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Comparison of the efficacy of pretreatment protection against adrenal necrosis induced by 7-hydroxymethyl-12-methylbenz(*a*)anthracene and by 7-methyl-12-methylbenz(*a*)anthracene in rats

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DESTRUCTION of the zona fasciculata and zona reticularis of the adult rat adrenal cortex follows intragastric or intravenous administration of a single dose of 7, 12-dimethylbenz(*a*)anthracene (DMBA).[1] It has been shown in a number of laboratories that the toxic and adrenocorticolytic effects of DMBA can be prevented or markedly reduced by stimulation of hepatic drug-metabolizing enzymes with a variety of different compounds.[2–9] The pretreating substances act protectively not only by increasing the overall rate at which DMBA is metabolized but also by (a) increasing the proportion of the metabolized molecules which undergo ring hydroxylation and (b) by promoting the further hydroxylation of molecules (both in ring and the second side chain positions) which have been hydroxylated in one of the methyl side chains. Since DMBA does not appear to be the active adrenocorticolytic or toxic substance but requires metabolism in the liver,[10] it is inferred that the essential feature of pretreatment protection is the accelerated removal of an adrenocorticolytic metabolite before it has time to damage the adrenal cortex, or the diversion of metabolism of DMBA away from that pathway which leads to the formation of the adrenocorticolytic intermediate. Although our earlier studies showed that hydroxylation of DMBA in the 7-methyl position produced a potent adrenocorticolytic substance which was active in animals with impaired liver function,[11,12] more recent experiments with the microsomal drug-metabolizing enzyme inhibitor β-diethylaminoethyl-diphenyl-*n*-propyl acetate (SKF 525-A) indicated that 7-hydroxymethyl-12-methylbenz(*a*)anthracene (7-OHM-MBA) is not the adrenocorticolytic metabolite but could be an intermediate which requires further metabolism.[13]

Preliminary experiments have shown that pretreatment of rats with substances which give protection from DMBA-induced adrenal necrosis also prevent 7-OHM-MBA from damaging the adrenal cortex. This suggests that stimulation of drug metabolism can hasten the detoxification of exogenous 7-OHM-MBA in a similar manner to DMBA. Differences in protection against the two were found and these could help to identify the relevance of 7-OHM-MBA in the adrenocorticolytic phenomenon.

Experimental. Sprague–Dawley female rats of 50 days of age and weighing 140–170 g were used. They were fed commercial rat cube and given water *ad libitum*. From five to ten rats were used in each group and many of the experiments have been repeated several times with consistent results. Pretreatments were invariably given intraperitoneally, a single dose of the pretreatment substance being injected 24 hr before the challenge dose of adrenocorticolytic agent (excepting phenobarbital sodium which was given for 5 consecutive days). The substances used as pretreatments are listed in Table 1. 3-Methylcholanthrene (3-MC), 1-hydroxy-3-methylcholanthrene (1-OH-3-MC), *p*-dimethylaminoazobenzene (butter yellow), 1-(*p*-phenylazophenylazo)-2-naphthol (Sudan III), DMBA and its hydroxymethyl derivatives, α-(*p*-aminophenyl)-α-ethylglutarimide (aminoglutethimide, Elipten(R), CIBA), α-ethyl-α-phenylglutarimide (glutethimide, Doriden(R), CIBA) and 2,2-*bis* (2-chlorophenyl-4-chlorophenyl)-1, 1-dichloroethane (*o*, *p*′-DDD) were dissolved in arachis or olive oil and given in a volume of 0·4 ml or less. 2-chloro-10-(3-dimethylaminopropyl)-phenothiazine (Chlorpromazine), phenobarbital sodium and salt were given in aqueous solution in volumes of 1·0 ml or less.

Challenge with adrenocorticolytic doses of DMBA or 7-OHM-MBA were the following:

(i) DMBA dissolved at 30 mg/ml in olive oil was injected by stomach tube (dose 1 ml = 30 mg dose).
(ii) DMBA in a 15% oil emulsion at 5 mg/ml was injected into a lateral tail vein (dose, 1 ml = 5mg).
(iii) DMBA overdose, i.v. injection of DMBA in oil emulsion (dose, 3 ml = 15 mg).
(iv) 7-OHM-MBA dissolved in olive oil at 10 mg/ml was introduced by stomach tube (dose, 1·5 ml = 15 mg).
(v) 7-OHM-MBA, in a freshly prepared 20% olive oil in water emulsion at a final concentration of 2 mg/ml was injected into a lateral tail vein (dose, 1·5 ml = 3 mg).

(vi) 7-OHM-MBA overdose, either 1·5 ml of a 50% oil emulsion i.v. (5 mg/ml, dose = 7·5 mg) or 3 ml of the oil solution i.g. (10 mg/ml, dose = 30 mg).

It was repeatedly established that treatment of rats with the vehicles did not produce adrenal necrosis. The challenge doses of DMBA and 7-OHM-MBA were carefully selected on the basis of past experience so that the adrenocortical-damaging potentials of (i), (ii) and (iii) corresponded closely to those of (iv), (v) and (vi) respectively. Adrenal damage for the purposes of these experiments was assessed on an all-or-none basis, adrenal glands only being considered protected where no evidence of parenchymal cell damage or vascular congestion could be detected in histological sections.

Results and discussion. The results of the different pretreatments are shown in Table 1. 3-MC and Sudan III, which have been found to be the most effective protectors against DMBA,[11,15] not only

TABLE 1. COMPARISON OF THE PROTECTIVE ACTION OF DIFFERENT TYPES OF PRETREATMENTS AGAINST ADRENAL NECROSIS INDUCED BY DMBA AND BY 7-OHM-MBA

Pretreatment	Dose	DMBA			7-OHM-MBA		
		Standard challenge		Overdose	Standard challenge		Overdose
(i.p.)	(mg)	30 mg i.g.	5 mg i.v.	15 mg i.v.	15 mg i.g.	3 mg i.v.	7·5 mg i.v. or 30 mg i.g.
3-MC	1	*	*	*	*	*	*
Sudan III	1	*	*	*	*	*	*
1-OH-3-MC	1	n.t.	*	—	†	n.t.	n.t.
DMBA	1	*	*	—	†	†	—
12-OHM-MBA	1	n.t.	*	†	‡/†	†	—
7-OHM-MBA	1	*	—	—	—	—	n.t.
7-OHM-12-OHM-BA	1	—	—	n.t.	—	—	n.t.
Chlorpromazine	2	*	*	—	n.t.	*	n.t.
Butter yellow	20	n.t.	*	‡	n.t.	†	n.t.
Phenobarbital	5 × 90 mg/kg	n.t.	*	n.t.	—	—	n.t.
Glutethimide	20	n.t.	‡/†	n.t.	n.t.	—	n.t.
Aminoglutethimide	20	—	—	n.t.	—	—	n.t.
o,p'-DDD	10	n.t.	—	n.t.	n.t.	n.t.	n.t.
oil or emulsion		—	—	—	—	—	—
saline or none		—	—	—	—	—	—

Key: * complete protection of adrenal glands of all pretreated rats, † more than half of rats protected, ‡ less than half the rats protected, — none of the rats protected, n.t. not tried.

protect against the more commonly employed challenge dose of DMBA and 7-OHM-MBA (i, ii, iv, v) but also protect against overdoses of these substances (iii and vi). Although the pretreatment of rats with 3-MC or Sudan III markedly stimulates the ability of the liver microsomal drug-metabolizing enzymes to detoxify DMBA and 7-OHM-MBA,[7] it is inconceivable that this can permit the inactivation of large amounts of i.v. administered DMBA or 7-OHM-MBA before the adrenal cortex has been exposed to them. We deduce therefore, that 7-OHM-MBA has no direct toxic action upon the adrenal cortex. This conclusion agrees with the previous findings with the microsomal drug-metabolizing enzyme inhibitor SKF 525-A which prevents 7-OHM-MBA from producing adrenal necrosis.[13] It would appear that 7-OHM-MBA must be further metabolized to the adrenocorticolytic principle. In rats treated with 3-MC or other substances, protection is probably achieved by more rapid inactivation of the adrenocorticolytic metabolite and/or by alteration of the pathway of 7-OHM-MBA metabolism away from the pathway leading to the formation of the toxic metabolite. This applies not only to the adrenocorticolytic phenomenon but also to the systemic toxicity and lethality of DMBA or 7-OHM-MBA which are completely prevented by pretreatment of rats with 3-MC or Sudan III.

It was generally found that agents such as butter yellow, DMBA itself, the hydroxymethyl derivatives of DMBA, 1-hydroxy-3-MC, and phenobarbital, which have moderate enzyme inducing properties compared with 3-MC or Sudan III (Boyland and Sims, 1967; Grover and Sims, unpublished), were less effective against 7-OHM-MBA-induced adrenal necrosis than against DMBA-induced necrosis.

Furthermore, these less powerful inducers proved ineffective against the adrenocorticolytic action of overdoses of DMBA or 7-OHM-MBA although they would protect against standard challenge doses of DMBA. Mortality from the overdoses was considerably reduced, however, by the less powerful protectors. It is noteworthy that the induction of enzymes which detoxify polycyclic compounds can be stimulated more intensely by 3-MC than by DMBA itself, showing that the process of enzyme induction involved here lacks strict specificity . During the present investigation, the hydroxymethyl derivatives of DMBA were employed as pretreatments to see whether a greater specificity of enzyme induction would be achieved by these substances before 7-OHM-MBA challenge. However, it can be seen from Table 1 that of the DMBA series, DMBA itself and the 12-hydroxymethyl derivative were both protective against standard challenges of DMBA but were less effective against 7-OHM-MBA challenge. The 7-hydroxymethyl gave slight potection against DMBA but not against itself, and the dihydroxymethyl derivative was completely ineffective. Their protective abilities correlate with their enzyme-inducing abilities (Grover and Sims, unpublished) and also with their lipid solubilities.

Lipid solubility is not, however, a prerequisite for the protective action of enzyme inducers. Chlorpromazine and phenobarbital in aqueous solutions both showed protective ability. However, the enzyme system(s) induced by a polycyclic hydrocarbon may differ considerably from that induced by phenobarbital.[16,17] The ability of phenobarbital to protect against DMBA-induced adrenal necrosis but not against 7-OHM-MBA presents some difficulties in interpretation in view of previous studies which showed that this narcotic increased hydroxylation of the methyl groups of DMBA without significantly altering the amount of ring hydroxylation.[7] It can only be surmised at this stage that nascent hydroxymethyl derivatives of DMBA are more likely to be further hydroxylated or detoxified to more polar compounds in the phenobarbital-stimulated microsomal enzyme systems than relatively large quantities of exogenous 7-OHM-MBA which are suddenly introduced into the body by intravenous injection. Possible alterations in the routes of metabolism of DMBA and its metabolites in rats receiving different pretreatments are depicted in Fig. 1, the schemes being based on the findings reported in refs. 6, 7 and 8 in relation to those reported here.

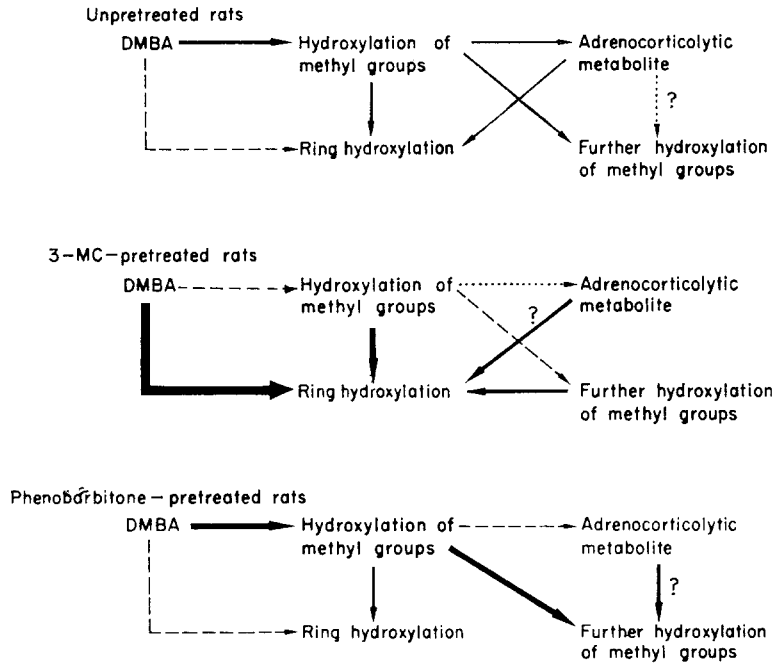

FIG. 1. Possible alterations in DMBA metabolism which may account for protection against adrenal necrosis after different types of pretreatment. Sizes of arrows give a guide to the relative intensities of the various reactions, not being absolutely proportional to intensity.

To establish that enzyme stimulation as opposed to inhibition is responsible for protection, the effective pretreatments listed in Table 1 were administered 30 min instead of 24 hr before challenge doses of DMBA or 7-OHM-MBA. In no instance was protection achieved. This contrasts with the time of action of the enzyme inhibitor SKF 525-A[13] or with CCl_4[18] which protect when given just before or at the same time as DMBA. Furthermore, *dl*-ethionine given 2 hr before and 2 hr after protectors abolished their effectiveness as 24 hr pretreatments. This schedule of ethionine administration was necessary to abolish the powerful protective action of 3-MC in our rats but the actions of weaker protectors could be abolished by a single 25 mg dose of ethionine 2 hr before the administration of the pretreatment. The results of experiments with 3-MC, DMBA and butter yellow as protective agents in the presence of ethionine are shown in Table 2.

TABLE 2. ABOLITION OF PROTECTION WITH *dl*-ETHIONINE

Dose of ethionine i.p. (mg)	Time of admin. (hr) in relation to "protector" (− before, + after)	24 hr pretreatment	Challenge	No. rats developing adrenal necrosis
25	−2, −1, +1 or +2	3-MC (1mg)	5 mg, i.v.	0/5 (each group)
50	−2, −1, +1 or +2	3-MC (1mg)	5 mg, i.v.	0/5 (each group)
50 + 25	−2 *and* +2	3-MC (1mg)	5 mg, i.v.	3/5
50 + 50	−2 *and* +2	3-MC (1mg)	5 mg, i.v.	7/10
50 + 50	−2 *and* +2	3-MC (1mg)	7-OHM-MBA 15 mg, i.g.	1/5
50 + 50	−2 *and* +2	3-MC (1mg)	7-OHM-MBA 3 mg, i.v.	2/5
25	−2	DMBA (1mg)	DMBA 5 mg, i.v.	5/5
25	−2	DMBA (1mg)	7-OHM-MBA 15 mg, i.g.	5/5
25	−2	Butter yellow (20 mg)	DMBA 5 mg, i.v.	4/5
25	−2	Butter yellow (20 mg)	7-OHM-MBA 15 mg, i.g.	2/5

In conclusion, the results of this investigation, in the light of previous findings,[13] strongly indicate that 7-OHM-MBA is not in itself adrenocorticolytic: exogenous 7-OHM-MBA requires further metabolism in the body before it exerts a damaging action of the adrenal cortex. Although the 7-hydroxymethyl derivative has been indentified as a product of DMBA metabolism by the rat liver, it cannot be definitely concluded from the available evidence that the conversion of DMBA to this metabolite is an obligatory intermediate step in the formation of the adrenocorticolytic substance. The nature of the toxic metabolite and how it is transported from the liver to the adrenal cortex remains to be established. The possibility exists that 7-OHM-MBA or a more chemically reactive species arising from the metabolism of either DMBA or 7-OHM-MBA requires conjugation in the liver before it can reach and enter the adrenal cortex in a soluble form. The sensitivity of the adrenal cortex to such a complex may be related to a peculiar ability of this tissue to release an active moiety from the conjugated or otherwise inactivated form.

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Department of Pathology,
University of Aberdeen
and
Chester Beatty Research Institute
Fulham Road,
London, S.W.3

D. N. WHEATLEY*

PETER SIMS

*Present address: McArdle Laboratory for Cancer Research, Medical Centre, Madison, Wisconsin 53706, U.S.A.

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Synthesis of 5-fluorouridine 5′-phosphate by a pyrimidine phosphoribosyltransferase of mammalian origin—II. Correlation between the tumor levels of the enzyme and the 5-fluorouracil-promoted increase in survival of tumor-bearing mice*

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IT IS now generally agreed that 5-fluorouracil (FU), the pyrimidine analog introduced by Duschinsky *et al.*,[1] must first be converted to 5-fluoro-2′-deoxyuridine 5′-phosphate (F-dUMP) before it can exert its carcinostatic effect. The latter compound has been shown to be a potent inhibitor of the enzyme thymidylate synthetase.[2,3] The inhibition of this enzyme interferes with DNA synthesis because of the resulting deficiency of available thymidylate. Current knowledge relating to the fluorinated pyrimidines has been summarized in a recent review by Heidelberger.[4]

Previous studies dealing with the development of tumor resistance to FU have indicated that more than one biochemical mechanism may be responsible for this phenomenon. Thus, insufficient levels of uridine phosphorylase,[5] uridine kinase[6] and orotidylate decarboxylase,[7] as well as the presence of an altered thymidylate synthetase[8] insensitive to inhibition by F-dUMP, have all been implicated as being determinants of resistance in a variety of transplantable mouse tumors.

This paper reports that cell-free extracts from a wide spectrum of transplantable mouse tumors catalyze the formation of 5-fluorouridine 5′-phosphate (F-UMP) from FU and 5-phosphoribosyl 1-pyrophosphate (PP-ribose-P). Furthermore, there is a statistically significant correlation between the specific activity of the enzyme responsible for this reaction in extracts from the various tumors

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