THE USE OF NITROSAMINES FOR THE INDUCTION OF TUMORS

(UDC 616-006-02 : 615.778.24)

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(Presented by Acting Member of the Academy of Medical Sciences of the USSR I. V. Davydovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 5, pp. 100-102, May, 1966
Original article submitted November 20, 1964

A large number of foreign publications has been devoted to the carcinogenic effect of nitrosamines first described in 1956 [14]. However, the Soviet literature on nitrosamines has so far been limited to the publications from a single laboratory [1-3].

Nitrosamines selectively, and with an almost 100% certainty, induce tumors of the liver [1-3, 5, 13, 16]; they were also instrumental in producing tumors of the trachea and lungs [6, 7, 9, 13], esophagus [10, 17], stomach [10-17], urinary bladder [11], kidneys [3, 15, 18] and nasal passages [12, 13]. The high carcinogenic activity of nitrosamines permits the production of tumor growth in different experimental animals, including the highly tumor-resistant guinea pigs [5]. It is of interest that methylnitrosourea selectively produces brain tumors [8].

The purpose of this investigation was to work out methods for the synthesis of nitrosamines which would make it possible to obtain these substances under relatively uncomplicated laboratory conditions, and to study morphological changes characteristic of the carcinogenic effect of action of nitrosamines.

EXPERIMENTAL METHODS

Two derivatives, dimethylnitrosamine (DMNA) and diethylnitrosamine (DENA) were obtained in the synthesis of nitrosamines. A method described in 1938 [4] was used by us with certain modifications.

About 50 g of dimethylamine hydrochloride, 25 ml of water, and about 2 ml of concentrated HCl were placed into a 500 ml round-bottom flask equipped with a mechanical mixer. The solution was stirred vigorously and heated in a water bath (70-75°C); a suspension of sodium nitrite in water (47 g per 30 ml) was gradually added to the solution during a one-hour period. An acid reaction was maintained throughout this process by periodic addition of concentrated HCl and by checking with litmus. After all the sodium nitrite had been added to the solution, it was heated for 2 h more. Following this the flask was evacuated with the aid of a water vacuum pump, water was removed, and dimethylnitrosamine was collected on a water bath, until an almost dry residue appeared on the bottom of the flask. This residue was discarded. The distillate was saturated with potash and left overnight. The fluid over the potash, dimethylnitrosamine, was decanted off carefully and twice distilled, each time collecting the fraction which boiled at 153°C. This process yielded 34 g of the product. Its physicochemical properties were as follows: a yellow fluid with a specific weight of 1.010_4^{20}, boiling point of 153°C, n_D^{18} of 1.432, soluble in water, alcohol, and ether.

The other derivative, diethylnitrosamine, was obtained in the same manner, but the starting material was 50 ml of diethylamine. The other components were used in the same amounts as for dimethylnitrosamine. The succession of procedures was basically the same; distillation was made at 177°C on Wood's alloy. Thirty-seven g of diethylnitrosamine were obtained. Its properties were as follows: a fluid somewhat more deeply yellow than dimethylnitrosamine, specific weight 0.938, boiling point 177°C, n_D^{18} of 1.438, soluble in water, alcohol, and ether.

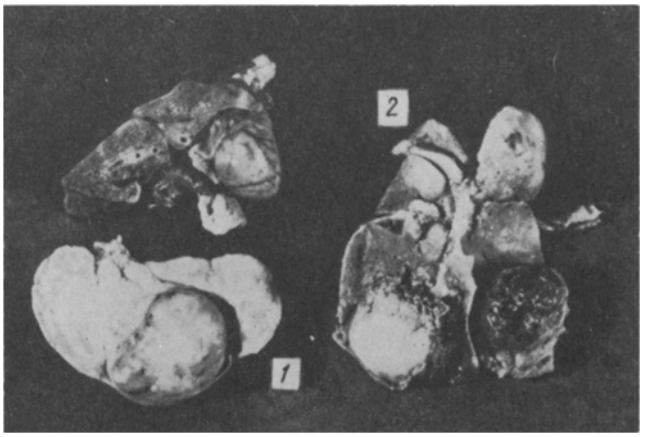

Fig. 1. Tumor in the liver, with metastases in the lungs (rat No. 1). Massive necroses and hemorrhagic changes of the tumor foci in the liver (rat No. 2).

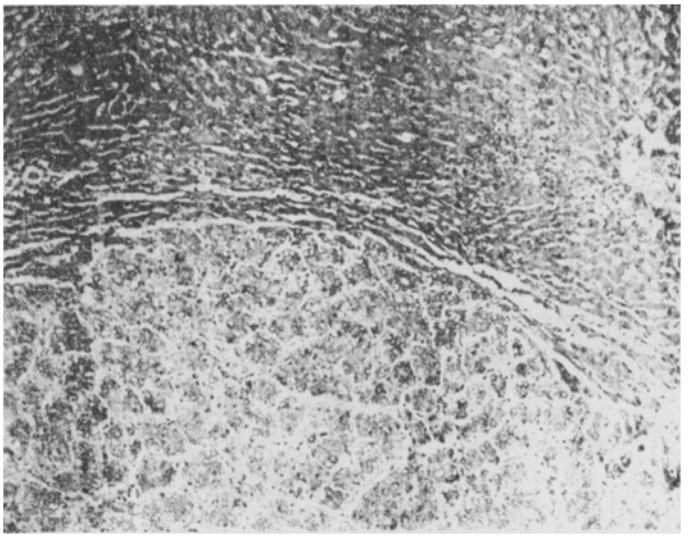

Fig. 2. Extensive growth of a trabecular hepatoma, accompanied by atrophy of adjacent liver tissue. Tumor cells aggregated into islands of rosettes and contain fatty inclusions. Hematoxylin-eosin, magn. × 56.

The substances obtained were given to nonpedigreed male rats weighing 100-120 g in drinking water in doses of 1.5-2.0 mg per 1 kg of body weight. Thus the preparations were being given continuously. Because the minimum doses of the carcinogenic substances for the induction of tumor growth had not been determined, the substances were given over a long period of time, so that the total doses exceeded 80-90 ml per animal.

RESULTS

In 11 of 15 rats killed 8 months after the beginning of administration of DMNA and DENA there were malignant tumors of the liver. Macroscopically these were discreet large nodules protruding from the surface of the organ and deforming it. In addition there were some considerably smaller nodules, also easily visible on the surface of the liver. On the surface and in the sections of the large tumor nodules there were white and dark-red foci produced by necrotization and by hemorrhagic changes in the parenchyma of the neoplasm (Fig. 1). The dark-red color of the tumors was also related to their histological structure (vascular tumors, rich in blood). The necroses and hemorrhages in the larger tumors indicated their rapid growth, and therefore their malignant nature. The de-

cisive proof of malignization was metastasis of tumors. There were metastases in the lungs as numerous roundish foci, protruding from underneath the visceral pleura (Fig. 1). In section the metastatic tumors appeared the same as the primary nodules; they were partially necrotized and partially invaded by hemorrhages.

In the material examined there was polymorphism of histological structure of the liver tumors. Hepatocellular cancer and angiosarcoma were identified. The structure of the hepatocellular cancer (hepatoma, hepatoadenoma) was trabecular (Fig. 2). The tumors had a tendency to dedifferentiate into adenocarcinoma and into microcellular cancer. Angiocarcinoma was represented by connective tissue tumors, containing numerous vessels of different diameters, filled with blood. This type of neoplasm was found in the liver as well as in its ligaments. The structure of metastases was the same as that of the primary nodules; they were surrounded by a zone of inflammation.

The carcinogenic effect and the histological structure of tumors obtained in our experiments corresponded to the published information. It is therefore rational to use nitrosamines in experimental oncology.

SUMMARY

Methods are described which are used for the synthesis of dimethylnitrosamine and diethylnitrosamine with a view to employing these substances for induction of tumors. The preparations were fed in drinking water to male albino rats of common breed. Eight months after the beginning of application of these preparations the experimental animals developed malignant tumors of the liver metastasizing to the lungs. The tumors are variable for their histological structure.

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