

ORGANIZATION OF CELL JUNCTIONS IN THE PERITONEAL MESOTHELIUM

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ABSTRACT

Intercellular junctions in the mesothelium of the visceral (mesentery and omentum), and parietal (diaphragm, pre-aortic, and iliac region) peritoneum were examined in rats and mice by using freeze-cleaved preparations. In addition to usual intercellular junctions (cell body junctions), special junctions are found between cell processes and the surface of the neighboring cell (cell process junctions). Cell body junctions are provided with tight junctions and communicating (gap) junctions. The former consist of one to two junctional strands which show a characteristic staggered arrangement, and focal discontinuities. In cell process junctions, the strands form loops or appear as short, free-ending elements; their polymorphism suggests considerable lability, probably in connection with their assembly and disassembly. The existence of free-ending strands indicates that such structures can be used as attachment devices without being concomitantly involved in the formation of occluding zonules. In both types of junctions, the strands can be resolved into bars, ~80–100 nm long, frequently provided with terminal enlargements and intercalated particles which occur singly or in small clusters. These particles are morphologically similar to those present in communicating (gap) junctions. The mesothelium is also provided with isolated composite macular junctions. Throughout the mesothelium, the cleavage plane follows the outer contour of junctional strands and particles, suggesting that strand-to-strand interactions in the apposed membranes are weaker than interactions between each strand and underlying cytoplasmic structures. In their general geometry and cleavage characteristics, the mesothelial junctions resemble the junctions found in the venular endothelium.

Earlier interest in the mechanisms of transport of solutes (3, 5, 6, 8, 21, 9, 32), particulate material (12, 10, 16), and cells across the mesothelium has been recently renewed after the success of peritoneal dialysis as a maintenance technique in chronically uremic patients (7, 23). Despite past investigations, current views concerning the structural substrate of the high permeability of the mesothelial membrane are still markedly divergent. According to a number of studies, water-soluble mol-

ecules cross the mesothelium by passive diffusion presumably along intercellular junctions (5, 21, 9, 12, 13, 10), whereas according to other investigations transport across the mesothelium is an active process (35, 32) effected primarily via vesicles (30, 42, 19, 17). These divergent views have broader implications than are immediately apparent, since it has been postulated that the mesothelium, due to its being morphologically and functionally similar to the vascular endothelium, can

serve as a model for predicting the effects of various chemical and physical agents on capillary permeability (5, 21, 14).

Ultrastructural studies agree as to the existence of a large population of plasmalemmal vesicles in the mesothelium, but are not in agreement as to the character of its intercellular junctions. Several investigators have detected closed as well as open junctions (29, 12, 13, 10, 4), while others have claimed that the mesothelium is provided with adhering and occluding junctions which generally seal the intercellular spaces (16). In the frog mesothelium, frequent desmosomes have also been encountered (22). Recently, a combined TEM and SEM study detected the existence of relatively large "fenestrations" along the mesothelial junctions, some of which are permeated by large particles such as carbon black and latex spheres (25).

The present paper reports the results of an inquiry on the structure of intercellular junctions in the peritoneal mesothelium as revealed by the freeze-fracture procedure. The study provides information on: (a) the intramembranous organization of these junctions in the visceral and parietal peritoneum and (b) similarities and dissimilarities between mesothelial and endothelial junctions.

MATERIALS AND METHODS

Animals

For these observations, we used 24 young adult male C₃H mice weighing 30–35 g, and 38 adult male rats of the Sprague-Dawley and Wistar-Furth strains weighing 120–220 g. Before the experiments, the animals were kept for 14 days under standardized conditions of housing and feeding. The animals were lightly anesthetized with ether to allow fixation *in situ* before collection of tissue specimens.

Tissue Processing

The fixation *in situ* was performed by injecting intraperitoneally 2 ml/100-g body weight of a solution of 3%

glutaraldehyde in 0.1 M HCl-Na cacodylate buffer, pH 7.2–7.4, warmed to 38°C.

Collection of specimens was carried out by opening the abdominal cavity after 15- to 20-min fixation *in situ*. Samples were collected from the visceral peritoneum (mesentery and omentum) and from the parietal peritoneum (caudal aspect of the diaphragm, surface of the iliopsoas muscle, and ventral aspect of the abdominal aorta). The specimens were transferred to the same fixative solution for 10–15 min and then immersed for 2 h at 4°C in 25% glycerol in 0.1 M HCl-Na cacodylate buffer, pH 7.2–7.4. Subsequently, the specimens were mounted on metal tissue carriers and further prepared for freeze-fracturing as previously described (24, 36).

The number of samples used for this study is given in Table I.

In order to study the general appearance of mesothelial junctions in sections, samples of peritoneal serosa were collected from the locations mentioned and processed for electron microscopy as indicated in reference 37 except that after osmication tissue blocks were mordanted with low molecular weight gallotannin (39).

RESULTS

General Procedure

Specimens were fixed *in situ* by intraperitoneal injection of fixative solution without opening the abdominal cavity, since exposure of the peritoneum to air leads to structural modification of the mesothelium, namely high frequency of open junctions.

Identification of Mesothelial Membrane

A reliable identification of mesothelial cells in freeze-cleaved preparations is necessary because of the proximity of other cell types, e.g., vascular endothelium, and muscle fibers which, according to information obtained on sectioned specimens, are expected to have comparable morphological features. In fact, we found that the cleavage faces of the mesothelial cell membranes can be easily

TABLE I
Number of Samples Examined

Sampling	Visceral peritoneum		Parietal peritoneum			Total
	Mesentery	Omentum	Diaphragm	Iliac region	Pre-aortic	
Animals*	19	17	14	5	7	62
Replicas examined‡	29	28	26	14	12	109
Junctions§	66	57	48	28	39	238

* Includes both mice and rats

‡ Aggregate areas of mesothelial cell membranes surveyed = ~4.2 mm².

§ Length of junctions investigated = ~2,450 μm. (This figure does not take into account the variable sinuosity of the junctional line. The great majority of specimens examined showed both cell body and cell process junctions.)

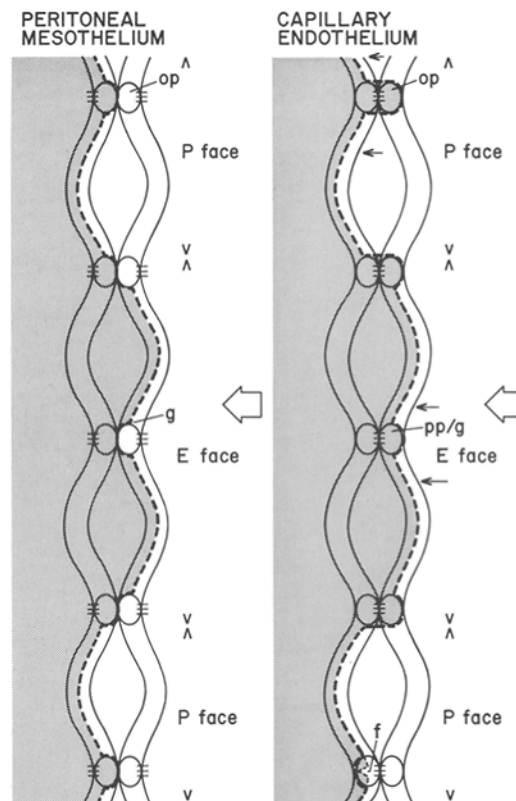
identified on account of the low frequency, random distribution, and the extensive variability in size of their vesicular openings. The latter have, in general, the same morphology as in the vascular endothelium: they appear as craters on the E faces and papillae on the P faces, and they vary in diameter from $\sim 200 \text{ \AA}$ to $1,400 \text{ \AA}$ (range of diameters for endothelial vesicular openings = $\sim 200\text{--}400 \text{ \AA}$). The cleavage faces of mesothelial cell membranes have no vesicle-free parajunctional zones as they do in the endothelium, and no linear arrays of vesicular openings as found in muscle cells. In addition, the identification of the mesothelium is facilitated by the fact that the cell boundaries were found to follow an irregular, serrate course with many fingerlike processes extending from the free surface of a cell to the free surface of its neighbors (Fig. 2). These processes vary widely in length ($\sim 0.1\text{--}1 \mu\text{m}$), width ($\sim 0.2\text{--}3 \mu\text{m}$), and appear to be more frequent in the visceral than in the parietal peritoneum. Such processes have no counterpart in any adjoining cell type.

Cleaving Characteristics

In the majority of cases, the cleavage plane exposes junctional areas in their entirety, on either the P or the E face of the plasmalemma (Figs. 1, 3, and 4). In some cases, however, the cleavage fractures the outer membrane leaflet of one cell along a junctional strand, when it shifts from the membrane of one cell to that of its neighbor. In such cases, the junctional strand regularly remains on the P face (Fig. 2). Unlike the situation described in the vascular endothelium (38), it appears that in the mesothelium the cleavage plane passes, as a rule, around the outer contour of the intramembranous strands of the junctions between the latter and the outer membrane surface (Fig. 1). As a result, junctional strands are consistently confined to the P faces, and their complementary grooves on the E faces are devoid of either particles or strands (Fig. 4).

General Organization of Mesothelial Junctions

Our observations are by necessity limited to junctional elements seen on cleaved faces of cell membranes. They give no information on the situation of the intercellular spaces at the level of these elements, which means that these spaces might be closed or opened irrespective of the



Key to Symbols

- cbj*, cell body junction
- cj*, communicating junction (gap junction)
- cpj*, cell process junction
- E*, E face of the mesothelial plasmalemma
- g*, grooves on the E face
- jb*, junctional bars (tight junction)
- ip*, intercalated particles
- m*, fractured microvilli
- P*, P face of the mesothelial plasmalemma
- s*, junctional strands (tight junction)
- v*, vesicular opening

FIGURE 1 Diagrammatic representation of the usual position of the cleavage plane in mesothelial junctions as compared to endothelial junctions (capillary). *op*, Occluding junction particle or strand; *pp*, occluding junction particle protruding in an E-face groove; *g*, groove on the E face; *f*, shallow furrow on the P face. In the mesothelial cell membrane, the cleavage plane leaves the junctional strands and particles on the P face ridges. The arrangement suggests a relatively weak interaction between the two sets of particles of the joint membranes. In the cell membrane of the capillary endothelium, the cleavage plane leaves the junctional particles on the E face grooves. This suggests a relatively strong interaction between the two sets of particles of the joined membranes.



FIGURE 2 Visceral peritoneum (mesentery). General view of an intercellular junction in which the cleavage plane reveals a cell body junction (*cbj*) and the cell process junctions (*cpj*) associated with it. The processes can be recognized as hillocks (*h*) emerging from the margin of cell *C*₁ and extending as fingerlike projections under the membrane of cell *C*₂. The junctional elements appear as discontinuous or interconnected strands (*s*) on the P face (*P*), and as complementary grooves (*g*) on the E face (*E*). A staggered arrangement of grooves can be seen at *sg*. Note that the outer leaflet of the plasmalemma of *C*₂ has fractured preferentially along the junction groove (arrows). Note also that the junctional elements of the cell process show free ends (*e*). Vesicular openings of various sizes and of random distribution are marked *v*. × 23,000.

presence of intramembranous elements at the same level. Under these circumstances, our findings neither confirm nor refute the existence of large openings or fenestrations detected by other procedures in the mesothelium (29, 22, 12, 13, 25).

Our observations indicate that mesothelial cells are generally linked to one another by tight junctions and communicating junctions,¹ which occur either between apposed cell bodies or between the processes of one cell and the body of its neighbor. Since the morphology is different and distinctive in the two cases, we shall refer to the first type as cell body junctions, and to the second as cell process junctions. These two types of junction are regularly found in continuity with one another. In addition, completely isolated patches of combined tight and communicating junctions are occasionally encountered (composite macular junctions).

Tight Junctions

Irrespective of their location, the tight junctions consist of strands $\sim 80\text{--}90$ Å in width, which appear to be formed by the end-to-end association of relatively short bars of 80 to 100-nm average length (range: $\sim 40\text{--}300$ nm) (Figs. 3, 5, and 6). The bars have a straight appearance and form sharp angles at their connecting points (Fig. 6). The ends of the bars are often marked by an enlargement or a discrete particle comparable in size to those found in communicating junctions (Figs. 5, 6, and 8). The degree of separation between these intercalated particles and the bars varies considerably, and in some cases their place is taken by small clusters of three to six particles which, again, are morphologically comparable to those found in communicating junctions (Figs. 5 and 6).

In cell body junctions, the number of strands varies from 1 to 5; they are usually arranged parallel to one another at an average spacing of 80–110 nm and are rarely connected by transverse bars (Table II and Fig. 6). Discontinuities and staggered arrangements of the junctional strands are commonly associated with uninterrupted lanes, ranging in width from 30 to 250 nm, extending from one side of the junction to the other (Figs. 3 and 4). Discontinuities in the junctional strands are particularly frequent in the visceral

peritoneum where they are present in about 75% of the junctions examined.

In cell process junctions, the strands vary in number from 1 to 8 (Table II). When few (1–2), they characteristically show free ends which can reliably be located under fractured process tips (Figs. 7 and 8). When more numerous, they form rather elaborate, multiple loops which, in some instances, still show discontinuities and free-ending spurs (Fig. 9). Such loops are encountered in $\sim 75\text{--}80\%$ of the cell process junctions (Table III). Between the two extremes illustrated by Figs. 7 and 9, a wide variety of appearances is encountered.

Communicating Junctions

Communicating junctions consist of aggregates of particles in widely variable numbers which form patches or plaques varying from ~ 20 to 600 nm in size. These junctions were found to be slightly more frequent (by $\sim 20\%$) in the visceral than in the parietal peritoneum. In both locations, they were more numerous on the cell processes ($\sim 60\%$) than on the cell bodies ($\sim 40\%$). Aggregates of $\sim 90\text{--}100$ Å particles occur either isolated or associated with tight junctions. In the latter case, they are roughly aligned with junctional strands (Figs. 3, 5, and 6) or interpolated in their framework. The interpolated version is characteristic for cell process junctions. The possible relations of these aggregates to occluding and communicating junctions will be covered in the Discussion.

The degree of order in the aggregates of particles within communicating junctions varies, and, schematically, three basic patterns can be distinguished: (a) regular lattice (close hexagonal packing), (b) random distribution, and (c) mixed (combination of the two previous types). In both visceral peritoneum and parietal peritoneum, the communicating junctions on the cell body preferentially appear as random aggregates of particles, whereas on cell processes regular lattices prevail.

Macular Junctions

These focal junctions are isolated structures completely surrounded by large areas of cleaved faces free of other junctional elements. They consist of a communicating junction of variable size (in which the particles usually form a regular lattice), framed by one to five more or less complete rings of tight junctional strands. The latter are

¹ We prefer the term communicating junction (*macula communicans*, *maculae communicantes*) instead of "gap junction" for reasons explained in reference 37.

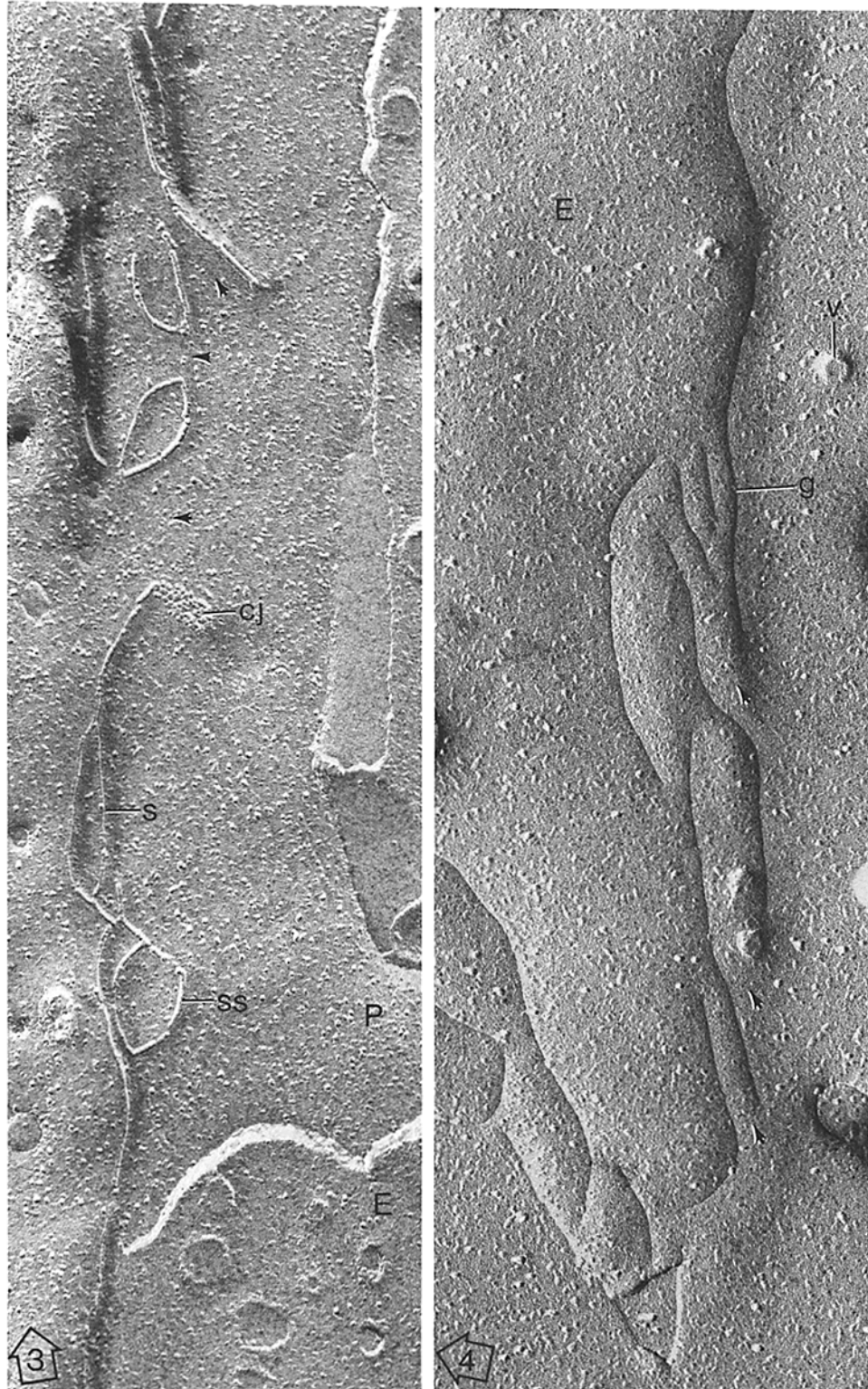


FIGURE 3 Parietal peritoneum (pre-aortic). The tight junction consists of a discontinuous strand (*s*) with frequent focal interruptions (arrowheads), and, at their level, continuous lanes extend from one side of the junction to the other. Secondary strands (*ss*) form loops along the main strand of the junction. $\times 76,00$.

FIGURE 4 Visceral peritoneum (omentum), E face. The tight junction is represented by a series of discontinuous particle-free grooves (*g*) which have a staggered distribution, leaving between them free lanes (arrowheads). $\times 95,000$.

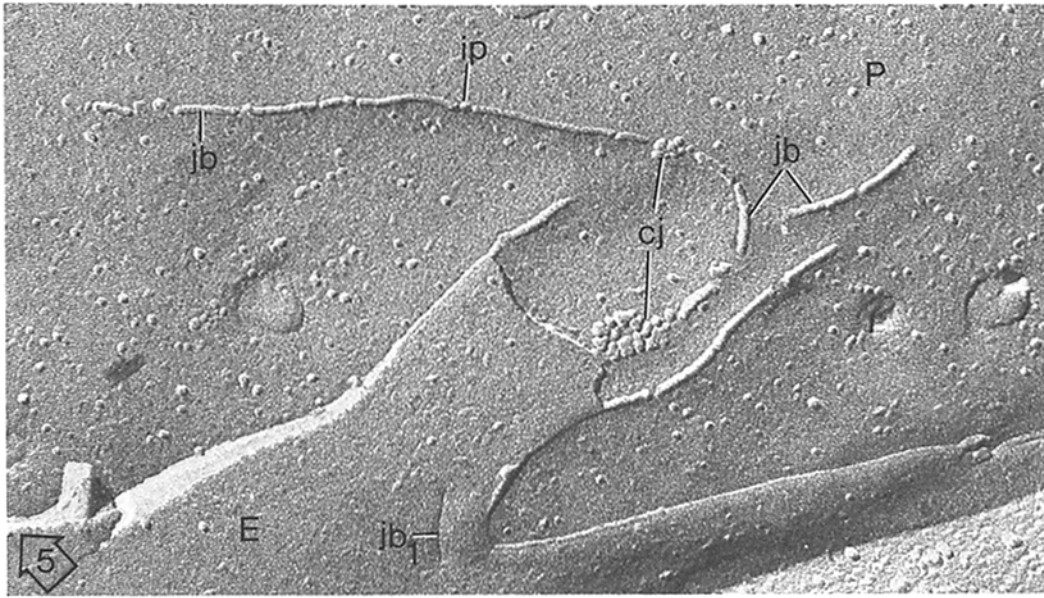


FIGURE 5 In this tight junction, the isolated bars which constitute the strands can be seen at *jb*. Singly intercalated particles appear at *ip*, and small clusters of particles located between bars in the continuity of the junctional strands can be seen at *cj*. At *jb₁*, a short groove may represent a complementary E-face image of a junctional bar. These clusters are morphologically similar to small communicating junctions. $\times 165,000$.

FIGURE 6 In this composite junctional structure, numerous communicating junctions (*cj*) of various sizes appear intercalated within the line of junctional strands (*s*) immediately or adjacent to it. Note the small aggregates formed by two to three particles (arrows) and the frequent occurrence of solitary intercalated particles (*ip*). Some of the junctional bars show a terminal enlargement (*te*). $\times 104,000$.

TABLE II
Average Number and Spacing of Junctional Strands in Freeze-Cleaved Preparations of Mesothelial Junctions*

	Visceral peritoneum		Parietal peritoneum		
	Mesentery	Omentum	Diaphragm	Iliac Region	Pre-Aortic
Cell body junctions					
Mean number ± SD	1.6 ± 2.5	1.4 ± 2	2.4 ± 2	2.0 ± 2.5	1.9 ± 2.5
Mean spacing (nm ± SD)	95 ± 75	105 ± 92	80 ± 90	110 ± 105	85 ± 80
Cell process junctions					
Mean number ± SD	5.4 ± 3	4.8 ± 3.5	3.0 ± 3.5	3.4 ± 2	2.8 ± 3
Mean spacing (nm ± SD)	85 ± 62	100 ± 70	85 ± 65	95 ± 70	120 ± 82

To facilitate the comparison with the endothelial junctions, the counts and measurements were made as indicated in reference 36. For cell body junctions, a standard band of ~250-nm width oriented perpendicular to the direction of the junction was used. For cell process junctions, the measurements were carried out on the entire length of the process, and perpendicular to its long axis. Counts and measurements were made irrespective of the orientation of the junctional strands which frequently were randomly distributed.

* The number of junctions examined was that presented in Table I.

TABLE III
Relative Frequency of Various Patterns of Cell Process Junctions in the Peritoneal Mesothelium

	Visceral peritoneum	Parietal peritoneum
Mesothelial junctions examined (238)	123	115
Cell process junctions encountered (346)	209	137
Free ending strands	33 (16%)	17 (12%)
Loops	156 (75%)	110 (80%)
Macular junctions*	20 (9%)	11 (8%)

* The relation of this appearance with the cell process is less certain (for comments, see Discussion).

occasionally connected by transverse bars (Fig. 10). Their possible relationship with cell process and cell body junctions is discussed in the next section.

DISCUSSION

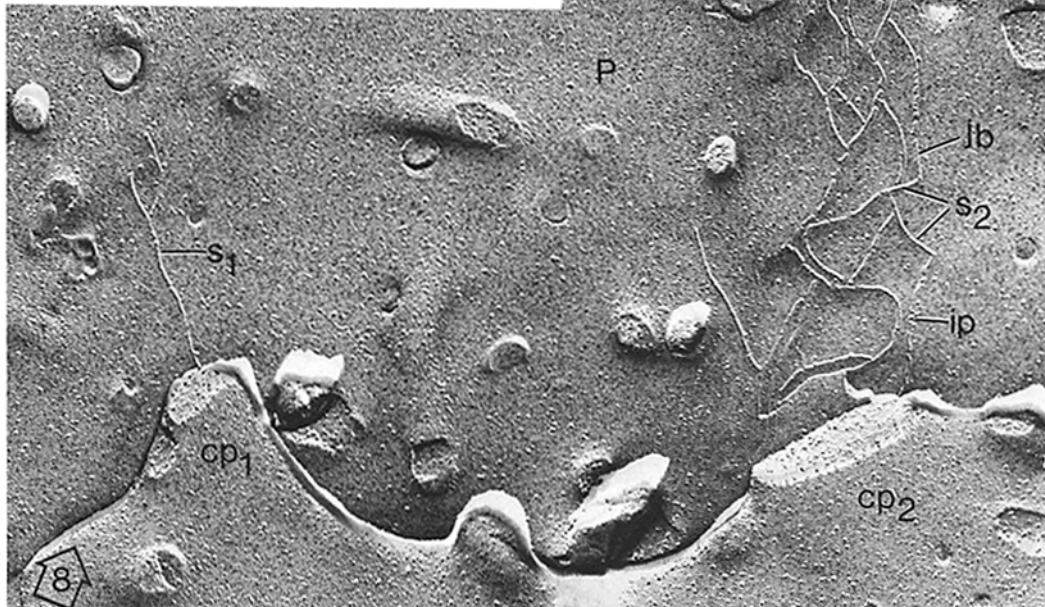
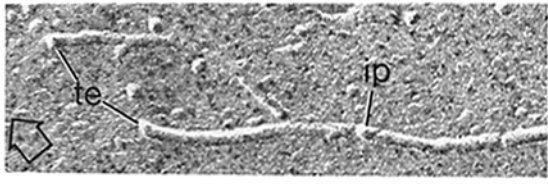
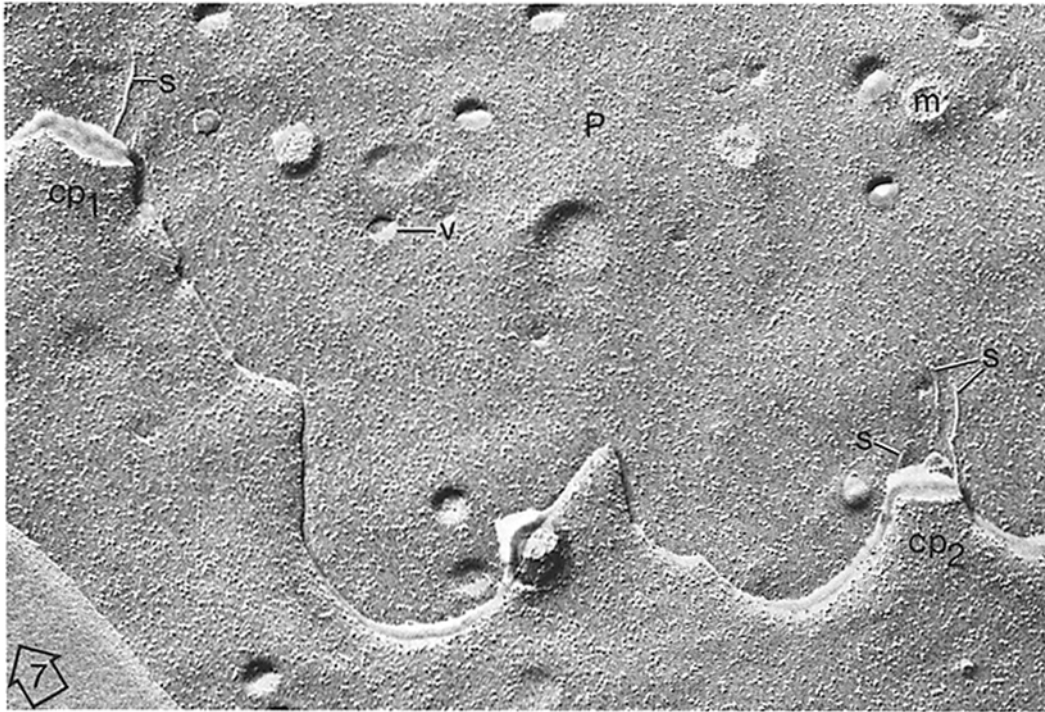
The frequent occurrence on cell process junctions of free-ending junctional strands indicates that these structures, in some instances, function simply as attachment devices, in contrast to the general assumption that they are used exclusively for the construction of continuous occluding junctions. The extensive polymorphism of cell process junctions may be an expression of their lability and may be suggestive of successive stages in junctional assembly or disassembly.

There is suggestive evidence that the building element of the tight junctional strands in the mesothelium is a short bar (~80-100 nm length)

frequently provided with a terminal enlargement. The ends of these bars appear to have a special affinity for particles 90-110 Å in diameter. As a result, the barrier represented by the junction consists of bars and particles intercalated among the latter either singly or in small clusters. Rows and clusters of particles which progressively merge into strands have been described in developing tight junctions in amphibian embryo (15), chick embryo (34), and fetal rat liver (28). Except for some details, e.g., absence of rows of particles in the mesothelial junctions, our findings are comparable to those recorded in those cases. The intercalated particles, especially when clustered, are morphologically similar to the particle encountered in communicating junctions. But, with the information so far available, we cannot ascertain whether they represent special elementary units in the formation of tight junctions or whether they are communicating junctional particles distributed in small numbers and in unusual locations.

Remnants of disorganized strands of the type reported in osmotically disrupted junctions (44), and after incorporation in lysosome-like vesicles (41), are not generally encountered in the vicinity of the composite macular junctions of the mesothelium. This finding suggests that in this case the two cells remain in contact at the level of the junctions, and that their partial detachment is relatively slow and progressive. Comparable composite macular junctions have occasionally been reported in a few cases in intact tissues, e.g., ovarian granulosa cells (2, 18), thyroid follicles (43), and hepatoma (31).

Our findings and findings already reported in



FIGURES 7-9 Visceral peritoneum (omentum). Series of micrographs suggesting the existence of stages in the formation of breaking of mesothelial cell process junctions.

FIGURE 7 Two fractured cell processes (cp_1 and cp_2) reveal the presence of short free-ending strands (s) running parallel to the axis of the cell processes. $\times 54,000$.

FIGURE 8 Under the fractured process to the left (cp_1) appears a single junctional strand (s_1) that terminates after bifurcation into two free ends. The junction under the fracture process to the right (cp_2) exhibits an irregular network of strands (s_2) with intercalated particles (ip) and free-ending spurs. $\times 52,000$; inset, $\times 194,000$.

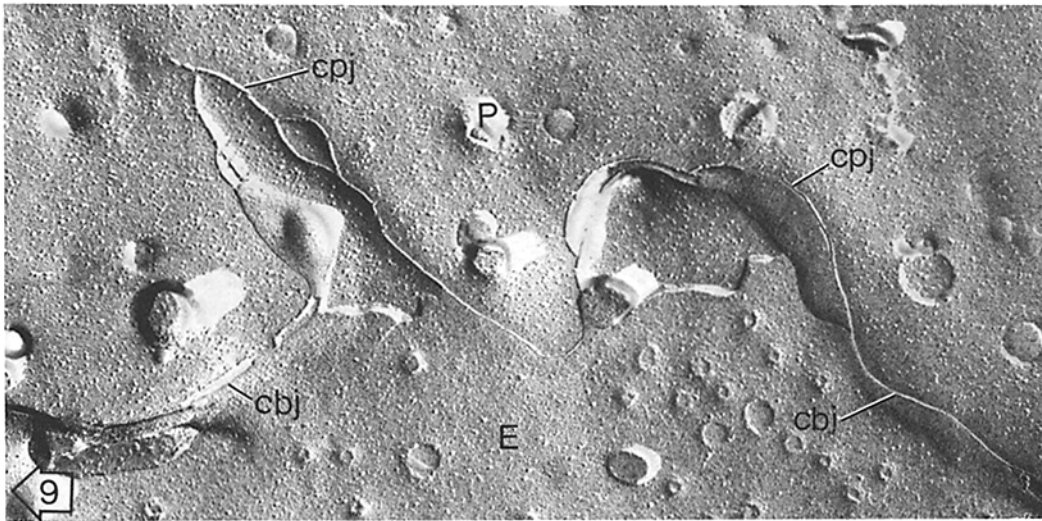


FIGURE 9 The two looplike extensions marked *cpj* may represent the final outcome of the incorporation of the two cell process junctions into the cell body junction (*cbj*), with which they were originally associated. $\times 47,000$.

FIGURE 10 Visceral peritoneum (omentum). Isolated, complex junction consisting of a relatively large communicating junction (*cj*) of regular lattice pattern, which is surrounded almost completely by one or two junctional strands (*s*). $\times 66,000$.

the literature indicate that three types of tight junction can be distinguished on the basis of the morphology of their junctional strands which appear as (a) continuous fibrils, e.g., in the epithelia of the gastro-intestinal tract (11, 41), (b) bars, e.g., in the developing junctions (15, 28, 34) and in the mesothelium (present paper), or (c) rows of particles, e.g., in the arteriolar and capillary endothelium (37, 38), stria vascularis (33), and among Sertoli cells (20). Forms transitional from one type to another are, however, occasionally encountered (1, 20). In the case of the first two types, the cleavage plane usually leaves junctional strands and bars on P faces, and simple grooves on E faces. Since cleavage follows the plane of least resistance within membranes, it can be assumed that, in all these cases, strand-to-strand interactions are weaker than strand-to-underlying cytoplasm (fibrillar framework) interactions. In the last case (type [c] from above mentioned), the junctional particles (and short occasional bars formed by the latter) are preferentially left on the E face along the bottom of the grooves; few particles mark the furrows on the complementary ridges of the P faces. In these tissues, the interactions between paired junctional particles appear to be generally stronger than the interactions between each particle and the underlying protoplasmic structures. These interactions may be influenced to some extent by the fixation process, since it has been reported that in some instances the cleavage plane follows a different path in fixed vs. fresh tissues (41). In fixed tissues, however, the differences mentioned are obvious and consistent as illustrated by our observations made on intestine and mesentery specimens in which within the same region junctions of (a) type (epithelium), (b) type (mesothelium), and (c) type (arteriolar and capillary endothelia) were encountered. On account of these considerations, the behavior of the cleavage plane should be considered an important criterion in classifying the tight junctions.

Considering the structural features described in this paper and in those of other investigators, marked differences became apparent between the mesothelium and the capillary endothelium. They concern not only differences in size, frequency, and distribution of plasmalemmal vesicles, but also dissimilarities in the intramembranous organization of the corresponding junctions. Thus, there is little ground for equating structurally the two types of epithelium and for using the mesothelium as a convenient model of relevance for capillary

permeability studies. By their general geometry, and in part by the behavior of the cleavage plane at their level, the mesothelial junctions are similar to those found in the venular endothelium (37). The venular junctions are known to be permeable and labile under normal (40) and pathological conditions (26, 27). The mesothelium, like the venular endothelium, reacts to 5-hydroxytryptamine by showing increased permeability to large molecules and particles, and, in the case of the endothelium, this increase has been traced to the appearance of focal separations along the intercellular junctions (26).

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