# TISSUE SOURCES OF SEX STEROIDS IN RAT OVARIES IN THE PREOVULATORY PERIOD

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To determine the degree to which the various structures of the ovaries participate in the preovulatory synthesis of sex hormones quantitative histoenzymologic analysis was used. Activity of 3β-, 17β-, and 20α-steroid dehydrogenases, glucose-6-phosphate dehydrogenase, and NAD- and NADP-diaphorases was investigated. Sex hormone synthesis was shown to take place through the combined function of all structures of the gland. In early proestrus increased estrogen synthesis occurs in the follicles, interstitial glands, and old corpora lutea. Young corpora lutea and follicles are active sources of synthesis of progesterone and 20α-hydroxypregn-4-en-3-one (middle proestrus) whereas the old corpora lutea at this time are chiefly synthesizing a progesterone derivative.

KEY WORDS: *sex cycle*; *ovary*; *sex hormone synthesis*.

Biochemical investigations in the last decade have shown that in the preovulatory period (animals in proestrus) the ovaries begin to secrete large quantities of estrogens and gesagens. Although clear ideas have been formed of the cycle fluctuations in the level of the sex hormones, the question of the tissue structures which are the sources of steroid synthesis in the ovaries still remains unanswered. Help with finding an answer to this question can be given by cytospectrophotometric analysis of the activity of enzymes localized in the various ovarian structures and concerned with steroid formation [3, 4].

The object of the quantitative histoenzymologic investigations described below was to determine the role of the various structures of the rat ovary in the preovulatory synthesis of sex hormones. Choice of this species of animal was determined by the fact that the endocrine status of rats has been studied most completely, so that morphological and biochemical data can be more definitely compared. In addition, the function of the corpora lutea in animals of this species is prolonged, so that the ovary of rats in this respect resembles the human gonad.

## EXPERIMENTAL METHOD

Experiments were carried out on 24 Wistar rats weighing 130-150 g, kept under standard conditions of light and darkness (in light from 3 a.m. to 5 p.m.), and with a regular 4-day cycle, established on the basis of vaginal smears. The animals were killed in diestrus (8 a.m.), in early (7-9:30 a.m.), middle (11:45 a.m.-1:45 p.m.), the late (3:15-7:30 p.m.) proestrus, and in estrus (3 h). The degree of edema of the uterus was estimated from its linear dimensions. Material was taken, kept, and investigated with the necessary precautions [2]. Activity of glucose-6-phosphate dehydrogenase (G6PD), of 3β-, 17β-, and 20α-steroid dehydrogenases (3β-, 17β-, and 20α-OH-SD), and NAD- and NADP-diaphorases was determined in 10-μ cryostat sections. Enzyme activity in the various structures of the ovary (Fig. 1) was estimated quantitatively with the MUF-5 scanning microspectrophotometer, with subsequent computer analysis of the numerical results in accordance with a special program [5]. Estimates of mathematical expectations and dispersions and the standard deviation of these estimates were calculated. The significance of differences between the means was judged with respect to 95% confidence limits. Histograms of distribution of probability were analyzed and the degree

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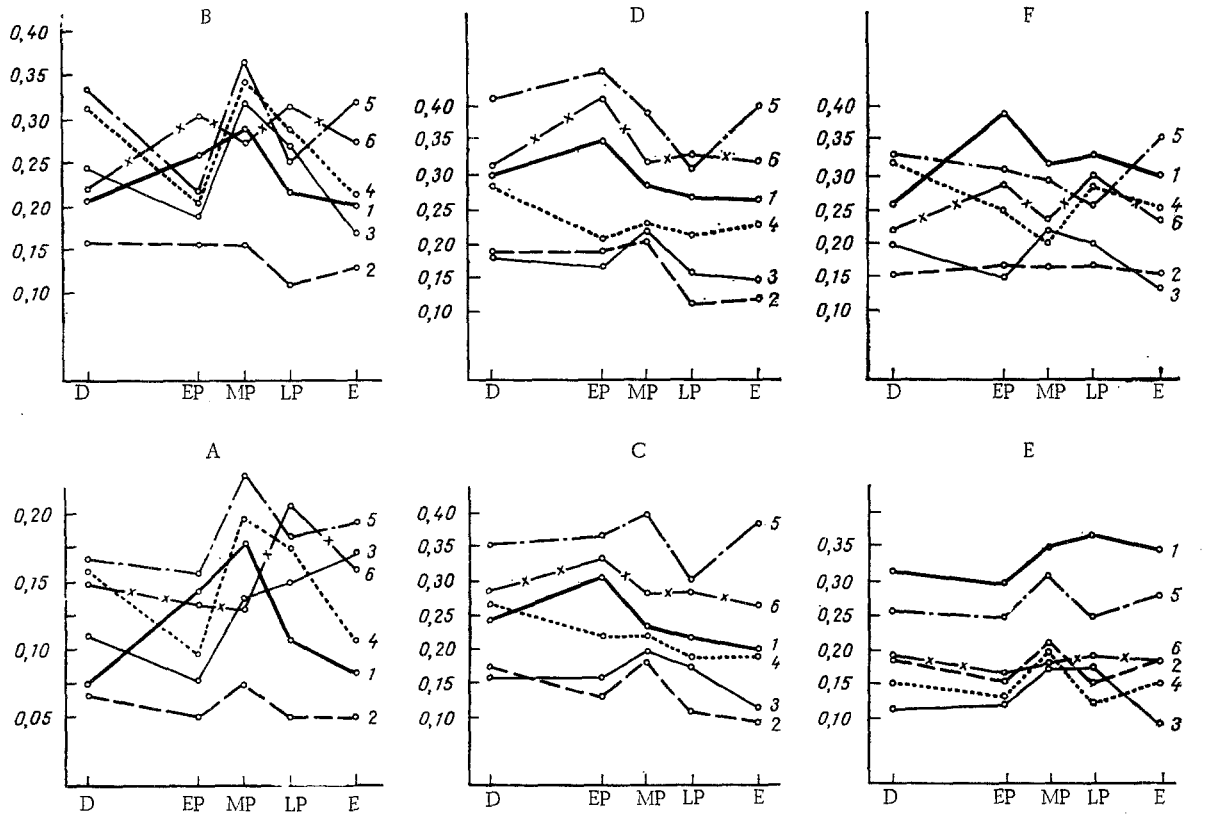


Fig. 1. Changes in enzyme activity in ovarian structures during preovulatory period: A and B) granulosa-cell and inner tecal membranes of large vesicular follicles respectively; C and D) young (with a cavity) and old (without a cavity) interstitial gland respectively; E and F) young (1st generation) and old (2nd-3rd generations) corpora lutea. 1, 2, and 3) 3β-, 17β-, and 20α-OH-steroid dehydrogenases respectively; 4) glucose-6-phosphate dehydrogenase; 5, 6) NAD- and NADP-diaphorases respectively. Ordinate, optical density of reaction products (in relative units); abscissa, phases of sex cycle: D) diestrus, EP) early proestrus; MP) middle proestrus; LP) late proestrus; E) estrus.

of their difference determined in accordance with a conventional index reflecting the minimal percentage of cells whose activity changed during the transitions between the successive phases of the cycle. The relative volumes of the ovarian structures were determined in the sections by the writer's own method [1, 4].

## EXPERIMENTAL RESULTS

Under these experimental conditions the uterus of the animals began to enlarge in early proestrus, indicating increased secretion of estrogens [6, 7]. Other evidence that early proestrus is the period of increased estrogenic function of the gonads in rats is also given by determination of the hormone in the ovarian vein [7, 8, 10, 11]. Consequently, during the period of diestrus and early proestrus considerable synthesis of estrogens takes place in the ovaries. Histoenzymologic investigation of the ovaries showed that during this period there are considerable morphological and functional changes in all the endocrine structures except the young corpora lutea. In the membranes of the vesicular follicles, the interstitial glands, and the old corpora lutea activity of 3β-OH-SD (an enzyme of progesterone synthesis) and of NADP-diaphorase was increased (Fig. 1). The granulosa-cell membrane was the most labile of all the structures: 52% of granulosa cells, tagged for 3β-OH-SD, passed into the region of higher optical densities in the period from diestrus to early proestrus. At this time the direction of the changes in G6PD in the structures examined was opposite in character. A high level of activity of this enzyme and also, evidently, the accumulation of NADP, a cofactor necessary for subsequent hydroxylation of the steroid molecules, was observed in diestrus, i.e., it preceded the period of increased estrogen synthesis. The level of the reaction for 20α-OH-SD either fell (old corpora lutea and follicles) or did not

change (interstitial tissue), thus indicating a decrease in the utilization of progesterone (the precursor of estrogens) for the formation of the other gestagen 20α-hydroxypregn-4-en-3-one. Considering the changes in enzyme activity it can be postulated that several structures of the ovary act as the active sources of estrogens in early proestrus: the interstital glands, the granulosa-cell and inner fetal membranes of the vesicular follicles, and the old corpora lutea.

The peak of the blood estrogen level is known to precede the gestagen peak by several hours [8, 9, 11-13]; the latter arises in response to sharp gonadotropic stimulation of the adenohypophysis [11-13]. What ovarian structures receive and act on these hypophyseal signals? Analysis of the cytospectrophotometric data showed that in middle proestrus all parts of the ovary undergo changes, but they differ in degree and direction. For instance, the continuing rise in the activity of 3β-OH-SD in the follicular membranes is accompanied by activation of 20α-OH-SD, NAD-diaphorase, and G6PD. Sharp activation of the reactions for all steroid dehydrogenases, NAD-diaphorase, and G6PD is found in the young corpora lutea. In the old corpora lutea, on the other hand, the intensity of the histoenzymologic reactions fell, except that for 20α-OH-SD. In the interstitial glands 17β- and 20α-OH-SD were activated. In the last stages of the sex cycle (late proestrus, estrus) activity of the specific dehydrogenases fell considerably, reflecting the depressed steroid-synthesizing function of the gonads [7, 8, 10-12].

It can thus be postulated on the basis of these results that in middle proestrus all structures of the ovary participate in the increased synthesis of gestagens; the membranes of the large follicles and the young corpora lutea, moreover, are active sources of both progesterone and 20α-hydroxypregn-4-en-3-one, whereas the interstitial tissue and the old corpora lutea synthesize mainly a progesterone derivative. In middle proestrus, activation of 17β-OH-SD, known to be evidence of increased interconversion of estrogen fractions (estradiol and estrone), also takes place in the granulosa, young interstitial, and lutein cells. As regards the old corpora lutea, the cyclic variations in enzyme activity which were observed must be emphasized; these fluctuations are attributable to changes in the functional state of 17-41% of the cells. It can accordingly be concluded that in lutein tissue undergoing involution not only is the steroidogenic function preserved, but it is actually increased at certain stages of the sex cycle.

Determination of the relative volumes of the structures by stereometry, taking into account the activity of their key enzyme of steroid production (3β-OH-SD), suggests that young corpora lutea play the main role in ovarian synthesis in general, and the old interstitial cells, old corpora lutea, young interstitial cells, and membranes of preovulatory follicles are involved in the process to a lesser degree.

This investigation thus showed that preovulatory synthesis of estrogens and gestagens takes place through the combined function of all structures of the ovary; a contribution of each of them to the production of steroids of a particular type is different.

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