BIOCHEMICAL AND HISTOCHEMICAL STUDY OF LACTATE DEHYDROGENASE ACTIVITY IN OVARIES OF MICE WITH HIGH AND LOW INCIDENCE OF SPONTANEOUS CANCER

V. I. Boikova and I. S. Zaretskaya

UDC 618.11-056.4:616-006.6-092.9]-008.931:577.158.347

Biochemical studies have shown that lactate dehydrogenase activity in ovaries of C3H mice, with a high incidence of cancer, is higher than in C57BL mice, with a low incidence. Histochemical studies have shown that the activity of this enzyme in C3H mice is higher in the corpora lutea, and in C57BL mice in the interstitial tissue.

* * *

The work of Kanekar and Bhide [7], Nagasawa Hiroshi and co-workers [8], and our own investigations [3] have demonstrated differences in the morphological structure and metabolic state in the mammary gland of mice of lines with high and low incidence of spontaneous cancer.

To understand the factors facilitating and preventing the development of mammary gland tumors, it is important to study the function of several endocrine organs, notably the ovary, participating in regulation of the state of the mammary gland.

In the present investigation the activity of lactate dehydrogenase (LDH), an enzyme connected with glycolysis and the most active of the oxidation-reduction enzymes in the ovary [2], was studied. Results obtained by biochemical and histochemical methods were compared, thus giving a more complete picture of the state of glycolysis in the investigated organ.

EXPERIMENTAL METHOD

Investigations were carried out on sexually mature virgin female mice of line C3H, with a high incidence of cancer, responding to injection of diethylstilbestrol by a more rapid rate of development of tumors, and on mice of line C57BL, in which carcinoma of the mammary gland practically never developed despite injection of diethylstilbestrol in the dose given below.

Starting from the age of 3 months the experimental animals received diethylstilbestrol once every week by subcutaneous injection of a suspension containing 200 μg in 0.1 ml physiological saline per animal. The mice were sacrificed one month later by decapitation.

The biochemical investigation of LDH activity was carried out by the method of Birkbeck and Stewart [4]. The ovaries were homogenized in a type RT-1 tissue micromincer for 30 sec, using a glass homogenizer in phosphate buffer, pH 7.4 (ratio between weight of ovary and volume of buffer solution 1:200). The homogenate was centrifuged for 10 min on a TsUM-1 centrifuge at 5000 rpm.

LDH in the supernatant was determined as follows: to 2.6 ml phosphate buffer, pH 7.4, 0.3 ml NAD·H_2 (0.8 μmole) and 0.05 ml of the supernatant fractions were added. After incubation for 5 min and immediately before spectrophotometry, 0.1 ml pyruvic acid (0.05 mole) was added to the cuvettes. The optical density was measured in the SF-4A spectrophotometer every 2 min for 10 min.

Activity of the enzyme was expressed in μmoles NAD·H_2 oxidized by 1 ml of the supernatant fraction of ovary in the presence of pyruvic acid per minute.

Histochemical investigation of mice of the high- and low-cancer lines was carried out on the left ovary (the right ovary being used for biochemical examination) and all the ovaries were mounted in the same piece

Pathophysiological Laboratory, P. A. Gertsen Moscow Oncologic Research Institute (Presented by Academician V. V. Parin). Translated from Byullenten' Éksperimental'noi Biologii i Meditsiny, Vol. 67, No. 5, pp. 58-60, May, 1969. Original article submitted October 5, 1968.

TABLE 1. LDH Activity in Ovaries of Mice of High- and Low-Cancer Lines at the Age of 4 Months

Line of mice	Control			Expr.		
	No. of animals	$M \pm m$	P	No. of animals	$M \pm m$	P
C3H	13	33,2±1,7	<0,01	10	35,6±1,1	0,01
C57BL	19	26,1±1,3		10	29,8±1,7	

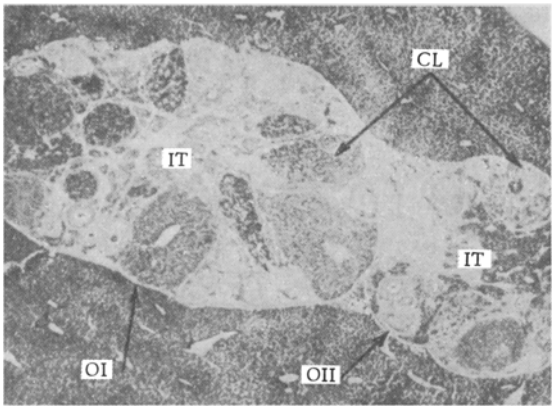

Fig. 1. Photomicrograph. Reaction for LDH. OI) ovary of mouse of high-cancer line C3H, OII) ovary of mouse of low-cancer line C57BL; CL) corpus luteum; IT) interstitial tissue, 56 ×.

of liver. In this way adequate conditions were maintained and comparison of the histochemical picture of the ovaries from mice of the two lines was facilitated. Freezing was carried out at the temperature of dry ice. Sections were cut to a thickness of 12 μ in a freezing microtome at −16°. LDH was determined histochemically by the method of Hess and co-workers using nitro-BT [5]. LDH activity was estimated from the number of diformazan granules located in the cell.

EXPERIMENTAL RESULTS

Control Group. Biochemical examination of the ovaries of intact animals revealed interlinear differences in the LDH content in these organs of the mice. As Table 1 shows, the LDH level was higher in the ovaries of C3H mice, with a high incidence of cancer.

The histochemical study revealed LDH activity in all structural elements of the ovary, highest activity being observed in the lutein cells of the corpora lutea, the interstitial tissue, and the theca interna of the follicles. Activity was lowest in the membrana granulosa of the follicles; developing follicles were also found in which the granulosa cells were completely free from diformazan granules. The size and character of deposition of the granules varied in cells of the different ovarian structures. In the granulosa cells of follicles at different stages of ripeness, for example, the diformazan granules were very small and uniform in size, whereas in the cells of the theca interna, intersitital tissue, and corpora lutea they were larger and frequently polymorphic. This state of the granules was found in the ovaries of mice of both lines.

In atretic follicles, depending on the degree of atresia, an increase in the number of cells with large polymorphic diformazan granules was observed from the center toward the periphery.

Comparison of ovaries of mice of the high- and low-cancer lines showed that LDH activity in the corpora lutea was higher in the C3H mice. Meanwhile, activity of this enzyme in the mice of low-cancer line C57BL was highest in the interstitial tissue (Fig. 1). Cells with polymorphic diformazan granules were more frequently found in the interstitial tissue of the ovaries of C57BL mice than of C3H mice.

Experimental Group. Biochemical investigation of the ovaries of mice of the experimental group (receiving diethylstilbestrol) revealed some increase in the LDH content in mice of both lines, although differences between the experimental and control data for C3H mice were not statistically significant. Interlinear differences still remained. As in the control, LDH activity was higher in the C3H mice (Table 1).

Histochemical investigation showed that LDH activity was greatest after injection of diethylstilbestrol into mice of the high-cancer line in the corpora lutea, whereas in the ovaries of mice of the low-cancer line C57BL activity was mainly increased in the interstitial tissue cells. The increase in activity was accompanied not only by an increase in the number of diformazan granules, but also by an increase in the number of cells with nonhomogeneous polymorphic granules. The same pattern was observed in the control, mainly in the interstitial tissue of the ovaries of C57BL mice.

The results of this investigation, in conjuction with previously published results of determination of the activity of oxidation-reduction enzymes of the Krebs cycle [1], indicate that the level of metabolism in the ovaries of mice of high-cancer line C3H (whether intact or receiving diethylstilbestrol) is higher than in mice of low-cancer line C57BL.

The histochemical investigations, demonstrating LDH activity separately in the different ovarian structures, showed that this activity is highest in the corpora lutea of C3H mice. According to reports in the literature, activity of oxidation-reduction enzymes is linked with hormone synthesis [6]. For this reason, the high level of synthesis associated with production of steroid hormones, especially progesterone, in the ovaries of mice of the high-cancer line C3H can be regarded as one of the factors responsible for their increased susceptibility to the formation of spontaneous mammary gland tumors.

LITERATURE CITED

1. V. I. Boikova, and M. I. Bronshtein, in: Proceedings of the 9th Junior Scientific Conference of the P. A. Gertsen Moscow Oncologic Research Institute [in Russian], Moscow (1966), p. 71.
2. N. T. Raikhlin, Oxidation-Reduction Enzymes in Tumors [in Russian], Moscow (1967).
3. I. P. Tereshchenko, V. I. Boikova, et al., in: Proceedings of the 25th Scientific Session of the P. A. Gertsen Moscow Oncologic Research Institute [in Russian], Moscow (1967), p. 166.
4. J. A. Birkbeck and A. G. Steward, Canad. J. Biochem., 39, 257 (1961).
5. R. Hess, D. G. Scarpelli, and A. G. E. Pearse, J. Biophys. Biochem. Cytol., 4, 753 (1958).
6. M. Ikonen, M. Niemi, et al., Acta Endocrinol. (Kbenhavn), 38, 293 (1961).
7. S. A. Kanekar and S. V. Bhide, Proceedings of the 8th International Cancer Congress [in Russian], Vol. 3, Moscow–Leningrad (1963), p. 416.
8. H. Nagasawa et al., Gann, 58, 45 (1967).