

EFFECT OF PREDISPOSITION TO MAMMARY  
GLAND TUMORS ON LACTATE DEHYDROGENASE  
ISOENZYME SPECTRUM IN THE MOUSE OVARYI. S. Zaretskaya, V. I. Boikova,  
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More intensive synthesis of the M-subunits of lactate dehydrogenase was demonstrated biochemically in the ovaries of mice with a high predisposition to cancer than in animals with a low predisposition, although in both strains of mice the common direction of glycolysis in the ovary was aerobic. Differences in the activity of M- and H-subunits of the enzyme were detected histochemically in the various structures of the ovary in intact animals and in mice receiving hexestrol.

KEY WORDS: lactate dehydrogenase of the mouse ovary; mammary gland tumors; glycolysis; hexestrol.

It has now been established that the isoenzyme spectrum of lactate dehydrogenase (LD) in the tissues of malignant tumors and in the blood serum of tumor carriers is characterized by high activity of the cathodic fractions [2, 4, 6]. The isoenzyme spectrum of LD in the various organs and systems of animals with tumors has received much less study. Nevertheless, the accumulation of experimental evidence in this field is essential for the elucidation of the pathogenesis of neoplastic growth.

Considering the important role of ovarian hormones in the production of mammary gland tumors and the dependence of steroid formation on the state of metabolism, the investigation described below was carried out to study the characteristics of glycolysis in the ovaries (as reflected in the LD isoenzyme spectrum) of mice with different degrees of predisposition to the formation of mammary gland tumors (strains C3H and C57BL), receiving or not receiving hexestrol.

## EXPERIMENTAL

Mice of strain C3H with a high predisposition to the formation of spontaneous mammary gland tumors and mice of strain C57BL, resistant to such tumors even despite long administration of hexestrol, were used. Experiments were carried out on 58 virgin females, 32 of which (16 of each strain) were intact, while 12 C3H and 14 C57BL mice were treated with hexestrol. Starting from the age of 3 months the experimental animals received hexestrol by weekly subcutaneous injection of a dose of 200  $\mu$ g in 0.1 ml physiological saline per animal. After 1 month the mice were decapitated and the ovaries isolated.

For the biochemical determination of the LD isoenzyme spectrum the method of electrophoretic fractionation in agar gel was used [3, 7]. The isoenzymes were demonstrated histochemically by the method of Ikava [5] and Nartsissov [1]. As a first step the optimal concentrations of urea (2.0 M for detection of the anodic fractions) and sodium lactate (1.0 M for the cathodic fractions) for the ovaries of the strains of mice used were determined.

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# RESULTS AND DISCUSSION

Five LD fractions were found in the ovaries of the intact mice of the strains studied. The electrophoretic analysis showed that the fast-migrating isoenzyme LD<sub>1</sub> had the highest activity; combined estimation of the H- and M-subunits revealed predominance of the anodic fractions in the ovaries of both strains of mice. Comparison of the isoenzyme spectra revealed differences between the strains as regards the activity of individual fractions: in the C3H mice the activity of LD<sub>1</sub> and LD<sub>2</sub> was lower than in the C57BL mice; activity of LD<sub>4</sub> and LD<sub>5</sub>, on the other hand, was higher in the ovaries of the C3H mice. The differences in the LD isoenzyme spectrum in the ovaries of the C3H and C57BL mice were confirmed also by the ratio between the totals of the M- and H-subunits (Table 1).

In the histochemical tests the activities of the LD isoenzymes of the M and H type were estimated from the deposition of diformazan granules in the various structures of the ovary.

In the intact mice of both strains, activity of the anodic LD fractions appeared in all structural components of the ovary, but to different degrees. In the cells of the stratum granulosum and theca interna of the ripe follicles the intensity of diformazan deposition was higher. Somewhat weaker activity was found in the granulosa cells and theca interna of the growing follicles. Activity of the H type of LD in the corpora lutea was distributed irregularly: in the pale cells (i.e., cells in the third stage of the secretory cycle – the phase of liberation of the secretion) the deposition of diformazan was more marked than in the dark cells (cells in preparation for and synthesizing hormones). Differences between the strains of mice as regards LD activity of the H type were observed in the pale lutein cells of the corpora lutea and the granulosa cells of the ripe follicles. The activity of the anodic fractions was higher in these structural components in the C3H mice.

The study of the cathodic LD fractions in preparations of the ovary revealed a positive reaction in all the structures of this organ. By the degree of the decrease in activity of LD isoenzymes of the M type the ovarian structures of C3H mice could be arranged in the following order: dark lutein cells of the corpora lutea, cells of the stratum granulosum and theca interna of the growing follicles, interstitial tissue, stratum granulosum and theca interna of ripe follicles.

So far as the C57BL mice are concerned, higher activity of the cathodic fractions in their ovaries was observed in the cells of the interstitial tissue.

Hexestrol affected the relative quantities of the LD fractions in the ovaries of the strains studied. Reliable evidence of an increase in the anodic (LD<sub>1</sub> and LD<sub>2</sub>) and a decrease in the cathodic (LD<sub>4</sub> and LD<sub>5</sub>) fractions in the C3H mice of the experimental group compared with intact animals was obtained. In the ovaries of the C57BL mice a tendency toward an increase in the LD<sub>1</sub> fraction and a decrease in LD<sub>5</sub> was observed, but the differences were not significant.

Histochemical investigation of the LD isoenzymes in the mice of the experimental group showed an increase in the LD activity of H type in the C3H animals in all structural elements of the ovaries, but especially in the dark cells of the corpora lutea and theca interna of the follicles. Hexestrol increased the activity of the isoenzymes of this type in the ovaries of the C57BL mice mainly in the cells of the interstitial tissue, but in the stratum granulosum of the follicles, on the other hand, less diformazan was deposited.

A decrease in LD activity of the M type in the ovaries of the experimental C3H mice compared with the control was observed chiefly in the interstitial tissue and pale cells of the corpora lutea. In the C57BL mice hexestrol produced no significant changes in the activity of LD isoenzymes of the M type.

TABLE 1. LD Isoenzymes in Ovaries of Mice with High and Low Predisposition to Cancer, Aged 4 Months (M ± m)

Strain of mice	No. of animals	Total LD activity (μmoles/ml/min)	LD isoenzymes (in %)					V:I	Total H	Total M	M:H
			I	II	III	IV	V				
C3H: Intact	16	33,2 ± 1,7	26,1 ± 0,9	17,4 ± 0,9	21,6 ± 0,5	15,3 ± 0,8	18,1 ± 1,0	0,7	53,8	44,7	0,83
Experimental	12	35,6 ± 1,1	39,7 ± 0,9	20,1 ± 0,8	21,9 ± 0,8	11,0 ± 0,8	13,5 ± 0,9	0,44	59,6	37,7	0,63
C57BL: Intact	16	26,1 ± 1,3	29,4 ± 0,8	21,5 ± 0,6	22,2 ± 0,6	10,0 ± 0,5	14,0 ± 0,5	0,5	59,1	38,9	0,65
Experimental	14	35,6 ± 1,1	31,1 ± 1,2	20,9 ± 0,6	20,3 ± 0,4	10,9 ± 0,7	13,3 ± 0,9	0,42	59,9	36,9	0,61

The differences in the changes of the LD isoenzyme spectrum in the ovaries of mice with high and low predisposition to cancer in response to administration of hexestrol can evidently be attributed to the low lability of the regulatory systems of the body in C3H mice, in which, by contrast with the resistant C57BL mice, the action of the hormone led to increased synthesis of subunits of the H type.

A study of the LD isoenzyme spectrum in the ovaries of intact C3H and C57BL mice thus revealed synthesis predominantly of H-subunits and the considerable magnitude of the anodic fractions. Differences between the strains were expressed as increased production of M-subunits in mice with high predisposition to cancer compared with the resistant strain. Administration of hexestrol led to different changes in the LD isoenzyme spectrum depending on the predisposition of the animals to form mammary gland tumors. The results suggest that in mice with a high predisposition to form spontaneous mammary gland tumors, slight but definite changes in metabolic processes develop as early as in the pretumor period, and they affect distant organs connected functionally with the organ in which the tumor is formed.

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