Proc. Nat. Acad. Sci. USA
Vol. 71, No. 11, pp. 4356–4360, November 1974

Effects of Inducers and Epoxide Hydrase on the Metabolism of Benzo[*a*]pyrene by Liver Microsomes and a Reconstituted System: Analysis by High Pressure Liquid Chromatography

(carcinogen metabolism/microsomal cytochrome *P*-450)

GERALD HOLDER*, HARUHIKO YAGI*, PATRICK DANSETTE*, DONALD M. JERINA*, W. LEVIN†, ANTHONY Y. H. LU†, AND A. H. CONNEY†

* National Institute of Arthritis & Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014; and † Department of Biochemistry and Drug Metabolism, Hoffmann-LaRoche Inc., Nutley, New Jersey 07110

Communicated by Sidney Udenfriend, August 12, 1974

ABSTRACT **The mobilities of 24 potential metabolites of benzo[*a*]pyrene were examined with high pressure liquid chromatography. Twelve phenols, five quinones, four dihydrodiols, and three oxides were studied. The chromatographic procedure employed allowed the separation and quantitation of benzopyrene metabolites into three major groups consisting of phenols, quinones, and dihydrodiols. Two of the benzopyrene oxides were unstable during chromatography, whereas the third oxide was more stable and chromatographed in the quinone fraction.**

Treatment of rats with phenobarbital or 3-methylcholanthrene enhanced the metabolism of benzopyrene by liver microsomes and altered the relative amounts of the various metabolites formed. In the absence of epoxide hydrase (EC 4.2.1.63), benzopyrene was metabolized primarily to phenols and quinones but was not appreciably metabolized to dihydrodiols by a solubilized, reconstituted cytochrome *P*-448 monooxygenase system. Addition of partially purified epoxide hydrase resulted in the formation of benzopyrene dihydrodiols with a concomitant decrease in the formation of phenolic metabolites, indicating that benzopyrene undergoes metabolism via arene oxides that are precursors for dihydrodiols and phenols.

The widespread occurrence of benzo[*a*]pyrene (BP) as an environmental contaminant (1) and the carcinogenic effects of BP in experimental animals (2) have stimulated interest in this compound for some 40 years. This interest is manifested by numerous metabolic studies of BP both *in vivo* (3–9) and *in vitro* (10–17), the testing of BP and its metabolites for their carcinogenic and mutagenic potency (18, 19), and binding studies of BP and its metabolites to nucleic acids and polynucleotides (15, 20–23). The growing evidence that arene oxides are ultimate carcinogens of polycyclic aromatic hydrocarbons (24, 25), and the established intermediacy of arene oxides in the metabolism of several of these hydrocarbons (26–29), make a critical examination of the metabolism of BP imperative. Recent studies of BP metabolism *in vitro* have led to the postulation of BP 4,5-oxide as an intermediate in rat liver microsomal systems (14) and in Syrian hamster liver microsomes (15), but none of the studies have taken fully into account the multiple pathways for metabolism of BP (Table 1). Due to the magnitude of the analytical problems, and the difficulties inherent in the synthesis of all potential BP metabolites, direct comparisons of possible metabolites with authentic reference materials have not been forthcoming. Product identifications have been based on limited comparisons of chromatographic, ultraviolet, and fluorescence data for metabolites with a relatively small number of standards which are sometimes of questionable origin.

Reported metabolic data (Table 1) suggest that BP 1,2-, 2,3-, 4,5-, 7,8-, and 9,10-oxides may be involved in biotransformations of BP. Arene oxides at ring junctures are considered less likely, based on profiles of metabolites from naphthalene (26) and substituted benzenes (30, 31). Also unlikely is the 8,9-oxide, since its stable tautomeric oxepin form (32) should have been detected previously. Lack of synthetic reference standards may account, in part, for the absence of suggested metabolism at the 11,12-K region. The present availability of synthetic reference compounds, which include twelve phenols, five quinones, three arene oxides, and four *trans*-dihydrodiols of BP, enables a more realistic interpretation of the metabolism of BP than had been possible in the past. The use of suitable reference compounds for product identification should lead to the identification of the bioactivated ultimate carcinogen(s) formed from BP. A recent report has described the use of high pressure liquid chromatography for separation of metabolites of BP (33). Independent studies in our laboratory have utilized similar methodology for the separation of BP metabolites. The present study describes the chromatographic mobility of 24 potential BP metabolites and describes the effects of induction by phenobarbital and 3-methylcholanthrene on the profile of BP metabolites formed by rat liver microsomes. In addition, the effects of epoxide hydrase [glycol hydro-lyase (epoxide-forming), EC 4.2.1.63] on the metabolism of BP by a partially purified mixed-function oxidase system (34) has been evaluated.

Abbreviations used: BP, benzo[*a*]pyrene; 1-HOBP, 1-hydroxybenzo[*a*]pyrene; 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, and 12-HOBP, other BP phenols; BP 1,6-quinone, benzo[*a*]pyrene 1,6-quinone; BP 3,6-quinone, BP 4,5-quinone, BP 6,12-quinone, and BP 11,12-quinone, other BP quinones; BP 1,2-dihydrodiol, *trans*-1,2-dihydroxy-1,2-dihydrobenzo[*a*]pyrene; BP 2,3-, 4,5-, 7,8-, 9,10- and 11,12-dihydrodiol, other dihydrodiols of BP; BP 4,5-oxide, benzo[*a*]pyrene 4,5-oxide; BP 7,8- and 9,10-oxide, other BP oxides; HPLC, high pressure liquid chromatography.

MATERIALS AND METHODS

Incubations. Incubations with hepatic microsomes obtained from immature male rats (Long-Evans strain) contained 100

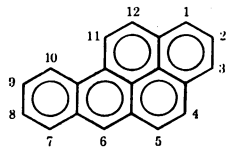
TABLE 1. *Reported metabolites of benzo[a]pyrene (BP)**

Metabolite	Refs.	Metabolite	Refs.
1-HOBP	3, 5, 10, 37	BP 2,3-dihydrodiol	4†, 6†
3-HOBP	3, 5, 6†, 10, 33	BP 4,5-dihydrodiol	6†, 9, 16, 33
5-HOBP	8	BP 7,8-dihydrodiol	11, 13, 15, 33
6-HOBP	6†, 7	BP 9,10-dihydrodiol	11, 12, 15, 33
9-HOBP	16, 33	1,6-dihydroxybenzo-[a]pyrene	6†
BP 1,6-quinone	2, 3, 10	3,6-dihydroxybenzo-[a]pyrene	6†, 10
BP 3,6-quinone	2, 3, 10	6,12-dihydroxy-benzo[a]pyrene	6†
BP 6,12-quinone	33	6-hydroxymethyl BP	17

* These references are representative but not complete. Many of the structural assignments are tentative, and some, such as the 2,3-dihydrodiol (37), have been shown to be in error.

† These metabolites are reported as water-soluble conjugates.

The numbering system for BP used in this paper is:



μg of microsomal protein, 0.5 μmol of NADPH, 3 μmol of magnesium chloride, 100 μmol of potassium phosphate buffer (pH 7.4), 95 nmol of [^{14}C]BP (specific activity 10.99 nCi/nmol added in 0.05 ml of acetone) per 1.0 ml of final volume, and were agitated in diffuse light under air for 10 min at 37°. The cytochrome *P*-450 or *P*-448 content of the microsomes from control, phenobarbital-pretreated, and 3-methylcholanthrene-pretreated animals was 0.66, 1.76, and 1.01 nmol/mg of protein, respectively. Incubations with the solubilized and reconstituted systems contained 0.2 nmol of cytochrome *P*-448 (34), 120 units of NADPH-cytochrome *c* reductase, 0.1 mg of lipid, 0.5 μmol of NADPH, 3 μmol of magnesium chloride, 100 μmol of potassium phosphate buffer (pH 6.8), epoxide hydrase (0, 72, 216, or 726 units, as indicated) (35), and 95 nmol of [^{14}C]BP as above per 1.0 ml of incubation. The samples were agitated in diffuse light under air for 5 min at 37°. The reactions were terminated by vortexing for 30 sec after the addition of 1 ml of acetone and 2 ml of ethyl acetate. More than 99% of the radioactivity was found in the organic phase after centrifugation.

Analysis. Extracts from two identical incubations were pooled. Two-milliliter portions of the organic phase were taken from each flask and evaporated in a stream of nitrogen in the dark. After addition of 4–5 nmol of BP 4,5-, 7,8-, and 9,10-dihydrodiol, BP 3,6-quinone, 3-HOBP, and 9-HOBP as reference compounds, the residue was dissolved in 5–10 μl of dioxane and injected onto the high pressure liquid chromatography (HPLC) column. Fractions were collected at 0.5- or 1.0-min intervals. Radioactivity was measured by scintillation spectrometry.

HPLC was conducted on a DuPont 830 instrument equipped with a 254-nm photometer. Metabolites were separated from BP into diol, quinone, and phenol fractions on two coupled 1 m × 2.1 mm DuPont analytical ODS columns operated with a linear gradient from 35 to 85% methanol in water at 2500 psig and flow rates of 0.7–1.1 ml/min. The column was operated at room temperature to prevent possible destruction of arene oxides and labile phenols such as 1- and 6-HOBP. Retention times of the reference standards are shown in Table 2.

TABLE 2. *High pressure liquid chromatography retention times of reference standards*

Compound*	R_t†	Compound*	R_t†
1-HOBP	44.5	BP 1,6-quinone	30.5
2-HOBP	41.0	BP 3,6-quinone	31.0
3-HOBP	45.0	BP 4,5-quinone	31.5
4-HOBP	45.0	BP 11,12-quinone	35.0
5-HOBP	45.0	BP 6,12-quinone	37.0
6-HOBP	39.0	BP 4,5-dihydrodiol	20.0
7-HOBP	44.0	BP 7,8-dihydrodiol	22.0
8-HOBP	41.0	BP 9,10-dihydrodiol	8.0
9-HOBP	42.0	BP 11,12-dihydrodiol	19.5
10-HOBP	44.0	BP 4,5-oxide	35.0
11-HOBP	45.5	BP 7,8-oxide	‡
12-HOBP	45.0	BP 9,10-oxide	‡
		BP	53.0

Two coupled 1 m × 2.1 mm analytical DuPont ODS columns were used with a linear gradient of 35–85% methanol in water over 50 min, an inlet pressure of 2500 psig, and a flow rate of 0.7–1.1 ml/min.

* The compounds have been prepared by previously published procedures or unequivocal *de novo* synthesis. The materials are analytically pure and free of chromatographically or spectroscopically detectable impurities. Details of the procedures employed have been submitted for publication or are in press. The 7,8- and 9,10-dihydrodiols were obtained by the action of epoxide hydrase on the corresponding arene oxides (32).

† Retention times (in minutes) vary from run to run by ±2%.

‡ These arene oxides are unstable under the conditions employed and decompose to afford phenols. The 7,8- and 9,10-oxides isomerize predominantly to 7-HOBP and 9-HOBP, respectively.

Results obtained from the microsomal and soluble systems were corrected for zero-time experiments analyzed as described above. The limit of detection of each metabolite fraction in this procedure was 0.01% of incubated BP.

RESULTS AND DISCUSSION

Chromatographic Properties of BP Metabolites. The HPLC procedure employed is highly efficient for separation and quantitation of phenols, quinones, and dihydrodiols produced from BP (Fig. 1). It is apparent, however, that rigorous identification of individual components within each group will require further study, as evidenced by the co-chromatography for many of the reference compounds presently available. The 9,10-dihydrodiol separates readily from the three other reference dihydrodiols, which overlap. Structural assignment to the metabolic dihydrodiols cannot be made with certainty until the unknown 1,2- and 2,3-dihydrodiols are available, or until further spectroscopic criteria can be applied. Three of the five available quinones (1,6-, 3,6-, and 4,5-) emerge from the column as a single badly tailing peak. The tail of this peak contains BP 6,12- and 11,12-quinones as well as BP 4,5-oxide. Mobility of the numerous other potential quinones is pres-

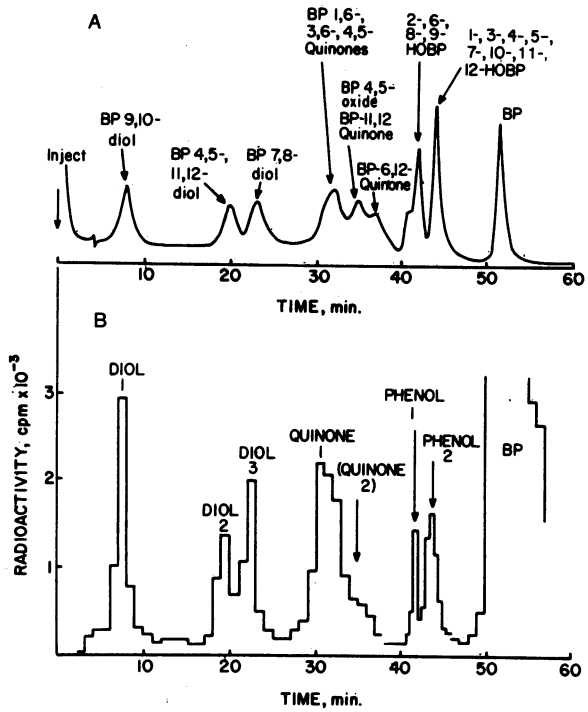


FIG. 1. Chromatographic profile of benzo[*a*]pyrene metabolites. (A) Composite high pressure liquid chromatography profile obtained with available reference compounds. (B) Chromatographic distribution of metabolites obtained from Exp. 6. The various "metabolite" regions are indicated.

ently unknown. The phenols are well separated from the quinones. However, they separate into only two overlapping peaks, the first containing 2-, 6-, 8-, and 9-HOBP, and the second containing 1-, 3-, 4-, 5-, 7-, 10-, 11-, and 12-HOBP. A composite plot of the separation of the available standards is shown (Fig. 1A).

Effect of Inducers on Microsomal Metabolism of BP. The microsomal mixed-function oxidase system is responsible for the detoxification, as well as the activation, of polycyclic hydrocarbons. Microsomal metabolism results in the formation of arene oxides, which are highly reactive metabolites that covalently bind to macromolecules and which are more active than the parent hydrocarbon in causing malignant transformations of cultured cells. Treatment of animals with compounds which induce this enzyme system can result in either increased or decreased toxicity of the hydrocarbon. Quantitative differences between the activity of liver microsomes from control, phenobarbital-pretreated, and 3-methylcholanthrene-pretreated animals are seen in both the percentage of metabolism and in the profile of metabolites formed (Table 3). Since structures for the metabolites cannot be assigned with certainty, the quantitative data obtained from metabolic studies with [^{14}C]BP are expressed only as diols 1, 2, and 3, quinones 1 and 2, and phenols 1 and 2 (Tables 3, 4; Fig. 1B). While quinone 2 is a metabolite fraction from control microsomes, it is not detectable as a separate peak with preparations from induced animals. Diol 1 predominates with control and 3-methylcholanthrene-induced microsomes, while diol 2 predominates with phenobarbital-induced microsomes. Phenol 1 is virtually absent with phenobarbital-induced microsomes. Direct comparison of the present results for microsomes from 3-methylcholanthrene-pretreated animals with a recent application of HPLC to the metabolism of BP (33) is not possible, since higher protein concentrations, longer incubation times, and microsomes from a different strain of rat were employed.

Differences in the overall catalytic activity of the microsomal preparations by the present assay were comparable to results observed previously with an assay that measures only fluorescent phenolic metabolites (36). Microsomes from 3-methylcholanthrene-pretreated animals were about 4.5 times more active per nmol of hemoprotein than microsomes from control animals, and microsomes from phenobarbital-pretreated animals were slightly less active per nmol of hemoprotein than the control.

Effect of Epoxide Hydrase on the Metabolism of BP by a Partially Purified Cytochrome P-448-Containing System. Hepatic epoxide hydrase is an important enzyme for the metabolism of aromatic compounds, since it converts the intermediate arene oxides formed by the mixed-function oxidase system to the corresponding dihydrodiols, which are nontoxic. Thus, the separation, purification, and reconstitution of the mixed-function oxidase system, and the removal of epoxide hydrase activity from this system (34, 35), permit a study of the central role of the oxidase and hydrase in controlling the levels of cytotoxic and carcinogenic metabolites of

TABLE 3. *Rat liver microsomal metabolism of benzo[a]pyrene*

Experiment	% Metabolized	Diol			Quinone		Phenol		Totals
		1	2	3	1	2	1	2	
1. Control	0.6	13.8*	9.2	9.7	18.8	16.5	6.1	26.6	100.7
		0.11†	0.07	0.08	0.15	0.13	0.05	0.21	0.80
2. 3-methylcholanthrene-pretreated	4.8	19.5*	8.7	11.8	23.4	—	2.3	24.8	90.5
		0.79†	0.35	0.48	0.94	—	0.09	1.00	3.65
3. Phenobarbital-pretreated	1.5	8.3*	11.8	4.6	32.1	—	1.4	25.0	83.3
		0.06†	0.09	0.03	0.23	—	0.01	0.18	0.60

Incubation mixtures contained 100 μg of microsomal protein, 0.5 μmol of NADPH, 3 μmol of $MgCl_2$, 100 μmol of potassium phosphate buffer (pH 7.4), 95 nmol of [^{14}C]BP per 1.0 ml of final volume.

* The upper row of numbers for each experiment is the percent of each metabolite expressed as proportion of total radioactivity eluting before BP.

† The lower row of numbers of the specific activity of preparation, nmol of product formed/nmol *P*-450 or *P*-448 per min.

TABLE 4. *Metabolism of benzo[a]pyrene by reconstituted liver microsomal system from 3-methylcholanthrene-pretreated animals*

Experiment	% Metabolized	Diol 1	Diol 2	Diol 3	Quinone 1	Quinone 2	Phenol 1	Phenol 2	Totals
4. 0.2 nmol of *P*-448	4.9	1.2*	1.0	1.4	32.4	11.0	18.4	27.7	93.1
		0.06†	0.05	0.07	1.54	0.52	0.87	1.31	4.42
5. 0.2 nmol of *P*-448, 72 units epoxide hydrase‡	5.5	11.3*	7.9	10.9	31.6	—	11.9	19.0	92.6
		0.59†	0.41	0.57	1.66	—	0.63	1.00	4.86
6. 0.2 nmol of *P*-448, 216 units epoxide hydrase‡	5.6	15.5*	9.2	11.9	31.9	—	6.8	17.8	93.1
		0.79†	0.47	0.61	1.62	—	0.35	0.90	4.74
7. 0.2 nmol of *P*-448, 725 units epoxide hydrase‡	5.2	24.6*	10.0	11.6	28.7	—	2.7	15.3	92.9
		1.19†	0.48	0.56	1.39	—	0.13	0.74	4.49

Incubation mixtures contained 0.2 nmol of *P*-448, 120 units of NADPH cytochrome *c* reductase, 0.1 mg of lipid, 0.5 μmol of NADPH, 3 μmol of $MgCl_2$, 100 μmol of potassium phosphate buffer (pH 6.8), and 95 nmol of [^{14}C]BP per 1 ml.

* The upper row of numbers for each experiment is the percent of each metabolite expressed as proportion of total radioactivity eluting before BP.

† The lower row of numbers is the specific activity of preparation, nmol of product formed/nmol of *P*-448 per min.

‡ One unit epoxide hydrase produces 1 nmol of styrene glycol per 15 min (38) from styrene oxide.

polycyclic hydrocarbons. Studies with the solubilized and reconstituted monooxygenase system (34) showed that very low levels of diols 1, 2, and 3 were formed from BP in the absence of added epoxide hydrase (Table 4). This is consistent with the negligible level of epoxide hydrase in the highly purified cytochrome *P*-448 (34, 35). The results obtained from these incubations in the absence of epoxide hydrase showed the presence of a peak for quinone 2, which accounted for one-quarter of the total quinones. On addition of epoxide hydrase to the system, diols 1, 2, and 3 appeared, while the radioactive peak in the region of BP 4,5-oxide and BP 6,12- and 11,12-quinone (quinone 2 fraction) was lost. Formation of diols 2 and 3 maximized with the addition of 72 units of partially purified epoxide hydrase, while further addition of this enzyme resulted in continued increase of diol 1. The total amount of phenols and quinone 2 fraction decreased in concert with increased production of dihydrodiols. These results suggest that the dihydrodiols and phenolic metabolites of BP share arene oxides as common precursors. The decrease in the quinone 2 fraction after the addition of epoxide hydrase may be principally due to hydration of BP 4,5-oxide that survives the conditions of incubation and chromatography. While the production of phenols was not completely blocked by addition of epoxide hydrase, metabolites in phenol peaks 1 and 2 were reduced by 85% and 45%, respectively. The data suggest that diol 1 is formed at the expense of both phenol 1 and 2, since only diol 1 continues to increase with increasing amounts of epoxide hydrase. The co-chromatography of the BP 9,10-dihydrodiol with diol 1, the 4,5- and 11,12-dihydrodiols with diol 2, and the 7,8-dihydrodiol with diol 3 suggests either that phenol 2 contains 10-HOBP, or that diol 1 is radiochemically heterogenous, containing either or both the BP 1,2- and 2,3-dihydrodiols. The latter dihydrodiols would be produced at the expense of 1-, 2-, and 3-HOBP (see Fig. 1A and Table 2). Preliminary isomerization studies of the 9,10-oxide (Table 2) have shown a preponderance of 9-HOBP under all conditions, and thus favor radiochemical heterogenicity in the diol 1 fraction. Addition of increasing amounts of epoxide hydrase does not result in a significant decrease in the total amount of quinone formed. Either the arene oxides that ultimately result in quinone production are not substantially hydrated, or a non-arene-oxide pathway to the metabolites in quinone 1 fraction pertains.

The activity of the reconstituted system (Exp. 4), measured radiochemically by phenol 2 production *only*, is about equivalent to the activity as measured by fluorimetry in terms of 3-HOBP (34). The comparison calculated from phenol 2 alone fails to take into account fluorescent 2- and 9-HOBP, which appear in the phenol 1 fraction. When both phenol peaks are considered, total phenol production is higher than when measured by fluorimetry. Although the fluorimetric assay for metabolism of BP has provided an adequate assay for the production of fluorescent phenols, it is inadequate when attempting to critically correlate metabolism with cytotoxicity and carcinogenicity of BP, since it fails to differentiate between the various fluorescent phenols formed or to take into account the nonphenolic metabolites (80% in Exp. 7).

BP metabolism results have sometimes been misintepreted due to incomplete characterization of metabolites (11–13). Despite an early warning by Berenblum and Schoental (37), numerous mistakes in describing the metabolism of BP have been made through over-reliance on spectroscopic techniques such as fluorimetry, ultraviolet spectra, and even mass spectra, which provide limited structural information in the absence of adequate reference standards. Application of the techniques described in this paper, followed by additional chromatographic and spectroscopic methods involving the widest possible array of standards, should provide comprehensive and definitive answers to metabolic questions associated with BP.

We thank Mrs. MaryAnn Augustin and Mrs. Cathy Chvasta for their help in the preparation of this manuscript. G.H. is currently at the National Institutes of Health on sabbatical leave from the University of Sydney, and supported in part by a Fogarty International Fellowship. P.D. is a Guest Worker at the National Institutes of Health, under the auspices of CNRS and NATO postdoctoral fellowships.

1. Committee on the Biologic Effects of Atmospheric Pollutants (1972) *Particulate Polycyclic Organic Matter*, (Nat. Acad. Sci., Washington, D.C.).
2. Cook, J. W., Hewett, C. L. & Hieger, I. (1933) "The isolation of a cancer-producing hydrocarbon from coal tar. Part II. Isolation of 1:2- and 4:5-benzpyrene, perylene, and 1:2-benzanthrene," *J. Chem. Soc.* 396–398.
3. Berenlbum, I. & Schoental, R. (1946) "The metabolism of 3,4-benzpyrene into 8- and 10-benzpyrenols in the animal body," *Cancer Res.* 6, 699–706.

4. Weigert, F. & Mottram, J. C. (1946) "The biochemistry of benzpyrene. II. The course of its metabolism and the chemical nature of the metabolites," *Cancer Res.* **6**, 109–120.
5. Cook, J. W., Ludwiczak, R. S. & Schoental, R. (1950) "Polycyclic aromatic hydrocarbons. Part XXXVI. Synthesis of the metabolic oxidation products of 3:4-benzpyrene," *J. Chem. Soc.* 1112–1121.
6. Falk, H. L., Kotin, P., Lee, S. S. & Nathan, A. (1962) "Intermediary metabolism of benzo[a]pyrene in the rat," *J. Nat. Cancer Inst.* **28**, 699–724.
7. Pihar, O. & Spaleny, J. (1956) "Biooxidation studies. IV. 6-Hydroxybenzo[a]pyrene, a new metabolite of benzo-[a]pyrene," *Chem. Listy* **50**, 296–301.
8. Tarbell, D. S., Brooker, E. G., Seifert, P., Vanterpool, A., Claus, C. J. & Conway, W. (1956) "Studies on the metabolic products obtained from mouse skin after painting with 3,4-benzpyrene," *Cancer Res.* **16**, 37–47.
9. Raha, C. R. (1972) "Metabolism of benzo(a)pyrene at the 4,5-position," *Ind. J. Biochem. Biophys.* **9**, 105–110.
10. Conney, A. H., Miller, E. C. & Miller, J. C.(1957) "Substrate-induced synthesis and other properties of benzpyrene hydroxylase in rat liver," *J. Biol. Chem.* **228**, 753–766.
11. Sims, P. (1967) "The metabolism of benzo[a]pyrene by rat-liver microsomes," *Biochem. Pharmacol.* **16**, 613–618.
12. Sims, P. (1968) "The synthesis of 8- and 9-hydroxybenzo-[a]pyrene and the role of the products in benzo[a]pyrene metabolism," *J. Chem. Soc.* (C), 32–34.
13. Waterfall, J. F. & Sims, P. (1972) "Epoxy derivatives of aromatic polycyclic hydrocarbons," *Biochem. J.* **128**, 265–277.
14. Grover, P. L., Hewer, A. & Sims, P. (1972) "Formation of K-region epoxides as microsomal metabolites of pyrene and benzo[a]pyrene," *Biochem. Pharmacol.* **21**, 2713–2726.
15. Borgen, A., Darvey, H., Castagnoli, N., Crocker, T. T., Rasmussen, R. E. & Wang, I. Y. (1973) "Metabolic conversion of benzo[a]pyrene by Syrian hamster liver microsomes and binding of metabolites to deoxyribonucleic acid," *J. Med. Chem.* **16**, 502–506.
16. Kinoshita, N., Shears, B. & Gelboin, H. V. (1973) "K-Region and non-K-region metabolism of benzo(a)pyrene by rat liver microsomes," *Cancer Res.* **33**, 1937–1944.
17. Flesher, J. W. & Sydnor, K. L. (1973) "Possible role of 6-hydroxy-benzo[a]pyrene as proximate carcinogen of benzo-[a]pyrene and 6-methylbenzo[a]pyrene," *Int. J. Cancer* **11**, 433–437.
18. Lubet, R. A., Brown, D. Q. & Kouri, R. E. (1973) "The role of 3-OH benzo[a]pyrene in mediating benzo[a]pyrene induced toxicity and transformation in cell culture," *Res. Commun. Chem. Pathol. Pharmacol.* **6**, 929–942.
19. Nagata, C., Tagashira, Y., Kodama, M., Ioki, Y. & Oboshi, S. (1973) "Effect of hydrogen peroxide, Fenton's reagent, and iron ions on the carcinogenicity of 3,4-benzopyrene," *Gann* **64**, 277–285.
20. Gelboin, H. V. (1969) "A microsome-dependent binding of benzo(a)pyrene to DNA," *Cancer Res.* **29**, 1272–1276.
21. Lesko, S. A., Jr., Ts'o, P. O. P. & Umans, R. S. (1969) "Interaction of nucleic acids. V. Chemical linkage of 3,4-benzpyrene to deoxyribonucleic acid in aqueous solution," *Biochemistry* **8**, 2291–2298.
22. Meunier, M. & Chauveau, J. (1973) "Binding of benzo(a)-pyrene metabolite(s) to main and satellite calf thymus DNA's *in vitro*," *FEBS Lett.* **31**, 327–331.
23. Wang, I. Y., Rasmussen, R. E. & Crocker, T. T. (1972) "Isolation and characterization of an active DNA-binding metabolite of benzo(a)pyrene from hamster liver microsomal incubation systems," *Biochem. Biophys. Res. Commun.* **49**, 1142–1149.
24. Grover, P. L., Sims, P., Huberman, E., Marquardt, H., Kuroki, T. & Heidelberger, C. (1971) "*In vitro* transformation of rodent cells by K-region derivatives of polycyclic hydrocarbons," *Proc. Nat. Acad. Sci. USA* **68**, 1098–1101.
25. Marquardt, H., Kuroki, T., Huberman, E., Selkirk, J. K., Heidelberger, C., Grover, P. L. & Sims, P. (1972) "Malignant transformation of cells derived from mouse prostate by epoxide and other derivatives of polycyclic hydrocarbons," *Cancer Res.* **32**, 716–720.
26. Jerina, D. M., Daly, J. W., Witkop, B., Zaltzman-Nirenberg, P. & Udenfriend, S. (1970) "1,2-Naphthalene oxide as an intermediate in the microsomal hydroxylation of naphthalene," *Biochemistry* **9**, 147–156.
27. Selkirk, J. K., Huberman, E. & Heidelberger, C. (1971) "An epoxide is an intermediate in the microsomal metabolism of the chemical carcinogen, dibenz(a,h)anthracene," *Biochem. Biophys. Res. Commun.* **43**, 1010–1016.
28. Grover, P. L., Hewer, A. & Sims, P. (1971) "Epoxides as microsomal metabolites of polycyclic hydrocarbons," *FEBS Lett.* **18**, 76–80.
29. Jerina, D. M. & Daly, J. W. (1974) "Arene oxides: A new aspect of drug metabolism," *Science*, **185**, 573–582.
30. Jerina, D. M., Kaubisch, N. & Daly, J. W. (1971) "Arene oxides as intermediates in the metabolism of aromatic substrate: Alkyl and oxygen migrations during isomerization of alkylated arene oxides," *Proc. Nat. Acad. Sci. USA* **68**, 2545–2548.
31. Kaubisch, N., Daly, J. W. & Jerina, D. M. (1972) "Arene oxides as intermediates in the oxidative metabolism of aromatic compounds. Isomerization of methyl-substituted arene oxides," *Biochemistry* **11**, 3080–3088.
32. Jerina, D. M., Yagi, H. & Daly, J. W. (1973) "Arene oxides-oxepins," *Heterocycles* **1**, 267–326.
33. Selkirk, J. K., Croy, R. G. & Gelboin, H. V. (1974) "Benzo-[a]pyrene metabolites: Efficient and rapid separation by high-pressure liquid chromatography," *Science* **184**, 169–171.
34. Levin, W., Ryan, D., West S. & Lu, A. Y. H. (1974) "Preparation of partially purified, lipid-depleted cytochrome P-450 and reduced nicotinamide adenine dinucleotide phosphate-cytochrome *c* reductase from rat liver microsomes," *J. Biol. Chem.* **249**, 1747–1754.
35. Dansette, P. M., Jerina, D. M., Daly, J. W., Levin, W., Lu, A. Y. H., Kuntzman, R. & Conney, A. H. "Assay and partial purification of epoxide hydrase from rat liver microsomes," *Arch. Biochem. Biophys.*, in press.
36. Alvares, A. P., Schilling, G., Levin, W. & Kuntzman, R. (1968) "Alteration of the microsomal hemoprotein by 3-methylcholanthrene: Effects of ethionine and actinomycin D," *J. Pharmacol. Exp. Ther.* **163**, 417–424.
37. Berenblum, I. & Schoental, R. (1955) "Metabolism of 3,4-Benzopyrene," *Science* **122**, 470.
38. Oesch, F., Jerina, D. M. & Daly, J. (1971) "A radiometric assay for hepatic epoxide hydrase activity with [7-^{3}H]styrene oxide," *Biochim. Biophys. Acta* **227**, 685–691.