

ORGAN CULTURE OF KIDNEYS FROM MOUSE EMBRYOS  
EXPOSED TO THE TRANSPLACENTAL ACTION  
OF 7,12-DIMETHYLBENZ[a]ANTHRACENE (DMBA)

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The transplacental action of 7,12-dimethylbenz[a]anthracene (when administered to pregnant females) has been demonstrated in organ cultures of mouse embryonic kidneys. The survival rate of experimental organ cultures was higher than that of controls, and several structures (cysts, hyperchromic glomeruli, tubular growths and projections), characterized by hyperplasia of epithelial cells and not encountered in the control, appeared.

The transplacental action of certain carcinogenic compounds has been demonstrated in work the first of which was published in 1945 [3-6]. Experimenting on mice of the Swiss breed, Tomatis [7] has recently found that if 7,12-dimethylbenz[a]anthracene (DMBA) is administered to a pregnant female, the incidence of tumors in the progeny is increased. Using line A mice, Kolesnichenko [2] showed that adenomas appear in organ cultures through the transplacental action of urethane. É. E. Smetanin later found that administration of nitrosamines to pregnant C3HA mice causes the development of adenoma-like growths in organ cultures of the embryonic lungs.

The object of this investigation was to examine the transplacental action of DMBA in organ cultures of mouse embryonic kidneys.

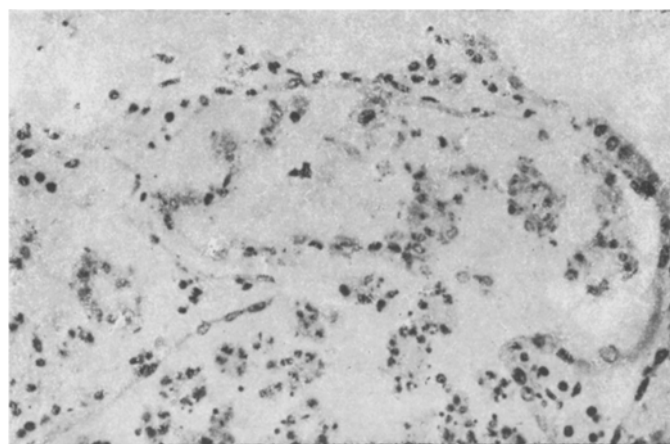


Fig. 1. Tubular structures inside cysts after administration of 2 mg DMBA. Hematoxylin-eosin, 140 $\times$ .

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## EXPERIMENTAL METHOD

Female C57 black mice were crossed with CBA males. Explants were taken from 19- to 21-day embryos, and from newborn and day-old hybrids (C57BL  $\times$  CBA) $F_1$ . DMBA was given to the females by mouth through a tube in a single dose of 2 mg 24 h before the experiment or on several occasions (3 and 5 times) in the last week of pregnancy (total doses 6 and 10 mg). The method of organ culture, as developed in the writer's laboratory by Adil'gireeva [1] and Kolesnichenko [2], was used. The explants were fixed 4, 7, 11, 14, and 19 days after the beginning of the experiment, in Bouin's fluid. Paraffin sections, 2-3  $\mu$  thick, were stained with hematoxylin-eosin.

## EXPERIMENTAL RESULTS

On the 4th day of the experiment the control group of explants had become spherical in shape and some of them had started to degenerate in the center. In the peripheral part of the explants, corresponding to the renal cortex, most of the glomeruli were intact. Morphologically they were almost indistinguishable from those observed in the embryonic kidney: the epithelial cells of the glomerulus were large, in close contact with one another, and hardly any capillaries could be seen; the epithelium of Bowman's capsule was cubical. In some glomeruli, however, the first signs of degeneration were visible: in the space between Bowman's capsule and the actual glomerulus, detritus was present. The convoluted tubules were particularly well preserved during organ culture. Many mitoses were observed in their epithelium. Of the straight tubules, the collecting tubules were the least viable: of all elements of the kidney they were the first to die, and all that

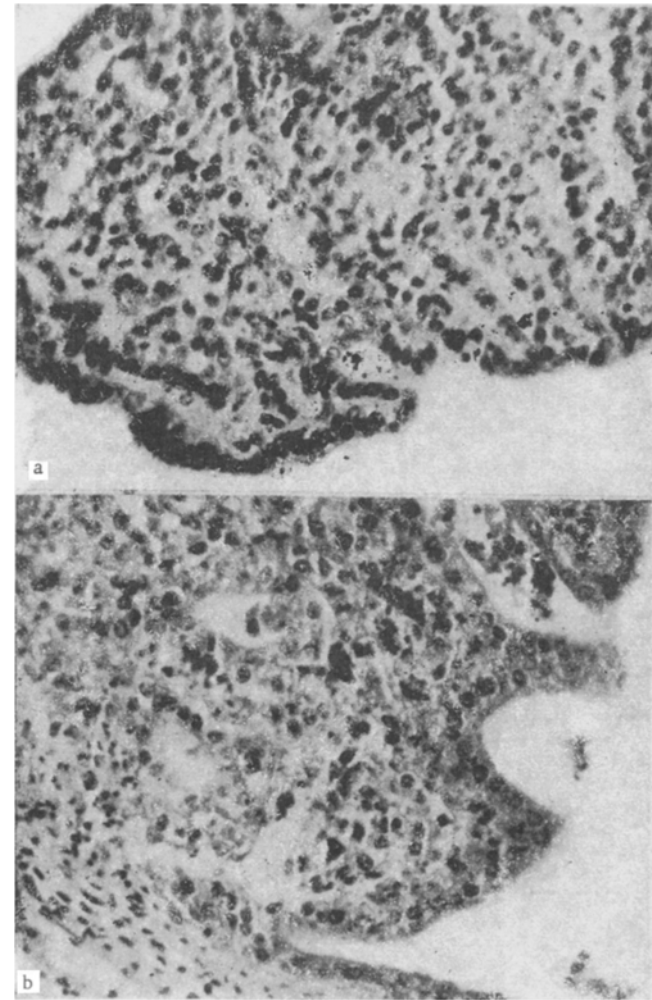


Fig. 2. Proliferation of hyperplastic epithelium on surface of explant (process; a) and inside it (b). Hematoxylin-eosin, 140 $\times$ .

remained of them were ghosts of the epithelial nuclei. The ascending and, in particular, the descending tubules persisted much longer. A considerable amount of blood was present in and between them. Macrophages were everywhere. A homogeneously stained protein material, detritus, and groups of desquamated cells appeared in the lumen of all the tubules.

After 7 days the central part of the explants was almost completely necrotic. Against the background of clearly defined destructive changes, a few descending tubules remained intact, although distended with detritus, and shadows of the collecting tubules were visible. The cortical part of the explants was narrowed. Many glomeruli were dead, and had either undergone lysis, leaving empty Bowman's capsules, or had disintegrated into masses of detritus. The convoluted tubules were changed to a lesser degree. Some of them ran along the medullary rays almost throughout the explant. Sometimes mitoses were visible in the epithelium of the convoluted tubules. In a few cases a capsule, consisting of cubical epithelium and probably the wall of a convoluted tubule, appeared at the periphery of the explant in certain areas.

Later, after 11-14 days, degenerative changes were well defined throughout the explant. Nearly all the glomeruli and tubules had degenerated. Some glomeruli were replaced by connective tissue. All that remained of the tubules were some of the convoluted tubules, mainly on the surface of the explants, and solitary epithelial cells left behind from the dying tubules. In the medulla complete degeneration was observed, but sometimes solitary descending tubules were still preserved, against the background of fibrosis.

TABLE 1. Effect of Various Doses of DMBA (Acting Transplacentally) on Survival of Explants in Organ Cultures of Mouse Kidneys

Duration of explantation (in days)	Control		DMBA			
	number of ex-plants	% of surviving explants	2 mg		6-10 mg	
			number of surviving explants	% of surviving ex-plants	number of surviving explants	% of surviving ex-plants
4	24	100	51	74,5	21	100
7	59	32,2	83	96,4	18	88,9
11	4	50,0	29	86,2	6	66,7
14	4	0	—	—	18	88,9
19	4	0	—	—	3	66,7
Total	95		163		66	

Administration of various doses of DMBA led to changes which were not found in the control.

Four days after administration of 2 mg DMBA fewer of the explants survived than in the control (Table 1). Necrotic changes were more pronounced, although degeneration followed the same lines as in the control. However, differences were present, and not only with respect to the degree of survival. They were concerned with the appearance of many structures not found in the control, or occurring only in isolated cases. In 13 of 51 explants multiple cysts appeared. They developed in place of the glomeruli, in which case their wall consisted of Bowman's capsules. Others were cystic dilatations of tubules with flattened epithelium.

After 11 days, when in most of the control explants only a few living tubules and epithelial cells remained alive, and the explants themselves were virtually completely necrotic, the degree of survival was higher in the experimental groups. Many viable structures remained (glomeruli, convoluted and straight tubules). In some cases hyperchromic glomeruli were observed. In 10 cases interesting structures were observed, consisting of cysts whose walls were formed by a single layer of greatly elongated epithelial cells. Such cysts contained tubular structures resembling remains of tubules. These latter structures could be viable, consisting of chains of well preserved epithelial cells; this epithelium was frequently in different stages of degeneration (Fig. 1).

Administration of larger doses of DMBA (6 and 10 mg) also led to an increase in the degree of survival of the explants (Table 1). Some of them survived until the 19th day (compared with the 11th day in the control). Signs of degeneration, although taking place more slowly, were of the same type as in the other previous groups. As well as cysts, hyperchromic glomeruli, and tubular structures, other quite distinctive structures were seen. In many explants areas were observed which differed sharply from the rest of the explant. They were usually located at the periphery of the explants and were covered with a clearly defined capsule, consisting of cubical or cylindrical epithelium. Sometimes these areas grew outside the explants and looked like processes (Fig. 2a). Some formations of this type remained inside explants (Fig. 2b). In their structure, these formations are divided into two types. Some consisted of tubules of equal diameter, resembling ascending tubules, loosely arranged in the connective-tissue stroma. Detritus was found very rarely between these tubules. Others consisted of tubules which were more closely packed, formed from intensely hyperchromic, sometimes cylindrical epithelium, in which mitoses were occasionally seen. Much detritus was present in the processes of this type, between the closely packed tubules. These formations must be distinguished from areas of ordinary explant tissue, projecting above its surface and covered with cubical epithelium, which were observed in both the experimental and control groups.

Organ cultures of the kidney were thus successfully obtained. They served as an object for testing the transplacental action of the most powerful carcinogenic hydrocarbon, DMBA. This action was manifested first by an increase in the degree of survival of organ cultures of the kidneys (especially in large doses), and second by the appearance of various structures (cysts, hyperchromic glomeruli, tubular growths, processes) not observed in the control. All these phenomena are the result of well marked hyperplasia of the epithelium.

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