A FREEZE-FRACTURE AND LANTHANUM TRACER STUDY OF THE COMPLEX JUNCTION BETWEEN SERTOLI CELLS OF THE CANINE TESTIS

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ABSTRACT

What appear to be true septate junctions by all techniques currently available for the cytological identification of intercellular junctions are part of a complex junction that interconnects the Sertoli cells of the canine testis. In the seminiferous epithelium, septate junctions are located basal to belts of tight junctions. In thin sections, septate junctions appear as double, parallel, transverse connections or septa spanning an ~90-Å intercellular space between adjacent Sertoli cells. In *en face* sections of lanthanum-aldehyde-perfused specimens, the septa themselves exclude lanthanum and appear as electron-lucent lines arranged in a series of double, parallel rows on a background of electron-dense lanthanum. In freeze-fracture replicas this vertebrate septate junction appears as double, parallel rows of individual or fused particles which conform to the distribution of the intercellular septa. Septate junctions can be clearly distinguished from tight junctions as tight junctions prevent the movement of lanthanum tracer toward the lumen, appear as single rows of individual or fused particles in interlacing patterns within freeze-fracture replicas, and are seen as areas of close membrane apposition in thin sections. Both the septate junction and the tight junction are associated with specializations of the Sertoli cell cytoplasm. This is the first demonstration in a vertebrate tissue of a true septate junction.

KEY WORDS   junction · Sertoli cells · testis · canine · freeze-fracture · lanthanum tracer

It has been known since the beginning of this century that many substances do not penetrate into the lumen of the seminiferous tubules from the interstitial fluid (39). The permeability barrier in the testis is not located in the capillary endothelial cells, as is the case for the blood-brain barrier, nor is it in the boundary tissue surrounding the tubules. The blood-testis barrier is located at the tight junction between adjacent Sertoli cells in the seminiferous tubules (11, 17). In addition to tight junctions, there are rudimentary desmosomes, gap junctions, and structures identified as septate junctions between adjacent Sertoli cells of the canine testis (4, 5, 7). Septate junctions (septate desmosomes) were described originally by Wood (44) in the epithelium of hydra and have been identified frequently between cells of invertebrates (21). Junctions that resemble septate junctions have been described in thin sections in a number of vertebrate tissues (9, 20, 21); however, none of these junctions has been substantiated as septate by freeze-fracture data (20).

This study demonstrates for the first time the presence of a true septate junction in vertebrate tissue. This junction is septate in appearance in freeze-fracture replicas, in thin sections, and in lanthanum tracer experiments. In this study a correlation will be made between the structure of the components of the complex junction between Sertoli cells and some of the known functions of the Sertoli cells within the germinal epithelium.

MATERIALS AND METHODS

The surgical method developed for the perfusion of canine testicular tissue using either aldehyde or aldehyde-lanthanum fixation has been described previously (9). 12 sexually mature mongrel dogs obtained from local suppliers were used in this study. Freeze-fracture replicas were prepared from cryoprotected fixed tissue (8), lightly fixed tissue (1 h in 1% Karnovsky's fixative [29] and cryoprotected with 20% glycerol in 0.1 M cacodylate buffer, pH 7.2, for 4–8 h), and from unfixed tissue not cryoprotected with glycerol. 1-mm cubes of tissue mounted on 3-mm cardboard disks were frozen in Freon 22 (Phillips Manufacturing Co., Chicago, Ill.) suspended in liquid N_2. The tissues were fractured at −115°C in a Balzers BA 360M freeze etch unit (Balzers High Vacuum Corp., Santa Ana, Calif.) and coated with platinum-carbon. Residual tissue was digested from the replicas with ~6% sodium hypochlorite, and the washed replicas were mounted on copper grids and viewed in a Philips 300 electron microscope.

The different methods of fixation used before freeze-fracturing gave essentially the same results; however, the morphology of tight and septate junctions was best observed after in vivo perfusion with formaldehyde-glutaraldehyde-picric acid (28), and gap junctions were revealed more frequently after immersion in Karnovsky's fixative. The poorest replicas were obtained from tissues that were not fixed or cryoprotected. Inasmuch as the results relating to the spatial arrangement of particles within the junctional complex are similar regardless of the procedure, all freeze-fracture data will be considered together.

The terminology and theory of the intramembranous location of the fracture plane proposed by Branton et al. (2) will be followed in describing the intramembranous specializations revealed by the freeze-fracture technique.

The various classes of junctions are identified on the basis of the following inclusive criteria that are applicable to both vertebrate and invertebrate tissues:

(a) Tight (occluding) junctions are defined as junctions that appear in thin sections as regions of close approximation of adjacent plasma membranes that do not permit the penetration of lanthanum tracer. In freeze-fracture replicas, these junctions appear as single rows of individual or fused particles arranged in an interlacing pattern.

(b) Septate junctions are defined as junctions that appear in thin sections as electron-dense, transverse connections (septa) between adjacent membranes. These intercellular septa exclude the passage of electron-dense tracers such as lanthanum. The intercellular distance is less than that found in nonjunctional regions but is greater than that found between plasma membranes in gap or tight junctions. In freeze-fracture replicas, the septate junction appears as rows of individual or fused particles in parallel array. These particles are in continuity with septa that span the intercellular space, and the distribution of the particles correlates with the distribution of the septa.

(c) Rudimentary desmosomes are defined as junctional specializations characterized in thin sections by the presence of electron-dense filamentous materials that are in register in adjacent cells. These electron-dense filaments may or may not be associated with obvious electron-dense plaques in the cytoplasm or with increased intercellular densities between the cells. The intercellular distance is not decreased, and in freeze-fracture replicas irregular arrays of particles are seen that are not reflected as pits on the complementary face of the adjacent cell membrane.

(d) Gap junctions are defined as intercellular specializations that permit the passage of tracers such as lanthanum within an ~20-Å intercellular space. In en face sections of gap junctions from lanthanum-perfused specimens, the intercellular components of the junction appear as particles outlined by the electron-dense lanthanum. In freeze-fracture replicas, the gap junction appears as aggregates of closely packed intramembranous particles that have pits of similar arrangement on the complementary face of the membrane of the adjacent cell. These particles are believed to be part of a complex that interconnects the two cells.

RESULTS

Junctions between Sertoli Cells

In the seminiferous epithelium, only the Sertoli cells extend from the basement membrane to the lumen. Sertoli cells are attached to the basal lamina by hemidesmosomes and to each other by rudimentary desmosomes located a few micra luminal to the basal lamina (4). Typical-appearing gap junctions (see Materials and Methods) are infrequently observed in thin sections; in freeze-fracture replicas, plaques of particles characteristic of gap junctions as well as linear aggregations of particles are noted occasionally. The linear aggregations of particles are usually seen around highly contoured regions of membranes and only rarely reveal pits in the complementary membranes of the adjacent cells. This junction will be described in detail in a later publication. In addition to
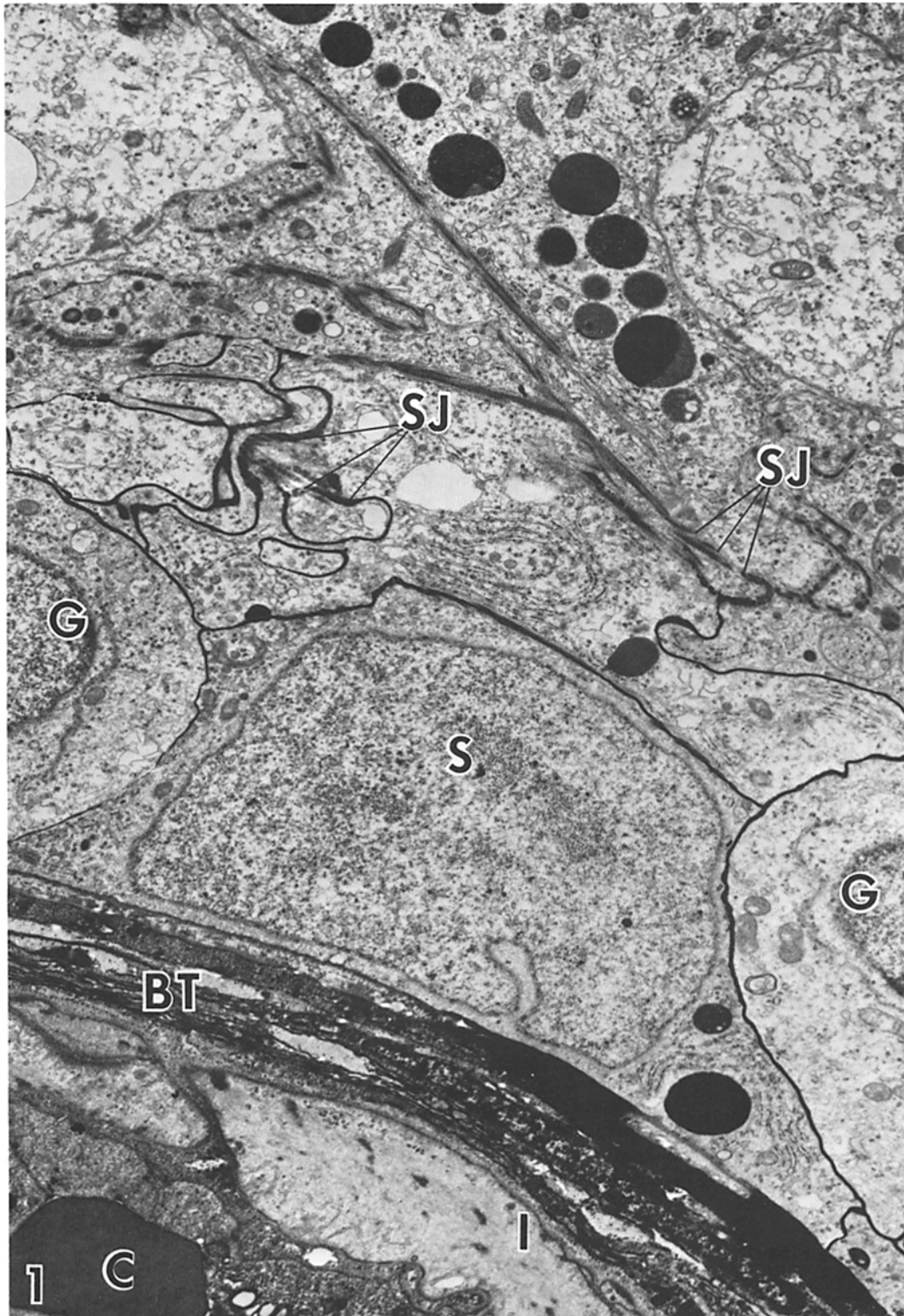
FIGURE 1 In lanthanum-aldehyde-perfused specimens, lanthanum moves from the interstitial capillaries (C) to the region in between the cells of the interstitial tissue (I) and between the myoid cells of the boundary tissue (BT) before penetrating into the seminiferous tubules. Within the basal regions of the tubules, the lanthanum moves between Sertoli cells (S) and between Sertoli cells and diploid germinal cells (G). More luminally, lanthanum is partially excluded from the region between Sertoli cells by septate junctions, resulting in a striated appearance of the lanthanum tracer (SJ). Luminal to the septate junction, lanthanum is completely excluded by belts of tight junctions. Unstained. × 8,400.

these junctions, two other types of junctions link Sertoli cells together. These inter-Sertoli junctions are identified as septate junctions and tight junctions, and they are always associated with specific cytoplasmic structures that will be described.

The Complex Inter-Sertoli Junction

In lanthanum-aldehyde-perfused specimens, the intercellular lanthanum in the basal region of the inter-Sertoli junction has a striated appearance resulting from the partial exclusion of lanthanum from the intercellular space (Fig. 1). These regions of exclusion are the result of intercellular connectors (Figs. 2–4) that act as barriers to the movement of the lanthanum tracer. The bands of lanthanum exclusion are ~85 Å-wide (range ~60–~120 Å) and usually appear in unstained preparations as pairs of lucent, intercellular connectors on the electron-dense background of intercellular lanthanum. Usually, members of a pair of lucent connectors are arranged in parallel and regularly spaced at ~100-Å distances (range ~85–~130 Å) from each other. Occasionally, they are widely separated from each other, appearing at infrequent intervals along the adjacent plasma membranes. The distance between successive pairs is highly variable. Sometimes, they appear to be spaced at uniform intervals (see the left side of Fig. 4), whereas in other cases they appear to be widely separated from each other (see the right side of Fig. 4). Because the interdigitating Sertoli cell membranes are highly contoured (Fig. 1), a series of true cross sections of the connectors are rarely seen (Fig. 3). In *en face* sections of lanthanum-aldehyde-perfused specimens, this portion of the inter-Sertoli junction

appears as electron-lucent lines on an electron-dense background of lanthanum (Fig. 4). These beaded-appearing electron-lucent lines continue parallel to the cell surface for some distance, indicating that the exclusion of lanthanum really is the result of intercellular septa that join the adjacent Sertoli cells. There are indications of electron-lucent "links" between some of the pairs of septa (Fig. 4) that may act to maintain the ~100-Å distance between the members of a pair.

In routine thin sections from tissues that have been stained *en bloc* with uranyl acetate and stained with hot uranyl acetate and lead citrate, the more basal of the two types of inter-Sertoli junctions appears as electron-dense septa, usually arranged in pairs that span an ~90-Å intercellular space (Fig. 2) between adjacent Sertoli cells, or their processes. The distance between the two members of a set is ~100 Å (range ~89–~106 Å), and the spacing between successive pairs of septa is variable. The width of the septa is ~75 Å as compared to ~85 Å in the lanthanum-aldehyde-perfused specimens, suggesting that only part of the structure is seen in routinely stained thin sections. The number of rows of septa within the inter-Sertoli junction is variable, ranging from only a few to over 40, depending upon the stage of spermatogenesis associated with the Sertoli cells and thus on the spatial relationship of the junctional complex to the basal lamina.

Freeze-fracture replicas of this region of the inter-Sertoli junction (Fig. 5) show a junctional specialization on the particle-rich surface of the P face that appears as rows of ~100-Å particles or fused particles that are recessed within furrows that are located on the crests of slight elevations

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FIGURE 2   Electron-dense septa (arrows) span the ~90-Å interspace between adjacent Sertoli cells. Bands of filaments (*f*) adjacent to the junction are bordered by a subsurface cisterna (*SSC*) of the endoplasmic reticulum. × 99,000.

FIGURE 3   In this unstained thin section, the angle of section varies from transverse to slightly oblique as can be noted by the appearance of the bands of filaments (*f*) bordering the junction. In a transverse section, the bands of filaments are cross-sectioned, are fairly evenly placed along the plasma membrane, and are in register from one cell to the other. Lanthanum is partially excluded from the intercellular space by pairs of septa (arrowheads) so that the septa appear as electron-lucent lines on an electron-dense background of lanthanum. The subsurface cisterna (*SSC*) is fenestrated (*) and borders the inter-Sertoli junction. × 133,000.

FIGURE 4   This unstained thin section shows an *en face* view of a lanthanum-aldehyde-perfused septate junction. The sets of intermembranous septa appear as paired electron-lucent lines with occasional short electron-lucent lines connecting the members of a pair (arrows), suggesting that the pairs of intercellular septa may be linked together. The subsurface cisternae (*SSC*) are seen in *en face* sections. × 75,000.
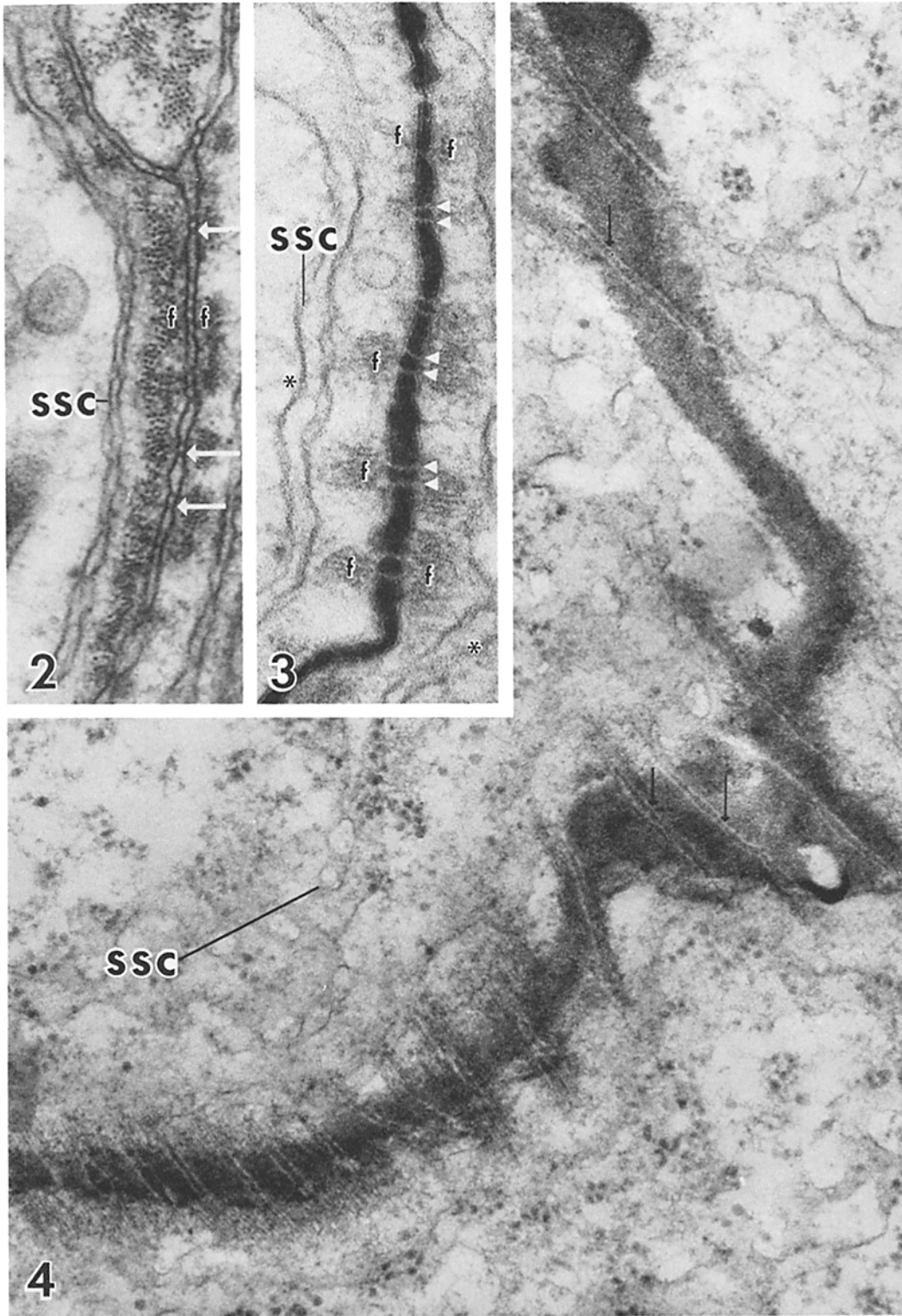
or ridges of this membrane face. These rows are usually arranged in parallel, double rows, and, sporadically, particles are seen within the ~100-Å space that separates the members of a pair (Fig. 6). These particles may act to maintain the distance between the parallel rows. The pairs of rows do not always run parallel to adjacent pairs, but may diverge from each other, intersect with their neighboring sets, or terminate abruptly (Fig. 7, 11). On the E face, individual or fused particles are recessed within furrows located at the base of grooves (Figs. 6-8), and the membrane faces immediately surrounding the junctions are relatively particle-free (Fig. 9). The furrows, in which the junctional particles usually are recessed, appear as crevices when devoid of particles (Fig. 8). The membrane faces between the rows usually are not contoured but are relatively flat. In replicas in which both E and P faces are exposed, parallel, double rows of particles are present on both faces (Figs. 7, 8, 10, 11). These rows are in register from the E face of one plasma membrane to the P face of the adjacent Sertoli cell (Figs. 7 and 10). Not only are these rows of particles in register from one cell to another, but they appear to be in continuity with strands that traverse the intercellular space (Fig. 10). Thus, these are structures that extend from within the plasma membrane of one cell and across the intercellular space, and into the plasma membrane of the adjacent cell. This junction appears to have all the ultrastructural characteristics peculiar to septate junctions (see Materials and Methods), and therefore represents the vertebrate counterpart to the invertebrate septate junction.

The septate portion of the junction, located basally, is semipermeable, but luminally the junction is impermeable to lanthanum (Figs. 1 and 12). The region of lanthanum exclusion corresponds to the location of the tight junction. The transition from the region of septate junctions to the region of tight junctions is gradual rather than abrupt (Figs. 5 and 11) so that in the area of transition the arrangements of particles characteristic of both septate and tight junctions are present (Fig. 11). The double parallel rows of particles in the septate portion of the inter-Sertoli junction may branch and continue as single rows of particles (Fig. 11). These single rows are continuous with the single interlacing rows of particles within the tight junction region. Fields of tight junctions are often bordered by septate junctions (Fig. 15). Both the septate and tight junctions are a part of the continuum of the inter-Sertoli junction.

The individual or fused particles that comprise the intramembranous component of the Sertoli cell tight junction have the characteristic interlacing arrangement of particles seen in other tight junctions. They are seen as interlacing rows of discrete or fused particles recessed within furrows at the crest of ridges on the particle-rich P face (Fig. 11), or within furrows in depressions on the particle-poor E face (Figs. 11 and 15). The strands of particles on the E face are in register with those on the P face of the adjacent cell (Fig. 11). In replicas of cross fractures through Sertoli cells, the rows of intramembranous particles converge at the site of close apposition of the adjacent cell membranes (Fig. 15). In freeze-fracture replicas, the plasma membranes adjacent to the tight junc-

FIGURE 5 This micrograph of a freeze-fracture replica shows the same distribution of septate and tight junctions as the micrograph in Fig. 1. The boundary tissue (BT), composed of myoid cells (M) and collagen (c), encloses the seminiferous tubules. Both intramembranous fractures as well as cross fractures of Sertoli cells (S and S') are shown. Basally, a portion of the septate junction (SJ') is revealed. It appears as rows of particles located on ridges of the P face (P) and corresponds to the striated region of the junction in Fig. 1. Luminal to this junction is a region of transition from septate junction (SJ) to tight junction (TJ). The septate junction is arranged as parallel rows of particles, and the tight junction as interlacing rows of particles with contoured membranes between the interlacing rows as shown here on the E face (E) of the Sertoli cell (S). The subsurface cisterna (SSC) and its fenestrae (*) are revealed in the more basally located Sertoli cell (S'). Near the top of the micrograph, another tight junction (TJ) is seen on an E face. The tight junctional regions shown in this replica correspond to the regions of lanthanum exclusion in Fig. 1. × 25,000.

FIGURE 6 Double rows of individual and fused particles are characteristic of the freeze-fracture appearance of the canine septate junction. These rows of particles appear to be intermittently interconnected by particles between the double rows (arrowheads). This tissue is from a dog treated with 1,000 IU of human chorionic gonadotropin for 1 h before fixation. × 118,000.
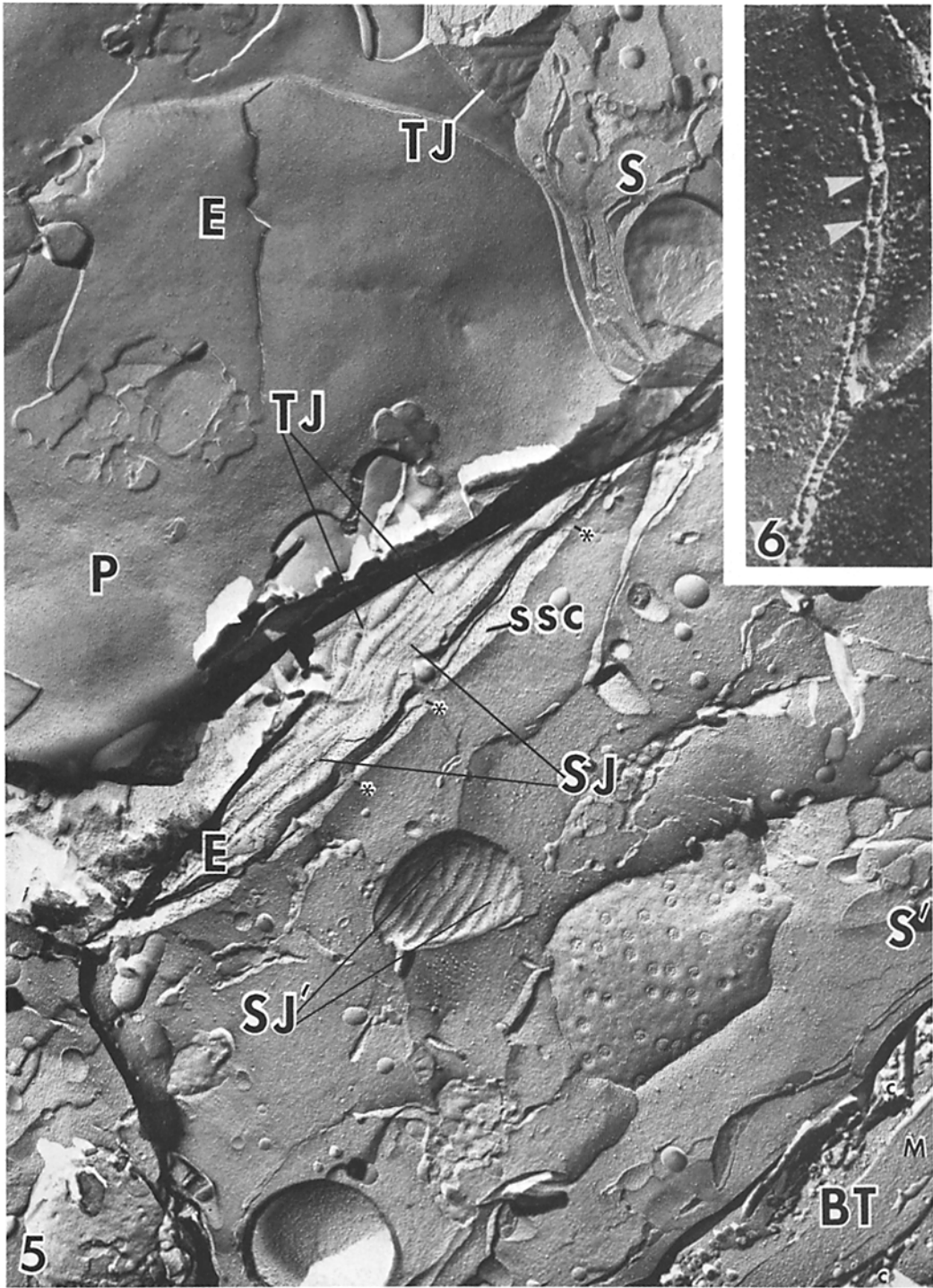
tions appear to bulge in, and thus the P face appears concave, whereas the E face appears convex (Figs. 5, 11, 15). Because the particles on the P face and the E face are recessed, either in grooves (E face) or on the crest of ridges (P face), these regions of plasma membrane may actually be pulled closer together than the distance characteristic for nonjunctional membranes. This would account for the fact that the total width of the tight junctional regions is often less than or equal to the sum of the dimensions of the component nonjunctional plasma membranes. The bulging of the plasma membrane is evident in thin sections as well, and this fact often can be used to differentiate regions of tight junctions from regions of septate junctions at relatively low magnification (cf. Figs. 12-14).

In thin sections, the plasma membranes of the tight junction do not appear fused although they are closely approximated. In transmission electron micrographs taken at high magnification, the adjacent membranes sometimes appear to be brought together by crisscrossing membranous extensions (Fig. 13) that may be continuous with the plasma membranes. Sertoli cell plasma membranes are ~100 Å-wide, and the total width of the cell contact at the tight junction measures ~200 Å at its narrowest. Tight junctions are commonly observed at the union of three plasma membranes (Fig. 14).

Cytoplasmic Specialization
Associated with the
Inter-Sertoli Junction

The inter-Sertoli junction begins a few micra luminal to the basal lamina (Fig. 1). Within the inter-Sertoli junction the normal intercellular distance of ~250 Å widens to ~350 Å and then narrows to ~90 Å in the region of the septate

junction. The intercellular space is obliterated by the tight junction, but the intercellular space between adjacent tight junctions is often exaggerated (Figs. 12, 13). Filaments ~60 Å in diameter (~90 Å in lanthanum-aldehyde-perfused tissue) are arranged in bands ~0.15- to ~0.60-μm wide adjacent to the plasma membrane and running parallel to the cell surface at the level of the inter-Sertoli junctions, i.e., the septate and tight junctions (Figs. 2 and 14). The distance between adjacent bands varies from ~0.14 to ~0.18 μm, and the bands are usually in register within adjacent cells.

In the cytoplasm, approximately parallel to the bands of filaments and the intercellular junctions, is an elaboration of the endoplasmic reticulum (Figs. 2-4, 14). This subsurface cisterna (SSC)[1] is located ~0.06-~0.14 μm from the plasma membrane and occasionally has ribosomes on its surface. The ribosomes are seen more commonly on the face of the cisterna directed toward the interior of the cell, but they are present on both surfaces (Fig. 14). The SSC is continuous with the endoplasmic reticulum system of the Sertoli cell (Fig. 14), and is seen in some sections to be continuous with the nuclear envelope (not shown). The SSC itself may be fenestrated, as can be noted in Figs. 3 and 4. It has bulbous extensions that appear to abut directly on the plasma membrane (Fig. 14).

A tentative model of the complex junction between Sertoli cells is presented diagrammatically in Fig. 16. This model implies no knowledge of the biochemical nature of the septa or the intercellular connectors of the tight junction. It does not imply congruity of the intercellular structures of septate or tight junctions or identity of

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[1] *Abbreviations used in this paper:* SSC, subsurface cisterna.

FIGURE 7 The septate portion of this inter-Sertoli junction is close to the basal lamina and basal to the Sertoli cell nucleus. A series of double, parallel rows of individual and fused particles are located either in grooves on the E face (arrows) or on the crest of ridges on the P face (arrowhead). The particles are in register from the E face to the P face of the adjacent Sertoli cell. Note that the distance between the sets of junctional particles varies; some sets run parallel, some converge, and other diverge from their neighboring sets. The subsurface cisterna (*SSC*) is indicated. × 57,500.

FIGURE 8 The individual and fused particles that constitute the intramembranous portion of the inter-Sertoli junction appear to be recessed within clefts in grooves on the E face (white arrow) or within ridges on the P face (black arrows). The recessed areas appear as narrow crevices when the particles are absent (arrowhead). × 127,500.
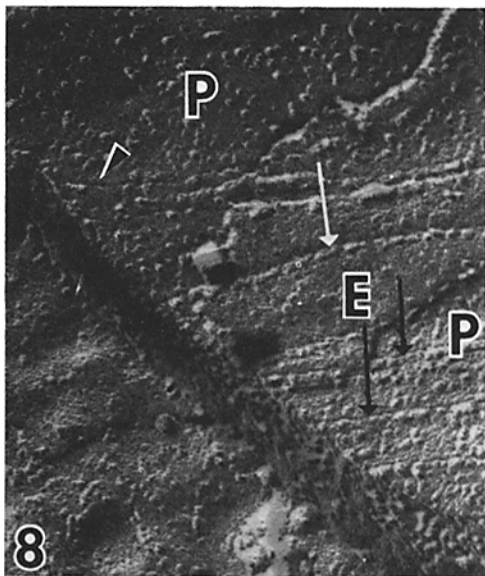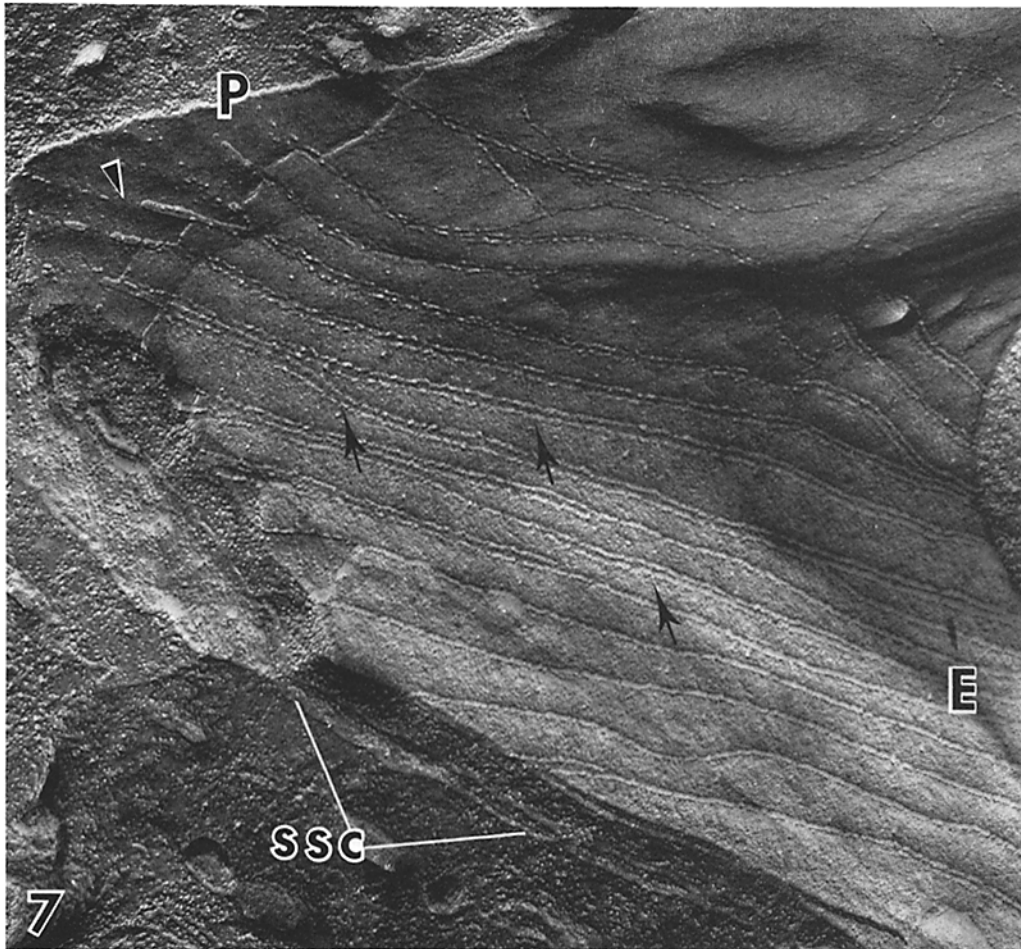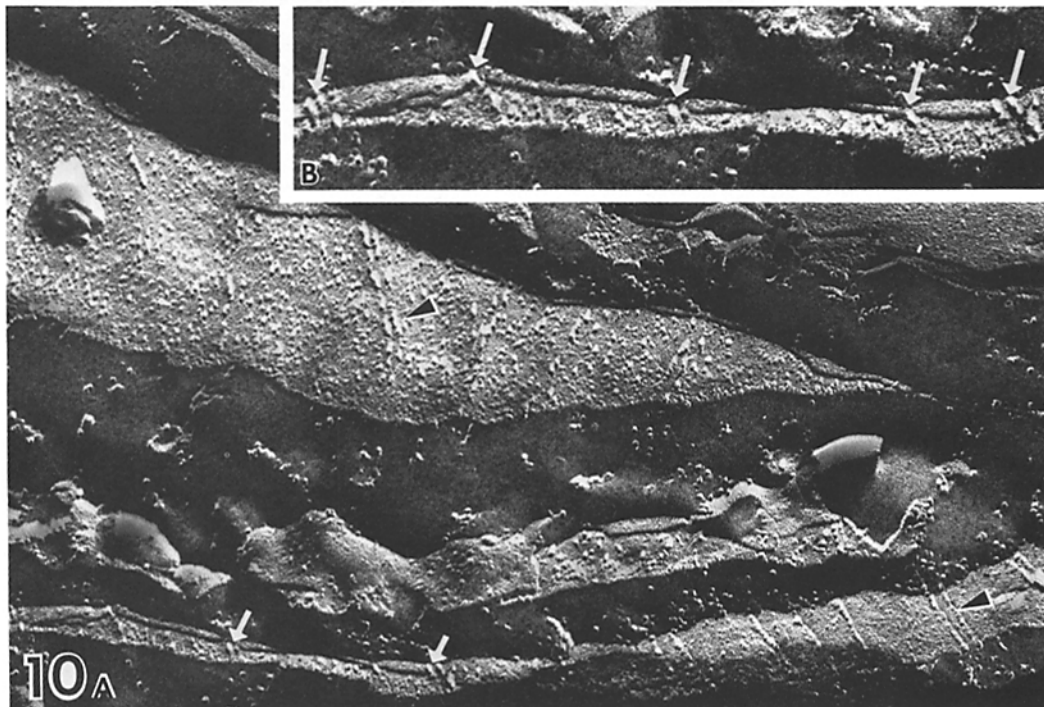
FIGURE 9   Sertoli cells have many intricately interdigitating processes that are joined by septate and/or tight junctions. In this freeze-fracture replica, a Sertoli cell process is shown that is incompletely encompassed by septate junctions (arrows). The subsurface cisterna (*SSC*) is indicated. × 78,000.

FIGURE 10   (*A*) Double, parallel rows of intramembranous particles on the E face (white arrows) and P face (arrowheads) are in register from one face to the other (white arrows) and appear to be in continuity with strands that seem to span the intercellular space. × 75,000. (*B*) The *inset* shows the strands from the E to P faces across the intercellular space at higher magnification. × 118,000.

the particles that make up the intramembranous components of these junctions. The continuity of the double parallel rows of particles of the septate portion of the inter-Sertoli junction with the single interlacing rows of particles of the tight junction is not shown in this diagram (see Fig. 11). This model is based entirely on cytological observations and is presented to correlate the data obtained from thin sections with that from freeze-fracture replicas.

DISCUSSION

The Sertoli cell occupies a unique position in the seminiferous epithelium as the only cell type extending from the basal lamina to the lumen. Canine Sertoli cells are anchored to the basal lamina by hemidesmosomes and to each other by rudimentary desmosomes located just apical to the basal lamina (4). Fawcett (14, 16) has reported that there are no junctions between Sertoli cells and spermatogonia or between Sertoli cells and spermatocytes. This is not the case in the dog. In the canine testis, Sertoli cells are attached to spermatogonia by rudimentary desmosomes (4), particularly on their adluminal face, and to spermatocytes and spermatids by the vertebrate counterpart of the septate junction. The inter-Sertoli junction will be discussed here, and the relationship between germinal elements and Sertoli cells will be explored in a future publication.

Tight junctions (zonulae occludentes) are the most luminal of the elements of the junctional complex (13) that link epithelial cells together. Tight junctions seal together Sertoli cells of the seminiferous epithelium and prevent the interchange of substances between the lumina of the seminiferous tubules and the interstitial fluid that permeates the spaces between the Sertoli cells and diploid germinal cells. Gilula et al. (23) suggest that the inter-Sertoli tight junction is unique because of its basal location. However, the location of the tight junction may only appear basal because of the numerous extensions of Sertoli cell cytoplasm which surround the clones of haploid germinal cells that bulge into the lumen of the seminiferous tubules. If one considers these cytoplasmic extensions to be an elaboration similar to other cytoplasmic extensions such as microvilli or cellular interdigitations, then the location of this tight junction is similar to that in other epithelia such as intestine (42) or liver (25). Gilula et al. (23) also suggest that the occluding junction between rat Sertoli cells is unique be-cause of the large number of particle rows that do not anastomose. Large numbers of particle rows that do not anastomose are seen in the canine Sertoli junction; however, as shown here, they represent septate junctions and not tight junctions.

In this study intramembranous particles were associated with both faces of the junctional membranes and were arranged as intersecting rows of discrete or fused particles recessed in ridges on the P face and as discrete or fused particles recessed in grooves on the E face. Although double replicas have not yet been done, this arrangement of particles in this tissue seems similar to that in other tight junctions (41); however, the fact that the particles are recessed within crevices or furrows on both faces has not been noted previously in mature junctions. Gilula et al. (23) found a preferential association of intramembranous particles with the E face of rodent Sertoli cells. In the dog inter-Sertoli junction, the particles appear to be more commonly associated with the E face (or perhaps the junction is more readily identifiable on this face because of the relatively particle-free background).

The bands of filaments bordering the junctions may impart a certain rigidity to that region of the cytoplasm so that increased intercellular fluid pressure would cause only the nonjunctional membranes to bulge. This could explain the scalloped appearance of membranes adjacent to tight junctions. However, the tight junction itself may cause membrane tension by bringing the leaflets of the plasma membrane closer together. By stitching the adjacent plasma membranes together in a quiltlike fashion, a seal is formed that prevents the entry of intercellular probes into the region containing the haploid cells. These pockets of fluid are apparently undisturbed by normal intercellular flow of fluids. Materials that are released in small quantities could be concentrated in these pockets and taken up by the adjacent Sertoli cell membrane. In testicular tissue of adult and prepubertal dogs stimulated with human chorionic gonadotropin, pinocytotic vesicles are dramatically increased in number within the membrane region enclosed by tight junctions (8).

The data presented here indicate that there are two separate but interrelated structures within the inter-Sertoli junction (Fig. 16). One, the tight junction, has just been discussed, and the other has many of the cytological characteristics of the invertebrate septate junction. This vertebrate
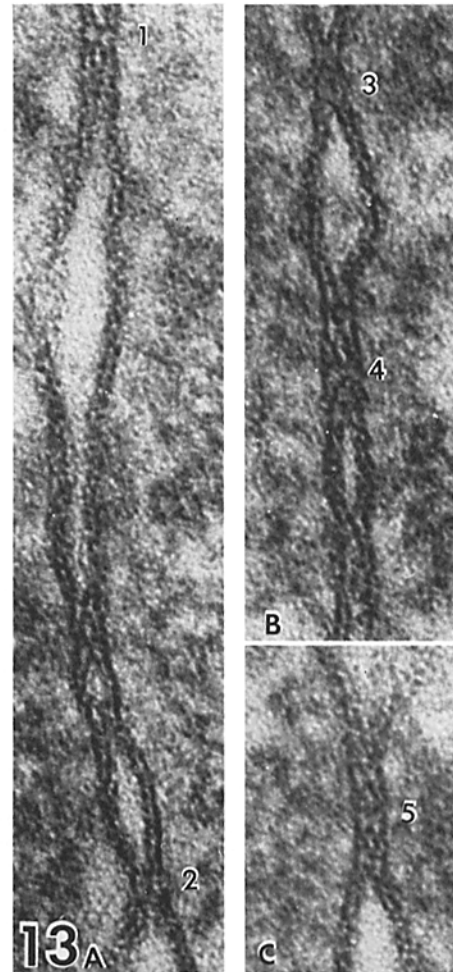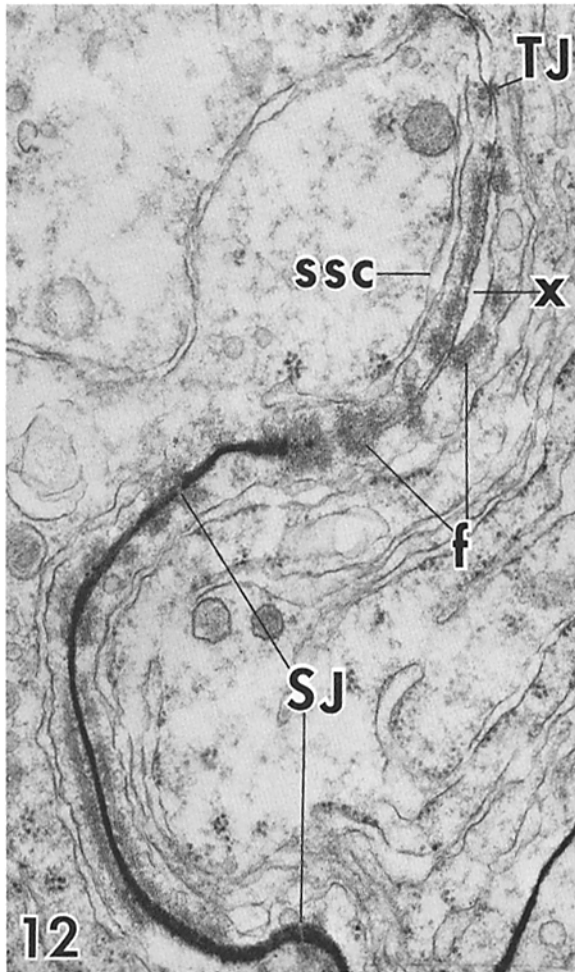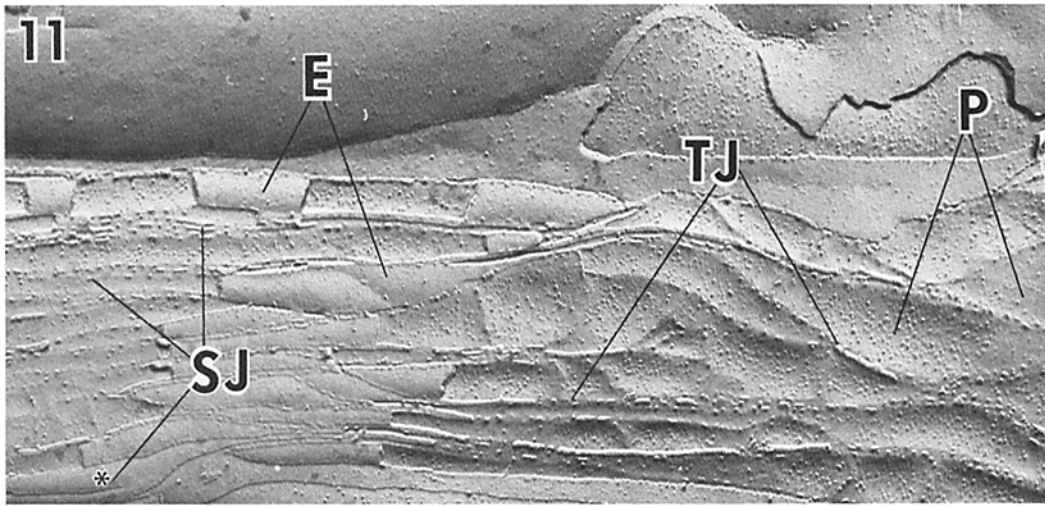
junction is classified as septate because septa are seen in thin sections linking adjacent cells, because areas of lanthanum exclusion are arranged in a pattern identical to that of the septa seen in thin sections, and because one sees in freeze-fracture replicas strands of particles arranged in parallel arrays that are in register from one face to the other and are in continuity with strands that appear to bridge the intercellular space.

A hypothesis incorporating the thesis that septate junctions can be converted into tight junctions or vice versa might be valuable in understanding just how the blood-testis barrier is maintained during the continuing process of spermatogenesis. The continuity of the septate and tight junctions within the inter-Sertoli junction might imply that the two junctions are intertransformable, or that the septate junction is developmentally essential for the formation of the tight junction. Preliminary data from a study of the genesis of the inter-Sertoli junction in prepubertal dogs (7) indicate that septate junctions do form before tight junctions, but that immature tight junctions are present in regions not occupied concurrently by septate junctions. Therefore, there is at present no experimental data to support a hypothesis that

septate junctions may be transformed into tight junctions or vice versa.

Junctions described in thin sections as "septate-like" have been noted in a number of endocrine tissues (9, 12, 20); however, there has been no freeze-fracture confirmation of septa in these cases or in any other vertebrate tissue until now. Recently, Gilula et al. (23) noted that the rat inter-Sertoli junctions combine certain features of the invertebrate septate junction and certain features of the vertebrate tight junction. A number of the junctions identified by Gilula et al. as occluding may be septate junctions instead. Although the magnification of Fig. 12 of Gilula et al. (23) does not permit positive identification of the type of junction present, the close but parallel arrangement of the membranes and the presence of densities spanning the intercellular space suggest that the junctions may be septate. The linear arrays of particles shown in freeze-fracture replicas of the rat inter-Sertoli junction (reference 15, Fig. 17; reference 23, Fig. 6) are consistent with the appearance of septate junctions and not with occluding junctions. Nagano and Suzuki (31) also show regions of junctions in replicas that appear to be septate (31; Figs. 2, 4), but are labeled as

FIGURE 11   As shown here, the transition from a region of septate junctions (SJ) to a region of tight junctions (TJ) is gradual and part of the continuum of the inter-Sertoli junctions. Note that the parallel rows of particles making up the septate junctions may branch, forming single rows of particles that then come together to form the characteristic interlacing pattern of a tight junction (TJ). Note also that some of the sets intersect or terminate abruptly (*). The relatively flat membrane surface between the rows of septate junctions is in contrast to the highly contoured membrane surfaces seen within the tight junctional region. For orientation purposes, the base of the seminiferous tubule is to the left, and the P and E faces are indicated. × 31,000.

FIGURE 12   Tight junctions (TJ) prevent the movement of lanthanum tracer into the luminal compartment of the seminiferous tubules. Unlike the ~90-Å intercellular space that is characteristic of the septate junction (SJ), the intercellular space between belts of tight junctions is apparently obliterated. Because the Sertoli cells are highly contoured, some regions of the junction are not cut in transverse section and thus not all of the tight junctions are clearly seen in this section. However, their presence can be inferred by the lack of penetration of the tracer and by the presence of regions of enlarged intercellular spaces (X) within the complex junction. The cytoplasmic constituents of this complex junction, bands of filaments (f), and subsurface cisterna (SSC) are adjacent to both the septate and tight junctions. × 43,500.

FIGURE 13   Sertoli cell tight junctions seen in thin sections appear to be regions of close membrane apposition adjacent to widened intercellular space. At the points of apposition a number of images can be seen: Fig. 13A. (1) bulbous enlargements with electron-lucent cores, (2) bulbous, electron-lucent enlargements with electron-dense cores; Fig. 13B: (3) a single, electron-dense structure with an electron-dense center, (4) extensions that appear to connect one membrane to the other; Fig. 13C. (5) as well as crisscrossing extensions with electron-lucent cores that appear to join one cell membrane to the other. × 264,000.
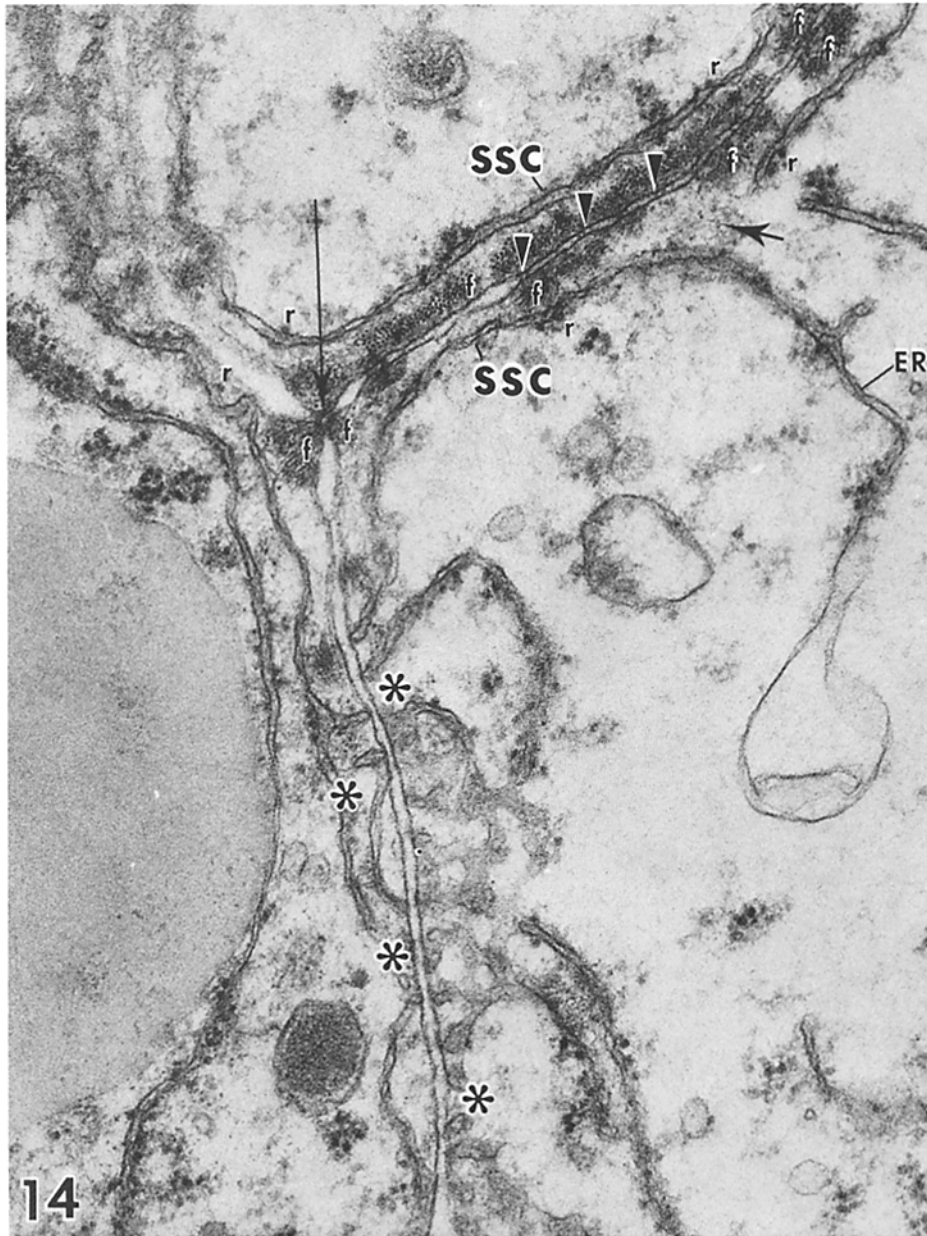
FIGURE 14 Tight junctions (arrow) between Sertoli cells are commonly seen at the union of three plasma membranes. Bands of filaments (f) border both the tight (arrow) and septate junctions (arrowheads). Subsurface cisternae (SSC) border the inter-Sertoli junction and are continuous with the endoplasmic reticulum (ER) of the cell. Note that the filaments are disorganized where the SSC does not directly border the plasma membrane (short arrow). Ribosomes (r) are more commonly seen on the cytoplasmic surface of the SSC. An unusual feature of the ER is the appearance of bulbous dilations of fenestrated smooth ER abutting directly on the plasma membrane (*). × 82,000.

occluding. However, they also show a replica (31; Fig. 3) with intersecting strands of particles, characteristic of the appearance of tight junctions (20). Neaves (32), using lanthanum hydroxide tracer in the rat testis after ligation of the ductus deferens and the ductuli efferentes, obtained en
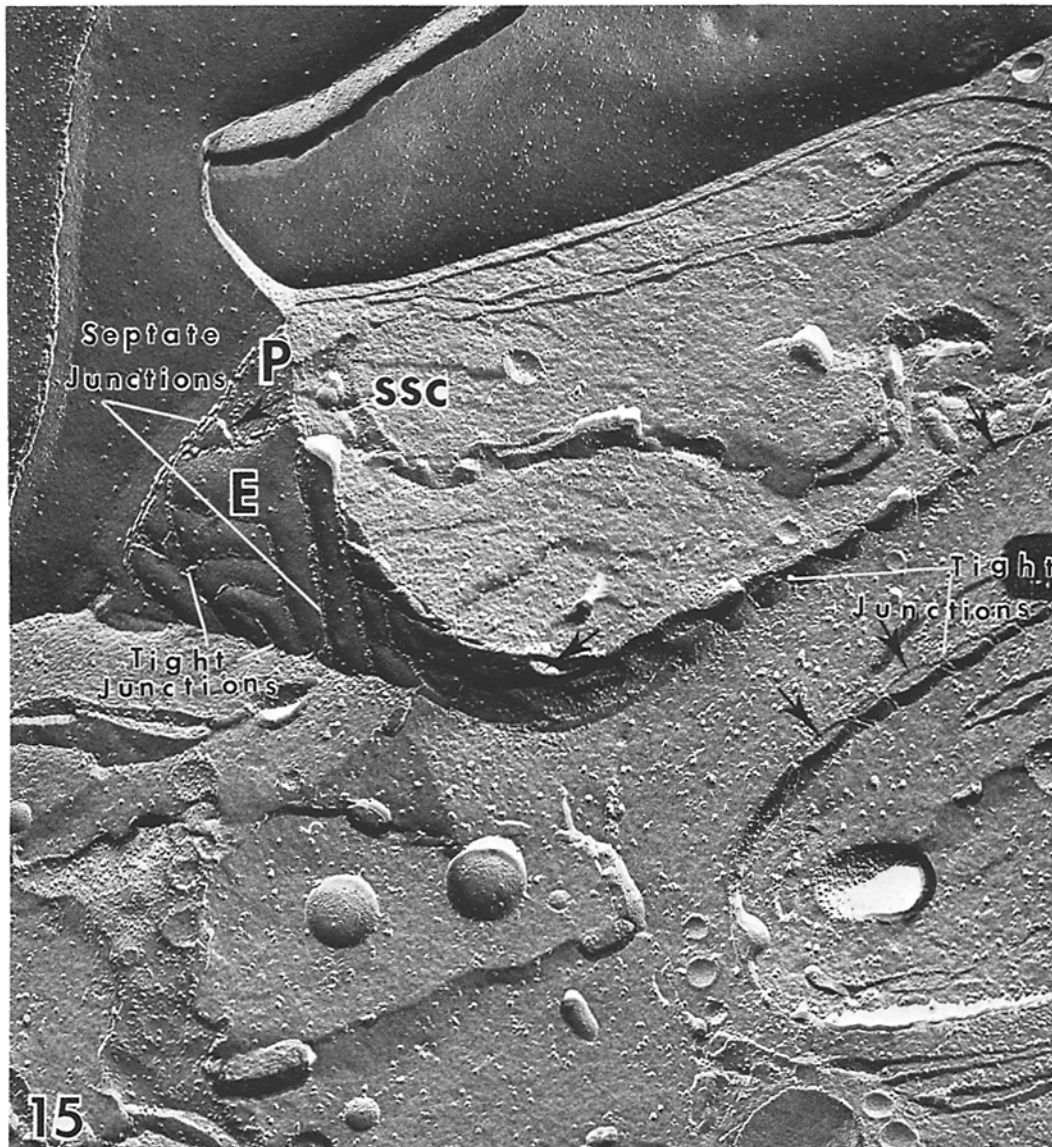
FIGURE 15 Interlacing rows of individual and fused particles are typical of the inter-Sertoli tight junction. Short arrows indicate the intercellular space between tight junctions. Note the inward bulging of the plasma membrane adjacent to the tight junction. The tight junction does not completely surround these Sertoli cell processes. A portion of the subsurface cisterna (*SSC*) is revealed adjacent to the junctional region. Septate junctions bordering the tight junction region, the P face (*P*), and the E face (*E*) are all indicated. × 59,500.

face sections that seem to have the appearance of typical invertebrate septate junctions (27). However, Neaves attributed these discontinuities in the lanthanum tracer to membrane fusions, i.e., tight junctions. It is probable that these junctions also are septate. Septate junctions have been identified in the rabbit inter-Sertoli junctions (C. J. Connell, unpublished results).

The functions of the vertebrate septate junction are not well established. Structurally, this junction provides increased intercellular adherence, but physiologically its role is untested. The vertebrate
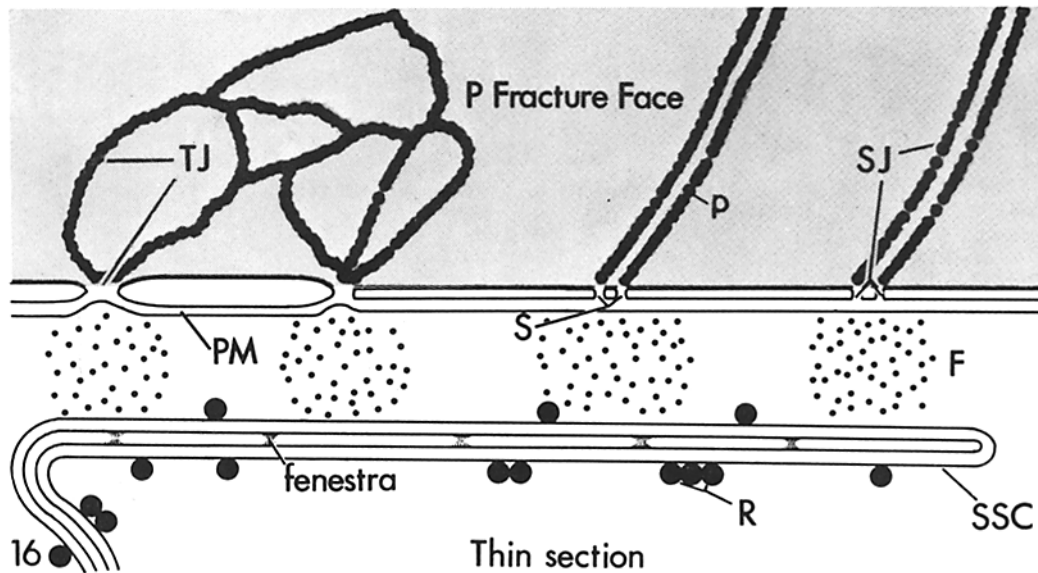
P Fracture Face

Thin section

FIGURE 16 This model presents, diagrammatically, the inter-Sertoli junction as it appears in thin sections and in freeze-fracture replicas. The lower, unstippled portion of the diagram represents a thin section, whereas the upper, stippled portion represents a freeze-fracture replica in which the interior face (P face) has been exposed. Cytoplasmic as well as plasma membrane specializations occur within the inter-Sertoli junction. The subsurface cisterna (SSC), an elaboration of the endoplasmic reticulum, is fenestrated, and ribosomes (R) are located preferentially on its cytoplasmic face. Between the SSC and the plasma membrane (PM) are bands of filaments (F) that border the intercellular junctions. The septate junctions (SJ) within the inter-Sertoli junction are basal to the tight junctions (TJ). The septa (S) span a $\sim$90-Å intercellular space and only partially impede the movement of fluid toward the lumen of the seminiferous tubules. At the region of the tight junction the plasma membranes are closely applied, and the luminal movement of tracer is prohibited. In freeze-fracture replicas, the septate junction appears as individual and fused "particles" (p) in linear array, usually as parallel, double rows. These rows are in continuity with the intercellular septa. In freeze-fracture replica, tight junctions appear as interlacing rows of individual and fused particles. These particles appear to stitch the adjacent membranes tightly together. The structures are drawn in proportion to their size, but are not drawn exactly to scale, and the interconnections between septa in thin sections and the particles located between the parallel rows of particles of the septate junction seen in freeze-fracture replicas have not been indicated.

septate junction could be considered restrictive in the sense that the intercellular space is diminished and the flow of fluids between the cells may be impeded. However, these junctions could also be considered permissive because the materials inside the septate corridors may be maintained there for a longer time and in higher concentration than within the unrestricted regions between the plasma membranes. The invertebrate septate junction was at first thought to play a role in intercellular communication (3, 22); however, this role was proposed before the presence of gap junctions in invertebrates was determined (27). Because gap junctions have been implicated in both ionic and metabolic coupling (1, 24), the role of invertebrate septate junctions in intercellular commu-

nication is now in doubt. It should be noted that gap junctions are seen between Sertoli cells, although not in abundance. Within invertebrate tissues, septate junctions are common and tight junctions are rare (21), whereas in vertebrate tissues tight junctions are common and septate junctions are presumably rare. The invertebrate septate junction also has been implicated as an occluding junction (30, 43). It will be of interest to learn whether vertebrate septate junctions are chemically homologous to invertebrate septate junctions and thus represent conservation of a structure during evolution.

Structurally, the canine septate junction resembles most closely the invertebrate continuous junction (18, 33). The Noirots (33) have proposed

that the invertebrate continuous junction would be found in regions of epithelial cell turnover that have extensive changes of cell to cell positions. Consistent with this hypothesis, the vertebrate counterpart of the invertebrate continuous junction occurs in a region of cell renewal that requires considerable alteration of plasma membrane interrelationships. Sertoli cells have a complex architecture that is subject to remodeling during the process of spermatogenesis as clones of haploid cells are moved luminally during their maturation. The structure of the vertebrate septate junction, i.e., parallel, double rows of septa occasionally linked by cross connections, should allow some side to side movement while retaining the alignment of filaments and SSC between the adjacent Sertoli cells. The specific intercellular relationship of the specialized regions of cytoplasm apparently must be maintained during the process of spermatogenesis, as the SSC and their associated bands of filaments appear to represent an internal membrane specialization essential to the integrity of the junction between Sertoli cells and between Sertoli cells and spermatocytes and spermatids. The SSC may be involved in the production and maintenance of the unique ionic, enzymatic, and nutritive milieu necessary for the differentiation of the germinal cells, and probably it is instrumental in regulating the agents that activate the movement of the ~60-Å filaments that are an essential part of this complex.

SSC are found in a variety of vertebrate and invertebrate cells (35). Many of these cells are irritable or sensory in nature (26, 35, 40), and some are connected by septate junctions as well (10, 38). It has been suggested that these SSC may be involved in regulating cation concentrations in a manner similar to that which occurs in the sarcoplasmic reticulum of muscle (34, 35). Inasmuch as bands of filaments that presumably are contractile and responsive to variations in calcium concentration are interposed between the SSC and the inter-Sertoli junction, a theory that links the SSC to the contraction of the filaments is certainly attractive. During the process of spermatogenesis, the germinal cells must be moved from the basal compartment to the luminal compartment, that is, luminal to the blood-testis barrier. Later, during stages of spermatid elongation, these haploid cells are moved closer to the basal lamina, but still above the blood-testis barrier, and then just before spermiation the spermatids are returned to a luminal position.

Throughout this process the clones of germinal cells are attached to the Sertoli cells by a variety of hold-fast devices (6, 16, 19, 36, 37), including septate junctions. The bands of filaments in the cytoplasmic specialization of Sertoli cells may be involved in the movement of the Sertoli cells and secondarily in the movement of spermatocytes and spermatids.

SUMMARY

Both septate and tight junctions are present between the specialized cytoplasmic regions of the inter-Sertoli junction. The tight junctions (zonulae occludentes) are the more luminal of these junctions. Basal to the septate junctions, a junctional type that could be considered a variant type of zonulae adherentes, are located rudimentary desmosomes (maculae adherentes). It seems, therefore, that the inter-Sertoli junction of the canine seminiferous epithelium is functionally a typical, epithelial complex junction (13). All of the junctions present between Sertoli cells contribute to intercellular adhesion, but each has a special structural function as well. The tight junction functions as a seal between the interstitial fluid and seminiferous fluid. This prevents the influx of interstitial fluid into the highly specialized luminal environment and prevents the efflux of specific antigens from haploid cells into the interstitial compartment. The septate junction is no doubt necessary for the maintenance of the highly specialized architecture of the Sertoli cell and serves not only to link Sertoli cells together, but also to link the haploid germinal elements to the Sertoli cell during the highly regulated process of spermiogenesis. This is the first demonstration in a vertebrate tissue of a junction that is septate in appearance in freeze-fracture replicas, in lanthanum tracer experiments, and in routine thin sections.

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