bility. It would seem more likely that the difference in activity resulted from a difference in the immediate environment around the enzyme. These differences between the soluble enzyme and the particle-bound enzyme also indicate that care should be taken in the interpretation of results when whole homogenates are used for the testing of tyrosine hydroxylase inhibitors.

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Metabolism of 7-hydroxymethyl-12-methylbenz(a)anthracene-12-^{14}C *in vitro**

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IT IS NOW well established from the experiments of Huggins *et al.*[1–3] and Dao and Tanaka[4, 5] that pretreatment with any of several polycyclic hydrocarbons protects rats from adrenal necrosis and mammary cancer induced by DMBA.* There is also evidence[6, 7] that the proximal necrotic agent is the 7-hydroxymethyl derivative of DMBA (7-OHM-12-MBA) and that treatment of rats with certain polycyclic hydrocarbons alters the metabolism of DMBA by hepatic tissue from side-chain to ring hydroxylation.[8, 9] It was therefore considered of interest to study the metabolism of 7-OHM-12-MBA by rat liver microsomes and also adrenal tissue and to determine the effect of compounds which protect against adrenal necrosis on the metabolism of this biologically active metabolite of DMBA.

Mature (55–70 days old) male hooded or Sprague–Dawley rats, with free access to food (Purina Labena) and water, were used. The polycyclic hydrocarbons (10 mg) dissolved in sesame oil (1 ml) by gentle heating were administered by stomach tube or by intraperitoneal injection 24 hr before killing the animals.

Radioactive 7-hydroxymethyl-12-methylbenz(a)anthracene (7-OHM-12-MBA-12-^{14}C) was prepared by the method of Boyland and Sims[10] from DMBA-12-^{14}C (0·2 mc in 5·5 mg), obtained from the Radiochemical Centre, Amersham, and diluted with 150 mg "cold" DMBA. The acetoxy derivative, however, was not isolated but hydrolyzed by refluxing with 2% (w/v) methanolic KOH before

* The following abbreviations are used: DMBA, 7,12-dimethylbenz(a)anthracene; 7-OHM-12-MBA, 7-hydroxymethyl-12-methylbenz(a)anthracene; 12-OHM-7-MBA, 12-hydroxymethyl-7-methylbenz(a)anthracene; 7,12-DiOHM-BA, 7,12-dihydroxymethylbenz(a)anthracene; MC, 3-methylcholanthrene.

column chromatography on silica gel in benzene. Each fraction was monitored for radioactivity and examined by thin-layer chromatography in benzene:ethanol (19:1) followed by autoradiography as described previously.[8] Fractions which contained predominantly 7-OHM-12-MBA were pooled, the solvent was removed at 40° under N_2 and the residue was recrystallized from ethanol and then benzene. The product (m.p. 161–162°) was free of any radioactive contaminants detectable by chromatography and autoradiography.

A microsomal fraction (8000 *g* supernatant) from 50 mg liver and a 1% homogenate of the adrenal glands was prepared as described previously.[8] The tissue preparations were incubated at 38° for 1 hr under O_2 with DMBA-12-^{14}C (0·11 μc in 3 μg) or 7-OHM-12-MBA-12-^{14}C (0·013 μc in 10μg), NADP (0·3 mM) and glucose 6-phosphate (3 mM) in 0·1 M potassium phosphate buffer, pH 7·4; total volume was 4 ml. The incubation mixtures were extracted three times with equal volumes of peroxide-free ether and the amount of ^{14}C in the ether-soluble fraction and the residual aqueous medium was determined by liquid scintillation counting.[8] For quantitative ^{14}C assay of the ether-soluble metabolites, the radioactive areas were scraped off the thin-layer sheets into vials, scintillation fluid was added and counted in the usual manner with 75–90 per cent recoveries.

Table 1 shows the effect of pretreatment with polycyclic hydrocarbons on the metabolism of DMBA and its 7-hydroxymethyl derivative by rat liver microsomes. Both substrates gave rise to water-soluble products, whose yield could be increased by giving the animals an oral dose of 10 mg MC or DMBA 24 hr beforehand. It is known that pretreatment of rats with low doses of DMBA stimulates its own hepatic metabolism[8, 9] and blocks the adrenal necrosis caused by a subsequent high dose of this compound.[5]

TABLE 1. EFFECT OF PRETREATMENT WITH POLYCYCLIC HYDROCARBONS ON THE CONVERSION OF RADIOACTIVE DMBA AND 7-OHM-12-MBA TO WATER-SOLUBLE PRODUCTS BY RAT LIVER MICROSOMES*

Pretreatment	per cent of added ^{14}C remaining in aqueous medium after extraction with ether	
	DMBA-12-^{14}C	7-OHM-12-MBA-12-^{14}C
Control (oil)	19·7 (20·3)	22·9
MC	59·5	60·9
DMBA	51·3 (48·2)	57·6
7-OHM-12-MBA	22·0 (25·8)	23·5

* The hydrocarbon (10 mg) in oil was given orally or by i.p. injection 24 hr before removing the liver. Each value is the mean of 2 experiments with the same range as observed previously[8] and those in parentheses are from rats injected intraperitoneally. Conditions are as described in text. See footnote in text for abbreviations.

These results also confirm the findings of Levin and Conney[9] that pretreatment with MC enhances the metabolism of ^{3}H-labeled monohydroxymethyl-DMBA, which they prepared biologically. They too observed an increase in the water-soluble metabolites and also in the polar products extracted from the incubation medium by acetone–hexane.

Pretreatment with 7-OHM-12-MBA, however, did not appreciably influence the hepatic metabolism of either DMBA-12-^{14}C or 7-OHM-12-MBA-12-^{14}C. Similar results were obtained after intraperitoneal injection of this hydrocarbon, showing that the lack of stimulatory effect by 7-OHM-12-MBA was not due to its lack of absorption by the alimentary tract. This is supported by the fact that 7-OHM-12-MBA produces adrenal necrosis by both routes of administration and that its distribution after an oral dose is similar to that of DMBA in most rat tissues.[11]

The ether-soluble products formed from DMBA and its hydroxymethyl derivative by rat liver microsomes are shown in Fig. 1. No metabolites less polar than the substrate were found and 7-OHM-12-MBA-12-^{14}C gave rise to a product which behaved like 7,12-DiOHM-BA chromatographically.

No significant metabolism of 7-OHM-12-MBA-12-^{14}C by adrenal homogenates of normal or pretreated rats was observed.

These results show that treatment with MC or DMBA increases the hepatic conversion not only of DMBA but also of its more active 7-hydroxymethyl metabolite to polar products. This would provide a further means of protecting the animals against adrenal necrosis.

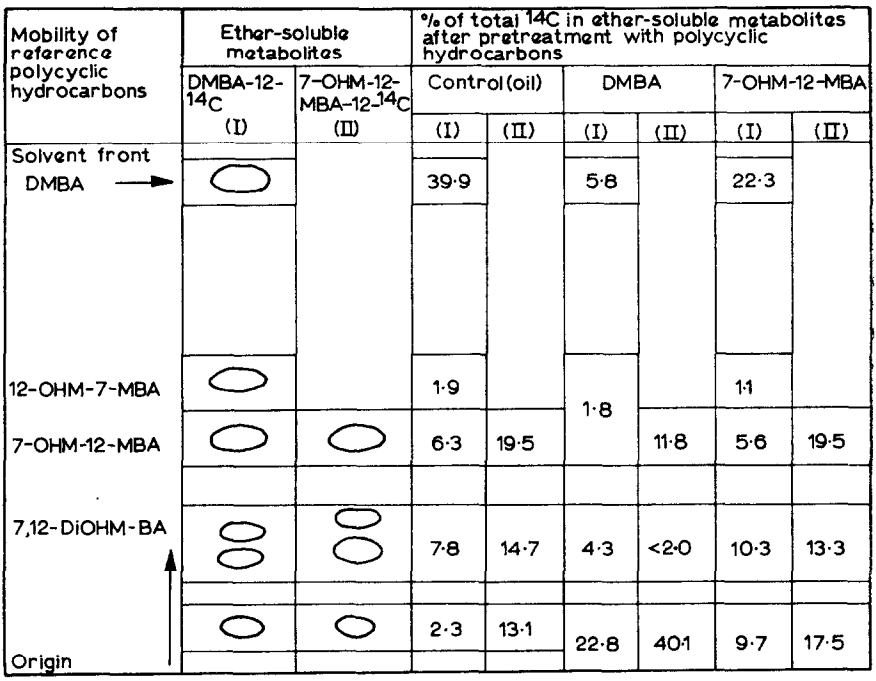

FIG. 1. Effect of pretreatment with polycyclic hydrocarbons on the metabolism of radioactive DMBA (I) and 7-OHM-12-MBA (II) by rat liver microsomes. The metabolites were separated by TLC chromatography, localized by autoradiography and counted as described in the text. The hydrocarbons (10 mg) were given orally 24 hr before removing the liver. The diagram above shows the R_f values of the metabolites and the distribution of radioactivity on the chromatograms. (See footnote in text for abbreviations.)

Studies are in progress to determine whether the decreased formation of 7-OHM-12-MBA from DMBA after pretreatment with polycyclic hydrocarbons is due to increased ring hydroxylation of DMBA at the expense of side-chain hydroxylation[8] or to increased metabolism of the 7-hydroxymethyl derivative produced.

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