SEGMENTAL DIFFERENTIATIONS OF CELL JUNCTIONS IN THE VASCULAR ENDOTHELIUM

Arteries and Veins

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ABSTRACT

A systematic survey of endothelial junctions in elastic (aorta) and muscular (mesenteric) arteries and in medium (renal and mesenteric) and large (cava inferior) size veins has been carried out in the rat using freeze-cleaved preparations.

The arterial endothelium is provided with a complex of occluding and communicating junctions (gap junctions) comparable to, though less elaborate than, that described in arterioles. The particles of the occluding junctions behave like "single unit" particles and have the tendency to remain on B faces upon membrane cleavage.

In the venous endothelium the junctions take the form of long occluding junctions with few associated communicating junctions (maculae communicantes). As in arterial endothelium, the junctional particles appear preferentially on B faces in cleaved preparations. These structures. although continuous over long distances, are interrupted focally by areas in which the junctional elements are similar to those found in venules: the ridges and grooves are short, discontinuous, randomly distributed along the general line of cell contact, and often particle-free.

In muscular arteries two unusual types of junctions are encountered. Both are disposed in loops over short distances along the perimeter of the cell. One type appears to be a stretched-out version of the usual combination of occluding and communcating junctions of the arterial endothelium (this type is also occasionally encountered in the venous endothelium). The other type is reminiscent of the septate junctions found in the epithelia of invertebrates but the apparent similarity remains to be checked by further work.

In a previous paper (29), we have shown that the endothelium of each segment of the microvasculature, i.e., arterioles, capillaries, and venules, has distinct and characteristic intercellular junctions. We have recently extended the inquiry to the endothelium of large vessels, namely arteries of different types and veins of different sizes. The observations reported in this paper were made on freeze-cleaved preparations as in (29). They refer, therefore, to structural elements within the endothelial plasmalemma at the level of the intercellular junctions, rather than to surface features directly involved in the junctions. Nonetheless our findings suggest that some of these elements extend to the cell surface where they are probably involved in the formation of intercellular junctions.

In the large vessels, the endothelium acts as a partition that separates the blood plasma from the interstitial fluid of the subendothelial spaces, and mediates all the exchanges required for the metabolism of the cellular elements of most of the vascular wall. Available data on structural aspects of endothelial permeability in large vessels are limited and in part conflicting. Among the structures implicated as actual or potential passageways for water-soluble molecules across the endothelium are the intercellular junctions. They have received a certain amount of attention (9, 10, 15, 16, 34) but systematic information concerning their organization is still lacking. The present report provides part of the missing information.

MATERIALS AND METHODS

Animals

The 36 Wistar-Furth male rats (weighing 150-250 g) used for these observations were kept under standardized conditions of housing and feeding for 8 days before the collection of specimens.

Processing for Freeze-Fracturing

Under ether anesthesia, the vessels to be investigated were fixed either *in situ*, or by perfusion, as indicated in (29). For general information on the freeze-fracture technique used, see (21). Fixed specimens to be processed for freeze-fracturing were taken from: (a) the abdominal and thoracic aorta, (with the exclusion of the aortic arch) as examples of elastic arteries; (b) the mesenteric artery as a representative muscular artery; (c) the mesenteric and renal veins; and (d) the vena cava inferior. In each case, the samples were collected away from the ostia of efferent or afferent vessels. A cylindrical segment of each
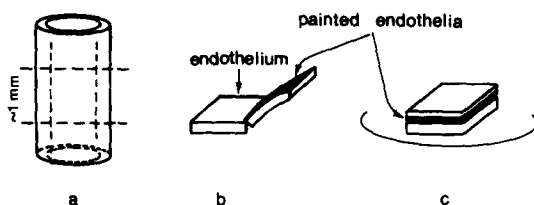

FIGURE 1 Diagram illustrating the experimental procedure used to process vascular specimens for freeze fracturing. (a) Two opposing square pieces of ~ 0.5 to 1 mm² each are cut out from a flattened cylindrical vascular segment; (b) one of the endothelial surfaces is painted dark with a marker (waterproof, smearproof); (c) the painted square is put back over its counterpart so that the two endothelial linings face each other. The paint-layer helps in guiding the knife through the endothelia at the time of fracturing.

TABLE I

Number of Samples Examined

| Sampling | Arteries | | Veins | | | |
| | Elastic | Muscular | | | | |
	Aorta (thoracic and abdominal)	Mesenteric artery	Renal vein	Mesenteric vein	Inferior cava	Total no.
Animals	13	11	6	7	9	46
Examined replicas	52	64	31	42	64	253
Junctions	30	49	28	51	32	190

vessel was cleared of its blood content (if needed), gently flattened, and incubated at 4°C for about 2 h in 25% glycerol. A square sample of about 0.5-1 mm² was cut through both sides, so that the endothelial surfaces of the two squares were facing each other. The upper half was usually recut slightly smaller and its endothelial surface, kept continuously wet, was painted with a waterproof ink (Blue Sharpie pen, waterproof, smearproof) (Fig. 1). This simple procedure made possible the recognition of the level of the endothelia (each of < 0.5 μm in thickness), through which the microtome knife of the Balzers apparatus had to be led (under microscopic control) at the time of fracturing. Control experiments with specimens unpainted before freeze-cleavage showed that the ink does not alter the morphology of the junctions. Table I gives the number of replicas examined and the number of endothelial junctions found in each type of vessel studied.

RESULTS

General

The cells of the endothelium of the large blood vessels are connected to one another by occluding and communicating junctions (gap junctions).[1] The degree of development of each type of junction and the extent of their association with one another vary consistently with the type of vessel so that each vascular segment has a characteristic organization of its endothelial junctions.[2] In general, the junctions of the arterial endothelium are similar to those found in arterioles, while the junctions of the venous endothelium are in part reminiscent—though more complex—than those

[1] For reasons explained in ref. 29, we use in this paper the term "communicating junctions" ("macula communicans") in preference to the term "gap junction".

[2] The morphology of the junctions was the same irrespective of the type of fixation used: *in situ* or by perfusion.

described in venules (29). Unlike the endothelium of the microvasculature, that of certain large blood vessels is fitted with two additional types of intercellular junctions of still obscure significance.

Arteries

ELASTIC ARTERIES (THORACIC AND ABDOMINAL AORTA): In these vessels, the endothelial cells are linked to one another by a combination of occluding and communicating junctions. The occluding junctions appear as networks of interconnected 2 to 4 ridges on A faces or 2 to 4 grooves on B faces (Figs. 2 and 4). The networks form continuous bands in which the ridges are usually 120–150 nm apart (Table II). Free-ending ridges or grooves are relatively rare (Fig. 4). The midlines of the grooves on the B faces are marked by continuous or quasi-continuous rows of particles which are either globular or rodlike in shape, measure ~ 100–400 Å in their longest diameter,[3] and occasionally appear fused into long strands. Similar particles are less frequently seen on the ridges of the A faces. The general impression is that particles in phase in the two joined membranes behave like "single units" (29) which are left by the plane of cleavage much more often in the grooves of the B faces than on the ridges of the A faces. This interpretation, which assumes that particles seen on ridges correspond to "missing" particles on the complementary grooves, remains to be substantiated in the future by the examination of paired (complementary) replicas. In keeping with the "single unit" behavior, rows of particles are occasionally found to continue uninterrupted from grooves to ridges (see Fig. 9 in ref. 29) and to project a longer shadow on A faces than on B faces (Fig. 7). The occasional presence of fine furrows on some particle-free ridges of A faces is another indication of "single unit" behavior. The preferred plane of cleavage found in arterial junctions is diagrammed in Fig. 23.

Many of the meshes in the network of occluding junctions are usually occupied by relatively large communicating junctions (Fig. 2), but well-separated junctions of each type are also occasionally encountered (Figs. 3 and 5). The particles that form the plaques of the communicating maculae measure ~ 80 Å in diameter and their arrange-

ment varies from close hexagonal packing (Fig. 5) to apparently random clustering (Fig. 3). In some cases, similar particles form linear arrays of one to three parallel rows which can be followed over long distances (up to 1 μm) and usually end in a regular communicating junction (Figs. 2 and 3). Except for their characteristic dimples or protruding particles, the plaques of the communicating maculae have a rather even appearance generally free of bends or other deformations, which suggests that they are relatively rigid. When they are framed by occluding junctions, the peripheral grooves appear very shallow or absent on the B faces, but the protruding particles that usually mark the midline of the grooves are present even in the grooves' absence (Fig. 2). Moreover, the rows of particles show fewer discontinuities than in the grooves of simple occluding junctions (Figs. 2 and 4). The corresponding ridges on the A faces are even more difficult to see than the grooves on the B faces around the communicating plaques, and occluding junction particles are generally absent around the A face-part of their perimeter (Fig. 2). Occasionally, a fine, double band (shadow and metal deposit; shadow first or last, depending on the direction of shadowing) is seen in their place (Fig. 2, see also Fig, 15). We interpret this band as the depression left by the avulsed particles of the surrounding occluding junction.

Irrespective of the presence or absence of intercalated communicating junctions, the tight junctions of the aortic endothelium form continuous, uninterrupted networks across the entire areas exposed by the cleavage planes over distances which in our specimens measure up to 10 μm. Accordingly, they appear to have the geometry of occluding zonules in other epithelia.

MUSCULAR ARTERIES (MESENTERIC ARTERY): The endothelial junctions of these vessels are entirely similar to those already described in the aorta (Figs. 6 and 7), except that the mean number of ridges or grooves appears to be slightly higher and their spacing smaller than in elastic arteries (Table II).

In addition, two different types of special junctions have been encountered rather frequently in the endothelium of these vessels. (a) The first type seems to be derived from the usual combination of occluding and communicating junctions of the arterial endothelium, except that they are not part of a belt system, but form complex loops limited to relatively small areas (Figs. 8–10). In detail, they

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[3] The longer particles may be oligomers of the 100 Å units.
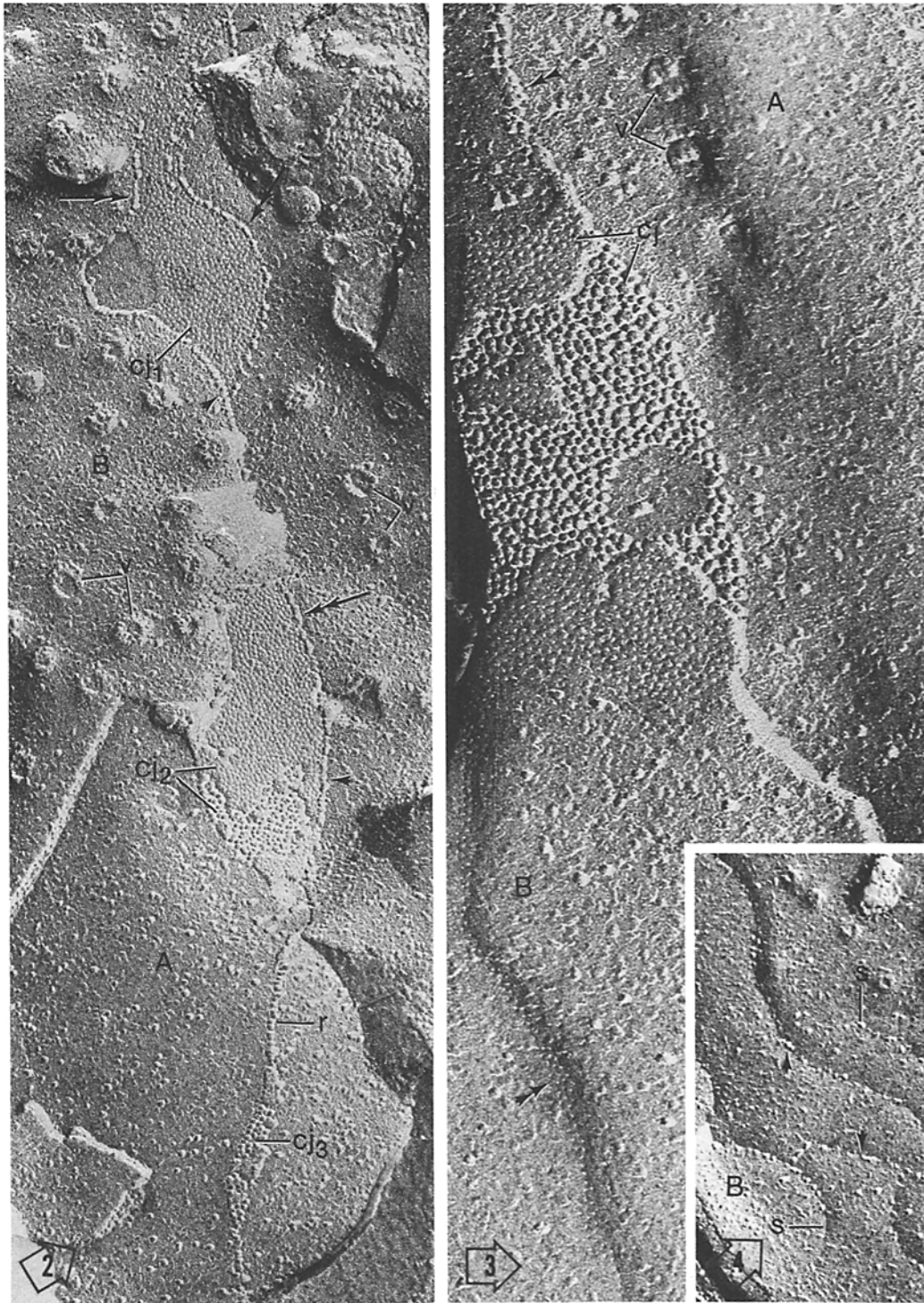
appear to consist (on the B faces) of parallel, closely spaced shallow grooves marked by particles similar in structure to those seen in regular occluding junctions. The intervening spaces are occupied by narrow, communicating maculae (Figs. 8 and 9). Occasionally, the general geometry is more irregular with ribbon-like as well as patch-like communicating junctions intercalated in the system. Typical occluding junction strands are frequently found subdividing patches and ribbons of the communicating junctions on both B and A faces (Figs. 8 and 10). The prominent feature of this special junction is the existence of parallel, closely spaced rows of particles which belong to both junctional types (occluding as well as communicating), and which occasionally appear mixed within the same band or plaque (Figs. 8 and 9). (b) The second type is more complex and more difficult to interpret. The general configuration is again that of multiple loops distributed over relatively limited areas rather than organized in belts (Fig. 11). Most of the component particles have the dimensions of the usual particles of communicating junctions, but appear to be arranged in tightly packed parallel rows. In addition, strands of globular to elongated particles appear in parallel arrays at a spacing of 30–100 Å (Fig. 11) in an arrangement which is reminiscent of that

described for septate junctions in invertebrates (6, 7, 11, 12, 14) and occasionally in vertebrates (13).

Large Veins

RENAL AND MESENTERIC VEINS: In the two veins examined, the general organization of the occluding junctions is characterized by long, continuous ridges or grooves which run parallel with the general direction of the band of cell contact (Fig. 12). The number of ridges is small (1–3) (Table II), but occasionally they branch to form spurs and relatively elaborate networks (Figs. 13 and 16). The arrangement is occasionally interrupted by regions in which the junctional ridges or grooves are short, straight, branching at sharp angles, and distributed at random in relation to the general line of cell to cell contact. This pattern is reminiscent of that already described in the venular endothelium, except that the discontinuities in the system of grooves or ridges are considerably less frequent. The junctional regions of this type appear to be rare, separated by long stretches of continuous ridges or grooves, and distributed at random along the perimeter of the endothelial cells.

In fine detail, the long continuous grooves of the B faces are marked by quasi-continuous rows of

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FIGURE 2 Aortic endothelium. The cleavage plane exposes Ia occluding junction with two large and one small communicating junctions (cj_1, cj_2, cj_3) fitted within its meshes. On the B face (B) the grooves of the occluding junction (arrowheads) surrounding the plaques of the communicating junctions are extremely shallow and their midlines are marked by protruding, elongated particles which tend to form continuous rows or strands (double arrows). Note the difference between the B face and A face part of the perimeter of the cj_2: along the latter there are no boundary particles. A single row of communicating junction particles is seen on the A face at r. In a few places on the left side of the row and of cj_3 there is a suggestion of fine grooves which presumably were left behind by avulsed occluding junctional strands. The appearance is comparable to that seen on the left side of cj_3 except that the angle of shadowing is less favorable for the demonstration of the furrow. Fractured necks of open plasmalemmal vesicles (v) appear as craters on the B face. × 90,000.

FIGURE 3 Aortic endothelium. A large isolated communicating junction (cj) appears as a plaque of clustered particles (on the A face) or small dimples (on the B face). The plaque is continued by single or double rows of particles (on the A face) or dimples (on the B face) (double arrowheads). Fractured necks of open vesicles (v) appear on the A face as circumvallate papillae. × 150,000.

FIGURE 4 Aortic endothelium. Small segment from a simple occluding junction. The grooves on this B face form a continuous network (arrowheads) with a few free spurs (s). By comparison with the grooves surrounding the communicating plaques of the composite junction in Fig. 2, they are deeper and their midline is marked by less continuous rows of particles. × 70,000.

protruding particles as in the arterial endothelium. The ridges have fewer particles and in some specimens they display thin furrows presumably left behind by particles removed with the outer leaflet of the membrane (Fig. 14). In those areas in which the general morphology of the junctions is reminiscent of that found in venules, the grooves are shallow and generally free of particles, and the
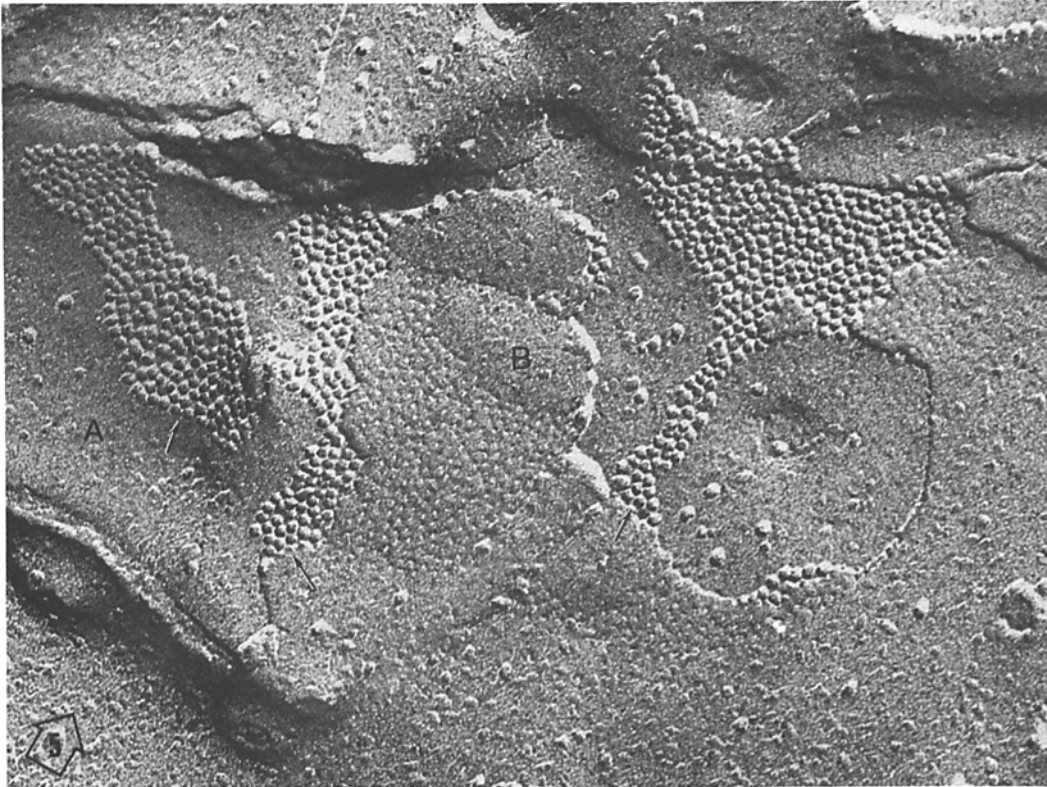

FIGURE 5   Aortic endothelium. An independent communcating junction of highly irregular outline appears on the A and B faces of the two cell membranes involved in this junction. The arrangement of junctional particles on the A face approaches in places (arrows) a close hexagonal packing. Note the absence of grooves or ridges along the margins of the plaque on both faces. Note also the presence of a few scattered particles of similar morphology in the vicinity of the plaque. × 160,000.

FIGURE 6   Mesenteric artery. A simple occluding junction is seen on a B face (B) as an elongated, continuous network of grooves (arrowheads) marked along their midline by quasi-continuous rows of particles. A single small communicating junction appears at cj. A number of plasmalemmal vesicles (v) open within the meshes of the occluding junction. Along the left margin of the figure, the fracture cuts through the endothelial cytoplasm (cy) of the adjoining cell and exposes a number of plasmalemmal vesicles. × 70,000.

FIGURE 7   Mesenteric artery. Composite junction with communicating plaques intercalated within the meshes of an occluding junction (arrowheads). The continuity of the occluding junction between the two asterisks is uncertain. A small independent communicating junction appears at cj and a linear extension of an intercalated plaque is seen at r on the A face. The occluding junction shows two free spurs (s) on the B face. The difference in depth and distribution of midline particles between simple grooves and grooves associated with communicating plaques is clearly visible in this figure. As in Fig. 2, there are no, or few, occluding junction particles along the perimeter of communicating plaques on the A face. The B face of cj_1 is outlined by occluding junctional particles on both sides; note that the boundary particles project a longer shadow on the A face (double arrow) than on the B face. × 90,000.

TABLE II

### Average Number and Spacing of Occluding Junctional Elements* in Freeze-Cleaved Preparations

	Aorta thoracic	Aorta abdominal	Mesenteric artery	Renal vein		Mesenteric vein		Vena cava inferior	
				Continuous junctions	Discontinuous junctions	Continuous junctions	Discontinuous junctions	Continuous junctions	Discontinuous junctions
Mean no (range)	3 (2-4)	3 (2-4)	4 (2-6)	2.5 (1-4)	2 (0-4)	2.5 (1-4)	2.5 (0-5)	2 (1-3)	2 (0-4)
Mean spacing (nm) (range)	120 (100-210)	140 (50-220)	100 (40-230)	100 (70-180)	170 (40-280)	110 (60-170)	200 (70-310)	100 (50-180)	210 (60-330)

The sampling used for these measurements is indicated in Table I.
The counts were carried out on a standard band of 250 nm width oriented perpendicular to the direction of the junctions. For arteries and for the continuous type of junctions of the large veins, the spacing between two neighboring ridges/grooves were measured perpendicular to the direction of the junctions. For the discontinuous type of junctions of the large veins, the spacings are minimal values measured between neighboring ridges/grooves irrespective of their orientation (since the latter are randomly distributed).

* Includes ridges (A faces) and grooves (B faces) with or without particles.
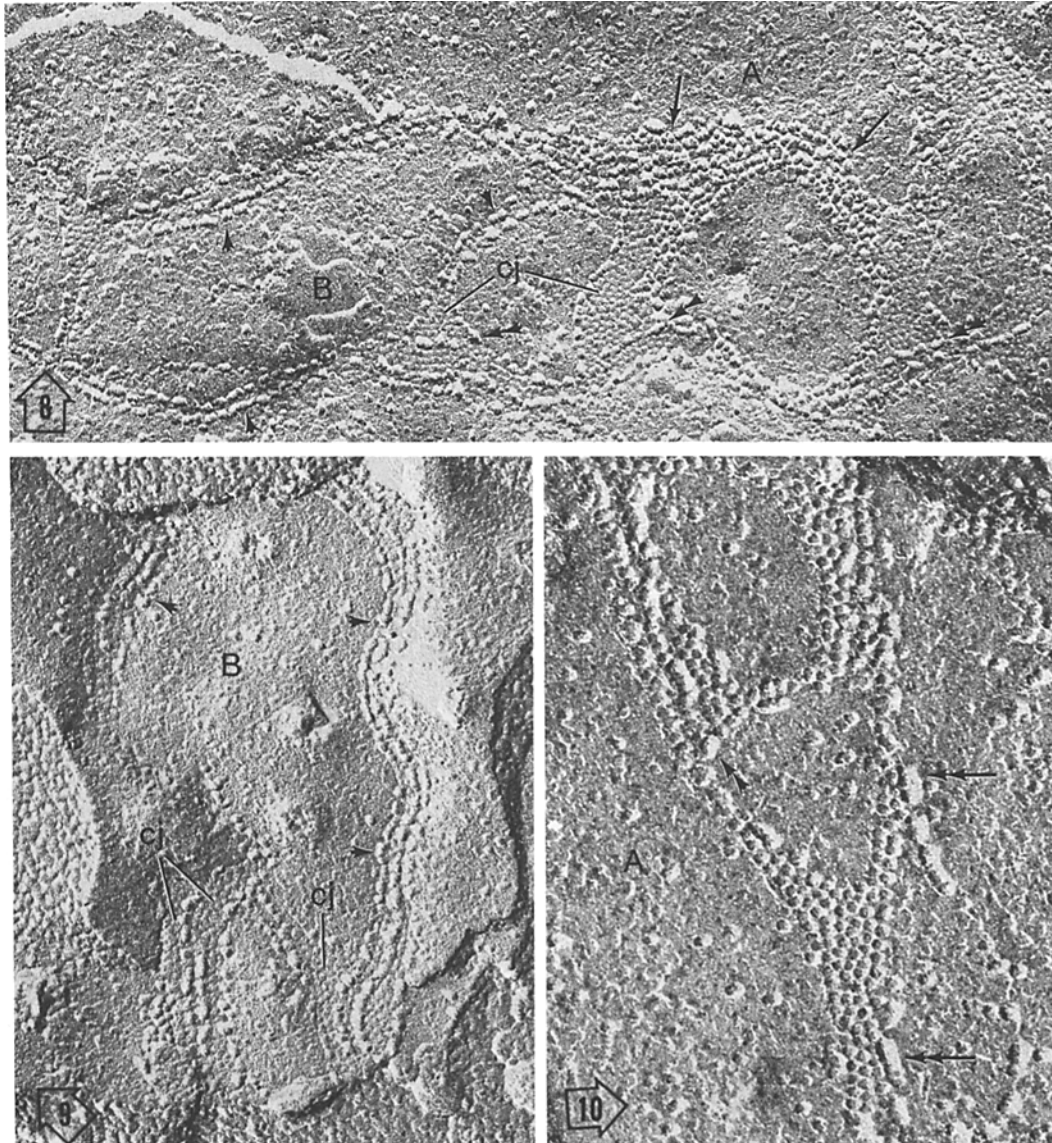
FIGURE 8   Mesenteric artery. Special endothelial junction, type 1. This structure appears as an intricate complex of occluding and communicating junctions. On the B face (*B*) the occluding junction consists of parallel, closely spaced, shallow grooves marked by large protruding particles along their midlines (arrowheads), while the communicating junctions (*cj*) appear as plaques or ribbons of pits intercalated in between the strands of the occluding junctions. Some of the communicating plaques are subdivided by typical occluding strands (double arrowheads). On the A face, the structure appears as a band (arrows) formed by mixed rows of small and large particles. The small ones have the morphology of communicating junction particles; the large ones have the size and the high prominence of occluding junction particles. × 105,000.

FIGURE 9   Mesenteric artery. Special endothelial junction, type 1. On this B face, the junction consists of parallel, closely spaced, shallow grooves marked by typical occluding junctional particles (arrowheads); most of the spaces in between the grooves are occupied by narrow, ribbon-like communicating junctions (*cj*). × 115,000.

FIGURE 10   Mesenteric artery. Special endothelial junction, type 1. On this A face, the structure consists of rows of communicating junction particles, alternating with (double arrowhead) or bordered by (double arrows) discontinuous rows of occluding junction particles. Note the difference in the length of the shadow projected by the two kinds of particles. × 180,000.

713

ridges have a low profile and are either free of, or marked by few, particles which form discontinuous rows. Their morphology is, therefore, similar to that of the negative and positive creases found in venular junctions. Transitions from one type of grooves to the other are seen occasionally within the same junction (Figs. 16 and 17). The endothelium of these vessels is also provided with commu-

nicating junctions (Figs. 12, 13. and 15) which appear either free or associated with the occluding junctions. In the latter case, they are usually contiguous to the occluding junctions rather than intercalated in the latter's meshes.

VENA CAVA: The endothelium of the vena cava inferior (abdominal part) is provided with a junctional system similar to the one described for
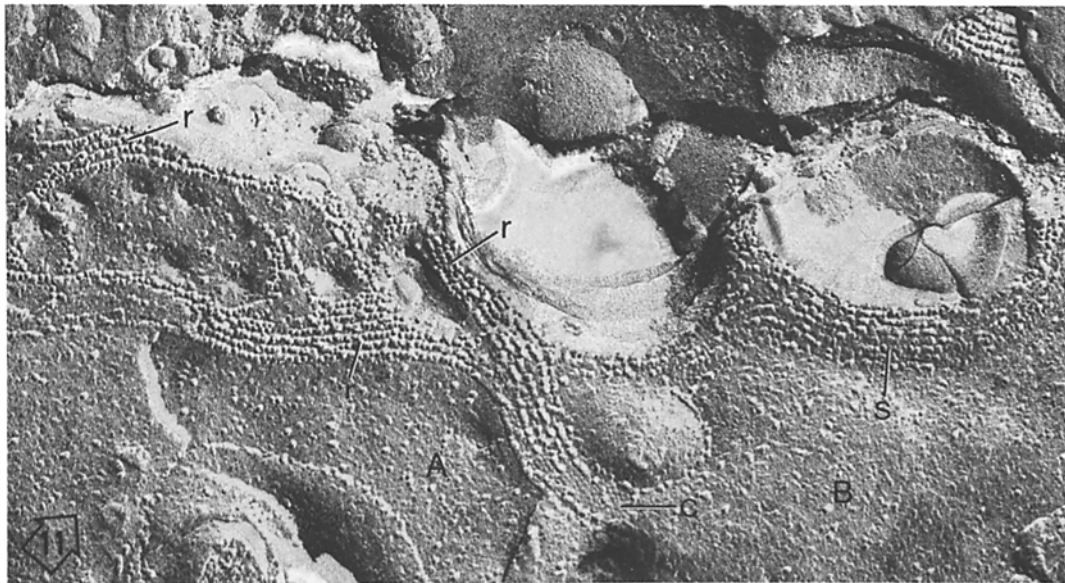

FIGURE 11   Mesenteric artery. Special endothelial junction, type 2. On the A face, the junction is seen as a series of loops formed (in the exposed areas) primarily by tightly packed parallel rows of communicating junction particles (r). On the B face seen in this specimen the structure appears either as parallel, closely spaced rows of occluding junctional particles with intercalated ribbon-like communicating junctions (c) or as parallel rows of large, relatively irregular particles reminiscent, in their arrangement, of a septate junction (s). × 80,000.

FIGURE 12   Renal vein. The cleavage plane exposes large areas of the B face of an endothelial cell (B) separated by strips and patches of the A face of its neighbor (A) and, in places, cytoplasmic regions populated by plasmalemmal vesicles (cy_1, cy_2). The occluding junction is represented by two or more parallel ridges marked by few particles on the A face (arrows), and by similarly arranged grooves marked by more numerous particles on the B face (arrowheads). Note the striking tendency of the membrane to fracture along the lines of junctions (between *) and the occurrence of an associated network of junctional grooves at g. Two communicating plaques (cj) appear to be contiguous with, rather than intercalated within, the occluding junction. × 60,000.

FIGURE 13   Mesenteric vein. On this A face, the occluding junction is represented by a series of low-profile ridges free of, or marked by only a few, particles. Two small, irregular communicating plaques (cj) are located in contiguity with the elements of the occluding junction. Note the presence of narrow furrows (f) along the crest of some parts of the ridges. × 115,000.

FIGURE 14   Mesenteric vein. The two ridges of an occluding junction seen on this A face have their crests marked by almost continuous fine furrows (f) which can be seen more clearly by rotating the figure ~ 90° (either way) from the direction of shadowing. A small communicating macula appears at cj. × 140,000.
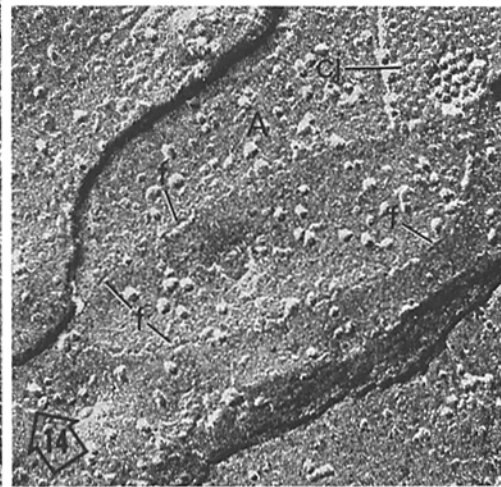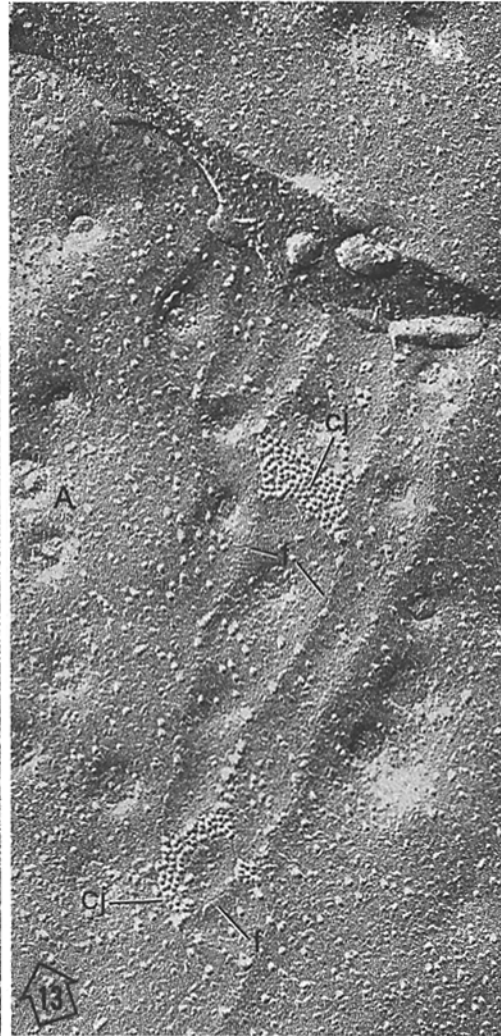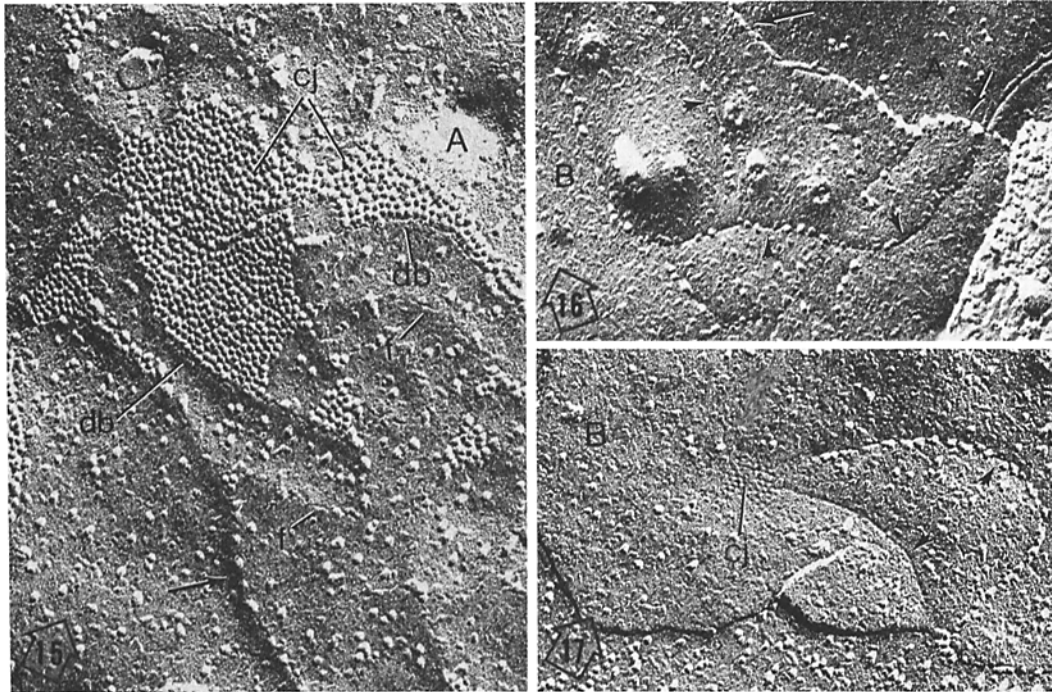
FIGURE 15 Mesenteric vein. A face. Composite junction which consists of low profile ridges for the occluding junction and plaques of irregular outline for the communicating junction (*cj*). Note that the ridges are marked by discontinuous rows of particles (arrow) and, in places, by fine furrows (*f*). Note also the narrow light (shadow)–dark (metal) bands which appear perpendicular to the direction of shadowing along occluding junctional ridges on the perimeter of the communicating plaques (*db*) × 115,000.

FIGURE 16 Mesenteric vein. Network of grooves of an occluding junction marked by discontinuous rows of particles (arrowheads). The arrows indicate the line of a junctional groove along which the fracture jumps from the membrane of one cell to that of its neighbor in the junction. Note that the shadow-cast on the A face (*A*) by the junctional particles located on the fracture line is more than twice longer than the shadow cast by the same particles on the B face (*B*). × 90,000.

FIGURE 17 Renal vein. Structural detail in a composite junction showing a small communicating junction and two adjacent grooves (B face) of an occluding junction (arrowheads). One of the grooves is entirely free of particles, the midline of the other is marked by usual junctional particles. × 92,000.

the renal and mesenteric veins, except for a higher frequency of regions in which the junctions have venular morphology and except for a lower frequency of communicating junctions (Figs. 18–20). In addition to the usual junctions already mentioned, special junctions similar to, but simpler than, those described as type 1 in the arterial endothelium have been occasionally encountered in the cava (Figs. 21 and 22). They appear to be essentially modified communicating junctions with minor contributions from occluding junction elements.

The preferred cleavage plane in venular endothelium and in certain limited regions of the venous endothelium is diagrammed in Fig. 25 and described in some detail in the corresponding legend. The diagrams in Figs. 23 and 24 and their legends allow a comparison of the cleavage faces of the intercellular junctions in arterial, capillary, venous, and venular endothelia.

DISCUSSION

The endothelium of the large arteries examined (aorta and mesenteric artery) has composite intercellular junctions which consist of occluding junctions with intercalated communicating maculae. The junctional system is comparable to that found
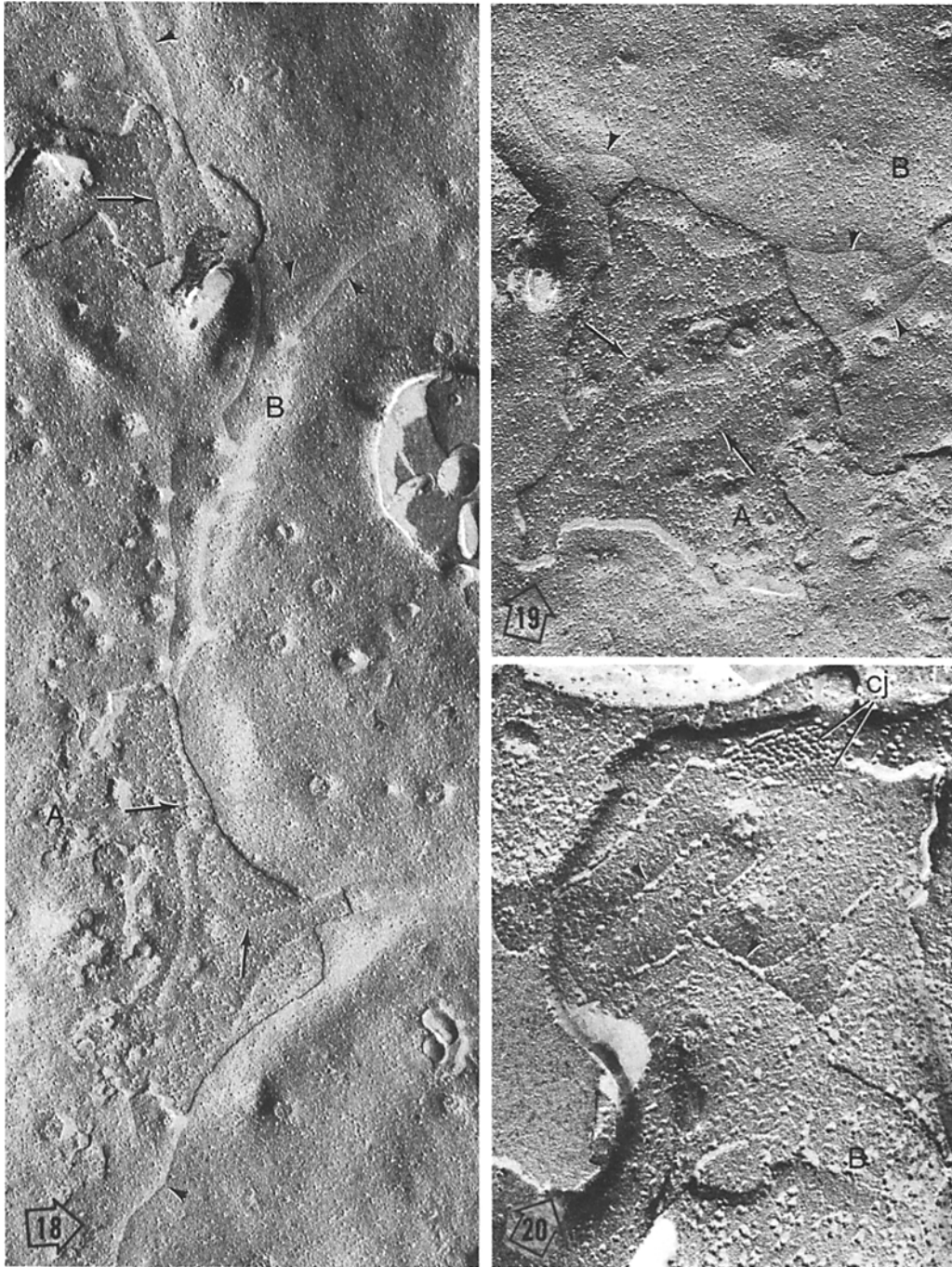
FIGURES 18–20   Vena cava inferior.

FIGURE 18   Fragment (4 μm) of a long ($\sim$ 15 μm) occluding junction which consists of particle-free grooves on the B face (arrowheads), and low-profile ridges marked by few particles on the A face (arrows). Note the relatively irregular orientation of the grooves and the presence of discontinuities in the system of grooves and ridges. × 45,000.

FIGURE 19   Another fragment of the same junction which illustrates the occurrence of networks of grooves (B face) (arrowheads) and low profile ridges (A face) (arrows) which occur focally along the occluding junctions of the venous endothelium. × 50,000.

FIGURE 20   Composite junction which consists of staggered grooves (occluding junction) marked by rows of particles (B face) (arrowheads) and a contiguous communicating plaque (cj). This field comes from the same replica as Figs. 18 and 19. × 100,000.

in arterioles (29) except that it is less elaborately organized in depth and that the number of occluding elements (grooves or ridges) is smaller than in the arteriolar endothelium.

In veins, the occluding junctions of the endothelium appear less elaborate than in arteries, but they have the tendency to be continuous over long distances. They are associated with contiguous rather than intercalated communicating junctions occurring at considerably less frequency than in the arterial endothelium. The venous occluding junctions show small focal areas in which the junctional grooves or ridges have the arrangement and the fine structure previously described in venular junctions, except that discontinuities in the junctional network are less frequent than in venules.

In the occluding junctions of the arterial endothelium and in the continuous regions of those of the venous endothelium, junctional particles are found more frequently along the midline of the B face grooves rather than along the crests of the A face ridges. The latter often exhibit fine furrows complementary in location to junctional particles, but considerably smaller. These furrows are probably left behind by the particles removed with the outer leaflet of the membrane. The explanation assumes that, in the process of cleavage, shadowing and replication, the width of the furrows is considerably reduced. A similar reduction in dimensions is incurred by the dimples of the communicating plaques. As previously discussed (29), the preferential association of the junctional particles with the outer membrane leaflet suggests that the interaction between particles paired in phase within the joined membranes are stronger than their interactions with the rest of the membrane and the cytoplasm. As a result, each pair of particles behaves as a "single unit" which preferentially deflects the cleavage plane along its inner contour as shown in Figs. 23-24. A comparable situation has been described in occluding junctions in other epithelia (2, 30, 33) except that in this case the particles (strands or fibers) are preferentially associated with the A face.

In veins, the focal regions of endothelial junctions of venular morphology are provided with shallow grooves or low-profile ridges (negative or

FIGURES 21-22 Vena cava inferior. Special endothelial junctions. The junctions consist essentially of irregular communicating junctions, most of them of elongated shape, in which the corresponding junctional particles tend to be arranged in parallel rows. The junction is reduced in places to a double row (r_2) or a single row (r_1) of such particles. Few particles of the type seen in occluding junctions appear in this area in the B face grooves or along fracture lines (arrows). × 105,000.

FIGURE 22 See legend under Figs. 21–22. × 105,000.

positive creases) free or nearly free of particles. The implications of these findings in terms of membrane organization have been discussed in a previous paper (29). As in venules, it may be tentatively assumed that these regions represent areas of increased paracellular permeability.

The communicating junctions often extend into rows of particles, occasionally single rows, which connect one communicating plaque to another. Such structures probably supplement to some extent the occluding junctions, but their permeability properties remain completely unknown.

In arteries, as well as in large veins, we have detected special junctions which seem to belong to two different types: the first appears as a locally amplified version of the usual combination of occluding and communicating junctions; the second, more complex in nature, is in part reminiscent of septate junctions. Both types of special junctions are characterized by the tendency of their particles to form parallel, closely spaced rows, instead of networks or plaques. These special junctions occur at relatively low frequency and are restricted to small sectors of the perimeter of the endothelial

cells. Their functional significance is unknown and their possible relation, if any, with septate junctions is an intriguing question which remains to be answered by future work.[4]

Our morphological findings suggest that the endothelial cells of the large vessels are securely attached to one another by two types of junctions: occluding and communicating. The attachment appears particularly tight in arteries, and less strong in veins, especially in areas provided with junctions of venular type. In view of the high frequency of communicating junctions, it can be assumed that the endothelial cells of the large vessels, especially those of arteries are, extensively coupled to their neighbors, as seems to be the case in other epithelia (23).

Notwithstanding the segmental differentiations described, the junctions of the endothelium appear to be variations on a common theme which includes in all cases the tendency of junctional particles and strands to separate upon cleavage with the outer leaflet of the membrane and in which communicating junctions are often intimately associated (included within or contiguous with) occluding junctions.

In addition, it is already known from studies of sectioned material that the junctional complexes of the endothelium do not have desmosomes and have no or poorly developed elements of the adhering zonulae type.

The literature on endothelial junctions in large vessels is limited to a small number of observations made primarily on sectioned specimens of the aorta and a few large arteries. These observations have established the existence of occluding and communicating junctions in the arterial endothelium (10, 16, 17, 27), but have not revealed the extent and intimacy of the association of the two types of junctions, features that are so evident in freeze-cleaved preparations.

The permeability of the arterial endothelium has been studied in a number of species with a variety of tracers (horseradish peroxidase [HRP]), ferritin, lactoperoxidase and catalase, of which only HRP appears to permeate in some cases the endothelial junctions. There is, however, no agreement on the behavior of this tracer: according to a number of investigators the junctions are not

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[4] The existence of septate junctions has been described in a few instances in thin-sectioned specimens of vertebrate tissues, but at least in one case their presence has not been confirmed in freeze-cleaved preparations (D.S. Friend and N.B. Gilula, 1972. *J. Cell Biol.* **53**:148).

(a)   (b)
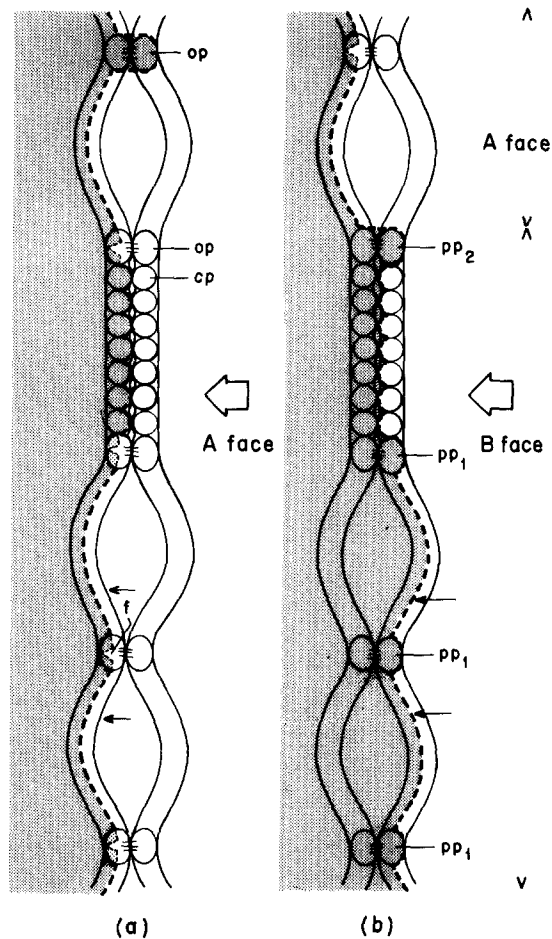
FIGURES 23–25 Diagrammatic representations of the usual positions of the cleavage plane in endothelial junctions. op, occluding junction particle (~ 100Å); pp_1, occluding junction particle protruding in a B face groove; pp_2, occluding junction particle protruding at a transition from a B to an A face; cp, communicating junction particle (~ 80Å): f, furrow on the A face ridges.

FIGURE 23 Composite junction of arterial type (found in arterial endothelium, and fully developed in arteriolar endothelium). The cleavage plane exposes a communicating junction intercalated between two occluding strands, and (at some distance) additional elements of the latter type. Slight infoldings in between occluding elements of the cell membranes involved in the junction give ridges on the A face (a) and grooves on the B face (b) (in between the arrows). Note that the grooves and ridges adjacent to the communicating junction are shallow and one-sided. At the level of the plaques of communicating junctions, the cleavage plane follows the inner contour of the occluding particles (found at the periphery of the plaque) and the outer contour of the communicating particles for the rest of the junction. A jump of the cleavage plane from the membrane of one cell to that of its partner (b)
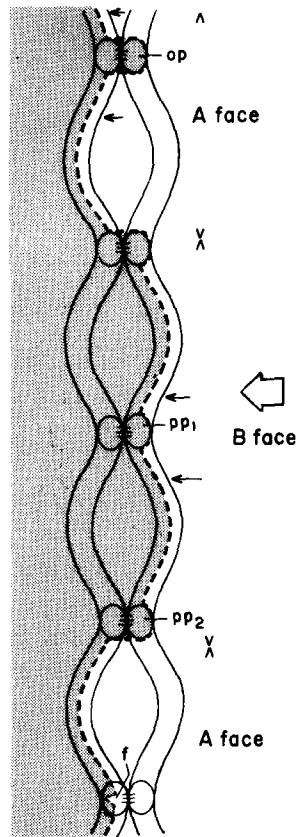
FIGURE 24   Junction of capillary type. This type, primarily found in capillaries, is reduced to occluding junctional elements similar to those found in the junctions of arterial type. Communicating junctions are generally absent. Slight outfoldings of the joint cell membranes in between occluding strands produce grooves on the B faces and ridges on the A faces (in between arrows). On the B faces occluding particles (strands) are left in the midline of the grooves (pp_1). On the A faces secondary alterations which follow the removal of the occluding strands may lead to the appearance of narrow furrows (f). As in arterial junctions, the jump of the cleavage plane from one membrane to its partner at the level of the occluding strands (pp_2) gives a ridge which protrudes high above the A face.
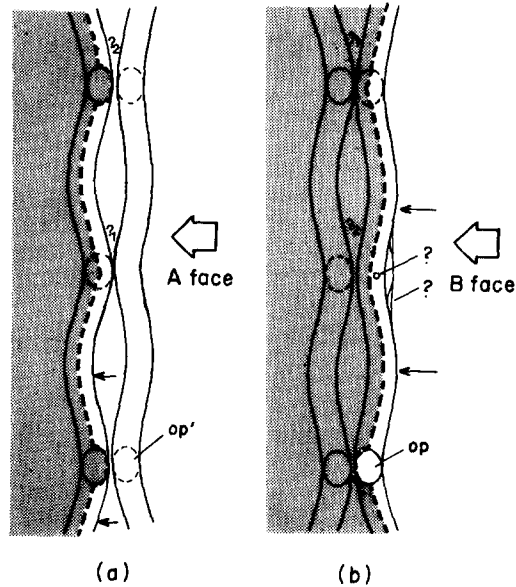

FIGURE 25   Junction of venular type. This type is found in pericytic and muscular venules (in the latter case being closely associated with rare communicating junctions). As in the junctions of the other types, outfoldings of the cell membranes in between junctional elements produce low-profile ridges on the A face (a) and shallow grooves on the B face (b). But, in this case, occluding particles are rare, often missing over long distances, and apparently interact weakly with their partners. As a result, the junctional elements are preferentially left on the ridges of the A face, and many ridges and grooves are free of particles over long distances and appear as simple negative or positive membrane creases. It is possible that the latter are maintained by still unresolved structural elements within the membrane (fine strands?) or on the inner aspect of the membrane (connecting strands?). op, and op', occluding particles in the plane of the diagrammatic section and at some distance from the latter, respectively. From the evidence at hand, it can not be decided whether the intercellular space is occluded $(?_1)$ or open $(?_2)$ at the level of the creases. Junctions of venous type consist of domains morphologically identical to venular junctions and domains comparable to arterial junctions except that the communicating elements are contiguous to, rather than intercalated in between, occluding elements.

gives (at the periphery of the plaque) an occluding particle (pp_2) protruding high above (and hence projecting a long shadow on) the A face of the partner's plasmalemma. The furrows (f) left behind on the A face by avulsed occluding particles are narrower than expected, presumably as a result of partial obliteration during the preparation procedure. On the B face (b), the cleavage plane leaves behind in the midline of the grooves occluding particles (strands) presumably because of their strong interactions (represented by connecting lines) with similar particles in the membrane of the adjoining cell.

permeable under normal conditions (10, 31), while according to others the junctions are normally penetrated by HRP (5, 17, 28). The permeability of the junctions has been found to increase with hypertension (4, 19, 32) and ischemia (24), to vary from one region of the endothelium to another (10, 18, 20, 25), and to increase at the level of atheromatous plaques (1, 8).

Our findings indicate that the arterial endo-

thelial junctions are continuous and well organized and suggest that they have a low degree of permeability, but, for reasons already explained in the previous paper (lack of information concerning the situation at the true cell surface at the level of the junctions), no reliable correlation can be established at present between the type of structure seen on cleaved membrane faces and the degree of paracellular permeability in the vascular endothelium, arteries included.

It would be of interest to use freeze-cleaved preparations to find out whether the organization of the junctions in the arterial endothelium is modified when the permeability is experimentally or pathologically increased. It would also be of considerable interest to establish the precise level at which the transition from venular to venous type of junctions takes place. It has been shown that the special reactivity of the venular endothelium to local mediators, i.e., histamine, serotonin and bradikinin (3, 22, 26), is restricted to venules smaller than 80 μm in diameter. These venules probably correspond to the pericytic and muscular venules in which the venular type of occluding junctions was originally described (29).

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