TRANSPLACENTAL ACTION OF VARIOUS DOSES OF 7,12-DIMETHYLBENZ(a)ANTHRACENE IN VITRO

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The transplacental action of DMBA in various doses (1, 4, 6, and 8 mg) and of its noncarcinogenic analogue anthracene (8 mg) was studied in organ cultures of mouse embryonic kidneys. The survival rate of the organ cultures in all groups with DMBA was higher than in the group with anthracene, and it was higher in the latter than in the control. The frequency of hyperplastic changes in the anthracene group was the same as in the group with 1 mg DMBA. The intensity and frequency of the hyperplastic changes were directly dependent on the dose of the carcinogen.

In the first investigation of transplacental carcinogenesis in vitro undertaken in this laboratory, a clear correlation was found between the frequency of adenomas in embryonic lung cultures and the dose of urethane [1]. A previous investigation [2] showed that 7, 12-dimethylbenz(a)anthracene (DMBA), if administered to pregnant females by gastric tube, induces hyperplastic changes in the epithelium in organ cultures of the kidneys of the mouse embryos.

The object of the present investigation was to study the transplacental action of DMBA in organ cultures of mouse embryonic kidneys depending on the dose of carcinogen administered subcutaneously. As well as the biological control, a parallel control was carried out with anthracene, a noncarcinogenic analogue of DMBA.

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 were injected subcutaneously in vegetable oil in the last week of pregnancy, the former in doses of 1, 4, 6, and 8 mg, the latter in a dose of 8 mg. The method developed in this laboratory was used for the organ cultivation. Explants were fixed in Bouin's fluid 4-30 days after the beginning of cultivation. Paraffin sections, 2-3 μ in thickness, were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Control cultures in this series of experiments did not survive longer than 14 days. Three outgrowths of definitive structure were observed in these cultures. After exposure to DMBA the rate of survival of the organ cultures was higher than in the control and also than in the group with anthracene (Table 1). It rose as the dose increased. The differences were particularly marked after the 14th day: the survival rate of the control series was 4.7%, of the anthracene group 20.9%, and in the DMBA group it rose from 38% (1 mg) to 71.1% (8 mg).

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TABLE 1. Survival Rate of Organ Cultures of Embryonic Kidneys after Transplacental Action of DMBA and Anthracene

Duration of expt. (in days)	No. of explants											
	control		anthracene, 8 mg		DMBA							
					1 mg		4 mg		6 mg		8 mg	
	total	living	total	living	total	living	total	living	total	living	total	living
4	10	10	16	16	22	22	20	20	—	—	—	—
7	23	23	42	42	26	26	66	65	64	64	11	11
11	23	22	37	37	27	22	90	90	43	43	25	25
14	23	5	47	24	20	15	15	15	30	30	22	22
18	25	—	24	12	17	7	20	—	47	16	34	32
22	60	—	30	2	15	5	—	—	12	4	—	—
26	—	—	30	—	19	—	10	—	10	2	20	—
30	—	—	50	—	—	—	—	—	17	17	—	—
Total	164 (36,6%)	60	276 (48,2%)	133	146 (66,4%)	97	221 (86%)	190	223 (78,9%)	176	112 (80,4%)	90
Total after 14 days	108	5	181 (20,9%)	38	71 (38%)	27	45 (33,3%)	15	116 (59,8%)	69	67 (71,1%)	54
			<0,001		<0,001		<0,001		<0,001		<0,001	

TABLE 2. Number of Hyperplastic Growths of Epithelium in Organ Cultures of Embryonic Kidneys after Transplacental Action of DMBA and Anthracene*

Duration (in days)	DMBA														Anthracene, 8 mg		
	1 mg			4 mg			6 mg					8 mg					
	1	2	3	1	2	3	1	2	3	4	5	1	2	3	1	2	3
4	2	9	1	6	—	1	—	—	—	—	—	—	—	—	5	3	3
7	7	—	1	12	13	1	2	—	2	—	—	6	11	11	7	16	—
11	—	1	4	15	20	21	—	1	28	—	—	3	7	25	4	—	2
14	3	—	—	—	10	—	11	4	10	—	—	3	6	7	2	—	—
18	—	—	—	—	—	—	—	2	7	—	—	4	8	22	—	—	—
22	—	—	—	—	—	—	—	—	4	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	14	17	3	4	—	—	—	—	—	—
Total	28 (28,9%)			99 (52,1%)			109 (61,8%)					115 (127,8%)†			42 (31,6%)		
P	<0,001			<0,001			<0,001					<0,001			<0,001		

Legend: 1) outgrowth of definitive structure; 2) diffuse hyperplasia of tubules; 3) epithelial layers; 4) focal proliferation of tubular epithelium; 5) papillary outgrowth.

*Free outgrowths of definitive structure were found in the control (on the 7th and 11th days).

†Different types of hyperplasia were found in the same explants.

During the first week, in the group receiving a dose of 1 mg, diffuse hyperplasia of the tubules was observed at the periphery of the explant, and some tubules projected above the surface of the explant (Fig. 1).

When higher doses were given, in the first week extratubular growth of the epithelium was observed, the structure of the organ was obliterated (Fig. 2), and continuous sheets of cells were formed (in the group with a dose of 8 mg these were observed in all explants). The hyperplasia of the individual tubules was so intensive that they projected above the surface everywhere, forming small buds and outgrowths. Hyper-

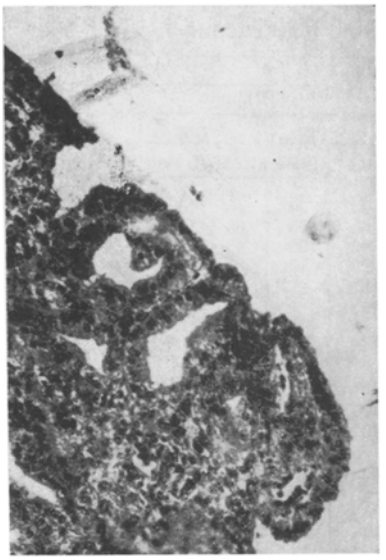

Fig. 1

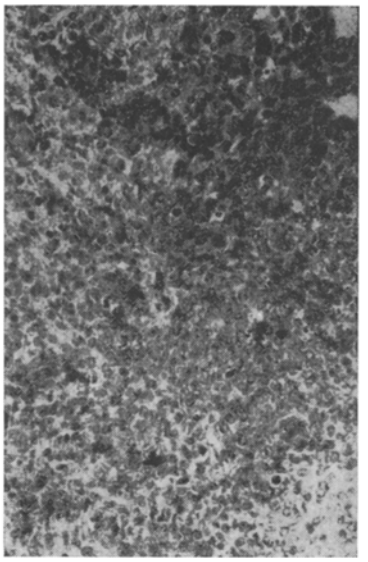

Fig. 2

Fig. 1. Hyperplasia of epithelium of convoluted tubules on surface of explant (dose of DMBA 1 mg, 4 days after explantation). Hematoxylin-eosin, ocular, 12.5, objective 25.

Fig. 2. Obliteration of tubular structure of organ and formation of continuous layer of epithelium (dose of DMBA four mg; four days after explantation). Hematoxylin-eosin, ocular 12.5, objective 25.

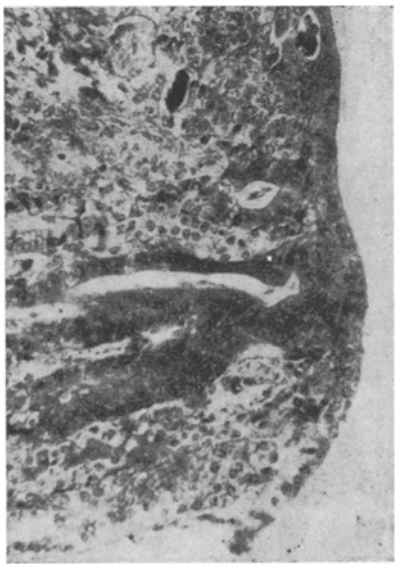

Fig. 3. Focal proliferation of tubular epithelium (DMBA given in dose of 6 mg, 30 days after explantation). Hematoxylin-eosin, ocular 12.5, objective 25.

plasia of the epithelium of the efferent ducts also was observed; sometimes their lumen was completely closed because of the proliferating transitional epithelium. A characteristic feature of the groups in which doses of 4 and 6 mg DMBA were given was the presence of large hyperchromic glomeruli with a very greatly enlarged vascular part. The outgrowths which were found in the epithelium were atypical and cylindrical, and atrophic cysts were frequently seen.

In the cultures 11-14 days after administration of DMBA in a dose of 1 mg, necrosis was severe; in four cases layers of relatively inactive epithelium were observed. Conversely, by the 11th day after administration of the higher doses, the number of structureless layers of cells had reached its maximum, and the larger the dose the greater the number of these layers. After 14 days the layers were visible in the series with doses of 6 and 8 mg. The atypy of the epithelium was clearly defined in the outgrowths.

In the later observations after administration of DMBA in a dose of 1 mg, only a few explants survived in a state of paranecrosis. In the series with doses of 6 and 8 mg, after 18 days excessive growth of the epithelium was still observed as before, with the formation of continuous sheets of cells, hyperplasia of the convoluted tubules, and the formation of outgrowths.

In all explants 30 days after administration of 6 mg DMBA, the tubular structure of the kidney was obliterated because of the rapidly proliferating epithelial cells. Against the background of diffuse hyperplasia, tubules with intensive proliferation of the epithelium of their walls could be seen (Fig. 3). The epi-

thelium lining such tubules was strongly hyperchromic, cubical, and stratified. It contained numerous mitoses. Frequently the lumen of these tubules was closed. Papillary outgrowths projected from the surface of the cultures.

After administration of anthracene, considerable extratubular growth of the epithelium, hyperplasia of individual tubules, fairly numerous outgrowths, and occasional continuous layers of cells were seen in the first week of cultivation. After 11-14 days, growth of the epithelium became weaker; most of the explants were in a state of paranecrosis.

The distribution of the various types of hyperplastic changes is shown in Table 2. The number of epithelial layers increased steadily from the series of experiments with anthracene to the series with the highest dose of the carcinogen (from 3.8 to 74.4%). The incidence of diffuse hyperplasia of the tubular epithelium differed only slightly in the anthracene group and in three of the DMBA group (1, 4, and 6 mg). However, in the group with the maximal dose of DMBA (8 mg), the incidence rose sharply (to 35.4%). The total number of outgrowths was greater than in the control, but there was no difference between the individual experimental groups. Only when doses of 6 mg were given, could well-marked focal proliferation of the convoluted tubules and papillary outgrowths be seen.

Subcutaneous administration of DMBA thus induced a series of hyperplastic changes in organ cultures of the embryonic kidneys. The intensity and frequency of these changes were directly dependent on the dose of the carcinogen. Whereas only a very slight increase in growth of the extratubular epithelium was observed after a dose of 1 mg, with an increase in the dose the hyperplastic changes grew steadily more marked. At the last time of observation (30 days) after administration of a dose of 6 mg, focal proliferation of the epithelium of the walls of the convoluted tubules and the presence of papillary outgrowths were observed.

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