Biochemical Pharmacology. Vol. 27, pp. 1865–1871.

0006-2952/78/0715-1865$02.00/0

STUDIES ON THE HEPATIC MICROSOMAL METABOLISM OF [^{14}C]PHENANTHRENE

S. CHATURAPIT and G. M. HOLDER*
Department of Pharmacy, University of Sydney, N.S.W. 2006, Australia

(*Received* 15 *September* 1977; *accepted* 28 *February* 1978)

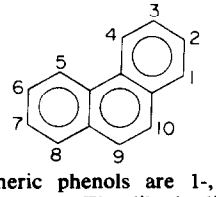
Abstract—[^{14}C]Phenanthrene was metabolished *in vitro* by hepatic microsomes obtained from untreated, sodium phenobarbital (PB), and 3-methylcholanthrene (MC) pretreated Wistar rats, guinea pigs, SW and DBA/2J mouse strains. Metabolite profiles were obtained by comparison of thinlayer radiochromatographs of the products with R_fs of authentic reference compounds. *trans*-dihydrodihydroxyphenanthrenes (dihydrodiols) were the major metabolites in all preparations. In every case, except with microsomes from MC pretreated guinea pigs, *trans* - 9,10 - dihydro - 9,10 - dihydroxyphenanthrene predominated. With microsomes from MC pretreated guinea pigs, the 1,2-dihydrodiol was the major metabolite. Addition of cyclohexene oxide (5.0 mM) to the incubation mixture inhibited dihydrodiol formation in rat and mouse preparations by 90 per cent, and in guinea pig preparations by 70 per cent. Phenols and phenanthrene - 9,10 - oxide were produced instead.

The prevalance of polycyclic aromatic hydrocarbons as environmental pollutants, and the potency of some of these compounds as carcinogens in experimental animals[1] have led to considerable interest in the matabolism of these compounds and their interaction with cellular constituents. One theory accounting for the carcinogenicity of these compounds that has received considerable experimental support is that of the *in vivo* production of reactive intermediates. These intermediates are thought to interact with cellular macromolecules and subsequent transformation of the cell to malignancy follows[2, 3]. The compound perhaps most widely studied is benzo[a]pyrene. Detailed *in vitro* metabolism profiles have appeared for preparations of rat and mouse liver, and mouse lung[4–6], and evidence has accumulated that a dihydrodiol epoxide may be the ultimate carcinogen[7]. Phenanthrene is not carcinogenic, but, as the simplest angular polycyclic aromatic hydrocarbon, its structure resembles those of benz[a]anthracene, dibenz[a,h]anthracene and benzo[a]pyrene. Benz[a]anthracene is weakly carcinogenic while dibenz[a,h]anthracene and benzo[a]pyrene are strongly carcinogenic. *In vivo* metabolic studies of phenanthrene have been conducted in rats and rabbits, and the five possible phenols and three dihydrodiols† have been identified as metabolites. These metabolites were excreted as sulphuric acid or glucuronic acid conjugates. Catchols were also identified[8–11]. *In vitro* studies include identification of phenanthrene-9,10-oxide[12] and a quantitative study using rat liver microsomes[13].

In the present study the metabolism of [^{14}C]phenanthrene by liver microsomes from Wistar rat, SW and DBA/2J mouse strains, and guinea pig were examined using untreated, phenobarbital pretreated, and 3-methylcholanthrene pretreated animals. Profiles of primary metabolites obtained are compared to those published using benzo[a]pyrene[4–6] as substrate, and to those obtained using generally tritiated phenanthrene[13].

*To whom all correspondence should be sent.

†The structure of phenanthrene is



The five isomeric phenols are 1-, 2-, 3-, 4- and 9-hydroxyphenanthrene. The dihydrodiols are *trans* - 1,2 - dihydro - 1,2 - dihydroxyphenanthrene (abbreviated phenanthrene - 1,2 - dihydrodiol) and the 3,4- and 9,10-isomers (abbreviated phenanthrene - 3,4 - dihydrodiol and phenanthrene - 9,10 - dihydrodiol respectively). Other abbreviations used are MC, 3-methylcholanthrene; PB, sodium phenobarbital; CHO, cyclohexene oxide.

MATERIALS AND METHODS

Materials. 9-[^{14}C]Phenanthrene was synthesised from [^{14}C]barium carbonate[14, 15] and was obtained with a specific radioactivity of 5.80 mCi/mmol after purification by preparative thin layer chromatography on silica gel. The plate was developed with 7.5% dioxan in light petroleum. Fieser's method was used to prepare 2- and 3-hydroxyphenanthrene[16]. Other standards were generous gifts from Dr. D. M. Jerina of NIH, Bethesda, Md, and were 1-, 4- and 9-hydroxyphenanthrene, phenanthrene-1,2-, 3,4- and 9,10-dihydrodiol, and phenanthrene-9,10-oxide. Thin layer chromatography of metabolites was conducted on silica gel using 0.1% triethylamine in benzene (solvent A) or 0.1% triethylamine in ethyl acetate:cyclohexane (1:1) (solvent B).

Incubation of 9-[^{14}C]phenanthrene with hepatic microsomes. Pooled microsomes were prepared[17] from the livers of the following male animals: Wistar rats (100–150 g), SW-mice (20–25 g), DBA/2J-mice (20–25 g) and guinea pigs (~ 300 g). Animals of each species were randomly divided into three groups each containing 3, 15 or 2 animals for rats, mice or guinea pigs respectively. Those of one group were dosed intraperitoneally

with sodium phenobarbital (PB) (100 mg/kg) in distilled water daily for three days, those of the second group received 3-methylcholanthrene (MC) (20 mg/kg) in arachis oil daily for 3 days, and those in the control third group were untreated. Animals received water *ad lib.* and were starved 24 hr before sacrifice. Microsomes were assayed for their protein[18] and cytochrome P450 (or cytochrome P448) content[19]. The incubation mixture contained microsomal protein (1.0 mg), NADPH (2 μmoles), magnesium chloride (6 μmoles) and potassium phosphate buffer pH 7.4 (150 μmoles) in 2.0 ml. 9-[^{14}C]-Phenanthrene (400 nmoles) in methanol (40 μl), and the epoxide hydratase inhibitor, cyclohexene oxide (CHO) (10 μmol) in methanol (0.1 ml), or pure methanol (0.1 ml), was added to each flask. The incubation mixtures were shaken at 37° in air for 10 min and the reaction stopped by the addition of acetone (2.0 ml).

Estimation of 9-[^{14}C]*phenanthrene metabolites.* Metabolites and unchanged phenanthrene were extracted into ethyl acetate (5.0 ml) by vortexing for 1 min, and the organic layer was separated. After drying with anhydrous sodium sulphate, the bulk of the ethyl acetate was removed under a stream of dry nitrogen, and the residue was divided into three parts and applied to three silica gel thin layer plates together with appropriate standards. Phenanthrene - 9,10 - oxide, the hydroxyphenanthrenes and the dihydrodiols were standards. Phenanthrene - 9,10 - oxide, the hydroxyphenanthrenes and the dihydrodiols were estimated in developing systems I, II and III respectively (Table 1) by measurement of the radioactivity cochromatographing with each standard. This silica gel layers were sectioned into strips, the silica gel suspended in scintillation cocktail (10 ml) using Cab-o-Sil (400 mg) (Packard Instrument Co.), and the radioactivity was measured by liquid scintillation counting. The scintillation cocktail contained 2,5-diphenyloxazole (1.5 g), 1,4 - bis - 2 - (4 - methyl - 5 - phenyloxazolyl)benzene (50 mg), ethanol (100 ml) and toluene to 1 l. Results were corrected using boiled and incubated enzyme blanks.

Table 1. Thin layer chromatographic R_f values of primary metabolites of phenanthrene

Metabolite	R_f in systems* I	II	III
Phenanthrene	0.82	SF†	SF
1-Hydroxyphenanthrene	0.24	0.51	SF
2-Hydroxyphenanthrene	0.16	0.37	SF
3-Hydroxyphenanthrene	0.19	0.41	SF
4-Hydroxyphenanthrene	0.32	0.63	SF
9-Hydroxyphenanthrene	0.25	0.56	SF
Phenanthrene 1,2-dihydrodiol	BL‡	0.05	0.5
Phenanthrene 3,4-dihydrodiol	BL	0.04	0.39
Phenanthrene 9,10-dihydrodiol	BL	0.06	0.68
Phenanthrene 9,10-oxide	0.52	isomerised	SF

* I—Single development with solvent A.
II—Triple development with solvent A.
III—Triple development with solvent B.
See text for details.
† SF—at solvent front.
‡BL—at baseline.

Standards of phenanthrene oxides isomeric with phenanthrene-9,10-oxide, phenanthrene-1,2-oxide and phenanthrene-3,4-oxide, were not used because they isomerise to phenols very much more rapidly than the 9,10-oxide[20] and would not be expected to survive the incubation and analysis conditions. The 9,10-oxide itself could not be chromatographed twice without isomerisation. In the analysis of the hydroxyphenanthrenes, although different R_f values for all five compounds were recorded in system II, separations of 2- from 3-hydroxyphenanthrene, and of 1, from 9-hydroxyphenanthrene were not sufficient to enable each compound to be reliably measured. Several other chromatographic systems tried gave even poorer separation so the two pairs of hydroxyphenanthrenes were measured together.

In replicate determinations of metabolic profiles, results agreed within 5 per cent when measured as percentages of total ethyl acetate soluble metabolites formed. Metabolic profiles presented in Tables 2–5 are means of two identical determinations. Total phenanthrene metabolism was determined as the sum of the water-soluble radioactivity and the organosoluble radioactivity separable from that of phenanthrene in chromatographic system II (Table 1). The results were corrected using the results from boiled incubated enzyme experiments. Preliminary experiments showed that total metabolism measured this way was linear with time for 10 min.

RESULTS

Results of incubations of [^{14}C]phenanthrene with liver microsomes prepared from untreated, PB treated and MC treated rats and guinea pigs are shown in Tables 2 and 3 respectively. Tables 4 and 5 show results with SW strain and DBA/2J strain mouse microsomes. The latter is of interest because animals of this strain are genetically "nonresponsive" to the induction of aryl hydrocarbon hydroxylase by MC[21]. Incubations were performed with all microsomes in the presence and absence of the epoxide hydratase inhibitor CHO[22]. In all incubations the radioactivity was monitored in both the aqueous and ethyl acetate phases, although only the organic phase was used in the subsequent metabolite profile analysis. Residual radioactivity in the aqueous phase after correction for boiled enzyme experiments did not exceed 2.1 per cent of the total and was usually less than 0.7 per cent. Re-extraction of the aqueous phase with a second portion of ethyl acetate failed to significantly decrease this water soluble radioactivity. This radioactivity, which is presumably due to water soluble metabolites, was not examined further. Table 6 shows a summary of the metabolism for all animals studied as total dihydrodiols, total phenols and arene oxide expressed as a proportion of the total ethyl acetate extractable metabolites.

In incubations with microsomes from untreated rats (Tables 2 and 6) dihydrodiols were the predominant metabolites, the 9,10-isomer being the major product. Only small amounts of the arene oxide and phenols were formed. While metabolite

Table 2. [^{14}C]Phenanthrene metabolism by hepatic microsomal fractions prepared from Wistar rat*

Experiment	Per cent metabolised†	Dihydrodiols 1,2-	3,4-	9,10-	Phenols 2- and 3-	1- and 9-	4-	9,10-Oxide	Total‡ per cent
Untreated	3.9	14.7	9.4	64.1	3.4	1.1	1.1	<0.1	93.8
	2.68§								
Untreated + CHO	4.8	0.9	0.4	3.3	0.5	12.9	11.8	56.5	86.3
	3.26§								
PB	13.8	4.1	1.6	83.1	4.0	0.8	1.1	1.2	95.9
	3.92§								
PB + CHO	11.2	0.8	0.3	6.7	4.7	4.9	5.5	73.0	95.9
	3.19§								
MC	18.3	16.1	9.7	65.3	1.9	0.9	2.0	0.8	96.7
	7.40§								
MC + CHO	10.9	1.2	0.6	4.3	6.9	11.7	13.1	59.4	97.2
	4.61§								

*Incubations were carried out as described in the Methods, and metabolic profiles are presented as normalised percentages of the total ethyl acetate soluble metabolites.

†Percentage of incubated [^{14}C]phenanthrene metabolised.

‡Percentage of ethyl acetate soluble metabolites accounted for by cochromatography with standards.

§Enzyme activity of microsomes expressed as nmoles phenanthrene metabolised/min/nmole cytochrome P450 (or cytochrome P448). Microsomes from untreated and PB pretreated animals contained 0.63 and 1.50 nmoles cytochrome P450/mg protein respectively, and microsomes from MC pretreated animals contained 1.04 nmole cytochrome P448/mg protein.

Table 3. [^{14}C]Phenanthrene metabolism by hepatic microsomal fractions prepared from guinea pig*

Experiment	Per cent metabolised†	Dihydrodiols 1,2-	3,4-	9,10-	Phenols 2- and 3-	1- and 9-	4-	9,10-Oxide	Total‡ per cent
Untreated	8.4	21.7	7.8	56.7	2.9	2.3	0.5	<0.1	91.9
	4.60§								
Untreated + CHO	7.1	3.7	1.3	16.3	9.3	20.1	5.8	34.6	91.1
	3.85§								
PB	23.8	3.2	2.7	88.3	1.8	0.4	0.1	0.3	96.8
	8.26§								
PB + CHO	23.3	1.1	0.6	33.6	2.5	5.3	1.7	53.8	98.6
	8.06								
MC	10.4	51.7	16.2	18.9	3.9	1.7	0.3	0.4	93.1
	4.62§								
MC + CHO	7.4	5.8	3.7	8.9	11.2	39.2	4.1	11.5	84.4
	3.38§								

*Incubations were carried out as described in the Methods, and metabolic profiles are presented as normalised percentages of the total ethyl acetate soluble metabolites.

†Percentage of incubated[^{14}C]phenanthrene metabolised.

‡Percentage of ethyl acetate soluble metabolites accounted for by cochromatography with standards.

§Enzyme activity of microsomes expressed as nmoles phenanthrene metabolised/min/nmole cytochrome P450 (or cytochrome P448). Microsomes from untreated and PB pretreated animals contained 0.75 and 1.16 nmoles cytochrome P450/mg protein respectively, and microsomes from MC pretreated animals contained 0.92 nmole cytochrome P448/mg protein.

profiles for microsomes from MC pretreated animals were virtually identical to those from control microsomes, MC microsomes showed greater enzymic activity. This increased activity is analogous to results obtained with rat liver microsomes and benzo[a]pyrene using a radiochromatographic assay[4, 5, 23] or the fluoroescence assay[24, 25].

Incubations carried out in the presence of the epoxide hydratase inhibitor, CHO, were expected to show depressed dihydrodiol formation and lead to the formation of greater amounts of phenols and arene oxides. This was confirmed with incubations of [^{14}C]phenanthrene with liver microsomes prepared from untreated and MC pretreated

Table 4. [^{14}C]Phenanthrene metabolism by hepatic microsomal fractions prepared from SW-mice*

Experiment	Per cent metabolised†	Dihydrodiols 1,2-	3,4-	9,10-	Phenols 2- and 3-	1- and 9-	4-	9,10-Oxide	Total‡ per cent
Untreated	6.7 3.95§	9.0	6.2	17.5	6.2	8.3	17.1	27.3	91.6
Untreated +CHO	6.1 3.57§	0.7	0.6	1.8	9.7	15.8	18.4	41.0	88.0
PB	13.9 6.12§	5.9	3.9	38.1	3.7	5.0	8.4	31.6	96.6
PB +CHO	11.9 5.25§	0.6	0.2	1.1	5.5	8.5	9.5	66.2	91.6
MC	13.6 8.34§	8.5	4.2	10.1	6.8	12.8	15.5	33.4	91.3
MC +CHO	13.3 7.59§	1.0	0.4	1.3	10.0	14.8	19.2	37.7	84.4

*Incubations were carried out as described in the Methods, and metabolic profiles are presented as normalised percentages of the total ethyl acetate soluble metabolites.

†Percentage of incubated [^{14}C]phenanthrene metabolised.

‡Percentage of ethyl acetate soluble metabolites accounted for by cochromatography with standards.

§Enzyme activity of microsomes expressed as nmoles phenanthrene metabolised/min/nmole cytochrome P450 (or cytochrome P448). Microsomes from untreated and PB pretreated animals contained 0.75 and 0.93 nmoles cytochrome P450/mg protein respectively, and microsomes from MC pretreated animals contained 0.76 nmole cytochrome P448/mg protein.

Table 5. [^{14}C]Phenanthrene metabolism by hepatic microsomal fractions prepared from DBA/2J mice*

Experiment	Per cent metabolised†	Dihydrodiols 1,2-	3,4-	9,10-	Phenols 2- and 3-	1- and 9-	4-	9,10-Oxide	Total‡ per cent
Untreated	7.7 5.42§	6.7	6.7	44.2	5.0	4.4	8.4	13.8	89.2
Untreated +CHO	7.4 4.95§	0.5	0.3	2.1	8.4	12.4	16.2	54.8	94.7
PB	19.4 4.75§	5.6	5.6	64.1	3.9	3.6	2.9	4.6	90.3
PB +CHO	14.8 3.64§	0.9	0.8	2.4	3.4	8.3	8.1	67.7	91.6
MC	8.2 5.27§	8.4	7.2	25.3	7.0	7.8	20.5	17.8	94.0
MC +CHO	7.6 4.38§	0.8	0.5	1.0	9.1	14.7	25.3	41.9	93.3

*Incubations were carried out as described in the Methods, and metabolic profiles are presented as normalised percentages of the total ethyl acetate soluble metabolites.

†Percentage of incubated [^{14}C]phenanthrene metabolised.

‡Percentage of ethyl acetate soluble metabolites accounted for by cochromatography with standards.

§Enzyme activity of microsomes expressed as nmoles phenanthrene metabolised/min/nmole cytochrome P450 (or cytochrome P448). Microsomes from untreated and PB pretreated animals contained 0.58 and 1.65 nmoles cytochrome P450/mg protein respectively, and microsomes from MC pretreated animals contained 0.63 nmole cytochrome P448/mg protein.

rats in the presence of CHO. Both the increases in amounts of phenols and arene oxide and the relative proportions of phenols formed in the presence of CHO were consistent with amounts of dihydrodiols formed in its absence. Liver microsomes from PB pretreated rats showed an enzymic activity intermediate between those or control and MC microsomes. In terms of totals of metabolite classes (Table 6), the metabolite profile of PB microsomes was similar to those from the other rat liver microsomes. However, within the dihydrodiols very low levels of the 1,2- and 3,4-isomers were present and the 9,10-isomer predominated to an extent greater than in the di-

Table 6. Summary of metabolism of [^{14}C]phenanthrene by liver microsomal fractions prepared from various animals

		Normalised per cent metabolites found*					
		No addition			CHO		
Species	Microsomes / Metabolite†	Control	PB	MC	Control	PB	MC
Wistar Rat	DHDO	88.2	88.8	91.1	4.6	7.8	6.1
	phenols	5.6	5.9	4.8	25.2	15.1	31.7
	oxide	<0.1	1.2	0.8	56.5	73.0	59.4
Guinea Pig	DHDO	86.2	94.2	86.8	21.3	35.3	18.4
	phenols	5.7	2.3	5.9	35.2	9.5	54.5
	oxide	<0.1	0.3	0.4	34.6	53.8	11.5
SW Mouse	DHDO	32.7	47.9	22.8	3.1	1.9	2.7
	phenols	31.6	17.1	35.1	43.9	23.5	44.0
	oxide	27.3	31.6	33.4	41.0	66.2	37.7
DBA/2J Mouse	DHDO	57.6	75.3	40.9	2.9	4.1	2.3
	phenols	17.8	10.4	35.3	37.0	19.8	49.1
	oxide	13.8	4.6	17.8	54.8	67.7	41.9

*Metabolic profiles are presented as groups of metabolites as normalised percentages of the total ethyl soluble metabolites.

†DHDO comprise *trans*-1,2, 3,4- and 9,10-dihydrodihydroxyphenanthrene; phenols comprise 1-, 2-, 3-, 4- and 9-phenanthrol; oxide is phenanthrene-9,10-oxide.

hydrodiols produced by microsomes from untreated and MC pretreated animals. When CHO was present in PB microsomal incubation mixtures, dihydrodiol formation was depressed and the 9,10-oxide was produced in proportions greater than those found in similar experiments with control and MC microsomes. In all cases CHO inhibited dihydrodiol formation by more than 90 per cent, and for microsomes from PB and MC pretreated animals total metabolism was also reduced.

Differences between rat and guinea pig hepatic microsomal metabolism of phenanthrene are not apparent on inspection of totals of metabolite classes (Table 6). Thus dihydrodiols constitute about 90 per cent of the total metabolites with each animal. The compositions of the dihydrodiol fraction for microsomes from PB pretreated guinea pigs (Table 3) and rats were virtually identical. In contrast to incubations with microsomes from MC pretreated rat, the 1,2-dihydrodiol was the major metabolite formed in microsomes from MC pretreated guinea pigs, and was present in markedly higher proportion for control guinea pig microsomes than for those of the rat. The ratio of 1,2- to 3,4-dihydrodiols in MC and control microsome profiles was about 3 for the guinea pig and less than 2 for the rat. The total metabolism and enzyme activity of microsomes from PB pretreated guinea pigs were about twice those of control and MC guinea pig microsomes.

Metabolic profiles obtained in the presence of CHO showed decreased amounts of dihydrodiols and increased amounts of phenols and 9,10-oxide compared to incubations conducted in the absence of the epoxide hydratase inhibitor. For microsomes from PB pretreated guinea pigs the 9,10-oxide was the predominant metabolite, while microsomes from MC pretreated animals afforded predominantly the 1- and 9-hydroxyphenanthrene fraction. The proportions for control microsomes were intermediate between PB and MC microsomes. These results obtained in the presence of CHO were consistent with the metabolic profiles obtained when epoxide hydratase was not inhibited. The 1- and 9-hydroxyphenanthrene fraction formed from control and MC pretreated guinea pig microsomes when epoxide hydratase was inhibited must be mainly the 1-isomer. This follows from the experiments with PB microsomes when the 88 per cent of 9,10-dihydrodiol formed in the absence of CHO is compared with 34 and 54 per cent of the 9,10-dihydrodiol and 9,10-oxide respectively in the presence of CHO. In rat PB microsomes, CHO caused only a slight increase in the proportion of 1- and 9-hydroxyphenanthrene. Thus little isomerisation of the 9,10-oxide to 9-hydroxyphenanthrene occurs in the absence of active epoxide hydratase. Kinetic parameters determined for this oxide support this conclusion[20].

Dihydrodiols comprised a smaller proportion of metabolites formed by hepatic microsomes prepared from the mouse than from the rat (or guinea pig) (Table 6). This indicates lower levels of epoxide hydratase activity present in the mouse. The proportions of dihydrodiols formed by SW mouse microsomes were lower than those formed by DBA/2J mouse microsomes for all three animal treatments, and in both strains they were increased by PB and decreased by MC compared to untreated controls.

In all phenanthrene metabolite profiles from mouse microsomal preparations (Tables 4 and 5), the reduced epoxide hydratase activity was more noticeable in the reduced proportion of the usually predominating 9,10-dihydrodiol rather than the other dihydrodiols. Oxidative attack at the 3,4-position of phenanthrene, as measured by the sum of the proportions of the 3,4-dihydrodiol and 4-

hydroxyphenanthrene, or by the proportions of 4-hydroxyphenanthrene alone, was greater for all microsomal preparations of mouse liver than for hepatic microsomal preparations of similarly treated rats or guinea pigs. No substantial differences between MC inducible SW mouse microsome metabolite profiles and SW mouse control profiles, or between MC pretreated (and genetically "nonresponsive") and untreated DBA/J2 mouse profiles were found. In both mouse strains, microsomes obtained after PB pretreatment afforded metabolite profiles in which oxidation products resulting from attack at the 9,10-position of phenanthrene were more predominant than in profiles from control of MC microsomes. This is analogous to results obtained with the rat and guinea pig microsomes. The effects of the addition of CHO to the incubation mixtures for each strain were similar to results obtained with rat microsomal incubations. Compared to incubations in the absence of the epoxide hydratase inhibitor, the proportions of phenols and 9,10-oxide increased and that of dihydrodiols decreased. The effect was most noticeable for the 9,10-oxide. PB and MC pretreatment increased total phenanthrene metabolism about 2 fold in SW-mouse liver microsomes, but with DBA-mice induction of metabolism was only observed in microsomes from PB pretreated animals.

DISCUSSION

The formation of arene oxides in biological systems is of great importance in carcinogenesis as these substances, at least in some instances, may be the ultimate carcinogens[2, 3]. Because isomeric arene oxides derived from carcinogenic hydrocarbons display varying degrees of tumorigenicity[26] it is important to describe in detail the oxidative metabolism of the parent hydrocarbon. In the current work the liver microsomal oxidative metabolism of [^{14}C]phenanthrene, a noncarcinogenic hydrocarbon, is reported. Metabolic profiles of [^{3}H]phenanthrene, previously obtained with hepatic microsomes from the Chester Beatty strain rat[13], are very similar to those reported herein for the Wistar rat. A slight difference emerges in the predominance of the 9,10-dihydrodiol in the present study, whereas in the earlier study equal amounts of the 1,2- and 9,10-isomers were produced with both control and MC microsomes. Of the hepatic microsomal metabolites, the proportion of total phenols obtained with phenanthrene was less than that obtained with dibenz[a,h]anthracene[13] in the rat and with benzo[a]pyrene in rat and mouse[4, 5, 13, 23], but comparable to that obtained with benz[a]anthracene with the rat[13]. The increased proportion of 9,10-dihydrodiol formed in microsomes from all PB pretreated animals parallels the predominance of the K-region* dihydrodiol of benzo[a]pyrene formed by microsomal preparations from PB pretreated rats. In contrast to benzo[a]pyrene studies with rats[4], MC pretreatment of rats and mice failed to produce significant alterations in microsomal phenanthrene metabolite profiles compared to control microsome profiles. Microsomes from MC pretreated guinea pigs however afforded different profiles, phenanthrene - 1,2 - dihydrodiol being the major metabolite. Evidence is accumulating that dihydrodiol epoxides of polycyclic aromatic hydrocarbons with the epoxide in the bay region,* such as *trans* - 7,8 - dihydro - 7,8 - dihydroxy - 9,10 - epoxybenzo[a]pyrene, are more mutagenic than isomeric diol epoxides[27–30]. Replacement of the rat liver 9000 *g* supernatant used in the *Salmonella typhimurium* test systems[31] with 9000 *g* supernatant from MC pretreated guinea pigs may increase the proportion of mutagenic metabolites and the number of revertant colonies with polycyclic aromatic hydrocarbons.

Levels of dihydrodiol metabolites found in hepatic microsomes from rat and mouse probably reflect different levels of epoxide hydratase present. Rat liver microsomes have been shown to have two to three times the hydratase activity of mouse microsomes when styrene oxide was used as substrate[32, 33]. The proportions of dihydrodiols formed from benzo[a]pyrene in microsomes of the rat[4, 5, 13, 23], mouse[5] and hamster[34] are well below that produced from phenanthrene and are consistent with the greater activity of microsomal epoxide hydratase for the K-region oxide of phenanthrene than that of benzo[a]pyrene[35]. Pretreatment of rats with PB causes greater increases in the levels of epoxide hydratase of liver microsomes than those observed when MC was used as the inducer[25, 36, 37]. The reverse is the case for aryl hydrocarbon hydroxylase when enzyme activities are greater in microsomes from MC pretreated animals[25]. In both strains of mice the greater proportion of dihydrodiols formed by PB microsomes, and the smaller proportion formed by MC microsomes compared to control microsomes, are probably due to the differential effects of the two inducers on epoxide hydratase and aryl hydrocarbon hydroxylase. In rats and guinea pigs this effect of the inducers is not observed because of the higher basic levels of epoxide hydratase.

Modifications of hydratase ability of *in vitro* metabolic systems have involved either the addition of solubilized epoxide hydratase to the reconstituted mixed function oxidase[4] or the use of added epoxide hydratase inhibitors[12, 34]. In the latter study, 2.0 mM cyclohexene oxide produced less inhibition of epoxide hydratase than that reported herein using 5.0 mM CHO, and in both the reduction of the proportion of dihydrodiols was equal to the sum of the increases in the proportions of phenols and arene oxide. The presence of CHO usually resulted in a reduction of total phenanthrene metabolism and may be in part due to direct inhibition of mixed function oxidase by CHO or the reduction of K-region arene oxides to the parent hydrocarbon[38, 39]. Whilst this liver microsomal reduction proceeds optimally under an atmosphere

*The K-region is the 9,10-position of phenanthrene and 4,5-position for benzo[a]pyrene; the bay region is that between ring carbon numbers and 3,4 and 5,6 in phenanthrene, and 9,10 and 11,12 in benzo[a]pyrene.

of nitrogen, air is not completely inhibitory, and reduction of phenanthrene-9,10-oxide which accumulated in the presence of CHO may account for the present observed inhibition. In a recent study of the effect of epoxide hydratase inhibitors on benzo[a]pyrene metabolism inhibition of oxidation was also observed, and was thought to be due to arene oxide reduction[40].

In this work phenanthrene metabolism has not been directly related to aryl hydrocarbon hydroxylase measured by the fluorescence assay using benzo[a]pyrene as substrate[41], but the effects of the inducers PB and MC suggest that assays using these two substrates are a measure of the same or similar enzymic activities. Compared to control microsomes, increases in phenanthrene metabolism occurred with microsomes from all PB pretreated animals and with microsomes from MC pretreated SW mice and rats. Similar induction of aryl hydrocarbon hydroxylase is well documented[24, 25, 42, 43].

Acknowledgements—This work was supported by financial assistance from the National Health and Medical Research Council.

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