MECHANISM OF THE LEUKEMOGENIC ACTION OF SEX HORMONES

N. A. Grigorovich

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In mice of both sexes injected with leukemogenic doses of estradiol monobenzoate, a dynamic study was made of the high-elastic deformation of oriented desoxyribonucleoprotein strands isolated from animal hematopoietic tissue. It was concluded from the changes discovered that estradiol monobenzoate acts on the tertiary structure of the desoxyribonucleoprotein, rupturing the bond between DNA and protein in its molecule.

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The ability of the sex hormones to induce tumors and leukemias has been known for a long time [4, 7-10]. An extensive literature devoted to analysis of mechanisms of tumor development under the influence of estrogens at the level of the complete organism has accumulated [2, 3]. However, little or no work has been done to study the intimate disturbances produced by estrogens in cells leading to their malignant transformation.

The latest findings in experimental oncology suggest that the process of malignant transformation is due primarily to changes in the macromolecular structure of the biochemical substratum of the chromosomes, desoxyribonucleoproteins (DNP). No information concerning the action of leukemogenic doses of estrogens on DNP of hematopoietic tissue could be found in the literature.

In view of the importance of this problem for the understanding of mechanisms of the oncogenic action of estrogens, in the present investigation we studied the state of the macromolecular structure of DNP isolated from hematopoietic tissue of mice receiving massive doses of estradiol monobenzoate (EMB), producing leukemias and lymphomas in experimental animals [7].

EXPERIMENTAL METHOD

Experiments were carried out on sexually immature noninbred albino mice weighing 14-16 g obtained from the nursery of the Academy of Medical Sciences of the USSR. Two groups of experiments were carried out on 250 animals, 125 in each group. In group 1 the action of EMB was studied on the macromolecular structure of DNP of male hematopoietic tissue. In group 2 the same investigations were carried out on females. EMB was injected subcutaneously in a dose of 250 μg into the animals once weekly. In the course of the experiment the mice received from 250 to 2200 μg of the preparation. According to data in the literature, administration of these doses of estrogens causes the development of leukemias and lymphomas in mice after 3-4 weeks [7, 10]. The animals were sacrificed 1, 2, 7, 14, 30, and 60 days after the first injection of the preparation. The spleen was immediately removed in the cold. The method of isolation of DNP from splenic tissue, together with its physicochemical and biochemical properties, have been described previously [1]. The state of the macromolecular structure of DNP was estimated from the results of a study of the behavior of the oriented strands which it forms [5]. The results were subjected to statistical analysis. Only statistically significant differences ($P \leq 0.05$) of the high-elastic deformation of oriented strands will subsequently be considered.

EXPERIMENTAL RESULTS

In the doses mentioned above, EMB had a marked effect on the experimental animals. The mice lost weight 3-4 weeks after the beginning of administration of the hormone. Postmortem examination of the females revealed an enlarged uterus in every case. In males atrophy of the testes was sometimes present.

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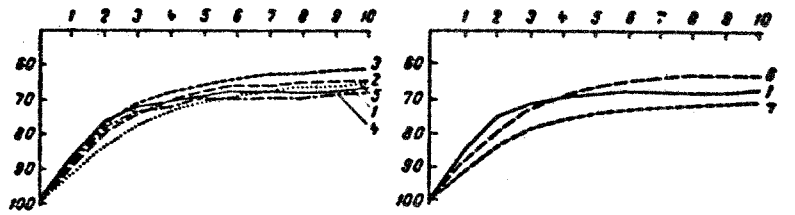

Fig. 1. Relaxation of oriented strands from splenic DNP of healthy male mice (1) and animals sacrificed 1, 2, 7, 14, 30, and 60 days after beginning of EMB administration (curves 2, 3, 4, 5, 6, 7 respectively). Here and in Fig. 2, curves are plotted from results of 2-6 experiments and reflect relaxation of 20-80 strands. Ordinate, length of strands (in %); abscissa, time (in min).

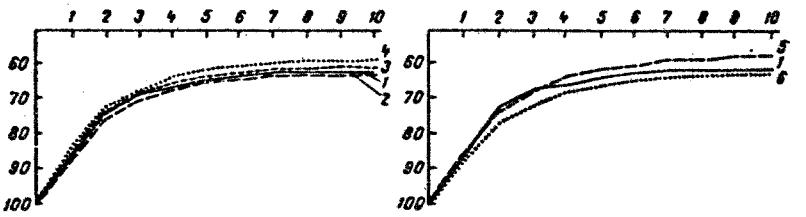

Fig. 2. Relaxation of oriented strands of splenic DNP from healthy female mice (1) and mice sacrificed 1, 7, 14, 30, and 60 days after the beginning of EMB administration (curves 2, 3, 4, 5, and 6 respectively).

The results of investigation of the rheologic properties of DNP isolated from DNP of the hematopoietic tissue of the mice at various times after the beginning of EMB administration are given in Figs. 1 and 2. Toward the 10th min of observations, oriented strands from splenic DNP of males sacrificed 24 h after the beginning of hormone administration were reduced in length by 35%. Strands of splenic DNA from healthy mice shortened during the same period by only 33% (Fig. 1, curves 1, 2, and 3).

The high-elastic deformation of strands from splenic DNA of the males was reduced 7 and 14 days after the beginning of EMB administration. Strands formed from splenic DNP of mice sacrificed 7 days after the beginning of EMB administration relaxed to a lesser degree throughout the period of observation than strands of splenic DNP from control mice, their length being reduced by 31% (Fig. 1, curves 1 and 4). Relaxation of strands of splenic DNP from males sacrificed 14 days after the first injection of hormone took place less intensively than that of strands from splenic DNP of healthy animals. During the first 3 min of testing their length shortened by 23%, whereas strands of splenic DNP from healthy mice shortened by 28% in the same time (Fig. 1, curves 1 and 2).

Strands of splenic DNP from males sacrificed 30 days after the first injection of hormone relaxed more intensively than strands of DNP from control mice. Toward the end of observation their length was reduced by 37%, whereas strands of splenic DNP from healthy animals shortened by 33% during this period (Fig. 1, curves 1 and 6). Ability of strands of splenic DNP of males receiving EMB for 60 days to undergo high-elastic deformation was reduced. Toward the end of the observation these strands were shortened by 30%. Their relaxation took place particularly slowly during the first 3 min. In this period these strands shortened by 21%. Strands of DNP from control animals shortened by 28% during the same period (Fig. 1, curves 1 and 7).

The results of investigations of the rheologic properties of DNP isolated from the spleen of female mice sacrificed at various times after the first injection of estrogens are given in Fig. 2. Strands of splenic DNP from mice starting to receive EMB the day before relaxed less intensively during the first 4 min than strands of splenic DNP from control animals. Their length was reduced by 24% 2 min, and by 30% 3 min after the beginning of the contraction. Strands of splenic DNP from control mice shortened by 26 and 32% respectively in the same period (Fig. 2, curves 1 and 2).

The high-elastic deformation of oriented strands of splenic DNP from females sacrificed 7, 14, and 30 days after the beginning of EMB administration increased (Fig. 2, curves 1, 3, 4, and 5). Strands of splenic DNP from mice sacrificed 7 days after the first injection of hormone shortened by 40%, and from animals sacrificed 14 and 30 days after the first injection, by 42%. Strands of splenic DNP of control animals shorted by only 38% during this time.

The study of the rheologic properties of DNP isolated from the spleen of female mice 60 days after the first injection of hormone showed a decrease in high-elastic deformation of oriented DNP strands (Fig. 2, curves 1 and 6). Although toward the 10th min of observation the length of the strands of splenic DNP was reduced almost equally in the experimental and control animals (by 37 and 38% respectively), at first (for 2-8 min) relaxation of the strands of splenic DNP from mice receiving the hormone took place less intensively than that of strands from splenic DNP of healthy animals.

For 14 days after the first injection of EMB, the behavior of oriented strands formed from DNP of the hematopoietic tissues of males and females differed, as the results described above show. In the later stages, 30 and 60 days after the first injection of EMB, the changes in rheologic properties of the oriented DNP strands from the hematopoietic tissue of males and females were identical in character. The high-elastic deformation of oriented strands of splenic DNP from mice of both sexes receiving EMB for 30 days increased, but was decreased 60 days after the first injection of the hormone. The results described above indicate changes in the macromolecular structure of DNP isolated from the hematopoietic tissue of mice at various times after the first injection of EMB. As shown experimentally by D. M. Spitkovskii and coworkers [5], the high-elastic deformation of oriented DNP strands reflects the flexibility of DNA molecules composing them. Flexibility of DNP macromolecules in turn is attributable to the fact that hydrogen bonds in the DNP molecule participating in formation of the secondary structure of DNA are broken and transferred to protein, thus causing the development of a quasisingle-helical state of the DNA in the DNP. The increase in high-elastic deformation of oriented DNP strands, like its decrease, must therefore reflect to some extent a change in the state of the bonds between DNA and protein.

It may be concluded from the above remarks that initial changes in the genetic apparatus of the hematopoietic cells caused by leukemogenic doses of estrogens are associated with disturbance of the tertiary structure of the complex protein (DNP) forming it. Probably changes take place in the number of hydrogen bonds between DNA and proteins in the DNP molecule. The possibility of such changes in nucleoprotein as a result of estrogen treatment has been demonstrated not only by this investigation, but also by other work showing that these hormones interact with the histones of DNP and modify its template activity [11-14].

Changes in the tertiary structure of DNP of hematopoietic cells of mice produced by EMB may lead to repression of functioning cistrons of DNA and to derepression of previously inactive cistrons, and this must be reflected in decrease or cessation of synthesis of certain cell proteins and the appearance of new proteins characteristic of tumor cells.

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