TRANSPLACENTAL ACTION OF 7,12-DIMETHYLBENZ(a)ANTHRACENE AND ITS NONCARCINOGENIC ANALOG ANTHRACENE IN ORGAN CULTURES OF EMBRYONIC MOUSE KIDNEYS

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The transplacental action of 7,12-dimethylbenz(a)anthracene (DMBA) and of its noncarcinogenic analog, anthracene, was studied in organ cultures of embryonic mouse C57BL×CBA) F_1 kidneys. The rate of survival was higher in both experimental groups than in the control. Hyperplastic changes in the epithelium (continuous sheets, hyperplasia of the tubules, projecting growths) after administration of DMBA and anthracene were of uniform type, but their intensity and frequency were higher (30.9%) in the group with the carcinogen than in the group with its noncarcinogenic analog (15.6%).

The study of the transplacental carcinogenic action of urethane in vitro in organ cultures of embryonic mouse lungs was commenced in the writer's laboratory in 1966 [2]. This work was subsequently continued with other carcinogenic agents in organ cultures not only of lungs [3], but also of kidneys [1, 4-6].

In a previous report [4] the writer showed that after administration of 7,12-dimethylbenz(a)anthracene (DMBA) to pregnant females by gastric tube, a series of changes appeared in organ cultures of the kidneys taken from the embryos which were classed as hyperplastic changes of the epithelium.

The object of the present investigation was to continue the study (in greater detail) of the transplacental action of DMBA in organ cultures of kidneys. At the same time, an experiment was undertaken with anthracene, the noncarcinogenic analog of DMBA, which was given under the same conditions and in the same doses to pregnant mice.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL mice mated with CBA males. Kidneys from 19-21-day embryos were used for organ cultivation. DMBA and anthracene in vegetable oil were injected into the stomach during the last week of pregnancy in four doses each of 2 mg, giving a total dose of 8 mg. The method of organ cultures developed in the writer's laboratory was used. Explants were fixed in Bouin's fluid 4, 7, 11, 14, 19, 22, 26, and 30 days after the beginning of cultivation. Paraffin sections 2-3 μ in thickness were stained with hematoxylineosin. The morphological picture observed during cultivation of the embryonic kidney of (C57BL×CBA) F_1 mouse hybrids under normal conditions and after the transplacental action of DMBA in the early stages were described previously [4], so that they will not be specially mentioned here. Only the hyperplastic changes in the epithelium after the action of DMBA and anthracene will be compared in this paper.

In the case of the transplacental action of DMBA and anthracene, the rate of survival of the cultures was incomparably higher than in the control, although no differences were observed between the experimental groups themselves. Starting from the 22nd day of cultivation (the last day on which living explants

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TABLE 1. Survival of Cultures and Hyperplastic Changes in Epithelium after Action of DMBA and Anthracene

Duration of experiment (in days)	Control					Anthracene					DMBA				
	number of explants		hyperplasia			number of explants		hyperplasia			number of explants		hyperplasia		
	total	surviving	1	2	3	total	surviving	1	2	3	total	surviving	1	2	3
4	35	35	—	—	—	12	12	2	—	3	11	11	2	—	2
7	131	130	—	—	9	23	23	—	—	1	16	16	7	—	4
11	45	43	—	—	—	43	42	3	3	6	25	25	6	6	7
14	23	10	—	—	—	24	24	—	—	4	36	36	1	2	6
18	14	9	—	—	—	36	28	—	—	1	29	22	—	—	1
22	19	2	—	—	—	31	17	—	—	1	13	5	—	—	3
26	17	—	—	—	—	14	6	—	—	—	25	12	—	—	1
30	10	—	—	—	—	22	2	—	—	—	24	9	—	—	—
Total No. . .	294	229	—	—	4	205	154	5	3	16	179	136	16	8	18
					1,8			3,2	2,0	10,4			11,7	5,8	13,4
% . . .	1,8%					15,6%					30,9%				

Note: 1) Epithelial sheets; 2) Hyperplasia of tubules; 3) Projecting growth.

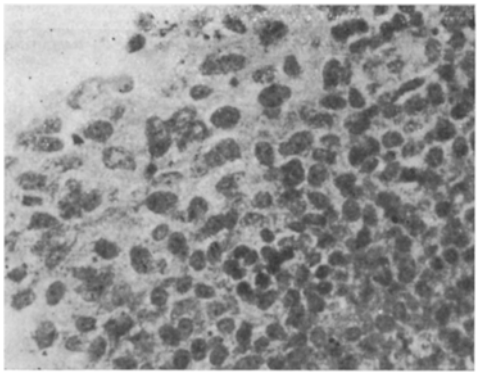

Fig. 1. Sheet of hyperplastic epithelium on the surface of an explant (DMBA; 11th day of cultivation). Hematoxylin-eosin, 500 ×.

remained in the control), 4.4% of explants survived in the control group, 37.4% in the group with anthracene, and 41.5% in the group with DMBA (Table 1).

Hyperplastic changes in the epithelium observed after administration of DMBA and of anthracene were on the whole similar in type, but their intensity and frequency were greater in the group with the carcinogen (30.9%) than in the group with its noncarcinogenic analog (15.6%). The hyperplastic changes observed were divided into three types. These will now be examined in detail.

After the action of the carinogen and its analog, considerable growth of the extratubular epithelium was observed. Against the background of these irregularly growing epithelial cells, hyperchromic tubules were usually seen. Later, extensive sheets developed from these chaotically growing epithelial cells. The cells composing these sheets were undifferentiated, large, with pale cytoplasm and a large, hypochromic nucleus (Fig. 1). Mitoses were frequent in them. Often atrophic cysts could be seen in the sheets. These sheets were most commonly found on the surface of the explant where they often covered up to half its area. As regards the time of their appearance, these sheets were distributed equally in the two experimental groups, but in the group with DMBA they were found more frequently (11.7%) than in the group with anthracene (3.2%).

Starting with the 11th day of cultivation, definite hyperplasia of the convoluted tubules was observed in both experimental groups. The epithelium lining these tubules was hyperchromic stratified, and cubical or cylindrical. Sometimes these hyperplastic tubules projected above the surface of the explant.

Finally, the third type of hyperplastic changes consisted of outgrowth. Some of these were projecting areas of the explant with a more or less clearly defined renal tubular structure, covered with a membrane consisting of cubical or cylindrical epithelium. Others were a continuation of the renal tubules which had grown beyond the boundaries of the explants; in these cases the epithelium lining the tubules was hyper-

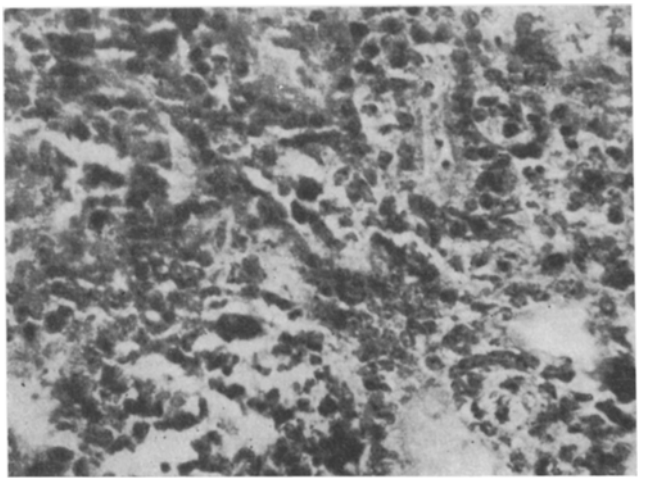

Fig. 2. Hyperplasia of the tubules (DMBA; 7th day of cultivation). Hematoxylin-eosin, 500 ×.

plastic. A third type consisted of epithelial cells alone, and appeared as deposits of epithelium with no signs of tubules. The fourth group consisted of dense, fibrotic areas covered with cubical or cylindrical epithelium. Besides the projections described above, intermediate and mixed forms were found, and no regular pattern was observed in the distribution of the various types of projections. The projections described were found between the 4th and 26th days of cultivation.

Hyperplastic changes observed in these experiments were characterized by different degrees of atypism of the epithelium. The epithelium of the sheets and of some structureless projections was the most atypical and the least differentiated. As the degree of atypism of the epithelium increased, the organ-specificity of the kidney with its tubular structure was lost, and a histiotypical growth of the epithelium began to predominate. Of the three types of hyperplastic changes, only projections were observed in the control group. In one case a projection of tubular type was observed, and in three others the projections were similar to the projecting areas of the explant covered by an epithelial membrane. Neither sheets nor hyperplastic tubules were observed in the control cultures of the mice of this line.

After the transplacental action of DMBA and anthracene, the survival rate of the cultures was thus the same in the experimental groups, but higher than in the control. The hyperplastic changes where observed were uniform in type, but in the group with DMBA they were observed more frequently (30.9%) than in the group with anthracene (15.6%).

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