EXPERIMENTAL MODEL OF CARCINOMA OF THE ESOPHAGUS

N. N. Litvinov, V. I. Govorchenko, and V. N. Kurilev

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Experiments on rats demonstrated the powerful and specific carcinogenic action of N, N^1-dimethylethylenedinitrosamine. In rats receiving the compound for four months by intraesophageal administration in a dose of 5 mg/kg, carcinoma of the esophagus developed after 5-6 months in 100% of cases. By the use of this model, the various stages of formation of the malignant growth can be studied.

Carcinoma of the esophagus is one of the commonest human malignant neoplasms, particularly in men [5, 6]. Nevertheless, the conditions of development, the morphogenesis, and the precancerous changes of esophageal neoplasms have received less study than those in other situations [8]. This is evidently because of the inadequate study of experimental neoplasms of the esophagus. Relatively few references to this subject are to be found in the literature [1-3, 7, 10, 12, 13, 15-17]; the workers cited give information regarding the possible development of esophageal neoplasms under the influence of chemical carcinogens.

This paper describes a study of the carcinogenic properties of N, N^1-dimethylethylenedinitrosamine, a compound which, as the work of Druckrey et al. [14] and the surveys of Shvemberger [11] and Ostrovskaya [4] show, possesses carcinogenic activity. The carcinogenic properties of this compound have not previously been investigated in the Soviet union.

EXPERIMENTAL METHOD

Experiments were carried out on 105 noninbred male albino rats which received N, N^1-dimethylethylenedinitrosamine daily (except on Sundays) for 4 months via esophageal tube in a dose of 5 mg/kg of the aqueous solution. The initial body weight of the animals was 110-130 g. The rats were sacrificed five at a time, 1, 2, 3,and 4 months after the beginning of the experiment. In the course of 2-6 months, 17 rats died, and the remaining 68 animals were sacrificed 5 and 6 months after the beginning of the experiment.

Pieces of the esophagus and the principal viscera were taken from all the animals, embedded in paraffin wax, and sections from them were stained with hematoxylin-eosin, and by the methods of Van Gieson, Feulgen, Brachet, and McManus.

EXPERIMENTAL RESULTS

No changes were found in the esophagus of the animals sacrificed one month after the beginning of the experiment. In all five rats examined after two mos., microscopic examination revealed diffuse or focal thickening of the esophageal mucous membrane on account of an increase in the number of layers of epithelial cells and their intensified keratinization. The size and structure of the epithelial cells were unchanged, and only a slight increase in the number of mitoses in them was recorded.

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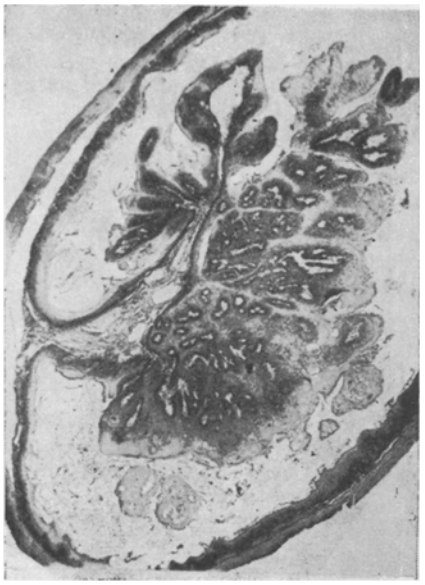

Fig. 1

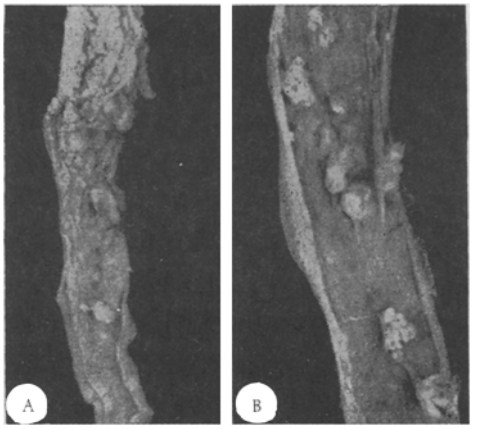

Fig. 2

Fig. 1. Transverse section through esophagus with a malignant tumor filling its lumen and resting on a narrow base (6 months after beginning of the experiment). Van Gieson, 25×.

Fig. 2. Segments of esophagus of two rats after receiving the compound for four months: multiple papillomatous growths and malignant nodules of the mucous membrane: A) 150 days after beginning of experiment (5×); B) 180 days after beginning of experiment (8×).

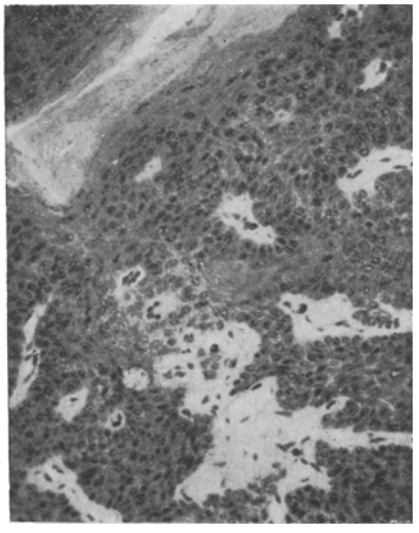

Fig. 3. Carcinoma of the esophagus consisting mainly of prickle cells with solitary epithelial pearls (6 months after beginning of experiment). Hematoxylin-eosin, 200×.

In the rats sacrificed after three mos., tiny whitish areas, almost invisible with the unaided eye, were raised above the rest of the surface of the mucous membrane (foci of leukoplakia). Microscopic examination showed that these raised areas of the mucous membrane were foci of hyperplastic epithelium with evidence of intensified growth of the cells and hyperkeratosis. No signs of inflammation were present.

Later (after four months) the total number of areas of hyperplasia of the mucous membrane rose appreciably, and their size increased. Bands of connective tissue with blood vessels penetrated from the submucosa into the hyperplastic epithelium. In this way, multiple papillomatous growths were formed, some of them on a thin connective-tissue pedicle, hanging in the lumen of the esophagus, while others had a wider base and were more like a cauliflower in appearance. By using serial sections from different parts of the esophagus of many animals, the subsequent evolution of the structure of the benign epithelial tumors could be traced. The process followed a somewhat different course in the tumors with a wide and with a narrow base.

During growth of the papillomas of the first type, invasion of the submucosa of the esophagus by epithelial cells with the formation of characteristic cell bands infiltrating the subjacent connective tissue was more frequently observed. The epithelial cells of the tumor in the zone of proliferation and infiltration showed signs of atypism (they were larger in size, irregular in shape, their cytoplasm was more strongly basophilic, and the structure of the nucleus was altered). In the tumors resting on a narrow pedicle, active growth of the epithelial cells showing atyp-

ical features was observed into the lumen of the esophagus without disturbance of the integrity of the basement membrane (Fig. 1). During development of the tumor, the regular arrangement of the epithelial layers was disturbed, and increased keratinization of the cells took place with the formation of numerous epithelial pearls.

In the animals examined five and six mos. after the beginning of the experiment besides the multiple tiny papillomatous growths of the esophageal mucous membrane, larger tumor nodules (measuring 1.5 × 1 cm) were found, along the whole length of the esophagus (Fig. 2), but mostly in its upper third. Some of the tumor nodules showed superficial necrosis and ulceration. Their microscopic study showed that they consisted of typical squamous-cell carcinomas, usually with keratinization (Fig. 3). Mitoses were numerous among the proliferating cells. Malignant tumors consisting mainly of "prickle" cells also were found.

Altogether 75 rats (68 killed and 7 dying) were examined 5 and 6 months after the beginning of the experiment, and multiple nodules of squamous-cell carcinoma were found in the esophagus of all the animals.

No primary neoplasms or metastases of esophageal carcinoma were found in the other organs.

Hence, when administered for a long period by the oral route in a dose of 5 mg/kg, the compound N, N^1-dimethylethylenedinitrosamine is an exceptionally active carcinogen with a specific type of action. Malignant tumors of the esophagus arise in all animals 5-6 months after the beginning of the experiment (in the 75 rats examined at these times). Multiple primary carcinomatous nodules develop along the whole length of the esophagus against the background of widespread papillomatosis.

It will be noted that other chemical carcinogens induce the development of esophageal tumors in a much lower percentage of cases [2-4, 7, 11, 13-17].

The type of tumor produced in these experiments serves as a very convenient model for experimental cancer research. In particular, even the preliminary study shows that carcinoma of the esophagus is preceded by certain tissue changes corresponding on the whole to the precancerous processes which have been described [9, 10] in relation to neoplasms of other organs. The earliest changes consist of a diffuse or focal thickening of the esophageal mucous membrane, on the basis of which multiple nodules of intensified proliferation of epithelial cells appear.

This is then followed by the formation of benign tumors or papillomas, some of which rapidly turn malignant, with infiltration of the underlying tissue while others become malignant more slowly, with the development of large growths projecting into the lumen of the esophagus.

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