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EFFECT OF DL-TRYPTOPHAN ON INDUCTION OF TUMORS BY DIETHYLSTILBESTROL IN *Rana esculenta*

V. V. Eliseev and V. V. Khudolei

UDC 615.277.4:547.757

Diethylstilbestrol, injected subcutaneously into frogs (Rana esculenta), induced leukemias and tumors of the liver in 21.4% of animals. After combined administration of diethylstilbestrol and DL-tryptophan, tumors of these sorts developed in 52.6% of frogs. The differences in the frequency of leukemias in the animals of these groups were significant ($P < 0.05$). Administration of tryptophan alone did not induce tumors.

KEY WORDS: frogs; diethylstilbestrol; tryptophan; hemocytoblastosis.

Information on the potentiating effect of tryptophan with respect to the action of certain carcinogens [2, 9, 10, 13] and on the ability of its metabolites to induce leukemia [1, 3, 11] has been published. One of us (V. V. Khudolei) showed previously [6] that diethylstilbestrol, if given parenterally, induces hemocytoblastosis and hepatocellular carcinoma in the frog Rana temporaria.

The object of this investigation was to study the combined action of diethylstilbestrol and DL-tryptophan on Rana esculenta.

EXPERIMENTAL METHODS

The experiments were carried out on pond frogs (Rana esculenta), which are tailless amphibians, of both sexes aged 1-1.5 years. The 25 animals of group 1 received a subcutaneous injection of 100 μg diethylstilbestrol once a week in the dorsal region. The 40 frogs of group 2 received 75 mg of DL-tryptophan in 0.2 ml water by gastric tube once a day (5 times a week). The 40 frogs of group 3 received diethylstilbestrol and DL-tryptophan in the same way. The longest period of observation on the animals was 58, 122, and 65 days respectively. The liver, kidneys, and spleen from animals dying at different periods were subjected to morphological examination. Material was fixed in Bouin's fluid and paraffin sections were stained with hematoxylin-eosin. The numerical data were subjected to statistical analysis by Fisher's exact method and by the Wilcoxon–Mann–Whitney U criterion.

EXPERIMENTAL RESULTS

The experimental results are given in Table 1.

Tumors of the hematopoietic system and liver were found in some of the animals in groups 1 and 3. When the hematopoietic organs were affected, the greatest macroscopic changes were observed in the spleen. The

Laboratory of Chemical Carcinogenic Agents, N. N. Petrov Scientific-Research Institute of Oncology, Ministry of Health of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 8, pp. 197-199, August, 1979. Original article submitted July 17, 1978.

TABLE 1. Results of Experiments to Study Effect of DL-Tryptophan on Carcinogenic Action of Diethylstilbestrol

Substances	Number of animals surviving until first tumor	Leukemia		Carcinoma of the liver	
		absolute number of animals	%	absolute number of animals	%
Diethylstilbestrol	14	2	14,3	2	14,3
Diethylstilbestrol + tryptophan	19	9	47,4*	6	31,6

*P < 0.05.

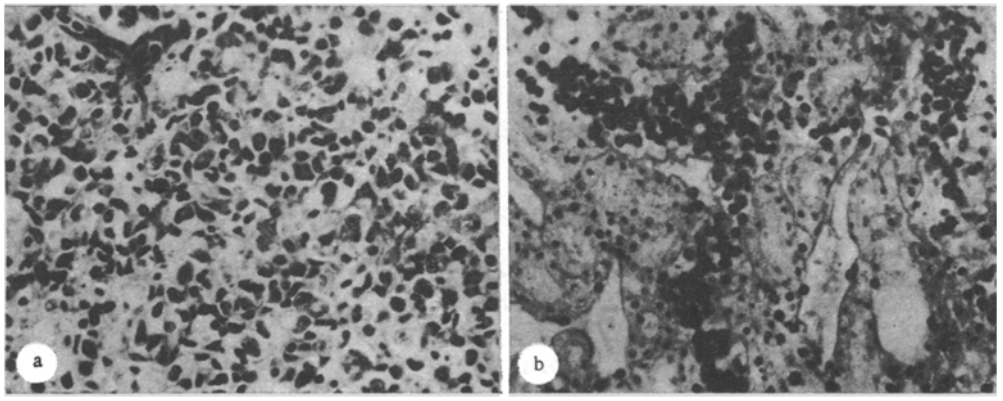

Fig. 1. Hemocytoblastosis in frogs. Hematoxylin-eosin, 440×. a) Lesion of the spleen; b) foci of hematopoiesis in the kidney.

organ was enlarged to between twice and four times its normal size and the tissue on section appeared juicy and a cherry-red color. Neoplastic changes in the hematopoietic tissue were classified as hemocytoblastosis and consisted of diffuse or focal leukemic growths of undifferentiated cells of hemocytoblast type in the spleen, kidneys, and liver. The spleen was characterized by a marked disturbance of the normal structure of the organ (Fig. 1a), a decrease in the number of lymphocytes, and disappearance of the lymphoid follicles. In the liver and kidneys, foci of proliferation of atypical small basophilic cells were usually located along the blood vessels (Fig. 1b).

Carcinoma of the liver was found in the form of multiple small nodules in the thickness of the organ. Histologically the tumors were diagnosed as hepatocellular carcinoma of trabecular, tubular, or, more rarely, anaplastic type. No metastases were found.

In group 1, 14 animals survived until the appearance of the first tumor (40 days). Neoplasms were found in three of them (21.4%): Leukemia twice and carcinoma of the liver twice. The tumors coexisted in one animal. The mean latent period of development of the neoplasms was 49 days. The maximal dose of diethylstilbestrol injected was 900 μg. During the combined administration of diethylstilbestrol and tryptophan (group 3) the first tumor was found after 36 days. By that time 19 frogs remained alive, and 10 of them developed tumors (52.6%). In nine cases leukemia and in six cases carcinoma of the liver were found. The tumors coexisted in five animals. The mean latent period was 46 days. The maximal dose of diethylstilbestrol was 1000 μg and of tryptophan 3450 mg. No tumors were found in the animals of group 2. The total dose of tryptophan received by the frogs was 6526 mg.

The investigation revealed a significant increase in the frequency of leukemia in Rana esculenta as a result of the combined administration of large doses of diethylstilbestrol and tryptophan. The frequency of liver tumors in the experimental groups compared did not differ statistically significantly.

Diethylstilbestrol has a broad spectrum of carcinogenic action, causing tumors of different organs in mammals [12]. The carcinogenic action of diethylstilbestrol on Rana temporaria, expressed as induction of

malignant tumors of the hematopoietic system and liver, was demonstrated previously. Similar neoplasms are found extremely rarely in the Anura under natural conditions of existence [5, 8]. The induction of leukemia by diethylstilbestrol in frogs is evidently due to the species-specificity of this class of vertebrates. Tumors of the hematopoietic system in mammals have been induced by various carcinogens, among which at the present time great importance is attached to indole and aromatic derivatives of tryptophan, which behave as endogenous carcinogenic agents [7]. There is information in the literature on the stimulating effect of tryptophan on hematopoiesis in animals [4]. Meanwhile the potentiating action of a tryptophan diet on the action of various carcinogens is well known. It is assumed that chemical carcinogens inhibit enzyme systems which participate in tryptophan metabolism, as a result of which intermediate products of tryptophan metabolism accumulate, some of which have the property of inducing tumors, including leukemia. Injection of tryptophan alone does not cause tumors [1, 13]. During the combined administration of tryptophan and diethylstilbestrol, which gives rise to hemocytoblastosis in amphibians, the carcinogenic action of the estrogen may be potentiated. There is no information in the literature on the intermediate metabolism of tryptophan in amphibians. Further investigations of this sort may make a useful contribution to the understanding of the nature and general features of processes of carcinogenesis in animals at different levels of evolution.

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