TRANSPLACENTAL ACTION OF 3-METHYLCHOLANTHRENE AND BENZ[α]PYRENE ON FOUR GENERATIONS OF MICE

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Small doses of carcinogens produce a state of increased sensitivity to chemical carcinogens in the progeny of experimental female mice. In four generations of transplacentally sensitized mice, an increase in the rate of appearance of papillomas and carcinoma was observed after applications of the carcinogen to the skin by comparison with control mice not receiving 3-methylcholanthrene or benz[α]pyrene in utero.

Much information regarding the transplacental action of various groups of chemical carcinogens has now been obtained. Carcinogenic hydrocarbons [16], aminoazo- compounds [4], nitroso-compounds [2, 11, 12, 14, 18], and urethane [5, 6, 17] have been injected into pregnant female rats, mice, and hamsters, and the formation of tumors in the progeny has been observed. If the progeny of mothers to which the carcinogen had been given was injected in the adult state with the same compound, the number of tumors formed was much higher than that usually observed during induction of tumors by this carcinogen [7].

Information concerning the effects of chemical carcinogens on several generations of animals has been obtained. Shabad [10] applied coal tar to mice and observed in the parent generation adenomas of the lungs in 25% of the animals (compared with 5% in the intact control), while in generations 1-4, to which the same tar was applied, adenomas developed in 50-60% of cases. Napalkov [12] injected 6-methylthiouracil into pregnant rats for 17 generations and observed a fluctuating incidence of tumors of the thyroid in the progeny.

In the investigation described below the effect of administration of 3-methylcholanthrene and of benz[α]pyrene to pregnant female mice on their progeny is studied.

EXPERIMENTAL METHOD

One drop of 0.5% solution of 3-methylcholanthrene or benz[α]pyrene in benzene was applied to the skin of pregnant noninbred female mice twice a week from the time of copulation. Eight weeks after birth of the progeny, the males were segregated and once a week the same carcinogen (methylcholanthrene or benz[α]-pyrene) as the mother received was applied weekly to the skin. This group of males was described as the first generation. The females were mated with males from the same litter and of the same age, and the carcinogens were applied to their skin in exactly the same way as to their mothers' skin. The generation born from these females was described as generation two, and from the age of 8 weeks the males began to receive methylcholanthrene or benzpyrene by application to the skin. The females were mated with a new batch of males and the carcinogens applied to their skin twice a week. The ensuing generation (the third) was distributed and treated just as the second generation. The same procedure was repeated with the fourth generation.

These experiments were thus carried out on males of four generations. Males whose mothers were not treated with the carcinogens acted as the control. The carcinogens were applied to their skin in the adult stage in exactly the same way as to that of the experimental animals.

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TABLE 1. Transplacental Sensitization with Carcinogens in the First to the Fourth Generations of Mice

	3-Methycholanthrene								Benz[α]pyrene							
	1st gen.		2nd gen.		3rd gen.		4th gen.		1st gen.		2nd gen.		3rd gen.		4th gen.	
	sensitization	control	sensitization	control	sensitization	control	sensitization	control	sensitization	control	sensitization	control	sensitization	control	sensitization	control
No. of mice at time of appearance of first papilloma	23	21	15	24	18	24	13	20	19	23	20	22	25	27	16	29
Mean latent period of appearance of papillomas (in weeks)	11,3	15,4	5,6	16,2	9,2	14,9	14,7	16,4	12,0	19,9	8,8	19,1	11,8	18,8	16,4	20,4
Difference between experiment and control (in weeks) for papillomas P	4,1 <0,001		10,6 <0,001		5,7 <0,001		1,7 <0,05		7,9 <0,001		10,3 <0,001		7,0 <0,001		4,0 <0,001	
Mean latent period of appearance of carcinoma (in weeks)	21,9	27,5	17,2	30,3	20,3	29,7	28,1	30,7	27,9	38,1	19,8	37,8	27,4	33,2	33,5	**34,4**
Difference between experiment and control (in weeks) for carcinoma P	5,6 <0,001		13,1 <0,001		9,4 <0,001		2,6 <0,001		10,2 <0,001		18,0 <0,001		5,8 <0,001		0,9 <0,2	
Percentage of mice with papillomas: weeks of experiment:																
10th	48	—	100	8,3	61,1	4,2	—	5	15,8	—	70	—	36	—	—	—
15th	87	66,7		33,3	100	58,3	61,5	50	100	13	100	9,1	88	22,2	43,7	17,2
20th	100	100		91,7		100	100	90		52,2		59,2	100	63	81,25	55,2
Percentage of mice with tumors: weeks of experiment																
20th	34,8	4,8	93,3	—	55,6	—	—	—	—	—	40	—	—	—	—	—
25th	82,6	28,6	100	16,7	94,4	16,7	15,4	25	21	—	100	—	24	3,7	—	—
30th	100	90,5		54,2	100	58,3	84,6	60	84,2	43,5		18,1	76	29	25	20,7

EXPERIMENTAL RESULTS

The results are given in Table 1. In the males of the first generation, exposed to the transplacentor action of methylcholanthrene, papillomas and carcinoma developed much more rapidly than in the unsensitized control mice. The mean difference was 4.1 weeks for the papillomas and 5.6 weeks for the carcinoma.

Papillomas and carcinoma developed even earlier in the second generation. The difference between the times of their appearance in the experimental and control animals was 10.6 weeks for papillomas and 13.1 weeks for carcinoma.

A similar picture was observed when the animals were sensitized transplacentally with benzpyrene, and in this case the difference in the time of appearance of papillomas and carcinoma was even more marked than in the experiments with methylcholanthrene (Table 1).

A slight decrease in the sensitivity to transplacental sensitization by carcinogens was observed in the third generation by comparison with the second. The difference between the times of formation of papillomas and carcinoma of the skin in the experimental and control animals was approximately the same as in the first generation after sensitization with both methylcholanthrene and benzpyrene.

In the fourth generation the difference between the experimental and control animals in sensitivity to carcinogens was even less than in the third generation. Papillomas appeared after sensitization with methylcholanthrene 1.7 weeks earlier, and after sensitization with benzpyrene 4 weeks earlier.

In the study of the difference between the latent periods of formation of cancer (2.6 weeks after sensitization with methylcholanthrene and 0.9 week in the experiment with benz[α]pyrene), in the case of benzpyrene all that could be deduced was a tendency for the lesions to appear earlier, because the difference is not significant.

Transplacental effects of chemical carcinogens were therefore studied in four generations of noninbred male mice. In all four generations, the appearance of papillomas and carcinoma after application of carcinogens was observed earlier than in control animals not receiving methylcholanthrene or benz[α]pyrene in utero. However, this more rapid development of carcinogenesis was not uniform in all generations. Increased sensitivity was observed in the progeny of the first generation; in the second generation this increase reached a maximum, while in the third and, in particular, in the fourth generations the increased sensitivity gradually diminished, although even in these generations it still remained higher than the sensitivity of the unsensitized control animals.

Investigations by several workers [9, 13, 15], including the present writers [3], have demonstrated the possibility of an increase in the sensitivity of animals to chemical carcinogens if small doses of the substances, hardly sufficient to cause the development of tumors, were administered to the animals. In the present investigation the method of transplacental sensitization was used, for in this case only a small quantity of the carcinogen can reach the fetus. Under these conditions also, the small doses of the carcinogen administered by the transplacental route evidently create a state of increased sensitivity to the chemical carcinogens in the progeny of the experimental females. As a result, the tumors develop earlier than after administration of ordinary effective doses of the carcinogens in the postnatal period.

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