ACTION OF NITROSOMETHYLUREA AND DIMETHYLNITROSAMINE IN ORGAN CULTURES OF MOUSE EMBRYONIC LUNGS

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During the first 2 weeks of cultivation, nitrosomethylurea (NMU; 0.05 mg/ml) or dimethylnitrosamine (DMNA; 0.02 mg/ml) was added to the nutrient medium. Altogether 613 explants were studied. In the experiments with NMU, starting from the 11th day and until the final stages of cultivation (38 days), adenomatous growths of the epithelium, mainly bronchiolar, were observed. DMNA proved very toxic for the cultures. The epithelial hyperplasia was less marked in the experiments with DMNA.

In a previous paper [2] the author described changes in the bronchiolar epithelium in organ cultures of embryonic lung tissue from mice of lines C3HA and A due to the transplacental action of dimethylnitrosamine (DMNA) and nitrosomethylurea (NMU). Adenomatous growths developing under these conditions in the cultures were observed after the 14th day and until the final stages of cultivation (35th day). These investigations were continued. The period of cultivation of individual experimental viable explants was in some cases prolonged to 50 days. After the 9th day and throughout the rest of the cultivation period, foci of marked hyperplasia of the bronchiolar epithelium, of adenomatous type, were observed.

The next step in the investigation was to study the action of NMU and DMNA on organ cultures of mouse embryonic lungs when the compounds were administered in vitro, i.e., added directly to the nutrient medium of the cultures. Administration of the carcinogens in vitro ruled out any possible conversions of these compounds which could take place in the maternal organism.

EXPERIMENTAL METHOD

Embryonic lungs of C3HA mice were taken for the organ cultivation experiments on the last day of the antinatal period. The same watch glass technique [5] as in the previous investigation [2] was used. The carcinogens were diluted extempore directly in the nutrient medium in the proportion of 0.05 mg NMU/ml medium and 0.02 mg DMNA/ml medium. Incubation with the carcinogens took place from the 1st to 14th days of cultivation, after which the explants continued in cultivation in "pure" nutrient medium, i.e., without the addition of the carcinogen. The medium was changed as a rule twice a week. Cultures of embryonic lungs of intact C3HA mice which were cultivated in "pure" nutrient medium were used as the control.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1 and Figs. 1-3. It is clear from Table 1 that the cultivation period in the experiments with NMU was 38 days and in the experiments with DMNA 28 days. Morphological changes in the control explants throughout this period were similar to those described by Kolesnichenko [1] and by the present author [2].

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TABLE 1. Action of NMU and DMNA on Organ Cultures of Embryonic Lung Tissue

Day	Treatment with NMU				Treatment with DMNA				Control			
	No. of explants	No. with morphological changes			No. of explants	No. with morphological changes			No. of explants	No. with morphological changes		
		deg.	nec.	hyp.		deg.	nec.	hyp.		deg.	nec.	hyp.
0	10	0	0	0	8	0	0	0	10	0	0	0
4 th	—	—	—	—	39	22	0	0	26	16	2	0
7 th	62	17	1	0	25	8	8	3 (12)	20	9	1	0
11 th	18	0	0	4 (22)	11	4	6	0	21	8	1	0
14 th	—	—	—	—	23	3	13	3 (13)	28	4	0	0
21 st	20	0	1	13 (65)	36	3	19	7 (20)	28	8	1	0
24 th	25	3	2	19 (76)	11	0	9	0	16	4	2	0
28 th	—	—	—	—	12	2	10	0	13	12	0	0
33 rd	61	2	2	54 (90)	—	—	—	—	25	20	1	0
38 th	38	4	0	32 (84)	—	—	—	—	27	24	13	0
Total	234	26	6 (2,6)	122 (75)	165	42	65 (42)	13 (11)	214	95	21 (10)	0

Legend: deg. denotes explants with degenerative changes, nec. those with necrotic changes, hyp. those with focal or diffuse hyperplasia of the epithelium.

Note. Figures in parentheses are percentages.

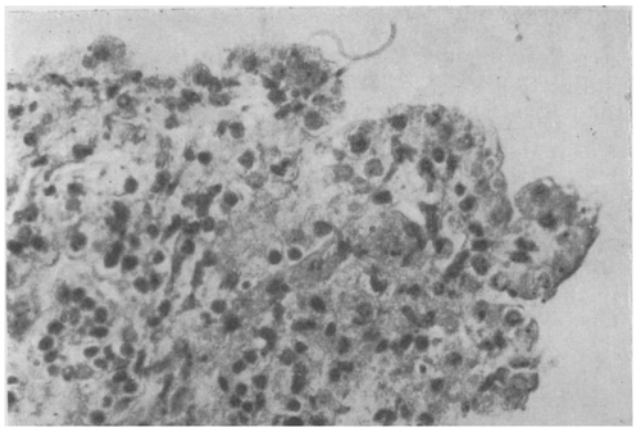

Fig. 1. Focal hyperplasia of bronchiolar epithelium on surface of explant. 11th day of cultivation, local administration of NMU, hematoxylin-eosin, 500×.

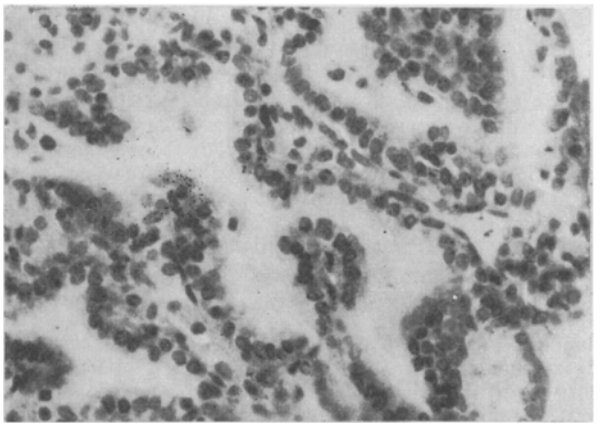

Fig. 2. Hyperplasia of bronchiolar epithelium inside explant. Experiments with NMU, 38th day of cultivation, hematoxylin-eosin, 300×.

Under the influence of NMU and under the experimental conditions used, marked stimulation of growth of the epithelium was observed in the lung tissue cultures. Adenomatous focal or diffuse hyperplasia, predominantly of the bronchiolar epithelium, was observed both inside the explants and on their surface from the 11th day of cultivation (Fig. 1). The number of explants with adenomatous proliferation increased during subsequent cultivation (Fig. 2). Altogether, starting from the 11th day of cultivation, hyperplasia was found in 122 of the 162 explants (75%). Evidence in support of stimulation of hyperplasia of the epithelium by NMU was given by the fact that in the experimental cultures there were significantly fewer necrotic explants than in the control (Table 1). No hyperplasia was observed in any of the control explants.

Unlike NMU, under the present experimental conditions DMNA gave rise predominantly to necrosis of the explants rather than to stimulation of their growth. After addition of DMNA to the nutrient medium in the course of cultivation, 65 of the 155 explants (42%) were necrotic (compared with 2.6% in the experiments with NMU). Focal or diffuse hyperplasia of the epithelium of the lung was detected in only 13 explants (Fig. 3). However, the fact was noted that the first growths were found on the 7th day of cultivation. In the

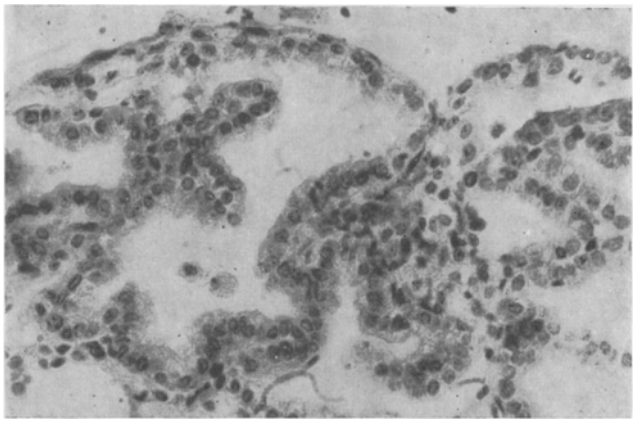

Fig. 3. Hyperplasia of bronchiolar epithelium after local application of DMNA, 21st day of cultivation, hematoxylin-eosin, 300×.

experiments with NMU, however, and also when the action of DMNA and NMU was by the transplacental route, the first growths were observed somewhat later (on the 9th-11th day). The character of hyperplasia of the epithelium in these experiments was similar to the picture described following the transplacental action of DMNA and NMU [2]. Investigations to study the local action of carcinogenic nitroso-compounds both in vivo and in vitro are described in the literature. In these cases various tumors were obtained in animals at the site of injection of NMU [3, 5, 6]. However, the results of the experiments in vitro are interpreted by the authors cited as negative or doubtful. For instance, after incubation of monolayer cell cultures of rat and hamster fibroblasts in a solution of nitrosomethylurethane, no significant differences were found in the growth of the experimental and control cells [8]; no marked specific morphological changes were found in cells after incubation in vitro with NMU [7].

The experiments to study the local action of NMU and DMNA by direct addition to the nutrient medium of organ cultures of mouse embryonic lungs thus showed that NMU, without possessing any marked toxic action, has a clear stimulating effect mainly on the bronchiolar epithelium, in which it gives rise to focal or diffuse adenomatous growths in most explants starting from the 11th day of cultivation. Stimulation of hyperplasia of the bronchiolar epithelium in organ cultures by the action of DMNA is less marked. This fact can evidently be attributed to the strong toxic action of this compound on the organ cultures.

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