,2- and 3,4-dihydrodiol had increased substantially, while the formation of DBA 5,6-dihydrodiol could not clearly be established. With epoxide hydrase the polar fractions a–c also appeared.

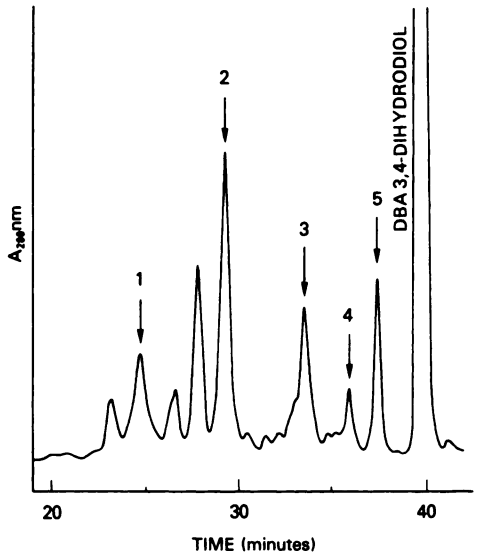


FIG. 4. *HPLC profile of metabolites obtained from DBA 3,4-dihydrodiol with liver microsomes from 3-methylcholanthrene pretreated rats*

For details see MATERIALS AND METHODS. The uv peaks indicated with arrows gave fluorescence spectra identical to 4-hydroxy-$H_4$-DBA. A gradient of 50% to 100% methanol in water at a rate of change of 1% per min was used. Peak 2 cochromatographs with the 1,2-dihydrodiol and peak 5 cochromatographs with the 5,6-dihydrodiol in this system.

Results from experiments with different amounts of added epoxide hydrase (42–334 units) are illustrated in Fig. 6. The total amount of DBA 1,2-dihydrodiol continued to increase with increasing amounts of epoxide hydrase, while the formation of DBA 3,4-dihydrodiol maximized with the addition of 42 units of epoxide hydrase. The small amount of the DBA 5,6-dihydrodiol fraction remained fairly constant. The phenol-1 and -2 fractions were reduced by 53% and 60%, respectively. Phenol-2 showed a maximal decrease with the addition of 42 units of epoxide hydrase, while further addition of this enzyme resulted in a continued decrease of phenol-1. The continued increase in the 1,2-dihydrodiol and decrease in the phenol-1 fraction with increasing amounts of epoxide hydrase argues that DBA 1,2-oxide isomerizes mainly to 2-

[3] D. R. Thakker, W. Levin, H. Yagi, S. Turujman, D. Kapadia, A. H. Conney, and D. M. Jerina, Absolute stereochemistry of the trans dihydrodiols formed from benzo[a]anthracene by liver microsomes. *Chem.-Biol. Interact.*, in press.

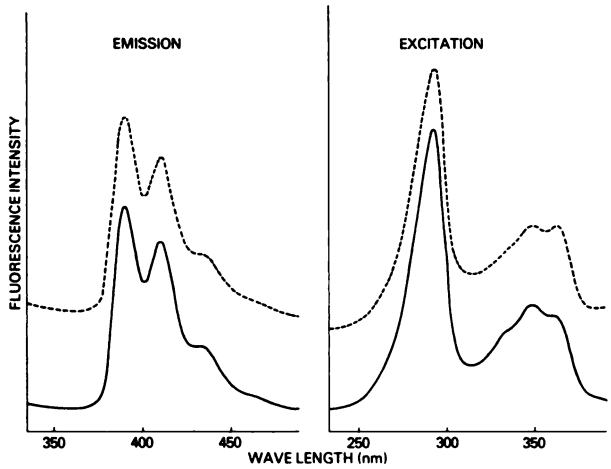


FIG. 5. *Comparison of the fluorescence emission and excitation spectra (methanol) of the least polar of the DBA 3,4-dihydrodiol metabolites (- - -, peak 5 in Fig. 4) with that of 4-hydroxy-$H_4$-DBA*

The other four peaks indicated with arrows gave identical spectra. The uv spectra of the five metabolites were also identical to the reference compound.

HODBA (phenol 1-fraction), and that DBA 1,2-oxide is not a particularly good substrate for epoxide hydrase. The presumed 2-HODBA was the major dehydration product of the 1,2-dihydrodiol (see MATERIALS AND METHODS). Since the total amounts of metabolites of DBA formed by 0.05 nmol/ml of cytochrome P-448 were slightly increased by the addition of epoxide hydrase (Fig. 6), there was some indication that phenols may inhibit the metabolism of DBA. Previously phenols of BA were shown to inhibit the metabolism of BA (20).

## DISCUSSION

A substantial body of evidence has now accumulated which either implicates or in some cases proves that bay-region diol epoxides are ultimate carcinogenic metabolites of the hydrocarbons BP (38, 39), BA (40, 41), DBA (33), 7-methyl BA (42), 7,12-dimethyl BA (43–46), 3-methylcholanthrene (47–50), chrysene (51), and 5-methylchrysene (52). Based on quantum mechanical calculations, the bay-region theory predicted that 3,4-diol-1,2-epoxides of BA should be chemically more reactive than those of DBA and would presumably have higher biological activity. Yet, DBA is more carcinogenic than BA. Presumably, a higher percentage of the critical 3,4-diol-1,2-epoxides are formed from DBA than from BA, a factor which the theory makes no attempt to take into account. The present study explores this possibility through an examination of the metabolism of DBA.

As has been the case for other polycyclic aromatic hydrocarbons, pretreatment of animals with either 3-methylcholanthrene or Aroclor results in a 2- to 3-fold stimulation in the overall rate of metabolism of DBA per nmol of cytochrome P-450. Pretreatment of rats with phenobarbital results in a marked reduction (50%) in the rate of metabolism (nmol product formed/nmol cytochrome P-450/min) compared to microsomes from untreated animals. This contrasts with much smaller decreases in rate of metabolism of BP (27) and BA (20) upon phenobarbital-pretreatment. The profile of metabolites from DBA obtained with microsomes from control or pretreated animals consists of 36–43% dihydrodiols, 33–47% phenols, and a total of 15–32% of several minor secondary oxidative metabolites (Table 1, Fig. 2). No significant amount of 7,14-quinone could be detected. The most unusual feature of the metabolism of DBA is the substantial amount of secondary oxidative metabolites (15–17%) formed at very low (5%) substrate conversion (Table 1). In contrast to the metabolism of BP and BA where 10–40% of the total metabolites represent K-region dihy-

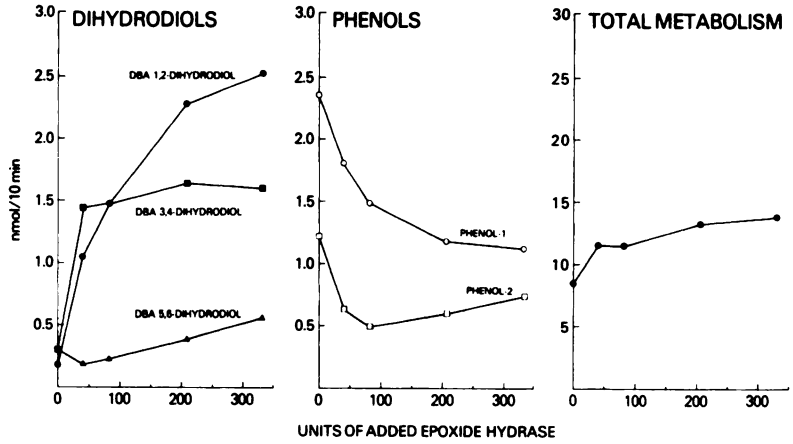


FIG. 6. *Effect of epoxide hydrase on the metabolism of DBA by a purified cytochrome P-448 containing system*

DBA (50 μM) was incubated for 10 min with a reconstituted system containing 0, 42, 83, 209 and 334 units of epoxide hydrase, respectively. Metabolites were quantitated from the radioactivity associated with the corresponding HPLC peaks. Total metabolism was calculated from the proportion of radioactivity above blank which eluted before DBA from the column.

drodiols (20, 27), only trace to minor amounts (<5% after phenobarbital-pretreatment) of the K-region DBA 5,6-dihydrodiol are formed.

In an attempt to establish the nature and origin of the secondary oxidative metabolites, both extensive metabolism of DBA (Fig. 3) and metabolism of the predominant 3,4-dihydrodiol (Fig. 4) were examined. At very high percent conversion of DBA to its metabolites, substantial depletion of phenols accompanied mainly by increases in the polar metabolites which chromatograph prior to the 1,2-dihydrodiol and peak j (*cf.* Fig. 2B) occurred. Although the percentage of the 3,4-dihydrodiol was only slightly decreased (from 25% to 22%), evidence is presented in the RESULTS section which indicates that 3,4-diol-1,2-epoxides are formed when either the 3,4-dihydrodiol or DBA is used as the substrate.

Examination of the metabolism of DBA by a highly purified and reconstituted system containing cytochrome P-448 in the presence of epoxide hydrase indicated a 50% increase in the total unknown minor metabolites formed, compared to incubations in the absence of epoxide hydrase (Fig. 2C and 2D). These results suggest that dihydrodiols are precursors for a significant portion of these unidentified minor metabolites. Since 5% of the metabolites, in the absence of epoxide hydrase (Table 1), emerge in the region of the 3,4-dihydrodiol, the amount of this metabolite from microsomes may be slightly overestimated due to contamination by this material. As was the case for the metabolism of BP (27), addition of very large amounts of epoxide hydrase to the reconstituted system failed to completely deplete the phenol fractions (Fig. 6). Since the amount of 3,4-dihydrodiol remained constant from low to very high epoxide hydrase levels, the 3,4-oxide appears to be a good substrate for epoxide hydrase. The 1,2-oxide appears to be a relatively poor substrate since the amount of 1,2-dihydrodiol continued to increase and actually exceeded the 3,4-dihydrodiol with large amounts of added epoxide hydrase. DBA 5,6-oxide is at best only a very trace metabolite in this system. Although this arene oxide is a poor substrate for epoxide hydrase (53, 54), as much as 40 nmol of 5,6-dihydrodiol/10 min could have been produced (53) compared to the 0.6 nmol found (Fig. 6) with the 220 units of enzyme used.

The specific activity of both control and induced microsomes is several fold lower toward DBA compared with BA and BP (Table 2). This does not necessarily argue for lower carcinogenicity of DBA, since

most of the administered hydrocarbon is probably metabolized at the low doses used in tumor experiments. The high regiospecificity of the cytochrome P-450 system at the 3,4-position of DBA and the good activity of epoxide hydrase toward the 3,4-oxide combine to produce from 6 to 20 times more of the critical 3,4-dihydrodiol with the bay-region 1,2-double bond than is produced from the very weak carcinogen BA. For the potent carcinogens BP (36) and DBA (present study), evidence for the formation of bay-region diol epoxides is present in metabolism profiles at high conversion of substrate which would normally occur *in vivo*. Such bay-region diol epoxide metabolites of BA could not be detected (20, 55) due to the very low level of BA 3,4-dihydrodiol formed from BA at high conversion. Taken together, these metabolism studies amply explain why DBA is much more tumorigenic than BA despite the prediction (6–9) that the 3,4-diol-1,2-epoxides of BA would be somewhat more reactive than those of DBA.

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## REFERENCES

1. Kennaway, E. L. and I. Hieger. Carcinogenic substances and their fluorescence spectra. *Brit. Med. J.* **1**: 1044–1046, 1930.
2. Miller, E. C. and J. A. Miller. In *The Molecular Biology of Cancer* (Busch, H., ed.), Academic Press, New York, 1974, 337–402.
3. Jerina, D. M. and J. W. Daly. Arene oxides: A new aspect of drug metabolism. *Science* **185**: 573–582, 1974.
4. Heidelberger, C. Chemical carcinogenesis. *Ann. Rev. Biochem.* **44**: 79–121, 1975.
5. Sims, P. and P. L. Grover. Epoxides in polycyclic aromatic hydrocarbon metabolism and carcinogenesis. *Adv. Cancer Res.* **20**: 165–274, 1974.
6. Jerina, D. M. and J. W. Daly. Oxidation at carbon. In *Drug Metabolism—from Microbe to Man* (Parke, D. V. and Smith, R. L., eds.), Taylor and Francis, Ltd., London, 1976, 13–32.
7. Jerina, D. M., R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, and A. H. Conney. Mutagenicity of benzo[a]pyrene and the description of a quantum mechanical model which predicts the ease of carbonium ion formation from diol epoxides. In *In Vitro Metabolic Activation in Mutagenesis Testing* (Deserres, F. J., Fouts, J. R., Bend, J. R., and Philpot, R. M., eds.), Elsevier/North Holland Biomedical Press, Amsterdam, 1976, 159–177.
8. Jerina, D. M. and R. E. Lehr. The bay-region theory. A quantum mechanical approach to aromatic hydrocarbon-induced carcinogenicity. In *Microsomes and Drug Oxidations* (Ullrich, V., Roots, I., Hildebrandt, A., and Estabrook, R. W., eds.), Pergamon Press, New York, 1978, 709–720.
9. Jerina, D. M., R. E. Lehr, M. Schaefer-Ridder, H. Yagi, J. M. Karle, D. R. Thakker, A. W. Wood, A. Y. H. Lu, D. Ryan, S. West, W. Levin, and A. H. Conney. Bay region epoxides of dihydrodiols: A concept which explains the mutagenic and carcinogenic activity of benzo[a]pyrene and benzo[a]anthracene. In *Origins of Human Cancer* (Hiatt, H., Watson, J. D., and Winsten, I., eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1977, 639–658.
10. Wood, A. W., W. Levin, P. E. Thomas, D. Ryan, J. M. Karle, H. Yagi, D. M. Jerina and A. H. Conney. Metabolic activation of dibenzo[a,h]anthracene and its dihydrodiols to bacterial mutagens. *Cancer Res.* **38**: 1967–1973, 1978.
11. Boyland, E., A. A. Levi, E. H. Mawson, and E. Roe. Metabolism of polycyclic compounds 4. Production of a dihydroxy-1:2:5:6-dibenzanthracene from 1:2:5:6-dibenzanthracene. *Biochem. J.* **35**: 184–191, 1941.
12. Dobriner, K., C. P. Rhoads, and G. I. Lavin. Conversion of 1,2,5,6-dibenzanthracene by rabbits, rats and mice. Significance in carcinogenesis of this conversion. *Proc. Soc. Exp. Biol. Med.* **41**: 67–69, 1939.
13. Heidelberger, C., H. I. Hadler, and G. Wolf. The metabolic degradation in the mouse of 1,2,5,6-dibenzanthracene-9,10-$C^{14}$. III. Some quinone metabolites retaining the intact ring system. *J. Am. Chem. Soc.* **75**: 1303–1308, 1953.
14. Boyland, E. and P. Sims. The metabolism of benz[a]anthracene and dibenz[a,h]anthracene and their 5,6-epoxy-5,6-dihydro derivatives by rat-liver homogenates. *Biochem. J.* **97**: 7–16, 1965.
15. Sims, P. Qualitative and quantitative studies on the metabolism of a series of aromatic hydrocarbons by rat-liver preparations. *Biochem. Pharmacol.* **19**: 795–818, 1970.
16. Grover, P. L., A. Hewer, and P. Sims. Epoxides as microsomal metabolites of polycyclic hydrocarbons. *Fed. Eur. Biochem. Soc. Lett.* **18**: 76–80, 1971.
17. Selkirk, J. K., E. Huberman, and C. Heidelberger.

An epoxide is an intermediate in the microsomal metabolism of the chemical carcinogen, dibenz[a,h]anthracene. *Biochem. Biophys. Res. Commun.* **43:** 1010–1016, 1971.

18. Daly, J. W., D. M. Jerina, and B. Witkop. Arene oxides and the NIH shift: The metabolism, toxicity and carcinogenicity of aromatic compounds. *Experientia* **28:** 1129–1149, 1972.
19. Dipple, A. Polynuclear aromatic carcinogens. In *Chemical Carcinogens*, ACS Monograph Series, No. 173 (Searle, C. E., ed.), American Chemical Society, Washington, D.C., 1976, 245–314.
20. Thakker, D. R., W. Levin, H. Yagi, D. Ryan, P. E. Thomas, J. M. Karle, R. E. Lehr, A. H. Conney, and D. M. Jerina. Metabolism of benzo[a]anthracene to its tumorigenic 3,4-dihydrodiol. *Mol. Pharmacol.* **15:** 174–189, 1979.
21. Karle, J. M., H. D. Mah, D. M. Jerina, and H. Yagi. Synthesis of dihydrodiols from chrysene and dibenzo[a,h]anthracene. *Tetrahedron Lett.:* 4021–4024, 1977.
22. Dansette, P. and D. M. Jerina. A facile synthesis of arene oxides at the K regions of polycyclic hydrocarbons. *J. Am. Chem. Soc.* **96:** 1224–1225, 1974.
23. Bhargava, P. M., H. I. Hadler, and C. Heidelberger. Studies on the structure of the skin protein-bound compounds following topical application of 1,2,5,6-dibenzanthracene-9,10-$C^{14}$. I. 2-Phenylphenanthrene-3,2-dicarboxylic acid, a degradation product. *J. Am. Chem. Soc.* **77:** 2877–2886, 1955.
24. Cason, J. and L. F. Fieser. Synthesis of 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene and its relation to products of metabolism of the hydrocarbon. *J. Am. Chem. Soc.* **62:** 2681–2687, 1940.
25. Cook, J. W. and R. Schoental. Oxidation of carcinogenic hydrocarbons by osmium tetroxide. *J. Chem. Soc.* 170–173, 1948.
26. Ryan, D., P. E. Thomas, D. Korzeniowski, and W. Levin. Separation and characterization of highly purified forms of liver microsomal cytochrome P-450 from rats treated with polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. *J. Biol. Chem.* **254:** 1365–1374, 1979.
27. Holder, G., H. Yagi, P. Dansette, D. M. Jerina, W. Levin, A. Y. H. Lu, and A. H. Conney. Effects of inducers and epoxide hydrase on the metabolism of benzo[a]pyrene by liver microsomes and reconstituted system: Analysis by high pressure liquid chromatography. *Proc. Natl. Acad. Sci. USA* **71:** 4356–4360, 1974.
28. Lu, A. Y. H. and W. Levin. Partial purification of cytochromes P-450 and P-448 from rat liver microsomes. *Biochem. Biophys. Res. Commun.* **46:** 1334–1340, 1972.
29. Omura, T. and I. Sato. The carbon monoxide-binding pigment of liver microsomes. II. Solubilization, purification, and properties. *J. Biol. Chem.* **239:** 2379–2385, 1964.
30. Yasukochi, Y. and B. S. S. Masters. Some properties of a detergent-solubilized NADPH-cytochrome c (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J. Biol. Chem.* **251:** 5337–5344, 1976.
31. Lu, A. Y. H., D. Ryan, D. M. Jerina, J. W. Daly and W. Levin. Liver microsomal epoxide hydrase: Solubilization, purification and characterization. *J. Biol. Chem.* **250:** 8283–8288, 1975.
32. Thakker, D. R., H. Yagi, and D. M. Jerina. Analysis of polycyclic aromatic hydrocarbons and their metabolites by high-pressure liquid chromatography. In *Methods in Enzymology—Biomembranes,* Vol. 52 (part C) (Packer, L. and Fleischer, S., eds.), Academic Press, New York, 1978, 279–296.
33. Buening, M. K., W. Levin, A. W. Wood, R. L. Chang, H. Yagi, J. M. Karle, D. M. Jerina, and A. H. Conney. Tumorigenicity of the dihydrodiols of dibenzo[a,h]anthracene on mouse skin and in newborn mice. *Cancer Res.* **39:** 1310–1314, 1979.
34. Yagi, H., D. R. Thakker, O. Hernandez, M. Koreeda, and D. M. Jerina. Synthesis and reactions of the highly mutagenic 7,8-diol-9,10-epoxides of the carcinogen benzo[a]pyrene. *J. Am. Chem. Soc.* **99:** 1604–1611, 1977.
35. Thakker, D. R., H. Yagi, A. Y. H. Lu, W. Levin, A. H. Conney, and D. M. Jerina. Metabolism of benzo[a]pyrene: Conversion of (±)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene to highly mutagenic 7,8-diol-9,10-epoxides. *Proc. Natl. Acad. Sci. USA* **73:** 3381–3385, 1976.
36. Thakker, D. R.,H. Yagi, H. Akagi, M. Koreeda, A. Y. H. Lu, W. Levin, A. W. Wood, A. H. Conney, and D. M. Jerina. Metabolism of benzo[a]pyrene. VI. Stereoselective metabolism of benzo[a]pyrene and benzo[a]pyrene 7,8-dihydrodiol to diol epoxides. *Chem.-Biol. Interact.* **16:** 281–300, 1977.
37. Yang, S. K., D. W. McCourt, P. P. Roller, and H. V. Gelboin. Enzymatic conversion of benzo[a]pyrene leading predominantly to the diol-epoxide r-7,t-8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydrobenzo[a]pyrene through a single enantiomer of r-7,t-8-dihydroxy-7,8-dihydrobenzo[a]pyrene. *Proc. Natl. Acad. Sci. USA* **73:** 2594–2598, 1976.
38. Kapitulnik, J., W. Levin, A. H. Conney, H. Yagi, and D. M. Jerina. Benzo[a]pyrene 7,8-dihydrodiol is more carcinogenic than benzo[a]pyrene in newborn mice. *Nature* **266:** 378–380, 1977.
39. Kapitulnik, J., P. G. Wislocki, W. Levin, H. Yagi, D. M. Jerina, and A. H. Conney. Tumorigenicity studies with diol epoxides of benzo[a]pyrene which indicate that (±)-*trans*-7β,8α-dihydroxy-

9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene is an ultimate carcinogen in newborn mice. *Cancer Res.* **38:** 354–358, 1978.

40. Levin, W., D. R. Thakker, A. W. Wood, R. L. Chang, R. E. Lehr, D. M. Jerina, and A. H. Conney. Evidence that benzo[a]anthracene 3,4-diol-1,2-epoxide is an ultimate carcinogen on mouse skin. *Cancer Res.* **38:** 1705–1710, 1978.
41. Slaga, T. J., E. Huberman, J. K. Selkirk, R. G. Harvey, and W. M. Bracken. Carcinogenicity and mutagenicity of benz[a]anthracene diols and diol-epoxides. *Cancer Res.* **38:** 1699–1704, 1978.
42. Chouroulinkov, I., A. Gentil, B. Tierney, P. L. Grover, and P. Sims. The metabolic activation of 7-methylbenz[a]anthracene in mouse skin: High tumour-initiating activity of the 3,4-dihydrodiol. *Cancer Lett.* **3:** 247–253, 1977.
43. Moschel, R. C., W. M. Baird, and A. Dipple. Metabolic activation of the carcinogen 7,12-dimethylbenz[a]anthracene for DNA binding. *Biochem. Biophys. Res. Commun.* **76:** 1092–1098, 1977.
44. Malaveille, C., H. Bartsch, B. Tierney, P. L. Grover, and P. Sims. Microsome mediated mutagenicities of the dihydrodiols of 7,12-dimethylbenz[a]anthracene: high mutagenic activity of the 3,4-dihydrodiol. *Biochem. Biophys. Res. Commun.* **83:** 1468–1473, 1978.
45. Yang, S. K., M. W. Chou, and P. P. Roller. Potential proximate carcinogens of 7,12-dimethylbenz[a]anthracene: Characterization of two metabolically formed trans-3,4-dihydrodiols. *J. Am. Chem. Soc.* **101:** 237–239, 1979.
46. Huberman, E. and T. J. Slaga. Mutagenicity and tumor-initiating activity of fluorinated derivatives of 7,12-dimethylbenz[a]anthracene. *Cancer Res.* **39:** 411–414, 1979.
47. Thakker, D. R., W. Levin, A. W. Wood, A. H. Conney, T. A. Stoming, and D. M. Jerina. Metabolic formation of 1,9,10-trihydroxy-9,10-dihydro-3-methylcholanthrene: a potential proximate carcinogen from 3-methylcholanthrene. *J. Am. Chem. Soc.* **100:** 645–647, 1978.
48. Wood, A. W., R. L. Chang, W. Levin, P. E. Thomas, D. Ryan, T. A. Stoming, D. R. Thakker, D. M. Jerina, and A. H. Conney. Metabolic activation of 3-methylcholanthrene and its metabolites to products mutagenic to bacterial and mammalian cells. *Cancer Res.* **38:** 3398–3404, 1978.
49. Vigny, P., M. Duquesne, H., Coulomb, B. Tierney, P. L. Grover, and P. Sims. Fluorescence spectral studies on the metabolic activation of 3-methylcholanthrene and 7,12-dimethylbenz[a]anthracene in mouse skin. *FEBS Lett.* **82:** 278–282, 1977.
50. King, H. W. S., M. R. Osborne, and P. Brookes. The metabolism and DNA binding of 3-methylcholanthrene. *Int. J. Cancer* **20:** 564–571, 1977.
51. Levin, W., A. W. Wood, R. L. Chang, H. Yagi, H. D. Mah, D. M. Jerina, and A. H. Conney. Evidence for bay region activation of chrysene 1,2-dihydrodiol to an ultimate carcinogen. *Cancer Res.* **38:** 1831–1834, 1978.
52. Hecht, S. S., E. LaVoie, R. Mazzarese, S. Amin, V. Bedenko, and D. Hoffmann. 1,2-Dihydro-1,2-dihydroxy-5-methylchrysene, a major activated metabolite of the environmental carcinogen 5-methylchrysene. *Cancer Res.* **38:** 2191–2194, 1978.
53. Lu, A. Y. H., D. M. Jerina, and W. Levin. Liver microsomal epoxide hydrase. Hydration of alkene and arene oxides by membrane-bound and purified enzymes. *J. Biol. Chem.* **252:** 3715–3723, 1977.
54. Jerina, D. M., P. M. Dansette, A. Y. H. Lu, and W. Levin. Hepatic microsomal epoxide hydrase: a sensitive radiometric assay for hydration of arene oxides of carcinogenic aromatic hydrocarbons. *Mol. Pharmacol.* **13:** 342–351, 1977.
55. Thakker, D. R., M. Nordqvist, H. Yagi, W. Levin, D. Ryan, P. Thomas, A. H. Conney, and D. M. Jerina. Comparative metabolism of a series of polycyclic aromatic hydrocarbons by rat liver microsomes and purified cytochrome P-450. In *Carcinogenesis*, Vol. 4, *Polynuclear Aromatic Hydrocarbons* (Jones, P. W. and Freudenthal, R. I., Eds.), in press, 1979.