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## COURSE OF STRUCTURAL CHANGES IN THE MICROCIRCULATION OF GROWING AND ATRETIC OVARIAN FOLLICLES

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Synchronized populations of growing and atretic vesicular follicles were obtained in the ovaries of prepubertal mice by injection of serum gonadotrophin. Light-optical and ultrastructural analysis by morphometric methods revealed definite correlation between the size of the follicles and the degree of their vascularization during growth and atresia. The results suggest that the microcirculation plays the leading role in the initiation of atresia.

KEY WORDS: ovarian follicles; atresia; microcirculation.

The ovarian follicular blood vessels are known to appear in the course of formation of the specialized theca folliculi, and the density of the network of vessels increases with growth of the follicle [2]. The hypothesis that the follicular vessels contribute to the development of atresia of the follicles [3, 5] has not yet been confirmed.

In this investigation the pattern of vascularization of the theca interna at different stages of development of vesicular follicles and the dynamics of ultrastructural changes in the vessels of the microcirculation during induced growth and atresia of the follicles were studied.

## EXPERIMENTAL METHOD

Stimulation of synchronized growth and atresia of ovarian follicles in prepubertal animals was used as the model. Experiments were carried out on noninbred prepubertal female albino mice weighing 7-9 g, which were given a subcutaneous injection of 5 i.u. pregnant mare's serum (PMS). Control animals received an injection of physiological saline. Animals of this age were chosen because of data in the literature showing that the ovaries of prepubertal mice can respond adequately to injection of PMS, and the dose of gonadotrophin used corresponded to that used by other investigators [4, 7].

For the light-optical and electron-microscopic investigations 22 experimental and six control animals were used.

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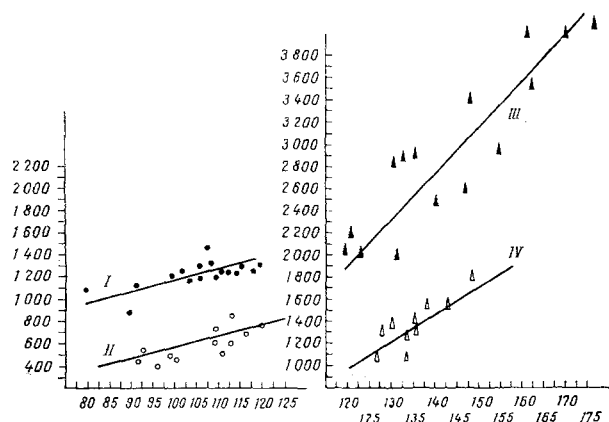


Fig. 1. Dynamics of vascularization of ovarian follicles during growth (I, III) and atresia (II, IV). Abscissa, perimeter of follicle (in relative units); ordinate, total area of lumen of microvessels of theca interna (in relative units). I, II) Follicles in 6th-7th stages of development. III, IV) Follicles in 8th stage of development.

Many normal vesicular follicles were found in the ovaries of the animals of one group (sacrificed 48 h after injection of PMS). In the animals of the other group (sacrificed after 96 h) the vesicular follicles were already undergoing atresia.

The ovaries were fixed in Carnoy's fluid and serial sections,  $5\ \mu$  thick, were stained with hematoxylin-eosin. Using maximal median sections through the vesicular follicles, the stage of their development [6] and their degree of atresia [5, 8] were determined.

A Reichert (Austria) microscope with projection screen was used for morphometric investigation of the structures of the follicles. The areas and length of the lines contained within the contours studied were measured by means of a light pen on the multipurpose morphometric grid of an electromagnetic plateau with magnifications of  $10 \times 10 \times 1.25$  and  $10 \times 100 \times 1.25$ . The results of the measurements were recorded by a MOP/AM-1 electronic counter (Kontron Mässgerett). The perimeters of the follicles and the total area of the microvessels of the theca interna were subjected to metric analysis. The numerical results were obtained in relative units and were subjected to statistical analysis (the rank correlation method and calculation of the standard error of confidence intervals of arithmetic mean values with assessment of significance by the Fisher-Student method).

Material for electron-microscopic study was prefixed in 2.5% glutaraldehyde and then postfixed in 1% osmium tetroxide solution. The subsequent stages followed the usual method. Trimming the pyramid to correspond to a definite follicle was ensured by examining semithin sections stained with toluidine blue.

#### EXPERIMENTAL RESULTS

Morphometric analysis of the course of vascularization of the follicles during their growth and atresia showed that growth of the follicles was accompanied by an increase in the total area of vessels of the theca interna. There was a particularly marked increase in the total area of the lumen of the microvessels during transition of the follicles from the 7th to the 8th stages of development. Determination of the rank correlation coefficient showed high positive correlation between growth of the follicles and their vascularization. The rank correlation coefficient for follicles at the 6th-7th stages of development was 0.640 ( $P < 0.01$ ) and for follicles in the 8th stage of development it was 0.840 ( $P < 0.01$ ).

To determine correlation between atresia and the state of the microcirculation, correlation analysis was made of the morphometric data obtained by investigation of normal and atretic follicles. It will be clear from Fig. 1 that the angles of slope of the axes of the point diagrams reflecting the level of vascularization of normal and atretic follicles in the 6th and 7th stages of development coincide; this indicates direct positive correlation between the total area of vessels of the theca interna and the perimeter of the follicles in the case of development of atresia also ( $p = 0.690$ ;  $P < 0.05$ ). However, the position of the correlation axis in the case of atretic follicles (closer to the abscissa) indicates a decrease in the total level of vascularization of the follicles during the development of atresia.

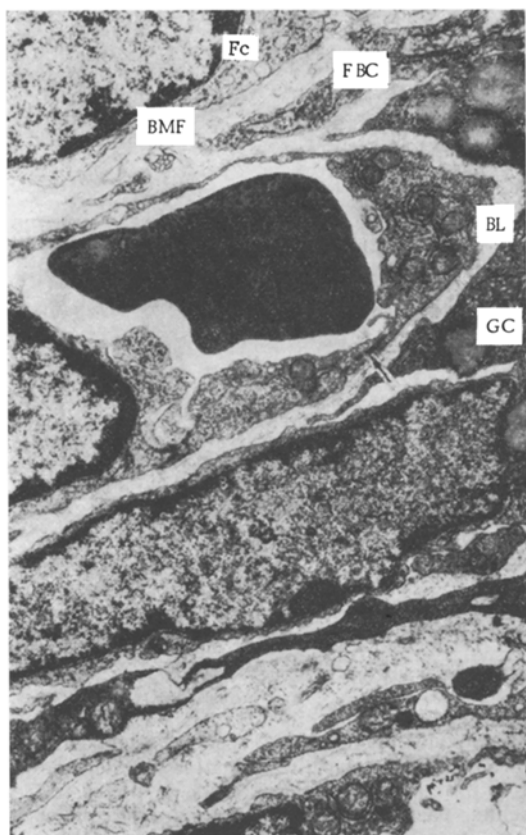


Fig. 2

Fig. 2. Capillary from theca interna of normal follicle in 7th stage of development. FC) Follicular cell; BMF) basement membrane of follicle; FBC) fibroblast-like cell; BL) basal layer of capillary; GC) processes of glandular cells. Arrows indicate endothelial cell junctions. 10,000 $\times$ .

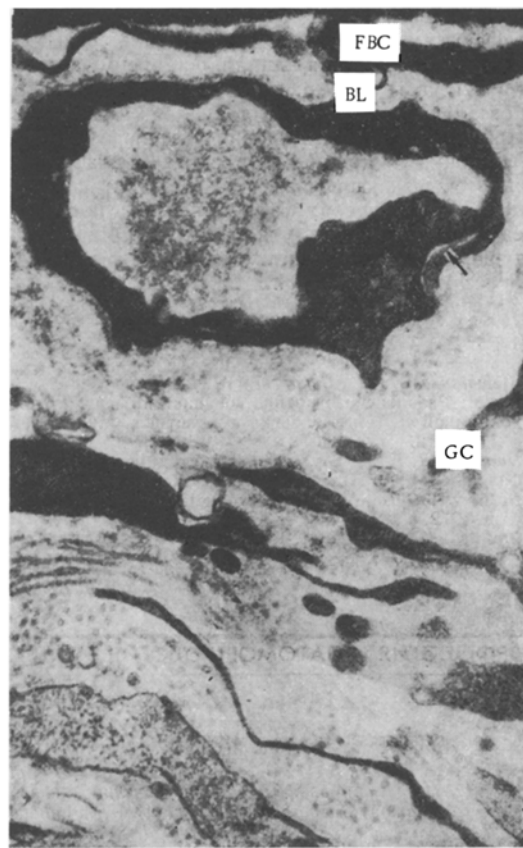


Fig. 3

Fig. 3. Capillary of theca interna of atretic follicle in 7th stage of development. Legend as in Fig. 1. 20,000 $\times$ .

Since spontaneous growth of follicles, which undergo total atresia in the 6th or 7th stages of development, is known to take place in animals of prepubertal age, it was considered important to study follicles in the 8th stage of development, so that follicles whose growth was stimulated entirely by exogenous gonadotrophin could be confidently evaluated. Comparison of the data on vascularization of the theca interna of normal follicles reaching the 8th stage of development 48 h after injection of PMS, and of the atretic follicles 96 h after injection (formed only after injection of PMS) showed that under the conditions of atresia of these follicles direct correlation continued to be observed between the size of the follicles and the degree of their vascularization (the total area of blood vessels of the theca interna). The rank correlation coefficient remained high at 0.782 ( $P < 0.01$ ). However, the results show that, besides a decrease in the indices of vascularization of the follicles, not only did the follicles cease to grow, but they actually decreased a little in size (Fig. 1).

The results of qualitative and quantitative light-optical analysis were confirmed and complemented by investigations of the ultrastructure of the microvessels of the theca interna (Figs. 2 and 3). During the development of atresia, besides marked signs of edema of the theca interna, considerable changes also took place in the principal component of the microcirculation, namely the capillaries. The cytoplasm of the endothelial cells became strongly osmiophilic, so that the structural components became difficult to distinguish. The matrix of the mitochondria became more electron-dense, elements of the cytoplasmic reticulum were hard to define, and in some areas of the cytoplasm of the endothelial cells microfilaments could be identified. Among the structures responsible for transcellular transport, mainly vacuoles could be seen, whereas micropinocytotic vesicles were very rare or absent. At the same time the structure of the endothelial cell junctions was simplified and the intercellular spaces were wider. The luminal surface of the endothelial cells showed considerable smoothness, but some areas of it were formed by undulating membranes, retaining large volumes of fluid. The

structure of the basal layer of the capillaries was much looser and unraveled, mainly in that part of the wall which faced the basement membrane of the follicle.

The morphological indices of structural changes in the microcirculation described above, which were considerably ahead of the development of degenerative changes in the structures of the follicles in time [1], suggest that this component plays a leading role in the initiation of development of atresia.

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