TRANSPLACENTAL ACTION OF BENZ(a)PYRENE AND PYRENE IN ORGAN CULTURES OF MOUSE EMBRYONIC KIDNEYS

Yu. D. Sorokina

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The transplacental action of benz(a)pyrene and of pyrene was studied in organ cultures of embryonic kidneys of (C57BL×CBA)F_1 mice. In the control series, hyperplastic changes in the epithelium were found in 1.8% of cases, in the series with pyrene in 17.8%, and in the series with benzpyrene 26.7% of cases. In almost half (45%) of cases of exposure to benzpyrene, a distinct but diffuse hyperplasia of the epithelium was observed in the late stages of cultivation, which was not found either in the control or after exposure to pyrene.

It was discovered in 1927-1928 by Shabad and by Lynch [7] that if coal tar was painted on the skin of the offspring of mice which had also been painted with the same tar, the number of adenomas of the lungs formed in several generations was increased. This phenomenon was most probably due to the presence of the carcinogenic polycyclic hydrocarbon benz(a)pyrene (BP) in the tar. The transplacental carcinogenic action of other polycyclic hydrocarbons has been demonstrated by other workers [6, 8, 9].

The transplacental carcinogenic action of some carcinogenic compounds was demonstrated in vitro for the first time in the writer's laboratory. Kolesnichenko [2] showed that adenomas are formed in organ cultures of mouse embryonic lungs by the transplacental action of urethane. Later, tumor-like changes in the lungs not only of mice, but also of rats, were discovered by the action of nitrosamines [3]. Later, hyperplastic changes in the epithelium were found in organ cultures of the kidneys through the transplacental action of 7,12-dimethyl-benz(a)anthracene, dimethylnitrosamine, orthotolidine, and dichlorobenzidine [1, 4, 5].

The object of the present investigation was to detect the possible carcinogenic action of BP by transplacental administration in organ cultures of mouse kidneys. A parallel experiment with the noncarcinogenic analog of BP, pyrene (P), was carried out as a control.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL mice crossed with CBA males. Explants were taken from 20-21-day embryos. BP and P in olive oil were injected intramuscularly in 4 separate doses (1 mg× 4) during the last week of pregnancy in a total dose of 4 mg per mouse. The method of organ cultivation developed in the writer's laboratory was used. The explants were fixed in Bouin's fluid 4-30 days after the beginning of cultivation. Serial paraffin sections, 2-4 μ in thickness, were stained with hematoxylin-eosin.

EXPERIMENTAL RESULTS

Morphological changes observed during cultivation of the embryonic kidney of this line of mice have been described previously [4], so that no special mention of them will be given in this report. Only the hyperplastic changes in the epithelium observed during cultivation of intact kidneys will be mentioned.

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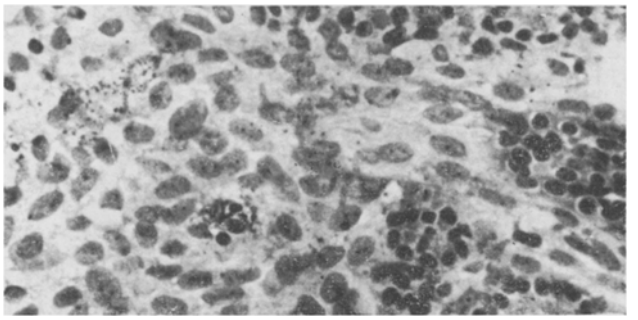

Fig. 1. Epithelial sheet with immured tubules (BP, 11th day of explantation). Hematoxylin-eosin, 400×.

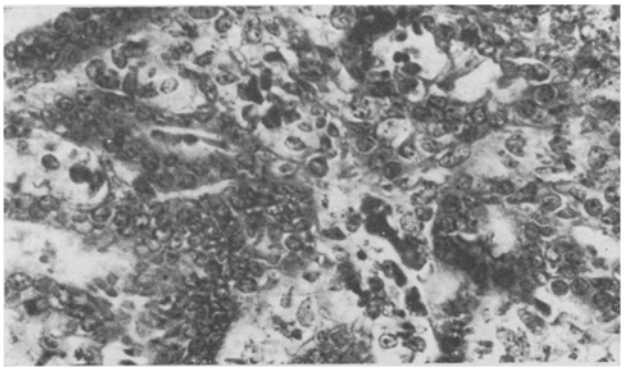

Fig. 2. Hyperplasia of convoluted tubules (BP, 11th day of explantation). Hematoxylin-eosin, 400×.

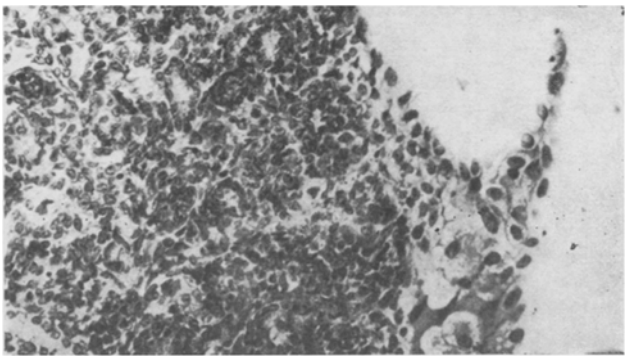

Fig. 3. Diffuse hyperplasia of epithelium in the form of a hyperchromic sheet. Formation of epithelial band on the surface (BP, 22nd day of explantation). Hematoxylin-eosin, 250×.

The so-called outgrowths were found in 4 explants 7 days after the beginning of cultivation. Three of these were large areas of kidney tissue spreading over the surface and covered by a relatively quiescent cubical epithelium. One was a tubule growing beyond the border of the explant; its epithelium was hyperplastic.

After the transplacental action of BP for 4 days the impression was obtained that, by contrast with the control, the extratubular scattered growth of the epithelium was more marked. In many explants growth of the epithelium, including the tubular epithelium, was so intensive that individual epithelial cells formed a characteristic palisade or tiny buds of several cells on the capsule, and in 6 cases, they formed whole sheets of cells (Table 1). Most of the observed outgrowths were epithelium with slight hyperplasia,

TABLE 1. Hyperplastic Changes in Epithelium Following Transplacental Action of BP and P in Organ Cultures of Mouse Embryonic Kidneys

Duration of experiment (in days)	Control	P								BP							
	number of explants with hyperplasia																
	total	living	epithelial sheets	hyperplasia of tubules	outgrowth	total	living	epithelial sheets	hyperplasia of tubules	outgrowth	total	living	epithelial sheets	hyperplasia of tubules	outgrowth		
4	35	35	—	—	—	11	11	3	1	—	61	61	6	—	19		
7	131	130	—	—	4	19	19	1	1	4	21	21	2	—	—		
11	45	43	—	—	—	18	18	6	2	4	82	82	17	4	10		
14	23	10	—	—	—	56	56	—	2	2	144	143	2	—	20		
18	14	9	—	—	—	59	55	—	—	7	28	13	1	—	3		
22	19	2	—	—	—	45	35	—	—	—	23	15	6	—	—		
26	17	—	—	—	—	26	2	—	—	—	5	2	—	—	—		
30	10	—	—	—	—	10	—	—	—	—	10	3	3	—	—		
Total	294	229			4 (1,8%)	244	196	10 (5%)	6 (3,1%)	19 (9,7%)	384	350	37 (10,6%)	4 (1,2%)	52 (14,9%)		
Grand total				1,8%				17,8%						26,7%			

but some were found which consisted of highly atypical epithelial cells, with no hint of tubular structure, or they were tubular outgrowths with well marked hyperplasia of the epithelium. Degenerative features were no more marked than in the control.

After 7 days the epithelial cells as before were spreading beyond the capsule and in 2 cases they formed sheets of cells. In most explants the capsule consisted of several layers of connective-tissue cells. Otherwise the explants were indistinguishable from the control.

After 11 days the cultures had lost all traces of necrosis in the center. However, a second wave of degeneration began from the surface: mostly the epithelial cells under the capsule died, and the capsule itself for a time remained intact and in some places was thickened. A few atrophic cysts lined with flat epithelium were observed. A characteristic feature of this period was the considerable growth of the extratubular epithelium. Structureless sheets of epithelial cells permeated all the explants. Cells forming these epithelial sheets as a rule were hypochromic, larger than normal, and outwardly similar to fibroblasts. Individual hyperchromic tubules were immured in the structureless mass of the epithelium (Fig. 1). In occasional explants individual epithelial bands were seen to leave the center of the explant in an outward direction together with the tubules immured in them. In 4 cases hyperplasia of individual convoluted tubules was observed (Fig. 2). All the observed outgrowths showed considerable hyperplasia of the epithelium. In the control at this period scattered growth of the epithelium was slight.

A connective-tissue capsule still remained after 14 days in only a few explants, and in the rest it had either completely disappeared, and the tubules emerged on the surface, or it was in a half-degenerated state. In the explants themselves the degeneration was fairly clearly defined. The outgrowths observed had considerable features of degeneration. Only in one, almost completely dead explant was an outgrowth of tubular type observed. It consisted of grossly hyperplastic, hyperchromic epithelial cells with clearly distinguishable mitoses. In some cases ordinary outgrowths emerged into extensive cysts lined with flat or cubical epithelium.

After 18 days only individual cultures still remained in a good condition; 3 outgrowths with definite structure and 1 epithelial sheet were observed in them.

On the 22nd day, among dead and half-dead explants, evidence of degeneration was completely absent in 6 cases, and diffuse hyperplasia of the epithelial tissue was well marked. Individual convoluted tubules were fairly clearly visible. The epithelium of these sheets was hyperchromic, and contained numerous mitoses. In addition, individual large epithelial cells formed bands on the surface of the explant (Fig. 3).

After 30 days, fields of epithelial cells with no clearly defined tubular structure, but with well-marked hyperplasia, still remained in only 3 explants. However, pycnosis of the nuclei was observed throughout these explants.

After injection of pyrene in the same dose as BP, individual epithelial cells also spread on the surface of the explants after 4-8 days, and in 3 cases they formed continuous sheets. In 1 explant focal hyperplasia of the tubules was observed.

After 11 days a varied pattern was observed, with outgrowths of every possible type and with extratubular growths of the epithelium. However, after 14 days, unlike in the group with BP, the connective-tissue capsule was well-preserved. It had completely disappeared after 18 days. After 22 days explants with severely degenerated outgrowths were still found. Only 1 explant still retained externally normal convoluted tubules, and there were neither mitoses nor epithelial bands growing outside the limits of the capsule. After 26 days, living tubules were seen in only 2 explants.

To summarize these observations, the rate of survival in both experimental groups was higher than in the control: cultures survived longer and in larger numbers. If the early periods (up to the 14th day inclusive) are compared, there were no differences between the survival rate of the experimental and control groups (Table 1). However, starting with the 22nd day, the control explants of the (C57BL×CBA)F_1 mice were virtually completely dead with the exception of 2 (4.4%), whereas 45.7% of explants survived after the transplacental action of P and 52.6% after that of BP. There were no substantial differences in this respect between the experimental groups themselves.

Hyperplastic changes in the epithelium observed in the control series consisted essentially of outgrowths with slightly atypical epithelium in 1.8% of the total number of explants. In both experimental groups the incidence of hyperplastic changes observed was much higher: 17.8% in the P group and 26.7% in the BP group. Hyperplastic changes in the epithelium were more varied, and the degree of atypia observed in them was much greater. Besides outgrowths with well marked atypia of the epithelium, hyperplasia of individual tubules and also structureless epithelial sheets were found. If all types of hyperplastic changes of the epithelium distinguished in this way were counted, a tendency was observed for their frequency to be greater in the experiments with BP. In the late stages of explantation, morphological differences were observed between the experimental groups. Well-defined and diffuse hyperplasia of the epithelium was present, the tubular structure was partly or completely obliterated, and structureless sheets were formed in 6 explants 22 days after exposure to BP and in 3 explants 30 days after exposure, i.e., in almost half the surviving cultures.

It can thus be concluded that well marked hyperplastic changes in the epithelium were present in organ cultures of mouse embryonic kidneys 22 and 30 days after the transplacental action of BP, although these changes were not observed either in the control or through the action of the noncarcinogenic analog P.

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