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On the ovarian bursa of the golden hamster. II. Intercellular connections in the bursal epithelium and passage of ferritin from the cavity into lymphatics*

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INTRODUCTION

A previous study demonstrated that the epithelium lining the inner surface of the hamster ovarian bursa is discontinuous and possesses gaps (Shinohara, Nakatani, Morisawa & Matsuda, 1986). These gaps may allow fluid to permeate directly into the connective tissue and facilitate its rapid drainage into lymphatic vessels. However, it is not known how the gaps are formed in the epithelium. According to Leak & Rahil (1978), similar gaps are present in the diaphragmatic mesothelium, and the discontinuous nature of intercellular junctions around the cells may be responsible for the easy separation of mesothelial cells, resulting in the formation of gaps. Although Martin, Sack & Talbot (1981*a*) have also reported the presence of gaps in the bursal epithelium of the golden hamster, they did not refer to details of intercellular connections.

The primary purpose of the present work is to examine whether the mechanism of formation of gaps in the bursal epithelium is related to intercellular connections, i.e. whether intercellular junctions in the bursal epithelium are discontinuous around the cells and labile as in diaphragmatic mesothelium. For this purpose, transmission electron microscopy studies were made of untreated (control) bursae and bursae with cationic ferritin injected into the cavity. In the course of the experiments, the routes by which ferritin drained from the bursal cavity into lymphatics were clarified.

MATERIALS AND METHODS

Animals

Female golden hamsters from 6 to 10 weeks of age were used in this study. The maintenance conditions for the animals have been described previously (Nakatani *et al.* 1985). Hamsters showed four days oestrous cycle and the day when they discharged a post-ovulatory vaginal secretion was designated as Day 1 of the cycle. Hamsters were used for the experiment on Day 4 of the cycle.

Transmission electron microscopy (TEM) of the control bursae

Anaesthetised hamsters were perfused with 0·2 M phosphate buffer solution, pH 7·4, and a fixative consisting of 6·5 % glutaraldehyde and 2·5 % acrolein in phosphate buffer (Sabatini, Bensch & Barrnett, 1963). The bursa was removed and cut into small blocks (1 × 1 × 1 mm) during immersion fixation in fresh fixative. After a rinse

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in phosphate buffer the tissues were postfixed in phosphate buffered 2% osmium tetroxide and stained *en bloc* with uranyl acetate (Revel & Karnovsky, 1967). Dehydration was performed in a graded series of ethanol solutions followed by embedding in Epon 812. Ultrathin sections were stained with uranyl acetate followed by lead citrate and viewed with a Nihondenshi 200CX electron microscope.

Transmission electron microscopy of bursae injected with cationic ferritin

Hamsters were anaesthetised and the ovarian bursa and attached oviduct were mobilised from the abdominal cavity through an incision made on the dorsal abdominal wall. A small incision was made in the ampulla of the oviduct. A 28 gauge needle attached to a microsyringe containing cationic ferritin was inserted into the ampullary lumen through the incision and threaded up to the level of the ostium tubae. Five μl of cationic ferritin (10 mg/ml in 0·15 M NaCl) were injected unilaterally into the bursal cavity. The contralateral (not injected) bursa was used as a control. These manipulations were carried out under a dissecting microscope. Based on results in preliminary examinations of the bursae 5, 10, 20 and 30 minutes after injection with ferritin (see Results), hamsters were killed 5 and 30 minutes later by perfusion with phosphate buffer, pH 7·4, and 2% glutaraldehyde in phosphate buffer. Both the injected and control bursae were isolated, cut into several small blocks, and immersed in fresh fixative. The blocks were rinsed, postfixed, and embedded as described previously. Ultrathin sections were stained only with lead citrate and were viewed with the electron microscope.

RESULTS

Results of preliminary experiments

There were two controls for the bursae injected with ferritin, one obtained from non-injected animals and the other obtained from unilaterally injected animals. The two controls did not differ from each other with respect to the structure of the bursal epithelium. Intervals of 5 and 30 minutes after injection with ferritin were chosen as appropriate times at which to observe the passage of ferritin. Diffusion of ferritin into connective tissue and lymphatic vessels occurred so rapidly that results obtained from any two times with a shorter interval between them tended to lose any clear cut differences; transcellular transport of ferritin did not change remarkably until 30 minutes after injection.

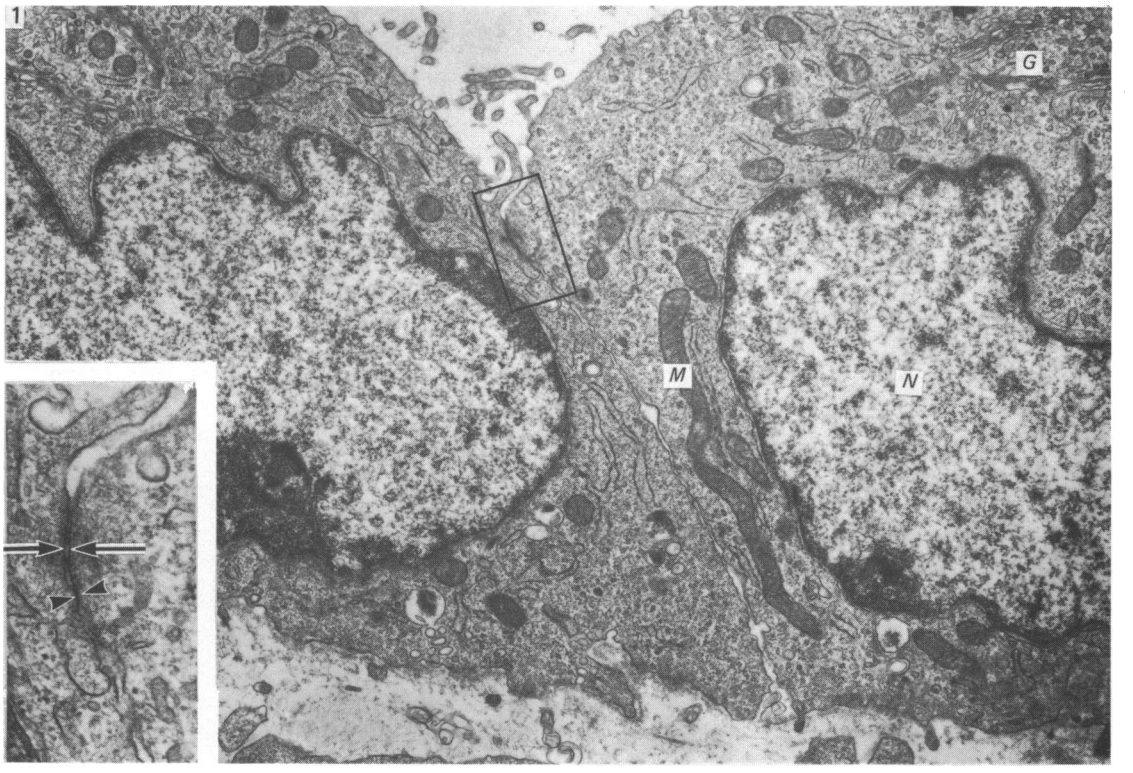
Fig. 1. Contiguous bursal epithelial cells form a zonula adhaerens at the apex of the intercellular space and rarely fuse with each other. Inset: below the zona adhaerens (arrows) membrane fusion is observed (arrowheads). *G*, Golgi complex; *M*, mitochondrion; *N*, nucleus. × 12000; inset: × 39000.

Fig. 2. An intercellular space. A zonula adhaerens is located at the apex (arrows), but the apposing plasma membranes are independent from each other along the entire length of the intercellular space. × 30000.

Fig. 3. A tight junction-like structure. Although it is often difficult to resolve fusion of the outer leaflets of the plasma membranes, bursal epithelial cells are adjoined with tight junction-like structures (inset, arrows) located at various levels of the intercellular space. The cytoplasm of the bursal epithelial cells often contains microfilaments (*Mf*) originating from the cell base. × 9700; inset: × 65000.

Fig. 4. Desmosome-like structures connecting adjacent cells. × 9700; inset: × 65000.

Fig. 5. Gap junctions are frequently encountered. They appear as a septilaminar structure consisting of two closely apposed trilaminar plasma membranes. The entire width of gap junctions measures approximately 18 nm. × 9700; inset: × 65000.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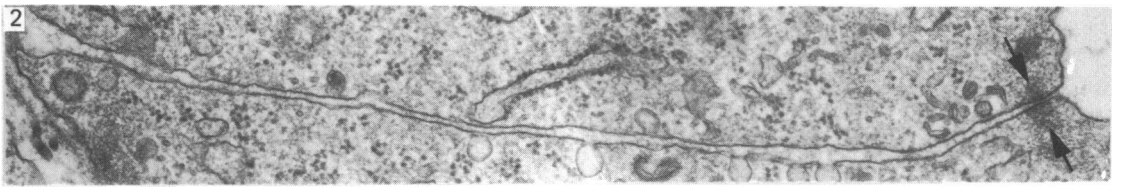
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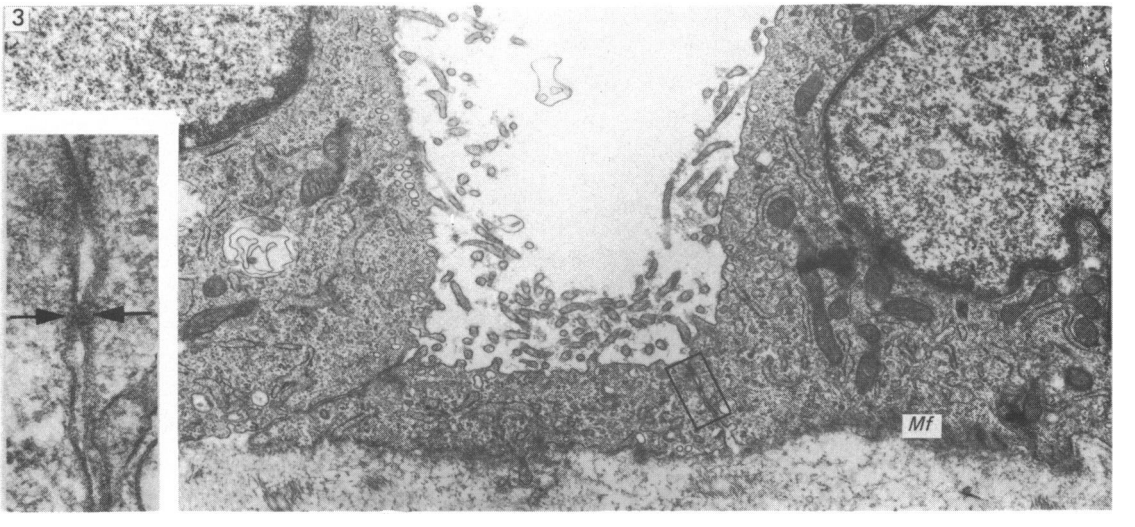
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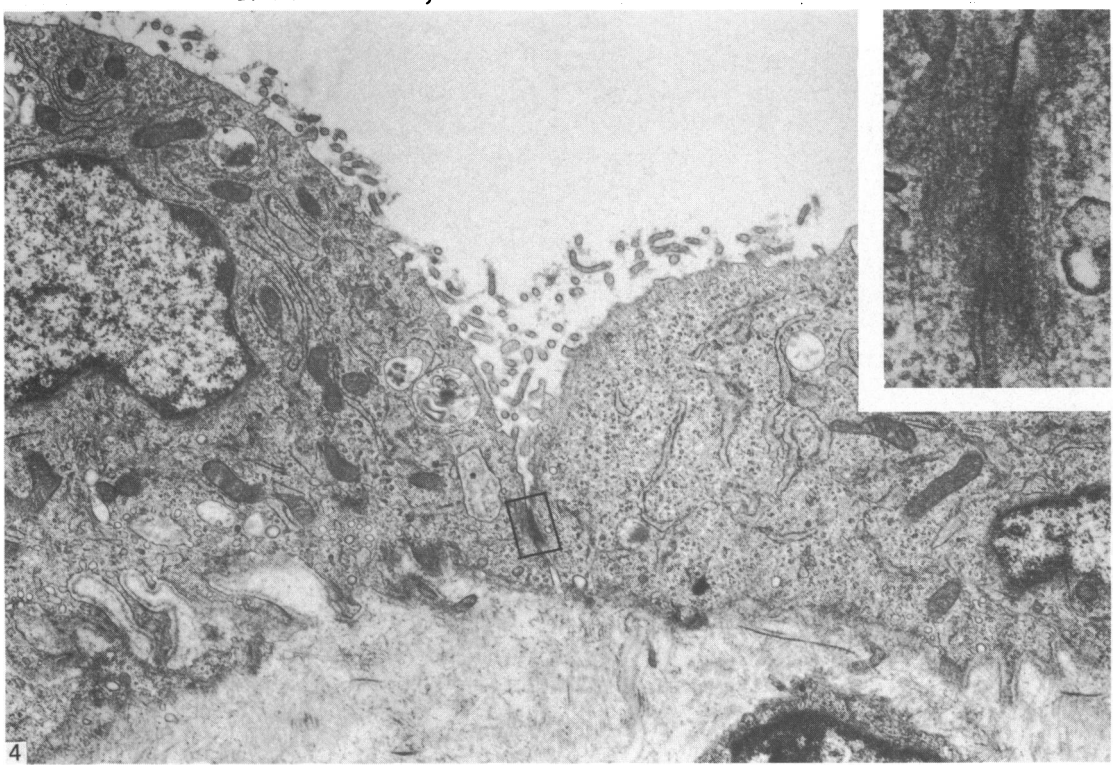

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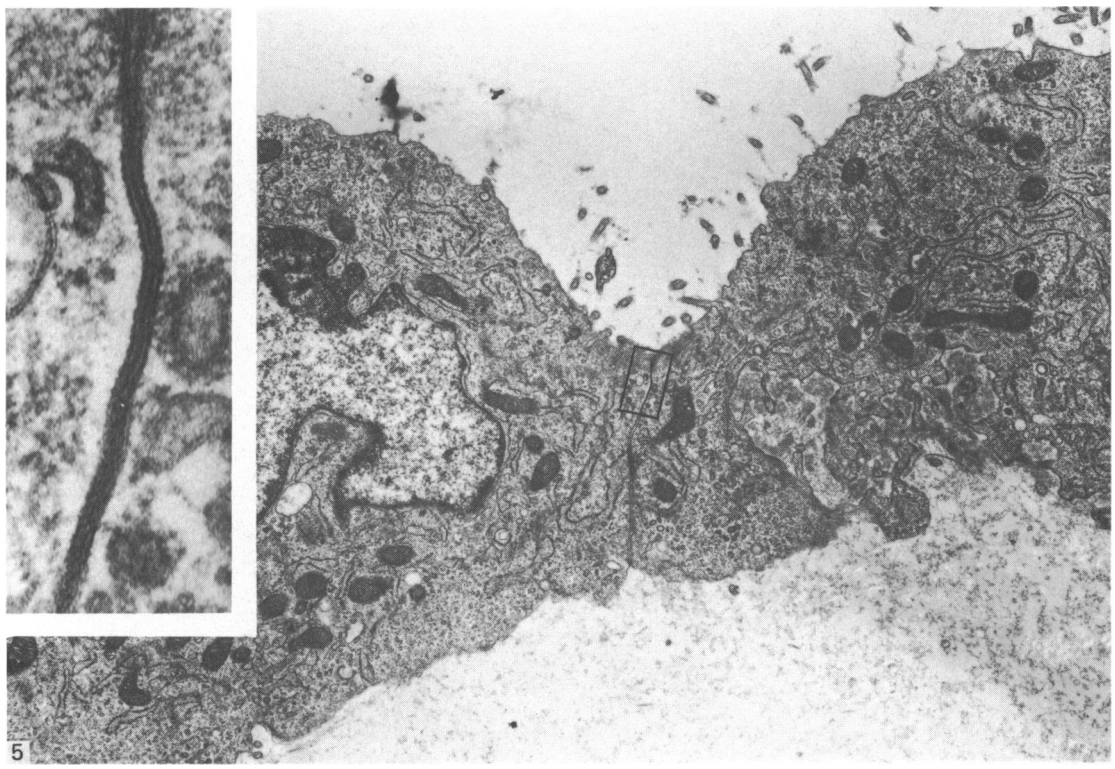

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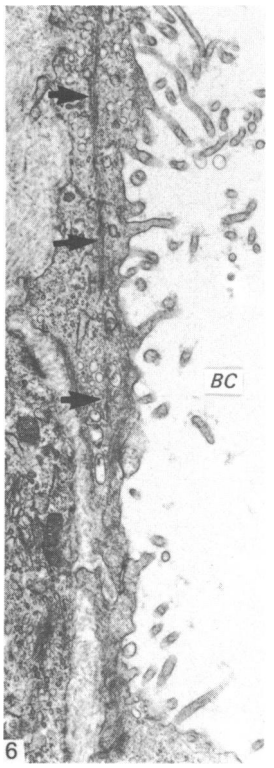

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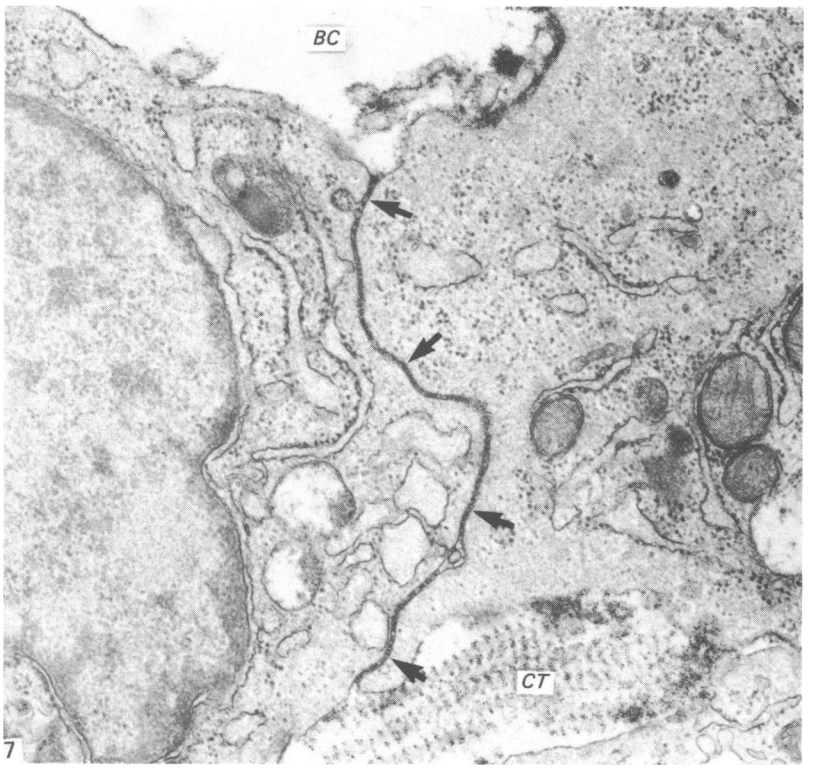

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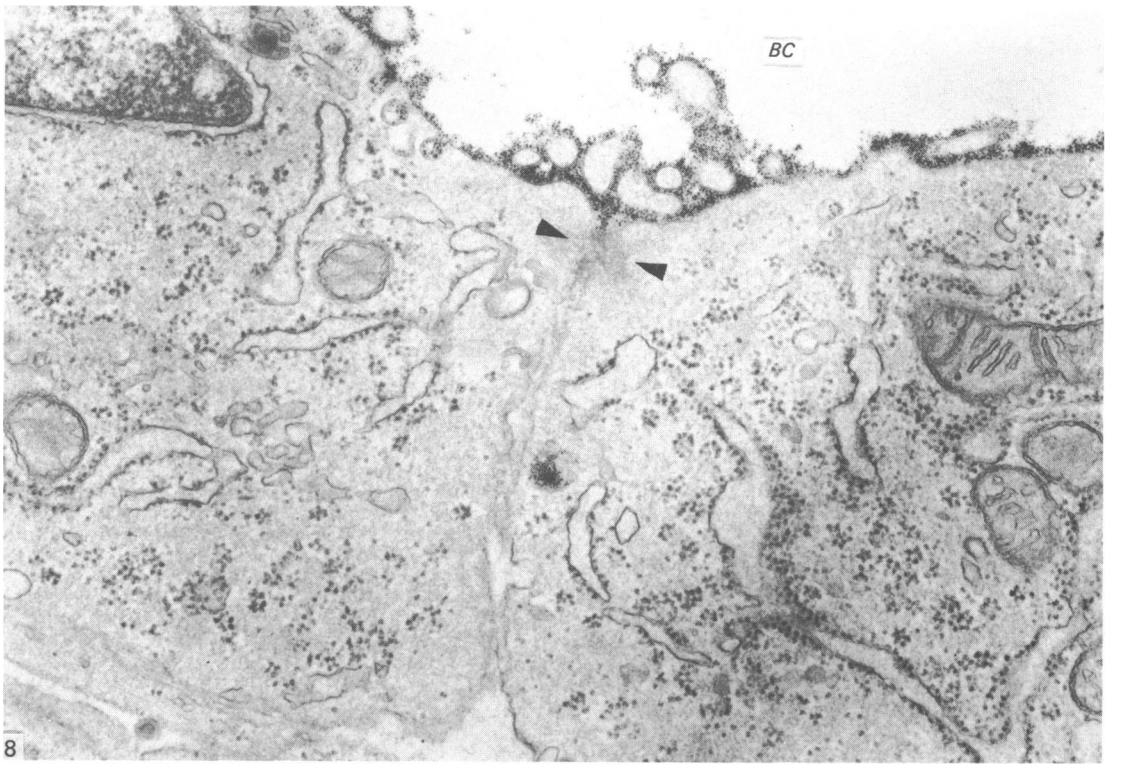

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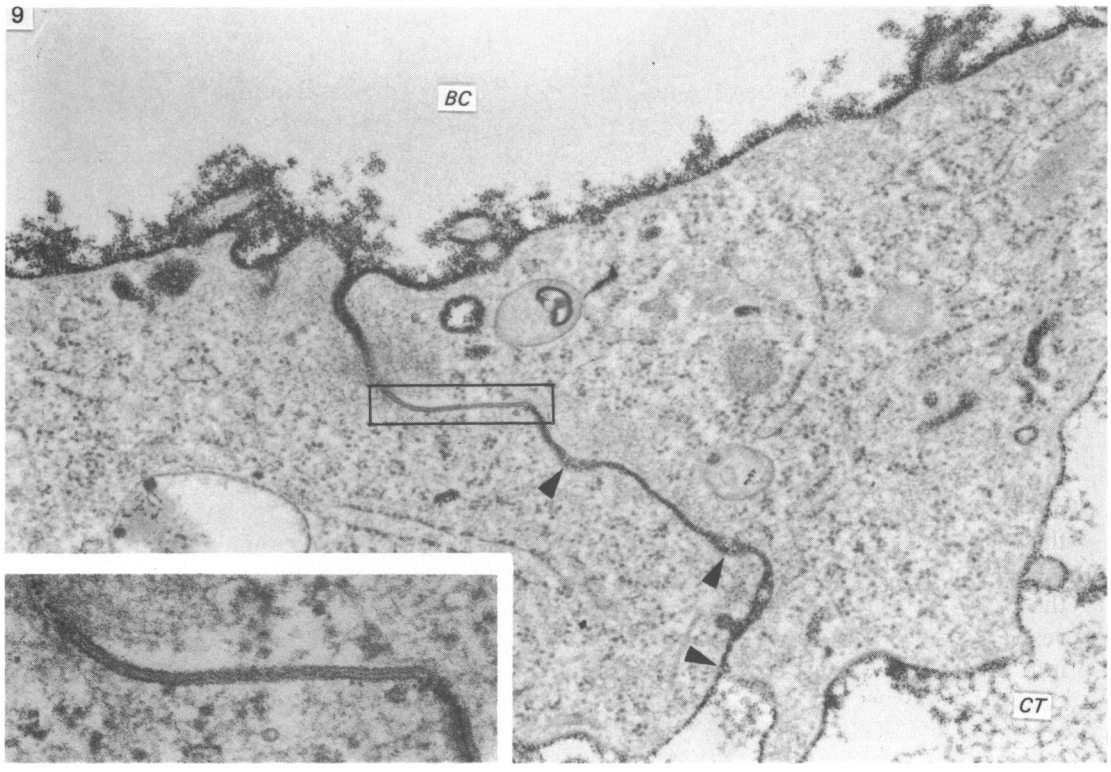
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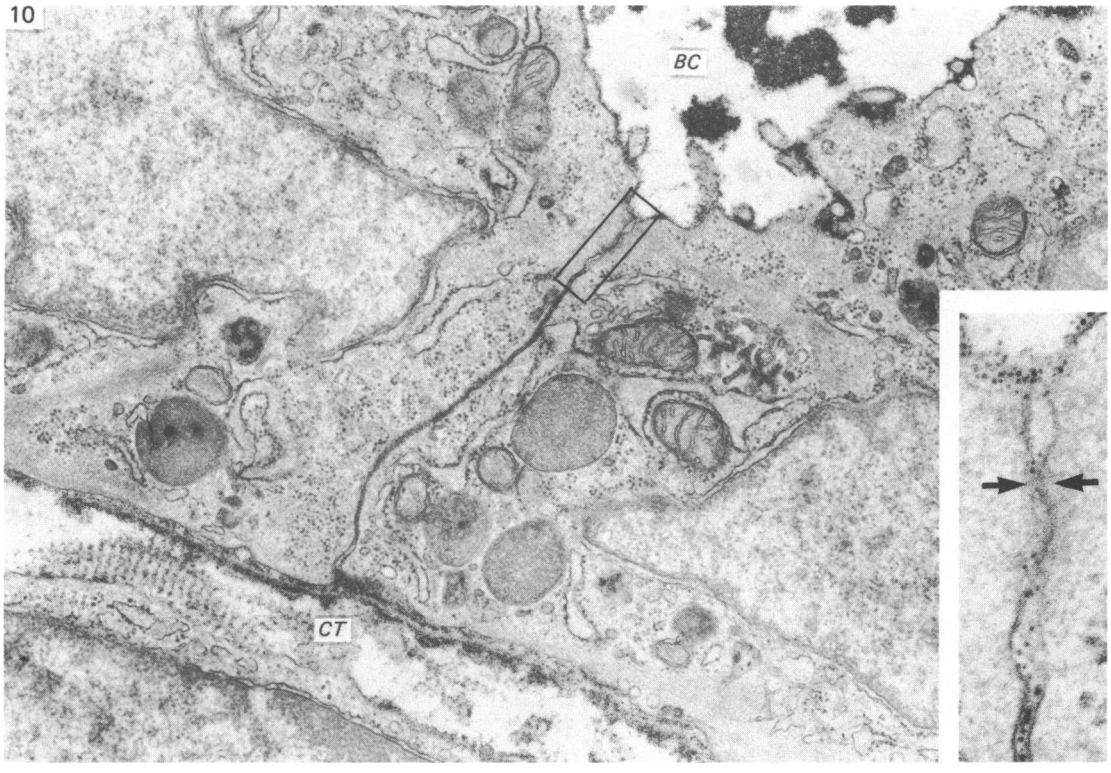
BC
CT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BC
CT

Control bursae

The cuboidal cells of the epithelium lay on a discontinuous basal lamina. Numerous microvilli and thick cytoplasmic processes projected from the free border of the cells into the bursal cavity. Thick processes also extended from the cell base into connective tissue. Adjacent cells abutted to form consistent junctions and, usually, simple intercellular spaces. Intercellular spaces formed by independent plasma membranes of adjacent cells were often encountered. The plasma membranes were closely apposed forming a zonula adhaerens at the apex, but did not always fuse for the entire length of the intercellular space (Figs. 1, 2). The intercellular clefts of the zonulae adhaerentes contained an area of moderate electron density 10–15 nm in width. Tight junctions and desmosome-like structures were located at any level from the apex to the base of the intercellular spaces (Figs. 3, 4). It was not uncommon for the epithelial cells to be joined by gap junctions (Fig. 5). At the site of contact, the junctions were resolved as septilaminar structures consisting of two trilaminar membranes separated by an electron-lucent space approximately 2 nm in width, and the entire width of the structure measured approximately 18 nm. The nuclei of cuboidal cells were indented. Round and elongated mitochondria were seen throughout the cytoplasm. The Golgi apparatus was located above the nucleus. Pinocytotic vesicles were present along the cell border and some opened into the bursal cavity, intercellular space or subcellular spaces. The cytoplasm of the epithelial cells often contained microfilaments (Figs. 3, 6). The microfilaments originated from and extended parallel to the base of the cell. The elongated cells did not differ remarkably from the cuboidal cells except in the following features: (1) they lay on a continuous basal lamina; (2) they rarely extended thick cytoplasmic processes from the cell border.

Bursae injected with cationic ferritin

The distribution of ferritin in intercellular spaces varied depending on the intercellular junctions. Ferritin continuously filled some intercellular spaces from the apex to the base (Fig. 7), while in others, junctions located at the apex blocked diffusion of ferritin (Fig. 8). When an intercellular junction prevented ferritin from filling an intercellular space basal to the junction, ferritin was often, but not consist-

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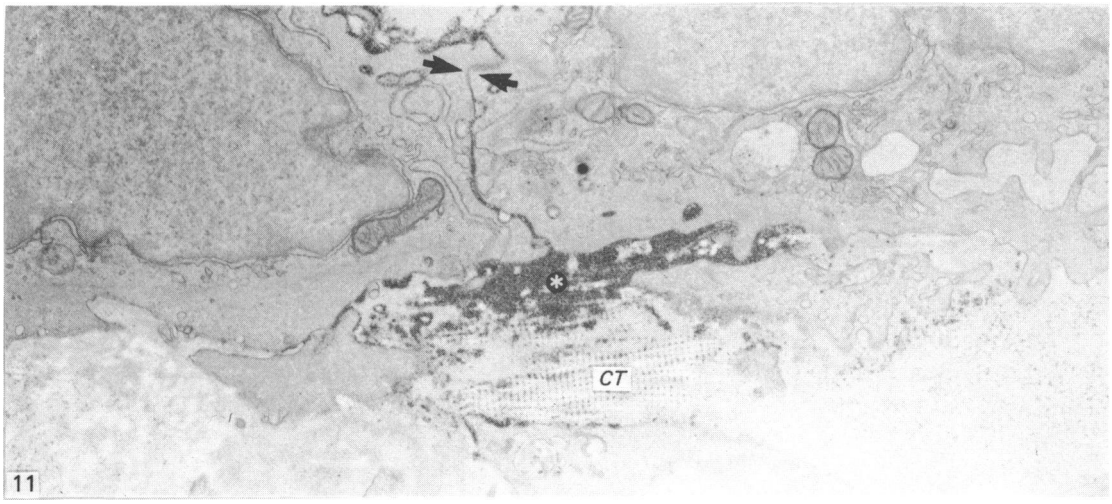
Fig. 6. Microfilaments (arrows) are often located in cytoplasmic processes extending from the lateral border of the epithelial cells. *BC*, bursal cavity. × 12000.

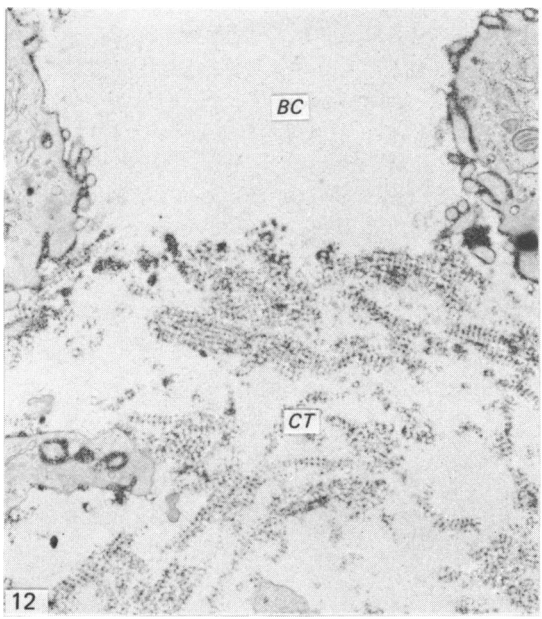
Fig. 7. As early as 5 minutes after injection into the bursal cavity, ferritin particles diffuse into the intercellular space (arrows) and reach the subepithelial connective tissue. Note that pinocytotic vesicles containing ferritin are very few. *BC*, bursal cavity; *CT*, connective tissue. × 30000.

Fig. 8. Thirty minutes after injection. A junction located at the apex of the intercellular space blocks diffusion of ferritin into the junctional area (arrowheads). Note that the intercellular space basal to the junction and the connective tissue near the intercellular space are free of ferritin. *BC*, bursal cavity. × 39000.

Fig. 9. Thirty minutes after injection. Ferritin has not diffused into the area of a (probably, gap) junction. However, the junction has failed to prevent filling of the intercellular space basal to the junctional area (arrowheads). *BC*, bursal cavity; *CT*, connective tissue. × 39000; inset: × 54000.

Fig. 10. Thirty minutes after injection. Another type of intercellular junction has not allowed diffusion of ferritin into the junctional area (inset, arrows), but has allowed filling of the intercellular space basal to the junction. Ferritin has accumulated in the subepithelial connective tissue. *BC*, bursal cavity; *CT*, connective tissue. × 19000; inset: × 95000.





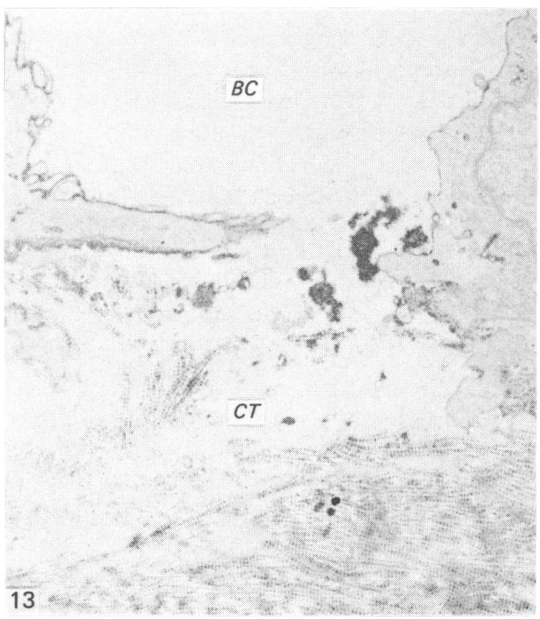
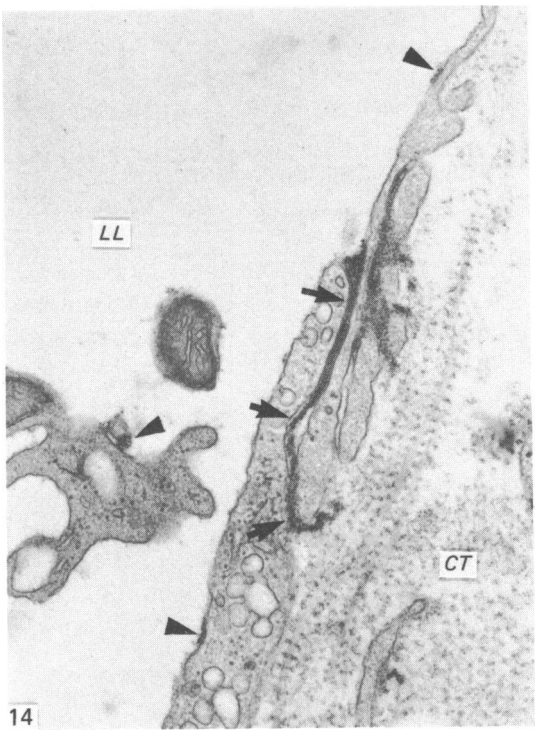


Fig. 11. Thirty minutes after injection. Occasionally, ferritin accumulates more densely in the connective tissue (*CT*) near an intercellular space (*) than the other connective tissue area. Arrows indicate junctional area. × 15000.

Figs. 12–13. Five minutes after injection. The bursal epithelium is discontinuous with gaps and pores in which the subepithelial connective tissue (*CT*) is directly exposed to the bursal cavity (*BC*). Ferritin diffuses rapidly into connective tissue by passing through these gaps (Fig. 12) and pores (Fig. 13). Fig. 12, × 14000; Fig. 13, × 10000.

ently, absent in the connective tissue near the intercellular space (Fig. 8). Although intercellular junctions did not allow permeation of ferritin into the junctional areas, ferritin often filled almost the entire length of an intercellular space except for a junctional area (Figs. 9, 10). In these cases, ferritin was consistently distributed in the connective tissue near the basal part of the intercellular space. Occasionally, ferritin accumulated more densely in the connective tissue near the intercellular



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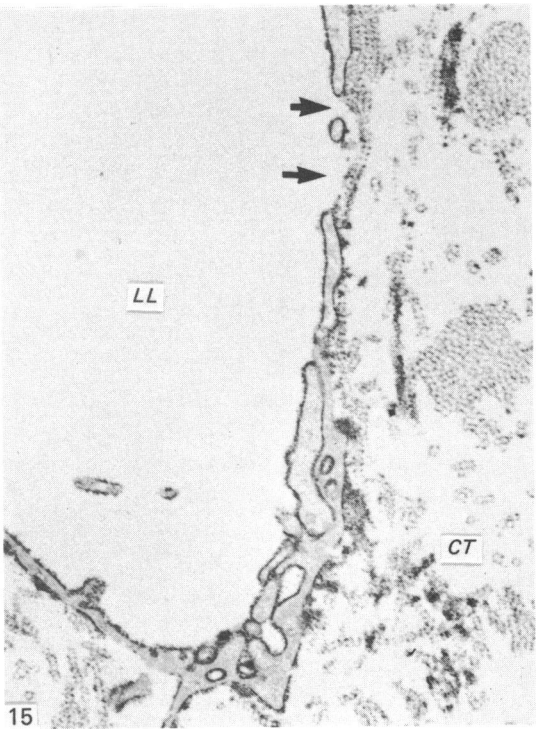

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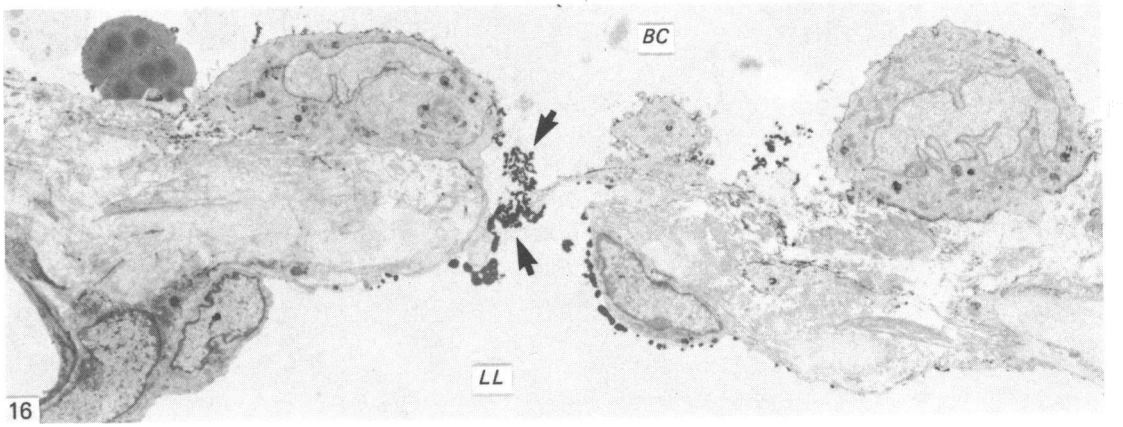

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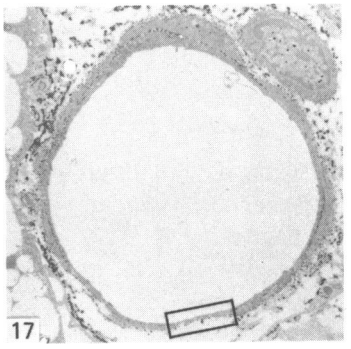
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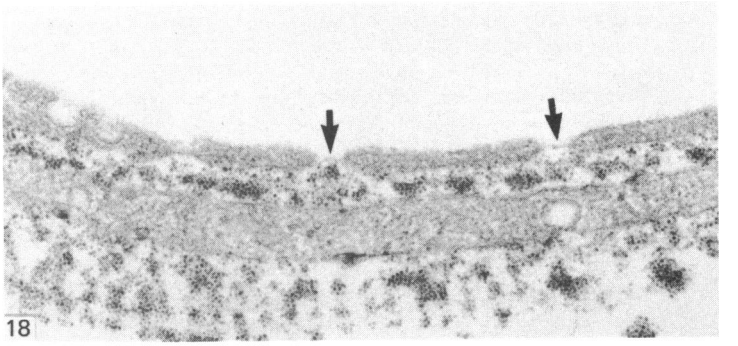
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space than in other connective tissue areas (Fig. 11). This suggested that the junctions might have occupied only part of the juxtaposed surfaces of adjacent cells so permitting ferritin to diffuse continuously through bypasses of the intercellular spaces. Pinocytotic vesicles containing ferritin particles were located along the free, lateral and basal borders of the epithelial cells. However, the number of vesicles in the cytoplasm which contain ferritin 5 minutes after injection was very small (Fig. 7) and the number did not increase remarkably until 30 minutes after injection (Figs. 8–11).

As early as 5 minutes after injection, ferritin diffused widely in the connective tissue by passing through gaps (Fig. 12) and pores (Fig. 13) in the epithelium. Ferritin tended to accumulate along the base of the epithelium and at the periphery of collagen fibrils.

Intercellular clefts in lymphatic endothelium began to be filled with ferritin as early as 5 minutes after injection (Fig. 14), and filling was more extensive 30 minutes after injection. However, all the ferritin might not have reached the surface by passing through intercellular clefts of the endothelium since ferritin could drain more rapidly into lymphatics via stomata (Fig. 16).

Numerous ferritin particles accumulated in the space between the endothelial cells of a fenestrated capillary and its pericytes (Fig. 17) 30 minutes after injection, but they did not enter the capillary lumen across the diaphragm closing the fenestrations (Fig. 18).

DISCUSSION

Intercellular connections in the bursal epithelium were variable as in the peritoneal mesothelium (Baradi & Hope, 1964). Tight junctions, desmosome-like structures, gap junctions and zonulae adhaerentes were common between adjacent epithelial cells. Although a number of tight junctions were encountered in peritoneal mesothelium, Cotran & Karnovsky (1968) believed that they might not be continuous along the entire circumference of the cell since horseradish peroxidase injected into the peritoneal cavity filled an intercellular space from its apex to its base irrespective of the presence of intercellular junctions. In the pericardium, too, horseradish peroxidase injected into the pericardial cavity filled an intercellular space basal to the intercellular junctions, and this was interpreted as suggesting a discontinuity

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Fig. 14. As early as 5 minutes after injection, ferritin has diffused into the intercellular space of the lymphatic endothelium (arrows) and patchy accumulations of ferritin particles are visible on the luminal surface of a lymphatic vessel (arrowheads). *LL*, lymphatic lumen; *CT*, connective tissue. × 31000.

Fig. 15. Numerous ferritin particles are consistently observed on the luminal surface (*LL*) of lymphatic vessels 30 minutes after injection. Intercellular spaces between adjacent endothelial cells are often widely open (arrows). *CT*, connective tissue. × 6200.

Fig. 16. By passing through a stoma, ferritin (arrows) has entered the lymphatic lumen (*LL*) 5 minutes after injection. Draining via stomata may occur immediately after injection of ferritin into the bursal cavity. × 2200.

Figs. 17–18. Numerous ferritin particles have been deposited in the connective tissue around the blood vessel, but none is visible in the lumen (Fig. 17) 30 minutes after injection. A portion of the blood vessel indicated by a rectangle is presented at higher magnification in Figure 18. This shows that ferritin fills the space between the endothelium and pericyte, but does not permeate the diaphragm closing the endothelial fenestrations (arrows). Fig. 17, × 3100; Fig. 18, × 58000.

of the junctions around the cell (Kluge & Hovig, 1968; Kluge, 1969). Indeed, Leak & Rahil (1978) have demonstrated the discontinuous nature of intercellular junctions in the diaphragmatic mesothelium by freeze-fracture techniques, and suggest that intercellular connections in the mesothelium may be labile due to the discontinuity. In the bursal epithelium examined in the present study, intercellular spaces have been seen which are formed by independent plasma membranes of adjacent cells. Ferritin frequently filled the entire length of an intercellular space in spite of the presence of intercellular junctions. Since ferritin had no effect on intercellular junctions as reported by other investigators (Burns & Palade, 1969; Dobbins & Rollins, 1970; Fedorko & Hirsch, 1971), the junctions in the bursal epithelium may indeed be discontinuous and the intercellular connection may be labile as in the diaphragmatic mesothelium.

Cotran & Karnovsky (1968) thought that horseradish peroxidase passed across the mesothelium principally by passive diffusion through intercellular spaces. Ferronha & David-Ferreira (1980) reported transcellular transport of horseradish peroxidase in the ovarian epithelium of the golden hamster. The present results show that transcellular transport of ferritin does not contribute much, if anything, to its passage across the bursal epithelium. In this respect, although the bursal epithelium is continuous with the ovarian epithelium and is not continuous with the peritoneal mesothelium, it shares the characteristics of the mesothelium.

Leak & Rahil (1978) postulated that cells in the diaphragmatic mesothelium may be easily separated due to the labile intercellular connection and hence that gaps may appear. The ovarian bursa of the golden hamster is markedly distended during ovulation and returns to the non-ovulatory state within several hours of the cessation of ovulation, as in mice (Sobotta, 1895) and rats (Long & Evans, 1922). Since ovulation takes place every four days in the golden hamster, the epithelium and connective tissue of the bursa periodically stretch and return to the non-ovulatory state. This periodic change may cause separation of bursal epithelial cells which are loosely interconnected and, thus, gaps may appear.

The exit of fluid from the ovarian bursa has received little investigation. Although Westman (1926) found that bursal fluid might enter lymphatic vessels distributed around the bursa, Wimsatt & Waldo (1945) in mice thought that the fluid drained via a slit-like foramen connecting the bursal and peritoneal cavities. Recently, Martin, Talbot & Pendergrass (1981*b*) hypothesised three possible exits for bursal fluid in the golden hamster: (1) blood vessels; (2) lymphatics; (3) oviducts. Since Evans blue injected into the cavity of the bursa did not enter the oviduct, Martin *et al.* abandoned the third possibility. They observed migration of lanthanum particles across the bursal epithelium and their accumulation in fenestrated blood vessels, indicating that the first possibility was most likely (Martin *et al.* 1981*a*). In the present experiments, ferritin particles injected into the bursal cavity reached lymphatic vessels via three routes: (1) via lymphatic stomata; (2) via gaps and pores; (3) via intercellular spaces. Ferritin may enter lymphatics via stomata almost simultaneously with injection into the bursal cavity and within 5 minutes by diffusion into connective tissue after passing through gaps and pores.

SUMMARY

Cell abutments in the ovarian bursal epithelium of the golden hamster included tight junctions, desmosome-like junctions, gap junctions and zonulae adhaerentes.

The plasma membranes of adjacent epithelial cells were often closely apposed forming a zonula adherens at the apex of the intercellular space, but these did not fuse along the entire length of the intercellular space. Gap junctions and other intercellular junctions did not allow diffusion of ferritin into the junctional areas, but failed to prevent ferritin from filling the intercellular space basal to the junction. This suggested that these junctions were not continuous around the entire circumference of the cell. In general, intercellular connections in the bursal epithelium may be labile and this lability, as well as periodic distention of the bursa, may give rise to separation of epithelial cells.

Ferritin injected into the bursal cavity reached lymphatics via three routes: (1) via stomata; (2) via gaps and pores; (3) via intercellular spaces. Transcellular transport did not make a major contribution to permeation of ferritin across the bursal epithelium, and ferritin particles did not enter fenestrated blood vessels within 30 minutes after injection. The variety of routes may facilitate rapid and complete drainage of fluid and cellular components from the bursal cavity.

REFERENCES

BARADI, A. F. & HOPE, J. (1964). Observations on ultrastructure of rabbit mesothelium. *Experimental Cell Research* **34**, 33–44.

BURNS, R. R. & PALADE, G. E. (1968). Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. *Journal of Cell Biology* **37**, 277–299.

COTRAN, R. S. & KARNOVSKY, M. J. (1968). Ultrastructural studies on the permeability of the mesothelium to horseradish peroxidase. *Journal of Cell Biology* **37**, 123–137.

DOBBINS, III, W. O. & ROLLINS, E. L. (1970). Intestinal mucosal lymphatic permeability: an electron microscopic study of endothelial vesicles and cell junctions. *Journal of Ultrastructure Research* **33**, 29–59.

FEDORKO, M. E. & HIRSCH, J. G. (1971). Studies on transport of macromolecules and small particles across mesothelial cells of the mouse omentum. *Experimental Cell Research* **69**, 113–127.

FERRONHA, M. H. & DAVID-FERREIRA, J. F. (1980). Uptake and transport of an exogenous protein (HRP) by the hamster ovary surface epithelium. *Journal of Submicroscopic Cytology* **12**, 61–71.

KLUGE, T. & HOVIG, T. (1968). Pericardial absorption of exogenous peroxidase in rats. An electron microscopical study. *Acta pathologica et microbiologica scandinavica* **73**, 521–536.

KLUGE, T. (1969). The permeability of mesothelium to horseradish peroxidase. A light and electron microscopic study, with special reference to the morphology of pericardial mast cells. *Acta pathologica et microbiologica scandinavica* **75**, 257–269.

LEAK, L. V. & RAHIL, K. (1978). Permeability of the diaphragmatic mesothelium: the ultrastructural basis for 'Stomata'. *American Journal of Anatomy* **151**, 557–594.

LONG, J. A. & EVANS, H. M. (1922). *Memoirs of the University of California*, vol. 6, pp. 16–32.

MARTIN, G. G., SACK, M. & TALBOT, P. (1981*a*). The structure of bursae ovaricae surrounding the ovaries of the golden hamster. *Anatomical Record* **201**, 485–498.

MARTIN, G. G., TALBOT, P. & PENDERGRASS, P. B. (1981*b*). An intrabursal injection procedure for the *in vivo* study of ovulation in hamsters. *Journal of Experimental Zoology* **216**, 461–468.

NAKATANI, T., SHINOHARA, H., TAKEDA, K., MORISAWA, S. & MATSUDA, T. (1985). Morphology of the intercapsular segment of the oviduct in the golden hamster with special reference to ovum-transit from ruptured follicles to the ampulla. *Experientia* **41**, 268–270.

REVEL, J. P. & KARNOVSKY, M. J. (1967). Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *Journal of Cell Biology* **33**, C7–C12.

SABATINI, D. D., BENSCH, K. & BARRNETT, R. J. (1963). Cytochemistry and electron microscopy: The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *Journal of Cell Biology* **17**, 19–58.

SHINOHARA, H., NAKATANI, T., MORISAWA, S. & MATSUDA, T. (1986). On the ovarian bursa of the golden hamster. I. Scanning electron microscopy of the inner surface and stomatal orifices. *Journal of Anatomy* **147**, 45–54.

SOBOTTA, J. (1895). Die Befruchtung und Fruchtung des Eies der Maus. *Archiv für mikroskopische Anatomie* **45**, 15–93.

WESTMAN, A. (1926). A contribution to the question of the transit of the ovum from ovary to uterus in rabbits. *Acta obstetrica et gynecologica scandinavica* **5**, 3–104.

WIMSATT, W. A. & WALDO, C. M. (1945). The normal occurrence of a peritoneal opening in the bursa ovarii of the mouse. *Anatomical Record* **93**, 47–53.