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AGE CHANGES IN OVARIAN STRUCTURE CORRESPONDING TO CHANGES IN FUNCTION

G. B. Koval'skii

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The constant succession of heterogeneous and heteromorphic structures taking place in the ovaries is linked with the development of the female sex cells and with cyclic changes in sex steroid secretion. Age changes in endocrine activity of the female sex glands may be a pathogenetic component of diseases arising in old age and, in addition, they may lead to disturbances of various aspects of reproductive function, the extreme manifestation of which is sterility. Cessation of the reproductive function in women in the menopause is connected with gradual inhibition of follicle production. However, the histophysiological mechanisms lying at the basis of these processes are not clear. The problem of the cellular and tissue substrates of age changes in ovarian hormone production and also the problem of the role of vessels of the microcirculatory system in involutional changes in structure of the gonads likewise remain unsolved.

The aim of this investigation was to study the histochemical features of various structures of the rat ovary in the initial stages of involution.

EXPERIMENTAL METHOD

Ovaries of albino rats were studied in stages of the estrous cycle taking place under two different conditions: I) in young sexually mature animals (32 rats aged 3-4 months) with a regular 4-day cycle, II) in animals in the early stages of involution (30 rats aged 12-14 months) with lengthening of the cycle (6-9 days) on account of the diestrus phase. In animals of this age group, according to data provided by the veterinary service of the "Rappolovo" nursery, Academy of Medical Sciences of the USSR, signs of age-associated depression of reproductive function are observed. Material was collected, kept, and studied in accordance with the necessary requirements [4]. Activity of NAD- and NADP-diaphorases, glucose-6-phosphate dehydrogenase (G6PDH), 3β- 17β-, and 20α-steroid dehydrogenases (3β-, 17β-, and 20α-SD), esterase, and acid and alkaline phosphatases were determined in frozen section 10 μ thick. The intensity of the enzyme histochemical reactions in the different ovarian structures was estimated with the MUF-5 instrument. Computer analysis of the data included, besides estimating mean values of optical density and dispersion, comparison of histograms so that the prob-

Department of Pathological Anatomy, I. P. Pavlov First Leningrad Medical Institute.
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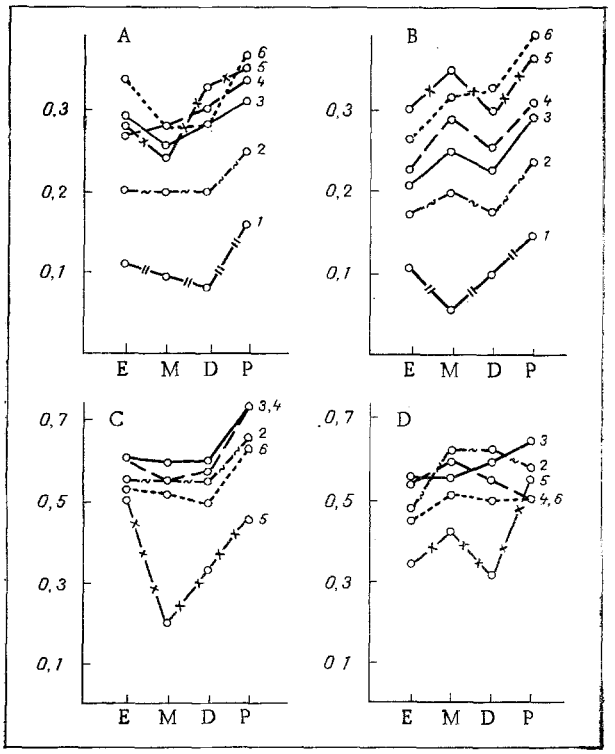

Fig. 1. Changes in enzyme activity in ovarian structures of young and aging rats. Abscissa, phases of estrous cycle: E) estrum, M) metestrus, D) diestrus, P) proestrus; ordinate, optical density of reaction products (in relative units). A, B) 3β-SD activity in ovarian structures of young and aging rats respectively; C, D) alkaline phosphatase activity in vessels of ovarian structures of young and aging rats respectively. 1, 2) Membrana granulosa and theca folliculi of large hollow follicles respectively; 3, 4) young (hollow) and old (solid) interstitial glands respectively; 5, 6) young (1st generation) and old (2nd-3rd generations) corpora lutea respectively.

able minimal number of cells which changed their state from one phase of the estrous cycle to the next could be calculated [4, 6]. To determine the degree of histophysiological integration of the membrana granulosa and theca folliculi [3, 8, 10, 11], coefficients of correlation were calculated for levels of activity of particular enzymes. The significance of differences between mean values was determined on the basis of 95% confidence intervals. Relative volumes of the ovarian structures were determined in the sections by the writer's won method [1, 5].

EXPERIMENTAL RESULTS

The data of stereometric analysis showed that the trend of cyclic transformation of ovarian structures were the same in young and aging rats. However, with age there was a redistribution of the volumes occupied originally by particular tissue components. In the gonads of aging animals the relative volume of lutein tissue was increased by about 1.5 times. This was accompanied by a reduction by almost half in the relative volume of the follicular apparatus and interstitial glands.

The results of the quantitative histochemical investigation showed that the cyclic pattern of changes in enzyme parameters and, in particular, that of 3β-SD (Fig. 1) in the steroid-synthesizing structures of the ovaries of the aging rats was similar to that in young rats, with preservation of the phase of morphological and functional maturation of the corpora lutea in metestrus and significant activation of all ovarian components in proestrus, as a reflection of gonadotrophic stimulation of the ovaries, on the one hand, and increased preovulatory synthesis of sex steroids, on the other hand [3, 6].

The level of alkaline phosphatase (AP) activity in the endotheliocytes of the microvessels (Fig. 1) gives an indirect estimate of the intensity of transport through the vessel wall, i.e., AP can be used as an indicator of the secretory capacity of ovarian structures [3, 4, 9]. AP activity in the walls of the small vessels was sharply increased in young rats in proestrus, i.e., "involvement" of the vessels coincides chronologically with involvement of the endocrine cells of the various structures in increased synthetic activity. In aging animals, on the other hand, in the period of increased steroid synthesis (proestrus) activity of transendothelial transport (reflected in AP) was observed in only two structures — young corpora lutea and interstitial glands. The follicular apparatus and old interstitial glands and corpora lutea apparently no longer took part in increased secretory activity. As already pointed out, the values and time course of the parameters of cell metabolism were maintained in the aging rats. Consequently, in the early stages of the structural changes in the ovaries with involution, morphological and functional changes in the microcirculatory system predominated. Particular attention must be paid to disturbances of transport processes in the vascular network of the follicles, i.e., in the system of the blood-follicle barrier [2].

Analysis of the histograms and dispersion values showed that the percentage of cells whose enzyme activity changed from one phase of the estrous cycle to the next decreased in aging rats in both membranes of the follicles, and this applied to all the parameters of cell metabolism investigated. In addition, considerable disturbances of cooperative relations between the membrana granulosa and theca folliculi of the large hollow follicles, at the stages of preovulatory growth and development, in the animals with age. For instance, in rats of early reproductive age clear correlation was observed between structural and metabolic changes in the two tissue elements of the follicles, as regards NAD- and NADP-diaphorase, G6PDH, and 3β- and 17β-SD. Only the coefficient of correlation calculated for changes in 20α-SD activity was low (0.23). However, the study of correlation in the theca–granulosa pair in aging rats showed that, despite preservation of high values of coefficients for NADP-diaphorase (0.94), 17β-SD (0.84), and G6PDH (0.8), correlation was considerably weakened in the case of mitochondrial NAD-diaphorase (0.29 compared with 0.65) and for the key enzyme of steroid production, 3β-SD (0.44 compared with 0.94), and in the case of 20α-SD the coefficient of correlation changed to negative (-0.65).

Granulosa cells and thecal cells, which differ in their tissue origin and functional specialization, are known to constitute a single follicular complex (histion), synchronized in time and equipped with a single structural-metabolic substrate, whose state largely determines the variations in endocrine and generative functions of the ovaries [3, 6, 7]. In animals in the early stages of evolution, there is no association between changes in endothelial, thecal, and granulosa cells, so that disturbances of the structural-metabolic mechanisms of integration of functions of the follicular complex can be postulated [2, 3, 8, 10, 11]. Desynchronization of the components of the ovarian "histion" is evidently one of the leading causes of age-associated disturbances of morphogenetic processes in the organ, and also of the sexual rhythm and reproductive function.

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