Lymphology 19 (1986) 55-64

# LYMPHATIC CAPILLARIES IN RABBIT OVARIES DURING OVULATION: AN ULTRASTRUCTURAL STUDY

**Y. Otsuki, S. Magari, O. Sugimoto**

Department of Obstetrics and Gynecology (YO, OS) and Anatomy (SM), Osaka Medical College, Takatsuki, Osaka, Japan

## ABSTRACT

*The fine distribution and ultrastructural changes of rabbit intraovarian lymphatics and blood vessels were compared in specimens obtained at accurately timed intervals after the injection of human chorionic gonadotropin (HCG).*

*At four and six hours after HCG injection, edema was observed around blood capillaries with a slight increase in the number of small fenestrae in the theca interna. Edema around the lymphatic capillaries in the theca externa occurred a little later. The lymphatic capillaries were markedly dilated with occasional wide openings between adjacent cells. At this stage, lysosome disappeared in the endothelial cells of the lymphatic capillaries, and macrophages with numerous peculiar lysosomes appeared around the lymphatic capillaries and some of them entered into the lumen. By eight hours after HCG injection, the edema around the lymphatic capillaries had disappeared. The form and structure of the lymphatic capillaries resumed their pre-injection appearance, but the blood capillaries showed large gaps or perforations in the endothelium.*

*At 11 and 18 hours after HCG injection, blood capillaries had penetrated into the membrana granulosa, but lymphatic capillaries had not.*

*Thus, the lymphatics appear to be modified at four and six hours after HCG injection and these modifications are consistent with the removal of edematous fluid.*

A number of studies have been carried out on the ultrastructural changes of intraovarian blood vessels during the estrous cycle (1-4). However, only a few reports have described lymphatic vessels (1,5,6). Steroid hormones have been measured in ovarian lymph (7-9); it is inferred that intraovarian lymphatic capillaries play a role during the ovulatory process and that this may be accompanied by changes in their fine structure. In the present work, changes in the fine structure of lymphatic capillaries surrounding the follicles observed at accurately timed intervals before follicle rupture, were compared with those in the blood capillaries.

## MATERIALS AND METHODS

Ovulation was induced in young, sexually mature rabbits weighing about 2 kg by an intravenous injection of HCG. Rabbits ovulate fairly regularly about 10-12 hours after HCG injection, and ovulation failure is extremely uncommon.

Fifteen rabbits were injected with HCG and 4, 6, 8, 11 and 18 hours later were anesthetized intravenously with Nembutal (Sodium Pentobarbital, 30 mg/kg body weight). Seven control rabbits received no HCG, and in three other rabbits India ink was injected into the lymphatic capillaries beneath the tunica albuginea of the ovaries.

All ovaries examined in this study, were performed in situ first with 0.85%

sodium chloride solution for two to five minutes to remove the blood, then fixed by perfusion with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M cacodylate buffer at pH 7.35 for five to ten minutes. Following perfusion fixation, the ovaries were removed, cut into small pieces and postfixed in 1% osmium tetroxide with 0.1M cacodylate buffer for 1.5 hours. The specimens were dehydrated in graded concentrations of ethanol and embedded in Epok 812 by the methods described by Luft (10). Sections were cut on a Porter-Blum MT-2 ultramicrotome. Serial semi-thin sections were stained with toluidine blue for light microscopy. Thin sections were double-stained with uranyl acetate and lead citrate (11), and observed in a transmission electron microscope.

For the quantitative study, 18 blood and lymphatic capillaries in 20 specimens were selected from Graafian follicles at each interval after HCG injection. Using an image analyzer (Videoplan, Kontron) the diameter, perimeter and endothelial thickness of both capillaries were evaluated on maps made from serial electron micrographs of similar magnification; that is, 3,000x in the diameter and perimeter, and 10,000x in the endothelial thickness. The endothelial thickness was measured in the nonnuclear cytoplasm.

## *RESULTS*

*Before HCG injection:* Blood capillaries formed two concentric dense networks surrounding Graafian follicles. These were located within the theca interna, and the innermost capillaries were seen just beneath the basal lamina of the membrana granulosa (*Fig. 1,2*). The blood capillaries averaged 6.8 μm in diameter and 22.0 μm in perimeter (*Table 1, 2*). The endothelial cells of the blood capillaries averaged 1.35 μm in thickness and contained a moderate number of mitochondria and some rough endoplasmic reticulum. Free ribosomes and polysomes were scattered throughout the cytoplasm. Several cytoplasmic projections and narrow flaps, termed marginal folds, extended into the lumen. Tight junctions were detected at the border of the adjacent

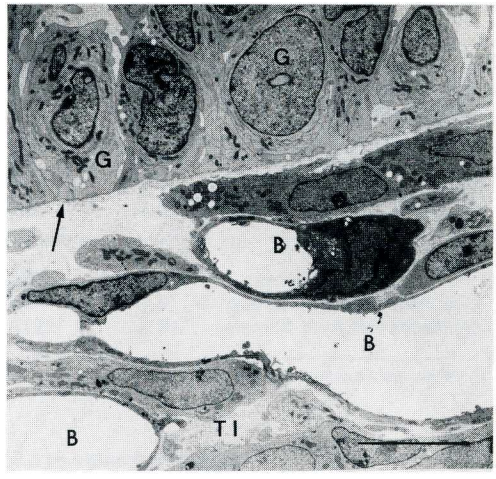


*Fig. 1: Part of a Graafian follicle of a rabbit before HCG injection. Granulosa cells (G) in the basal layer are closely attached to each other and are surrounded by a distinct, thin basal lamina (arrow). Longitudinal and cross-sectioned blood capillaries (B) are seen in the theca interna (TI). Lipid droplets can be seen in both the granulosa and theca interna cells. x2800, Bar = 10 μm*

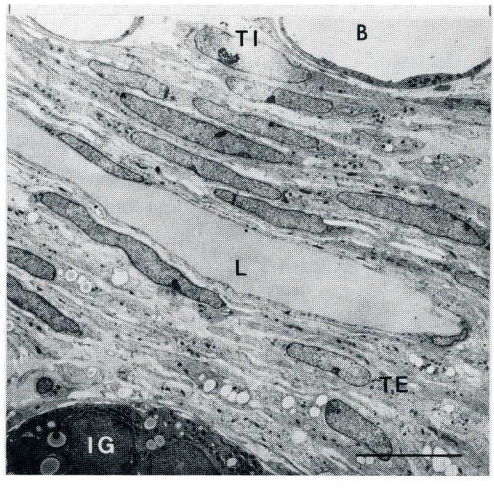


*Fig. 2: Outer part of the wall of the Graafian follicle shown in Fig. 1. The blood capillaries (B) in the theca interna (TI) shown in Fig. 1 can be seen at the top. A lymphatic capillary (L) of the theca externa (TE) is present in the middle. Interstitial gland cells (IG), which contain many mitochondria and lipid droplets, can be seen at the bottom. x2600, Bar = 10 μm*

**Table 1.**
**Mean Diameter of Blood and Lymphatic Capillaries[a]**

| Intervals after HCG injection | | 0 hr | 4 hrs | 6 hrs | 8 hrs | 11 hrs | 18 hrs |
|---|---|---|---|---|---|---|---|
| *Blood capillaries* | Mean diameter | 6.8±2.0 | 8.6±2.3 | 10.8±3.8 | 10.3±4.7 | 13.0±4.8 | 18.1±5.3 |
| | Minimal diameter | 4.5 | 5.0 | 7.0 | 4.7 | 6.2 | 13.3 |
| | Maximal diameter | 10.1 | 11.6 | 19.0 | 23.7 | 23.0 | 26.5 |
| *Lymphatic capillaries* | Mean diameter | 19.2±4.5 | 99.9±44.6 | 56.6±26.6 | 18.8±7.5 | 28.1±12.6 | 17.1±6.3 |
| | Minimal diameter | 14.4 | 45.6 | 37.8 | 10.2 | 12.5 | 10.7 |
| | Maximal diameter | 28.0 | 159.0 | 110.3 | 35.7 | 45.2 | 32.5 |

[a] Values are expressed in micrometers.

**Table 2.**
**Mean Perimeter of Lymphatic and Blood Capillaries[a]**

| Intervals after HCG injection | | 0 hr | 4 hrs | 6 hrs | 8 hrs | 11 hrs | 18 hrs |
|---|---|---|---|---|---|---|---|
| *Blood capillaries* | Mean diameter | 22.0±7.0 | 27.1±9.1 | 31.7±9.7 | 32.5±13.1 | 40.7±13.7 | 50.7±11.4 |
| | Minimal perimeter | 12.1 | 14.1 | 20.6 | 19.3 | 17.8 | 37.7 |
| | Maximal perimeter | 31.4 | 40.4 | 52.8 | 59.3 | 70.2 | 70.8 |
| *Lymphatic capillaries* | Mean perimeter | 56.5±13.4 | 289.3±36.0 | 180.3±48.0 | 54.8±15.3 | 69.1±21.6 | 62.6±16.5 |
| | Minimal perimeter | 40.8 | 199.8 | 116.3 | 32.6 | 38.2 | 40.3 |
| | Maximal perimeter | 81.5 | 418.7 | 294.2 | 82.3 | 95.4 | 97.7 |

[a] Values are expressed in micrometers.

endothelial cells. Fenestrae were not numerous; only one small fenestra closed by a thin diaphragm was seen in the inner capillary network among the 57 blocks from the seven non-injected rabbits. The abluminal surface of the blood capillaries was smooth and surrounded by a continuous basal lamina. Pericytes were occasionally seen. No blood cells or carbon particles were present in the lumen of the capillaries.

The lymphatic capillaries were found in the border region between the theca interna and externa surrounding the outer network of blood capillaries (*Fig. 2*). They varied from 14.4 to 28.0 μm in diameter and from 40.8 to 81.5 μm in perimeter, and averaged 19.2 μm and 56.5 μm respectively (*Table 1,2*). They formed a looser network than the blood capillaries and showed a variety of shapes and calibers. The endothelial cells averaged 0.7 μm in thickness and were thinner than those of the blood capillaries. The marginal folds seen in the blood capillaries were not observed on the luminal surface of the lymphatic capillaries. The endothelial cells extended small projections into the surrounding tissue in which fine intracellular filaments 6 nm, in diameter, were concentrated showing an electron-dense spot. Microfibrils about 10 nm in diameter, called "anchoring filaments" by Leak and Burke (12), were detected just beneath the projections. The features and distribution of mitochondria, free ribosomes, polysomes and rough endoplasmic reticulum in the endothelial cells of the lymphatic capillaries were similar to those of the blood capillaries. The Golgi complex was well developed. Filaments 6 nm in diameter and pinocytotic vesicles were prominent throughout the cytoplasm. One of the more significant features in the lymphatic endothelial cells was the presence of lysosomes (*Fig. 3*). Adjacent endothelial cells showed an overlapping or simple inter-

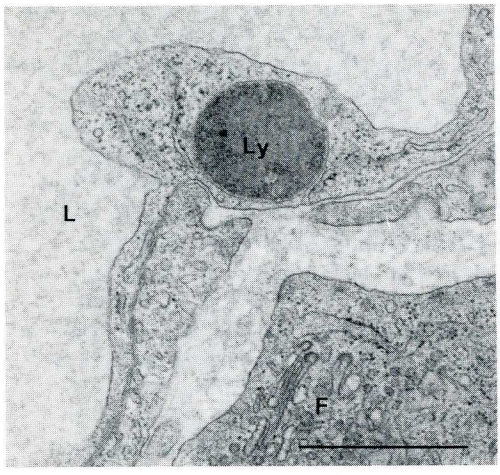


*Fig. 3: Part of a lymphatic capillary (L) in the theca externa before HCG injection. The adjacent endothelial cells show overlapping. Numerous pinocytotic vesicles and a lysosome (Ly) are seen in the endothelial cells. No basal lamina or pericytes can be seen beneath the endothelial cells. Fibroblast (F) x41,200, Bar = 1 μm*

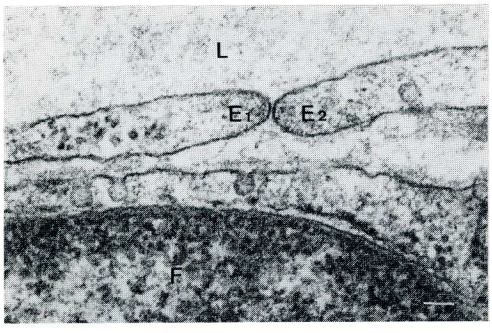


*Fig. 4: Part of a lymphatic capillary (L) in the theca externa before HCG injection. The adjacent endothelial cells (E1 and E2) are simply apposed to each other at a distance of about 10 nm. No specialized junction apparatus is seen, although a few intracellular filaments are present at the margins. Fibroblast (F) x73,600, Bar = 0.1 μm*

digitation. Macula adherens was commonly noted on the boundaries, but tight junctions, as seen in the blood capillaries, were absent. Occasionally the adjacent endothelial cells were simply apposed to each other and no specialized apparatus was detected (*Fig. 4*). Patent junctions were, however, not observed except when India ink had been injected into the ovarian lymphatics. The basal lamina and pericytes seen in blood capillaries were not present. The lumen contained uniformly gray flocculent material of moderate electron density. No edema was observed in the perivascular area.

*After HCG Injection:* 1) Four hours after injection: The blood capillaries averaged 8.6 μm in diameter and 27.1 μm in perimeter (*Table 1,2*), and were somewhat dilated and contained a few blood cells. Edema was noted beneath the basal lamina of the membrana granulosa (*Fig. 5*). Small closed fenestrae were sometimes detected in thin parts of the endothelial cells, but no gaps or perforations were observed.

The lymphatic capillaries averaged 99.9 μm in diameter and 289.3 μm in perimeter; that is, the lymphatic capillaries were five

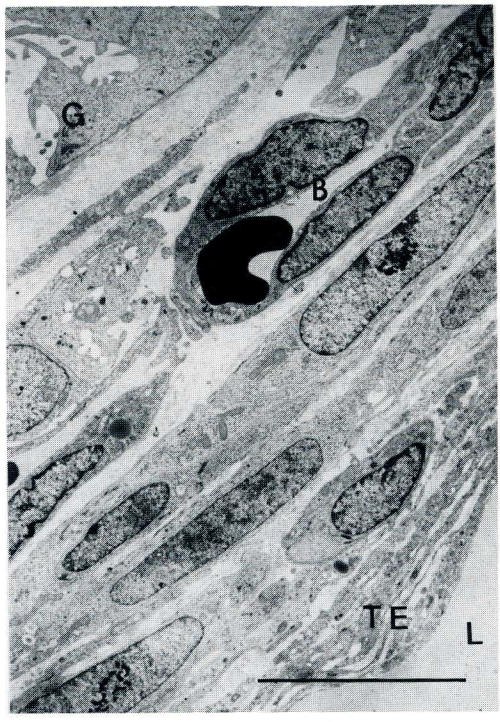


*Fig. 5: Apical region of a Graafian follicle four hours after HCG. The granulosa cells (G) are slightly dissociated, and edema beneath the basal lamina of the membrana granulosa is most prominent at this stage. The cells of the theca externa (TE) are compressed by a dilated lymphatic capillary (L). Blood capillary (B) x3600, Bar = 10 μm*

times the diameter and perimeter of those before HCG injection (*Table 1, 2*). Such dilated lymphatic capillaries were compressing the cells of the theca interna and externa in their vicinity. This compression was prominent in the innermost portion of the theca externa, in which the cells were extremely extended and closely packed (*Fig. 5*). The collagen fibers seen between the cells of the theca externa were difficult to distinguish. The endothelial cells averaged 0.43 μm in thickness and were about 3/5 of the endothelial thickness before HCG injection. Lysosomes were rarely seen in their cytoplasm. At this stage, macrophages appeared around the lymphatic capillaries (*Fig. 6*). They contained numerous larger dense bodies of irregular shape, as well as round lysosome-like bodies with an electron-lucent or dense appearance. Phagosomes filled with electron-dense granules were also visible in the cytoplasm. Some of the macrophages extended a projection into the intercellular cleft of the lymphatic capillaries. The cell body of a macrophage was constricted in the intercellular cleft (*Fig. 7*). The major part of the cytoplasm occupied by many dense bodies was in the lumen of the lymphatic capillary, while a smaller part containing no dense bodies remained in the interstitium beneath the endothelium. In another macrophage, almost all of the cell body was seen in the lumen of the lymphatic capillary

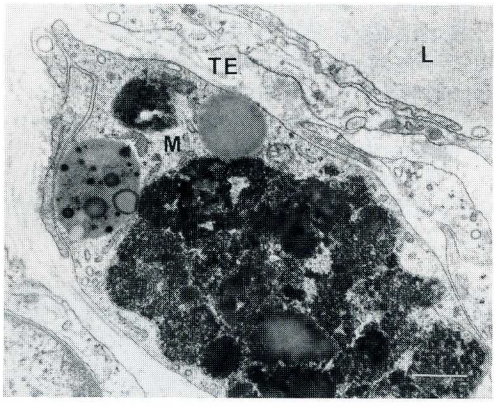


*Fig. 6: Part of a lymphatic capillary (L) and adjoining connective tissue of the theca externa (TE) four hours after HCG injection. The endothelial cell of the dilated lymphatic capillary is extremely thin and contains no lysosome-like dense bodies. On the other hand, a macrophage (M) containing peculiar lysosome-like dense bodies can be seen beneath the endothelial cell. x13,500, Bar = 1 μm*

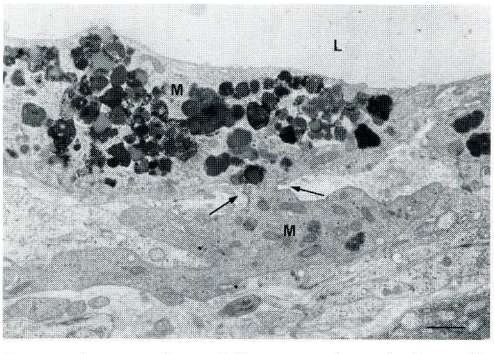


*Fig. 7: A macrophage (M) passing through the wall of a lymphatic capillary (L) in the theca externa four hours after HCG. The cell body of the macrophage is constricted in the intercellular cleft (arrows) of the endothelial cells and the major part of the cytoplasm, occupied by many electron-dense bodies, is seen in the lumen. x12,500, Bar = 1 μm*

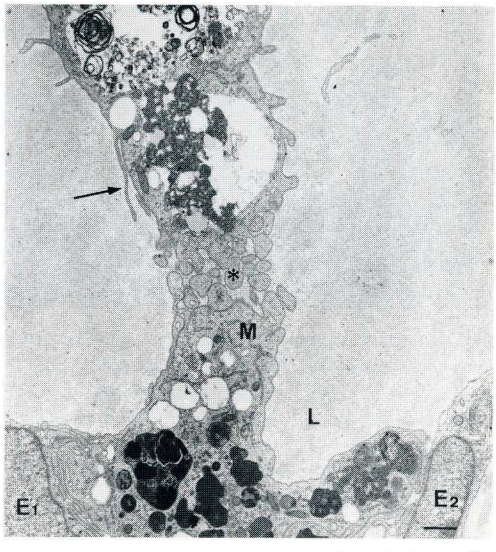


*Fig. 8: A macrophage (M) passing through the wall of a lymphatic capillary (L) in the theca externa four hours after HCG. The adjacent endothelial cells (E1 and E2) of the lymphatic capillary are separated by a major part of the macrophage, bearing numerous short, blunt folds (arrow) and projections (*). Lysosome-like dense bodies, electron lucent vacuoles and myelin figures are seen in the cytoplasm. x7200, Bar = 1 μm*

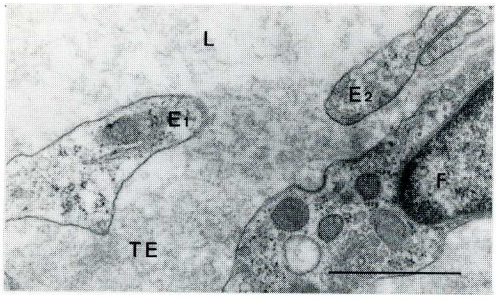


*Fig. 9: Part of a lymphatic capillary (L) in the theca externa (TE) six hours after HCG. Flocculent material seen beneath the endothelium of the lymphatic capillary can be followed into the lumen through a patent junction between apposing endothelial cells (E1 and E2). Fibroblast (F) x34,900, Bar = 1 μm*

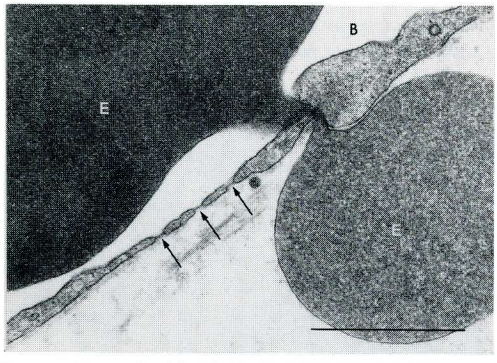


*Fig. 10: Part of a blood capillary (B) beneath the basal lamina of the membrana granulosa eight hours after HCG, showing the escape of an erythrocyte (E) through the endothelium. Closed fenestrae (arrows) are observed in thin parts of the endothelial cell. x53,400, Bar = 1 μm*

whose elongated cytoplasm bore many pseudopodia and a fine fringe (*Fig. 8*). Some inclusions with a myelin-like appearance were noted in addition to the commonly observed dense bodies in the cytoplasm. Several finger-like projections of this cell remained beneath the endothelium.

2) Six hours after HCG injection: The blood capillaries averaged 10.8 μm in diameter and 31.7 μm in perimeter (*Table 1,2*). Small closed fenestrae were increased in number in the endothelium of the blood capillaries. The lumen of the capillaries contained many blood cells, and some erythrocytes had escaped into the perivascular area, where moderate edema was evident.

The lymphatic capillaries averaged 56.6 μm in diameter and 180.3 μm in perimeter, and were similar to, but somewhat smaller than, those at 4 hours after HCG injection (*Table 1,2*). Some patent junctions, which were approximately 800 nm in diameter, appeared between the adjacent endothelial cells. Perivascular edema around the lymphatic capillaries was evident at this stage. The lumen of the lymphatic capillaries was devoid of cellular elements but filled with flocculent material. The electron-dense flocculent material seen in the perilymphatic edema could occasionally be followed through the intercellular clefts of the patent junctions into the lumen of the lymphatic capillaries (*Fig. 9*). Macrophages were more often present in these regions.

3) Eight hours after HCG injection: The blood capillaries averaged 10.3 μm in diameter and 32.5 μm in perimeter, and were similar to those at 6 hours (*Table 1,2*). Large but closed fenestrae, about 60 nm in diameter, were discernible in thin parts of almost all of the endothelial cells of the dilated blood capillaries. The passage of erythrocytes through the endothelium was more evident than at 6 hours after HCG injection (*Fig. 10*). Blood platelets, in addition to erythrocytes and leukocytes, were noted in the lumen of the capillaries.

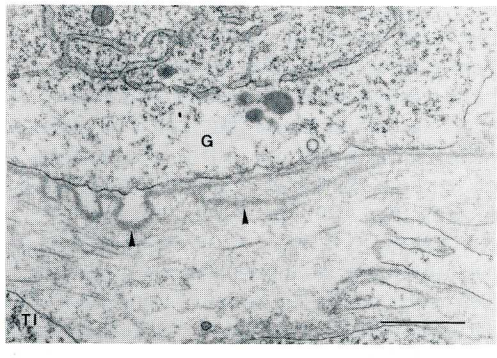


*Fig. 11: Part of a granulosa cell (G) and basal lamina eight hours after HCG. The granulosa cell contains well-developed endoplasmic reticulum. The basal lamina is occasionally duplicated and curved (arrow heads). Theca internal cell (TI) x29,000, Bar = 1 μm*

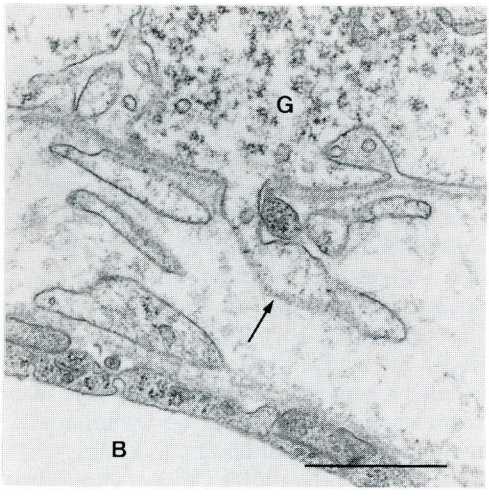


*Fig. 12: Part of two adjacent granulosa cells (G) eight hours after HCG. The basal lamina is penetrated by the protrusion (arrow) of a granulosa cell. Part of a blood capillary (B) is seen at the bottom. x35,100, Bar = 1 μm*

The lymphatic capillaries averaged 18.8 μm in diameter, 54.8 m in perimeter and 0.68 μm in thickness of the endothelial cells, and were similar to those before HCG injection (*Table 1,2*). The dilatation of the lymphatic capillaries and perivascular edema seen 4 and 6 hours after HCG injection, had disappeared. Lysosomes appeared again in their cytoplasm as before HCG injection, while the macrophages in the perivascular regions, seen at 4 and 6 hours, had disappeared. The edema just beneath the basal lamina of the membrana granulosa had also disappeared, and the basal lamina showed occasional duplications and small waves (*Fig. 11*). The protrusions of the granulosa cells frequently passed through the basal lamina into the theca interna (*Fig. 12*). The cells of the theca interna contained more lipid droplets, and occasionally one or several droplets together were surrounded by layers of concentrically arranged rough endoplasmic reticulum (*Fig. 13*).

4) Eleven hours after HCG injection (ovulatory stage): The blood capillaries averaged 13.0 μm in diameter and 40.7μm in perimeter; that is, the blood capillaries were two times the diameter and perimeter

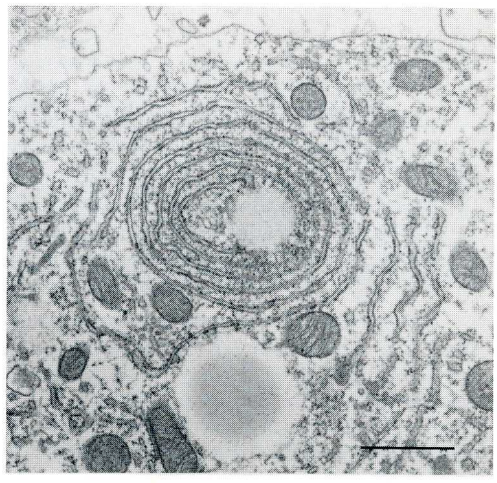

*Fig. 13: Theca interna cell eight hours after HCG, showing many lipid droplets in the cytoplasm. One lipid droplet is surrounded by concentrically arranged rough endoplasmic reticulum. x22,800, Bar = 1 μm*

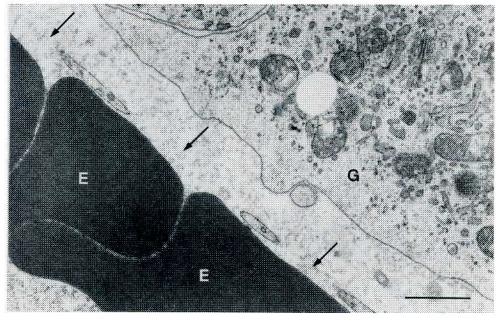


*Fig. 14: A blood capillary beneath the membrana granulosa 11 hours after HCG, showing a large perforation (arrows) of the endothelium. The lumen is filled with erythrocytes (E). The basal lamina of the granulosa cells (G) is completely lost. The granulosa cells contain many well-developed Golgi apparatuses, mitochondria, endoplasmic reticulum and lipid droplets. x22,800, Bar = 1 μm*

of those before HCG injection (*Table 1,2*). The blood capillaries contained many blood corpuscles and sometimes showed signs of thrombosis. Many large gaps or perforations, 1-3 μm in diameter, were present in the endothelium (*Fig. 14*), and through these gaps erythrocytes had escaped into the theca interna. Minute hematomas were commonly observed, especially in the periapical region. In addition, some protrusions of the endothelial cells of the blood

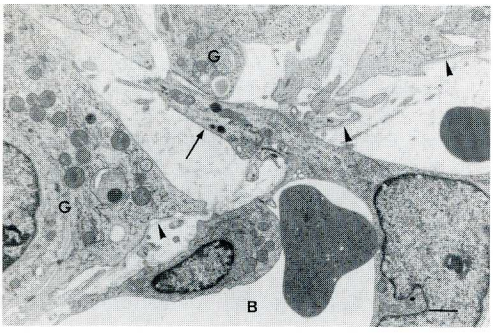


*Fig. 15: A blood capillary beneath the granulosa cells 11 hours after HCG. The dissociation between granulosa cells (G) is pronounced. A protrusion (arrow) of an endothelial cell of a blood capillary (B) penetrates into the membrana granulosa through the fragmented basal lamina (arrow heads). x10,400, Bar = 1 μm*

capillaries penetrated into the membrana granulosa through the fragmented basal lamina (*Fig. 15*). The lymphatic capillaries averaged 28.1 m in diameter and 69.1μm in perimeter (*Table 1,2*). The form and structure of the lymphatic capillaries were unchanged. That is, their appearance was the same as at eight hours and similar to that before HCG injection.

5) Eighteen hours after HCG injection: The blood capillaries averaged 18.1 μm in diameter and 50.7 μm in perimeter (*Table 1,2*). The invasion into the membrana granulosa by blood capillaries was most conspicuous at this stage. The small, closed fenestrae seen in the endothelia gradually decreased in number, although they were occasionally observed five days after HCG in our subsequent studies (13).

The lymphatic capillaries averaged 17.1 μm in diameter and 62.6 μm in perimeter (*Table 1, 2*). The lymphatic capillaries never penetrated the membrana granulosa and maintained the form and structure seen at 11 hours.

Blood vessels and lymphatics in the medulla showed no morphological changes during the ovulatory process.

## *DISCUSSION*

Intraovarian lymphatics have often been examined by light microscopy. It is generally accepted that lymphatics are present in the theca externa and absent in the membrana granulosa. However, controversy exists concerning the presence of lymphatics in the theca interna. Inohara (14), Morris and Sass (1) and Kutuna (15) report the presence of lymphatic capillaries, but Murata (5) stresses that they are not lymphatic capillaries, but blood capillaries. The controversy is due in part to the difficulty of correctly identifying lymphatics under light microscopy. Electron microscopy has revealed, however, that lymphatics possess specific characteristics which differentiate them from blood vessels (16-18). We used the electron microscope to examine intraovarian lymphatic capillaries and confirm that they first appear in the border region between the theca interna and externa, and that the capillaries seen within the theca interna are blood capillaries. Earlier electron microscopic observations (1,5) suggested that the junctions of the adjacent endothelial cells of the intraovarian lymphatics are often "open." However, with the careful fixation used in our study, no such "openings" are observed before HCG injection, except when India ink is injected into the lymphatics.

Ultrastructural changes were clearly demonstrated in both the blood and lymphatic capillaries after HCG injection. At four and six hours edema appeared around the lymphatic capillaries in the theca externa, corresponding to the dilatation of the blood capillaries in the theca interna, and the number of fenestrae in their endothelium was increased. The patent junctions of adjacent endothelial cells of lymphatic capillaries, which were not observed before HCG injection, appeared at these stages, and flocculent material in the edematous area followed into the lumen of the lymphatics through the patent junctions. Such findings suggest that lymphatic capillaries absorb excess interstitial fluid. At eight hours the openings between the adjacent endothelial cells of the lymphatic capillaries had disappeared, and large perforations were seen in the endothelium of the blood capillaries. At 11 hours, (ovulatory stage), the blood capillaries had

invaded the membrana granulosa. The lymphatic capillaries did not penetrate the membrana granulosa at this stage or later, suggesting that the main drainage system for excess fluid at these stages is not the lymphatics but direct channels outside and inside the follicular cavity, which are formed by the fragmentation of the basal lamina and the loosening of junctions between the granulosa cells.

Other notable findings of this study were that macrophages, with numerous peculiar lysosome-like dense bodies appeared in the edema fluid around the lymphatic capillaries at six hours, and some of them entered into the lumen of the lymphatic capillaries via intercellular clefts in the endothelium of the lymphatics. At this stage, lysosomes were not present in the endothelial cells of the dilated lymphatic capillaries. On the other hand, the absence of macrophages and the presence of lysosomes in the endothelial cells were noted eight hours after HCG injection, when the edema around the lymphatics had disappeared. Similar features have been seen after experimental ligation of the lymphatics (19,20).

From the preceding description the lymphatics appear to be modified at four and six hours after HCG injection, and these modifications are consistent with the removal of edematous fluid.

## *REFERENCES*


1. Morris, B, MB Sass: The formation of lymph vascular systems of the ovary. Proc. Roy. Soc. Ser. B. 164 (1966), 557.
2. Reynolds, SRM: Blood and lymph vascular systems of the ovary. Greep, RO and EB Astwood (Eds): Handbook of Physiology, Sec. U: Endocrinology. American Physiological Society, Washington, D.C. (1973), p. 261-316.
3. Bjersing, L, S Cajander: Ovulation and the mechanism of follicle rupture. VI Ultrastructure of theca interna and the inner vascular network surrounding rabbit Graafian follicles prior to induced ovulation. Cell Tiss. Res. 153 (1974), 31.
4. Okuda, Y, et al: An ultrastructural study of capillaries of rabbit ovarian follicles during ovulatory process. Acta Obst. Gynec. Jpn. 32 (1980) 739.
5. Murata, K: Fine distribution of lymph vessels within the ovaries. J. Tokyo Med. Coll. 34 (1976), 425.
6. Otsuki, Y, S Magari, et al: Fine distribution and ultrastructure of intraovarian lymphatics before ovulation. Jpn. J. Lymph. 7 (1984), 145.
7. Lindner, HR, MB San, B Morris: Steroids in the ovarian lymph and blood of conscious ewes. J. Endocrinol. 30 (1964), 361.
8. Lindner, HR: Participation of lymph in the transport of gonadal hormones. Proc. Symp. Hormonal Steroids, 2nd, Milan (1966), p. 821-827.
9. Yoffey, JM, FC Courtice: Steroids in ovarian lymph. Lymphatics lymph and the lymphomyeloid complex. In: Lymph Flow from Regional Lymphatics. Academic Press, London and New York, 1980, p. 206-355.
10. Luft, JH: Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. 9 (1964), 409.
11. Reynolds, ES: The use of lead citrate at high pH as in electron-opaque stain in electron microscopy. J. Cell. Biol. 17 (1963), 208.
12. Leak, LV, JF Burke: Ultrastructural studies on the lymphatic anchoring filaments. J. Cell Biology 36 (1968), 129.
13. Otsuki, Y, S Magari, et al: Fine distribution and ultrastructure of intraovarian lymphatics of rabbit. III Corpus luteum, Acta Anat. Nippon. 60, (1985) IN PRESS
14. Inohara S: Einige neue beitraege zur kenntnis der lymphgefaesse im ovariumparenchym. Zbl. Gynaek. 59 (1935), 98.

15. Kutuna, M: Anatomie des lymphsystems der japaner. Kanehara Shuppan Co., Tokyo and Kyoto (1968).
16. Casley-Smith, JR, HW Florey: The structure of normal small lymphatics. Quart. J. Exp. Physiol. 46 (1961), 101.
17. Leak, LV, JF Burke: Fine structure of the lymphatic capillary and the adjoining connective tissue area. Am. J. Anat. 118 (1966), 785.
18. Magari, S, et al: Morphological studies on liver lymphatics. Lymphology 12 (1979), 14.
19. Magari, S, S Asano: Regeneration of the deep cervical lymphatics light and electron microscopic observations. Lymphology 11 (1978), 57.
20. Magari, S, et al: Form, distribution, fine structure and function of hepatic lymphatics with special reference to blood vessels and bile ducts. Asian Med. J. 24 (1981), 254.