EFFECT OF DIMETHYLNITROSAMINE ON MOUSE EMBRYONIC KIDNEY TISSUE IN ORGAN CULTURE

S. P. Bogovskii and Yu. D. Sorokina

UDC 612.46 : 612.64-085.23 : 615.277.4

Addition of dimethylnitrosamine (DMNA) in various doses to the medium has a toxic effect in organic cultures of embryonic kidney tissue, which increases with an increase in the dose. Meanwhile, under the influence of DMNA, hyperplastic growth of the epithelium was observed in explanted tissue, and the higher dose of DMNA the more frequently this phenomenon was observed.

Dimethylnitrosamine (DMNA) induces tumors in various organs: the liver, lungs, kidneys, alimentary tract, nervous system, and so on [1, 3, 4]. Its harmful action is manifested during the first days after administration. In the writers' laboratory, Smetanin [2], after adding DMNA to the medium, observed hyperplastic changes in the epithelium in organ cultures of mouse embryonic lungs.

The object of this investigation was to study the action of DMNA, when added directly to the medium, on embryonic kidney tissue in organ cultures.

EXPERIMENTAL METHOD

Mice of line BALB/C were used. Kidneys of 19-21-day embryos were used for the organ cultures. DMNA was added to the nutrient medium in doses of 0.02, 0.01, and 0.002 mg/ml. The explants were kept

TABLE 1. Effect of DMNA on Organ Cultures of Mouse Embryonic Kidneys

Duration of explantation (in days)	No. of explants in control*		No. of explants exposed to DMNA (in mg/ml)											
			0,002				0,01				0,02			
					hyperplasia				hyperplasia				hyperplasia	
	total	alive	total	alive	diffuse	focal	total	alive	diffuse	focal	total	alive	diffuse	focal
7	17	16	12	12	—	—	30	30	3	7	8	2	—	—
11	29	26	23	23	2	17	26	18	1	3	30	28	4	14
14	30	8	28	26	1	8	31	20	—	11	29	18	2	16
18	30	23	10	6	—	—	27	10	—	8	25	0	—	—
21	12	8	14	5	—	—	30	0	—	—	30	0	—	—
24	10	0	28	0	—	—	30	0	—	—	30	0	—	—
Total	128	81	115	72	3	25	174	68	4	29	152	48	6	30
%	63,3		62,6		38,9		39,1		48,5		31,5		75,0	
P							<0,001		>0,1		<0,001		<0,001	

*No hyperplastic changes were observed in the control explants.

Laboratory of Prophylaxis of Carcinogenic Action, Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 75, No. 2, pp. 75-77, February, 1973. Original article submitted April 10, 1972.

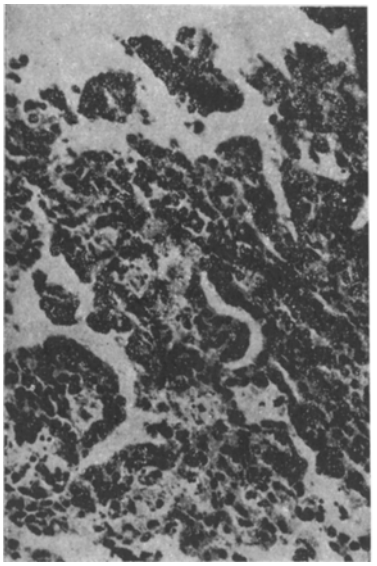

Fig. 1

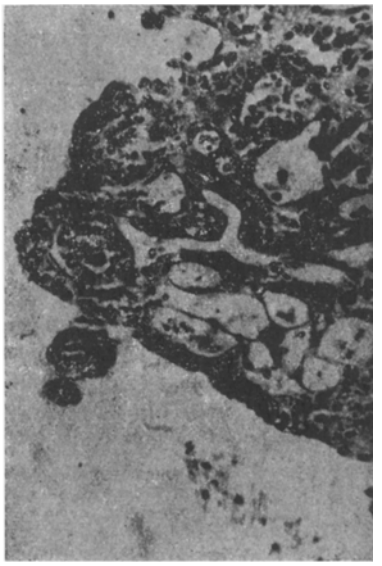

Fig. 2

Fig. 1. Culture with exposure to 0.01 mg/ml DMNA, 11th day of cultivation: hyperplasia of epithelium of convoluted tubules; debris visible between tubules. Here and in Figs. 2 and 3, hematoxylin-eosin, 140 ×.

Fig. 2. Cultivation with exposure to 0.01 mg/ml DMNA, 11th day of cultivation: atrophic cysts.

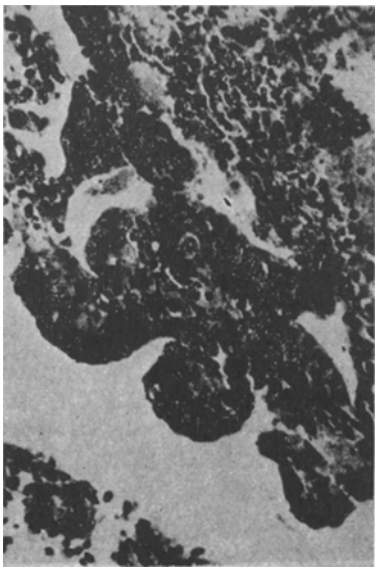

Fig. 3. Cultivation with exposure to 0.02 mg/ml DMNA, 11th day of cultivation: papillary projections on surface of explant.

in the medium with the carcinogen from the 4th to the 7th day of cultivation. They were then replaced in ordinary medium. Fixation with formalin was carried out 7, 11, 14, 18, 21, and 24 days after the beginning of cultivation. Paraffin sections 3-4 μ in thickness were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

When added to the nutrient medium, DMNA had a primarily toxic effect on the explanted kidney tissue and this was particularly marked in the early period of cultivation. Degenerative changes were observed in all explanted fragments of kidney tissue without exception, and when the large dose was used they were so severe that it was difficult to distinguish the structure of the kidney. With smaller doses masses of debris were scattered at random throughout the explant. Later, when most of the debris had disappeared, characteristically traces of it continued to remain in the interstitial connective tissue throughout the rest of the experiment. The survival rate of the explants was lower with the two larger doses than in the control ($P < 0.001$). The smallest dose did not affect survival (Table 1).

Although 11 days after the beginning of explantation the cultures were not completely freed from debris, the tubules were clearly visible in them. By contrast with the control, with these doses of nitrosamine, glomeruli were never observed. Distinct proliferation of the epithelium was present (Fig. 1). Many atrophic cysts could be seen (Fig. 2). In a few cases diffuse hyperplasia of the epithelium was observed and as a rule it extended throughout the explant (Table 1). In most cases, however, the proliferation of the epithelium was focal in character and affected only part of the explanted fragment of kidney. In other areas necrosis was usually observed during this period.

Hyperplastic changes in the epithelium consisted mainly of hypertrophy of the walls of convoluted tubules. Some of the proliferating tubules projected above the surface of the explant form distinctive papillary outgrowths (Fig. 3). Similar epithelial outgrowths could be seen in the atrophic cysts. Only rarely could islands of chaotically growing epithelium, not externally connected with the tubules, be seen. A characteristic feature of the growing epithelial tissue was the well-marked polymorphism of the nuclei. With rare exceptions, 2 weeks after the beginning of cultivation only individual fragments of the explant remained alive and were clearly demarcated from the remaining necrotic part. The morphological picture of these areas differed very little from that described at the previous period. A few explants consisting entirely of chaotically growing tubules, with a clearly defined hyperplasia of the epithelium, were exceptions to this rule. After 18 days individual surviving areas consisting of epithelium with marked signs of anaplasia still remained. At all other times of observation only isolated tubules could be seen among the necrotic tissue.

It is clear from the results described above that the survival rate of the embryonic kidney tissue was reduced by DMNA by an amount which increased with an increase in the dose of the carcinogen. Meanwhile, with an increase in the dose, the intensity of the proliferative changes in the epithelium (both diffuse and focal) increased in the remaining kidney tissue.

LITERATURE CITED

1. L. A. Ostrovskaya, in: Comparative Oncology [in Russian], Moscow (1967), p. 5.
2. É. E. Smetanin, On the Transplacental Carcinogenic Action of Nitrosomethylurea and Dimethylnitrosamine in Experiments on Mice and in Organ Cultures of the Lungs. Candidate's Dissertation, Moscow (1970).
3. L. M. Shabad and L. A. Savluchinskaya, Byull. Éksperim. Biol. i Med., No. 3, 76 (1971).
4. I. N. Shvemberger, in: The Cytology of Malignant Growth [in Russian], Moscow-Leningrad (1963), p. 76.
5. H. Druckrey, D. Steinhoff, R. Preussmann, et al., Naturwissenschaften, 50. 735 (1963).
6. G. Hard and W. Butler, Cancer Res., 30, 2806 (1970).