The Structure of Bursae Ovaricae Surrounding the Ovaries of the Golden Hamster

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ABSTRACT The ovaries of many mammals lie within membranous sacs called bursae ovaricae. In this study, we have examined the morphology of the bursa surrounding the hamster ovary using light and electron microscopy. The bursa is composed of three layers: (1) an inner, discontinuous bursal epithelium that faces the ovary; (2) a middle layer of connective tissue that contains fibroblasts, bundles of smooth muscle cells, and blood vessels; and (3) an outer, continuous epithelium that faces the peritoneal cavity. One side of the bursa has a thin layer of connective tissue, and because the ovary may be seen through it, we refer to this region of the bursa as the “window.” Elsewhere a thick layer of fat joins the connective tissue and blocks visualization of the ovary. Tracers (Evans blue and lanthanum) applied to the peritoneal surface do not penetrate beyond the peritoneal epithelium. Tracers injected into the bursal cavity penetrate all layers of the bursa, but do not pass through the peritoneal epithelium. Therefore, the bursa prevents tracer exchange between the bursal and peritoneal cavities, but exchange does take place between the bursal cavity and blood vessels within the bursa. We suggest that bundles of smooth muscle cells within the bursa may serve to regulate fluid volume and pressure within the bursal cavity. Possible functions of the complete bursa in the hamster are discussed.

The ovaries of some mammals are enclosed within membranous sacs called bursae ovaricae, which partly or completely isolate the ovary from the peritoneal cavity (Mossman and Duke, 1973). For convenience, we will refer to the wall of these sacs as the bursa and the chamber containing the ovary as the bursal cavity.

Although the function(s) of the ovarian bursa have not been clarified, investigators have suggested that it aids in collection of oocytes by the oviduct [see (Beck, 1972; Alden, 1942) for references] or is required for normal ovarian and/or follicular development. The former idea would seem to be correct at least in the case of the complete bursa which prevents oocytes from escaping into the peritoneal cavity. The latter idea is supported by the following experimental studies of Butcher (1947). First, when bursae were removed from immature ovaries, the ovaries did not develop normally. Second, when mature ovaries were transplanted with intact nerves and blood vessels, in between the muscle of the body wall and subcutaneous tissue, normal follicular development occurred. However, when bursae were surgically removed prior to transplantation, ovarian development was retarded and fecundity decreased. Butcher (1947), therefore, suggested that complete bursa may provide: (1) space that is necessary for follicles to develop and/or (2) specialized fluids required for normal ovarian development.

In some mammals with complete bursae, such as the albino and hooded rat, there are small pores in the bursae that allow direct communication between the bursal and peritoneal cavities.

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cavities. Alden (1942) demonstrated that the pores in the bursa of the albino rat may be functionally closed or plugged by the fimbriated tip of the oviduct at specific times during the estrous cycle. When he sutured the openings closed, the bursal cavity, but not the oviduct, became swollen. In some animals, ovaries treated in this manner became reduced in size and cyclic ovulation was disrupted. Alden concluded from these experiments that, “the presence of the connection between the bursa and abdominal cavity is essential to the normal physiology of the region.” Without these holes, fluid pressure builds within the bursal cavity and prevents follicle maturation.

Unlike the rat bursa, the complete bursae surrounding hamster ovaries do not contain pores (Clewe, 1965). We have taken advantage of this fact to develop a technique for injecting drugs into the bursal cavity of hamsters and using this technique we have studied effects of smooth muscle inhibitors on ovulation (Martin et al., 1981; Martin and Talbot, 1981b). In the course of this work, we became interested in the morphology of the bursa and its role in ovarian events. Therefore, we undertook the present study to describe the morphology and ultrastructure of the hamster ovarian bursa and to examine its effectiveness as a barrier between the bursal and peritoneal cavities.

MATERIALS AND METHODS

Sexually mature female golden hamsters (Mesocricetus auratus) 8–16 weeks old were used throughout this study. Experiments were conducted to determine if the morphology of the bursa changed during the estrous cycle. The day of the vaginal discharge was considered to be Day 1 of the cycle. Bursae from two hamsters were prepared for light and electron microscopy at noon and 11 PM on each day of the estrous cycle using the following procedure. Animals were sacrificed and the fat pad containing the ovarian end of the reproductive tract was exposed through a dorsal incision in the flank. Fixative (3% glutaraldehyde/1% acrolein in 0.1 M sodium cacodylate pH 7.4) was injected into the bursal cavity and also dripped onto the outer surface of the bursa for 5 min. The bursa was then rigid enough to be dissected free from the fat without distortion and was placed in fresh fixative at room temperature for 2 hr. During this time, bursae to be examined by light microscopy (LM) and transmission electron microscopy (TEM) were dissected into smaller pieces. Tissue was rinsed in 0.1 M sodium cacodylate, postfixed in 1% OsO₄ in the same buffer for 1 hr at room temperature, and dehydrated in acetone. Samples for LM and TEM were infiltrated and embedded in Spurr’s low viscosity plastic (1969). Thin sections were cut on a Porter Blum MT2-B ultramicrotome, stained 1 hr with uranyl acetate and 5 min with lead citrate, then examined in a Hitachi H-500 TEM. Samples for scanning electron microscopy (SEM) were critically point dried (Sandri PVT 3), coated with gold-palladium mixture in a Technics Hummer II, and examined with a JOEL JSM-35C SEM.

The permeability of the bursa was studied by following the movement of two tracers, Evans blue and lanthanum nitrate (LaNO₃). To test the former, the bursa was treated with Evans blue in the following two ways: (1) the fat pad containing the bursa was removed from anesthetized hamsters after ligating the oviduct and soaked in a 1% solution of Evans blue in normal saline, or (2) 50μl of this solution was injected into the bursal cavity of an anesthetized hamster. After 30 min, the bursa was fixed as described previously, and the distribution of the dye within the bursa was observed in unstained sections by light microscopy.

Three experiments were performed to determine the permeability of the bursa to lanthanum. First, fixative containing the ovary was immersed in fixative containing 1% LaNO₃ was injected into the bursal cavity while the fat pad containing the ovary was immersed in fixative without the tracer. After 3 hours the tissue was processed as described above. The bursa remained intact until the final dehydration in 100% acetone, at which time it was sliced into small (1-mm²) pieces.

In the second experiment, fixative was injected into the bursal cavity while the fat pad containing the ovary was immersed in fixative containing 1% LaNO₃. After 3 hr the fat pad containing the ovary was processed as described above except that 1% LaNO₃ was added to all solutions except the 100% acetone. During the final dehydration, the bursa was dissected from the fat pad and sliced into small pieces.

In the third experiment, strips of bursa were fixed for 12 hr at 4°C in fixative containing 1% LaNO₃. The tissue was washed for 3 hr in 0.1 M Cacodylate with 1% LaNO₃ at 4°C and postfixed in 1% OsO₄ in 0.1 M cacodylate (pH 7.2) with 1% LaNO₃ for 1 hr. Tissue was dehydrated series of acetones; all but the 100% acetone contained 1% LaNO₃. Tissue was further processed as described previously.
RESULTS

1. General observations on the structure of the bursa

The hamster ovary is enclosed within a sac called the bursa (Figs. 1 and 2) which is composed of the following three layers: (1) the bursal epithelium facing the ovary; (2) a connective tissue layer; and (3) the peritoneal epithelium. The connective tissue layer contains fat cells except for an oval area (5 × 6 mm) along one side. We refer to this part of the bursa that is free of fat as the “window” because it is thin and translucent, and the ovary can be visualized through it. The bursa is thinnest (50 μm) and most compact at the center of the “window.” It gradually thickens, due to an increase in extracellular space, toward the periphery of the window where the fat cells are located. The “window” of the bursa contains numerous blood vessels that are oriented primarily parallel to the anterior–posterior axis of the reproductive tract (Fig. 1). Parallel to these vessels are dense strips of tissue which have been shown by TEM to be bundles of smooth muscle cells (SMC). Blood vessels and bundles of SMC are also found in the connective tissue that surrounds the bursal cavity.

The ultrastructure of the bursa did not change during the estrous cycle; thus the following description pertains to the entire cycle.

2. Bursal epithelium

The bursal epithelium is a discontinuous layer of cells that rests on a dense mat of collagen fibers (Figs. 3 and 4). A basal lamina is seen beneath these cells, but it is absent or difficult to resolve between cells separated from one another. The epithelial cells vary in shape from spherical to elongate; isolated cells (see Fig. 4) are typically spherical. The nuclei in spherical cells are ovoid and highly indented, whereas in elongate cells they are cigar-shaped with only a few indentations.

The cell surface is covered with microvilli of a fairly uniform length. Short microvillar-like processes also project from the base of spherical cells into the collagenous matrix (see Fig. 4). Epithelial cells in contact with each other often share long expanses of plasma membrane separated by a 100 Å space and desmosome-like junctions are present (inset, Fig. 4).

The cytoplasm of the epithelial cells contains abundant rough endoplasmic reticulum (RER), Golgi bodies, mitochondria, free ribosomes, and vesicles of two different sizes. The small vesicles (0.06–0.12 μm diameter) are seen throughout the cell but are most abundant along the apical and lateral plasma membrane where numerous pinocytotic vesicles of similar size and morphology are located. The contents of both the pinocytotic and intracellular vesicles are electron lucent. The larger vesicles vary in diameter from 0.3 to 1.0 μm, and appear either empty or contain flocculent material of moderate electron density.

3. Peritoneal epithelium

The peritoneal surface is covered by a continuous layer of epithelial cells (Figs. 5 and 6) which rests on a filamentous basal lamina. The surface of most of these cells is covered by microvilli which are oriented approximately parallel to the cell surface. Cells occasionally have microvilli only along their perimeter (Fig. 5); in these instances the cell shape appears polygonal. Where microvilli are sparse, pits with 0.1 to 0.5 μm diameters are visible in the cell surface (Fig. 5).

The peritoneal epithelial cells are elongate and in most sections only thin (1-2 μm) extensions of these cells are seen. Sections through the nuclei of these cells show that they are also elongate, narrow (3–4 μm), and have a smooth outline. The cytoplasm contains numerous mitochondria, Golgi bodies, RER, and free ribosomes. Most of the organelles are clustered at the poles of the nucleus although they are also found in smaller numbers throughout the long extensions of these cells. Pinocytotic vesicles line the apical, lateral, and basal plasma membrane, and are similar to vesicles distributed throughout the cell. Occasionally, large vesicles partially filled with dense granular material are seen. In some micrographs the membranes near the apex of these cells appear to fuse (inset, Fig. 5) and probably represent tight junctions.

4. Connective tissue

The connective tissue of the bursa is composed of fibroblasts, collagen fibers, bundles of SMC, and blood vessels. At the center of the “window” (Fig. 7A), the connective tissue is compact; however, in the more peripheral regions of the “window,” there are extensive extracellular spaces containing only flocculent material.

The fibroblasts occur singly, are surrounded by bundles of collagen fibrils, and are found throughout the bursa (Figs. 6 and 8). They are either elongate and spindle shaped or stellate. These shapes most likely correspond to sections through different axes of the same type of cell. Both views display 2–4 long, thin arms extending from the nucleated region of the cell.
The cytoplasm is similar to that described for the epithelial cells in that it contains RER, free ribosomes, Golgi bodies, and mitochondria. Fibroblasts, however, differ from epithelial cells in having fewer vesicles (0.08 μm diameter), numerous coated vesicles, and secondary lysosomes. Junctions between fibroblasts were not observed, although occasionally the plasma membranes of adjacent cells were parallel for 2 μm and separated by 120 Å.

SMC are organized in bundles within the layer of connective tissue (Figs. 6, 7, and 9). In the “window,” two layers of SMC bundles are present. A thick layer adjacent to the bursal epithelium is oriented parallel to the anter-
ior–posterior axis, whereas a thinner layer adjacent to the peritoneal epithelium is oriented perpendicular to this axis. Bundles are composed of 4–12 SMC (Fig. 6), and each SMC is surrounded by a basal lamina. Collagen fibrils are found both singly and in small clusters within the SMC bundles. Both desmosomes and gap junctions were observed between SMC, although these junctions were rare. Two more commonly observed associations between SMC were: (1) narrow projections from one cell that passed into indented pockets in the adjacent cell (Fig. 9); and (2) regions of close apposition (~100 Å) between adjacent plasma membranes for distances up to 4 μm.

SMC of the bursa have the ultrastructural characteristics of “typical SMC.” The nucleus is sausage shaped with several deep and narrow indentations, indicating that the cells were contracted at the time of fixation (Lane, 1965; Bagby et al., 1971; Cooke and Fay, 1972; Fay and Delise, 1973). Accumulations of synthetic organelles, including RER, ribosomes, mitochondria, and Golgi bodies, are localized at the nuclear poles. The cytoplasm of the spindle-shaped SMC is filled with three types of filaments; thin (45–55 Å in diameter), intermediate (80–120 Å in diameter), and thick filaments (130–140 Å in diameter). Dense bodies are scattered throughout the filamentous regions of the cell. The thin filaments appear to terminate at dense attachment plaques that alternate along the plasma membrane with clusters of caveolae.

Macrophages (Fig. 10) and mast cells (Fig. 11) are present in connective tissue of the bursa, but are less abundant than fibroblasts. The former are large, spheroidal-shaped cells that are most numerous in large extracellular spaces in the ventral part of the bursa, that is, the side opposite the window. The nucleus is irregularly shaped and the cytoplasm contains numerous Golgi bodies, small amounts of RER, and mitochondria. Distributed throughout the cytoplasm are vesicles (0.5–2.0 μm in diameter) that are filled with an electron-lucent

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**Abbreviations**

- BC, Bursal cavity
- C, Collagen
- Ca, Caveolus
- D, Dense attachment plaque
- E, Endothelial cell
- EL, Elastin
- F, Fibroblast
- G, Mast cell granule
- N, Nucleus
- PC, Peritoneal cavity
- RBC, Erythrocyte
- SM, Smooth muscle
- V, Vesicle
Fig. 3. Scanning electron micrograph of the bursal epithelium showing its discontinuous nature and the bundles of collagen fibrils between epithelial cells. × 4400.

Fig. 4. Transmission electron micrograph of the bursal epithelium showing the round profiles of epithelial cells resting on a layer of collagen fibrils. Note the microvilli-like extensions from the base of these cells (arrows) and the lack of a basal lamina between adjacent cells. × 7000. Inset: A desmosome junction between two epithelial cells. × 12,000.
Fig. 5. Scanning electron micrograph of the peritoneal epithelium. These cells have microvilli either just along their perimeter or all across their apical surface. Note the pits (arrows) on the cell surface. $\times 6500$. Inset: Transmission electron micrograph of an apparent tight junction between two epithelial cells. $\times 95,000$.

Fig. 6. Transmission electron micrograph of a section through the bursa in the window region showing the peritoneal epithelium, a fibroblast surrounded by collagen, and a bundle of smooth muscle cells. $\times 7100$. Inset: Higher magnification ($\times 21,000$) transmission electron micrograph showing part of a peritoneal epithelial cell containing a vesicle and synthetic organelles.
Fig. 7. Light micrograph of the bursa near the center (A) and periphery (B) of the window region. Note the difference in density of the tissue in the two areas. × 300.

Fig. 8. Transmission electron micrograph of a "stellate" fibroblast surrounded by clusters of collagen fibrils. × 12,000.
Fig. 9. Transmission electron micrograph of a smooth muscle cell showing a process from one cell inserted into an indentation of an adjacent cell. × 75,000.

Fig. 10. Transmission electron micrograph of a macrophage containing vesicles of two different sizes. × 8200.

Fig. 11. Transmission electron micrograph of a mast cell. These are usually found adjacent to capillaries. × 12,000.
material and often contain one or two additional components of higher electron density. The cytoplasm also contains small electron-lucent vesicles similar in size to the numerous micropinocytotic vesicles that line parts of the plasma membrane. Other regions of the plasma membrane are drawn out into microvilli or lamellae that may be involved in engulfing extracellular material.

The mast cells are less common than macrophages and are usually found near capillaries. They are easily identified by their lobed nucleus and large (1.0–1.5 μm in diameter) homogeneous electron-dense granules that fill the cytoplasm.

5. Blood vessels

The bursa contains numerous blood vessels ranging in diameter from 4 to 60 μm. The morphology of the walls of these vessels is similar to those described in greater detail elsewhere (Bloom and Fawcett, 1975). Capillaries are lined by a single layer of irregularly shaped, fenestrated endothelial cells (Fig. 12). In addition to RER, free ribosomes and mitochondria, the cytoplasm of these cells contains microtubules, microfilaments, and two types of vesicles. Coated vesicles are rare, whereas small (0.1 μm in diameter), electron-lucent vesicles are abundant and similar to the micropinocytotic vesicles that line the plasma membrane. Adjacent cells share long expanses of interdigitated folds of the plasma membrane. Junctions other than desmosomes were not observed. Surrounding the endothelium is a filamentous basal lamina and an occasional pericyte. The entire tube is surrounded by bundles of collagen fibrils. The structure of the wall of venules is similar to that described for the capillaries except that the endothelium lacks fenestrations.

The arterioles are characterized by the following features: (1) the endothelial cells have few pinocytotic vesicles and microvilli; (2) the lateral surfaces of adjacent endothelial cells are not highly interdigitated and few cell junctions were observed; and (3) the endothelium and basal laminae are surrounded by layers of elastic fibers and SMC (Fig. 13). The morphology of the SMC is similar to the nonvascular SMC described under Section 4.

6. Permeability of the bursa

When lanthanum or Evans blue was applied to the peritoneal surface of the bursa, both formed a deposit along the apical surface of the peritoneal epithelial cells, lined invaginations of the plasma membrane, and penetrated a short distance into the intercellular space between cells (Figs. 14 and 15). Neither tracer was observed elsewhere within the bursa.

Both lanthanum or Evans blue, when injected into the bursal cavity, penetrated all layers of the bursa but did not pass beyond the peritoneal epithelium. When the bursa was treated for 3 hr with fixative containing lanthanum, most of the tracer was found along bundles of collagen fibrils bordering the bursal epithelium (Fig. 16). Although aggregations of lanthanum were observed in deeper parts of the bursa, their abundance was considerably less than layers adjacent to the bursal epithelium.

When strips of bursa were treated with lanthanum for 12 hr the tracer was found throughout the bursa (Fig. 17). Lanthanum was found bound to collagen fibrils, outlining all cells of the connective tissue layer, filling micropinocytotic vesicles of both epithelia and the caveolae of the SMC, and inside the lumen of blood vessels (Fig. 18).

**DISCUSSION**

We have described the morphology and ultrastructure of the hamster ovarian bursa and have examined its permeability using two tracers. Our results confirm the earlier observations of Clewe (1965), that is, the hamster ovary is surrounded by a morphologically complete bursa, and that exchange of materials between the bursal and peritoneal cavities is highly restricted. Both epithelial cell layers and all the intervening connective tissue layers have been described ultrastructurally in detail. Moreover, we have determined that the ultrastructure of the bursal cells and the organization of the bursa does not change during the estrous cycle. Smooth mus...

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**Fig. 12.** Transmission electron micrograph of a transverse section through a capillary in the window region of the bursa. Note the fenestrations (arrows) in the endothelium. ×8,800.

**Fig. 13.** Transmission electron micrograph showing part of an arteriole in the bursa. Note the elastin and smooth muscle layer surrounding the endothelium. ×4700.

**Fig. 14.** Transmission electron micrograph showing a peritoneal epithelial cell with lanthanum deposits along its apical surface. The tracer was only applied to the peritoneal surface of the bursa and did not penetrate beyond the epithelial layer. Unstained ×65,000.

**Fig. 15.** Transmission electron micrograph of a peritoneal epithelial cell not treated with lanthanum. Compare its apical plasma membrane with Figure 14. Unstained ×65,000.
cle cells were shown to be present in an ovarian bursa, and their presence suggests a non-passive role for the bursa.

The discontinuous bursal epithelium and underlying meshwork of collagen fibrils appear to provide little impediment for the movement of fluids between the bursal cavity and interior of the bursa. Indeed, this was shown to be the case using solutions of Evans blue and lanthanum. Both tracers moved freely from the bursal cavity into all layers of the bursal wall. A tight seal at the peritoneal surface is suggested, however, by the close apposition of peritoneal epithelial cells and the failure of the tracers to penetrate beyond this cell layer. Furthermore, in regions where the bursa is surrounded by fat, the adipose cells also appear to form an impermeable layer because dyes injected into the bursal cavity do not penetrate more than 1 mm into the fat. While the observations do not exclude possible fluid or molecular exchange across the membranes of the peritoneal layer (see Cotran and Karnovsky, 1968) or fat cells, they do at least suggest that such exchange is limited and that the bursa may isolate a specialized fluid around the hamster ovary. Recent evidence suggests that bursal fluid may be predominantly an ovarian exudate (Konicckx et al., 1980), although some may come from the oviduct (Battalia and Yanagamachi, 1979). It would be informative to know if the amount or composition of the bursal cavity fluid changes during the estrous cycle and to determine if this fluid regulates any ovarian processes, as suggested by Butcher (1947). Our observations also suggest that solutions injected into the bursal cavity would freely enter the bursa and be cleared through the bursal blood vessels. From previous data we know that when drugs are injected into the bursal cavity 6 hr before ovulation, 20% of the original drug concentration is still present at ovulation (Martin et al., 1980). While ovarian blood vessels may remove some portion of these drugs, much of this dilution probably occurs by clearance through the bursa.

The control of fluid volume and pressure within the bursa most likely depends on the exchange of fluids between the bursal cavity and the vasculature of the bursa. Our studies with lanthanum and Evans blue demonstrate that the movement of fluids between the bursal cavity and blood vessels is not prevented by any layer of the bursa. The SMC in the bursa may help to regulate fluid volume and pressure within the bursal cavity. That is, if the volume of the bursa increases, the SMC would be stretched, possibly triggering contraction of these cells. SMC surrounding many fluid-filled structures like the bladder, stomach, and blood vessels, respond to stretch by contracting (Kosterlitz and Watt, 1975). Contraction would increase the pressure within the bursal cavity and oppose or even counteract fluid leakage from the capillaries in the bursa. It is also possible that contraction of the SMC aids in movement of the cumulus mass into the oviduct; contraction after ovulation could flush bursal fluid containing the cumulus toward the infundibulum where it would be picked up and transported into the ampulla by the ciliated epithelium. Measurements of changes in pressure and volume within the bursal cavity throughout the ovarian cycle may help to answer these questions and provide insight into the function of the complete ovarian bursae of hamsters.

In summary, we have presented a description of the morphology of the bursa surrounding the hamster ovary, paying particular attention to the permeability of this layer. Ovarian bursae have received little attention, probably because many mammals lack these structures. However, the experiments presented in this and previous papers show that the bursa effectively isolate fluids within the bursal cavities in several rodents that are commonly used in studies of reproductive biology. In the hamster, this specialized environment may be important for normal ovarian function, egg transport into the oviduct, and possibly fertilization.

Fig. 16. Transmission electron micrograph showing lanthanum deposits around the bursal epithelial cells and in the adjacent connective tissue. Note the dense accumulation of lanthanum by the collagen fibrils. The bursa was fixed intact with glutaraldehyde and lanthanum for 3 hr. Unstained × 30,000.

Fig. 17. Transmission electron micrograph of a region of the bursa comparable to that shown in Figure 16, except that strips of the bursa were fixed with glutaraldehyde and lanthanum for 12 hr. Note the increased penetration of the tissue by the tracer. Unstained × 3500.

Fig. 18. Transmission electron micrograph of a capillary from the bursa prepared as in Figure 17. Note the dense accumulation of lanthanum around the vessel and the numerous deposits within the lumen. Unstained × 15,500.

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LITERATURE CITED


