TRANSPLACENTAL ACTION OF SOME NITROSO-COMPOUNDS ON ORGANOTYPICAL CULTURES OF WISTAR RAT EMBRYONIC KIDNEY TISSUE

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The transplacental action of dimethylnitrosamine (DMNA) and n-nitrosomethylurea (NMU) in organ cultures of Wistar rat embryonic kidneys was manifested as a higher rate of survival of the experimental cultures than the controls and intensified hyperplasia of the epithelium. The lesions included diffuse and focal proliferation, layers of atypical epithelium and, in the experiments with DMNA, cystic and papillary formations resembling cystadenomas. Hyperplastic changes were more marked in the experiment with DMNA than in the experiment with NMU.

One way to study the transplacental action of carcinogenic substances is the method of organ cultures [5-7, 10, 14]. Investigations in the writers' laboratory have shown that organ cultures of the kidneys can be used in research of this type [2-4, 8, 9, 12].

The object of this investigation was to study the transplacental action of dimethylnitrosamine (DMNA) and n-nitrosomethylurea (NMU) on organotypical cultures of Wistar rat embryonic kidney tissue.

EXPERIMENTAL METHOD

In the last week of pregnancy female rats received subcutaneous injections of DMNA and NMU in a dose of 30 mg/kg body weight in 0.2 ml water on two occasions, so that each animal received 60 mg/kg of the test substance. Cultivation was carried out by the method proposed by Chen [13] in Adil'gireva's modification [1], which is now used in the writers' laboratory. Altogether 373 control and 550 experimental explants were studied.

EXPERIMENTAL RESULTS

Investigation of the explants treated with DMNA and NMU showed a higher rate of survival of the experimental cultures than the controls. Whereas the control cultures died chiefly on the 21st-23rd day, in the experiments with both nitroso-compounds the number of surviving explants was about 70% at that particular period of cultivation. The experimental explants retained their viability as a rule until the 28th day of cultivation. Altogether, starting from the 18th day of explantation, 12.1% of cultures survived in the control group, 68% in the group treated with NMU, and 63% in the group treated with DMNA (Table 1).

A study of the control explants throughout the period of cultivation showed that in their morphological structure they resembled organ cultures of mouse kidney [3, 4, 8, 12]. It should be emphasized that cells forming structural elements in the rat kidneys appeared larger and areas of connective tissue were found much more often in them. The control cultures of rat embryonic kidneys passed through a certain cycle of changes during the period of explantation which was similar to that observed in experiments on mice of

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TABLE 1. Rate of Survival and Hyperplasia of Epithelium in Organ Cultures of Rat Embryonic Kidneys Exposed to Transplacental Action of DMNA and NMU

Duration of expt. (days)	Control			DMNA							NMU						
	no. of explants		1	no. of explants		1	2	3	4	5	no. of explants		1	2	3	4	5
	total	living		total	living						total	living					
4	72	72	—	75	75	4	—	—	—	—	42	40	2	—	—	—	—
7	73	73	2	81	70	6	5	—	4	—	35	30	2	—	—	—	—
10	58	55	—	65	40	—	3	6	8	—	31	25	—	3	1	2	—
14—15	63	35	—	41	28	—	1	4	4	5	29	20	—	3	2	3	—
18—20	52	11	—	31	21	—	—	1	3	4	33	23	—	—	—	3	—
21—23	31	2	—	26	18	—	1	—	3	2	24	18	—	—	—	—	—
25—28	24	—		19	9	—	—	—	—	—	18	10	—	—	—	—	—
Total . . .	373	248	2	338	261	10	10	11	22	11	212	166	4	6	3	8	—
Rate of survival after 18th day of cultivation	—	12,1%	—	—	63%	—	—	—	—	—	—	68%	—	—	—	—	—
No. of explants with hyperplasia of epithelium (in %)	—	—	0,8	—	—	3,8	3,8	1,2	8,4	4,2	—	—	2,4	3,6	1,8	4,8	—
						24,4							12,6				

Legend: 1) outgrowths; 2) diffuse hyperplasia of tubular epithelium; 3) focal proliferation of tubular epithelium; 4) layers of epithelium; 5) cystic structures resembling cystadenomas.

TABLE 2. Results of Action of DMNA and NMU on Organ Cultures of Wistar Rat Kidney

Distribution of hyperplastic lesions of epithelium by types	Control	DMNA	NMU
Outgrowths	0,8%	3,8% $P_{C-1}<0,01$	2,4% $P_{C-2}>0,05$
Diffuse hyperplasia of tubular epithelium	—	3,8% $P_{C-1}<0,01$	3,6% $P_{C-2}<0,05$
Focal proliferation of tubular epithelium	—	4,2% $P_{C-1}<0,01$	1,8% $P_{C-2}>0,05$
Epithelial layers	—	8,4% $P_{C-1}<0,001$	4,8% $P_{C-2}<0,01$
Cystic structures resembling cystadenomas	—	4,2% $P_{C-1}<0,01$	—
Total . .	0,8%	24,4% $P_{C-1}<0,001$	12,6% $P_{C-2}<0,001$

Legend: P) criterion of significance determined from the Fisher–Student table; PC-1) statistical significance of differences between action of DMNA and control; PC-2) statistical significance of differences between action of NMU and control.

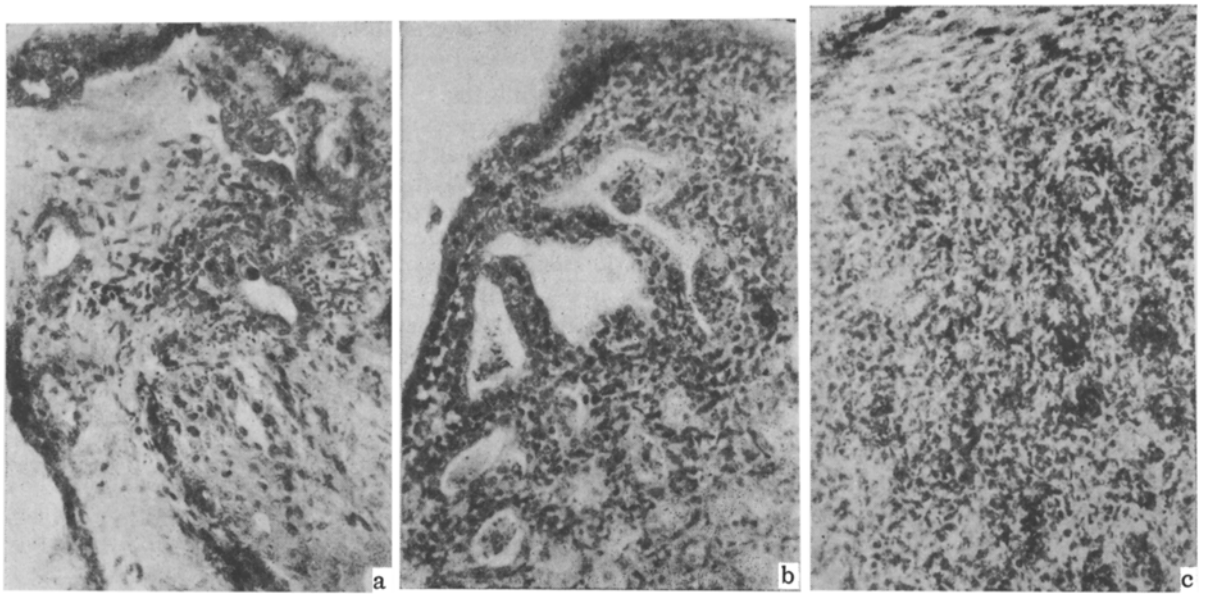

Fig. 1. Results of transplacental action of DMNA (60 mg, subcutaneously) on organotypical cultures of Wistar rat embryonic kidney tissue: a) focal proliferation of tubular epithelium (14th day of explantation; 150×); b) cystic-papillary focal structure resembling cystadenoma (18th day of explantation; 150×); c) epithelial layer consisting of atypical cells (18th day of explantation; 300×).

different strains, and which consisted essentially of some growth of the epithelium at the periphery of the kidney explant followed by progression of the degenerative, necrobiotic, and necrotic phenomena, causing death of the cultures after 2-3 weeks [12].

The results of the transplacental action of DMNA and NMU are compared in Table 1. In the experimental cultures morphological changes were found far more often than in the control. For instance, structural outgrowths were observed in 3.8% of explants treated with DMNA and in 2.4% treated with NMU, compared with only 0.8% in the control. In addition, in explants treated with nitroso-compounds hyperplastic lesions were found in the epithelium which did not occur in the control. These included diffuse and focal proliferation and layers of atypical epithelium.

In the experiment with DMNA diffuse hyperplasia began to appear on the 7th day of cultivation and these changes continued mainly until the 14th-15th day. In the experiment with NMU this process began rather later, on the 10th day, and it ended at the same time. The appearance of the first signs of hyperplasia was expressed in individual explants as an increase in the number of cells forming tubules, widening of their lumen, and the appearance of areas consisting of several layers of epithelial cells at the periphery of the fragments. Intensification of the hyperplasia led to gradual obliteration of the structure of the kidney and to disappearance of the boundaries between the tubules. In some explants the hyperplastic processes were so intensified that individual tubules spread beyond the surface of the fragment to form "buds" and small outgrowths.

Starting from the 10th day of cultivation in the experiments with DMNA and NMU foci of proliferation were observed (Table 1). These foci could be seen both at the periphery and in the center of the explants. Their characteristic feature was the presence of atypical, often hyperchromic, epithelium which in most cases was found at the site of the "hypertrophied" tubules. In some cases foci of proliferation spread over the whole explant and showed a great variety of forms of atypical epithelium. This variety in all probability was caused by the extension of the hyperplasia to affect all structural elements in the kidney tissue. As a rule in these foci both epithelial cells of the hypertrophied tubules and also small, hyperchromic cells which, in some cases, appeared on the surface of the explant, could be seen. In addition, large, pale, epithelial cells forming areas consisting entirely of these cells were quite often observed in the center of the explant (Fig. 1a).

In the experiments with both carcinogens distinctive epithelial layers often spreading over the whole explant and observed in conjunction with general necrosis of the whole fragment were found (Fig. 1b). In

some cases the epithelial cells emerging on the surface of the explant formed structures resembling papillary outgrowths. Under the influence of NMU, the layers formed by epithelial cells were more compact and, in some cases, they were separated by a distinct border from the rest of the explant. Finally, in some cases in experiments with DMNA cystic structures with papillary appendages, clearly demarcated from the surrounding tissue, could be observed in the organ cultures of the rat embryonic kidney (Fig. 1c). Neoplasms of this type can be considered to be cystadenomas. It may be recalled that cystadenomas of the kidneys have been induced in mice in vivo by the same carcinogen – DMNA [11].

These experiments thus showed that embryonic kidney tissue of Wistar rats can be explanted in organ cultures for 20-28 days. Control cultures pass through a certain cycle of changes under these conditions, consisting essentially of growth of the epithelium chiefly at the periphery of the explant followed by necrobiotic changes leading to death of the cultures after 20 days. Meanwhile, cultivation of embryonic kidney tissue after exposure to the transplacental action of carcinogenic nitroso-compounds gave a completely different picture. It must be emphasized that in the experimental explants increased growth of the epithelium and survival of the control cultures for 7-10 days were observed, together with the appearance of definite morphological changes (Table 2) which, except for the structural outgrowths, were evidently closely connected with the transplacental action of the nitroso-compounds. These changes were more numerous in the experiment with DMNA in which, besides focal and diffuse hyperplasia, structures resembling cystadenomas also were observed.

LITERATURE CITED

1. R. Kh. Adil'gireeva, Byull. Éksperim. Biol. i Med., No. 4, 115 (1964).
2. N. I. Golub', Byull. Éksperim. Biol. i Med., No. 11, 83 (1969).
3. N. I. Golub', Vopr. Onkol., No. 10, 67 (1971).
4. N. I. Golub', Byull. Éksperim. Biol. i Med., No. 3, 83 (1972).
5. T. S. Kolesnichenko, Vopr. Onkol., No. 12, 39 (1966).
6. T. S. Kolesnichenko, Vopr. Onkol., No. 7, 59 (1971).
7. É. E. Smetanin, Vopr. Onkol., No. 8, 48 (1969).
8. Yu. D. Sorokina, Byull. Éksperim. Biol. i Med., No. 1, 76 (1970).
9. Yu. D. Sorokina and S. P. Bogovskii, Byull. Éksperim. Biol. i Med., No. 8, 84 (1971).
10. L. M. Shabad, Pat. Fiziol., No. 2, 28 (1970).
11. L. M. Shabad and L. A. Savluchinskaya, Byull. Éksperim. Biol. i Med., No. 3, 76 (1971).
12. L. M. Shabad, J. D. Sorokina, N. I. Golub' (Golub) et al., Cancer Res., 32, 617 (1972).
13. J. M. Chen, Exp. Cell Res., 7, 518 (1954).
14. L. Lasnitzki, J. Endocrinol., 12, 236 (1955).