Carcinogenic Effects of Subcutaneous Administration of Benzo(*a*)-pyrene During Pregnancy on the Progeny (34993)

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Some chemical carcinogens, such as urethane and diethylnitrosamine, have been shown to produce carcinogenic effects on the fetus *in utero* (1, 2). In the case of the polycyclic hydrocarbon carcinogens, the data are quite unclear. A number of investigations have demonstrated that administration of polycyclic hydrocarbon carcinogens to pregnant animals will result in an increased number of tumors in their progeny (3–7). However, in these experiments, the offspring have been allowed to nurse on their own mothers. Since polycyclic hydrocarbon carcinogens will appear in the milk, a distinction cannot be made between prenatal and postnatal exposures (8). The studies to be presented were designed to distinguish between these two possibilities. The progeny of female mice which had received subcutaneous injections of benzo(*a*)pyrene (BP) during the latter half of pregnancy were delivered by caesarean section and nursed on foster mothers from a control group which had not received injections of any type. Thus, any carcinogenic effect which resulted from administration of the carcinogen would have occurred during the prenatal period. In the experiments to be reported the presence and number of pulmonary adenomas have been determined. The mouse lung is highly sensitive to chemical carcinogens, and such exposures result in formation of pulmonary adenomas (9). A second tissue which has received special attention is the skin. Weak carcinogenic effects in the skin, such as initiation, can be elicited or accelerated by subsequent topical application of croton oil (10) and this procedure has been employed. In addition to the study of these two sensitive indices of a carcinogenic effect, the presence of tumors in other tissues was also searched for at autopsy.

Materials and Methods. Female Ha/ICR mice obtained from Millerton Farms, N.Y. were used for the study of carcinogenesis. Groups of four females, 14 weeks of age, were housed with one male. They were examined daily for mucus plugs. On days 11, 13, and 15 of pregnancy mice were injected subcutaneously with 0.2 ml of carbowax-400 (Union Carbide Corp.) containing 4 mg of BP, vehicle only, or nothing. On day 20 of gestation, the progeny were delivered by caesarean section. Five of these offspring were placed with a foster mother and three of her own progeny. The tip of the tail of the experimental animals delivered by caesarean section was cut to identify them. Ha/ICR mice from Schmidt, Madison were used as foster mothers. The foster mothers received no injections of any type. When the experimental mice were 4 weeks of age, they were weaned. Males and females were housed separately, six mice to a cage. The backs of the mice were shaved and topical administrations of croton oil were begun. Two drops of 1% croton oil in acetone were dropped onto the back twice a week throughout the remainder of the experiment. Mice were sacrificed when they were 28 weeks of age, and a complete autopsy was performed. Pulmonary adenomas were counted according to the procedure of Shimkin (9).

Results. In Table I the marked effect of BP administration to pregnant mice on pulmonary adenoma formation in their progeny is presented. An increase in tumor incidence occurs. An additional parameter showing the effect of exposure to carcinogen is the number of adenomas per animal. In the 171 control animals only one mouse had more than a single adenoma. In contrast 19 of 55 mice which were the progeny of mothers injected with BP had three or more adenomas. Table II summarizes the effect of prenatal ex-

TABLE I. Effect of Benzo(*a*)pyrene Administration to Pregnant Mice on Pulmonary Adenoma Formation in the Progeny.

Experimental group	Material administered	No. of mice	No. of mice with adenomas[a]	% of mice with adenomas	No. of adenomas per group	No. of adenomas per mouse	No. of mice with multiple adenomas	
							3–6	7–9
Absolute controls	Nothing	82	6	7.3	7	0.09	0	0
Vehicle controls	Carbowax-400	89	10	11.2	13	0.15	1	0
Benzo(*a*)pyrene	Benzo(*a*)pyrene in carbowax-400	55	34	61.8	130	2.36	13	6

[a] Number of mice with pulmonary adenomas at 28 weeks of age.

posures to BP on skin papilloma formation. Again, both the tumor incidence and the number of tumors per animal are increased in the progeny of mothers injected with BP. The incidence of tumors of the lung and skin was very similar in both males and females so that these data are combined in the two tables. Aside from the effects on the lung and skin, no evidence of a carcinogenic effect of BP administration was found in any other tissue.

Discussion. The data obtained in the present study indicate that the progeny of pregnant mice injected subcutaneously with BP during the latter half of pregnancy have an enhanced tumor formation in the lung and skin. Since these animals were delivered by caesarean section and nursed on control foster mothers the carcinogenic effect was produced during the prenatal period. Under the conditions employed the two tissues showing a carcinogenic response are sensitive to the effects of chemical carcinogens. A carcinogenic effect was not observed in other tissues. However these experiments were of relatively short duration and it remains to be determined whether a neoplastic response will occur in other tissues if mice are followed for a total life span.

There is currently a widespread exposure of man to polycyclic hydrocarbon carcinogens present in the atmosphere, in cigarette smoke, and in food. The question arises as to whether these exposures could have a carcinogenic effect on the fetus *in utero*. The data presented in this paper suggest that such an effect is, indeed, possible. It remains to be determined whether it actually does occur under conditions of environmental exposures. The very high doses of carcinogen employed in the experimental work and the large species differences between mouse and man create uncertainties. Nevertheless, it has been shown by Welch *et al.* (11) that the placentas of women who smoke have an increased benzo(*a*)pyrene hydroxylase activity presumably from the inducing effects of polycyclic hydrocarbons in the cigarette smoke. Studies with polycyclic hydrocarbons in rats show such an inducive effect on the placenta

TABLE II. Effect of Benzo(*a*)pyrene Administration to Pregnant Mice on Skin Papilloma Formation in the Progeny.

Experimental group	No. of mice	No. of mice with papillomas[a]	% of mice with papillomas	No. of papillomas per group	No. of papillomas per mouse
Absolute controls	82	6	7.3	8	0.10
Vehicle controls	89	8	9.0	9	0.10
Benzo(*a*)pyrene	55	13	23.6	18	0.33

[a] Number of mice with skin papillomas after 24 weeks of topical treatment twice weekly with 1% croton oil. Mice were 28 weeks of age.

and in addition induction of increased benzo(*a*)pyrene hydroxylase activity in fetal tissues indicating transplacental passage of carcinogen or a metabolite (11, 12). Thus, further work is important in order to more precisely assess the hazards to the fetus of maternal exposures to polycyclic hydrocarbons.

1. Klein, M., J. Nat. Cancer Inst. **12**, 1003 (1952).

2. Mohr, U., Althoff, J., and Authaler, A., Cancer Res. **26**, 2349 (1966).

3. Schabad, L., C. R. Soc. Biol. **99**, 1550 (1928).

4. Strong, L. C., and Hollander, W. F., J. Nat. Cancer Inst. **8**, 79 (1947).

5. Shay, H., Weinhouse, S., Gruenstein, M., Marx, H. E., and Freidmann, B., Cancer **3**, 891 (1950).

6. Tomatis, L., Proc. Soc. Exp. Biol. Med. **119**, 743 (1965).

7. Shay, H., Gruenstein, M., and Weinburger, M., Cancer Res. **12**, 296 (1952).

8. Shay, H., Friedmann, B., Gruenstein, M., and Weinhouse, S., Cancer Res. **10**, 797 (1950).

9. Shimkin, M. B., Advan. Cancer Res. **3**, 223 (1955).

10. Berenblum, I., Advan. Cancer Res. **2**, 129 (1954).

11. Welch, R. M., Harmson, Y. E., and Conney, A. H., Science **160**, 541 (1968).

12. Welch, R. M., and Conney, A. H., Fed. Proc. **28**, 676 (1968).

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