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# FINE STRUCTURE AND MORPHOMETRIC ANALYSIS OF LYMPHATIC CAPILLARIES IN THE DEVELOPING CORPUS LUTEUM OF THE RABBIT

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## ABSTRACT

*The fine distribution and ultrastructural changes in the intraovarian lymphatics were studied in the developing corpus luteum of rabbits. Three days after human chorionic gonadotropin (HCG) injection, lymphatic capillaries were observed among theca lutein but not granulosa cells. This distribution persisted even on day 14. Edema appeared around the lymphatic capillaries, corresponding to dilatation of blood capillaries surrounding the membrana granulosa. The diameter and perimeter of the latter vessels were about 5 times greater than before HCG injection. Lymphatic capillaries were slightly dilated and about 2 times their original diameter and perimeter. Flocculent material migrated into the lumen of the lymphatics through the open junctions. Lysosomes and rough endoplasmic reticulum were increased in number in the lymphatic endothelial cells. Five and 7 days after HCG injection macrophages and sometimes loose, degenerated lutein cells were observed in the lymphatic capillaries. Fourteen days after HCG injection, the dilatation of blood capillaries disappeared, although lymphatic capillaries remained slightly dilated after day 3. Some lymphatic but not blood vascular endothelial cells began to degenerate.*

*The results suggest that lymphatic capillaries function to absorb and transport excess fluid and "hormones" in association with changes in ultrastructure.*

Lymphatics play an important role in both absorption and transport of excess interstitial fluid and solute. Moreover, steroid hormones have been detected in lymph as well as in ovarian venous blood during the estrus cycle (1-3) suggesting hormonal absorption by intraovarian lymphatic capillaries and ongoing ultrastructural changes. We have previously reported the ultrastructural changes of rabbit intraovarian lymphatic capillaries during ovulation (4). In the present study, the fine distribution and ultrastructure of lymphatic capillaries are compared with newly forming blood capillaries in the developing corpus luteum of the rabbit.

## MATERIALS AND METHODS

Ovulation was induced in 10 young sexually mature female rabbits weighing 2-3kg by an intravenous injection of human chorionic gonadotropin (HCG). Rabbits ovulated fairly regularly about 10-12 hours after HCG injection and were laparotomized 1, 3, 5, 7, and 14 days after the injection. All ovaries examined in this study were perfused *in situ* first with 0.85% sodium chloride solution for 2 to 5 minutes to remove the blood, than fixed by perfusion with 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1M cacodylate buffer at pH 7.35 for 5 to 10 minutes. Following perfusion-fixation, the ovaries were removed and the corpora lutea were cut into small pieces which were postfixed in 1% osmium tetroxide with 0.1M cacodylate buffer for 1.5

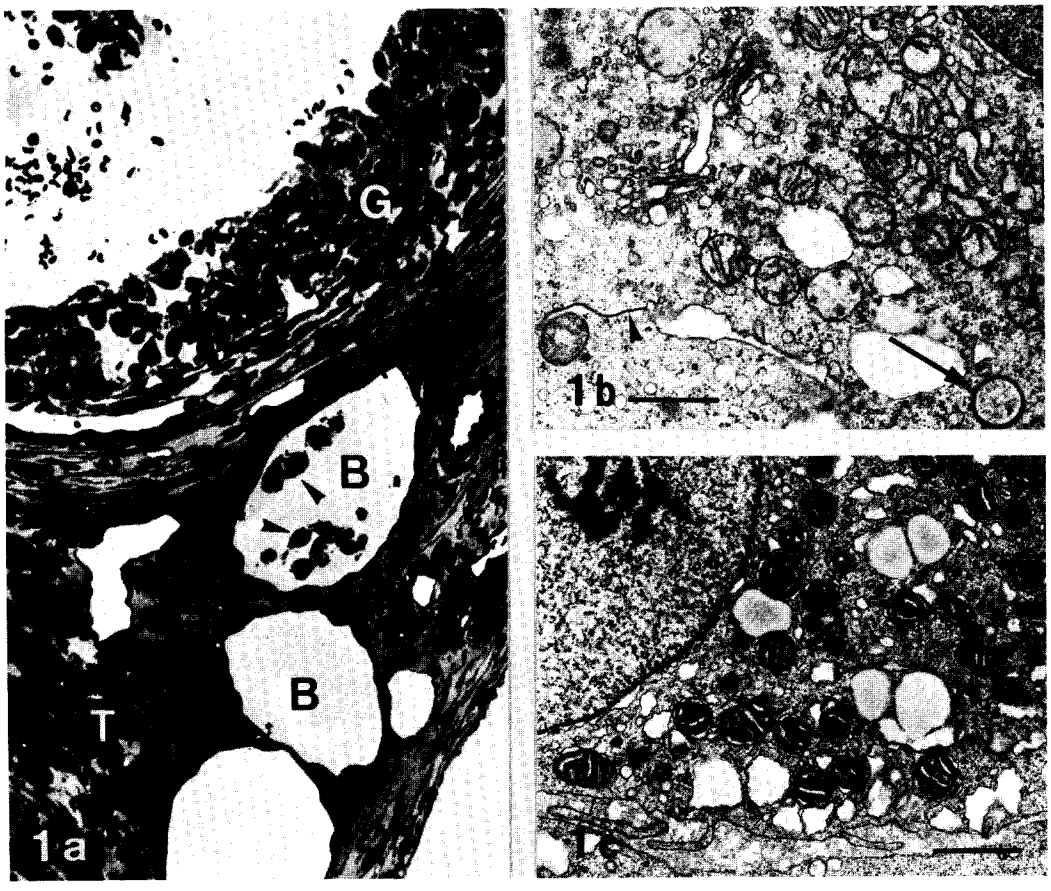


*Fig. 1. One day after HCG injection. a) Part of a ruptured follicle. Blood capillaries (B) surrounding the membrana granulosa (G) are slightly dilated and contain some large cells (arrowhead). Theca layer (T) (x106). b) Part of granulosa lutein cells. Annular nexus (arrow), abutment nexus (arrowhead). (x11,200) Bar: 1μm. c) Theca lutein cell showing many mitochondria with high electron-dense matrix in the cytoplasm. (x11,100) Bar: 1μm.*

hours. The specimens were dehydrated in graded concentrations of ethanol and embedded in Epok 812 by the methods described by Luft (5). Sections were cut on a Porter-Blum MT-2 ultramicrotome. Semithin sections, 1μm thick, were stained with toluidine blue for light microscopy. Serial thin sections, 60-80nm were double-stained with uranyl acetate and lead citrate (6) and examined in a transmission electron microscope.

For quantitative study, 15 blood and lymphatic capillaries in 8 specimens were selected from corpora lutea at each interval after HCG injection. With an image analyzer (Videoplan, Kontron) the diameter and perimeter of both types of capillaries were evaluated on maps made from serial electron micrographs of similar magnification (x3,000).

## RESULTS

### *One day after HCG injection*

The centers of the follicles after ovulation were filled with erythrocytes. The endothelial cells of blood capillaries penetrated the membrana granulosa through the fragmented basal lamina 11 hours after HCG injection (4). On the other hand, lymphatic capillaries were present in the theca externa layer but not in the membrana granulosa *(Fig. 1a)*.

Table 1
**Mean Diameter of Blood and Lymphatic Capillaries**

| *Intervals after HCG injection* | | 0 hr | 1 day | 3 days | 7 days | 14 days |
|---|---|---|---|---|---|---|
| *Blood capillaries* | Mean diameter | 6.8±2.0 | 9.0±2.1 | 37.2±9.1 | 38.1±10.0 | 7.5±1.7 |
| | Minimum diameter | 4.5 | 5.9 | 19.5 | 19.8 | 4.7 |
| | Maximum diameter | 10.1 | 13.0 | 50.1 | 53.5 | 10.3 |
| *Lymphatic capillaries* | Mean diameter | 19.2±4.5 | | 35.9±9.9 | 40.0±10.5 | 34.5±9.6 |
| | Minimum diameter | 14.4 | | 19.2 | 19.0 | 18.2 |
| | Maximum diameter | 28.0 | | 50.1 | 53.8 | 48.1 |

Values are expressed in micrometers.

Table 2
**Mean Perimeter of Lymphatic and Blood Capillaries**

| *Intervals after HCG injection* | | 0 hr | 1 day | 3 days | 7 days | 14 days |
|---|---|---|---|---|---|---|
| *Blood capillaries* | Mean perimeter | 22.0±7.0 | 26.9±8.7 | 105.7±27.1 | 109.5±28.6 | 22.2±5.0 |
| | Minimum perimeter | 12.1 | 12.0 | 52.9 | 55.1 | 14.3 |
| | Maximum perimeter | 31.4 | 39.8 | 149.3 | 150.9 | 30.3 |
| *Lymphatic capillaries* | Mean perimeter | 56.5±13.4 | | 105.4±29.5 | 114.7±31.5 | 101.0±32.9 |
| | Minimum perimeter | 40.8 | | 56.6 | 53.8 | 53.6 |
| | Maximum perimeter | 81.5 | | 150.3 | 158.8 | 139.6 |

Values are expressed in micrometers.

Blood capillaries surrounding the membrana granulosa averaged 9.0μm in diameter and 26.9μm in perimeter *(Tables 1,2)*, were slightly dilated and contained large cells in addition to erythrocytes, leukocytes, and blood platelets *(Fig. 1a)*. The cells sometimes showed an annular nexus or an abutment nexus which was a connection peculiar to granulosa cells.

Granulosa lutein cells were separated from theca lutein cells by a connective tissue layer and contained a fairly round nucleus with regularly distributed chromatin, enlarged nucleolus, a number of lipid granules, mitochondria with tubular cristae, saccular smooth endoplasmic reticulum, and Golgi apparatus *(Fig. 1b)*. On the other hand, theca lutein cells were distinguished from granulosa lutein cells by the high electron-dense matrix in their mitochondria and prominent dilated smooth endoplasmic reticulum *(Fig. 1c)*.

*Three days after HCG injection*

The blood capillaries surrounding the membrana granulosa averaged 37.2μm in diameter and 105.7μm in perimeter, that is, about 5 times greater than before HCG injection *(Tables 1,2)*. Such dilatation of the blood capillaries and edema around them was observed for 7 days. Mitotic figures and fenestrae were often seen in the endothelial cells of the blood capillaries among granulosa lutein cells. Fenestrae were especially noticeable in the thinned portion of the endothelial cells.

Lymphatic capillaries were present among the theca lutein cells form this stage on and averaged 35.9μm in diameter and 105.4μm in perimeter: about 2 times greater than before HCG injection *(Tables 1,2)*. Lysosomes of various sizes and rough endoplasmic reticulum were increased in number in the endothelial cells, and edema around the lymphatic capillaries was conspicuous at this stage *(Fig. 2a)*. The lumen of the lymphatic capillaries was devoid of cellular elements but filled with flocculent material. The electron-dense flocculent material seen with the perilymphatic edema was on occasion able to be traced through the intercellular clefts of the open junctions into the lumina of the lymphatic capillaries *(Fig. 2b)*, a phenomenon observed until day 14.

Theca lutein cells contained numerous rough endoplasmic reticulum, mitochondria with electron-dense matrix, lipid granules, and smooth endoplasmic reticulum which showed saccular shapes without dilatation. Granulosa lutein cells were similar to those on day 1. Thus, theca lutein cells apparently differed from granulosa lutein cells in several respects; that is, the density of the matrix in the mitochondria and the number of Golgi apparatus, but were difficult to distinguish from ovarian interstitial cells.

*Five and 7 days after HCG injection*

Blood capillaries infiltrating the membrana granulosa were arranged radially into the center of the ruptured follicle, and the mean diameter and perimeter were similar to those on day 3 *(Tables 1,2)*. Mitotic figures and fenestrae were still observed in the endothelial cells of the blood capillaries.

Proliferation of theca lutein cells was prominent and lymphatic capillaries were abundant among the theca lutein cells *(Fig. 3a)*. The mean diameter and perimeter of the lymphatic capillaries were as large as on day 3 *(Tables 1,2)*. At this stage, macrophages with lysosome-like dense bodies and finger-like projections *(Fig. 3b)*, in addition to degenerated lutein cells with myelin-like bodies and large lysosome-like dense bodies *(Fig. 3c)*, were observed both around and within lymphatic capillaries. The characteristic lysosomes and rough endoplasmic reticulum in the endothelial cells of the lymphatic capillaries as on day 3 were decreased and therefore, the fine structure of these endothelial cells resumed their preinjection appearance except for the presence of open junctions.

Granulosa lutein cells contained a number of electron-dense bodies and occasionally one or several bodies together were surrounded by layers of concentrically arranged smooth endoplasmic reticulum *(Fig. 3d)*. These bodies were not seen in theca lutein cells. Granules which showed vague outlines with moderate electron density were observed in the matrix of the mitochondria of both types of lutein cells *(Fig. 3e)*. The theca lutein cells at this stage were similar to ovarian interstitial cells. As noted above, many of both types of lutein cells possessed such characteristic ultrastructure, but some had a variety of degenerative features in the cytoplasm: lipid granules with various electron density, inclusions with a myelin-like appearance and lysosome-like dense bodies.

*Fourteen days after HCG injection*

The blood capillaries surrounding the membrana granulosa were no longer dilated, averaging 7.5μm in diameter and 22.2μm in perimeter, similar to those before HCG injection *(Tables 1,2)*. Degenerative change in the endothelial cells of the blood capillaries were not seen at this stage.

Lymphatic capillaries were present only among theca lutein cells and not within the granulosa. The mean diameter and perimeter were 34.5 and 101.0μm, respectively, similar to those on day 3 *(Tables 1,2)*.

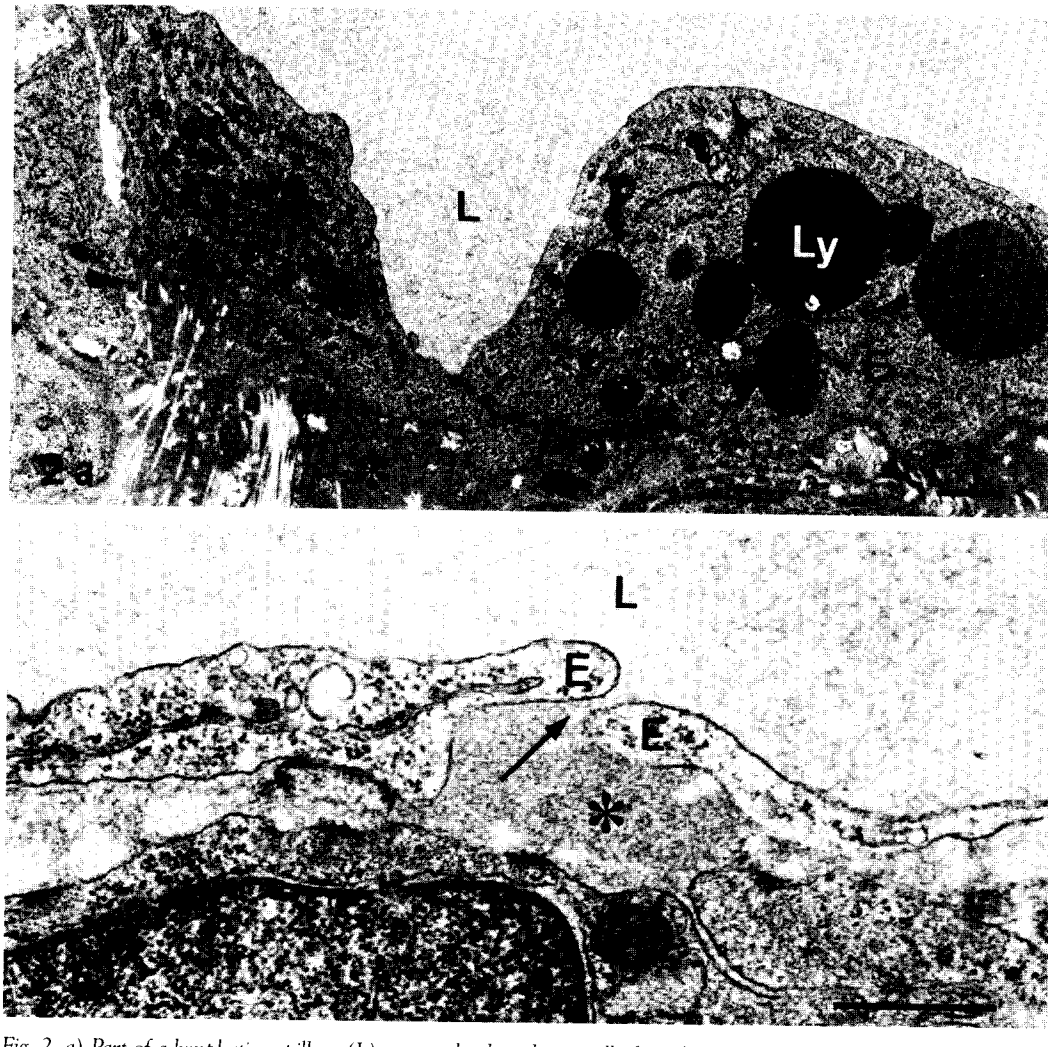


*Fig. 2. a) Part of a lymphatic capillary (L) among the theca lutein cells three days after HCG injection. Lysosomes (Ly) of various size and rough endoplasmic reticulum are increased in number in the endothelial cells (E) and dark amorphous material (asterisk) around the lymphatic capillary is conspicuous. (x7,700) Bar: 1μm. b) Part of a lymphatic capillary (L) among the theca lutein cells three days after HCG injection. Electron-dense flocculent material (asterisk) can be followed into the lumen through a patent junction (arrow) between apposing endothelial cells (E). (x20,100) Bar: 1μm.*

The endothelial cells which had begun to degenerate partially, contained lysosomes of various sizes *(Fig. 4)*. Macrophages appeared around the lymphatic capillaries more frequently.

The ultrastructure of both granulosa and theca lutein cells were the same as on days 5 and 7 but more degenerating lutein cells were seen at this stage in the corpus luteum.

## *DISCUSSION*

The lymphatic capillaries in the corpus luteum have often been examined by light microscopy. It is generally accepted that lymphatic capillaries as well as blood capillaries invade the corpus luteum. Morris and Sass (7) reported that sheep lymphatic capillaries which were previously in the thecal layers were present at the margins of the

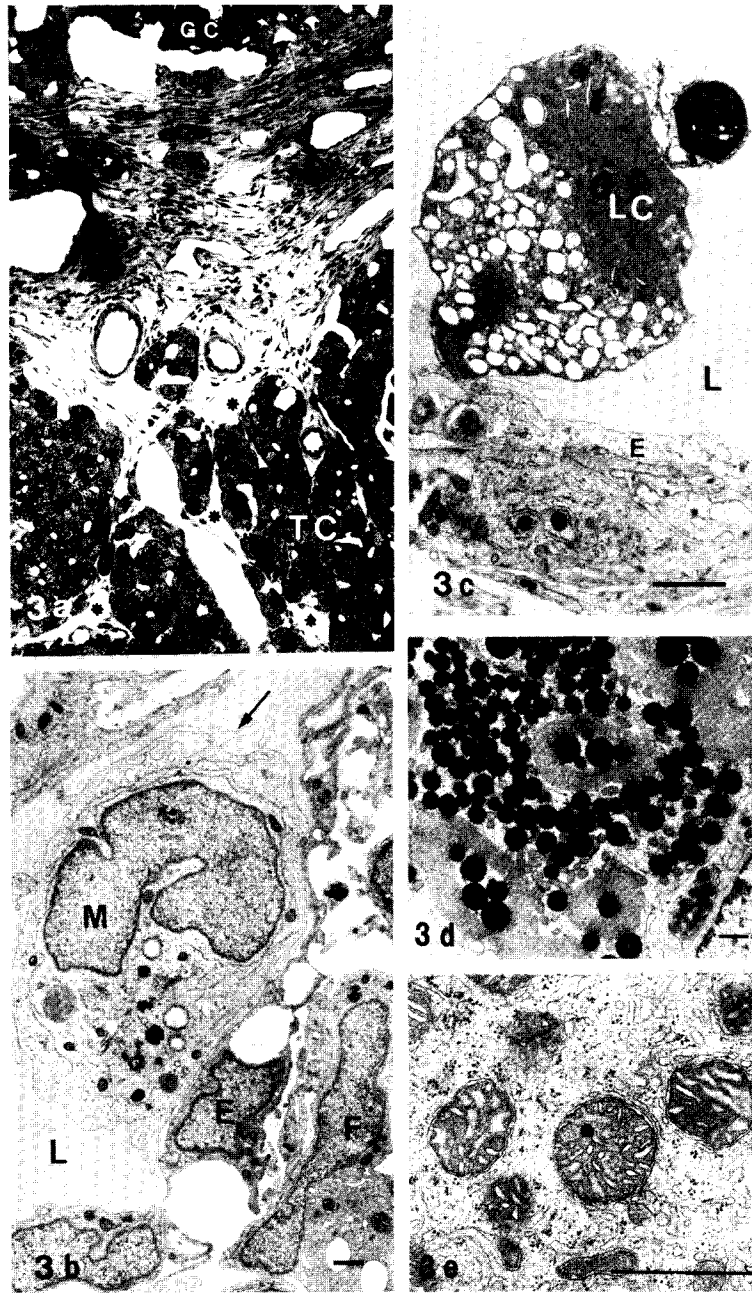


*Fig. 3. Five days after HCG injection. a) Part of a corpus luteum. Lymphatic capillaries (asterisks) are abundantly present among the theca lutein cells (TC), but not among the granulosa cells (GC) (x73). b) A macrophage (M) with fine "fingers) (arrow) and lysosome-like dense bodies in a lymphatic capillary (L) 7 days after HCG injection. Lymphatic endothelial cell (E), fibroblast (F). (x5,400) Bar: 1μm. c) A degenerating lutein cell (LC) showing both myelin-like and large lysosome-like bodies in a lymphatic capillary (L) 7 days after HCG injection. Lymphatic endothelial cells (E). (x11,900) Bar: 1μm. d) Part of a granulosa luteal cell. One or several electron-dense bodies are surrounded by layers of concentrically arranged smooth endoplasmic reticulum. (x4,900) Bar: 1μm. e) Part of a theca lutein cell. A granule with vague outlines is observed in the matrix of a mitochondria. (x24,400) Bar: 1μm.*

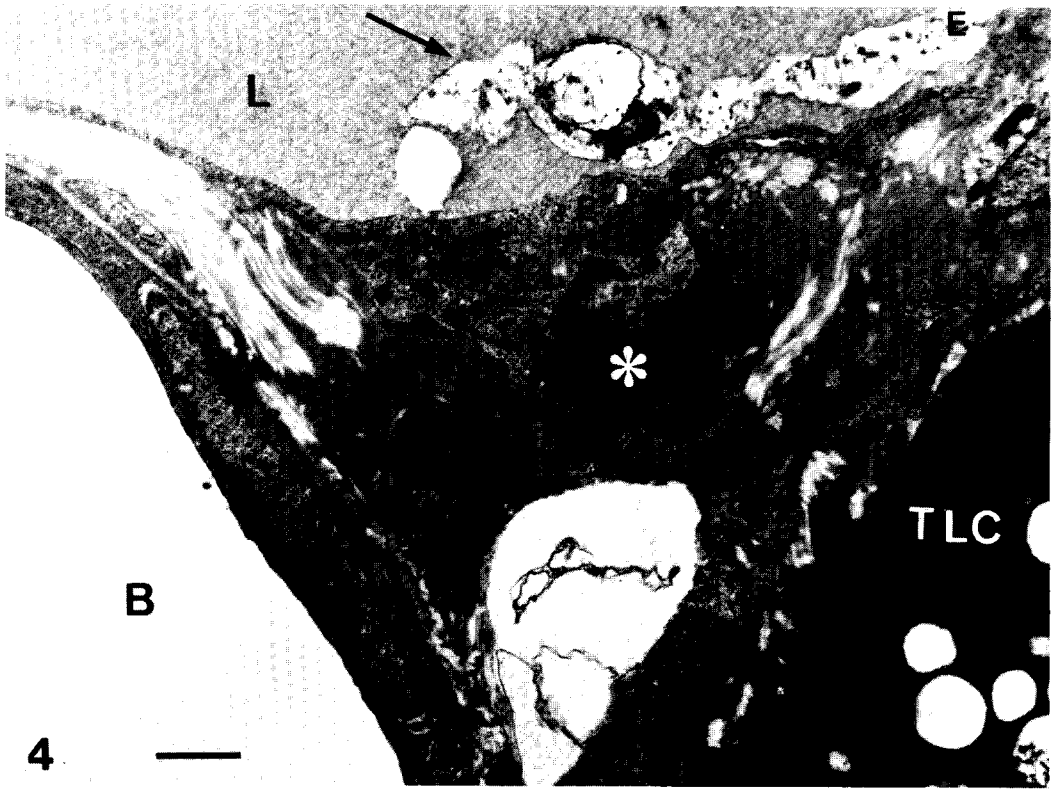


*Fig. 4. Part of a lymphatic capillary (L) and a blood capillary (B) 14 days after HCG injection. The endothelial cell (E) of the lymphatic capillary undergoes conspicuous degeneration (arrow), although that of the blood capillary shows no signs of regression. Dark amorphous material (asterisk) is present in the subendothelial area between the lymphatic capillary and the blood capillary. Theca Lutein cell (TLC) (x9,500) Bar: 1μm.*

corpus luteum, penetrated its substance within 48 hours of ovulation, and invaded radially from the thecal layer towards the center of the corpus luteum. Moreover, Murata (8) noted that such radial lymphatic capillaries in dogs joined to form the central sinus. In our previous study (4), however, rabbit lymphatic capillaries did not invade the membrana granulosa and were not expected to be present in the corpus luteum derived from the membrana granulosa even if they originated from the thecal layer. This discrepancy probably relates to species difference and the difficulty in distinguishing between granulosa and theca lutein cells on light microscopy. It has been shown that the origin of lutein cells and ultrastructural changes in the development of lutein hormones can be differentiated by transmission electron microscopy (9). Along these lines, in the present study, the ultrastructural differences between granulosa and theca lutein cells and the fine distribution of the lymphatic capillaries in the corpus luteum were clearly discernible by electron microscopy. The granulosa lutein cells contained numerous Golgi apparatus and electron-dense bodies, often surrounded by layers of concentrically arranged smooth endoplasmic reticulum and showed peculiar annular and abutment connections. On the other hand, theca lutein cells were similar to ovarian interstitial cells with both containing lipid granules and mitochondria with an electron-dense matrix. Blood capillaries first penetrated the membrana granulosa 11 hours after HCG injection (4) and were arranged radially in the center of the ruptured follicle. However, lymphatic capillaries appeared only in the corpus luteum

derived from the thecal layer on day 3 and did not form a central sinus as described in the corpora lutea of dogs (8). Finally, some of the endothelial cells of the lymphatic but not blood capillaries began to degenerate on day 14.

Several reports have described the ultrastructure of lymphatic vessels in the corpus luteum of rats (7) and dogs (8). These workers noted that the ultrastructure of the lymphatic capillaries in the corpus luteum were similar to those of other organs and that the most significant features of lymphatic capillaries were the open junctions between apposing endothelial cells. Although open junctions were not seen in rabbit intraovarian lymphatic capillaries before HCG injection, as we reported previously (10), they were detected from day 3 on in this study. The lymphatic capillaries in the corpus luteum, therefore, showed ultrastructural changes like those in the theca externa during ovulation (4). Thus, lysosome-like dense bodies and rough endoplasmic reticulum were increased in the endothelium cells of the lymphatic capillaries on day 3, and electron-dense flocculent material accumulated in the subendothelial area and migrated through the intercellular clefts into the lumen of the lymphatic capillaries. These findings suggest that lymphatic capillaries absorb excess interstitial fluid which leaks from the fenestrae of the dilated blood capillaries surrounding the membrana granulosa. On days 5 and 7 when some granulosa and theca lutein cells begin to degenerate, they migrate into the lumina of lymphatic capillaries with open junctions. It is reasonable to conclude that the lymphatic capillaries play an important role in the uptake and transport of hormones presumably secreted from the theca lutein cells as well as excess interstitial fluid and other solutes.

It has previous been shown that macrophages play a key role in the regressing corpus luteum of humans (11), hamsters (12), sheep (13), rats (14), and guinea pigs (15). On the other hand, Koering and Thor (16) report that macrophages and other connective tissue cells are not prominent during regression of the rabbit corpus luteum, suggesting that in the rabbit, unlike other species, macrophages are not critical, and that numerous lysosomes in the lutein cells facilitate luteolysis. In the present study, we often observed that both macrophages and degenerating lutein cells with cytoplasmic lysosomes appeared around and in the lymphatic capillaries and theca layer starting on day 5. Thus in rabbits, the digestion of the corpus luteum during luteolysis appears to involve both physiologic functions.

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