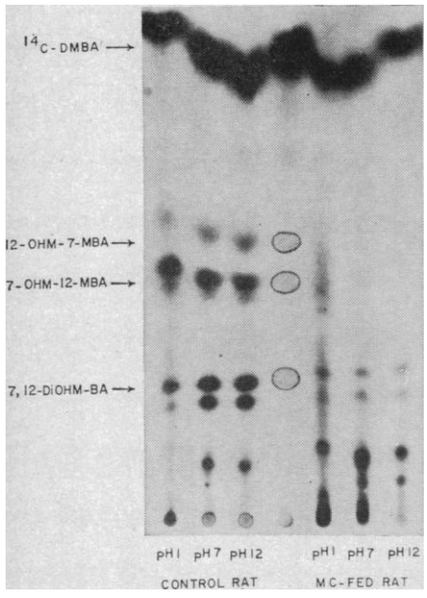

Fig. 1. Autoradiogram of ether-soluble ^{14}C-DMBA metabolites formed by rat liver microsomes before and after treatment with 3-methylcholanthrene (MC). The metabolites were separated with a mixture of benzene and ethanol (19:1) by thin-layer chromatography on silica gel (*6*). The incubation mixtures 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.

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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.

C3H inbred mice (*4*), bred in our laboratory by brother-to-sister mating since 1961, were used. They were housed in groups of ten in plastic cages with sterilized, granular cellulose bedding; and, with one exception, they were given Rockland diet in pellets and tap water ad libitum. Experimental and control groups originated from a number of litters born within a few days.

Isonicotinic acid hydrazide (*5*) was dissolved in drinking water to make a 0.1 percent solution and was given continuously during the life span of 30 female and 30 male C3H mice that were 5 weeks old when the experiment was started. Average daily consumption of INH per animal was 4.7 mg (in 4.7 ml) for females and 5.6 mg (in 5.6 ml) for males. Untreated C3H mice (30 female and 30 male) were controls.

Animals were checked and weighed at weekly intervals; they were allowed to die or were killed with ether when they were found to be in poor condition. All animals were necropsied. Histologic studies were made on organs showing pathologic changes and on at least four lobes of the lungs of each mouse. Sections were routinely stained with hematoxylin and eosin.

The change in average weekly weights of the mice (Fig. 1) indicate that INH caused a reduction of body weight. The survival rate (Table 1) shows a reduced survival only in the treated males.

The incidence of mammary tumors in mice and the time these tumors appeared are presented in Fig. 2. In the females of group 1, the five tumor-bearing mice had a total of six mammary tumors, and, in group 2, the 23 tumor-bearing mice had a total of 30 mammary carcinomas. In the treated males, four animals developed lung adenoma at the ages 52, 58, 60, and 68 weeks, and two animals each developed this lesion at 75 weeks; in addition, one treated female, 75 weeks old, had a lung adenoma. Only one lung adenoma, in a 70-week-old male, was seen in controls.

Mammary tumors in both groups were adenocarcinomas, predominantly with acinar structure, type A and, in a few instances, type B, with cells arranged in sheets or chords between cystic spaces as described by Dunn (*6*). In addition, we observed one granulosa cell tumor in the treated females and one papilloma of forestomach in the control males.

Table 1. Survival rate of INH-treated and control C3H mice.

Group	Sex and initial number of mice	Number of survivors (weeks)								
		10	20	30	40	50	60	70	80	90
1. INH-treated	30 ♀	27	24	24	24	22	17	12	0	0
	30 ♂	29	29	27	21	21	16	8	0	0
2. Control	30 ♀	30	30	30	28	22	12	6	2	0
	30 ♂	30	30	30	30	29	27	20	17	*

* Some of these animals are still alive.

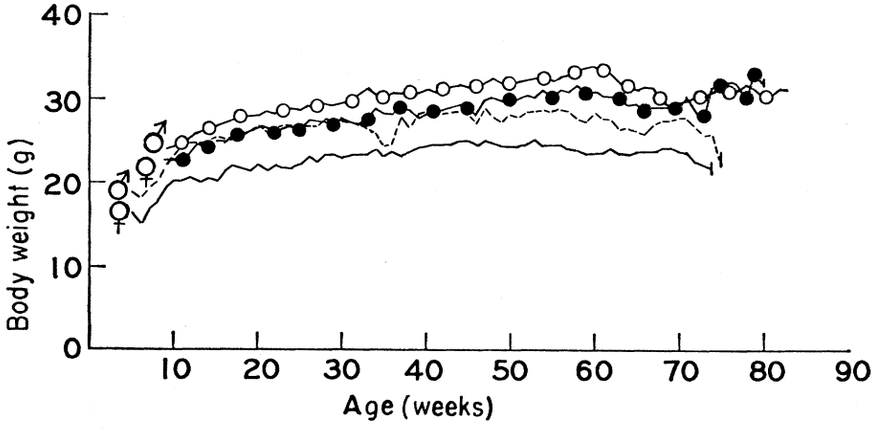

Fig. 1. Average body weight of INH-treated and control C3H mice. Solid line, female mice treated with INH; dashed line, INH-treated males; open circles, male controls; closed circles, female controls.

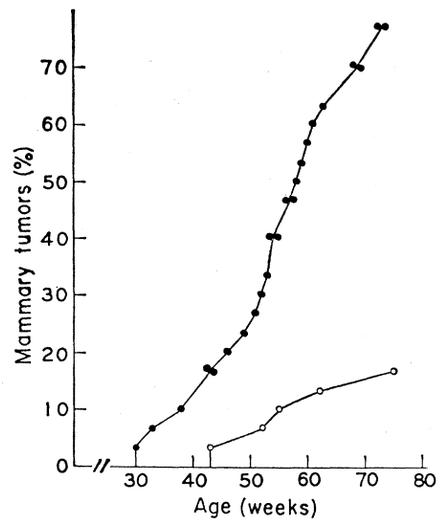

Fig. 2. Cumulative percentage of female mice with mammary tumors, together with the latent period for these tumors. Closed circles, untreated (76.6 percent); open circles, INH-treated (16.6 percent).

The results of this experiment are clear-cut: pulmonary adenomas are induced, as in prior studies, but the development of mammary tumors is inhibited. There is a slight weight loss caused by the treatment, which suggests that inhibitory action may be due to an effect similar to that demonstrated by Tannenbaum, who used caloric restriction. However, in Tannenbaum's studies, mammary tumors and pulmonary adenomas were equally inhibited by dietary factors (*7*). It is most unlikely that this effect could account for our results. We do not know of any example of a carcinogen that induces or enhances one type of tumor while it inhibits another. It seems clear that isonicotinic acid hydrazide has a specific effect on the mammary tumors of mice, but we cannot suggest a mechanism for this effect on these tumors which do carry the Bittner virus.

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