

# MICROPHOTOMETRIC INVESTIGATION OF OXIDATIVE ENZYME ACTIVITY IN ENDOMETRIAL EPITHELIUM OF RATS AFTER OVARECTOMY AND INJECTION OF TESTOSTERONE PROPIONATE

I. S. Kruglova and A. U. Cherkesova

UDC 618.11-089.87-092.9-07: 618.14-018.  
73-008.931-02: 615.357.631

Activity of oxidative enzymes in the epithelium lining the uterine cavity and in the glandular epithelium of ovariectomized rats in the period of climacteric disorders was investigated by a comparative microphotometric method. An increase in lactate dehydrogenase (LD) and glucose-6-phosphate dehydrogenase (G6PD) activity and a decrease in succinate dehydrogenase (SD) and NAD- and NADP-diaphorase activity were found in the epithelial cells lining the uterine cavity. LD activity was increased in the glandular epithelium but SD, and NAD- and NADP-diaphorase activity was sharply reduced. Injection of testosterone propionate caused a marked increase in the activity of the oxidative enzymes studied in the endometrial epithelium of the rats compared with their activity in control rats and in rats not receiving the hormone.

KEY WORDS: endometrium; ovariectomy; oxidative enzymes; testosterone propionate.

Hormones play an important role in the maintenance of homeostasis in animals and in man and in the development and irradiation of certain pathological processes. Male sex hormones are widely used for the treatment of certain endocrine disturbances in women. However, data on the effect of androgens on the structure and metabolism of cells of the target organs so far published are very few in number and contradictory in nature. According to some workers androgens, as antagonists of the female sex hormone, induce atrophic changes in the female reproductive organs and depress RNA and DNA synthesis considerably [4, 5]. Others regard androgens as bisexual hormones, promoting the growth and development of the female sex hormones and increasing RNA synthesis [7, 11].

Because of this state of affairs the object of the present investigation was to study changes in the activity of oxidative metabolic enzymes connected with glycolysis (lactate dehydrogenase - LD), the pentose pathway (glucose-6-phosphate dehydrogenase - G6PD), the Krebs' cycle (succinate dehydrogenase - SD), and the electron transport chain (NAD- and NADP-diaphorase) in the endometrial epithelium of rats after bilateral ovariectomy and prolonged administration of large doses of androgens to the animals.

## EXPERIMENTAL METHOD

The uterus of noninbred female rats aged 15-17 months (the period of climacteric disorders) served as the test object. Bilateral ovariectomy was performed by Zondek's method [9]. Daily for a month the rats were given subcutaneous injections of testosterone propionate in a dose of 3 mg/100 g body weight starting 2 weeks after castration, and at the end of the course they were killed by decapitation. The uterus of intact rats of the same age served as the control. For morphological investigation of the endometrium paraffin sections were stained with hematoxylin-eosin. Activity of the oxidative enzymes was determined in frozen sections 10  $\mu$  thick obtained from blocks from pieces of tissue of the control and experimental animals mounted in the same object-holder of the microtome, according to the instructions given in Pearse's textbook. Activity of the oxidative enzymes was estimated quantitatively by means of the TSIM-2 digital integrating microphotometer [6] by the method of comparative selective cytophotometry [1] in a monochromatic beam of light with a wavelength of 570 nm. The results were subjected to statistical analysis and differences were considered to be significant at a 0.95 level of probability.

---

Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. Department of Obstetrics and Gynecology, Daghestan Medical Institute, Makhachkala. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 85, No. 2, pp. 209-212, February, 1978. Original article submitted June 30, 1977.

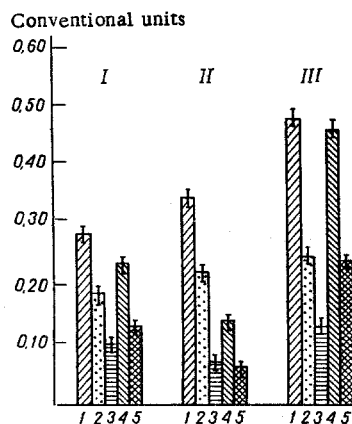


Fig. 1

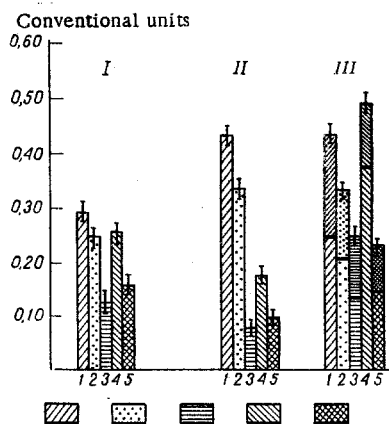


Fig. 2

Fig. 1. Changes in activity of oxidative enzyme in epithelium lining uterine cavity of rats during period of climacteric disorders. 1) LD, 2) G6PD, 3) SD, 4) NAD-diaphorase; 5) NADP-diaphorase. Abscissa: I) control, II) ovariectomy, III) ovariectomy plus testosterone propionate; ordinate, optical density (in conventional units).

Fig. 2. Changes in activity of oxidative enzymes in glandular epithelium of rat uterus during period of climacteric disorders. Legend as in Fig. 1. Closer shading denotes activity of enzymes in apical part of cell.

## EXPERIMENTAL RESULTS

One month after castration the weight of the uterus was reduced, its lumen constricted, and the stroma of the endometrium condensed. The height of the epithelium lining the uterine cavity was reduced. Comparative microphotometric investigation of the activity of the oxidative enzymes showed that it did not differ significantly in the epithelium lining the uterine cavity and in the glandular epithelium of the control animals.

Abolition of the hormonal effect of the ovaries (bilateral ovariectomy) caused changes in the activity of the oxidative enzymes in the lining epithelium and in the glandular epithelium to a different degree. For instance, in the cells of the lining epithelium LD activity was increased by 1.4 times and G6PD activity by 1.2 times compared with the control, whereas SD and NAD- and NADP-diaphorase activity was reduced (Fig. 1). An increase in LD activity and a marked decrease in SD and NAD- and NADP-diaphorase activity were observed (Fig. 2).

Injection of testosterone propionate into the castrated animals led to a sharp increase in SD (by 3 times), NAD-diaphorase (by 2.7 times), and NADP-diaphorase (by twice) activity in the cells of the epithelium lining the uterine cavity; in the apical part of the cells the activity of the oxidative enzymes was much higher than in the basal part. LD and G6PD activity were unchanged compared with their value in animals not receiving the hormone.

Under the same conditions LD activity in the cells of the glandular epithelium was increased by 1.3 times, SD activity by 1.8 times, and NAD- and NADP-diaphorase activity by 3 and 4 times respectively. An increase in the height of the epithelium lining the uterine cavity and an increase in the size and number of the endometrial cells were observed morphologically.

The quantitative cytochemical data are evidence that the removal of hormonal stimulation of the ovaries as a result of bilateral ovariectomy causes a sharp decrease in the activity of oxidative enzymes of the Krebs' cycle in the cells of the glandular and lining epithelium. This fact is evidently connected with atrophy of the reproductive organs after castration [3]. A decrease in mitotic activity in the endometrial epithelium of castrated animals [2, 3], a decrease in the glycogen content of the epithelial cells [13], and a decrease in acid and alkaline phosphatase activity [12] have been described in the literature.

When the level of estrogenic stimulation was low (castration) the intensity of metabolic processes in the epithelium of the uterine glands was lower than in the epithelium lining the uterine cavity, a fact which demonstrates differences in the sensitivity to estrogens of these endometrial structures. Following injection of testosterone propionate into the ovariectomized animals metabolic and synthetic processes were intensified

in the endometrial epithelial cells. These results are in good agreement with data in the literature on the development of proliferation and of adenocystic hyperplasia of the endometrium of castrated rats receiving testosterone propionate [10]. The development of adenocystic hyperplasia of the endometrium under the influence of large doses of androgens in castrated rabbits has also been described [4].

The results of quantitative cytospectrophotometry thus showed that ovariectomy in rats during the period of climateric disorders led to a sharp decrease in activity of the oxidative enzymes catalyzing metabolism in the Krebs' cycle and to the development of atrophic processes in the endometrium. Injection of testosterone propionate into the castrated animals caused a marked increase in the activity of the oxidative enzymes, which was evidently connected with the stimulation of synthetic and proliferative processes in the uterine epithelium.

#### LITERATURE CITED

1. G. G. Avtandilov, *Morphometry in Pathology* [in Russian], Moscow (1973).
2. O. I. Epifanova, *Hormones and Cell Multiplication* [in Russian], Moscow (1965).
3. S. S. Laguchev, *Hormonal Regulation of Proliferation of the Epithelium of the Uterus, Vagina, and Breasts* [in Russian], Moscow (1970).
4. R. Mirsaburova, "The action of testosterone on the DNA and RNA content and mitotic activity of the glandular epithelium of the endometrium in rabbits," Author's Abstract of Candidate's Dissertation, Dushanbe (1974).
5. E. M. Papitashvili, M. B. Kharabadze, G. E. Daneliya, et al., *Collected Transactions of the Research Institute of Obstetrics and Gynecology of the Georgian SSR* [in Russian], No. 9, 31 (1960).
6. B. L. Pereverzev, V. M. Andreev, S. I. Konovalov, et al., *Tsitologiya*, No. 8, 1050 (1974).
7. E. A. Popova, *Arkh. Patol.*, No. 8, 48 (1963).
8. I. A. Yudaev and B. V. Pokrovskii, *Biokhimiya*, No. 1, 72 (1970).
9. B. Zondek, *Hormones of the Ovary and Anterior Lobe of the Pituitary* [Russian translation ], Moscow (1938).
10. S. Aschheim and J. Varangot, *C. R. Soc. Biol.*, 130, 827 (1939).
11. J. Janata, O. Filip, and L. Starka, *Zbl. Gynäk.*, 87, 463 (1965).
12. J. P. Maning, B. G. Steinetz, T. Gliannina, et al., *Proc. Soc. Exp. Biol. (New York)*, 125, 508 (1967).
13. T. C. West and P. Cervoni, *Am. J. Physiol.*, 182, 287 (1955).