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QUANTITATIVE HISTOCHEMICAL STUDY OF ALKALINE PHOSPHATASE ISOZYMES IN THE UTERUS OF OVARIECTOMIZED GOLDEN HAMSTERS DURING ESTROGENIZATION

N. V. Grushchetskaya and A. S. Loktionov

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Activity of two isozyme forms of alkaline phosphatase in the uterus of ovariectomized golden hamsters was investigated. The animals of different groups received single daily injections of 10 μg estrogen (benzestrol) for 4 and 16 days. Administration of the estrogen did not affect total alkaline phosphatase activity in the uterine epithelium but reduced the activity of the enzyme in the stroma. It was also shown that with an increase in the duration of estrogenization the relative proportion of the alkaline phosphatase isozyme of intestinal type in the epithelial cells of the uterine cavity increased.

KEY WORDS: estrogenization; uterus; histochemistry.

Investigation of the isozyme spectra of various enzymes is nowadays widely used in oncologic practice [14]. An isozyme of alkaline phosphatase not normally encountered (the placental form of Regan's isozyme) is found in the blood serum of patients with certain forms of cancer and also in the tumor tissue [8]. The distinguishing features of this isozyme are its thermostability, its inhibitability by L-phenylalanine, its resistance to imidazole and levamisole, and its antigenic properties. In normal tissues only the alkaline phosphatase of intestinal epithelium (the intestinal type) resembles the placental type in its properties.

Since prolonged estrogenization is supposed to lead to the development of pretumor and tumor processes in the reproductive organs of women and female experimental animals, an attempt was made to study changes in the activity of two alkaline phosphatase isozymes in the uterus of ovariectomized golden hamsters during estrogenization of varied duration.

EXPERIMENTAL METHOD

Experiments were carried out on 48 ovariectomized golden hamsters, divided into four groups. The animals of group 1 received a single subcutaneous injection of the synthetic estrogen octestrol (benzestrol) in a dose of 10 μg (in 0.2 ml peach oil). The animals of group 2 received injections of 10 μg benzestrol daily for 4 days. The animals of group 3 received 10 μg benzestrol daily for 16 days. Animals of group 4 (control) received no hormones.

The hamsters were killed by decapitation 18-20 h after the last injection of the hormone. The uterine cornua, frozen in liquid nitrogen, were taken for examination. Alkaline phosphatase in frozen sections 10 μ thick was revealed by Burstone's method with naphthol AS-MX and fast blue (Reanal, Hungary). To determine the alkaline phosphatase isozymes, L-levamisole [6], an inhibitor of the isozyme of hepatic type, was added to the incubation medium in concentrations of 0.01, 0.05, and 0.5 mmole/ml medium [4].*

The intensity of the reaction was estimated quantitatively from a cytospectrophotometer with digital print-out (objective 40×, ocular 7×, wavelength 550 nm, probe area 4.9 μ^2. Enzyme activity was expressed in optical density units (o.d.u.). Enzyme activity in the presence of inhibitor was expressed as a percentage of

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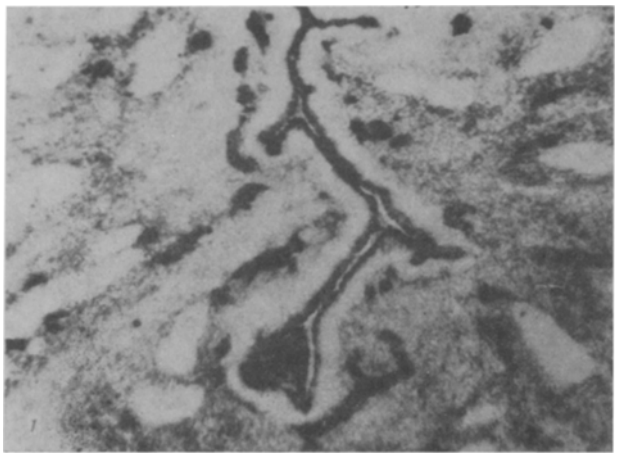

Fig. 1. Distribution of alkaline phosphatase activity in uterine epithelial and stromal cells of ovariectomized golden hamsters. Series 4, control. Burstone's method, 100×.

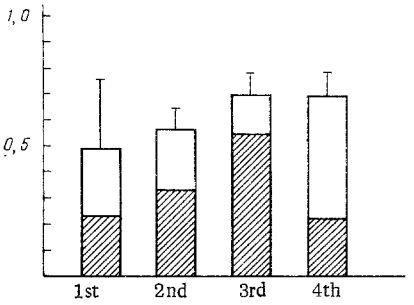

Fig. 2

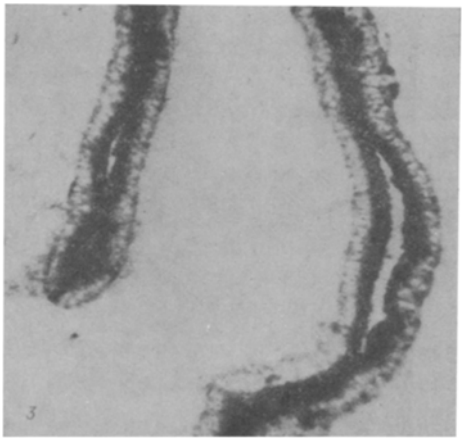

Fig. 3

Fig. 2. Activity of alkaline phosphatase isozymes in uterine epithelial cells of ovariectomized golden hamsters depending on duration of benzestrol administration. Abscissa, group of animals; ordinate, optical density (in o.d.u.). Shaded part represents intestinal form, unshaded form hepatic form of alkaline phosphatase.

Fig. 3. Alkaline phosphatase activity in uterine epithelial cells of ovariectomized golden hamsters of group 3 in the presence of L-levamisole. Burstone's method, 400×.

alkaline phosphatase activity in the same preparation without inhibitor. The data were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS

Alkaline phosphatase activity in the uterus is known to depend on the estrogen level in the body. This has been demonstrated both for man [9] and for experimental animals [10, 12, 13, 15].

The localization of alkaline phosphatase activity thus observed agreed in general with results obtained previously [10, 12, 13, 15]. Enzyme activity was most marked in the apical portions of epithelial cells lining the uterine cavity [13]. In the stroma the enzyme was distributed diffusely, but in gland cells it was virtually absent (Fig. 1). Quantitative assessment of alkaline phosphatase activity in the uterine epithelium revealed no significant differences between the groups of animals. This does not agree with data obtained previously

in rats [12, 13] and mice [15], according to which estrogens cause an increase in alkaline phosphatase activity in the uterine epithelium. However, enzyme activity in these investigations was not estimated quantitatively. Meanwhile, Hayashi and Fishman [10] found no significant fluctuations in alkaline phosphtase activity in the rat uterus under the influence of estrogens as a result of a quantitative investigation of enzyme activity. It is also possible that the results of the present experiments reflect species differences in hormonal regulation of alkaline phosphatase activity in the uterus of golden hamsters.

Analysis of changes in alkaline phosphatase activity in the uterine stroma showed that administration of benzestrol considerably reduced the activity of the enzyme. The mean value of alkaline phosphatase activity in the stroma were as follows: 0.37 ± 0.07 o.d.u. in the control group; 0.16 ± 0.03 o.d.u. in group 1, 0.22 ± 0.04 o.d.u. in group 2, and 0.18 ± 0.05 o.d.u. in group 3. Besides determination of total alkaline phosphatase activity, changes in the relative proportion of its two isozymes depending on the duration of estrogenic stimulation were analyzed.

Activity of the enzyme in the uterine epithelium was preserved after all concentrations of L-levamisole, but in the maximal concentration of inhibitor the percentage of levamisole-resistant isozyme of intestinal types differed for animals of different groups, depending on the duration of estrogenization (Fig. 2). The activity of this isozyme in the uterine epithelium of the control animals was on average 31.8% of the initial value. A single injection of benzestrol increased this index to 43.3%. The intestinal type of isozyme accounted for 58.7% of the total enzyme activity in the group of animals receiving benzestrol daily for 4 days. After administration of the hormones for 16 days the isozyme of intestinal type predominated (78.9%; Fig. 3).

Alkaline phosphatase activity in the cells can change depending on the phase of the cell cycle in which the cells are at that time [11]. The highest activity of this enzyme is observed in the S-period, especially during mitosis. Since estrogens increased the proliferative pool of uterine epithelial cell [2, 3] and, consequently they increased the proportion of cells in the S-period and in mitosis, an increase in alkaline phosphatase activity might be expected. However, as was stated above, under the influence of benzestrol total alkaline phosphatase activity was unchanged, but activity of the intestinal type of isozyme increased.

In the stroma, after administration of L-levamisole enzyme activity was virtually completely suppressed even by the minimal concentration of the inhibitor in all animals. This is evidence that the stromal cells contain only the hepatic type of isozyme.

Estrogenic stimulation thus leads to a reduction in the activity of the hepatic type of alkaline phosphatase isozyme both in the epithelial cells and in the stromal cells of the uterus.

A distinguishing feature of the uterine epithelial cells was an increase in the proportion of the intestinal type of isozyme despite the fact that total alkaline phosphatase activity remained unchanged.

Since estrogens act on target cells at the genetic level [5, 7] it can tentatively be suggested that the decrease in alkaline phosphatase activity in the stroma and the change in the relative proportions of its isozymes in the epithelium of the uterus observed under the influence of benzestrol are connected with changes in the activity of the genes responsible for synthesis of the above-mentioned isozyme forms. Meanwhile the possibility cannot be ruled out that the changes observed may also be to some extent connected with posttranslation regulation [5] of alkaline phosphatase isozyme activity.

Histochemical techniques do not give the exact answer to the question of the mechanism of the observed effect of benzestrol. More concrete data can be obtained by the use of biochemical methods.

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HETEROGRAFTING CULTURES OF HUMAN PANCREATIC ISLET CELLS IN RATS WITH EXPERIMENTAL DIABETES MELLITUS

V. I. Shumakov,* B. I. Shal'nev,
V. N. Blyumkin, N. N. Skaletskii,
R. A. Babikova, and M. A. Danilov

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Cultures of human fetal pancreatic islet cells were transplanted into the liver of rats with diabetes induced by alloxan. This heterografting led to a prolonged fall of the blood sugar. Histological examination of the recipients' liver revealed groups of implanted islet cells.

KEY WORDS: cell cultures; pancreatic islet cells; experimental diabetes; blood sugar.

It has now been shown that allografting of the pancreas into patients with severe diabetes mellitus as a rule gives unsatisfactory results, not only because of a rejection reaction, but also because of the high proteolytic activity of the exocrine part of the human cadaveric pancreas, leading to enzymic destruction of the transplanted organ [4, 5]. Accordingly in recent years considerable attention has been paid to the development of methods of isolation and culture of endocrine (islet) tissue of the human pancreas [10, 12]. Furthermore, the first reports of allografting of islet tissue into patients with severe diabetes have appeared. However, the results indicate an incomplete and brief effect of such an operation, which may be due either to rejection of the implanted B cells or to their insufficient number [4, 5, 13, 14].

The question of the use of human embryonic and fetal pancreas as the source of islet tissue for culture and subsequent transplantation has attracted special attention. In the fetus the endocrine portion accounts for up to 30% of the weight of the pancreas [7] and the exocrine portion has virtually no proteolytic activity. Moreover, B cells of the pancreatic islets of Langerhans are immunologically immature until a certain moment, a fact which must facilitate their survival in the recipient's body.

A new technique of obtaining cultures of human embryonic and fetal pancreatic islet cells has been developed in the writers' institute, with the use of the Soviet enzyme preparation collalytin [1, 3], and their insulin-forming activity has been studied during culture under various conditions [2-3]. Experiments have been carried out to study the effect of heterografting of cultures of human fetal pancreatic islet cells on the course of experimental diabetes in rats. These experiments also were regarded as an essential stage in the preparations for the clinical allografting of human pancreatic islet cell cultures.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar rats weighing 180-240 g. Experimental diabetes was induced by subcutaneous injection of alloxan (from Sigma, USA) in a dose of 200 mg/kg body weight. Rats with a blood glucose level of over 350 mg% for not less than 2 weeks after induction of diabetes were used as recipients. The blood sugar was determined by the orthotoluidine method using the "Bio-Lab-Test" kit (from Lachema, Czechoslovakia). Cultures of pancreatic islet cells from 16-20-week human fetuses were

*Corresponding Member, Academy of Medical Sciences of the USSR.

Laboratory of Pathomorphology, Research Institute of Transplantation and Artificial Organs, Ministry of Health of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 1, pp. 48-50, January, 1980. Original article submitted May 4, 1979.

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