Increased Incidence of Tumors in F1 and F2 Generations from Pregnant Mice Injected with a Polycyclic Hydrocarbon.* (30289)

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The establishment of strains of mice specially sensitive to carcinogenic agents has been described(1,2).

Strong(3,4) produced data suggesting that gastric cancer was a hereditary disease in mice that could be induced by the chemical carcinogen methylcholanthrene. Miller and Pybus(5,6) did not find evidence that methylcholanthrene actually induced a germinal mutation; the increased incidence of tumors that they found in untreated descendants of methylcholanthrene-treated mice seemed more likely to be the product of hybridization and segregation of a character. Strong(4) suggested that the influence of biologic variability might have been superimposed on a single primary embryologic lesion.

The appearance of malignant lymphomas in rats suckled by mothers receiving methylcholanthrene was reported by Shay *et al*(7). The same authors later reported a significantly increased incidence of tumors in F1 and F2 generations derived from female rats fed methylcholanthrene by stomach tube prior to conception(8).

Similar results were reported by Gelshtein (9) who obtained an increased incidence of liver and lung tumors in F1 and F2 generations derived from female C3HA mice painted with o-aminoazotoluene for various times, before, and/or during pregnancy, and after delivery.

In view of these previous reports it seemed of interest to investigate the possible effects on the offspring of the administration of the polycyclic hydrocarbon 7,12-dimethylbenz(a)anthracene (DMBA) to pregnant mice.

Materials and methods. Swiss mice originally obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and randomly bred in this laboratory for the past 14 years were used. The carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) (Eastman Organic Chemicals) was purified by chromatography on magnesia/celite. Pregnant females received one i.p. injection of purified DMBA. This was made in the midline of the upper abdomen by means of a 26 gauge needle on a tuberculin syringe. Care was taken not to perforate the abdominal organs. Delivery occurred between 12 hours and seven days after the injection. The length of gestation did not seem to have been affected.

The experimental animals were divided into 3 groups. In Group 1, 5 pregnant females received 500 γ of DMBA i.p. in 0.2 ml of tri-n-caprylin (Trioctanoin, Eastman Organic Chemicals), and from these animals a total of 33 siblings was delivered, of which 16 survived at weaning. In Group 2, 5 pregnant females were given 350 γ of DMBA i.p. in 0.2 ml of tri-n-caprylin, from which a total of 24 siblings was delivered and 19 of these survived at weaning. Group 3 was started by mating 5 females of the F1 generation of Group 2 with their brothers (Fig. 1).

Results. The injected mothers survived from 8 to 50 weeks after delivery. In Group 1 one had ovarian and lung tumors, one had lung tumors and a mesothelioma and in 3 no tumors were found. In Group 2, one had an adenoma of the salivary gland, one had a malignant lymphoma, one a sarcoma of the mesentery and in 2 no tumors were found. Cannibalism occurred quite frequently and since decomposition is very rapid in infant mice, few of the siblings which died before weaning were suitable for a complete histological examination. The histological findings suggested the presence of a severe cellular depletion of the thymus, spleen and bone marrow. Although the cause of death could not be ascertained, since the stomach was full of milk, starvation could be excluded in all of those examined. The results are summarized in Tables I, II and Fig. 1. Con-

* This work was supported by grant CA 9112 from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

sidering Groups 1 and 2 together, 31 (or 85%) of the 35 mice surviving at weaning later developed a total of 52 tumors. In Group 3, 27 (or 84%) of the 32 mice surviving at weaning later developed a total of 52 tumors. A considerable number of tumor bearing animals (t.b.a.) had more than one tumor, namely 16 out of 31 in Group 1 and 2, and 14 out of 25 in Group 3. More female animals developed tumors than male animals, and a larger number of females than males bore more than one tumor. The difference, however, is not statistically significant.

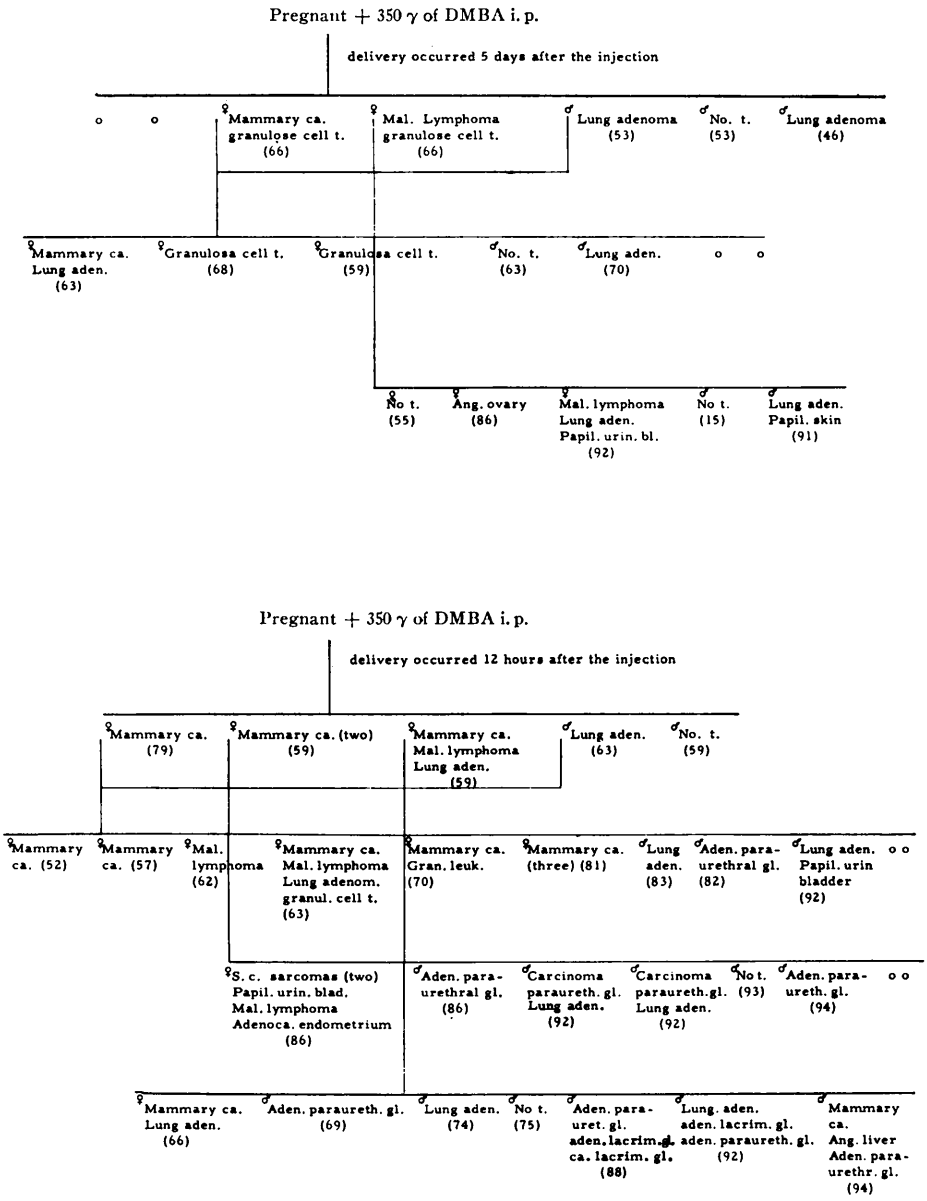

FIG. 1. Pedigree of F2 descendants (Group 3) from pregnant mice injected with 350 γ of DMBA i.p. The tumors occurring in each animal and the age at death, in weeks, are recorded. "No T." means that the animal died with no tumors. The symbol ° indicates the animals which died before weaning.

TABLE I. Survival Rate and Tumor Incidence in F1 Descendants from Pregnant Mice Injected with 7,12-Dimethylbenz(a)Anthracene.

Group	No. pregnant treated	Total born	Total surv at weaning	Survival rate (wk) 10	20	30	40	50	60	70	80	90	T.B.A.	Total No. tumors	Type of tumors
Group I (500 γ DMBA)	5	33	9♀	9	9	9	8	7	3	1	1	0	8 (88%)	13	6 lung adenomas 2 granulosa cell tum 2 skin carcinomas 1 angioma liver 1 mal lymphoma 1 adenoma lacrimal gl
			8♂	8	8	8	8	8	8	8	1	0	6 (75%)	6	6 lung adenomas
Group II (350 γ DMBA)	5	24	10♀	10	10	9	8	7	6	4	1	0	10 (100%)	24	6 mammary carcinomas 4 granulosa cell tum 5 lung adenomas 4 mal lymphomas 1 adenoma salivary gl 1 angioma liver 1 hepatoma 1 mesenchymal tum 1 lymphangioma
			9♂	9	9	9	9	8	4	2	1	0	7 (77%)	10	7 lung adenomas 2 mal lymphomas 1 adenoma paraurethral gl
Totals	10	57	36	36	36	35	33	29	21	15	4	0	31 (85%)	53	

TABLE II. Survival Rate and Tumor Incidence in F2 Descendants from Pregnant Mice Injected with 7,12-Dimethylbenz(a)Anthracene (DMBA) (Group 3).

Total born	Total surv at weaning	Survival rate (wk) 10	20	30	40	50	60	70	80	90	100	T.B.A.	Total No. tumors	Type of tumor
	14 ♀	14	14	14	14	14	11	4	4	2	0	13 (92%)	27	9 mammary carcinomas 4 granulosa cell tum 4 mal lymphomas 4 lung adenomas 1 papil urinary blad 2 s. c. sarcomas 1 angioma ovary 1 granulocytic leuk 1 adenocarcinoma endom
	18 ♂	18	18	17	17	17	17	15	12	6	0	14 (77%)	25	5 adenomas paraurethral gland 4 adenocarcinomas paraurethral gl 8 lung adenomas 2 adenomas lacrimal gl 1 carcinoma lacrimal gl 2 papillomas urinary bl 1 mammary carcinoma 1 angioma liver 1 papilloma skin
Totals 36	32	32	32	31	31	31	28	19	16	8	0	27 (84%)	52	

The most frequently occurring tumors were mammary, ovarian and lung tumors in the females, and lung adenomas and adenomas of the paraurethral gland in the males. What appeared more striking, however, was the wide distribution of the organs affected and the frequency in which more than one was affected in the same animal.

Our colony of randomly bred Swiss mice has a fairly high incidence of "spontaneous" tumors, the average incidence of t.b.a. in several years of observation being 36% in females and 21% in males. The incidence of mammary carcinomas, ovarian tumors, lung adenomas and malignant lymphomas in the females was 11%, 5%, 21%, and 16%, respectively. The incidence of lung adenomas, malignant lymphomas and adenomas of the paraurethral gland in the males was 13%, 12% and 1.1% respectively.

The average incidence of t.b.a. with more than one tumor, in the control groups, was 12% in the females and 5% in the males. The incidence of tumors was not altered in the control group that was inbred for one generation.

Discussion. It has been shown that newborn mice have a particular susceptibility to the tumorigenic action of DMBA, as well as many other carcinogens(10-16). The predominant tumors occurring in mice treated with DMBA within 24 hours of birth are malignant lymphomas, lung adenomas and subcutaneous sarcomas and these occur at an early age(10-16). In contrast DMBA given to pregnant mice resulted in the occurrence in both F1 and F2 generations of a wide variety of tumors, none of which appeared before the 55th week of age. The considerable length of the latent period, similar to that observed for the tumors occurring in untreated control groups, indicates a low degree of inherited susceptibility(17). The later in life the tumors appear, the more likely it is that non-genetic factors exert their influence.

The production of lung tumors in young mice following transplacental exposure to urethan has been reported by various authors, (18-21), and a possible transplacental carcinogenic action of diethylnitrosamine in hamsters was recently described(22). Polycyclic hydrocarbons, on the contrary, are said not to pass the placenta in effective quantities (19), although their effectiveness in the production of tumors in mice following the injection of 1,2,5,6-dibenzanthracene directly into the amniotic fluid was demonstrated by

Law(23). Pietra and Spencer, in recent experiments were unable to recover free DMBA or labeled material from mouse embryos 18, 24, 48, and 72 hours after the s.c. injection of a high dose of tritiated DMBA into pregnant mice at the 15th and 16th day of pregnancy (personal communication).

Though DMBA may have reached the fetuses through the placental barrier, it seems more likely that DMBA was transmitted to the siblings *via* the mothers' milk. The transmission of methylcholanthrene from lactating mothers to suckling rats has been demonstrated(7) and the same seems to occur with o-aminoazotoluene in mice(9). Moreover if the carcinogen had reached the fetuses through the placenta, some differences in the number and types of tumors and their latent period would be expected between the litters born 12 hours and those born 5 days after the injection of the carcinogen. This was not the case (Fig. 1).

A direct contact with the carcinogen could be reasonably excluded in the case of the F2 generation; more studies, however, would be required to completely exclude the transmission of some metabolite of DMBA. In the F2 descendants of DMBA treated pregnant mice there appears to be an enhancement of an inherited susceptibility to certain tumors.

Summary. Pregnant Swiss mice were injected i.p. with 500 or 350 γ of 7,12-dimethylbenz(a)anthracene (DMBA) in tri-n-caprylin. Delivery occurred between 12 hours and 7 days after the injection. Five females, descendants from 2 pregnant mice injected with 350 γ of DMBA, were mated with their brothers. In both F1 and F2 untreated descendants a high incidence of tumors occurring at various sites was recorded. The most frequently occurring tumors were mammary carcinomas, granulosa cell tumors of the ovary and lung adenomas in the females, and lung adenomas, adenomas and carcinomas of the paraurethral gland in males. Many of the tumor-bearing animals bore more than one tumor. The transmission of the carcinogen *via* the mother's milk probably occurs in the mice of the F1 generation. The enhancement of an inherited low susceptibility to certain tumors seems to be the cause for the high incidence of tumors in the mice of the F2 generation.

The author wishes to thank Dr. P. Shubik for his interest in this work and Dr. C. M. Goodall for his very helpful advice. The valuable technical assistance of Mrs. Birute Zidonis is gratefully acknowledged.

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Received April 8, 1965. P.S.E.B.M., 1965, v119.