J. Anat. (1986), **147**, pp. 45–54
With 13 *figures*
Printed in Great Britain

On the ovarian bursa of the golden hamster. I. Scanning electron microscopy of the inner surface and stomatal orifices

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(*Accepted* 26 *September* 1985)

INTRODUCTION

The presence of lymphatic stomata in the diaphragmatic peritoneum was initially reported by von Recklinghausen (1863), but their presence was confirmed ultrastructurally only recently by Leak & Rahil (1978) after more than a century of controversy (Hertzler, 1901; MacCallum, 1903; Chlopin, 1937; Baradi & Hope, 1964). Today, it is known that stomata are also present in the parietal pleura (Wang, 1975). These stomata, which connect the body cavities and lymphatic vessels are considered to play important roles in draining fluids from the cavities into the lymphatics, presumably in association with movements of the diaphragm (Wang, 1975; Bettendorf, 1978, 1979).

Very recently, it was found that the ovarian bursa of the golden hamster also has lymphatic stomata connecting the bursal cavity and lymphatic lumen (Shinohara, Nakatani & Matsuda, 1985). Since the ovarian bursa in the hamster is a completely closed capsule (Clewe, 1965), and since bursal fluid may not enter the oviduct, which opens to the bursal cavity (Martin, Talbot & Pendergrass, 1981; Nakatani *et al.* 1985), the existence of stomata is functionally important for draining bursal fluid as well as cell components. However, many problems remain to be clarified regarding stomata in the hamster bursa. For example, transmission electron microscopy of the bursa has revealed two types of stomatal orifices, one of which is formed between indented walls (or 'lips') of lymphatics and the other between valve-like flaps of lymphatic endothelial cells. However, it is not clear whether these structures imply the existence of two structural categories of stomatal orifices or simply reflect two different aspects of a uniform type of stomatal orifice, i.e. it is unclear whether the structural appearance of stomatal orifices is uniform or variable. Although the size of stomatal orifices that appeared in sections of the bursa varied up to 50 μm in diameter, the authors have noticed that orifices opening between valve-like flaps are generally small compared to those opening between lips. However, this relationship between morphology and size of the stomatal orifices has not been confirmed yet. Obviously, sectioning of the bursa is not an appropriate method with which to clarify these points.

In the present study hamster ovarian bursa have been studied by scanning electron microscopy to perform a more extensive survey of the inner surface of the ovarian bursa and to clarify the surface architecture of the orifices of stomata.

MATERIALS AND METHODS

Animals

Adult, female golden hamsters from 6 to 10 weeks of age were individually caged in an air conditioned room. Water and laboratory chow were supplied *ad libitum*. Lights were switched on at 05.00 hours and off at 19.00 hours. Under these conditions, the hamsters came into oestrus in the evening of every fourth day (Day 1 of the oestrous cycle). If a sticky and viscous secretion was detected in the vagina between 09.00 and 10.00, the animal was considered to be in Day 1 of the oestrous cycle and to have ovulated in the early morning between 01.00 and 04.00 (Harvey, Yanagimachi & Chang, 1961).

Scanning electron microscopy (SEM)

Hamsters were anaesthetised with ether vapour and perfused with 0·2 M phosphate buffer solution, pH 7·4, and 2 % glutaraldehyde in phosphate buffer. The ovarian bursa, which was approximately 3 mm in diameter, was cut into upper (rostral) and lower (caudal) halves. The tissues were immersed in fresh glutaraldehyde, rinsed in phosphate buffer, postfixed in 2 % osmium tetroxide in phosphate buffer, dehydrated in a graded series of ethanol solutions, and placed in isoamyl acetate. They were subjected to critical point drying in the gaseous–liquid phase of carbon dioxide and gold coated in an ion sputtering chamber. Sixteen bursae obtained from eight hamsters on Day 1 (10.00 hours) of the oestrous cycle were thoroughly examined using a scanning electron microscope.

Transmission electron microscopy (TEM)

To compare the structural appearance of stomatal orifices viewed by SEM and TEM, a few bursae were processed for TEM. Small blocks of ovarian bursae were obtained from two hamsters on Day 1 of the cycle after perfusion fixation. The blocks were rinsed, postfixed, and dehydrated as in SEM. They were embedded in Epon 812 and ultrathin sections were stained with lead citrate and uranyl acetate.

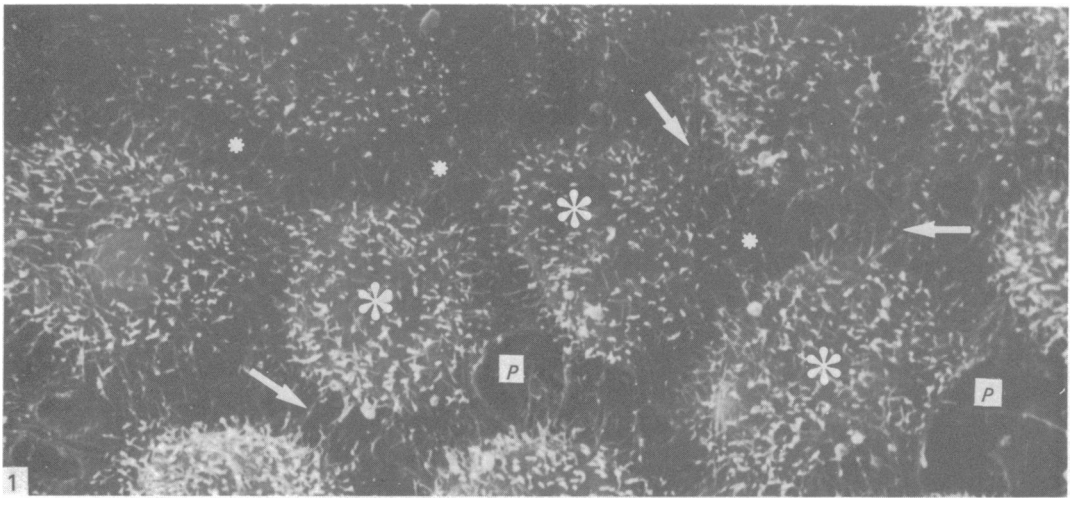
Fig. 1. Cuboidal epithelial cells lining the inner surface of the bursa consist of a dome shaped central region (*) and a perimeter (*). Microvilli are numerous in the central region, but sparse in the cell perimeter. Cytoplasmic processes extending from the lateral walls of adjacent central regions are interconnected forming a loose network on the surface of the cell perimeters (arrows). *P*, pore or circular defect in the epithelium. × 4800.

Fig. 2. The bursal epithelium is discontinuous due to the presence of crevice-like discontinuities or gaps (*G*) and pores (*P*). The gaps suggest that some epithelial cells have fallen off the surface exposing subepithelial connective tissue. Either a stomatal orifice (small arrow) or connective tissue (large arrows) is visible through the pores. Gaps and pores are often continuous. × 1200.

Fig. 3. The epithelial lining is also interrupted by the presence of smooth surfaced areas (*SSA*). These areas are not found in all bursae examined, but if they are present, circular (large arrows) or jagged (small arrows) stomatal orifices consistently open in them. × 240.

Fig. 4. A stomatal orifice (*S*) in the smooth surfaced area (*SSA*) is often separated into several smaller compartments by bridges (solid arrows). The smooth surfaced area is continuous from the floor of the lymphatic lumen to the inner surface of the bursa (open arrows). × 1200.

Fig. 5. Another smooth surfaced area (*SSA*) with stomata (*S*). Blood cell components (arrows) may enter the lymphatic lumen from the bursal cavity. Some red blood cells are already in the lymphatic lumen. × 1700.



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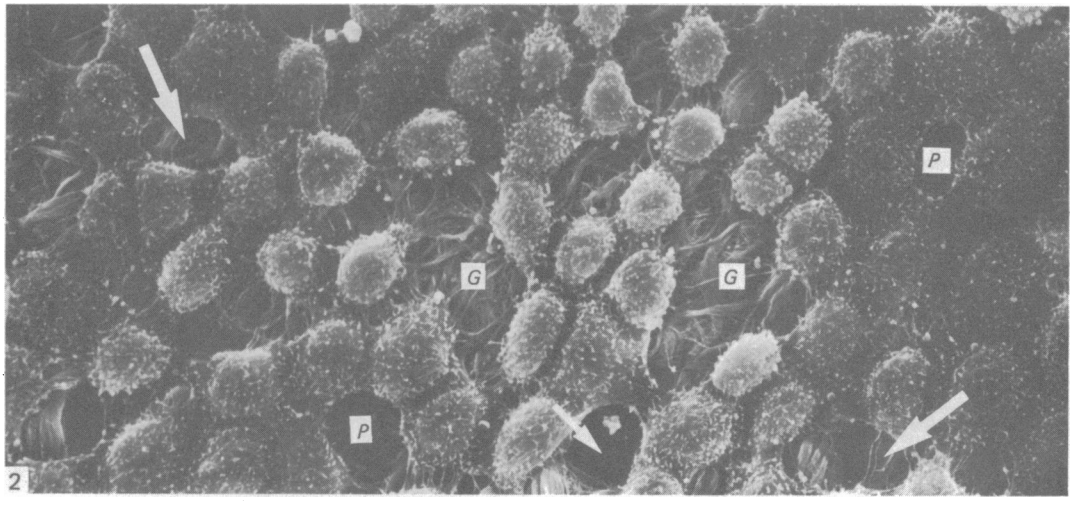

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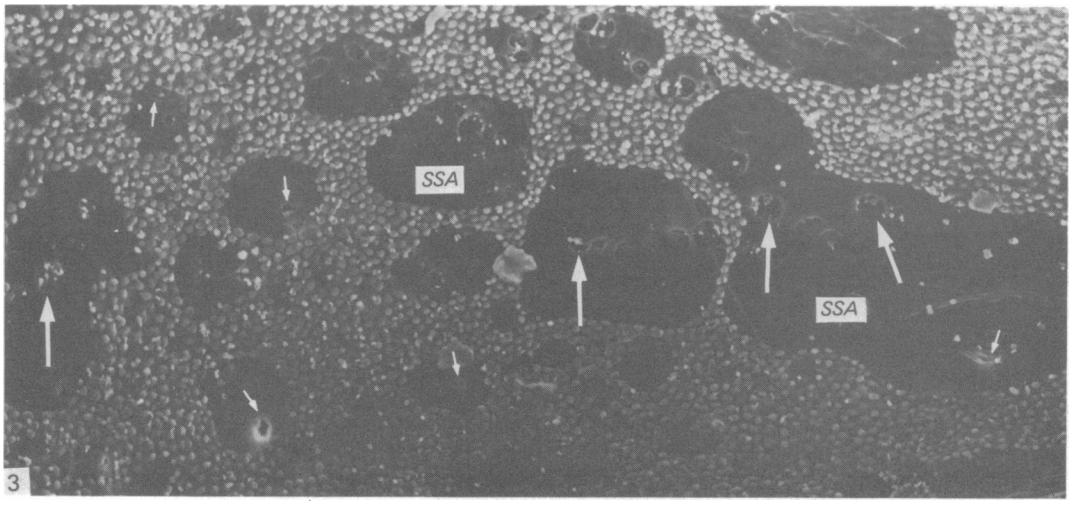

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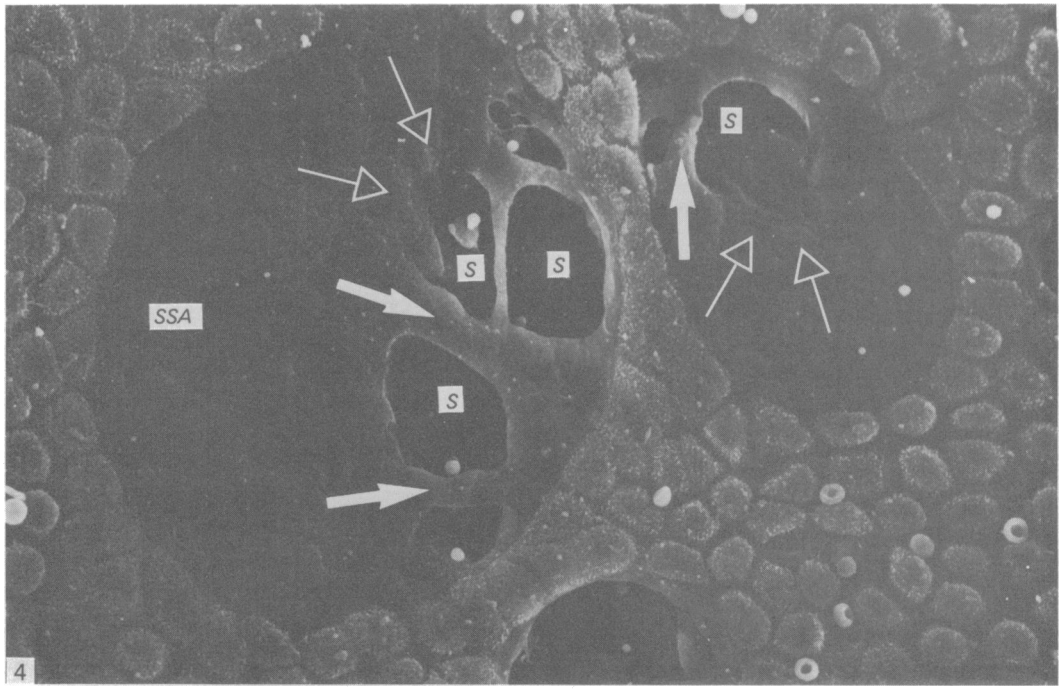

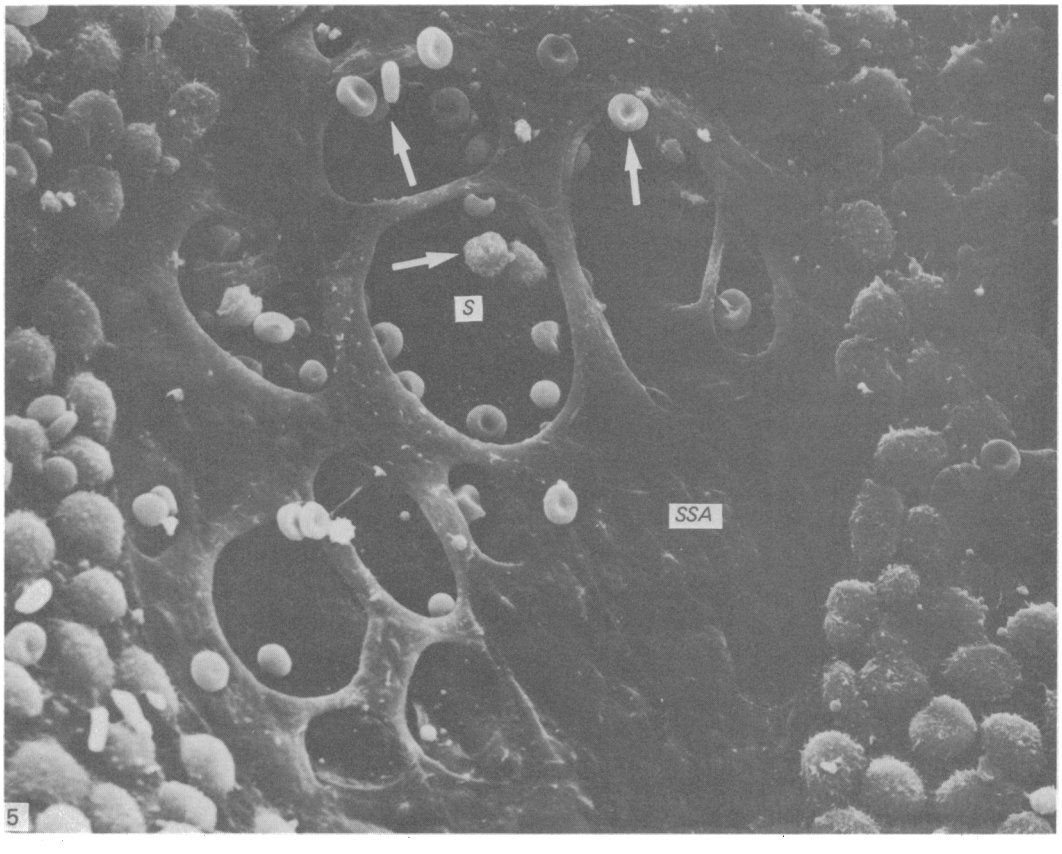

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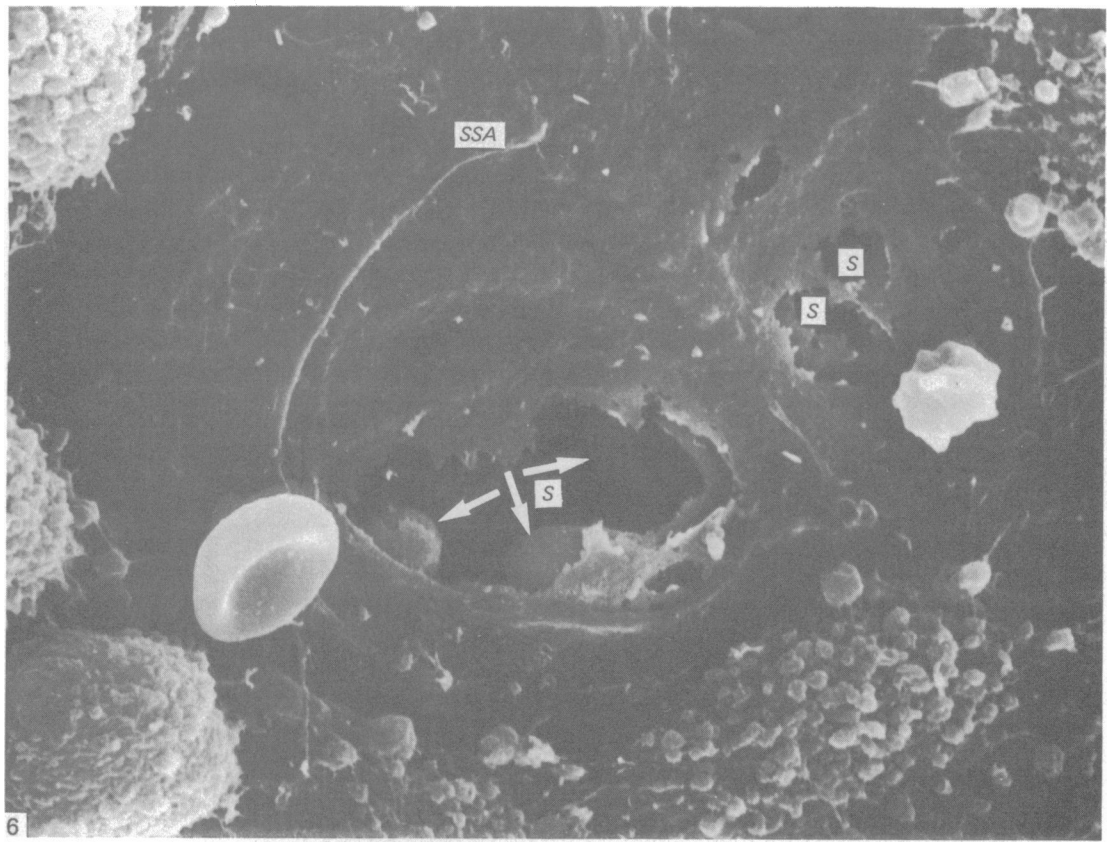

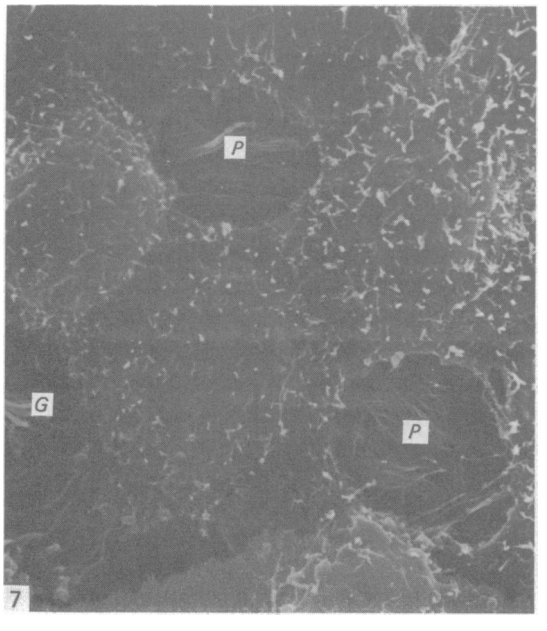

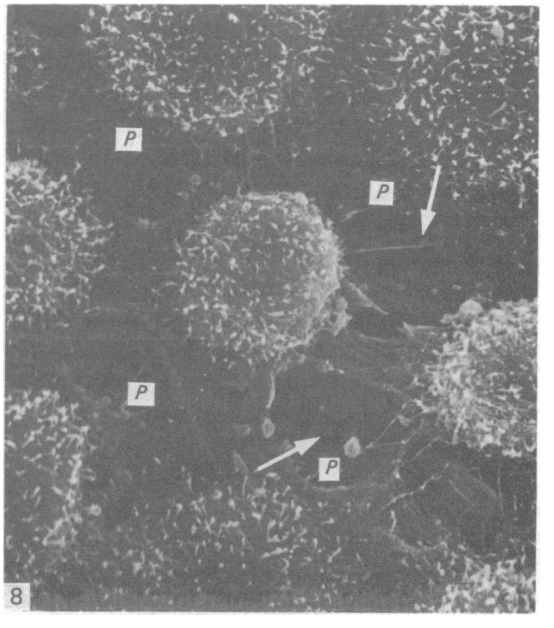

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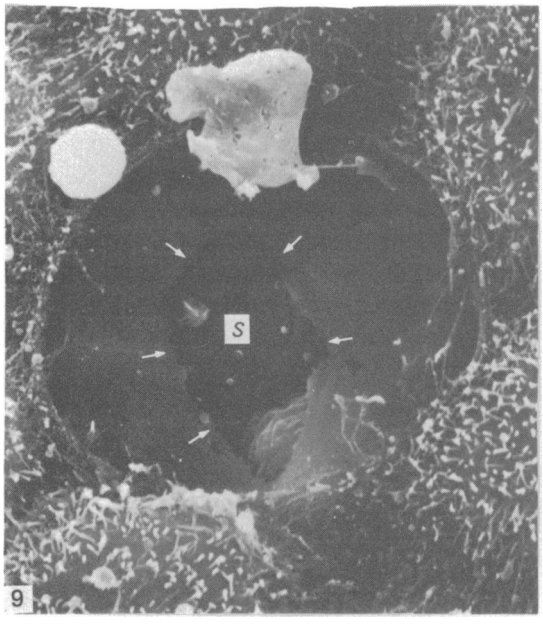

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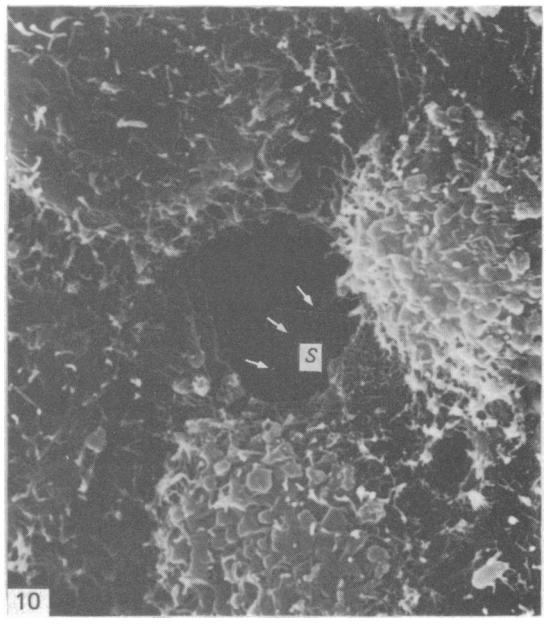

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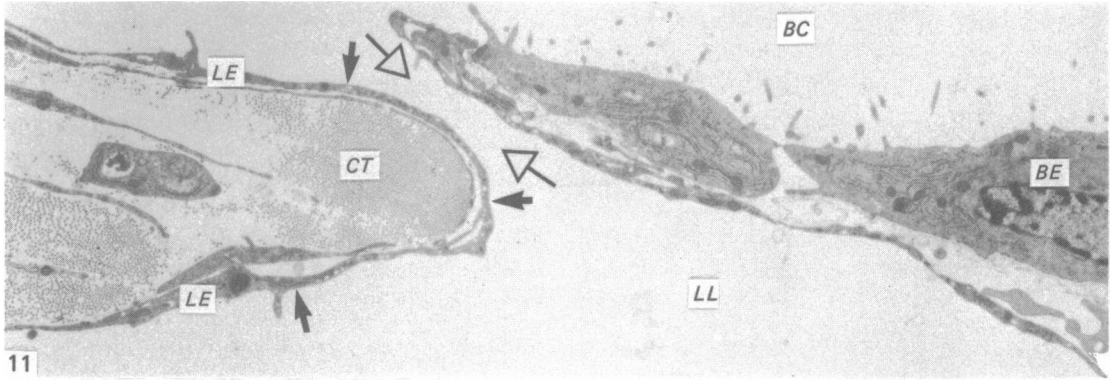

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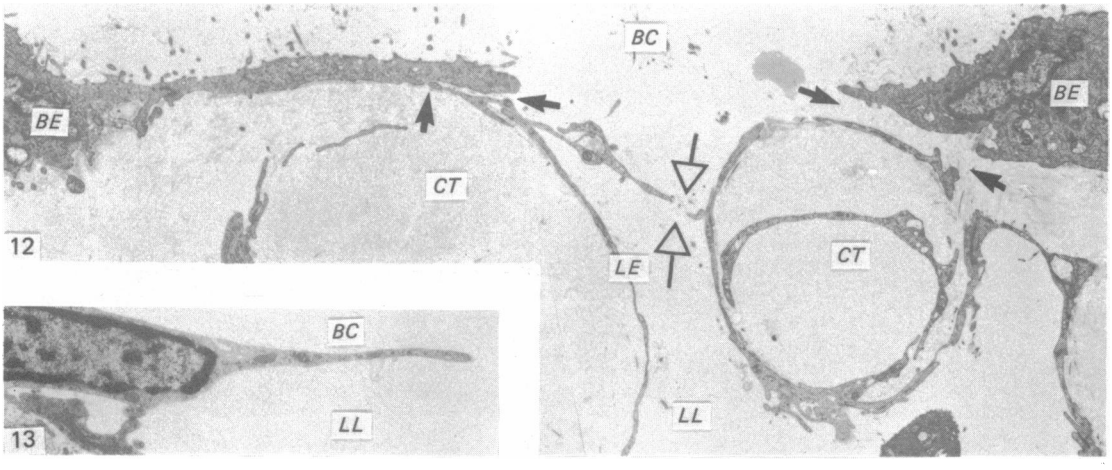

RESULTS

Scanning electron microscopy

A major part of the inner surface of the bursa was lined with epithelial cells with a dome shaped central region and a flatter perimeter (Fig. 1). The central region projected into the bursal cavity and the apical surface was studded with numerous microvilli. The perimeters of the cells, 'paved' the areas between adjacent central regions. Numerous cytoplasmic processes extended from the lateral walls of adjacent central regions and interconnected, forming a loose network on the relatively microvilli-free surfaces of the cell perimeters.

This lining of epithelial cells was discontinuous. Crevice-like discontinuities or gaps were formed between irregularly spaced epithelial cells (Fig. 2). It appeared as though some epithelial cells had detached from the surface to expose the subepithelial connective tissue to the bursal cavity. These gaps were found especially in the upper half of the bursa.

The epithelial lining was also interrupted by smooth surfaced areas (Fig. 3). These areas were usually circular in shape and more than 10 μm in diameter. The number of such areas in a bursa varied from 0 to 156 and differed even between two bursae obtained from the same animal. In two hamsters (4 bursae) no area like this was found. Either circular orifices with a round edge (Figs. 4, 5) or jagged orifices with a sharp edge (Fig. 6) consistently opened into the smooth surfaced areas. Most orifices were less than 50 μm in diameter. Since bursae were sampled several hours after ovulation had finished, numerous blood cells still remained within the cavity of the bursae. Blood cell components were frequently located on the floor of the lymphatic lumen, which was continuous with the bursal cavity through the orifices (Figs. 4–6).

Circular defects or pores in the epithelial lining were up to 20 μm in diameter. Either underlying connective tissue (Fig. 7) or a stomatal orifice (Figs. 8–10) was

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Fig. 6. Although stomatal orifices (*S*) opening in smooth surfaced areas (*SSA*) are predominantly circular, there are also jagged orifices as shown in this Figure. A few red blood cells that have already entered the lymphatic lumen (arrows) are visible through a jagged orifice. × 7100.

Fig. 7. Pores (*P*) exposing the subepithelial connective tissue. Pores are usually circular in shape and less than 20 μm in diameter. Their margins are made up mostly of the perimeters of cuboidal epithelial cells. *G*, Gap. × 7000.

Fig. 8. Jagged stomatal orifices (arrows) usually open in pores (*P*). They are less than 10 μm in diameter. × 4000.

Fig. 9. Another jagged stomatal orifice (*S*) opening in a pore. The floor of the lymphatic lumen is seen through the orifice. The orifice looks like the opening of a bicuspid valve (arrows). × 5500.

Fig. 10. A circular stomatal orifice (*S*) opening in a pore. Arrows indicate the margin of the orifice, which is partly hidden by the margin of the pores. This type of orifice is occasionally encountered. × 6800.

Fig. 11. Transmission electron micrograph of a stomatal orifice (indicated by open arrows). Note that the endothelium (LE) of the lymphatic vessel forms a continuous lining for the inner surface of the bursa (small arrows). This orifice corresponds to the circular stomatal orifices seen by SEM. *BC*, bursal cavity; *BE*, bursal epithelial cell; *CT*, connective tissue, *LL*, lymphatic lumen. × 5600.

Fig. 12. A stomatal orifice opening between valve-like flaps of lymphatic endothelial cells (indicated by a pair of open arrows). This type of orifice is usually small in size and its architecture appears to be compatible with that of the jagged stomatal orifices in SEM. Abbreviations as in Figure 11 × 4600.

Fig. 13. A higher magnification of another valve-like flap. Abbreviations as in Figure 11. × 8000.

visible through the pore. Stomatal orifices opening in pores were either circular or jagged, but the latter type of orifice was far more common. Parts of the circular orifices were often hidden by the margins of pores, so their sizes were not always measured accurately. Jagged orifices did not exceed 10 μm in diameter. Due to the number and variation in size of pores, the number of pores through which stomatal orifices were visible was not counted.

Transmission electron microscopy

Sections cut in a plane passing through the smooth surfaced area revealed that the circular stomatal orifices seen by SEM appeared as stomatal orifices formed between indentations or 'lips' of the lymphatic wall as seen by TEM; the smooth surfaced areas, in SEM, consisted of the endothelium extending continuously from the walls of the lymphatics to the inner surface of the bursa (Fig. 11).

A definite correspondence between the jagged orifices in SEM and orifices in TEM was technically difficult to determine because of their size. However, narrow orifices formed between valve-like flaps of lymphatic endothelial cells in TEM (Figs. 12, 13) were compatible with the jagged orifices judging from their size and frequency and from the absence of other types of stomatal orifices in TEM.

DISCUSSION

Stomatal orifices in the hamster bursa can be categorised into two types: (1) large, circular orifices opening predominantly in smooth surfaced areas; (2) small, jagged orifices, the majority of which open in pores. This difference in the size and shape of stomatal orifices seems to suggest differences in their nature and function. Circular orifices may allow passage of large particles as well as fluid, while jagged orifices may allow passage of fluid and only relatively small particles. Indeed, red blood cells pass frequently through circular orifices, but rarely through jagged orifices. However, circular orifices do not seem to be indispensable for draining blood cell components, and it does not appear that jagged orifices do not allow the passage of blood cells, because circular stomata were completely absent in two hamsters out of the eight examined. The stomatal orifices in these hamsters were mostly jagged orifices opening in pores. According to Leak & Rahil (1978), stomata in the diaphragmatic peritoneum are persistent structures that may open irrespective of the movements of the diaphragm. In contrast, Bettendorf (1978, 1979) suggested that stomata open to allow the passage of abdominal fluid and of latex beads injected into the peritoneal cavity during expiration and that they close during inspiration. Recently, Tsilibary & Wissig (1983) suggested that stomata in the peritoneum are not stable openings, but that their patency may vary in response to changes in the abdominal cavity; actin components in mesothelial and lymphatic endothelial cells may control their patency. The structural appearance of circular orifices in the hamster bursa suggests that they are stable openings and supports the view proposed by Leak & Rahil (1978). However, this may not be true in the case of jagged stomatal orifices. The correspondence in the structural appearance of jagged orifices seen by SEM with that of orifices formed between valve-like flaps of lymphatic endothelial cells seen by TEM suggests that the degree of opening in jagged orifices may change in response to the bursal fluid volume, intrabursal pressure and other factors in the bursal cavity. As in the ovarian bursa of the golden hamster, stomatal orifices in the diaphragmatic peritoneum may not be homogeneous in their structure and, possibly, function.

It was surprising to find smooth surfaced areas lined with lymphatic endothelium on the inner surface of the bursa. It is considered that the area may appear during development of the ovarian bursa since it was observed even in prepubertal hamsters 24 days postpartum (authors' unpublished data).

SUMMARY

The inner surface of the ovarian bursa in the golden hamster was observed by scanning electron microscopy. There were numerous discontinuities in the bursal epithelium. Crevice-like gaps were formed between irregularly spaced epithelial cells, and the subepithelial connective tissue was exposed to the bursal cavity through the gaps. Through circular defects in the epithelial lining or pores, which were less than 20 μm in diameter, either the subepithelial connective tissue or stomatal orifices were visible. There were smooth surfaced areas lined with lymphatic endothelium, instead of bursal epithelium, which was continuous from the wall of the lymphatic vessel to the inner surface of the bursa. These areas were not present in all bursae, but if they were present, stomatal orifices consistently opened in them. Stomata in the ovarian bursa had two types of orifices, (1) circular orifices opening predominantly in smooth surfaced areas and measuring up to 50 μm in diameter; (2) jagged orifices opening usually in pores and measuring less than 10 μm in diameter. Blood cell components derived from ovulation entered lymphatics via stomata. Bursal fluid and small particles may drain into lymphatics directly via stomata and indirectly by diffusion through gaps, pores and connective tissue. Judging from the structural appearance of the stomatal orifices, the degree of opening of jagged orifices may change in response to changes in the cavity, while circular orifices may be stable openings.

We thank Mr T. Horii, Miss H. Matsuda, Miss A. Masuda and Mrs M. Shinohara for technical assistance. We also thank Dr C. A. Mahi-Brown for revision of the manuscript.

REFERENCES

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

BETTENDORF, U. (1978). Lymph flow mechanism of the subperitoneal diaphragmatic lymphatics. *Lymphology* **11**, 111–116.

BETTENDORF, U. (1979). Electronmicroscopic studies on the peritoneal resorption of intraperitoneally injected latex particles via the diaphragmatic lymphatics. *Lymphology* **12**, 66–70.

CHLOPIN, N. G. (1937). Über Regenerationsprozesse im Mesothel und die Bedeutung der Serosadeckzellen. *Beitrage zur pathologischen Anatomie und zur allgemeinen Pathologie* **98**, 35–64.

CLEWE, T. H. (1965). Absence of a foramen in the ovarian bursa of the golden hamster. *Anatomical Record* **151**, 446.

HARVEY, E. B., YANAGIMACHI, R. & CHANG, M. C. (1961). Onset of estrus and ovulation in the golden hamster. *Journal of Experimental Zoology* **146**, 231–235.

HERTZLER, A. E. (1901). The morphogenesis of the stigmata and stomata occurring in peritoneal and vascular endothelium. *Transactions of the American Microscopical Society* **22**, 63–92.

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

MACCALLUM, W. G. (1903). On the mechanism of absorption of granular materials from the peritoneum. *Bulletin of the Johns Hopkins Hospital* **14**, 105–115.

MARTIN, G. G., TALBOT, P. & PENDERGRASS, P. B. (1981). 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**, 368–370.

SHINORAHA, H., NAKATANI, T. & MATSUDA, T. (1985). The presence of lymphatic stomata in the ovarian bursa of the golden hamster. *Anatomical Record* **213**, 44–52.

TSILIBARY, E. C. & WISSIG, S. L. (1983). Lymphatic absorption from the peritoneal cavity: regulation of patency of mesothelial stomata. *Microvascular Research* **25**, 22–39.

von Recklinghausen, F. (1863). Zur Fettresorption. *Archiv für pathologische Anatomie und Physiologie* **26**, 172–208.

WANG, N. (1975). The preformed stomas connecting the pleural cavity and the lymphatics in the parietal pleura. *American Review of Respiratory Diseases* **111**, 12–20.