CARCINOGENIC ACTION OF NITROSAMINES ON MICE

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After administration of nitrosamines (dimethylnitrosamine, diethylnitrosamine, nitrosomethylurea) to mice of lines A and C3HA, tumors developed in various situations in more than half the animals. The commonest tumors were adenomas and adenocarcinomas of the lungs and hepatomas. Precancer of the forestomach also was found, most frequently after administration of diethylnitrosamine, and adenomas of the kidneys occurred chiefly after administration of dimethylnitrosamine.

After the discovery in 1956 that dimethylnitrosamine (DMNA) can induce tumors [8], nitroso-compounds have frequently been investigated [2, 5-7, 9, 10]. Attention has been paid, in partiuclar, to the carcinogenic action of nitroso-compounds, particularly the possibility of obtaining neoplasms in particular organs by means of particular nitroso-compounds.

The problem of transplacental carcinogenesis by the action of nitroso-compounds is one of particular interest. Recently in the writer's laboratory, the transplacental action of various chemical compounds has been investigated by means of organ cultures. In 1966, Kolesnichenko [1] first obtained adenomas of the lungs in organ cultures by the transplacental action of urethane. Later, Smetanin [3] observed hyperplastic adenomatous changes in organ cultures of embryonic lung tissue by the transplacental action of two nitroso-compounds: DMNA and nitrosomethylurea (NMU). Smetanin also showed, in experiments on mice, that the offspring of mice receiving nitrosamines during pregnancy develop tumors.

Besides this work to study the characteristics of transplacental carcinogenesis, other work has been undertaken in the writers' laboratory to study the carcinogenic action of nitroso-compounds in experiments on adult animals. The action of three carcinogenic nitroso-compounds, DMNA, NMU, and diethylnitrosamine (DENA), has been studied.

EXPERIMENTAL METHOD

Experiments were carried out on 104 mice (54 males and 50 females) of line A and 117 mice (67 males and 50 females) of line C3HA, aged 2 months. The carcinogens were diluted to a concentration of 0.85% in physiological saline and were administered, either by subcutaneous injection twice a week for 3 weeks in a dose of 0.1 mg of each compound per injection, or through a gastric tube 3 times a week for 8-12 weeks in the same dose. All the mice were fixed at death and their internal organs investigated under the microscope.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show no significant differences between the action of the three carcinogens tested. The tumors obtained by the action of all three compounds will therefore be described.

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TABLE 1. Effect of Carcinogenic Nitrosamines on Tumor Development in Mice

Carcinogen tested	Line of mice and method of administration of carcinogen	Number of animals in experiment	Number of animals with tumors	Type of tumor							
				adenomas of lungs	adenocarcinoma of lungs	hepatoma	hepatocellular carcinoma	adenoma of kidney	adenocarcinoma of kidney	carcinoma of forestomach	other tumors
DMNA	C3HA by gastric tube	54	38/70.4	25	8	17	4	7			
	A by gastric tube	47	34/72.2	25	4	3	1		1	1	1
DENA	C3HA subcutaneously	23	8/34.7	2	3					1	2
	by gastric tube	13	12/92.3	6	2					7	
	A subcutaneously	7	4/57.1	2	1	1				1	1
	by gastric tube	20	10/50	6	3	2				1	3
NMU	C3HA subcutaneously	12	7/58.2	5	3	1				1	
	by gastric tube	15	11/73.3	8	2	3	1	1		2	1
	A subcutaneously	16	14/87.5	9	2	1					2
	by gastric tube	14	13/92.8	11	2		1				2

Note. Numerator shows number of animals with tumors, denominator percentage of total number of animals.

The first tumor (an adenocarcinoma of the lungs) in the C3HA mice was found 12 months after the beginning of the experiment in which the compound was given by subcutaneous injection, and the first tumor after administration via the gastric tube was observed in a line A mouse after 7 months. This was an adenoma of the lung. Most tumors appeared in the lungs and liver. In the lungs they were adenomas and adenocarcinomas, which sometimes were observed together in the same animal. As a rule the adenomas were multiple and of various sizes. On microscopic examination they were seen to be tubular and tubulopapillary, frequently of mixed type, and the degree of differentiation of the tumor was greater in its center. The cells forming them were juicy, and cubical and cylindrical, with a large, hyperchromic oval nucleus. An anaplastic tumor of the lung was diagnosed in one animal.

Most tumors of the liver were benign, either single or multiple, and were most commonly small hepatomas composed of large cells with hyperchromic nuclei, and sometimes exhibiting marked atypism. Most of them characteristically showed growth of cell-sheets. A hepatocellular carcinoma was found in 9 animals. These tumors were distinguished by their infiltrative growth, and their cells and nuclei were polymorphic. In one case the metastasis of a tumor of the liver was found in the lungs of a C3HA mouse.

Microscopic examination of the tumors of the forestomach revealed a squamous-cell keratinizing carcinoma. Keratinization and the formation of pearls and keratin cysts were well marked in all tumors, but varied in degree. DENA produced carcinoma of the forestomach rather more frequently (in 10 of 34 animals) than DMNA (in 1 of 72 mice) and NMU (in 3 of 45 animals).

Another interesting situation of the neoplasms was the kidneys, in which tumors were found in 10 mice: 8 animals had cystadenomas, 1 a large adenoma with hypernephroidization, and 1 mouse had a cystadenocarcinoma. This last tumor, like 7 of the cystadenomas and the large adenoma, was found in the experiments with DMNA, whereas in mice receiving NMU a cystadenoma was found in only 1 animal, and in the series of experiments with DENA no neoplasms of this type were found. In some of these tumors a curious lipid infiltration of the cells was observed, and regarded as "hypernephroidization" [4].

Besides the cystadenomas, careful microscopic examination of the kidneys in the mice receiving DMNA also revealed a characteristic, irregular hyperplasia of the convoluted tubules (Fig. 1). This hyperplasia progressed to focal proliferation, and then into adenomas and cystadenomas, the third stage of the process (Figs. 2 and 3). The fourth stage consisted of various types of adenocarcinomas and cystadenocarcinomas, developing by malignant transformation of the pre-existing adenomas and cystadenomas. All these growths occurred multicentrically and were accompanied by inflammation. These experiments thus supported the conclusions regarding stages in tumor development which were fully described previously [4].

In organ cultures of embryonic kidney tissues from BALB/C mice exposed transplacentally to the action of the known carcinogenic aminoazo compound o-aminoazotoluene in the writers' laboratory, hyperplastic changes in the epithelium and, in some cases, small but characteristic cystadenomas, very similar to those observed in the present investigation, were found (Fig. 2). This comparison shows that organ cultures provide suitable objects for the study of precancerous changes.

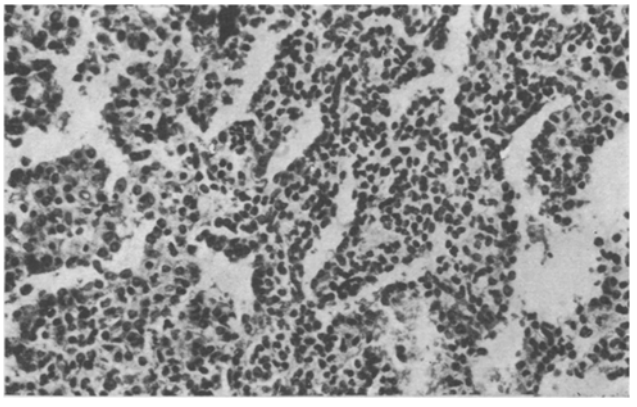

Fig. 1. Diffuse irregular hyperplasia of kidney tissue. Hematoxylin-eosin, 500 ×.

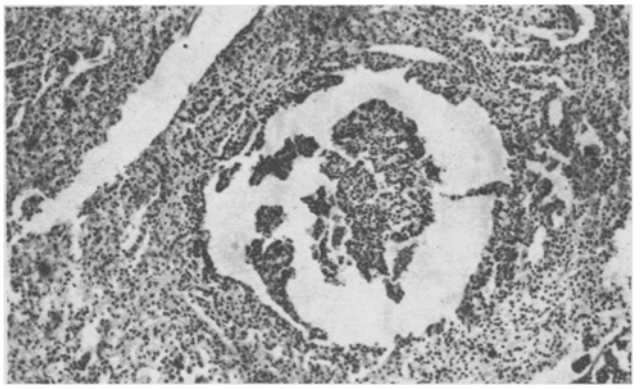

Fig. 2. Cystadenomas of the kidney with papillary outgrowths. Hematoxylin-eosin, 300 ×.

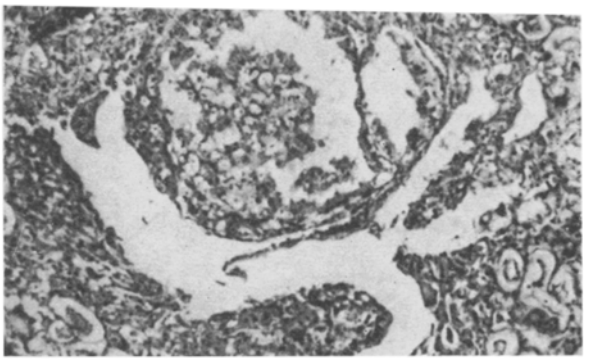

Fig. 3. Another cystadenoma of the kidney. Hematoxylin-eosin, 300 ×.

The results given in Table 1 show that in all series of these experiments tumors were obtained in a high proportion of animals (from 34 to 92.8 %). Neoplasms developed most commonly after administration of NMU. These results as a whole indicate that mice of lines A and C3HA are approximately equally susceptible to DMNA and NMU. However, mice of line C3HA were evidently sensitive to DENA, which produced carcinoma of the forestomach in 29.6% of these animals.

In a study of transplacental carcinogenesis in the writers' laboratory, Smetanin gave NMU and DMNA to pregnant C3HA mice and investigated their progeny at the age of 12-14 months. He found tumors of the lungs in 31% and hepatomas in 18% of cases. As Table 1 shows, in the present experiments about twice as many neoplasms were obtained. The most likely explanation of this phenomenon is that the mice investigated in this series survived until natural death, and they were thus much older than the animals in Smetanin's experiments.

LITERATURE CITED

1. T. S. Kolesnichenko, Vopr. Onkol., No. 12, 39 (1966).
2. N. P. Napalkov and V. A. Aleksandrov, in: Problems in Carcinogenesis and the Organization of Cancer Prevention [in Russian], Kiev (1967), p. 44.
3. É. E. Smetanin, Vopr. Onkol., No. 8, 48 (1969).
4. L. M. Shabad, Experimental Morphological Aspects of Precancer [in Russian], Moscow (1967).
5. I. N. Shvemberger, in: The Cytology of Malignant Growth [in Russian], Moscow-Leningrad (1963), p. 86.
6. H. Druckrey, R. Preussmann, D. Schmähl, et al., Z. Krebsforsch., 69, 103 (1961).
7. H. Druckrey, R. Preussmann, and D. Schmähl, Acta Un. Internat. Canc., 19, 510 (1963).
8. P. N. Magee and J. M. Barnes, Brit. J. Cancer, 10, 114 (1956).
9. P. N. Magee, Biochem. J., 64, 676 (1956).
10. P. N. Magee and R. Schoental, Brit. Med. Bull., 20, 102 (1964).