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Histological Observations on Post-Ovulatory Follicles in the Domestic Fowl

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(Text-figs. 1–12)

It is known that in bird the ovarian follicle shrinks immediately after ovulation and regresses gradually. This regression process of the follicle after ovulation has been studied morphologically by many researchers, PERAL and BORING[1] (1918), HETT[2] (1923), NOVAK and DUSCHAK[3] (1923), FELL[4] (1924), DAVIS[5] (1942), AITKEN[6] (1966), and GUZSAL[7] (1966). The present authors, however, observed many degenerative changes occurring in the tissue of the follicle-wall. The most striking one was the accumulation of so-called vacuolated cells in the empty follicular lumen remaining after ovulation. This change apparently resembles that of the follicle after ovulation in some species of mammal, in which the follicle is filled with lutein cells. Accordingly, several early workers used the same term corpus luteum for the follicle after ovulation in the bird. Recent workers, however, are unanimous in the opinion that the vacuolated cell of bird is not homologous to the corpus luteum of the mammal, histologically or functionally. Thus, DAVIS[5] (1942) has given the term post-ovulatory follicle to the follicle after ovulation. Nevertheless, the origin and function of the vacuolated cell is still a matter of speculation.

In this study, histological observations were made on the regression process of the post-ovulatory follicle of the chicken, including the fate of the vacuolated cell.

MATERIALS AND METHODS

White Leghorn hens laying regularly were used. Records had been kept on the laying of the birds prior to the experiment. The presence and location of eggs in the oviduct at the time of dissection were also recorded to determine the time of ovulation. The birds were sacrificed by breeding from the jugular veins, and the ovary was removed immediately from them. Then, post-ovulatory follicles in various stages of regression were excised and fixed in 10% formalin, BAKER's calcium formalin, ZENKER's, and BOUIN's solution. The tissue were made to paraffin sections 5–8 μ thick and occasionally to frozen sections. In addition to these sections, thin stripped membranous preparations were made from the follicle-wall by forceps under a dissection microscope, and subjected to examination. The stains employed in this study were hematoxylin and eosin, azan stain, and HEIDENHAIN's iron hematoxylin for usual observation, sudan black stain for lipids, and alcian blue (MOWRY[8], 1956), aldehyde fuchsin (HALMI[9], 1953), and periodic acid-Schiff (Mc-MANUS[10], 1948) for mucopolysaccharides.

RESULTS

After ovulation, the follicle shrank immediately to a flat, thin-walled sac with an opening widely ruptured at the stigma. At first, the follicle appeared reddish and

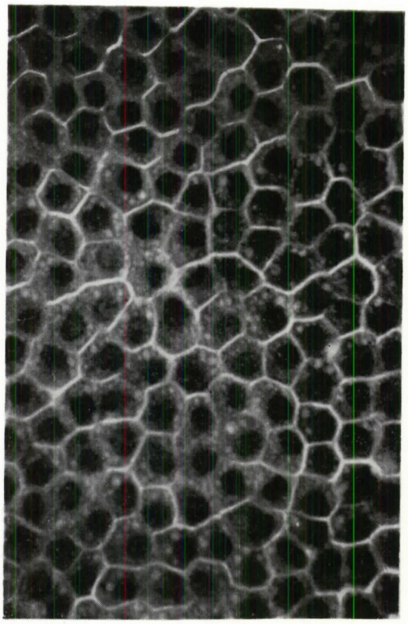

Fig. 1. Epithelial cells of the pre-ovulatory follicle in stripped preparation. They are polygonal in shape and have a clear outline. The cytoplasm is finely vacuolated. Hematoxylin and eosin stain. ×400.

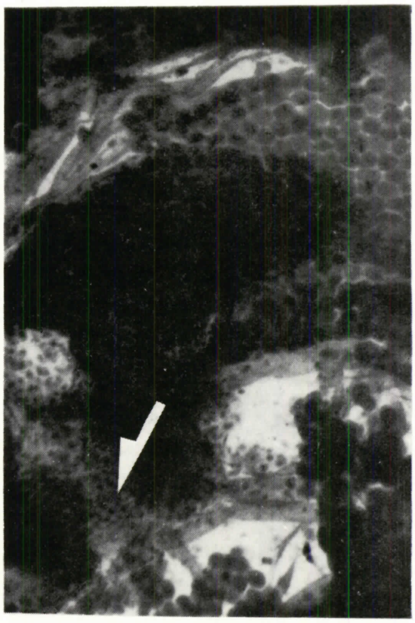

Fig. 2. Epithelium of the post-ovulatory follicle 24 hours after ovulation in stripped preparation. It has been partly stripped away. The basement membrane is folded. The epithelial cells are arranged into a single or stratified layer (dark area). Arrow shows hemorrhage. Hematoxylin and eosin stain. ×100.

measured about 13 mm long, 10 mm wide, 3 mm in thick. Thereafter, it went on shrinking gradually day by day and finally became a pale spindle-formed protrusion with short pedicle. About thirty days after ovulation, the atrophic follicle was so small in size that it was hardly visible to the naked eye and became ultimatedly embedded in the stroma of the ovary. The wall of the pre-ovulatory follicle was formed by the layers of the follicular epithelium, the basement membrane, the theca, and the capsule enveloping the follicle.

Follicular epithelium

The follicular epithelium constituted the innermost layer of the follicle-wall. In the mature follicle, the epithelium consisted of a single layer of low cuboidal

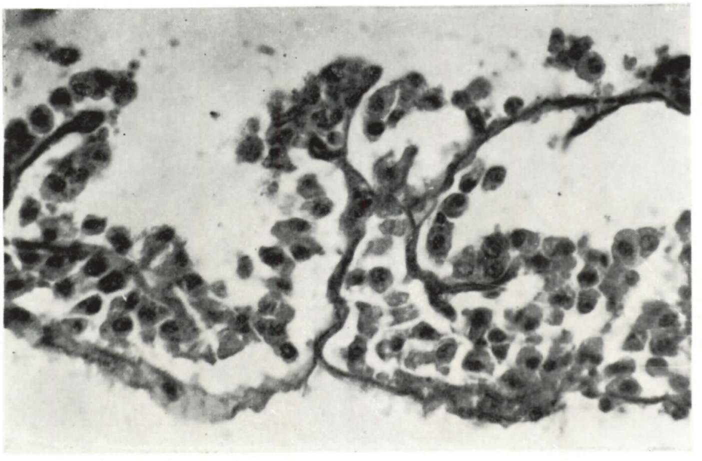

Fig. 3. Epithelial cells and basement membrane 24 hours after ovulation. The epithelial cells are round or spindle-shaped. The basement membrane is folded and stained well with alcian blue. Alcian blue stain. ×150.

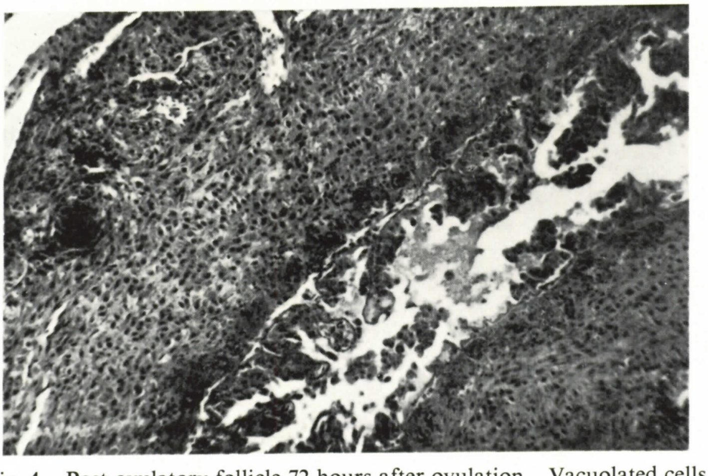

Fig. 4. Post-ovulatory follicle 72 hours after ovulation. Vacuolated cells gather considerably in the follicular lumen. Hematoxylin and eosin stain. ×100.

cells and adhered closely to the underlying basement membrane. The appearance of the epithelium was detected in stripped preparations better than in paraffin sections. Fig. 1 illustrates the stripped preparations of the epithelium. As is shown in the figure, the epithelial cells were polygonal in shape and had a distinct contour. They contained centrally a round nucleus riched in chromatin and had cytoplasm with fine vacuoles.

On the other hand, in the fresh post-ovulatory follicle just after ovulation, the epithelium was largely detached from the internal theca with underlying basement membrane. Some of the epithelial cells disappeared at the time of ovulation, but most of them remained on the surface of the basement membrane (Fig. 3). The remaining cells were modified to round or spindle-shape and arranged in a single or stratified layer (Figs. 2 and 3). Two days after ovulation, the epithelial cells began to show vacuolation. They were more advanced in vacuolation with the progress of regression (Figs. 4 and 5). Entirely vacuolated cells had a hypertrophic size, containing large vacuoles in the cytoplasm (Fig. 8). The vacuolated cells gathered into cellular masses which filled the lumen of the follicle completely (Figs. 6 and 7). The vacuoles were strongly stained by sudan staining method (Fig. 9). Few blood vessels were present in the cellular mass of the vacuolated cells. The aggregation of vacuolated cells was caused not by cellular proliferation, but by shrinkage of the follicle, since no mitosis was found to have occurred. From these findings, it seems clear that the vacuolated cells were derived from the epithelial cells.

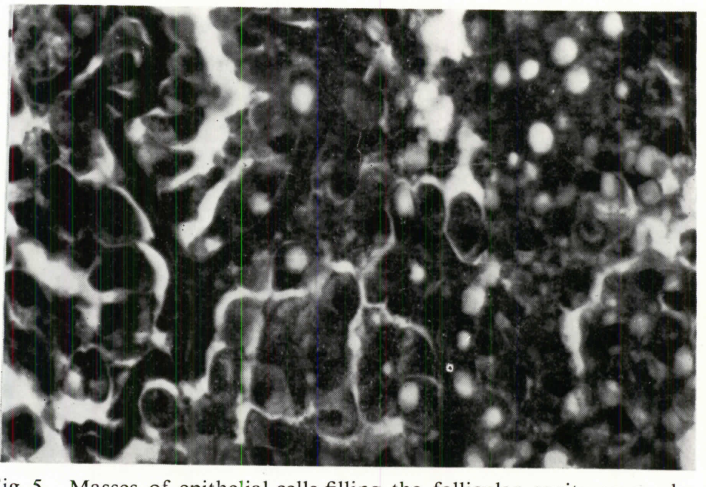

Fig. 5. Masses of epithelial cells filling the follicular cavity seven days after ovulation. Epithelial cells are considerably vacuolated. The thickened basement membrane is seen among these cells. Hematoxylin and eosin stain. ×400.

Basement membrane

The basement membrane of the pre-ovulatory follicle was in close contact with the internal theca. It was too thin to be seen even by the microscope. On the other hand, the basement membrane of the post-ovulatory follicle was mostly detached from the internal theca (Figs. 3 and 4). The membrane thickened greatly and wrinkled in a complicated manner (Figs. 3, 4, and 5), probably due to relax the tension of the follicle after ovulation. The membrane became as thick in a

Fig. 6. Post-ovulatory follicle about twelve days after ovulation. The follicular lumen is filled entirely with vacuolated cells. The central area is more advanced in changes than the peripheral area. Hematoxylin and eosin stain. ×100.

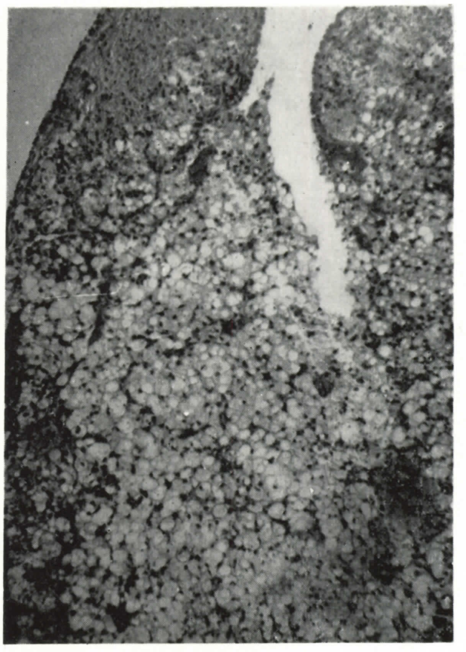

Fig. 7. Post-ovulatory follicle about twenty days after ovulation. Entirely vacuolated cells filled the follicular lumen. Hematoxylin and eosin stain. ×150.

follicle after artificial rupture by forceps as in a follicle after natural ovulation. The thickened membrane appeared homogeneous and structureless. The membrane was stained well by such stains as aldehyde fuchsin and alcian blue, and by the periodic acid-Schiff method (Fig. 3). In an old follicle, the membrane was swollen amorphously and reduced in stainability with the mucin stains. Ten days later, the basement membrane was no longer detectable in sections which had been stained with the usual stains, but it retained its slight stainability with the mucin stains.

Theca

The theca constituted the greater part of the follicle-wall and consisted of two layers, internal and external. There was no distinct border between the two layers. The internal theca was just beneath the basement membrane closely attached to it. The internal theca was constructed with fibrous connective tissue relatively riched in cell elements. These cell elements were fibrocytes and follicular cells located in the deep layer of the internal theca. The follicular cells had a large round nucleus and cytoplasm which appeared scanty (Fig. 11). The internal theca was supplied abundantly with capillary networks. The external theca was the broader part of the theca layer, consisting of compact fibrous connective tissue. This layer was very

Fig. 8. Entirely vacuolated cells. The mass of vacuolated cells consists of two zones. Between the two zones is seen a swollen septum of collagenous fibers. The upper area (peripheral zone of vacuolated cells) is less distinctly vacuolated than the lower area (central zone of vacuolated cells). Hematoxylin and eosin stain. ×400.

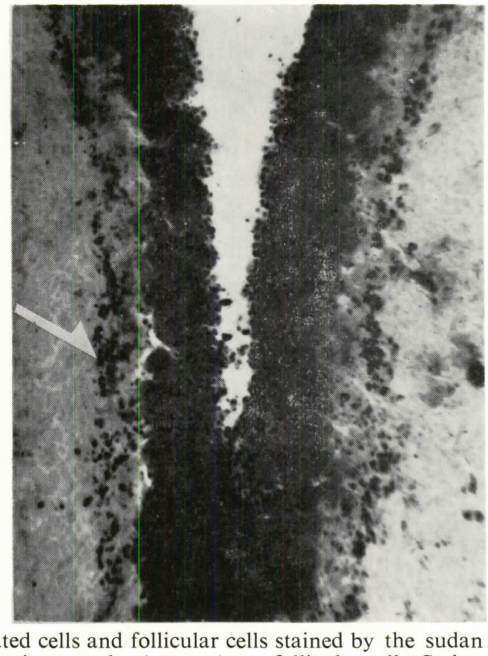

Fig. 9. Vacuolated cells and follicular cells stained by the sudan staining method. Vacuoles stain strongly. Arrow shows follicular cells. Sudan black stain. ×100.

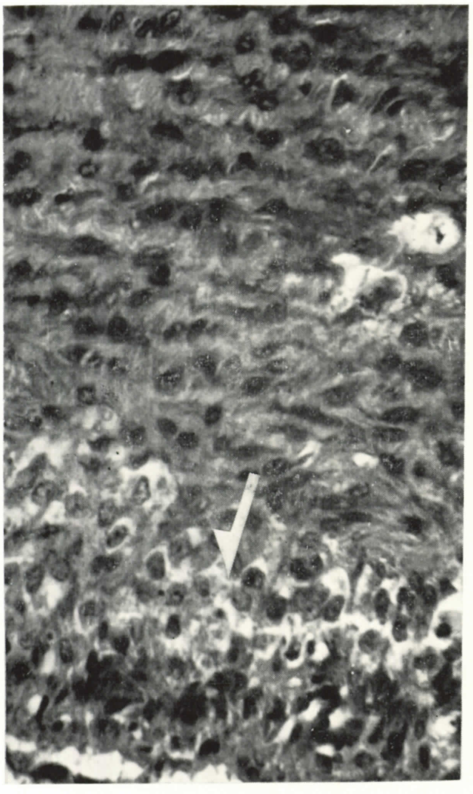

Fig. 10. The theca layer of the post-ovulatory follicle three days after ovulation. Collagenous fibers run parallel. Fibrocytes are arranged into a row. Arrow shows follicular cells. Compare with Fig. 11. Hematoxylin and eosin stain. ×150.

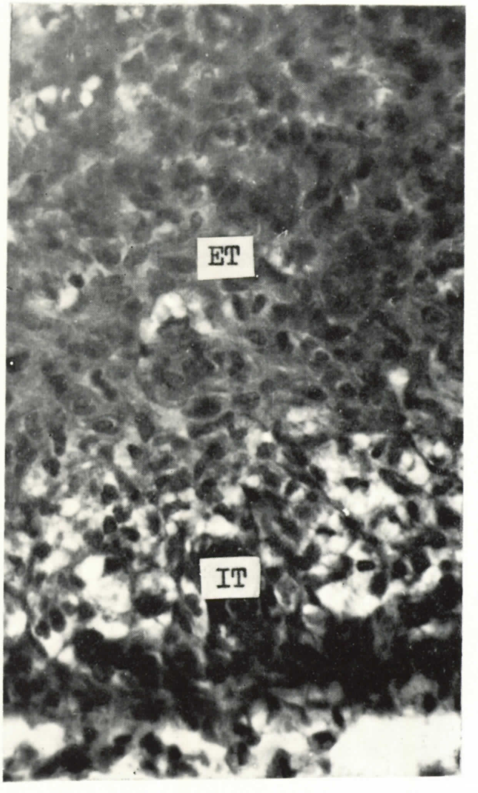

Fig. 11. The theca layer of the post-ovulatory follicle seven days after ovulation. ET and IT stand for the external and the internal theca, respectively. Collagenous fibers have mostly disappeared. Fibrocytes are rounded considerably and irregular in arrangement. Vacuolation is more advanced in the follicular cells than in those of Fig. 10. Hematoxylin and eosin stain. ×150.

rich in sinusoidal vessels.

Just after ovulation, the theca, especially the external theca, thickened largely due to relax of the tension. About five days later, the follicular cells of the internal theca became larger in size and began to have vacuoles, which were distinctly sudanophilic (Figs. 9 and 11). On the other hand, proliferation of collagenous fibers occurred in a narrow area along the inner surface of the lumen. Following the changes in the internal theca, marked changes occurred in the external theca too. First, the amount of collagenous fibers which were the main constituents of the external theca decreased progressively (Figs. 10 and 11). Secondly, the fibrocytes which were arranged parallel to the collagenous fibers became rounded and began to show vacuolation (Figs. 6 and 11). The vacuolated cells derived from the theca layer were less vacuolated than those from the epithelial cells, in the early stage of regression. Accordingly, each of the masses of vacuolated cells which filled the follicular lumen was composed of two portions, the central and the peripheral zone (Figs. 6 and 8). These two zones were separated by the band of collagenous fibers mentioned above (Fig. 8). In the old follicle, these zones of vacuolated cells were no longer distinguished because of the excessive vacuolation and the disappearance of the septum of collagenous fibers (Fig. 7).

Blood vessels

The blood vessels of the follicle formed a very much complicated network to

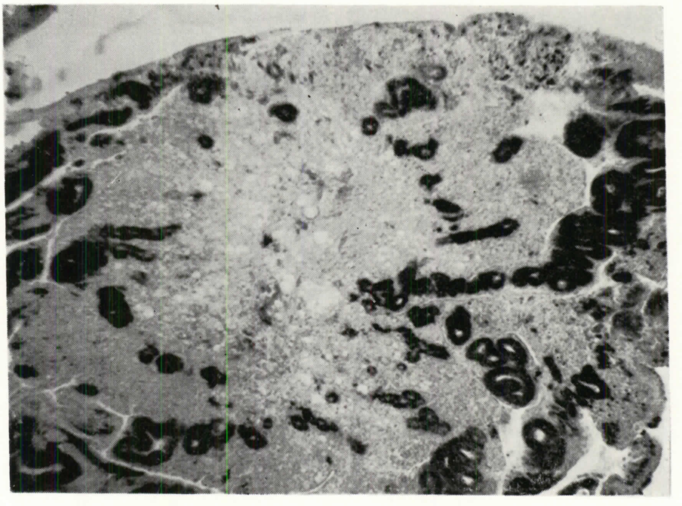

Fig. 12. Degenerated blood vessels of the old follicle after ovulation. The vessels are stained strongly by the aldehyde fuchsin method. Aldehyde fuchsin stain. × 100.

enclose the whole ovum, except the stigma. Just after ovulation, the vascular network shrank greatly and became atrophic. In the early stage of the follicle, varying degrees of hemorrhage were noted in the inner surface of the internal theca. In small lesions of hemorrhage, erythrocytes were scattered in the perivascular tissues and the follicular lumen. Large lesions of hemorrhage were visible grossly by opening the follicular lumen. The hemorrhage is considered to have been induced by the rupture of the capillary wall at the time when the ovum was expelled.

On the other hand, there was a proliferation of capillaries at the injured area along the basement membrane. The newly formed capillaries reached the basement membrane, but they did not extend through the basement membrane to the mass of vacuolated cells. Small vessels disappeared during the early stage of regression, but relatively large arteries remained for a long time after ovulation. In the course of regression of blood vessels, the vascular walls became thick and diffuse. These regressive vessels were stained well with such stains as aldehyde fuchsin and periodic acid-Schiff (Fig. 12).

DISCUSSION

The structure of the post-ovulatory follicle has been studied by such workers as cited above. DAVIS[5] (1942) divided the regression process of the post-ovulatory follicle into four stages. The first stage, or the period when eggs were contained in the oviduct, was characterized by the shrinkage of the follicle and the thickening of the theca. The second stage was noted for the rounding and vacuolation of the epithelial cells and swelling of the basement membrane. The third stage was characterized by the disappearance of the basement membrane and the extensive vacuolation of the epithelial cells. The fourth stage appeared about 4 days after ovulation, with the formation of a mass of vacuolated cells. Similar findings have been reported by AITKEN[6] (1966) who studied the post-ovulatory follicle of the hen. The present investigation gives results basically identical to those mentioned above. However, some supplementary findings were obtained.

The problem of the origin of the vacuolated cells which fill the follicular cavity is still a point of discussion amongst workers. PEARL and BORING[1] (1918) reported that these vacuolated cells were originated from larger vacuolated cells in the internal theca. FELL[4] (1924) and DAVIS[5] (1942) regarded those cells as modified epithelial cells. AITKEN[6] (1966) mentioned that they were derived from epithelial and theca cells. The present study indicates that the vacuolated cells are derived not only from epithelial cells, but also from follicular cells in the internal theca and fibrocytes in both theca layers. In it, the vacuolation process was observed to occur first among the epithelial cells, subsequently among the follicular cells, and lastly among the fibrocytes. Vacuolated cells of three different origins were distinguished from one another in the early stage of regression. Those derived from the epithelial cells were located centrally and more outstandingly vacuolated than those from the cells of the theca layers, which were located peripherally and not so remarkably vacuolated.

Much attention has been given to the function of the vacuolated cells. Many workers, including DAVIS[5] (1942), AITKEN[6] (1966), and GUZSAL[7] (1966) have denied, from a structural point of view, that the post-ovulatory follicle is homologous to the corpus luteum in some mammals. The reasons for their denial were that the vacuolated cells are not prolific, that the mass of vacuolated cells is avascular, and that the regression of the follicle is rapid. The results of the present study lend support to their opinion. Any way, the formation of vacuolated cells must be considered rather as a sign of degeneration in the follicle.

In the present study, hemorrhage and proliferation of blood capillaries were observed on the inner surface of the internal theca. The hemorrhage which has been reported by several workers is considered to be due to mechanical damage at the time of expulsion of the ovum. The proliferation of blood capillaries seemed to be not related to the formation of vacuolated cells, but it rather served to repair the injured area. Newly formed capillaries were transient and disappeared presently, none of them invading the mass of vacuolated cells.

SUMMARY

The post-ovulatory ovarian follicle of the hen was studied histologically for regressive changes. The results obtained are as follows.

1. After ovulation the follicle shrank to a flat thin-walled sac with an opening at the stigma. The ruptured follicle underwent progressive atrophy and disappeared ultimately into the stroma of the ovary.

2. After ovulation the empty follicular cavity was filled with an aggregative mass of vacuolated cells, the vacuoles of which were strongly sudanophilic. The vacuolated cells were derived from epithelial cells, follicular cells located in the internal theca, and fibrocytes present in the internal and external theca.

3. The basement membrane was detached from the internal theca at the time of ovulation. It thickened and wrinkled extensively after ovulation. It was rich in mucopolysaccharides.

4. Varying degrees of hemorrhage occurred in the inner wall of the follicle at the time of ovulation. Proliferation of capillaries was presented in the injured area of the inner wall of the follicle.

5. The blood vessels of the follicular wall underwent gradual atrophy after ovulation. In the course of regression, a large quantity of mucopolysaccharides was demonstrated in the vascular wall.

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ニワトリの Post-Ovulatory Follicle の組織学的観察

藤井俊策・田村達堂

ニワトリの Post-Ovulatory Follicle を組織学的に観察した．卵胞は排卵とともに収縮し，スチグマに破裂口を有するやや扁平な嚢状となる．その後次第に萎縮し瘤状となり，排卵 1 ヶ月後には卵巣実質に吸収されて，肉眼的には不明となる．

顕微鏡的に観察すると，排卵直後の卵胞腔は空虚であるが，卵胞腔にいわゆる Vacuolated Cell が現われる．この Vacuolated Cell が結局は卵胞腔を充塡する．Vacuolated Cell は強くズダン陽性である．Vacuolated Cell は卵胞上皮細胞，卵胞膜内層にある卵胞膜細胞および卵胞膜の線維細胞の変性によって生じたものである．

排卵に際して，卵胞上皮は基底膜とともに広範囲に卵胞膜から剝離する．基底膜は著しく厚くなる．また卵胞膜の内側に出血をきたす．