Definitive Evidence for the Existence of Tight Junctions in Invertebrates

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ABSTRACT Extensive and unequivocal tight junctions are here reported between the lateral borders of the cellular layer that circumscribes the arachnid (spider) central nervous system. This account details the features of these structures, which form a beltlike reticulum that is more complex than the simple linear tight junctions hitherto found in invertebrate tissues and which bear many of the characteristics of vertebrate *zonulae occludentes*. We also provide evidence that these junctions form the basis of a permeability barrier to exogenous compounds. In thin sections, the tight junctions are identifiable as punctate points of membrane apposition; they are seen to exclude the stain and appear as electron-lucent moniliform strands along the lines of membrane fusion in *en face* views of uranyl-calcium-treated tissues. In freeze-fracture replicas, the regions of close membrane apposition exhibit P-face (PF) ridges and complementary E-face (EF) furrows that are coincident across face transitions, although slightly offset with respect to one another. The free inward diffusion of both ionic and colloidal lanthanum is inhibited by these punctate tight junctions so that they appear to form the basis of a circumferential blood-brain barrier. These results support the contention that tight junctions exist in the tissues of the Invertebrata in spite of earlier suggestions that (*a*) they are unique to vertebrates and (*b*) septate junctions are the equivalent invertebrate occluding structure. The component tight junctional 8- to 10-nm-particulate PF ridges are intimately intercalated with, but clearly distinct from, inverted gap junctions possessing the 13-nm EF particles typical of arthropods. Hence, no confusion can occur as to which particles belong to each of the two junctional types, as commonly happens with vertebrate tissues, especially in the analysis of developing junctions. Indeed, their coexistence in this way supports the idea, over which there has been some controversy, that the intramembrane particles making up these two junctional types must be quite distinct entities rather than products of a common precursor.

It has been claimed that the cells of true invertebrates differ from those of chordates with respect to the kinds of junctions they are capable of forming. Whereas tight or occluding junctions have been recognized as existing between cells in a wide range of chordate cell types (2, 3, 8, 12, 13, 20, 45, 59, 66, 70), comparable *zonulae occludentes* have usually been considered to be absent between invertebrate cells (23, 53, 54, 62, 63, 73). Instead, the junctional type proposed to form the basis of permeability barriers restricting the paracellular entry of ions and molecules is the septate junction (14, 23, 46, 49, 54, 55, 67, 74), despite the considerable structural differences of septate junctions from vertebrate tight junctions. They have been said to be occluding, representing the invertebrate equivalent of the vertebrate tight junction (14, 23, 52, 55). However, the evidence for this assumption, based on physiological and tracer-uptake studies, is highly equivocal (see reference 35).

A number of "tight junction-like" structures have been reported to occur in the invertebrates, although they exhibit only certain of the features of vertebrate tight junctions. They include "continuous" or "smooth septate" junctions (16, 30, 35, 51), retinular cell junctions (6, 7, 34), reticular septate junctions (17, 33–35), the junctions found in regenerating crustacean sheath cells (65), and those occurring in the sheath of ticks (1) and the horseshoe crab, *Limulus* (25). However, although all these junctional types exhibit intramembrane particles fused into extensive ridges (hence reminiscent of vertebrate tight junctions), they have also been found to possess an intercellular cleft of some width, often as much as 10–20 nm or more, and

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in some cases atypical fracturing characteristics as well. Thus in each of these cases it has become clear that the junctions are not structurally equivalent to vertebrate *zonulae occludentes*, nor do they constitute a definitive permeability barrier.

There have been, however, a number of reports indicating that true tight-junctional structures among the Insecta are present in a variety of tissues (30, 35) that exhibit a permeability barrier, both with respect to the entry of tracers (32, 41, 42) and by electrophysiological criteria (69). The first investigations of this sort were on the avascular insect nervous system, in which the beltlike outer cellular sheath, the perineurium, was found to prevent the intercellular entry of tracer molecules beyond its inner limits (28, 36–38, 41, 42, 48). Earlier observations on the insect perineurium had incorrectly reported tight junctions (47, 68) that were actually gap junctions,[1] because without en bloc uranyl-acetate staining no clear distinction can be drawn between these two junctional types in thin sections (61). True punctate "tight" membrane appositions were subsequently found between perineurial cells in thin sections (28, 30, 35–39). Moreover, freeze-fracture observations reveal that tight junctions are indeed present in the perineurium, although these are frequently discontinuous and of a very simple nature (30, 36–39). They are also found relatively infrequently, and there has been no unequivocal demonstration of the occlusion of the intercellular cleft nor of the complementary nature of the ridge-groove system. For this reason, in some quarters (for example, see reference 23) there has been a lack of acceptance of the hypothesis that the invertebrates, represented by arthropods, do possess tight junctions.

In this report we now present unequivocal evidence for the existence in the invertebrates of tight junctions like those in vertebrates, within the CNS of the spider. Here the perineurial cells, which ensheathe the nerve cells and glia as a beltlike layer, exhibit no septate junctions but possess punctate intercellular membrane appositions in thin sections. These restrict the free entry of tracers when incubated under physiological conditions in vivo and must therefore be important in the functioning of the CNS, because they constitute a permeability or blood-brain barrier. These appositions exhibit an obliteration of the intercellular cleft, together with circumferential complementary P-face (PF) ridges and E-face (EF) grooves in freeze-fracture replicas; they therefore appear to be structures comparable to those that form the basis of vertebrate permeability barriers.

MATERIALS AND METHODS

Specimens of the house spider, *Tegenaria domestica* (Agelenidae, Araneidae), were collected as required. The tissue studied was the central nervous system (CNS) composed of a supraesophageal ganglion or "brain," and a subesophageal, fused, cephalothoracic ganglion.

Conventional Fixation, Embedding, and Sectioning

The tissues were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.2, plus 0.15 M NaCl. One drop of 1% $CaCl_2$ solution was added for each 10 ml of the final solution.

After fixation at room temperature (r.t.) for 1 h, the tissues were washed in three changes of buffer. Treatment with 1% osmium tetroxide in buffer followed for 60 min at r.t. After this, the tissues were rinsed in buffer and stained en bloc with 2% aqueous uranyl acetate for 30 min at r.t. Dehydration through an ascending series of ethanols and propylene oxide ensued, followed by embedding in Araldite. Sections were cut on a Cambridge Huxley Mark II Ultramicrotome (Cambridge Instrument Co., Inc., Ossining, N. Y.). Thick, 1-μm sections were stained with toluidine blue for examination under the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 300.

Lanthanum Incubation

The nervous tissues were treated with lanthanum in one of two ways:

(*a*) The ganglia were exposed, without fixative, and incubated in vivo for 1 h in 10 mM lanthanum chloride in a phosphate-free Ringer's containing 10 mM K, 140 mM Na, 2 mM Ca, 152.25 mM Cl, 5 mM HEPES, 6.8% sucrose (513.6 mOSM). This allows the uptake of ionic lanthanum to occur under "physiological" conditions. At the end of this time, the lanthanum solution was pipetted out from around the tissues and replaced by 2.5% glutaraldehyde made up in phosphate buffer to precipitate the lanthanum. The buffer used was the same as that for the conventional fixation, although no calcium was added. The tissue was then dissected out and placed in fresh fixative solution for 1 h. In an alternative procedure, the ganglion was removed from the spider and then incubated in vitro.

Most of the incubated tissue was then treated in the conventional fashion, as outlined above, but in a few cases the en bloc staining with uranyl acetate was omitted and the specimens were taken straight from the buffer washing, after osmium tetroxide, to the dehydration schedule. From this point, treatment was the same for all tissues.

Appropriate controls, with 1-h-long incubation in Ringer's without lanthanum, were also carried out.

(*b*) Tissue was fixed for 1 h in a solution of 2.5% glutaraldehyde in a 0.1 M cacodylate buffer, containing 0.1 M NaCl, to which 1% colloidal lanthanum hydroxide, prepared from lanthanum nitrate, had been added. The tissue was then treated in the conventional fashion, as described above, using a buffer of 0.1 M cacodylate (pH 7.2), 0.15 M NaCl and one drop of 1% $CaCl_2$ per 10 ml of final solution. This technique reveals details of the external membrane surfaces composing the various junctional complexes and the extracellular matrix.

Freeze-Fracture

The nervous tissue was dissected out and prepared for freeze-fracturing either without fixing or after brief fixation. Fixed tissues were treated with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, plus 0.15 M NaCl for 20 min at r.t. They were then washed in several changes of the same phosphate buffer and incubated for 15–20 min at r.t., in 20% glycerol made up in buffer. Unfixed tissues were treated with 20% glycerol made up in spider Ringer's (513 mOSM, as described earlier) for 15–20 min at r.t., to determine whether the distribution or preferential fracture face of the various populations of intramembrane junctional particles would change in the absence of chemical treatment before freezing (see reference 16). Both fixed and unfixed tissues were rapidly frozen in Freon cooled in liquid nitrogen (N_2) and stored in liquid N_2 until use. Material was fractured in a Balzers device (BA 360 M; Balzers Corp., Nashua, N. H.), without etching, at −100°C and at a pressure of 1.33×10^{-4} Nm^{-2} (1.5×10^{-6} torr). Shadowing was carried out using tungsten-tantalum followed by backing with carbon. The freeze-fractured replicas were cleaned with sodium hypochlorite or sulphuric acid, picked up on coated grids, and examined in a Philips EM 300. The micrographs are mounted so that the direction of metallic shadow is either from the bottom or side.

RESULTS

Conventional Fixation

The tissue under investigation is the sheath surrounding the cephalothoracic ganglionic mass. This sheath is in many respects similar to that in other arthropods (29, 30) and is overlain by an acellular neural lamella that is effectively a thick, collagen-containing, basal lamina (Fig. 1).

The perineurium is composed of modified glial cells that form a complete layer of variable thickness around the entire ganglionic mass of glial-ensheathed neurons. Its component cells interdigitate and are thrown into lateral folds so that the cell borders often run parallel to the outer cell surface (Fig. 1) on the external side. The perineurial cells display numerous hemidesmosomes with the neural lamella. These take the form of electron-dense plaques of material that lie on the inner

[1] Similar reports were also made about thin-sectioned preparations from tissues of a number of other invertebrates (see references in reference 35).

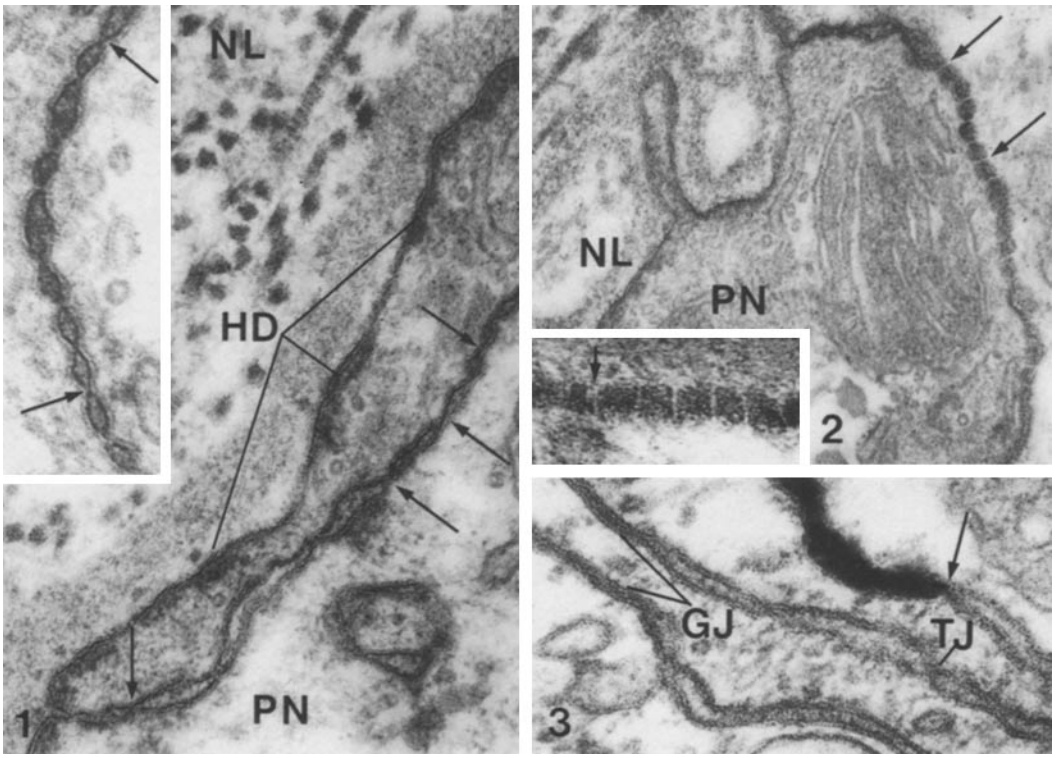


FIGURE 1 Figs. 1–13 are micrographs of the epithelial-like glial cell layer, the perineurium, that ensheaths the outer surface of the central nervous system of the adult spider, *Tegenaria*. Fig. 1 is a thin section showing the acellular collagenous neural lamella (*NL*) surrounding the outer border of the perineurium (*PN*) with extensive arrays of tight junctions (arrows) on the lateral cell borders, which may run parallel with the perineurial surface. The perineurial cells exhibit hemidesmosomes (*HD*) at their boundaries with the neural lamella. The *inset* shows the punctate nature of these junctions and the total occlusion of the intercellular space (arrows) at the points of membrane fusion. × 79,000; *inset*, × 125,000.

FIGURE 2 Thin section showing tight-junctional arrays cut in *en face* plane (arrows) to reveal the ridgelike structures in the plane of the membrane that occur at the points of fusion. *Inset* shows a higher power micrograph of similar structures; note the particulate nature of the junctional ridges and the double ridge (at arrow). *NL*, neural lamella; *PN*, perineurium. × 59,000; *inset*, × 83,500.

FIGURE 3 An ionic lanthanum preparation showing that the penetration of lanthanum is stopped by punctate tight junctions (arrow). Note the gap junctions (*GJ*) in close association with tight-junctional structures (*TJ*). Although cross-striations between adjacent membranes, reminiscent of septate junctions, are seen here, no distinct junctions of this type have been observed. × 93,000.

aspect of the outer membranes of the perineurial cells (Fig. 1). The membranes of adjacent perineurial cells are associated with one another by both tight junctions (or *zonulae occludentes*) and gap junctions. No distinct septate junctions have been observed, although occasional irregular cross-striations occur (Fig. 3).

TIGHT JUNCTIONS: The lateral membranes of the perineurial cells exhibit extensive arrays of punctate tight junctions that are found at fairly frequent intervals all along the interdigitating clefts of the cell borders (Fig. 1, *inset*). As is typical of tight junctions, they appear pentalaminar, and, at the point of membrane fusion, the total membrane width is only ~75% of the width of the two component cell membranes. Because of the punctate nature of tight junctions, identification of them is critically dependent on the section plane and angle. Although not anticipated, "uranium-calcium en bloc staining" (72) had the effect of staining the outer leaflet of the plasmalemma particularly heavily (Fig. 2). As a result, at the actual point of junctional contact, where membrane fusion occurs, the stain was excluded and revealed images characteristic of negatively stained tight junctions usually evident only in junctional areas that are "leaky" (19). This is especially clear in *en face* sections where oblique views of the linear ridges of membrane fusion can be seen (Fig. 2); in some cases, these may be so closely aligned as to appear double (Fig. 2, *inset*). Further examination at high resolution suggests that the points of fusion in uranyl-calcium-stained tangential thin sections are actually moniliform ridges seemingly composed of linear arrays of particles that in some cases appear fused together (Fig. 2, *inset*).

GAP JUNCTIONS: Gap junctions occur between apposing membranes throughout the depth of the perineurium. They exhibit the characteristic gap-junctional septilaminar structure with a 2- to 3-nm gap between adjacent membranes and may be closely associated spatially with the tight junctions (Fig. 3). After uranyl-calcium treatment, the intercellular gap appears wider because the heavy metal cannot be distinguished from the dense external membrane leaflet (as in Fig. 13, *inset*).

Lanthanum Penetration

In this study, both ionic and colloidal lanthanum were used as tracers to determine whether the perineurium of *Tegenaria* restricts the inward movement of exogenous molecules. Ionic-lanthanum-treated intact tissues show scattered dense deposits

in the neural lamella, (in particular, adhering to the collagen fibers). In the perineurium, lanthanum deposits are restricted to the extracellular spaces and are found near the external surface of the perineurium in the outermost intercellular clefts (Fig. 3). Although lanthanum may penetrate some of the more peripheral tight junctions, it is not seen to penetrate beyond the tight-junctional belt of the perineurial layer either in ionic lanthanum or in colloidal lanthanum preparations.

Freeze-Fractured Replicas

Freeze-fractured replicas of the supraesophageal and fused subesophageal ganglia of *Tegenaria* reveal that the peripheral circumferential layer of epithelial-like perineurial cells are associated by both tight and gap junctions on their lateral borders (Figs. 4 and 5). Unlike the insect perineurial layer (29, 30, 35), extensive surveys indicate that no septate junctions are to be found here. The perineurial borders of the spider CNS interdigitate in a rather complex manner (see Fig. 4, *inset*), as is typical of arthropods (35).

TIGHT JUNCTIONS: The tight junctions of *Tegenaria* display fracturing features many of which are comparable to those of vertebrates. When extensive expanses of membrane face are encountered (for example, Fig. 4), the network of tight junctions assumes a circumferential distribution around the lateral borders of each cell. In both fixed and unfixed material, the lateral membranes exhibit this reticulum of ridges that fracture onto the PF (Figs. 4 and 5). These display varying degrees of complexity, and this variability may be associated with the position they occupy with respect to the outward-facing perineurial surface and acellular neural lamella. Just below this neural lamella, the ridges are not always interconnected in a complete network. Here they may be loosely associated and may exhibit some discontinuous strands (Fig. 5). Below this region, the components of the junctional reticulum appear closely enmeshed in an extensive network (Fig. 4). Towards the base of the lateral perineurial cell borders, the ridges become discontinuous and then disappear, leaving unspecialized membrane areas (Fig. 12). Gap junctions are intercalated between these ridges throughout the depth of the junctional belt (Figs. 4, 5, and 12).

The ridges that compose the tight-junctional network are made up of intramembrane PF particles, ~8–10 nm in diameter (with a mean of 9.14 ± 0.97 nm), and these are fused together laterally into distinctly moniliform ridges (Fig. 12, *inset*) or fibrils. In some cases, discontinuities are present (Fig. 5) and the degree of lateral fusion varies. These ridges, which in some cases stand in considerable relief from the fracture plane, are undoubtedly complementary to the grooves that lie in the opposing membrane's EF (Figs. 6 and 7), because the ridges and grooves are coincident across face transitions (Figs. 6 and 7). At the transition point where the fracture plane shifts from the PF up to the EF, the PF ridges appear to lie just to one side of the EF grooves (Figs. 4 and 10). This suggests that the aligned ridges or fibrils in the two adjacent cells that appear to fuse to form the punctate apposition may be slightly offset with respect to one another, although this may be only an apparent displacement resulting from the angle of shadow. In some cases, the complementary nature of the PF ridges and EF

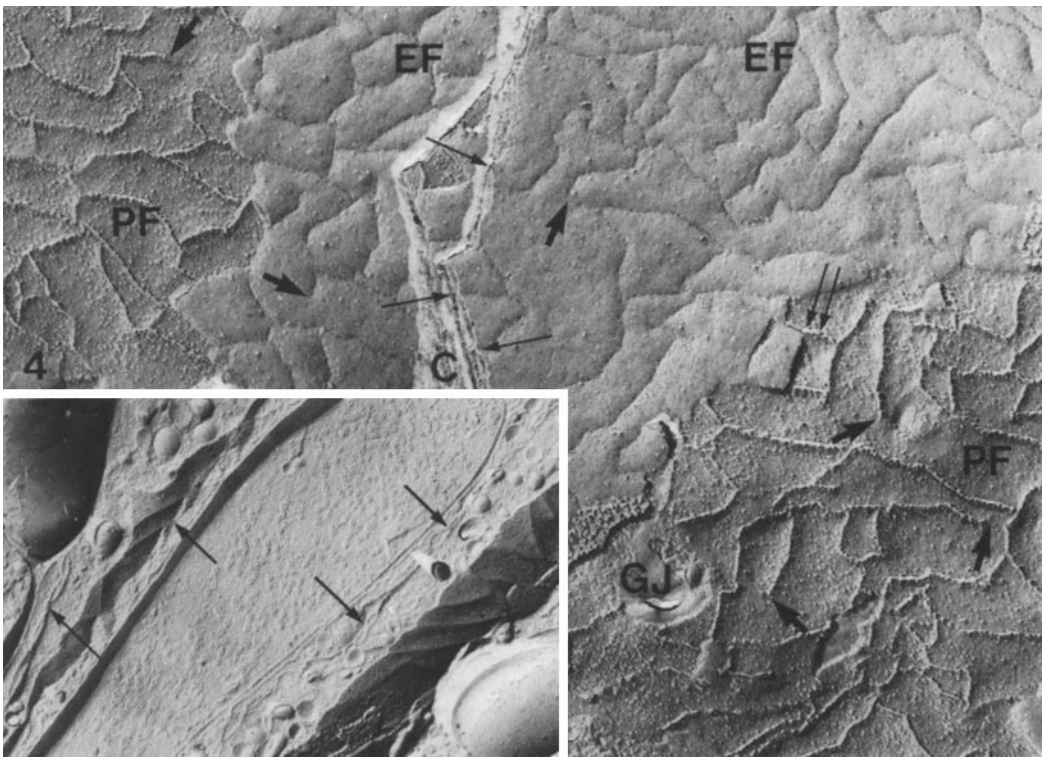


FIGURE 4 Freeze-fracture replica of the circumferential tight junctions that encompass the peripheral perineurial cells in the CNS of *Tegenaria*. This area shows the attenuated nature of the interdigitating cytoplasmic (*C*) processes along the lateral cell borders. Adjacent membranes are pinched together in punctate apposition (arrows). The P-face (*PF*) exhibits interconnecting ridges, whereas the E-face (*EF*) displays comparable grooves. Gap junctions (*GJ*) are intercalated between the tight-junctional elements, and possess the typical arthropod features of inverted complexes with EF particles and PF pits. The thick arrows show discontinuous ridges and grooves, while the double arrow indicates an area where the PF ridges are possibly offset with respect to the complementary EF grooves. The *inset* is a lower power micrograph illustrating the interdigitating lateral perineurial border with the high frequency of punctate membrane appositions (arrows) and complementary ridges and grooves. × 44,400; *inset*, × 22,800.

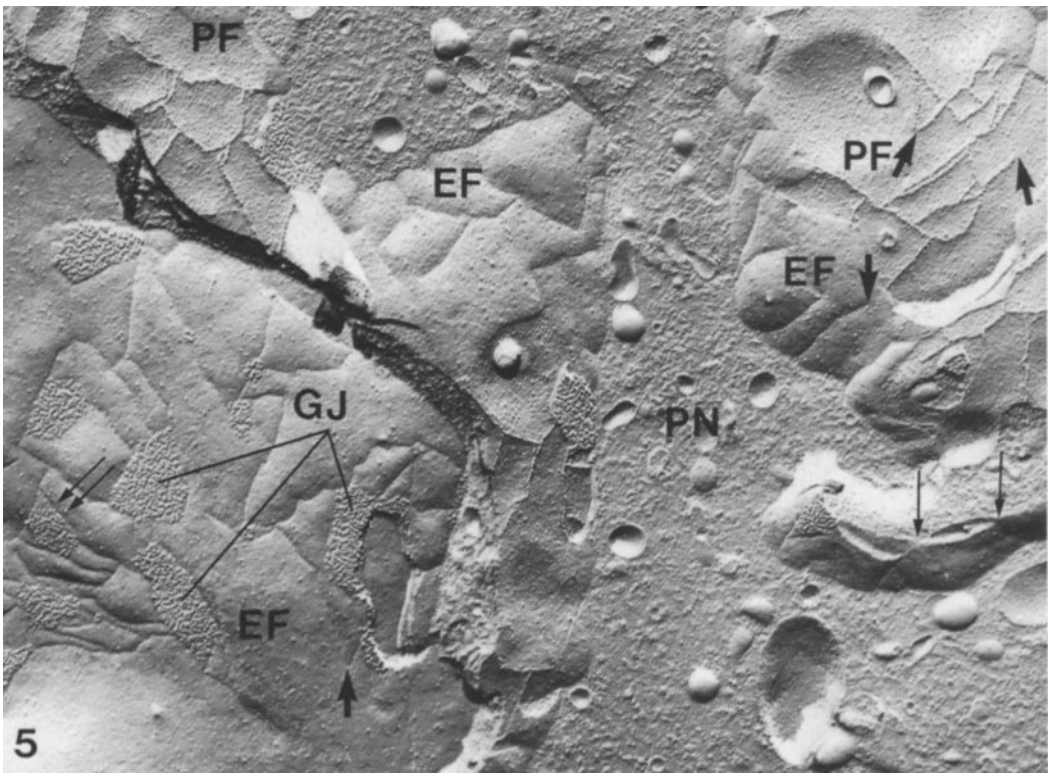
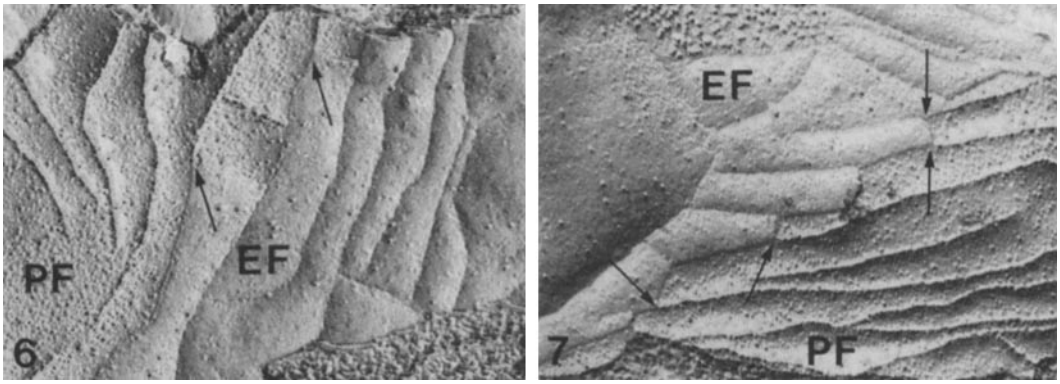


FIGURE 5 Perineurial cells (*PN*) demonstrating the arrays of P-face (*PF*) ridges and E-face (*EF*) grooves that comprise the circumferential tight junctions. Note that, unlike vertebrate *zonulae occludentes*, these exhibit discontinuities (at thick arrows) but, like them, they possess intercalated gap-junctional plaques (*GJ*); these, as in other arthropods, feature E-face particles. Punctate appositions between adjacent cell membranes are also evident (arrows). Double arrows indicate tight-junctional grooves lying alongside gap-junctional plaques. × 32,600.



FIGURES 6 and 7 These freeze-fracture replicas from the spider perineurium are from regions displaying the complementary nature (at arrows) of the P-face (*PF*) ridges and E-face (*EF*) grooves that comprise the tight-junctional network. The obliterated intercellular space is also evident here as is the occasional discontinuous ridge. Fig. 6, × 59,500; Fig. 7, × 57,900.

grooves is not clear-cut, because the ridges are discontinuous and a PF ridge may terminate just as the fracture plane shifts up to the EF (Figs. 5 and 6), so that no complementary groove is then present.

At the junctures where the plane of cleavage shifts from PF to EF, it can be seen that the intercellular space becomes negligible (Figs. 6, 7, and 8). This shows that, at these points, ridges in the membranes of the two adjoining perineurial cells fuse to occlude the intercellular space and give rise to the punctate membrane appositions seen in thin sections (as in Fig. 8); the PF ridges and EF grooves meet along these lines of membrane fusion (Fig. 8).

The "quilting" of the tight-junctional network shows that the PF ridges may sit on elevated parts of the membrane while the EF furrows are at the bottom of the membrane troughs (see Fig. 9); such an arrangement is consistent with the observed scalloping or undulating of the membrane into punctate fusions as seen in thin sections (Fig. 1) and in cross-fractured replicas (Figs. 8 and 10) and is similar to that observed in vertebrate tight junctions. In some cases, the tight-junctional ridges are very numberous and lie in close spatial association, often in parallel (Fig. 11).

The gap junctions that are intercalated in intimate relationship with the tight junctions are sometimes partly circumscribed by the ridges of the latter (double arrows in Fig. 5) or, more frequently, the ridges terminate slightly beyond the pe-

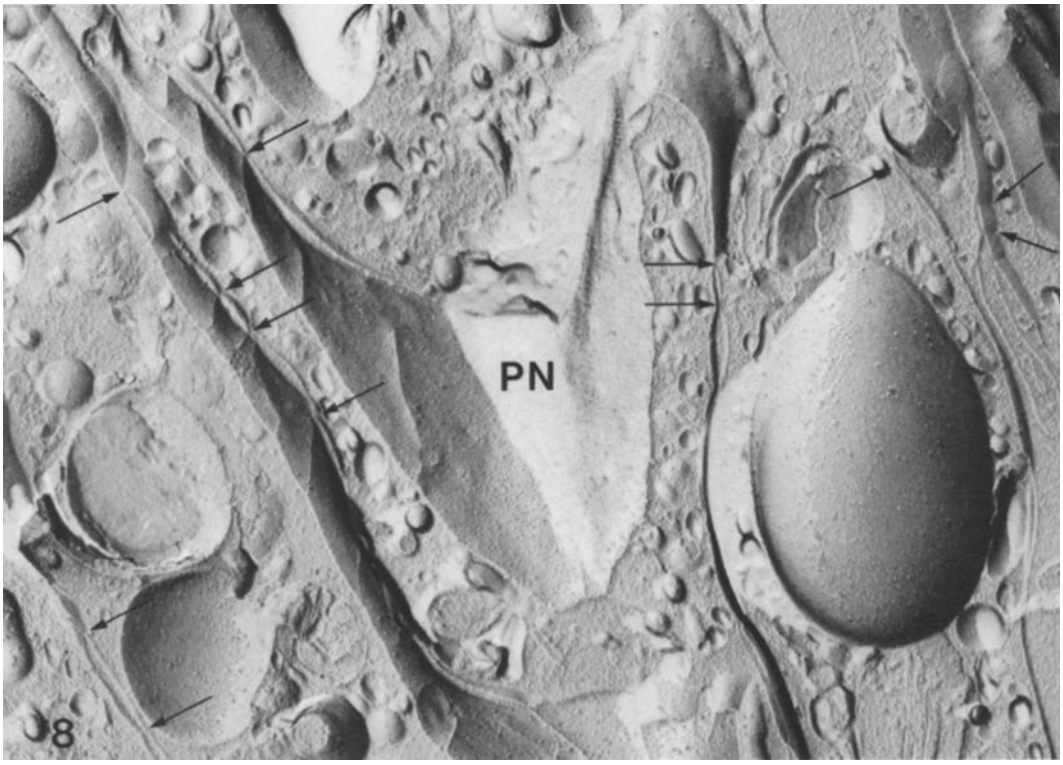


FIGURE 8 Low-power view of a replica of a perineurial region (*PN*) showing the undulating lateral membranes and especially the cross-fractures of the adjacent membranes to show the punctate tight-junctional appositions between cells (arrows). × 31,400.

riphery of the macular gap-junctional plaques (Fig. 12).

GAP JUNCTIONS: The gap junctions that coexist with the tight-junctional network are typically arthropodal in that they consist of plaques of EF particles with complementary PF pits (Fig. 12). The intercellular cleft is considerably reduced at the point of cleavage from EF to PF (Fig. 12). The junctional particles measure ~13 nm in diameter (range of 12–14 nm), although they sometimes lie close alongside one another (Fig. 5), and they exhibit a variable center-to-center spacing. At higher magnification, they may display a small central indentation, which is probably the pore through which ions and small molecules are exchanged when the cells are coupled. In some cases, they are very loosely packed (Fig. 13).

HEMIDESMOSOMES: The lateral border with peripherally located gap and tight junctions sometimes fractures from this edge out across the outward-facing perineurial surfaces. This membrane PF is highly enriched with intramembrane particles (IMP) as is also typical of other outward-facing perineurial membranes in arthropods (1, 35, 36).

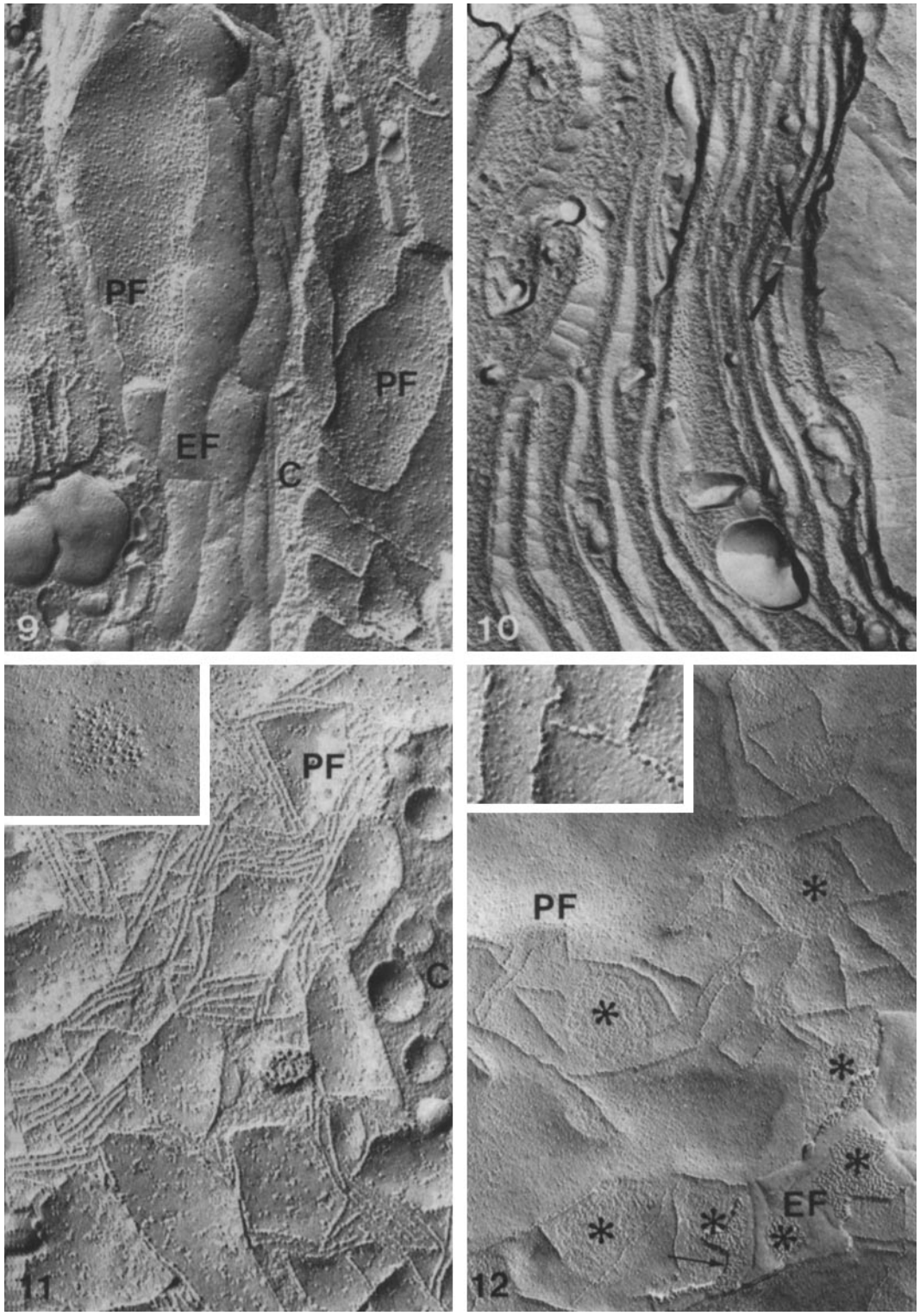
Dotted at random on this membrane face are clusters of somewhat larger IMP of variable diameter (Fig. 11, *inset*) which are quite distinct from the EF gap-junctional particles; they may be the freeze-fracture image of the hemidesmosomes that are found on this surface in thin sections (as in Fig. 1). In many other arthropod tissues (see references 35, 37–39), although desmosomes or hemidesmosomes may be present in profusion in thin sections they have no characteristic intramembrane profile visible after freeze-fracturing. However, in ver-

FIGURE 9 Two adjacent perineurial membranes showing the "quilting" effect whereby the E face (*EF*) shows troughs or depressions in which the tight-junctional grooves sit, whereas the opposite P face (*PF*) displays membrane waves, upon the crests of which sit the ridges. These correspond exactly to the expected topography for membranes that are fusing to form punctate appositions with a "scalloped" effect. *C*, perineurial cytoplasm. × 55,800.

FIGURE 10 Cross-fracture through a striking number of interdigitating perineurial processes showing a "scalloping" effect. This corresponds to the punctate membrane appositions seen in thin section (as in Fig. 1). Arrows indicate regions where the P-face ridges and complementary E-face grooves are coincident across face transitions. × 36,200.

FIGURE 11 Lateral border of a perineurial cell showing that the P-face (*PF*) ridges in some cases may be very numerous; this kind of image would be correlated with the frequent scalloping seen in thin section (as in Fig. 1) and in cross-fractures (as in Fig. 10). The *inset* shows a profile of a cluster of the irregular particles found in the outward-facing perineurial membrane; these probably represent the hemi-desmosomes seen in thin sections (as in Fig. 1). *C*, perineurial cytoplasm, × 65,900; *inset*, × 55,400.

FIGURE 12 Details of the gap junctions (asterisks) intercalated between the tight-junctional ridges (*PF*) or grooves (*EF*). The reduced intercellular cleft (arrow) is evident at the face transitions from E-face particles to P-face pits. Note that the tight-junctional elements seem to terminate just at the gap-junctional perimeter, if they are running at right angles into it. If the tight junctions are aligned parallel to the macular edge (as in the lower left-hand corner), the ridges then continue to run alongside the gap-junctional plaque. The *inset* shows that the ridges are composed of particle subunits (8–10 nm) aligned laterally into fibrils. × 37,100; *inset*, × 74,500.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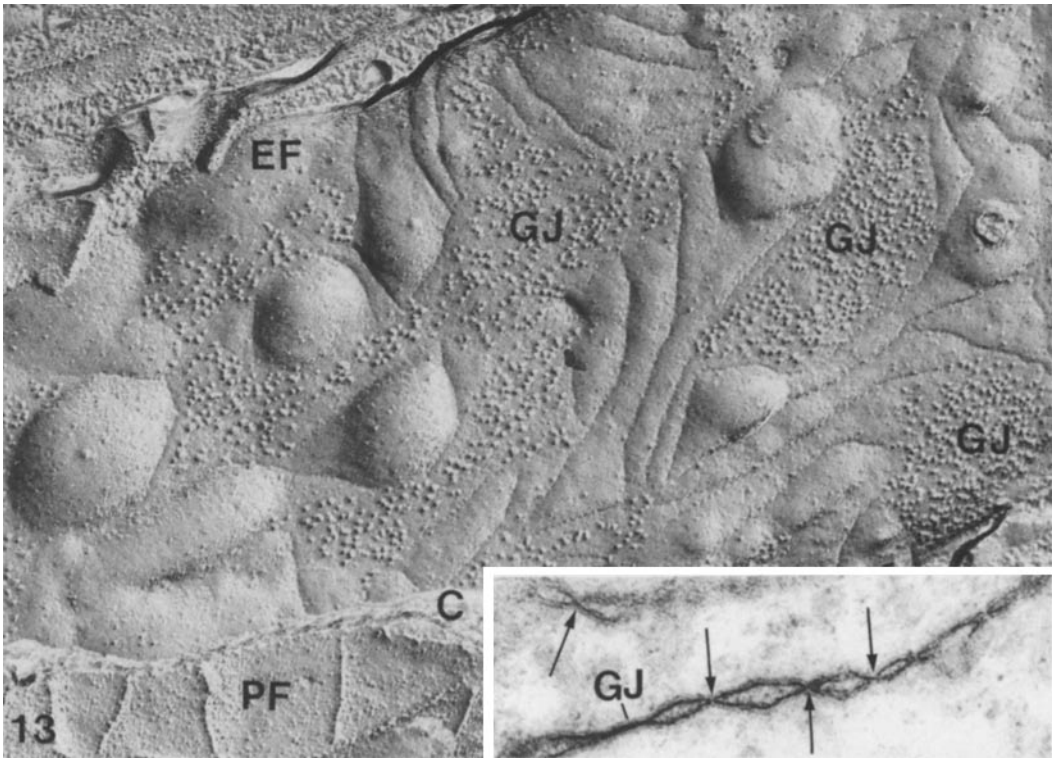

FIGURE 13 Replica through the lateral borders of perineurial cells (*C*) revealing 13-nm intramembrane E-face (*EF*) gap-junctional particles (*GJ*) that are arranged in very loose configurations; these arrays may reflect the degree of cell coupling. The P-face ridges and E-face grooves of the tight junctions would appear to hold these 13-nm particles in fairly restricted areas, preventing excessive translateral migration of intramembrane particles. The *inset* shows a thin-section correlate of this junctional coexistence with punctate tight junctions (arrows) and an adjacent gap junction (*GJ*) with its characteristic reduced cleft. × 55,200; *inset*, × 93,800.

tebrate tissues, their appearance is often comparable to that shown here (66), displaying clusters of intramembrane particles of widely different diameters.

DISCUSSION

For a junction to be categorized as a definitive *zonula occludens*, a number of criteria have to be met. These are all satisfied by the punctate appositions found between the perineurial cells that completely ensheathe the spider central nervous system. These punctate arachnid cell junctions exhibit a reduced width of apposed membranes at the points of fusion so that the intercellular space is obliterated. They have a circumferential distribution and inhibit the entry of exogenous tracers. Hence these tight junctions, which resemble those in vertebrates, constitute the morphological basis of a permeability or "blood-brain" barrier. In freeze-fractured replicas of spider CNS, the PF ridges are distributed as a network; these ridges are coincident across membrane face transitions with EF grooves and so appear to be complementary. It can no longer be claimed therefore that true *zonulae occludentes* occur only in the vertebrates (14, 23, 53, 54, 62, 63, 73) nor, as a corollary, can it be inferred either that septate junctions represent the invertebrate equivalent of the vertebrate tight junctions (14, 20, 23, 46, 54, 55, 74) or that the septate junctions must be the evolutionary forerunners of the vertebrate occluding junctions (22).

Comparison with Vertebrates

The fracturing characteristics of spider tight junctions are very similar to those of vertebrate tight junctions and reveal that the punctate membrane appositions seen in thin sections are likely to be the result of the PF ridges (in apposing cell membranes) fusing together in a way that obliterates the intercellular cleft. In both vertebrates and these spiders, the tight junctions occur near the peripheral or luminal part of an epithelial layer that forms a permeability barrier. As in mammalian tissues (19), the fact that the tracer appears to move in beyond some of the most peripheral of the spider punctate tight junctional appositions may be explained by the fact that sometimes the networks of ridges and grooves are discontinuous when close to the neural lamella (basement membrane). The lanthanum probably leaks in past these discontinuities (as in reference 19), to be stopped by the more extensive network of ridges farther into the system. The spider's sealing junctions may also function to segregate different populations of IMP between the outward facing membrane and the lateral borders, as has been suggested for vertebrate tight junctions (10, 26). However, in spiders, the lateral perineurial border is highly interdigitated (35), in contrast to the relatively straight border of the vertebrate terminal bar region (8, 13).

The intercalation of gap junctions between the tight-junctional quilting (as in Figs. 5, 12, and 13) is also comparable to vertebrate systems, in which such junctional coexistence is very common (see reference 60). In this respect, the only differences are (*a*) that the gap-junctional particles in spiders fracture not onto the PF but onto the EF, as is typical of arthropods (15, 30, 35); (*b*) that the tight-junctional ridges are commonly discontinuous around the gap junctions; and (*c*) that the gap-junctional arrays in spiders are occasionally more loosely packed than is generally the case for vertebrates. The first of these points is important with regard to the developmental stages in junctional formation; in this system, unlike tight

junctions of vertebrates, the developing PF tight junctions are clearly distinguishable from the EF gap-junctional arrays. The fact that gap junctions and extensive tight junctions coexist, but with different-sized component particles that fracture onto opposite membrane faces, enables them to be distinguished from each other (40), a feat which is not possible in vertebrates (60). This particle distinction does much to support the view that gap- and tight-junctional intramembrane particles are quite separate entities during development and do not arise from a common pool of precursor particles and diverge in the course of differentiation to perform two different functions depending on the stimulus (11).

The packing of the gap junctions into macular arrays in the spider tissue is sometimes so loose (Fig. 13) compared with that of vertebrates as to make them appear either immature and in the process of formation (30, 35) or in the process of dispersing (40). Because the tissues are from fully developed adults, these loose aggregates of junctional particles may signify junctional turnover (31) or the degree to which the cells are coupled; loose and close packing of comparable particles in other systems have been taken to mean coupled and uncoupled systems, respectively (56, 58), with fixation and handling affecting their packing density (58).

Possible Tight-Junctional Model

Vertebrate tight junctions have been analyzed with respect to their fracturing characteristics in attempts to construct models to explain their intramembrane structures. The "single fibril" model (71) suggests that the ridges from adjacent cells, strongly bound together in vivo, remain together, with the fracture excursion going around both fibrils during the cleaving process. The "off-set two fibril" model (4) suggests that the fused ridges break apart during fracturing, but still produce a ridge that is relatively prominent because of lipid collapse. Although some images indicate that the spider's junctional PF ridges are very high, which is consistent with the single fibril model (71), the height of ridges could be enhanced by both lipid collapse and the slight penetration of each ridge into the adjacent membrane (4); hence the double fibril model (4) may be the correct one for these arthropod tight junctions, given their offset appearance.

Comparison with Other Arthropods

In comparison with the very simple linear tight junctions reported in insects (see references 30, 35, and 36), the junctional ridges of spider tissues are much more numerous and form more elaborate reticular arrays. Hence, in thin sections, more punctate appositions are observed than in insect tissues. They are also encountered towards the peripheral part of the perineurial cell layer, rather than in its basal region, as is usually the case for insects (35). However, in both spider and insect tight junctions, the ridges are in some regions discontinuous. Hence the occasional apparent lack of complementarity (as in Fig. 6) could be the result of a discontinuous ridge or groove terminating abruptly just at the point where the fracture plane undergoes a transition from one fracture face to another.

Impact on the Role of the Septate Junctions

The discovery of unequivocal tight junctions between the cells of an invertebrate casts further doubt on the postulated "sealing" function of the septate junctions. They clearly can no longer be considered to be the occluding "invertebrate equivalent of the vertebrate tight junction", although they may act as a filtering device, for example in *Hydra* and other cnidarians (14, 24, 27, 43, 74). The function of septate junctions is more likely to be adhesive, particularly given their occurrence along epithelial borders when there is considerable external pressure (35) and given their observed patency to tracers (see references in reference 32). Moreover, in vertebrate tissues, similar septatelike junctions have been found to coexist with tight junctions; in these situations, the latter are assumed to be sealing and the former, adhesive (9, 18, 64).

The frequent coexistence of so-called tight and septate junctions in insect CNS (29, 35, 38) and compound eye (6, 35, 50) has hitherto made it difficult to determine the precise role of each in insect tissues, although tracer studies on rectal pads suggested that tight, and not septate, junctions were occluding (32). Because no septate junctions have been observed in the spider perineurium and yet a permeability barrier exists, it must be the observed tight junctions that are restrictive; their fine-structural features corroborate this supposition. In the insect perineurium, with the exception of the moth *Manduca sexta* (37–39), septate junctions as well as basal tight junctions are always found between adjacent cells. The absence of septate junctions in *Manduca* has suggested that they cannot play any significant role in occlusion, because this lepidopteran exhibits an efficient blood-brain permeability barrier, observed both electrophysiologically (57) and after incubation with tracers (28, 39, 48). Although the lack of tight junctions in systems other than those of arthropods suggests that certain septate junctions may substitute functionally for them, as more than one mechanism for controlling intercellular permeability may exist (75), the present results from the spider CNS would appear to support the contention that the main occluding structures to be found throughout the animal kingdom are tight junctions.

Thus it is evident that septate junctions are unlikely to be the evolutionary forerunners of the vertebrate tight junctions. In any case, this theory (22) must be held to be very dubious, given the differences in intercellular cleft dimensions between the two junctional types (see references 35 and 75) as well as the distinctions in function of the intramembrane junctional particles in septate junctions (possible septal insertion) as compared with those of tight junctions (sites of intramembrane ridge fusion). It is interesting, in a historical context, that septate junctions should have been taken (*a*) to be the communicating pathway between invertebrate cells (5, 21, 44) and (*b*) to be the occluding structures between them (14, 23, 46, 52, 54, 55, 74). It would now seem that in both instances they have been superseded, first, by gap junctions and, secondly, by tight junctions.

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