decrease in Q_{10} from 2 to 1 suggests some alteration of the enzyme which inhibits the thermochemically induced increase in rate. Such effects are normally observed as a result of heat-induced denaturation of an enzyme and may be expected at about 45–50°. More detailed temperature vs. activity studies are required to obtain a better understanding of this temperature-dependent phenomenon.

The finding of preferential inhibition of cerebral cortex PD by DOPAC and apomorphine was unexpected. Since apomorphine is thought to act on dopamine receptors,[6] and DOPAC is a metabolite of dopamine, one is forced to consider that a tissue specific PD may be an important component of a dopamine receptor.

Finally, it is clear that in order to evaluate the potency of various substances as inhibitors of PD, one must use enzymes prepared from several tissues.

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Effect of polycyclic hydrocarbons *in vitro* on aryl hydrocarbon (benzo[a]pyrene) hydroxylase

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ARYL hydrocarbon (benzo[a]pyrene) hydroxylase is an inducible enzyme system which has been found in many tissues of various mammalian species. It is part of the microsomal enzyme complex which is responsible for the metabolism of a variety of exogenous chemicals which include carcinogens, drugs and pesticides.[1–3] The level of activity of this enzyme system varies with the age, sex, species and environment of the animal.[4,5] This enzyme system hydroxylates benzo[a]pyrene to phenolic derivatives.[6,7] It is also capable of hydroxylating a variety of other polycyclic hydrocarbons.[5,8] The phenolic derivatives are generally relatively weak or inactive as carcinogens. Recent studies, however, have suggested that the hydroxylase enzyme complex may convert polycyclic hydrocarbons to a reactive form.[9–12] The evidence for this enzyme involvement in polycyclic hydrocarbon activation is: (1) the toxic effect of polycyclic hydrocarbons correlates with the level of enzyme in a variety of cells grown in culture;[9] (2) the toxicity of dimethylbenz[a]anthracene and benzo[a]pyrene is inhibited by an inhibitor of the enzyme system, 7,8-benzoflavone;[10] (3) the rat liver microsomal enzyme system catalyzes the formation of covalent complexes of polycyclic hydrocarbons with deoxyribonucleic acid and with protein.[11,12]

In this study we thought it would be of interest to examine the affinity of various carcinogenic and noncarcinogenic polycyclic hydrocarbons for this enzyme system. We have determined the effect of various polycyclic hydrocarbons on benzo[a]pyrene hydroxylation. The effect of the added hydrocarbon may be a measure of its activity as a competitor for the benzo[a]pyrene hydroxylation site on the enzyme.

The polycyclic hydrocarbons were obtained commercially and purified by recrystallization. Reduced nicotineamide adenine dinucleotide (NADPH) was obtained from Calbiochem or Sigma. An Aminco–Bowman spectro–photofluorometer (Model 4-8202) was used. Sprague–Dawley rats (150–200 g) were injected intraperitoneally with 6·8 mg methylcholanthrene in 0·5 ml corn oil. Control rats received 0·5 ml of corn oil. The rats were killed by exsanguination 16 hr later. The livers were placed in an ice-cold solution of 0·25 M sucrose, then minced and homogenized in 0·25 M sucrose–0·05 M Tris–chloride buffer, pH 7·5, with a tight-fitting Potter–Elvehjem glass–glass homogenizer. Microsomes were prepared as described earlier.[13] The previously described assay for measuring aryl hydrocarbon hydroxylase[14] was slightly modified: the reaction mixture, in a total volume of 1·00 ml, contained 50 μmoles of Tris–chloride buffer, pH 7·5, 0·54 μmoles of NADPH, 3 μmoles of $MgCl_2$, 0·10 ml of rat liver 105,000 *g* supernatant (containing approximately 1·5 mg of protein), 0·10 ml of liver microsomes (containing 0·15 to 0·20 mg of protein), and 80 mμmoles of benzo[a]pyrene in 0·040 ml of methanol. The mixture was shaken at 37° for 20 min in air. The reaction was stopped by addition of 1·0 ml of cold acetone. Enzyme activities were determined in duplicate or triplicate. The activity was compared to a blank to which acetone had been added prior to incubation. The various polycyclic hydrocarbons were dissolved in the appropriate solvent and added in 0·010 ml to yield a final concentration equimolar to the benzo[a]pyrene. Each hydrocarbon was also incubated without addition of benzo[a]pyrene. This blank gave either no fluorescent reading or insignificant readings at the wavelengths used for benzo[a]pyrene hydroxylation determinations. Similarly, the addition of the solvents for the various polycyclic hydrocarbons (0·010 ml) to the reaction mixture after the incubation period did not affect the subsequent fluorometric measurements. Solvents, however, do have some stimulatory or inhibitory effects on the activity *in vitro* of aryl hydrocarbon hydroxylase depending on the source of the microsomal enzyme preparation as described recently[15]

TABLE 1. EFFECT OF VARIOUS POLYCYCLIC HYDROCARBONS ON ARYL HYDROCARBON (BENZO[a]PYRENE) HYDROXYLASE ACTIVITY *IN VITRO*

Experiment	Additions	Solvent	Aryl hydrocarbon (benzo[a]pyrene) hydroxylase Units/mg proteins ($\pm 10^{-2}$) Untreated	Induced*
1	BP† +	Methanol	52‡ ± 6	309 ± 11
	BP + 7,12-DMBA†	Methanol	32 ± 4§ (− 38)‖	210 ± 33§ (− 32)
	BP + Naphthalene	Methanol	50 ± 2 (− 4)	310 ± 13 (± 0)
2	BP +	DMSO†	38 ± 2	287 ± 15
	BP + Dibenz(a,h)anthracene	DMSO	39 ± 2 (+ 3)	260 ± 15 (− 10)
	BP + Dibenz(a,c)anthracene	DMSO	34 ± 3 (− 10)	174 ± 31§ (− 40)
	BP + 3-Methylcholanthrene	DMSO	38 ± 1 (± 0)	141 ± 16§ (− 51)
3	BP +	Acetone	72 ± 1	320 ± 12
	BP + Phenanthrene	Acetone	50 ± 2§ (− 31)	268 ± 8§ (− 11)
	BP + Anthracene	Acetone	62 ± 2§ (− 14)	308 ± 17 (− 4)
	BP + Perylene	Acetone	72 ± 9 (± 0)	299 ± 8 (− 7)

* Microsomal preparations from 3-methylcholanthrene pretreated rats (see text).

† Abbreviations: BP = benzo[a]pyrene; DMBA = dimethylbenz[a]anthracene; DMSO = dimethylsulfoxide.

‡ Mean ± S.D. of duplicate or triplicate determinations from two to three separate experiments that were normalized to the mean of the appropriate solvent control. The range of the solvent controls were: "Untreated"—methanol = 44–61; DMSO = 35–41; acetone = 70–74; "Induced"—methanol 290–330; DMSO—270–310; acetone 310–340.

§ Significantly different from controls ($P < 0.001$).

‖ Per cent stimulation (+) or inhibition (−).

(see also Table 1). The inhibitory effect of a polycyclic hydrocarbon on benzo[a]pyrene hydroxylation relative to the appropriate solvent control appeared not to be related to the solvent used. One unit of aryl hydrocarbon hydroxylase activity is defined as the amount of enzyme catalyzing the formation

of hydroxylated metabolite with a fluorescence equivalent to 1 $\mu\mu$mole of 3-hydroxybenzo[a]pyrene per 30 min. Protein concentrations were determined by modification of the method of Lowry *et al.*[16] using ribonuclease A as standard.

As shown in Table 1, with microsomes from control rats, the addition of five of the polycyclic hydrocarbons used, at concentrations equimolar to the substrate benzo[a]pyrene, had no significant effect on benzo[a]pyrene hydroxylation. Three of the compounds tested, dimethylbenz[a]anthracene, phenanthrene, and anthracene, inhibited hydroxylation of benzo[a]pyrene by 38, 31 and 14 per cent. With microsomes from 3-methylcholanthrene pretreated rats, the addition of dimethylbenz[a]anthracene, dibenz[a,c]anthracene and 3-methylcholanthrene resulted in a marked inhibition of hydroxylation of benzo[a]pyrene. The addition of the other polycyclic hydrocarbons resulted in either negligible or no inhibition of benzo[a]pyrene hydroxylation. The relative inhibition by each compound may indicate the degree of competition for the benzo[a]pyrene hydroxylation enzyme site. With the enzyme from control rats only two of the eight compounds caused greater than a 25 per cent inhibition and none caused more than a 38 per cent inhibition. This suggests that none of these compounds has an affinity for the benzo[a]pyrene hydroxylation site equal to benzo[a]pyrene. With the microsome enzyme preparation from 3-methylcholanthrene pretreated rats, dibenz[a,c]anthracene and 3-methylcholanthrene caused a 40 and 51 per cent inhibition. Thus, these compounds compete more strongly with benzo[a]pyrene for the induced enzyme than for the control enzyme. This suggests that benzo[a]pyrene hydroxylating enzyme from hepatic microsomes of control rats is different from that derived from rats pretreated with 3-methylcholanthrene.

TABLE 2. EFFECT OF POLYCYCLIC HYDROCARBONS ON BENZO[a]PYRENE HYDROXYLATION

Compound	Carcinogenicity	Per cent activity	
		Control	Induced*
7,12-DMBA†	+	− 38	− 32
3-Methylcholanthrene	+	± 0	− 51
Dibenz[a,h]anthracene	+	+ 3	− 10
Naphthalene	−	− 4	± 0
Perylene	−	± 0	− 7
Dibenz[a,c]anthracene	−	− 10	− 40
Phenanthrene	−	− 31	− 11
Anthracene	−	− 14	− 4

* As in Table 1.
† Dimethylbenz[a]anthracene.

As shown in Table 2, with the enzyme from control rats there is no correlation between the carcinogenicity of these compounds and their ability to alter benzo[a]pyrene hydroxylation. Thus, dimethylbenz[a]anthracene and 3-methylcholanthrene have different effects although both are potent carcinogens. With the enzyme system from 3-methylcholanthrene pretreated rats, the potent carcinogens, 3-methylcholanthrene and dimethylbenz[a]anthracene, markedly inhibit the enzyme. The noncarcinogens, naphthalene, perylene, phenanthrene, and anthracene, have only negligible effects on the enzyme system. The dibenz(a)anthracenes, however, do not fit this pattern. The carcinogenic isomer has less inhibitory effect than the noncarcinogenic isomer.

The enzyme system hydroxylates polycyclic hydrocarbons in a variety of positions. Some of these hydroxylation intermediates may indeed be the active carcinogens. In order to clarify the role of the enzyme system in carcinogenic activation, it will be necessary to isolate and identify those active carcinogenic intermediates. The degree of this intermediate formation may correlate with the substrate affinity of these compounds for the enzyme system.

The present study shows that the microsomal enzyme system from control rats and from 3-methylcholanthrene-treated rats is affected differently by various hydrocarbons. This suggests that the control and induced enzymes have differing affinities for the various hydrocarbons. This is consistent with the difference in other properties of the control and induced microsomal enzyme system.[15,17–19]

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Failure to affect tissue reserpine concentrations by alteration of adrenergic nerve activity*

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RECENT studies[1,2] have affirmed the persistent presence of minute quantities of bound reserpine in several organs, and it has been shown that a correlation exists at certain times between the concentration of bound reserpine and the degree of norepinephrine (NE) depletion.[1] More recently, we have presented evidence pointing to the existence of two types of reserpine binding in rat tissues, a reversible phase lasting about 24–30 hr, and an irreversible phase persisting for many days.[3] It was suggested that the former is associated with the relatively short-lived inhibition of the granule amine carrier mechanism, while the latter is associated with irreversible alteration of the granule storage mechanism for the life span of the storage granule. The present study was designed to investigate the possibility that reserpine bound in adrenergic nerve terminals might be released by adrenergic nerve activity. Such information could help shed light on the site of reserpine binding.

In order to gain direct evidence that bound reserpine is localized in adrenergic nerve terminals, rats were pretreated with 6-hydroxydopamine, a compound which, in high dosage, leads to destruction of adrenergic nerve terminals in several organs.[4] Some of the rats were then given intravenous [^{3}H]reserpine, 200 μg/kg (572 or 424 mc/m-mole), and were killed 18 hr later. [^{3}H]reserpine concentrations were measured in heart and small intestine as described elsewhere,[1] and compared with those of control rats not pretreated with 6-hydroxydopamine. The same tissues of other rats given 6-hydroxydopamine were analyzed for their NE content[5] and compared with those of untreated controls. As shown in Table 1, 6-hydroxydopamine decreased the NE content of the tissues by about 70 per cent and the [^{3}H]reserpine content by about 60 per cent. These results suggest that the bulk, if not all, of bound reserpine is localized in adrenergic nerve terminals.

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