to rise while its concentration in plasma was declining, suggests existence of a drug-concentrating mechanism in plasmodia or in parasitized red cells in contrast to an exchange of CQ by free diffusion; only insignificant quantities of CQ were removed by washing parasitized erythrocytes in 0.15*M* NaCl.

A marked difference between concentrations of CQ accumulated in red cells parasitized by either susceptible or resistant *P. berghei* is shown in Fig. 3, in which these drug levels are represented as a function of the quantities of drug administered. Depending upon these quantities, concentrations of CQ were two to three times higher in red cells containing sensitive plasmodia than in those containing CQ-resistant *P. berghei*. Difference in the content of CQ per parasite is even greater, since erythrocytes parasitized with resistant *P. berghei* contain an average of 1.6 parasites to every erythrocytic CQ-sensitive parasite. Even at the nearly toxic dose of 40 mg/kg, the concentration of CQ attained with resistant *P. berghei* was no more than that attained with sensitive parasites upon administration of only 4 mg/kg. The greater accumulation of CQ in erythrocytes parasitized by sensitive *P. berghei* in comparison to lower levels attained in normal red cells, liver, and spleen explains the selective toxicity of CQ, upon which its usefulness as a chemotherapeutic drug is based. Furthermore, a linear relation exists between the degree of accumulation of CQ in tissues and normal red cells and the administered dose. In contrast, the uptake response of parasitized erythrocytes to graded doses of the drug more nearly resembles a correlation in which the logarithms of doses are proportional to the probits of the CQ concentrations attained. Such a specific accumulation of the drug in the parasite–red cell system may be ascribed to a mechanism that could be based upon the following conditions: (i) active transport of CQ, or (ii) free diffusion of CQ followed by reversible binding to intracellular sites.

Our experiments do not distinguish between these two mechanistic explanations; they also do not distinguish whether such a mechanism is intrinsic to the plasmodia or to the whole parasitized erythrocyte. The results do, however, suggest that CQ is selectively toxic because it attains higher concentrations in parasitized cells than in normal tissue cells, and that plasmodial resistance to CQ is based on an impairment of the mechanism by which such drug levels are accumulated.

PETER B. MACOMBER
Department of Experimental Pathology
RICHARD L. O'BRIEN
FRED E. HAHN
Department of Molecular Biology, Walter Reed Army Institute of Research, Washington, D.C. 20012

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Protective Action of Polycyclic Hydrocarbons against Induction of Adrenal Necrosis by Dimethylbenzanthracene

Abstract. *Treatment of rats with certain polycyclic hydrocarbons shifts the hydroxylation of dimethylbenzanthracene (DMBA) by liver microsomes from the side-chain to the ring. Protection by these hydrocarbons against dimethylbenzanthracene-induced adrenal necrosis is possibly achieved by decreasing the yield of the 7-hydroxymethyl derivative of dimethylbenzanthracene which may act as the necrotic agent by virtue of its structural resemblance to the adrenocortical steroids.*

The classical experiments of Huggins (*1*) and Dao (*2*) show that the administration of certain aromatic substances to rats prior to the feeding of 7,12-dimethylbenz(*a*)anthracene (DMBA) completely inhibits the induction of adrenal necrosis by DMBA and also gives some protection against the development of mammary cancer. Also the administration of a small dose of polycyclic hydrocarbon to rats induces a marked increase in hydroxylating enzymes in the liver (*4*). It has been shown that the gastrointestinal tract, lungs, and kidneys are also sites where hydroxylating enzymes increase after injection of polycyclic hydrocarbons (*5*). Boyland and Sims (*6*) have found that DMBA is metabolized in the rat mainly by oxidation of the methyl groups to the isomeric monohydroxymethyl derivatives, in contrast to phenanthrene, benz(*a*)anthracene, dibenz(*a,h*)anthracene and other unsubstituted aromatic hydrocarbons which are oxidized at reactive double bonds in the nucleus.

Several mechanisms have been pro-

Table 1. Effect of prior treatment with polycyclic hydrocarbons on the formation of ^{14}C-DMBA metabolites by rat liver microsomes. The polycyclic hydrocarbon (10 mg) dissolved in oil was given orally to rats (50 to 60 days of age), and the animals were killed 2 days later. The microsomes (8000*g* supernatant) from 50 mg of liver were incubated under oxygen for 1 hour at 37°C with DMBA-12-^{14}C (1.5×10^5 count/min in 3 μg), nicotinamide adenine dinucleotide phosphate (0.3 m*M*) and glucose-6-phosphate (3 m*M*) in 4 ml of phosphate buffer (0.1*M*), *p*H 7.4. The amount of radioactivity remaining in the aqueous medium after extraction with ether at *p*H 1 was then determined. The ether-soluble metabolites were separated by thin-layer chromatography and located by autoradiography.

Hydrocarbon	^{14}C in aqueous medium after extraction with ether (%)	Expts. (No.)	Ether-soluble fraction after chromatography		
			Unchanged DMBA (10^3 count/min)	Hydroxymethyl—MBA region (10^3 count/min)	Polar products near origin (10^3 count/min)
None (oil only)	27.1 ± 4.3	8	2.1	3.0	1.5
3-Methylcholanthrene	56.9 ± 8.4	8	1.0	0.6	2.7
Dibenz(*a,h*)anthracene	48.5 ± 9.0	5	1.3	.7	3.7
Naphthalene*	24.7 30.0	2	2.3	1.7	1.5

* Does not protect against adrenal necrosis (*2*).

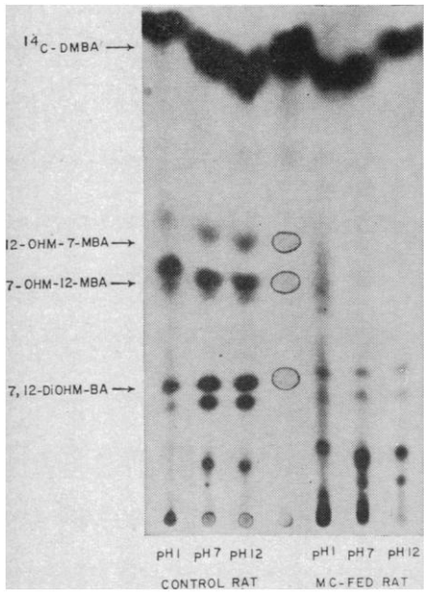

Fig. 1. Autoradiogram of ether-soluble ^{14}C-DMBA metabolites formed by rat liver microsomes before and after treatment with 3-methylcholanthrene (MC). The metabolites were separated with a mixture of benzene and ethanol (19:1) by thin-layer chromatography on silica gel (*6*). The incubation mixtures were extracted at different *p*H values as indicated, and the nonradioactive standards were located by their fluorescence. 12-OHM-7-MBA, 12-hydroxymethyl-7-methylbenzanthracene; 7-OHM-12-MBA, 7-hydroxymethyl-12-methylbenzanthracene; 7,12-DiOHM-BA-7,12-dihydroxymethylbenzanthracene.

posed (*7*) to explain the protective action of polycyclic hydrocarbons against selective damage of the adrenals by DMBA. One theory suggests that the markedly increased concentration of hydroxylating enzymes in the liver and other sites after treatment with polycyclic hydrocarbons makes possible the destruction of DMBA before it can reach the adrenal cortex in sufficient quantity to inflict damage upon the gland.

Our results (Table 1) support this theory, and we should like to extend it by proposing that the main effect may be due to a shift from the formation of the hydroxymethyl derivatives by the liver to ring-hydroxylated metabolites (Fig. 1). These phenolic products are subsequently converted to inactive water-soluble products by a reaction which may be analogous to the conversion of estrogens to water-soluble derivatives by rat liver preparations (*8*). A decrease in the yield of the hydroxymethyl derivatives of DMBA after treating rats with substances that induce increases in the microsomal enzymes has been reported (*9*), but no experimental data were given.

7,12-Dimethylbenz(*a*)anthracene causes a significant decrease in the corticosterone content of the adrenals (*1*, *10*) and a correlation was also reported (*11*) between the susceptibility of the adrenal cortex to DMBA-induced necrosis and its content of this steroid. In addition, rats have been protected (*12*) against DMBA-induced necrosis with SU4885, an amphenone analog that is known to inhibit 11β-hydroxylation and corticosterone synthesis. It was therefore postulated (*1*) that the cause of damage is related to the similarity in structure of DMBA and corticosterone.

In the light of the findings that DMBA, unlike other carcinogenic hydrocarbons, is oxidized preferentially in the side chain, it seems reasonable to suggest that it is a hydroxymethyl derivative, rather than the unchanged carcinogen, that interferes with corticosteroid metabolism. It is the presence of the hydroxymethyl group, characteristic of the adrenocortical steroids, which should make 7-hydroxymethyl-12-methylbenzanthracene into a more potent and selective inhibitor of corticosterone synthesis than its parent hydrocarbon. On the other hand, 12-hydroxymethyl-7-methylbenzanthracene which shows less structural similarity to the corticosteroids than the 7-hydroxymethyl analog should be less active as an adrenal necrotic agent. This, in fact, has been found (*9*) in that 5 mg of 7-hydroxymethyl-12-methylbenzanthracene caused about the same amount of adrenal damage as 30 mg of DMBA, and 12-hydroxymethyl-7-methylbenzanthracene was inactive even in 60-mg doses. It should also be possible to test this theory by studying the effect of DMBA and its metabolites on the biosynthesis of corticosterone in the rat.

The role of steroid hormones in the growth and differentiation of hormone-dependent tissues such as the uterus, breast, and prostate is now well established and there is also a close correlation between the induction of tumors by polycyclic hydrocarbons and the hormonal environment. Possibly, these carcinogenic hydrocarbons exert their effect on such tissues by mechanisms not too unrelated to the action of DMBA on the adrenal glands.

P. H. JELLINCK
BARBARA GOUDY
Cancer Research Centre, University of British Columbia, Vancouver, Canada

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Mammary Tumor Inhibition and Lung Adenoma Induction by Isonicotinic Acid Hydrazide

Abstract. *The occurrence of mammary adenocarcinomas in C3H mice is largely inhibited by prolonged administration of 0.1 percent isonicotinic acid hydrazide in drinking water. At the same time that this compound produced the inhibitory action, it also increased the incidence of pulmonary adenomas.*

Isonicotinic acid hydrazide (INH) induces pulmonary adenomas in a variety of mouse strains (*1*, *2*); it is also reported to cause an increased incidence of lymphomas and leukemias (*1*).

A recent study in this laboratory (*3*) confirmed the enhancement of the incidence of lung adenomas in Swiss mice that had been given a 0.1-percent solution of INH. In contrast, the appearance of mammary tumors and malignant lymphomas seemed to have been diminished in these animals. This last result could not be considered conclusive because too few tumors were involved, although in treated groups several developing mammary tumors regressed. We therefore studied this effect in a strain of mice with high incidence of mammary cancers. When the mice were treated with INH, development of mammary tumors in C3H female mice was inhibited and the incidence of lung adenomas was increased.