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Comparative metabolism of dimethylbenzanthracene-12-^{14}C *in vitro**

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IT IS now well established that the powerful carcinogen dimethylbenz(a)anthracene (DMBA),† which in high concentrations also produces adrenal necrosis in the rat,[1] has no effect on the adrenals of the hamster, guinea pig or mouse.[2] There is also evidence[3, 4] that the active adrenocorticolytic agent may be the 7-hydroxymethyl derivative rather than DMBA itself and that pretreatment with polycyclic hydrocarbons protects the rats from adrenal damage by changing the hepatic metabolism of DMBA from side-chain to ring-hydroxylation, thereby decreasing the yield of 7-OHM-12-MBA.[5]

It appears that the induction of adrenal necrosis is dependent on a functional adrenal[6] since immature or hypophysectomized rats showed no adrenal damage after treatment with DMBA but could be made susceptible by the administration of adrenocorticotrophic hormone.

It was therefore considered of interest to compare the metabolism of DMBA by hepatic and adrenal tissues of the mature and immature rat and the mouse to determine whether there were any differences which could account for the variation in response to this polycyclic hydrocarbon.

MATERIALS AND METHODS

Mature (60- to 90-day-old) or immature (10- to 15-day-old) rats from an inbred hooded strain and mature (90- to 120-day-old) CNZ mice were used. All animals had free access to food (Purina laboratory chow) and water and were killed by asphyxiation in carbon dioxide. A supernatant fraction (8000 *g*) from 50 mg liver and 5% homogenate of the adrenal glands was prepared as described previously[5] and the tissue preparation was incubated at 38° with 7,12-dimethylbenzanthracene-12-^{14}C (0·11 μc in 3 μg), NADP (0·3 mM), and glucose 6-phosphate (3 mM) in 0·1 M potassium phosphate buffer, pH 7·4; total volume, 4 ml. The incubation mixtures were extracted three times with peroxide-free ether and the amount of ^{14}C in the ether-soluble fraction and the residual aqueous medium determined by a standard method[5] in a Packard Tri-Carb liquid scintillation counter. A control tube containing DMBA-12-^{14}C was also incubated in the absence of tissue and each value given (Figs. 1 and 3) is the mean of two experiments. The incubations with mouse and rat adrenals were repeated many times with consistent results and, in addition, homogenates from adult and immature rat adrenals were mixed to be certain that the immature preparations did not possess an inhibitor or an enzyme capable of destroying NADP.

Purification of the substrate DMBA-12-^{14}C (from the Radiochemical Centre, Amersham), thin-layer chromatography in benzene:ethanol (19:1), and autoradiography were carried out as described previously.[5] The products formed by liver and adrenals were also examined by thin-layer chromatography on silica gel in chloroform or benzene:dichloromethane (7:3) and, in these systems, the main adrenal metabolite was shown to differ from 7-OHM-12-MBA. By this method it was also possible to exclude 7,12-DiOHM-BA as a metabolite of DMBA in adult rat liver.

RESULTS AND DISCUSSION

Although differences both in the rate of metabolism (Fig. 1) and in the nature of the products (Fig. 2) formed by the liver microsomes of mice and adult or immature rats were observed, the most striking result was the complete lack of DMBA-metabolizing enzymes in the adrenals of mice and infant rats. Perhaps it is significant that neither of these animals is susceptible to DMBA-induced adrenal necrosis.

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† 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.

The possibility that one of the DMBA metabolites formed exclusively in mature rat adrenals is the proximal necrotic agent is unlikely because the metabolism of DMBA in this tissue was not altered by pretreatment with protective doses of methylcholanthrene.[5] In addition, Dao and Varela[7] observed no increase in adrenal benzpyrene hydroxylase under these conditions. Furthermore, the effect of DMBA cannot be readily explained by differences in adrenocorticosteroid metabolism, since the mouse adrenal like that of the rat produces mainly corticosterone.[8]

The rate of formation of the two main metabolites of DMBA by mature rat adrenal homogenates is shown in Fig. 3 and the change-over to the adult metabolic pattern occurred earlier in this tissue

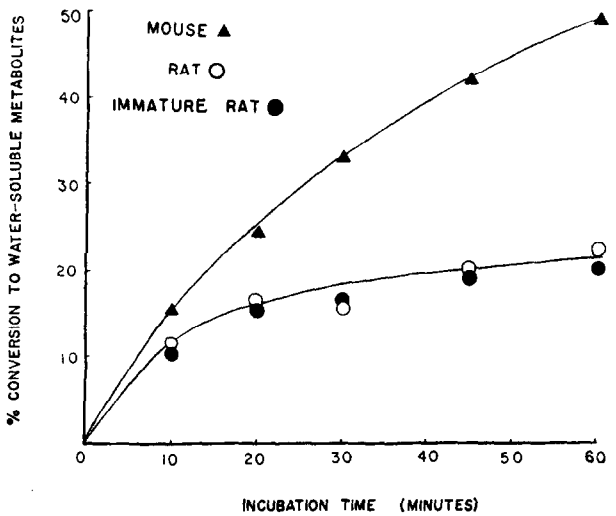

FIG. 1. Time curve for the conversion of DMBA to water-soluble metabolites by rat and mouse liver. DMBA-12-^{14}C (0·11 μc in 3 μg) was incubated with the 8,000 g supernatant fraction of liver (50 mg) together with NADP (0·3 mM) and glucose 6-phosphate (3 mM) as described in the text.

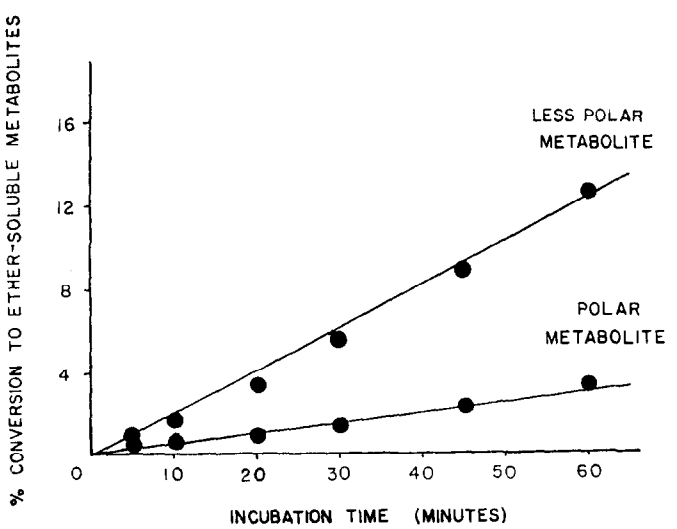

FIG. 3. Time curve for the conversion of DMBA to ether-soluble metabolites by adult rat adrenals. DMBA-12-^{14}C was incubated with a 5% homogenate of adrenals. Other conditions as described in the text.

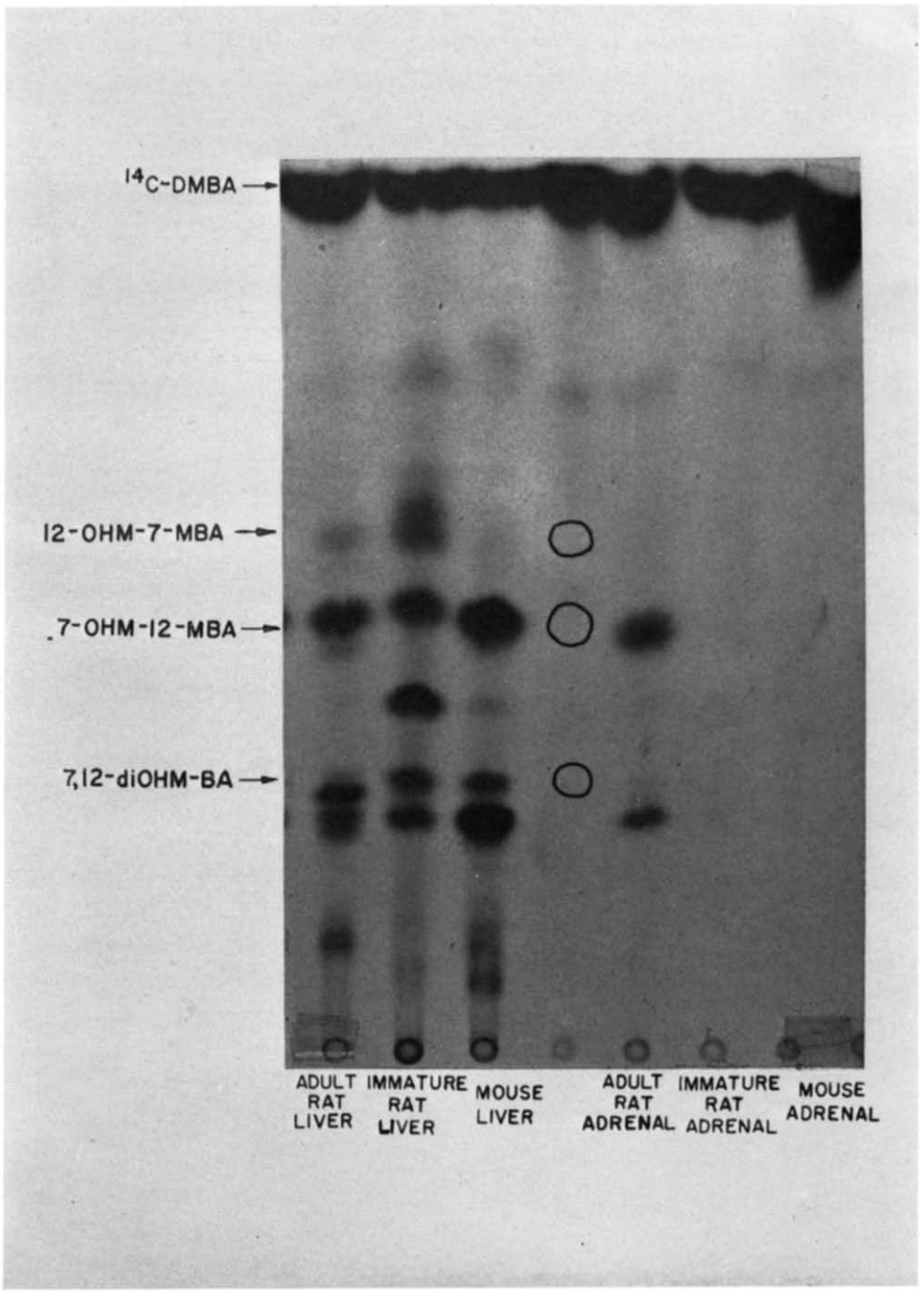

FIG. 2. Autoradiogram of the ether-soluble metabolites of DMBA formed by rat and mouse liver and adrenals. DMBA-12-^{14}C was incubated for 1 *hr* with the 8000 *g* supernatant fraction of liver or a 5% homogenate of adrenals, and the metabolites were separated by thin-layer chromatography on silica gel in benzene:ethanol (19:1). Other conditions as described in the text.

(at 35–40 days) than in the liver (at 50–55 days). The addition of adrenal preparations from immature animals did not inhibit the metabolism *in vitro* of DMBA by mature rat adrenals and, in all three cases, 7-OHM-12-MBA was formed by liver microsomes.

It is, of course, always dangerous to extend results *in vitro* to the situation in the intact animal. Nevertheless, it does appear that neither the metabolism of DMBA by the liver nor the presence of an adrenal gland actively producing corticosterone can account for the adrenocorticolytic effect of DMBA in the adult rat and yet not in the infant rat or mouse. Some other factor, such as the sensitivity of rat adrenal lysosomes to 7-OHM-12-MBA[9] or the susceptibility of the adrenal vascular bed in this species,[2] may have to be invoked to explain the highly selective action of this polycyclic hydrocarbon.

The findings of Huggins *et al.*[10] that marked depression in DNA synthesis in rat tissues and a great increase in the formation of liver menadione reductase were produced by doses of DMBA which, on a body weight basis, had little effect in mice, may also be important.

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Hyperthermia and elevated brain 5-hydroxytryptamine of rabbits in response to tryptophan and 5-hydroxytryptophan infusion*

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IT IS KNOWN that administration of 5-hydroxytryptophan (5-HTP) or L-tryptophan (L-Try) will cause hyperthermia in mammals and birds. This effect is presumed to be due to 5-hydroxytryptamine (5-HT). Feldberg and Myers[1, 2] have shown hyperthermia with 5-HT injected into the cerebral ventricals of cats in the area of the hypothalamus through an indwelling cannula.

It is the purpose of this investigation to compare various brain levels of 5-HT after infusion of 5-HTP and L-Try in monoamine oxidase-inhibited rats, and further to attempt to relate these changes to a physiological parameter, body temperature. The areas of the central nervous system examined are the hypothalamus, pons-medulla region, thalamus, and white and gray cortical matter. The peripheral organs examined are the liver and stomach.

MATERIALS AND METHODS

Male New Zealand white rabbits (1·6–2·1 kg) were used. Under light thiopental anesthesia, supplemented with procaine locally, a Teflon cannula was inserted retrograde into the external carotid

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