induced tumor antigens. Infection of the mice immediately after birth with Bittner virus specifically altered the immunological response to virus-induced antigens. These changes evidently consisted essentially of the development of immunostimulation of virus-transformed cells or of distortion of the immune response as in the enhancement phenomenon. Early thymectomy, with its immunodepressive action, reduced this immune response to virus-induced antigens of the tumor cells. When the tumor appeared in factorless mice and evidently contained no virus-induced antigens, thymectomy had no effect on its growth in either the MTV-S^+ or the MTV-S^- recipients.

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CARCINOGENIC ACTION OF DIETHYLSTILBESTROL ON FROGS

V. V. Khudolei and V. S. Ermoshchenkov

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Diethylstilbestrol in a dose of 480-4400 μg, injected subcutaneously into frogs (*Rana temporaria*), gave rise to hemocytoblastosis and hepatocellular carcinoma in eight of the 38 animals (21%) after 15.6 weeks.

KEY WORDS: diethylstilbestrol; frogs; hemocytoblastosis; hepatocellular carcinoma.

It is now firmly established that dyshormonal disturbances play an important role in the genesis of some tumors of man and animals. Large doses of estrogens, given *in vivo*, give rise to various neoplastic changes [6]. In the context of comparative oncological investigations in the laboratory, the study of the sensitivity of lower vertebrates to the carcinogenic action of hormones is of particular interest. Diethylstilbestrol, if given to trout with the food, is known to induce hepatomas in these fish [5]. No such investigations have been carried out on amphibians.

The object of this investigation was to study the action of diethylstilbestrol propionate on *Tana temporaria*.

EXPERIMENTAL METHOD

Experiments were carried out on tailless amphibians (*Rana temporaria*) of both sexes aged 1-1.5 years. For the duration of the tests 98 (46 female and 52 male) experimental animals

Laboratory of Chemical Carcinogenic Agents, N. N. Petrov Research Institute of Oncology, Ministry of Health of the USSR, Leningrad.

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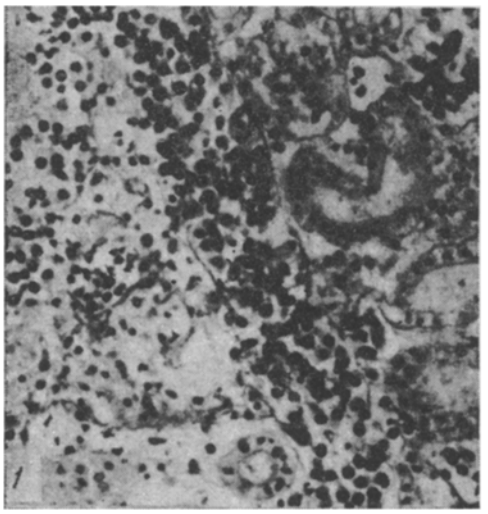

Fig. 1. Hemocytoblastosis. Lesion of the kidney. Here and in Fig. 2: hematoxylin–eosin, 440 ×.

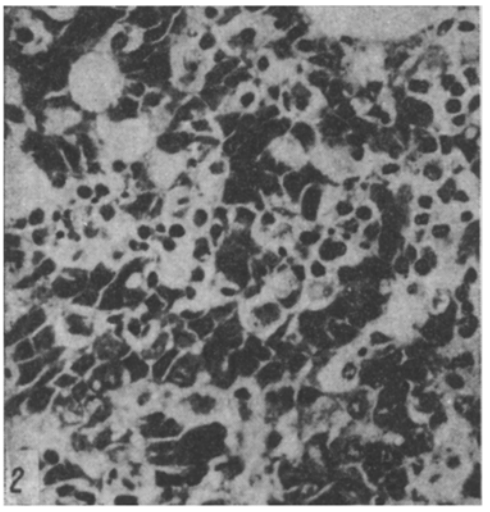

Fig. 2. Hepatocellular carcinoma against the background of degeneration.

received subcutaneous injections of 40-200 μg of a solution of diethylstilbestrol propionate in vegetable oil in the dorsal region once a week. The maximal duration of observation was 29.5 weeks. The liver, kidneys, spleen, and pituitary gland obtained from frogs dying at different times were studied morphologically. The material was fixed in 4% formalin solution and, after ordinary histological treatment, sections 5-8 μ in thickness were stained with hematoxylin–eosin, by Van Gieson's method (using saturn red instead of fuchsin), and oil red O.

EXPERIMENTAL RESULTS

On microscopic examination well-developed phenomena of large-droplet fatty degeneration were observed in the kidneys and, more especially, in the liver, accompanied by the presence of large cysts filled with fat (fat depots). In six of the 21 females (29.5%) and in two of the 17 males (11.2%) surviving more than 9.5 weeks (the time of discovery of the first neoplasm) neoplasms of the hematopoietic tissue (seven cases) and liver (two cases) were discovered. The mean latent period of development of the tumors was 15.6 weeks. The minimal dose of diethylstilbestrol causing tumor development was 480 μg and the maximal dose 4400 μg. Neoplastic changes of the hematopoietic system were classified as hemocytoblastosis (Fig. 1). They consisted of multiple foci of proliferation composed of small, atypical basophilic cells, arranged as a rule along the blood vessels in the liver, kidneys, and spleen. Against the background of these severe degenerative changes in the liver (in one case combined with hemocytoblastosis), an undifferentiated hepatocellular carcinoma developed (Fig. 2) and showed invasive growth. A high degree of polymorphism was observed, with pathological mitoses in some cells. No metastases of the tumor nor neoplasms in other situations, including the pituitary gland, were observed. In the control observations on 19 (11 female and eight male) frogs receiving vegetable oil only, no tumors were seen during the same period (31.5 weeks).

These experiments demonstrated for the first time that diethylstilbestrol has a carcinogenic action on amphibians and they revealed high sensitivity of these animals to large doses of the estrogen. In the present experiments neoplasms appeared in the hematopoietic system and liver. Tumors in these situations are very rarely observed spontaneously in amphibians living under natural conditions [2, 3]. However, such changes have been obtained by the use of nitrosamines [1, 7]. The response of the hematopoietic tissue and liver to various carcinogens is evidently determined by the species-specificity of the members of this class of vertebrates. In other vertebrates diethylstilbestrol can induce neoplasms of the mammary glands, testes, cervix uteri and vagina, lymphoid tissue, pituitary gland, urinary bladder (mice, rats), and kidneys (hamsters), neoplastic changes in the ovaries (dogs) and, finally, malignant mesotheliomas of the ovaries (dogs) and of the uterus (monkeys) [6]. It was concluded quite recently that high doses of hormonal preparations with estrogenic activity induce tumors of the liver in man [4]. Comparison of the sensitivity of different species of animals, sometimes quite widely separated on the scale of evolution, to the carcinogenic action of diethylstilbestrol thus revealed considerable differences in the localization of

the tumors in them. The essential features of this relationship between the species of animal and the tumor spectrum requires elucidation.

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IMMUNOCHEMICAL IDENTIFICATION OF A NEW EMBRYONIC ANTIGEN IN OVARIAN TUMOR TISSUE

V. V. Kalashnikov, S. A. Borisenko, and Yu. S. Tatarinov

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A new embryonic antigen was found in ovarian tumors by methods of immunodiffusion analysis. This antigen consists of two components with different electrophoretic mobilities: The slow peak migrates in the α_0-globulin zone, the fast peak in the prealbumin zone. It is immunologically different from α-fetoprotein and from the carcinoembryonic antigen of the large intestine. The antigen was found in fetal and neonatal blood serum and also in tissue extracts of ovarian tumors. It was not found in normal adult human tissues or in the blood serum of healthy donors and cancer patients.

KEY WORDS: ovarian tumor; tumor antigens; embryonic antigens.

Embryonic reversion of tumor tissue has now been demonstrated in tumors of the liver [1], large intestine [8], and pancreas [6]. The discovery of embryonic antigens in patients' blood serum is the basis for the serological diagnosis of some tumors [3, 6, 10]. The writers previously found both heterologous organ antigens [2] and the carcinoembryonic antigen of the large intestine [4] in ovarian tumor tissue.

This investigation is a continuation of earlier work aimed at finding embryonic antigens in ovarian tumor tissue.

EXPERIMENTAL METHOD

Tumor tissue (adenocarcinoma of the ovaries) was obtained during operations and it was processed within 2-3 h. A weighed sample of tissue with the addition (1:1) of Tris–glycine buffer containing detergent (Triton X-100) was homogenized with powdered glass. The resulting homogenate was frozen and thawed twice and then centrifuged at 12,000 rpm. The supernatant was lyophilized. Extracts from normal organs were prepared in the same way from the cadavers of persons dying from injuries. Extracts containing 50,000 μg protein/ml were used.

Department of Biochemistry and Problem Laboratory for Immunochemistry of Malignant and Embryonic Tissues, N. I. Pirogov Second Moscow Medical Institute.

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