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TRANSPLACENTAL ACTION OF BENZO(a)PYRENE AND PYRENE

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The transplacental and direct action of benzo(a)pyrene (BP) on mice of strains A and C57BL and of their progeny was studied. BP was found to represent a carcinogenic risk for the progeny. The greatest carcinogenic effect in progeny of strain A mice was exhibited by BP in a dose of 6 mg: The frequency of development of lung tumors was 76.8% compared with 12.3% in the control ($P < 0.001$). Liver tumors were found in the progeny of the C57BL mice (chiefly in males). Their frequency after a dose of 12 mg of BP was 31.6% in males and 9.1% in females, compared with only 1.2% in males in the control. No tumors of the liver were observed in females in the control. Pyrene, the noncarcinogenic analog of BP, had no carcinogenic effect.

KEY WORDS: *transplacental action; benzo(a)pyrene; carcinogen.*

Among carcinogenic agents polluting the external environment benzo(a)pyrene (BP) is particularly important. It can serve as an indicator of environmental pollution with polycyclic aromatic hydrocarbons (PAH) [2].

The carcinogenic action of BP on man has been proved by numerous observations on occupational cancer. The wide distribution of this substance in the environment presents the risk of its entering the human body and, in particular, the pregnant woman.

Nowadays the possibility of a transplacental carcinogenic action has been demonstrated for several substances, including BP [1, 3, 5-7]. The writers previously investigated the transplacental action of BP using the method of organ culture [4]. This paper gives the results of a study of the action of BP and pyrene on pregnant mice of strains A (with high risk of cancer) and C57BL (low risk) and their progeny.

EXPERIMENTAL METHOD

On the 18th to 19th day of pregnancy BP in 0.2 ml sunflower oil was injected subcutaneously into the experimental animals as a single dose of 4 and 6 mg or as two separate injections each of 6 mg, i.e., 12 mg per mouse. The action of pyrene was investigated in the maximal dose of 12 mg in strain A mice only. The young mice were weaned at the age of 4-5 weeks. The experimental mothers and their progeny were investigated 1 year later. Intact mice of both strains and mice of strain A receiving subcutaneous injections of pure sunflower oil in a dose of 0.4 ml (two injections, each of 0.2 ml), served as the control. The index of multiplicity was calculated for adenomas of the lungs and the numerical results were subjected to statistical analysis by Student's method.

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TABLE 1. Transplacental Action of BP on Mice

Dose of BP, mg	Sex	Strain A							Strain C57BL						
		number of animals	number of animals with tumors of						number of animals	number of animals with tumors of					
			lungs		mammary glands		liver			lungs		mammary glands		liver	
			absolute	%	absolute	%	absolute	%		absolute	%	absolute	%	absolute	%
4	Males	30	8	26,7					36	6	16,7			10	27.8
	Females	18	8	44,4	2	11,1	1	5,5	20	3	15,0			1	5.0
Total		48		33,3					56		16,1				19.6
P				<0,01							<0,01				
6	Males	55	49	89,1			4	7,3	31	5	16,1			7	22.6
	Females	53	34	64,1	11	20,7	1	1,9	17	8	47,0	4	23,5	1	5.9
Total		108		76,8				4,6	48		27,1				16.7
P				<0,001							<0,001				
12	Males	52	34	65,4					38	20	52,6			12	31.6
	Females	35	20	57,1	9	25,7			22	8	36,4	8	36,4	2	9.1
Total		87		62,1					60		46,7				23,3
P				<0,001							<0,001				<0.001
Control	Males	37	3	8,1					78	3	3,8			1	1,2
	Females	36	6	16,7	10	27,8			48			3	6,2		
Total		73		12,3					126		2,3				

TABLE 2. Direct Action of BP on Female Mice

Dose of BP, mg	Strain A							Strain C57BL				
	number of animals	number of animals with tumors of						number of animals	number of animals with tumors of			
		lungs		mammary glands		at site of injection			mammary glands		at site of injection	
		absolute	%	absolute	%	absolute	%		absolute	%	absolute	%
4	6	4	66,6	3	50,0	—	—	8	—	—	2	25.0
6	22	5	22,7	2	9,1	8	36,4	24	1	4,2	15	62.5
12	19	3	15,8	6	31,5	5	26,3	26	1	3,8	16	61.5
Control	18	3	16,7	8	44,4	—	—	18	—	—	—	—

Legend. No tumors of the lungs were found in the C57BL mice.

EXPERIMENTAL RESULTS

Through the transplacental action of BP on the mice of the two strains studied tumors developed in the lungs, mammary glands, and liver. The frequency of the neoplasms depended on the dose and on the strain and sex of the animals. Tumors in similar situations were found as a result of the action of BP on the experimental mothers also.

After injection of BP in a dose of 4 mg no statistically significant difference could be found between the frequency of adenomas of the lungs in males and females in the progeny of the mice belonging to strains A and C57BL (Table 1). However, the increase in the number of adenomas of the lungs in the mice of both strains compared with the control was statistically significant ($P < 0.05$).

A dose of 6 mg was most effective for the progeny of the strain A mice (Table 1). Adenomas of the lungs were found in these animals in 76.8% of cases, compared with only 12.3% in

the control ($P < 0.001$). They appeared in 49 of 55 males (89.1%, index of multiplicity 6.0), compared with 3 of 37 in the control (8.1%, index of multiplicity 1.0; $P < 0.001$). Adenomas of the lungs were found in 34 of 53 mothers (64.1%, index of multiplicity 6.3), but in only 6 of 36 mothers in the control (16.7%, index of multiplicity 1.3; $P < 0.001$). Tumors of the mammary glands developed in 20.7% of animals compared with 27.8% in the control, i.e., there was no increase in their frequency. Tumors of the liver were found in five mice (four males and one female).

This dose of BP had less effect on the progeny of the C57BL mice than on the progeny of the strain A mice. Adenomas of the lungs were found in 27.1% of cases, namely in 5 of 31 males (16.1%) and in 8 of 17 females (47.0%), but in only 2.3% of cases in the control ($P < 0.001$). The index of multiplicity was 2.2 for the males and 3.7 for the females, compared with 0.03 in the control. In this series of experiments adenomas of the lungs were more numerous in females. Mammary gland tumors appeared in four females, i.e., in 23.5% of cases compared with 6.2% in the control. Tumors of the liver were found in eight mice (one female and seven males).

After injection of BP in a dose of 6 mg, a higher frequency of adenomas of the lungs was thus found in females of the progeny of strain A mice than in males ($P < 0.001$). In the progeny of C57BL mice this difference was not significant. The number of adenomas of the lungs in males of the high-risk strain was greater than in males of the strain with a low cancer risk ($P < 0.001$). The frequency of mammary gland tumors in the progeny of strain A mice was virtually indistinguishable from the control, but in the progeny of the C57BL mice it was statistically significantly increased ($P < 0.05$). Tumors of the liver occurred more frequently in the progeny of the C57BL mice, chiefly in males.

BP in the maximal dose (12 mg) had its greatest action on the progeny of C57BL mice, in which it caused an increase in the number of lung and liver tumors compared with the dose of 6 mg. Liver tumors appeared chiefly in males (31.6%). The number of tumors in the strain A mice was smaller than with a dose of 6 mg, but the number of adenomas of the lungs was greater than in the control and in the female C57BL mice ($P < 0.05$).

Injection of BP in a dose of 12 mg thus did not potentiate the carcinogenic effect in strain A mice compared with the dose of 6 mg. This can be explained either on the grounds that only one injection gave a carcinogenic effect, i.e., 6 mg, or that the double dose of BP had a toxic action.

Pyrene, a noncarcinogenic analog of BP, in a dose of 12 mg, and pure sunflower oil caused no appreciable changes compared with the control in the frequency of tumors of the lungs and mammary glands. Under the influence of BP tumors appeared at the site of injection, and also in the lungs and mammary glands, in the mothers of the progeny (Table 2). The number of adenomas and their index of multiplicity were always lower for the mothers than for their progeny.

The experiments thus showed that BP, by transplacental administration to mice, has a carcinogenic action. The most effective dose of BP for strain A mice was 6 mg, which led to an increase in the frequency of tumors of the lungs, mammary glands, and liver. The frequency of lung tumors in the progeny of the strain A mice was 76.8%. In the progeny of the C57BL mice the observed carcinogenic effect depended clearly on the dose of the carcinogen. The number of tumors increased with an increase in the dose, but never exceeded that in mice of the high-risk strain. The general conclusion from these experiments is that the fetus is more sensitive to the carcinogenic action of BP: Despite the fact that very small amounts of the substance passed through the placenta, more tumors appeared in the progeny than in the parents. The appearance of liver tumors under the influence of BP, not previously recorded in the literature, is noteworthy.

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