

# The Direct Spectrophotometric Observation of Benzo(a)pyrene Phenol Formation by Liver Microsomes<sup>1</sup>

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## SUMMARY

Optical spectral repetitive scan analysis during the oxidative metabolism of benzo(a)pyrene by liver microsomal suspensions reveals the time-dependent formation of an intermediate(s) of which the visible spectra resemble those of several benzo(a)pyrene phenols. Liver microsomes from 3-methylcholanthrene-treated rats showed a greater rate of formation of the phenols than did microsomes from control animals; the rate of formation catalyzed by liver microsomes from phenobarbital-pretreated rats was intermediate. When 3-hydroxybenzo(a)pyrene was used as a standard for comparison of activity, the rates of formation of phenols were compared when measured by fluorometric, spectrophotometric, or high-pressure liquid chromatographic analytical techniques. An epoxide hydrase inhibitor, 1,1,1-trichloropropene-2,3-oxide, enhanced phenol formation regardless of the source of liver microsomes, and 7,8-benzoflavone inhibited control and 3-methylcholanthrene-induced microsomal metabolism of benzo(a)pyrene. 7,8-Benzoflavone did not effect benzo(a)pyrene metabolism by liver microsomes from phenobarbital-pretreated rats. The effect of inhibitors on the spectrophotometric assay correlates well with the results obtained from benzo(a)pyrene metabolite analysis using high-pressure liquid chromatography.

## INTRODUCTION

Benzo(a)pyrene and a number of polycyclic aromatic hydrocarbons induce cancer in many tissues of laboratory animals and humans (12), and interest in the molecular mechanism of polycyclic hydrocarbon carcinogenesis has generated considerable information relating to the metabolism of benzo(a)pyrene and 3-MC.<sup>2</sup> The oxidative metabolism of benzo(a)pyrene can be measured by 3 methods: fluorometric analysis which measures only phenol formation (2, 6, 10, 18), radiometric assays which measure total metabolism (3, 4), and HPLC or thin-layer chromatography which measures the various classes of metabolites (5, 14, 17). All 3 methods involve several steps during analysis, and

the rates of formation of metabolites cannot be observed directly. This report demonstrates that the formation of some benzo(a)pyrene phenols can be monitored directly, using spectrophotometric techniques. The rates of formation are affected by animal pretreatment, activators, or inhibitors in the same manner as the other methods available to measure the oxidation of benzo(a)pyrene.

## MATERIALS AND METHODS

NADH and NADPH were purchased from P-L Biochemicals, Milwaukee, Wis., and sodium isocitrate, isocitrate dehydrogenase (type IV), and Tris were purchased from Sigma Chemical Co., St. Louis, Mo. Benzo(a)pyrene, 3-MC, BF, and TCPO were obtained from the Aldrich Chemical Company, Milwaukee, Wis. [<sup>14</sup>C]-7,10-Benzo(a)pyrene was purchased from Amersham/Searle, Arlington Heights, Ill., and authentic benzo(a)pyrene metabolite standards (1-, 3-, 6-, and 9-phenols; 4,5-, 7,8-, and 9,10-diols; and 1,6-, 3,6-, and 6,12-quinones) were obtained from the National Cancer Institute, Bethesda, Md. Solvents were purchased from Burdick & Jackson Laboratories, Muskegon, Mich.

Male Sprague-Dawley rats (150 to 250 g) were obtained from the Charles River Breeding Laboratory, Wilmington, Mass.; microsome preparation was performed as previously reported (9). The animals were pretreated by i.p. injection of corn oil, PB in 0.9% NaCl solution (80 mg/kg), or 3-MC in corn oil (20 mg/kg) for 4 days and were starved for 18 hr prior to sacrifice. Protein concentration was determined by the method of Lowry *et al.* (8) and the cytochrome P-450 content of microsomes was measured by the method of Omura and Sato (11).

A standard reaction mixture, containing 5 mM sodium isocitrate, 5 mM MgCl<sub>2</sub>, 5 μM MnCl<sub>2</sub>, 80 μM benzo(a)pyrene (10 μl in acetone), 0.8 units isocitrate dehydrogenase per ml, and 0.3 mg microsomes per ml in 0.05 M Tris-chloride buffer, pH 7.4, was incubated at 37°. The fluorescent and liquid chromatograph assays were initiated by addition of NADPH (1 mM), and the reaction was terminated by addition of aliquots of the reaction mixture to an equal volume of chilled acetone. The specific enzymatic activity was obtained using the linear portion of several time points between 0 and 6 min. All experiments reported were the average of 3 preparations of liver microsomes, and the average error was less than 10% in all cases.

The fluorescence assay used was a modification of the method of Dehnen *et al.* (2), and the analysis of metabolites, using HPLC, was similar to the method reported by Selkirk

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<sup>2</sup> The abbreviations used are: 3-MC, 3-methylcholanthrene; HPLC, high-pressure liquid chromatography; BF, 7,8-benzoflavone; TCPO, 1,1,1-trichloropropene-2,3-oxide; PB, phenobarbital.

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et al. (14) or Holder et al. (5), except that a Waters Associates Model ALC-202/401 liquid chromatograph (Milford, Mass.) and a 0.25-in.  $\times$  1-ft.  $\mu$ Bondapak/C<sup>18</sup> column were used. The [<sup>14</sup>C]7,10-benzo(a)pyrene used in the HPLC assay had specific activities ranging from 0.6 to 3.2 mCi/mole. The metabolic reactions were monitored by removing 1-ml aliquots every 40 sec during the 1st 6 min and mixing with an equal volume of chilled acetone. Terminated reaction mixtures were extracted with ethyl acetate, filtered through 5- $\mu$ m solvent-inert Millipore filters, and evaporated to dryness with N<sub>2</sub>. The sample, in a small volume of methanol, was injected onto the column and eluted by a 60 to 80% methanol in water gradient run at a 0.67% change/min with a 1.4 ml/min flow rate (25°). Samples were collected and quantitated using liquid scintillation techniques. The radioactivity recovered in the ethyl acetate phase was >99%, and the total recovery on HPLC analysis was >93% in all cases.

Repetitive scan spectrophotometric analyses were performed using an Aminco DW-2 spectrophotometer in the split-beam mode thermostatted at 37°. The microsomal suspension containing all components except NADPH was carefully divided into the sample and reference cuvetts. After establishing a baseline of equal light absorption, NADPH (100  $\mu$ M final concentration) was added to the sample cuvet, and the spectra were scanned at a rate of 5 to 10 nm/sec. The absorbance contribution of cytochrome *b<sub>5</sub>* in the visible region of the spectra was compensated by addition of 100  $\mu$ M NADH to the contents of both cuvetts. The presence of NADH caused the rate of formation of benzo(a)pyrene metabolites to increase by less than 15%. The rates of formation could also be determined by monitoring, with time, the absorbance at 428 nm relative to 454 nm in the dual-beam mode.

## RESULTS AND DISCUSSION

During the enzymatic oxidation of benzo(a)pyrene by liver microsomes from 3-MC- or PB-treated animals, a time-dependent increase in absorbance at 400 to 460 nm can be observed using turbid sample difference spectrophotometric techniques (Charts 1 and 2). A decrease in absorbance was noted at wavelengths above 454 nm, which may be attributed in part to the conversion of benzo(a)pyrene to products in the sample cuvet; benzo(a)pyrene has an appreciable absorbance at these wavelengths. NADH was added to remove the contribution of reduced cytochrome *b<sub>5</sub>* from the difference spectra, and the effect of NADH as a synergist (1) of the NADPH-dependent rate of formation was less than a 15% increase. Certain preparations of microsomes elicited these typical spectra for the 1st 6 to 8 min and then showed a time-dependent decrease in absorbance at 418 nm. This phenomenon may be the result of further metabolism of benzo(a)pyrene metabolites and will be the subject of future research.

The absorbance increases at 400 to 460 nm (Charts 1 and 2) are similar to the absolute spectra of various benzo(a)pyrene phenols reported by Jerina et al. (7). A mixture of 3- and 9-hydroxybenzo(a)pyrene in the presence of liver microsomes and benzo(a)pyrene has a spectrum similar to those obtained during the oxidative metabolism of benzo(a)pyrene by liver microsomes (Chart 3A). The

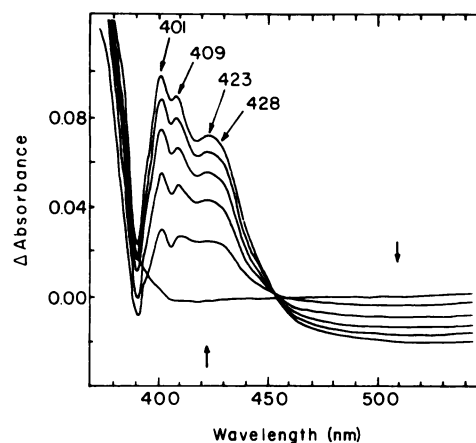


Chart 1. The oxidative metabolism of benzo(a)pyrene by 3-MC-induced liver microsomes. Repetitive scan analysis was performed with a new scan initiated every 2 min. The samples were adjusted to equal light intensity at 454 nm prior to initiation of the reaction (see "Materials and Methods"). Left ordinate, change in absorbance ( $\Delta$  Absorbance) resulting from the metabolism of benzo(a)pyrene upon addition of NADPH to the sample cuvet.

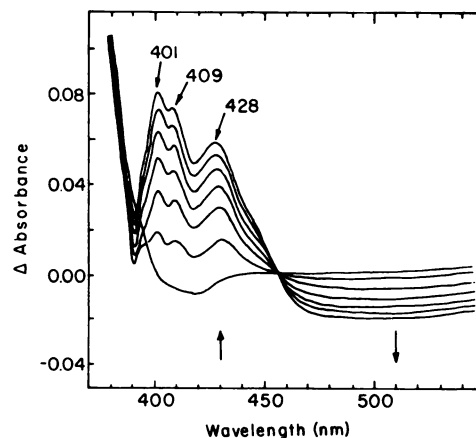


Chart 2. The oxidative metabolism of benzo(a)pyrene by PB-induced liver microsomes. The repetitive scan analysis was performed as described in Chart 1. Left ordinate, change in absorbance ( $\Delta$  Absorbance) resulting from the metabolism of benzo(a)pyrene upon addition of NADPH to the sample cuvet.

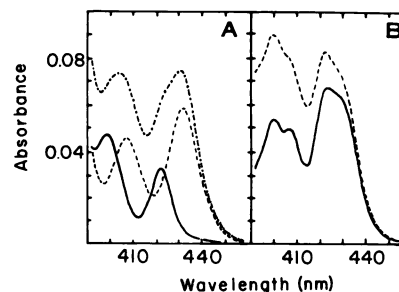


Chart 3. The spectra of benzo(a)pyrene phenols in the presence of 3-MC-induced liver microsomes. In A, a solution containing microsomes (0.3 mg/ml) and 80  $\mu$ M benzo(a)pyrene was placed in the sample and reference cuvetts. The samples were adjusted to equal light intensity at 454 nm, phenols were added, and spectra were recorded. —, 1.7  $\mu$ M 9-hydroxybenzo(a)pyrene; ---, 5.4  $\mu$ M 3-hydroxybenzo(a)pyrene; - · -, 1.7  $\mu$ M 9-hydroxy- and 5.4  $\mu$ M 3-hydroxybenzo(a)pyrene. In B, the samples were prepared as in A, and spectra were recorded after addition of phenols or benzo(a)pyrene. ---, 2.1  $\mu$ M 9-hydroxy- and 5.4  $\mu$ M 3-hydroxybenzo(a)pyrene; —, 2.1  $\mu$ M 9-hydroxy- and 5.4  $\mu$ M 3-hydroxybenzo(a)pyrene in the sample cuvet, and 2.0  $\mu$ M benzo(a)pyrene in the reference cuvet.

addition of a small amount of benzo(a)pyrene to the contents of the reference cuvet to simulate the loss of benzo(a)pyrene results in a spectrum that has multiple absorption maxima nearly identical to those obtained during benzo(a)pyrene oxidation (Chart 3b). In this case, a mixture of 3- and 9-hydroxybenzo(a)pyrene gives absorption maxima at 401, 409, and approximately 428 nm. The similarity between the absolute spectra of several benzo(a)pyrene phenols and the absorbance increases at 400 to 460 nm during metabolism suggests that the formation of some phenols can be directly monitored by spectrophotometric methods.

The effect of animal pretreatment on the rate of phenol formation was as expected (Table 1) from the fluorescent measurements on benzo(a)pyrene metabolism (9). The rates of product formation obtained were maximal with microsomes from animals treated with 3-MC, were intermediate for PB-induced microsomes, and were lowest with control microsomes. The rate measurements were obtained by monitoring the absorption at 428 nm under our conditions, relative to an isosbestic point at 454 nm, and the linear initial rate was converted to an activity assuming that 3-hydroxybenzo(a)pyrene has a differential extinction coefficient of  $13,200 \text{ M}^{-1} \text{ cm}^{-1}$ .<sup>3</sup> Of the metabolites available, only 3-hydroxybenzo(a)pyrene had an appreciable absorbance at 428 nm under our conditions, and we have assumed that the spectrophotometric assay method described measured principally the 3-hydroxybenzo(a)pyrene. However, Jerina *et al.* (7) suggest that, in methanol, several other phenols may have appreciable absorption at wavelengths above 420 nm. The absorbance spectra of all 12 benzo(a)pyrene phenols in microsomal suspensions have been recorded; only the 3-, 9-, and 11-phenols have major absorption transitions above 415 nm, and the 1- and 6-phenols have a low absorbance above 415 nm (unpublished results). The wavelength maxima for the 3-, 9-, and 11-phenols of benzo(a)pyrene in the region between 420 and 440 nm are 429, 421, and 421 nm, respectively. A comparison of the rates of formation of phenols determined by the fluorometric, spectrophotometric, and HPLC methods is also shown in Table 1. The 2nd phenol liquid chromatography peak, which had a retention time identical with that of 3-hydroxybenzo(a)pyrene, was used for comparison. The fluorescent assay, which under alkaline conditions can measure preferentially the 3- and 9-hydroxybenzo(a)pyrene (6), showed some differences, suggesting that the 2 assays may measure different populations of the benzo(a)pyrene phenols.

The rate of formation of the 2nd phenol peak obtained by HPLC separation agrees closely with the spectrophotometric assay illustrated in Chart 1. Selkirk *et al.* (15) recently have shown that the 2nd phenol peak may contain measurable amounts of the 1- and 7-hydroxybenzo(a)pyrene. Since all of the phenols have nearly identical extinction coefficients at 254 nm (7),<sup>4</sup> their results suggest that 1- and 7-hydroxybenzo(a)pyrene are minor components of the 2nd

HPLC peak. Based on these reports, the spectrophotometric assay probably measures largely 3-hydroxybenzo(a)pyrene formation, but the contribution of other phenols can not be definitively ruled out. When microsomes from 3-MC- or PB-treated rats are used, close agreement between all 3 methods exists when the rates are expressed based on mg protein or nmoles cytochrome P-450. This result suggests that the major component of the 2nd HPLC phenol peak probably is the maximum contributor to the phenol species being measured by the fluorescent and spectrophotometric assays. However, the low rate of formation of phenols measured by the spectrophotometric technique with control microsomes indicates that 3-hydroxybenzo(a)pyrene may not be the major metabolite under that condition.

**The Effect of Inhibitors on Benzo(a)pyrene Phenol Formation.** The effect of several inhibitors of microsomal reactions on the spectrophotometric assay is shown in Table 2. TCPO, an epoxide hydrase inhibitor, caused a 10 to 20% stimulation of the rate of phenol formation catalyzed by liver microsomes from all 3 pretreatments. BF (100  $\mu\text{M}$ ) inhibited phenol formation catalyzed by liver microsomes from 3-MC-treated rats but did not significantly inhibit the reaction of PB-induced microsomes. The NADPH-cytochrome c (P-450) reductase inhibitor, adenosine-2'-monophosphate, inhibited phenol formation catalyzed by all microsome preparations, suggesting that the flavoprotein reductase involved in phenol formation is NADPH-cytochrome c (P-450) reductase (13).

Table 1  
The rates of benzo(a)pyrene phenol formation by rat liver microsomes

Treatment	Assay		
	Fluorescent <sup>a</sup>	Spectrophotometric <sup>b</sup>	HPLC <sup>c</sup>
Control	2.30 (1.90)	1.03 (0.94)	1.73 (1.57)
PB	2.90 (0.95)	2.82 (0.94)	2.55 (0.83)
3-MC	3.70 (1.68)	3.46 (1.57)	3.30 (1.50)

<sup>a</sup> Rates were expressed as nmoles equivalent to 3-hydroxybenzo(a)pyrene formed per min per mg protein. Values in parentheses, nmoles/min/nmol cytochrome P-450. The fluorescence excitation and emission wavelengths were 467 and 518 nm, respectively.

<sup>b</sup> Rates were expressed as nmoles equivalent to 3-hydroxybenzo(a)pyrene formed per min per mg protein. Values in parentheses, nmoles/min/nmole cytochrome P-450. The fluorescence excitation and emission wavelengths were 467 and 518 nm, respectively and 454 nm; the latter was an isosbestic wavelength.

<sup>c</sup> Rates were expressed using the radioactivity in the 2nd phenol peak obtained by HPLC. Values in parentheses, nmoles/min/nmole cytochrome P-450.

Table 2  
Effect of TCPO and BF on phenol formation

	Rate <sup>a</sup>			
	Control	TCPO	BF	2'-AMP
Control	1.28	1.54		0.38
PB	3.06	3.85	2.95	0.92
3-MC	3.80	4.59	1.43	0.95

<sup>a</sup> Rates were expressed as nmoles equivalent to 3-hydroxybenzo(a)pyrene formed per min per mg protein.

<sup>3</sup> In the presence of liver microsomes, 0.3 mg/ml, 3-hydroxybenzo(a)pyrene has a molar absorptivity at 428 nm of  $13,200 \text{ M}^{-1} \text{ cm}^{-1}$ . This is similar to the value of 13,000 to 15,000  $\text{M}^{-1} \text{ cm}^{-1}$  reported by Jerina *et al.* (7) in methanol.

<sup>4</sup> From the data of Jerina *et al.* (7), the 12 benzo(a)pyrene phenols have extinction coefficients at 254 nm on the order of 26,000 to 39,000  $\text{M}^{-1} \text{ cm}^{-1}$ .



Selkirk *et al.* (16) have reported that TCPO enhances the 9-phenol formation by liver microsomes from 3-MC-induced animals as analyzed by HPLC, but they did not see a rate enhancement of the fluorescent analysis of benzo(a)pyrene phenols. TCPO (2 mM) caused a 50% inhibition of total metabolism and completely prevented formation of the 4,5-, 7,8-, and 9,10-dihydrodiols of benzo(a)pyrene. We have repeated their HPLC analysis and extended it to include benzo(a)pyrene metabolism by liver microsomes from control, PB-, and 3-MC-pretreated rats (Table 3). In agreement with results of Selkirk *et al.* (16), BF (100  $\mu$ M) inhibited total metabolism and the formation of each class of benzo(a)pyrene metabolite by 70 to 80% and TCPO-stimulated phenol formation catalyzed by microsomes from 3-MC-induced rats. Further, BF inhibited benzo(a)pyrene metabolism by control liver microsomes by 30 to 40% but had no effect on metabolism by microsomes from PB-treated animals. The effects of BF on benzo(a)pyrene metabolism are in agreement with studies using the fluorimetric assay (19). TCPO (2 mM) did not inhibit total metabolism of benzo(a)pyrene by liver microsomes from any pretreatment regimen; the suppression of dihydrodiol formation was compensated for by a concomitant increase in phenol and quinone formation. The 9-hydroxybenzo(a)pyrene HPLC peak (Peak 1) was increased by 200 to 400% in all cases, but the 3-hydroxybenzo(a)pyrene peak (Peak 2) was only slightly increased (10 to 20%). The differential effect of TCPO on phenol formation is consistent with the observation of Holder *et al.* (5) that incubation mixtures containing a reconstituted liver microsomal mixed function oxidase system and benzo(a)pyrene in the presence of added epoxide hydrase have diminished concentrations of Peak 1 phenols relative to Peak 2 phenols. These results support the suggestion that the dihydrodiols and phenol metabolites (particularly Peak 1 metabolites) share arene oxides as common precursors.

This report demonstrates that benzo(a)pyrene phenol formation can be monitored spectrophotometrically. The spectra obtained during metabolism resemble the known benzo(a)pyrene phenols, and the rates of phenol formation obtained by measuring the absorbance change at 428 nm

are nearly identical to those obtained by HPLC analysis of the 2nd phenol peak. Animal pretreatment alters the rates of phenol formation measured by the 2 methods to the same extent, and compounds like TCPO and BF have similar effects on results obtained with either assay method. Based on the metabolite analysis by HPLC recycle techniques (15) and the physicochemical data from the uniquely synthesized benzo(a)pyrene phenols (7), the spectrophotometric assay presented here may allow the direct observation of 3-hydroxybenzo(a)pyrene formation. The effect of compounds like TCPO or BF on the rate of phenol formation measured by the 3 methods further supports the contention that phenol measurement at 428 nm most probably reflects the concentration of 3-hydroxybenzo(a)pyrene.

HPLC analysis of benzo(a)pyrene metabolism demonstrates that TCPO does not affect the total metabolism of benzo(a)pyrene but enhances quinone and phenol formation at the expense of dihydrodiol formation. In agreement with the report of Selkirk *et al.* (16), the 1st phenol peak of reverse-phase HPLC analysis, which contains 9-hydroxybenzo(a)pyrene, is enhanced to a greater extent than is the 2nd phenol peak when the oxidation of benzo(a)pyrene occurs in the presence of 2 mM TCPO. Although BF does not effect benzo(a)pyrene metabolism by liver microsomes from control or PB-treated rats, it does inhibit the formation of all metabolites with microsomes from 3-MC-treated rats by 70 to 80%, suggesting that this compound inhibits equally the oxidation of the aromatic hydrocarbon at various sites on the molecule.

It has been suggested that multiple forms of purified cytochrome P-450 exhibit site specificity toward benzo(a)pyrene oxidation (20). Wiebel *et al.* (20) have shown that 3 forms of purified cytochrome P-450 catalyzed the formation of the 2 classes of benzo(a)pyrene phenols (defined by HPLC separation), and the relative rates of formation of the 2 classes of phenols depended on the form of cytochrome used. The equal inhibitory action of BF on the various site-specific oxidations of benzo(a)pyrene by liver microsomes from 3-MC-treated rats and the relative lack of inhibition of the oxidation by microsomes from control or PB-treated rats shown in this report suggest that the puri-

Table 3

*Effect of BF and TCPO on total benzo(a)pyrene metabolism*

The products were analyzed as described in "Materials and Methods." The rates were expressed as nmoles product formed per min per mg protein. The cytochrome P-450 content for control, PB- and 3-MC-induced microsomes were 1.0, 2.9, and 2.6 nmoles/mg protein, respectively.

Pretreatment	Inhibitor	Diol			Quinones	Phenols		Total
		1	2	3		1	2	
Control		0.40	0.11	0.21	1.49	0.33	2.18	4.72
	BF	0.14	0.07	0.13	1.15	0.27	1.09	2.85
	TCPO				1.74	0.73	2.09	4.56
PB		0.35	0.49	0.21	2.16	0.32	2.11	5.64
	BF	0.29	0.40	0.20	2.37	0.29	1.90	6.28
	TCPO				2.78	1.28	2.53	6.59
3-MC		1.64	0.68	0.78	1.92	1.46	3.53	10.01
	BF	0.53	0.18	0.31	0.89	0.43	1.01	3.35
	TCPO				2.74	3.15	3.79	9.68

fied forms described (20) may represent only a few of the classes of cytochrome P-450 molecules present in liver microsomes or that the membrane may alter the substrate site specificity of cytochrome P-450.

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## REFERENCES

- Cohen, B. S., and Estabrook, R. W. Microsomal Electron Transport Reaction. III. Cooperative Interactions between Reduced Diphosphopyridine Nucleotide and Reduced Triphosphopyridine Nucleotide Linked Reactions. *Arch. Biochem. Biophys.*, **143**: 54-65, 1971.
- Dehnen, W., Tomingas, R., and Roos, J. A Modified Method for the Assay of Benzo(a)pyrene Hydroxylase. *Anal. Biochem.*, **53**: 373-383, 1973.
- DePierre, J. W., Moron, M. S., Johannesen, A. M., and Ernster, L. A Reliable, Sensitive, and Convenient Radioactive Assay for Benzpyrene Monooxygenase. *Anal. Biochem.*, **63**: 470-484, 1975.
- Hayakawa, T., and Udenfriend, S. A Simple Radioisotope Assay for Microsomal Aryl Hydroxylase. *Anal. Biochem.*, **51**: 501-509, 1973.
- Holder, G., Yagi, H., Dansette, P., Jerina, D. M., Levin, W., Lu, A. Y. H., and Conney, A. H. Effects of Inducers and Epoxide Hydrase on the Metabolism of Benzo(a)pyrene by Liver Microsomes and a Reconstituted System: Analysis by High Pressure Liquid Chromatography. *Proc. Natl. Acad. Sci. U. S. A.*, **71**: 4356-4360, 1974.
- Holder, G., Yagi, H., Levin, W., Lu, A. Y. H., and Jerina, D. M. Metabolism of Benzo(a)pyrene. III. An Evaluation of the Fluorescence Assay. *Biochem. Biophys. Res. Commun.*, **65**: 1363-1370, 1975.
- Jerina, D. M., Yagi, H., Hernandez, O., Dansette, P., Wood, A. W., Levin, W., Chang, R. L., Wislocki, P. G., and Conney, A. H. Synthesis and Biological Activity of Potential Benzo(a)pyrene Metabolites. In: R. I. Freudenthal and P. W. Jones (eds.), *Symposium on Polynuclear Aromatic Hydrocarbons*. Battelle-Columbus Laboratories, October 15 to 17, 1975, Vol. 1, pp. 91-113. New York: Raven Press, 1976.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.*, **193**: 265-275, 1951.
- Matsubara, T., Prough, R. A., Burke, M. D., and Estabrook, R. W. The Preparation of Microsomal Fractions of Rodent Respiratory Tract and Their Characterization. *Cancer Res.*, **34**: 2196-2203, 1974.
- Nebert, D. W., and Gelboin, H. V. Substrate-inducible Microsomal Aryl Hydroxylase in Mammalian Cell Culture. *J. Biol. Chem.*, **243**: 6242-6249, 1968.
- Omura, T., and Sato, R. The Carbon Monoxide-Binding Pigment of Liver Microsomes. I. Evidence for Its Hemoprotein Nature. *J. Biol. Chem.*, **239**: 2370-2378, 1974.
- Particulate Polycyclic Organic Matter. Washington, D. C.: National Academy of Sciences, 1972.
- Phillips, A. H., and Langdon, R. G. Hepatic Triphosphopyridine Nucleotide-Cytochrome c Reductase: Isolation, Characterization and Kinetic Studies. *J. Biol. Chem.*, **237**: 2652-2660, 1962.
- Selkirk, J. K., Croy, R. G., and Gelboin, H. V. Benzo(a)pyrene Metabolites: Efficient and Rapid Separation by High-Pressure Liquid Chromatography. *Science*, **184**: 169-171, 1974.
- Selkirk, J. K., Croy, R. G., and Gelboin, H. V. High-Pressure Liquid Chromatographic Separation of 10 Benzo(a)pyrene Phenols and the Identification of 1-Phenol and 7-Phenols as New Metabolites. *Cancer Res.*, **36**: 922-926, 1976.
- Selkirk, J. K., Croy, R. G., Roller, P. P., and Gelboin, H. V. High-Pressure Liquid Chromatographic Analysis of Benzo(a)pyrene Metabolism and Covalent Binding and the Mechanism of Action of 7,8-Benzoflavone and 1,2-Epoxy-3,3,3-trichloropropane. *Cancer Res.*, **34**: 3474-3480, 1974.
- Wang, I. Y., Rasmussen, R. E., and Crocker, T. T. 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, 1972.
- Wattenberg, L. W., Leong, J. L., and Strand, P. J. Benzpyrene Hydroxylase Activity in the Gastrointestinal Tract. *Cancer Res.*, **22**: 1120-1125, 1962.
- Wiebel, F. J., Leutz, J. C., Diamond, L., and Gelboin, H. V. Aryl Hydrocarbon (Benzo(a)pyrene) Hydroxylase in Microsomes from Rat Tissues: Differential Inhibition and Stimulation of Benzoflavones and Organic Solvents. *Arch. Biochem. Biophys.*, **144**: 78-86, 1971.
- Wiebel, F. J., Selkirk, J. K., Gelboin, H. V., Haugen, D. A., Van Der Hoeven, T. A., and Coon, M. J. Position-Specific Oxygenation of Benzo(a)pyrene by Different Forms of Purified Cytochrome P-450 from Rabbit Liver. *Proc. Natl. Acad. Sci. U. S. A.*, **72**: 3917-3920, 1975.

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