content in all structures studied. The most significant quantitative changes in these substances were observed in FE and TI of the ovaries, and also in IE and GE of the uterus. The stimulating effect of 50,000 I.U. of RA on the epithelial and theca-glandular structures of the ovaries and endometrium was exhibited most strongly in those phases of EC that are characterized by the highest level of their functional activity.

Vitamin A in a dose of 80,000 I.U. inhibits protein synthesis in all structures of the ovaries and uterus described above. FE and O in the ovaries are more susceptible to the action of toxic doses of retinol whereas the theca-glandular structures exhibit greater resistance.

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## EFFECT OF HIGH DOSES OF RETINOL ACETATE ON 3-β-OL-STEROID DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITY IN MOUSE OVARIES


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Sex steroid production is the most important parameter of ovarian function. Processes of steroid production involve several ovarian structures, and no strict specialization in the production of any one hormone evidently exists among them. This is shown by the presence of a full complement of enzymes catalyzing the metabolic conversions of hormones of both estrogen and progesterone series in all the histophysiological components of the ovary that take part in steroid production [4, 9]. The data cited above indicate that the ovary is a system of hormonally active structures connected in a functional hierarchy and maintaining steroid homeostasis at an adequate level. The principal role among ovarian endocrine formations is probably played by follicles, which are not only independent steroid-producing structures, but also the sources of formation of the corpora lutea (CL) and, to some extent also, of the interstitial gland (IG) of the ovaries.

It is natural to suggest that factors with epitheliotropic action, which affect secretory processes and are characteristic of glandular structures, will take part in the realization of ovarian endocrine function. They include vitamin A. Data in the literature on the effect of retinol on the steroidogenic activity of ovarian structures are very incomplete [7, 8, 10].

The object of this investigation was to study the basic principles governing the effects of different doses of vitamin A on synthesis and secretion of female steroid hormones.

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## EXPERIMENTAL METHOD

Experiments were carried out on 55 female CBA × C57BL mice with a mean body weight of 18-20 g. A 3.44% oily solution of retinol acetate (RA) was used as the vitamin A preparation and was injected into animals of two groups in doses of 5000 and 8000 I.U. daily respectively for 10 days by means of a gastric tube. Animals receiving the RA solvent (soy oil) served as the control. Before the beginning and at the end of the experiment the time course of the estrous cycle was determined by the vaginal smear method. The animals were decapitated. Material was obtained and fixed and the histochemical reaction carried out in accordance with the rules of quantitative enzyme histology [3]. Frozen sections 10 μ thick were stained with diazonium salts by Burstone's method to determine alkaline phosphatase (AP) and with tetrazolium salts by Koval'skii's method [6] to reveal 3-β-ol-steroid dehydrogenase (3-β-ol-SD). Dehydroepiandrosterone at pH 9.0 was used as substrate in the tetrazolium reaction. The 3-β-ol-SD revealed under these conditions served as indicator of activity of progesterone synthesis. The AP detected in cells of the follicular epithelium (FE) and also in the capillary endothelium of the theca interna of the follicles (IT), in CL, in the corpora atretica (CA), and interstitial tissue (IT) was interpreted as an indicator of steroid hormone secretion [1, 2]. The optical density of the cell cytoplasm was determined on a cytospectrophotometer (from Reichert, Austria) by the single-wave, multiple-point method [5, 11]. AP and 3-β-ol-SD activity was expressed in conventional units.

## EXPERIMENTAL RESULTS

Comparative analysis of the 3-β-ol-SD activity in ovaries of control animals and animals receiving 50,000 I.U. of RA showed that activity of the enzymes in the experimental mice was increased in all hormone-producing structures, but with different levels of significance, so that the regularity of the observed changes could be judged.

Highly significant results in IT ($P < 0.001$) reflected an increase in the 3-β-ol-SD content in all phases of the estrous cycle (EC). The maximal increase was observed during proestrus (P), when the enzyme activity reached 24.05 ± 0.39, and during metestrus (M), when it was 15.81 ± 0.36, i.e., 18.3 and 19.8% higher respectively than the corresponding control values. The results demonstrate two peaks of enzyme activity in IT in the course of EC, which also were observed in the control.

Only the proestrus peak of 3-β-ol-SD activity was found with high significance ($P < 0.001$) in CA and TI. In CA it was 21.92 ± 0.21, in TI it was 21.18 ± 0.31. These values are 10.7 and 27.8% higher than the control values respectively.

An increase in the level of enzyme activity with a high degree of significance ($P < 0.001$) was observed in M (29.63 ± 0.25) and in diestrus (D) (27.2 ± 0.29), equivalent to an increase of 4.77 and 21.7% respectively compared with the control.

In FE the increase in the 3-β-ol-SD activity was not significant in any phase of EC except D ($P < 0.05$).

Administration of RA in a dose of 80,000 I.U. caused the cyclic changes in the ovaries to cease and led to a significant decrease of enzyme activity in them: to 4.33 ± 0.18 in FE, 10.47 ± 0.22 in TI, 10.43 ± 0.24 in CA, and 10.17 ± 0.22 in CL. Expressed as a percentage this reflected a fall in the 3-β-ol-SD level by 42.1% in FE, by 14.9% in TI, by 16.76% in CA, and by 30.3% in CL. In IT activity of the enzyme reached 10.52 ± 0.18, which corresponded to the mean values in the control during EC.

The AP level in the cytoplasm of cells of FE and the remaining endocrine structures and also in the capillary epithelium correlated with 3-β-ol-SD activity in the glandular cells following administration of different doses of vitamin A.

Administration of 50,000 I.U. of RA caused an increase in AP activity in all the structures mentioned above. The highest AP level in P was observed significantly ($P < 0.001$) in TI (67.11 ± 1.28), CA (55.99 ± 0.14), and IT (55.7 ± 0.4). Activity of the enzyme in FE although not so marked, showed the highest increase as a percentage compared with the control (43.68 ± 1.46; 29.19%; $P < 0.001$). Meanwhile, the corresponding increase in the remaining steroid formations was 20.44% for TI, 1.95% for CA, and 6.79% for IT. The maximal AP activity (70.23 ± 1.23) observed in M was not significant ($P > 0.05$).

Administration of 80,000 I.U. of RA caused a significant ($P < 0.001$) decrease in AP activity in FE (7.62 ± 0.36, 63.49%) and in TI (26.22 ± 1.37, 44%). The AP level in CA

and IT reached 48.94 ± 0.13 and 47.47 ± 0.32, which corresponded to the mean values in the control in the course of EC. The fall in AP activity in CL (23.37 ± 1.4) was not significant ($P > 0.05$).

The following conclusions can be drawn from the results of this investigation.

Vitamin A in a dose of 50,000 I.U. had a stimulating effect on the steroidogenic activity of the hormone-producing structures of the ovaries. Production of sex steroids was intensified principally in IT, CA, and TI — structures constituting a single endocrine formation — the IG.

Administration of 80,000 I.U. of RA depressed the steroidogenic activity of the ovaries as a whole. This was mainly due to the fact that epithelial structures (FE and CL) were exposed the most to the toxic effect of high doses of retinol. Meanwhile the cells of IG underwent less marked changes, especially CA and IT, whose secretory function remained within normal limits. This last result we interpreted as a compensatory-adaptive mechanism, aimed at maintaining the necessary level of sex steroids in the body at a time of sharp depression of the endocrine function of the ovarian follicular apparatus. However, this kind of compensation is very imperfect, for it cannot ensure an adequate level of sex steroids. This is shown by cessation of the cyclic changes in the reproductive system of the female animals which we observed in the presence of toxic hypervitaminosis A (80,000 I.U. of RA).

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