

TUMOR DEVELOPMENT IN THE BIVALVE MOLLUSK

Unio pictorum INDUCED BY N-NITROSO

COMPOUNDS

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Diethyl- and dimethylnitrosamines, dissolved in aquarium water in doses of 200-400 ppm, induced the formation of neoplasms of the digestive gland (basophil-cell tumors) in the mollusk *Unio pictorum* in 16 of 95 and 6 of 17 animals, respectively, which survived until the time of appearance of the first tumor (38-39 days), and also neoplasms of the hematopoietic system (lymphatic leukemia) in seven and one mollusks, respectively, mainly in conjunction with tumors of the digestive gland. Metylnitronitrosoguanidine caused only inflammatory changes at the site of the injection. The mollusks can be used with advantage as a biological indicator of pollution of the hydrosphere with chemical carcinogens.

KEY WORDS: mollusks; nitrosamines; tumors of the digestive gland; lymphatic leukemia.

A practical problem in comparative oncological research is the study of the possible use of various biological objects in order to determine the degree of pollution of the environment by carcinogenic agents [4]. Bivalve mollusks, animals with a filtration method of feeding and with an extensive territory of distribution, are particularly interesting from this standpoint. The development of neoplasms in mollusks has been shown to be quite definitely connected with pollution of the hydrosphere by various carcinogens [5, 6]. Nevertheless, it was only relatively recently that malignant and benign tumors of the foot and mantle were first successfully induced in the gastropod mollusk *Ampularia australis* by injection of methylcholanthrene and benzopyrene [7, 8]. In recent years the attention of investigators has been strongly attracted to the carcinogenic nitrosamines, compounds which can be discharged into reservoirs or synthesized de novo from their chemical precursors. However, no investigations have yet been carried out into the induction of tumors in mollusks by nitroso compounds.

The object of this investigation was to compare the sensitivity of members of this group of animals to the action of nitrosamines.

EXPERIMENTAL METHOD

Experiments were carried out on adult sexually mature individuals of the widespread mollusk *Unio pictorum*, belonging to the Bivalvia class. The animals were kept in 10-liter aquaria with continuous aeration at 18-22°C. No active feeding took place during the experiments. Altogether three series of experiments were carried out using the maximal tolerated doses of carcinogens as established in acute experiments. In series I 146 mollusks were exposed for 62-64 days to diethylnitrosamine (DNA), which was dissolved in the aquarium water in a dose of 200-400 ppm. A constant concentration was maintained by adding DNA daily. In series II dimethylnitrosamine (DMNA) was used in a constant concentration of 200 ppm, dissolved in the water of aquaria in which 30 mollusks were kept. The period of exposure was 51 days. In series III crystalline methylnitronitrosoguanidine (MNNG) was injected as a single dose of 100 µg into the region of the foot of 25 mollusks. After exposure to the compounds the mollusks were kept in aquaria containing pure water. The maximal duration of observation on all the animals was 6 months. A morphological study was made of the digestive gland, gonads, foot, gills, and other organs from mollusks which died or were killed after various times. The material was

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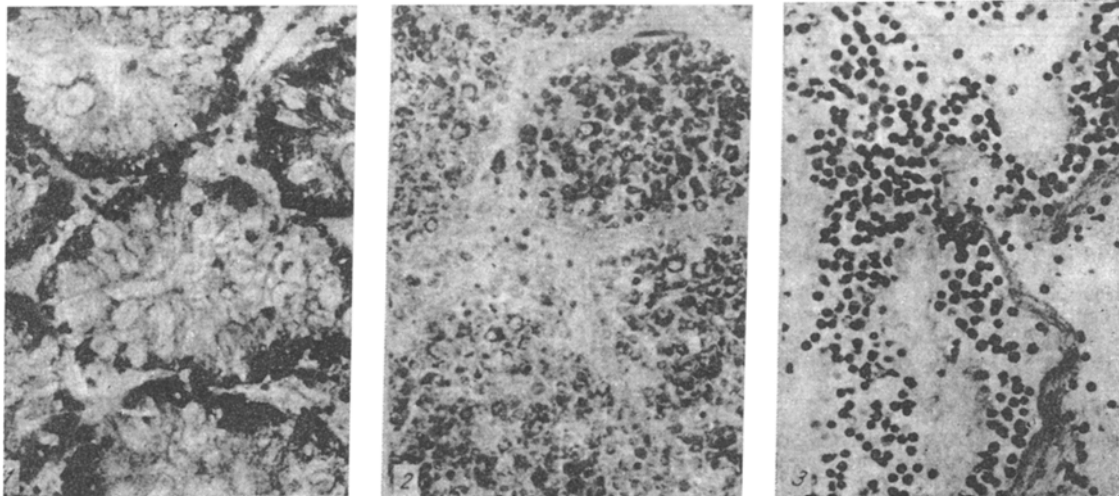


Fig. 1

Fig. 2

Fig. 3

Fig. 1. Normal structure of digestive gland of mollusk Unio pictorum. Here and in Figs. 2 and 3, hematoxylin-eosin, 440 \times .

Fig. 2. Basophil-cell tumor of digestive gland, 35 days of exposure to DENA.

Fig. 3. Lymphatic leukemia; 61 days of exposure to DENA.

fixed in 4% formalin solution and, after the usual histological treatment, sections were stained with hematoxylin-eosin and by Van Gieson's method.

EXPERIMENTAL RESULTS

The digestive gland of mollusks is the analog of the vertebrate liver. The normal structure of this organ is characterized by numerous diverticula, consisting of large digestive and basophilic cells (Fig. 1).

Series I and II. Changes in series I and II were of the same type. Many mollusks died in the course of 4-5.5 months and the rest were killed after 6 months. The digestive glands of mollusks which died in the early stages (4-5 weeks) were brownish in color and had an uneven surface. On microscopic examination proliferation of connective tissue resembling cirrhotic changes in fishes was observed between the diverticula [2]. The first tumors of the digestive gland were found in both series on the 38th-39th day. By this time 95 mollusks were still alive in the experiments with DENA and 17 in the experiments with DMNA. The digestive gland of all these mollusks was enlarged by 1.5-2.5 times, firm in consistency, nodular, and brownish-red in color. In most of the mollusks which survived over 38-39 days a well-marked but chaotic proliferation of the basophilic cells was observed, so that these cells filled the lumen of the diverticula and displaced the digestive cells which formerly were present there. Increased polymorphism and atypia of the cells, as well as the presence of giant deformed basophilic cells, were extremely characteristic of these foci of proliferation (Fig. 2). The walls of the diverticula were invaded by tumor cells and mitotic figures, which are extremely rare in intact mollusks, were observed. These zones of proliferation, which were observed in 16 mollusks in series I and 6 in series II, were classified as basophil-cell tumors. Increased activity of the hemocytes, which appears in excessive numbers in the connective tissue between the diverticula, was often observed in such cases. Similar changes have been described in mollusks during prolonged starvation [11]. However, in seven cases in series I and one case in series II (mainly in conjunction with basophil-cell tumors) rapid proliferation of hemocytes with atypical growth was observed, and was extraordinarily reminiscent of lymphatic leukemia in oysters [9]. Blood cells with large oval or irregularly shaped nuclei were found in the digestive gland, kidneys, gills, and gonads; they were distributed mainly close to the lacunar tissue. These changes were regarded as leukemia (Fig. 3). In one animal in series I focal hyperplasia of the connective tissue of the inner gill membranes also was observed.

Series III. After 7-10 days reduction of the foot and ulceration of the tissue at the site of injection were observed in all 25 mollusks. Destruction of muscle fibers and the appearance of hemocytes around the areas of necrosis were observed. These findings indicated a definite inflammatory reaction. Toward the end of the experiment, slight epithelization of the ulcer was observed. No changes were found in the other organs.

These investigations showed for the first time the high sensitivity of the digestive gland of mollusks to the carcinogenic action of nitrosamines. Spontaneous tumors in mollusks are widely known [8, 9], but changes such as these had not previously been described. The tumors of the digestive gland obtained in the present experiments were very similar in their morphology and clinical picture (atypia, polymorphism, invasive growth, the presence of mitoses) to liver tumors in mammals [1] and lower vertebrates [3] induced by nitrosamines. Recently the attention of research workers has been drawn to leukemias in mollusks, whose onset has been connected with pollution of the water by carcinogens [9]. The analogous changes discovered by the present writers have been obtained for the first time experimentally.

Considering the relatively intensive carcinogenic response of the bivalve mollusk Unio pictorum, this animal can advantageously be used together with fishes and amphibians [3, 10] as a biological indicator of pollution of the hydrosphere by carcinogenic nitrosamines. Mollusks have several advantages over the vertebrates just mentioned: economy, universal distribution, simplicity of upkeep, and so on. Considering the short period of development of the neoplasms and the relative ease of reproduction of the results, the possibility of the use of mollusks for testing chemical carcinogens must be considered. Controls based on the frequency of development of tumors in mollusks could play an important role in the analysis of the state of the external environment and could assist with the prevention of tumors in animals and man.

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