TRANSPLACENTAL ACTION OF DIMETHYLNITROSAMINE (DMNA) IN KIDNEY ORGAN CULTURES

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The transplacental action of dimethylnitrosamine (DMNA) was studied in organ cultures of embryonic kidneys of C3HA mice. As a result of administration of DMNA to pregnant females, hyperplasia of the tubular epithelium, initially diffuse and later focal, was observed, and papillary outgrowths consisting of epithelial cells of uniform type appeared in the organ cultures. The observed changes in the epithelium are possibly the initial stages of a precancerous process.

The transplacental action of certain nitroso-compounds has been demonstrated by several workers [1, 4, 8, et al.]. Recently in the writer's laboratory, the transplacental tumor-producing action of a number of carcinogenic substances has been detected in vitro – in organ cultures. This effect was first studied by Kolesnichenko in 1966 [3]. She showed that after injection of urethane to pregnant mice of line A, adenomas appeared in organ cultures of the lungs taken from the embryos. Later, Smetanin [5] found that injection of dimethylnitrosourea and dimethylnitrosamine into female C3HA mice is followed by the development of adenoma-like growths in organ cultures of embryonic lungs. Later, Kolesnichenko discovered preneoplastic changes in the epithelium and connective tissue of organ cultures of the lungs of VD-9 rats after administration of dimethylnitrosourea.

TABLE 1. Transplacental Action of DMNA in Organ Cultures of Mouse Embryonic Kidneys

Duration of explantation (in days)	Control* (number of explants)	DMNA				
		number of explant	hyperplastic changes in epithelium			
			epithelization of explant		outgrowths	
			complete	partial	structural	papillary
4	23	6	2	2	—	—
8	16	6	2	4	—	—
15	9	17	2	13	5	—
19	6	6	—	—	—	6
22	8	10	—	5	—	—
26	2	5	—	—	—	—

*No hyperplastic changes were observed in the control.

During investigations with another object – the embryonic kidney – to study transplacental action on organ cultures, Golub' [2], using orthotoluidine and 3,3-dichlorobenzidine, and Yu. D. Sorokina [6], using 7,12-dimethylbenz(a)anthracene, found hyperplastic changes in the epithelium.

In the present investigation the transplacental action of DMNA was studied in organ cultures of mouse kidneys.

EXPERIMENTAL METHOD

Experiments were carried out on C3HA mice. DMNA was injected into pregnant females in a dose of 0.25 mg/kg subcutaneously 2 days before explantation. Kidneys from 19-21-day embryos were used for organ cultivation. The method of organ cultures developed in the writer's laboratory by T. S. Kolesnichenko was used. Explants were fixed with Bouin's fluid 4, 8, 15, 19, 22, and 26 days after preparation of the cultures. Paraffin sections, 2-3 μ in thickness, were stained with hematoxylin-eosin.

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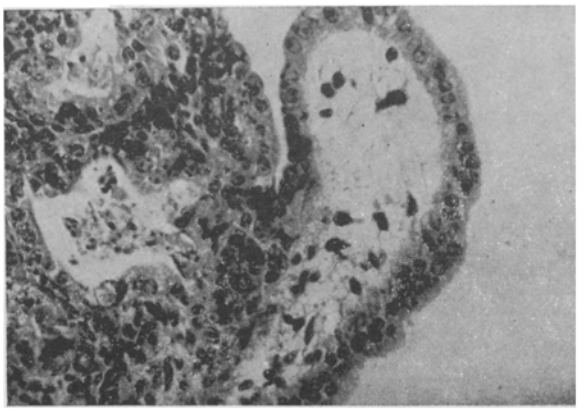

Fig. 1. Cyst outgrowth 15 days after administration of DMNA. Hematoxylin-eosin, 200 ×.

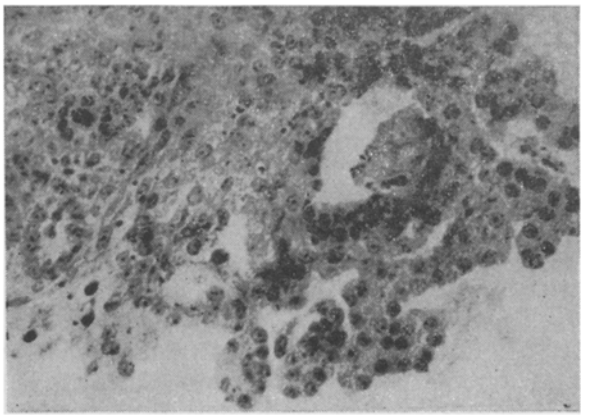

Fig. 2. Papillary outgrowth 19 days after administration of DMNA. Hematoxylin-eosin, 200 ×.

EXPERIMENTAL RESULTS

After 4 days the explants of the control series became rounded in shape and were covered by a connective-tissue capsule consisting of one or several layers. The basic kidney structure was well preserved. At the center of the explant, hyalinosis was observed in individual tubules and glomeruli. After 8 days, necrotic processes were more evident. Although the great majority of glomeruli and tubules in the peripheral part remained intact, the central part of the explants was occupied either by continuous hyaline masses, or by debris. Sometimes pycnosis of all the nuclei were observed on the surface of the explant. Some tubules were greatly dilated, and necrotic masses were present in their lumen.

After 15 days necrotic changes affected a considerable part of the explant. The connective-tissue membrane had disappeared. The zone of normal tubules and glomeruli was narrowed, but in some of them the beginning of hyalinosis also was observed. Later, after 19 days, the outlines of the tubules in the remaining part of explants were indistinct and, in addition, many epithelial cells lay outside the tubules, so that as a result the general structural pattern was obliterated. Only glomeruli with signs of hyalinosis could be distinguished more or less clearly. The zone of cells with pycnotic nuclei on the surface was widened.

After 22 days a narrow zone of tubules with apparently healthy epithelium could be seen. Karyorrhexis was well marked not only in the center, but also at the periphery of the explant. By the end of the period of observation, the 26th day, only individual living tubules and epithelial cells could be seen against the background of general necrosis. By contrast with the control, 4-8 days after injection of DMNA chaotic growth of epithelium was observed in some explants outside the tubules (Table 1). The tubules themselves were ill-defined. As a result, sometimes the whole explant consisted of a continuous sheet of epithelium. Meanwhile other explants showed a much more severe degree of degeneration than in the control.

After 15 days, in the experimental explants degeneration was extremely slight compared with the control. Some glomeruli were considerably enlarged. In some explants there were areas in which the epithelium was growing as individual bands. In other parts, fibrosis of the tubules and glomeruli was observed. In 5 cases, outgrowths projected from the usually smooth surface of the explants. Some of them appeared more like cysts. The walls of such a cyst consisted of one or several layers of cubical or cylindrical epithelial cells. The inner part of such a process contained a few stellate fibroblasts (Fig. 1), or the connective tissue was more compact and resembled areas of fibrosis. Outgrowths of this type, as careful examination of serial sections showed, were the continuation of separate renal tubules beyond the limits of the explant. In such cases, when outside the ordinary tissue structure of the kidney, the tubules formed curiously shaped structures, and the uniformity of their epithelial lining was disturbed. Along with these outgrowths others were seen which consisted of a projecting area of the explant with an ill-defined kidney structure (among connective-tissue epithelial cells the tubules could not be clearly distinguished), covered by simple or stratified epithelium. Outgrowths of the latter type probably arose on account of the uneven surface of the explant used for the culture. Outgrowths observed at this time were thus structural in character.

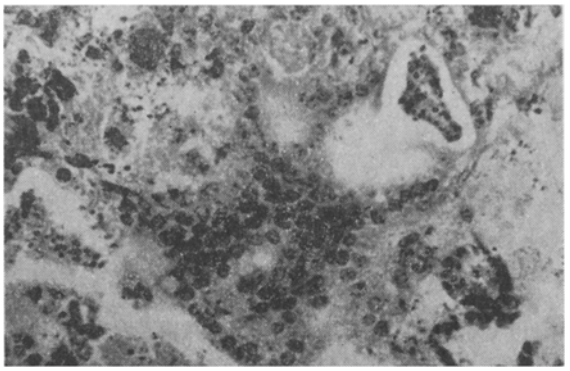

Fig. 3. Hyperplasia of epithelial cells 22 days after administration of DMNA. Hematoxylin-eosin, 200 ×.

After 19 days epithelization of the explant was still more evident. Basically the explants consisted of a continuous sheet of epithelium, and only occasionally could individual tubules be faintly distinguished. In all explants, zones of proliferation of epithelium were observed on the surface. Some of them consisted of papillary outgrowths of different shapes, covered by a uniform cubical epithelium. Inside them, against the background of an indistinct structure, individual epithelial cells could be seen. Other outgrowths were formed entirely of epithelial cells, also of uniform type (Fig. 2).

After 22 days the edges of the explants were no longer smooth, as in the control, but winding on account of death of the interstitial tissue and the appearance of tubular structures on the surface. The necrotic changes were weaker than in the control. In some explants, even those which were completely necrotic, areas of intensive hyperplasia of epithelial cells were encountered (Fig. 3). Some explants were so well preserved that glomeruli could be seen in them. Almost complete necrosis was observed after 26 days, just as in the control.

To sum up, after administrationof DMNA, hyperplasia of the tubular epithelium and, to some extent, of the glomerular epithelium was clearly observed during the first two weeks from the beginning of explantation. This was manifested as continuous or partial epithelization of most of the explants. At this stage of development of the organ cultures, the hyperplasia was diffuse in character. Subsequently, against the background of this diffuse hyperplasia, papillary outgrowths appeared, consisting of foci of proliferation of hyperplastic epithelium. In addition, the transplacental action of DMNA was revealed by a higher level of survival of the explants, especially in the late periods of observations.

Hence, the transplacental action of DMNA was manifested by the appearance of diffuse, followed by focal,hyperplasia of the renal epithelium of mouse embryos in organ cultures. According to the views of Shabad [7] on the development of tumors as a multistage process, the diffuse and focal hyperplasia observed in the organ cultures described above are the first stages of preneoplastic changes.

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