SEGMENTAL DIFFERENTIATIONS OF CELL JUNCTIONS IN THE VASCULAR ENDOTHELIUM

The Microvasculature

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ABSTRACT

Small vascular units consisting of an arteriole, its capillaries, and the emerging venule (ACV units) were identified in the rat omentum and mesentery. They were fixed *in situ* and processed for electron microscopy either as whole units or as dissected segments. Systematic examination of the latter (in thin sections, as well as in freeze-cleaved preparations) showed that the intercellular junctions of the vascular endothelium vary characteristically from one segment to another in the microvasculature. In arterioles, the endothelium has continuous and elaborate tight junctions with interpolated large gap junctions. The capillary endothelium is provided with tight junctions formed by either branching or staggered strands; gap junctions are absent at this level. The pericytic venules exhibit loosely organized endothelial junctions with discontinuous low-profile ridges and grooves, usually devoid of particles. No gap junctions were found in these vessels. The endothelium of muscular venules has the same type of junctions (discontinuous ridges and grooves of low profile); in addition, it displays isolated gap junctions of smaller size and lower frequency than in arterioles. The term communicating junction (*macula communicans*) is proposed as a substitute for gap junction, since the latter is inappropriate, in general, and confusing in the special case of the vascular endothelium.

The structure of the intercellular junctions in the endothelium of small vessels, especially capillaries, is still an unsettled issue. The presence of occluding junctions (3, 7, 33) as well as of open junctions (18, 19) has been recorded in the endothelium of vessels assumed to be blood capillaries in thin sections. However, there is no agreement about the relative frequency of these two types: for instance, in muscular capillaries open junctions are described as rare by some investigators (3) and as frequent by others (18). Among the variables which could explain the lack of consensus is the location of the observed junctions, since often the exact nature of the vascular segments cannot be ascertained beyond question in sectioned tissues. Another possible reason for disagreement is the limited amount of information obtained from such specimens, which reveal only the presence or absence of close membrane aposition or fusion over distances limited to the thickness of the sections. More detailed information covering larger areas can be obtained from freeze-cleaved specimens, but so far

this approach has been used only sporadically (8, 20, 52) on small vessels of questionable identification. Precise observations on this general topic are needed, since intercellular junctions, especially open junctions, are still presumed to represent the small pore system of the capillary wall (18, 19, 53).

In the work reported in this paper we have used identified segments of the microvasculature and have examined their intercellular junctions in sections as well as in freeze-cleaved preparations. Sequential microvascular segments were recognized in arteriole-capillaries-venule units (ACV units) in the omentum and mesentery of the rat. The results indicate that the organization of the junctions varies characteristically from one segment to the other of the peripheral vascular bed.

MATERIALS AND METHODS

Animals

As in our previous studies (41–43, 45), we have used Wistar-Furth rats known to be genetically resistant to histamine-releasing factors. All animals were kept under standardized conditions of housing and feeding for at least 10 days before the experiments. 26 mature males were used for the isolation of ACV units and 32 for the examination of vessels in various tissues.

Isolated Vessels: ACV Units

The basic experimental procedure is schematically presented in Fig. 1. ACV units have been isolated from the omentum and the mesentery, organs whose local microcirculation has been extensively studied in various species (5, 16, 21, 54, 55). These organs have been selected because the segments of their microvasculature can be identified reliably and dissected easily after fixation *in situ*. Their capillaries, which are known to have a continuous endothelium (17), and their other small vessels are morphologically similar to their counterparts in skeletal muscle.

Experience in identifying the different parts of the vascular beds was acquired by examining the exteriorized omentum in the living animal. Recognition of the same segments after fixation was made on the basis of diameters and patterns of distribution of the vessels. When the ACV units were dissected, small vessels of uncertain nature were eliminated from the sample finally processed. The identification made at dissection was rechecked by electron microscopy and was generally found to be satisfactory. We used most frequently the omentum in which the minute vessels form simply organized units made up of an arteriole that branches into a few true capillaries, which in turn are drained by a single venule (Fig. 2). We purposely avoided the vascular beds of more complex geometry usually found within the local lobulated adipose tissue, since their segments cannot be satisfactorily separated, and we worked mostly with simpler units (e.g. Fig. 2) from which segment separation could reliably be achieved.

The abdominal cavity was opened under ether anesthesia by a small incision, and the omentum was fixed with a 2% solution of glutaraldehyde in 0.1 M HCl-Na cacodylate or Na arsenate buffer, pH 7.2–7.4, applied topically for 10–15 min. At the end of this period, the omentum was removed as a whole, immersed in the same fixative, and examined under a dissecting microscope at × 50. The blood vessels of the omentum are largely restricted to branching cords of tissue. Arterioles and venules tend to run closely, but not strictly in parallel to one another. From the terminal part of an arteriole, an average of 6–10 capillary loops arise and, after a course of 50–200 μm, converge into an emerging venule. An excised ACV unit measures 2–3 mm in length (Fig. 2). Such units were either processed and embedded as a whole, or dissected into three segments (arteriole, capillaries, venule) which were separately prepared for thin sectioning and freeze-fracturing. For the present study, a number of 28 ACV units were examined.

Vessels in Tissue

Diaphragm, pancreas, and jejunum specimens were fixed *in situ* as in ref. (42), (43), and (41) respectively. The heart was fixed by perfusion with a Harvard pump at a rate of 10 ml/min using the same fixative as above.

Processing for Thin Sectioning

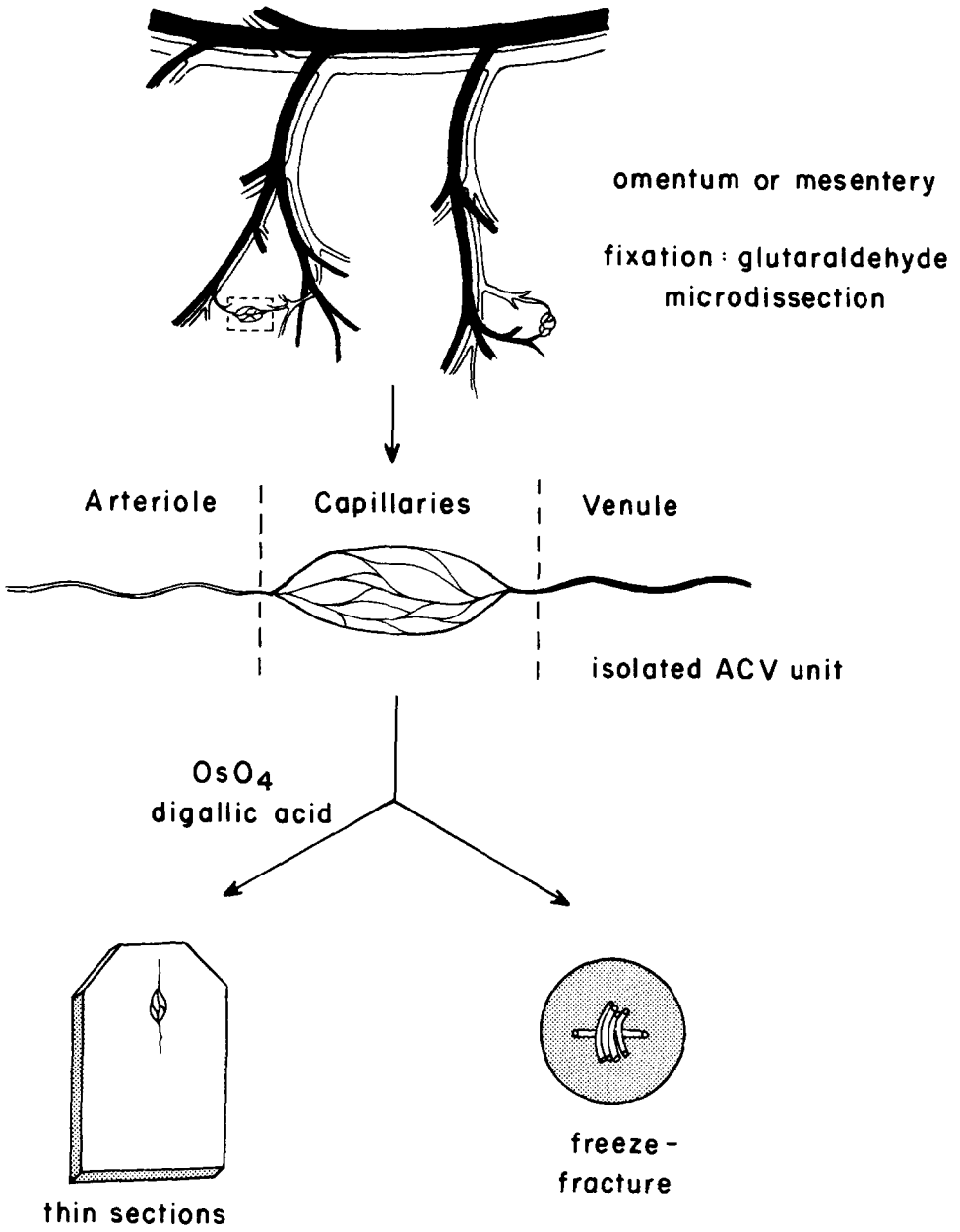
After the initial fixation (*in situ* or by perfusion), isolated vessels as well as tissue specimens were further fixed by immersion in the same fixative for 60–90 min at room temperature. The specimens were postfixed for 90 min at room temperature in 2% O_8O_4 in 0.1 M HCl-Na cacodylate buffer pH 7.2–7.4 and then treated in block for 30 min at room temperature with 1% digallic acid ($C_{14}H_{10}O_9$, Tannic acid-1764, Mallinckrodt Chemical Works, St. Louis, Mo.) in 0.05 M HCl-Na arsenate buffer, pH 7.0 (44).[1] The blocks were subsequently dehydrated in alcohol and embedded in Epon.

Thin sections (500 – 700 Å thick) were cut on a Porter Blum MT2B ultramicrotome, stained for 3–5 min with 0.4% aqueous solution of lead citrate and examined with a Philips-301 electron microscope, operated at 80 kV, and provided with apertures in the condenser (300 μm) and the objective (50 μm).

Processing for Freeze-Fracturing

Isolated vessels and tissue specimens, fixed *in situ* as indicated above, were collected and further fixed by

[1] Simionescu, N., and M. Simionescu. 1975. Digallic acid as mordant in electron microscopy. *J. Cell Biol.* **67** (2, Pt. 2):802 *a*. (Abstr.).



Abbreviations used in legends:

a, adventitia;
A_b, A face of the endothelial plasmalemma on the blood front;
A_t, A face of the endothelial plasmalemma on the tissue front;
B_b, B face of the endothelial plasmalemma on the blood front;
B_t, B face of the endothelial plasmalemma on the tissue front;
bm, basement membrane;
e, endothelium;
ej, endothelial junction;
ie, internal elastic membrane;
l, vascular lumen;
n, nucleus;
pc, pericyte;
sm, smooth muscle cell.

On all micrographs of freeze-fracture replicas the direction of metal shadowing is given by the arrow put on the number in the lower left corner of each figure.

FIGURE 1 Diagram illustrating the experimental procedure used to isolate and process ACV units for thin sectioning and freeze-fracturing.

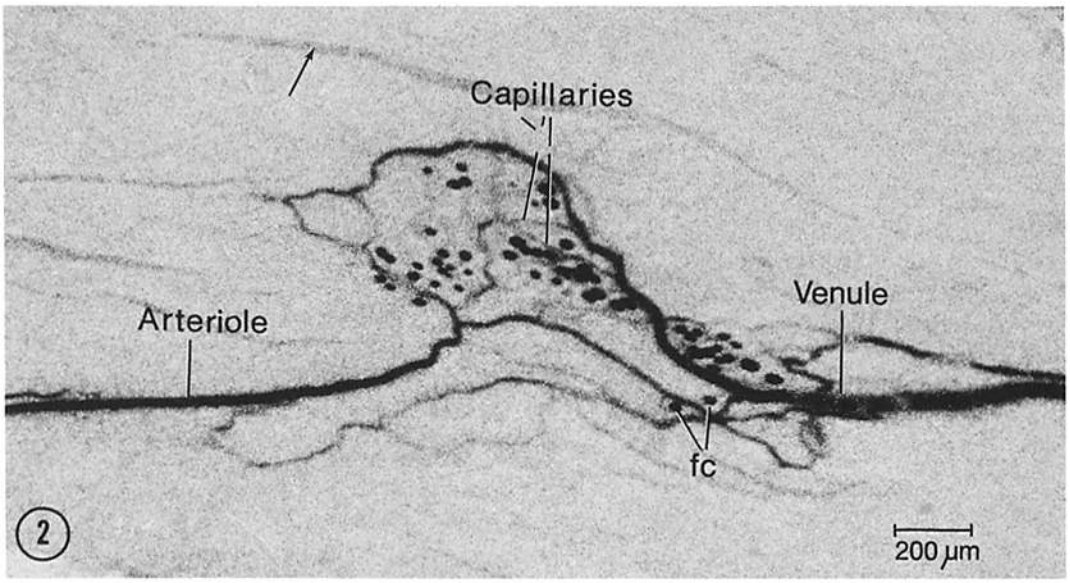


FIGURE 2 ACV unit isolated from a rat omentum. The arteriole branches into a few capillaries which converge into a venule. Fat cells *fc;* peritoneal fold, arrow. × 56.

immersion for 20–30 min in the same fixative. All specimens were prepared for freeze-fracturing as described previously (43). For fracturing dissected vessels, it was found convenient to mount them in small bent bundles on the specimen carrier of the Balzers microtome (Fig. 3). In bent bundles the fracture plane often cuts through the lumen, the endothelium and all the other elements of the vascular wall, thereby facilitating recognition of the different structures by their position. Table I gives the number of endothelial junctions examined in freeze-fracture preparations of each tissue.

Densitometry

22 micrographs of endothelial junctions in sectioned blood capillaries were scanned with a Joyce-Loebl Mark III microdensitometer, at × 10, using a 1-mm width slit on the photomultiplier, and 1.5 OD optical wedge. Two examples of microddensitometric tracings are given in Figs. 11 and 12.

RESULTS

General Procedure

Segments isolated from ACV units and separately processed were first examined in thin sections to check the identity of the vessels by determining their average diameter, and establishing the structure of their wall. In this way it was possible to identify, in addition to arterioles and capillaries, two successive types of postcapillary venules which we designate as pericytic and muscular. In general, the organization of the different segments of the microvasculature of the omentum and mesentery is similar to that so far described in other locations such as muscle fascia (37, 38), nervous system (10, 28) and gingiva (32).

Identical segments processed through freeze-cleavage were then investigated to establish the organization of their intercellular junctions. Whenever possible, we chose replicas of vessels which, by their dimensions and structures, conformed to the information already available from thin sections. This survey showed that each segment has a characteristic system of intercellular junctions in its endothelium.

At the next step, the inquiry was extended to freeze-fractured preparations of intact tissue to see if the same patterns of junctional organization occur *in situ*. Here again, we relied primarily on replicas in which the identity of the vessels examined could be checked by some other independent feature, e.g., the organization of the wall and occasionally the diameter.

Except for blood capillaries, thin sections of isolated vascular segments and of tissues were used primarily for the identification of the vessels rather than for a detailed study of their endothelial junctions. Hence, only in the case of capillaries have we attempted to correlate the observations

FIGURE 3 Segments of venules (~50 μm diameter), isolated from ACV units and piled on a tissue carrier for freeze-fracturing. × 32.

TABLE I

Number of Endothelial Junctions Examined in Freeze-Fractured Preparations

	Arterioles	Capillaries	Venules
Isolated vessels*	16	27	38
Vessels in tissue‡	13	99	30

* ACV units from omentum or mesentery.
‡Diaphragm, heart, pancreas, jejunum.

made on the two types of preparations (thin sections and replicas of freeze-cleaved specimens).

General Findings

The general picture that has emerged from this inquiry shows that in the vascular endothelium there are no usual junctional complexes of the type encountered in other epithelia (11). The cells are connected to one another only by tight (occluding) junctions and gap junctions. The appearance of such junctions in freeze-fractured preparations in other epithelia has been described in a large number of papers and has been reviewed in (30) and (48).

The relative development of the two types of junctions in the vascular endothelium and the extent to which they are associated with one another result in characteristic junctional patterns for each segment of the microvasculature as described below.

ARTERIOLES: In these vessels, which have a diameter of 30–100 μm and a continuous layer of smooth muscle cells in their media (Fig. 4), the cells of the endothelium are joined together by a combination of tight and gap junctions. Depending on the cleavage surface examined, the tight junctions appear as a system of two to six ridges (A face) or grooves (B face) (Table II) which form a continuous network. By themselves, the ridges[2] are rather low and the grooves shallow, but they are marked by protruding particles (diam. ≃80–100 Å) which are aligned in quasi-continuous rows or strands and which appear much more frequently in the grooves of the B faces (Fig. 5 *a*, *b*) than on the ridges of the A faces (Fig. 6). Many of the meshes of the tight junction's network are fully or partly occupied by gap junctions of usual appearance, except for their location, (Fig. 5 *a*, and *b*). Around them, the grooves and especially the ridges of the tight junctions are almost completely leveled off; only the tight junction particles are clearly seen. "Free" gap junctions, not framed by tight junctions and not clearly integrated in the network, are only occasionally encountered (Fig. 6). As in other epithelia, the gap junctions consist of aggregates of particles; the degree of order in the aggregates varies from crystalline lattices (close hexagonal packing) to apparently random distribution. Often the particles form linear arrays. Some of the meshes of the tight junctions are occupied by vesicular openings. Whenever found, the tight junctions are continuous over the entire exposed areas of the endothelium over distances which could measure up to 10–12 μm. There are no interruptions affecting all of their ridges and there are few ridges with free ends; the networks are

[2] The term "ridge" is used in the text with a connotation different from that found in most papers dealing with junctions. It refers to a linear bend or crease in the membrane not necessarily marked by particles. The difference will be explained more fully in the Discussion.

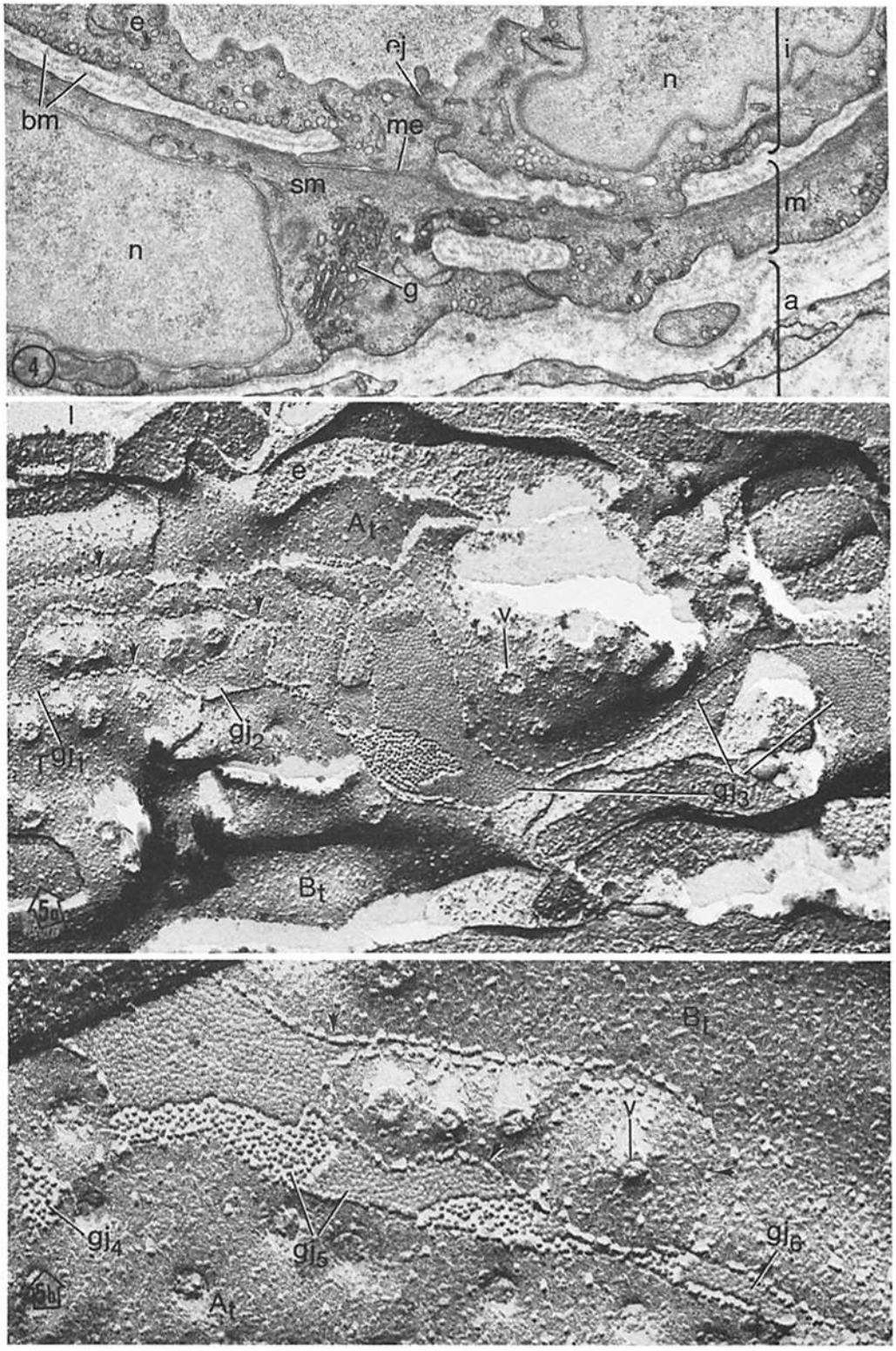

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TABLE II

Average Number and Spacing of Junctional Strands (Freeze-Fractured Preparations)

	Arteriole	Capillary	Pericytic venule	Musuclar venule
Mean no.	3.7 (2–6)*	3.1 (2–5)*	1.8 (0–4)*	2.3 (0–5)*
Mean spacing (nm)	70 (45–155)	75 (40–165)	125 (75–310)	110 (65–240)

For each type of vessel, 15 endothelial junctions ranging from 2 to 5 μm width, were examined. The counts were made on a standard band of 250 nm width oriented perpendicularly to the direction of the junctions. For arterioles and capillaries, the spacings between two neighboring strands (ridges/grooves) were measured perpendicularly to the direction of the junction, whereas for venules they were measured between strands irrespective of their orientation (since the strands are randomly distributed).

* Range in parentheses.

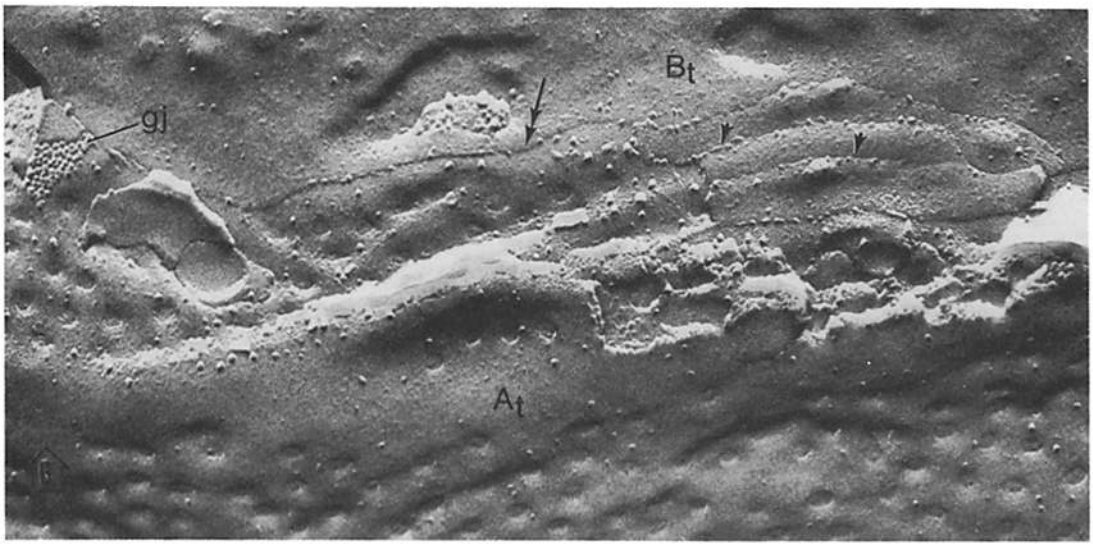


FIGURE 6 Rat diaphragm; small vessel, probably an arteriole. The cleavage plane exposes an A face (A_t) and a B face (B_t) both on the tissue front of the endothelium, and reveals an occluding junction partially associated with a gap junction (*gj*). On the B face, the tight junction appears as 2–4 parallel grooves partially marked by discontinuous rows of particles (arrowheads). Note the tendency of the membrane leaflets to fracture along the ridges or grooves of the occluding junctions (double-arrow). × 82,000.

FIGURE 4 Rat omentum. Small sector of an isolated arteriole showing the basic structure of the vascular wall: tunica intima (endothelium), *i;* tunica media (smooth muscle cell), *m;* tunica adventitia, *a*. Note the myoendothelial junction (*me*) and the two layers of basement membrane (*bm*) between the endothelium and the smooth muscle cell. Golgi apparatus, *g*. × 20,000.

FIGURE 5 *a* and *b* Rat omentum; isolated arteriole. Two fragments of an endothelial junction exposed over a long distance (11 μm out of which 3 μm are shown in these figures). The tight junction appears on the B face (B_t) as a network of continuous interconnected grooves marked by rows of particles (arrowheads). Gap junctions (gj_{1-6}) occupy some of the meshes of the tight junction network. At gj_3 and gj_5, two large gap junctions exhibit their A and B faces and are surrounded over most of their perimeter by strands of the occluding junctions. Note the opening of a number of plasmalemmal vesicles (*v*) within the meshes of the occluding junctions. In its widest part, the complex of occluding and gap junctions measures about 0.5 μm which represents about one-third of the height of the endothelium at the level of the intercellular space. (*a*) × 75,000; (*b*) × 120,000.

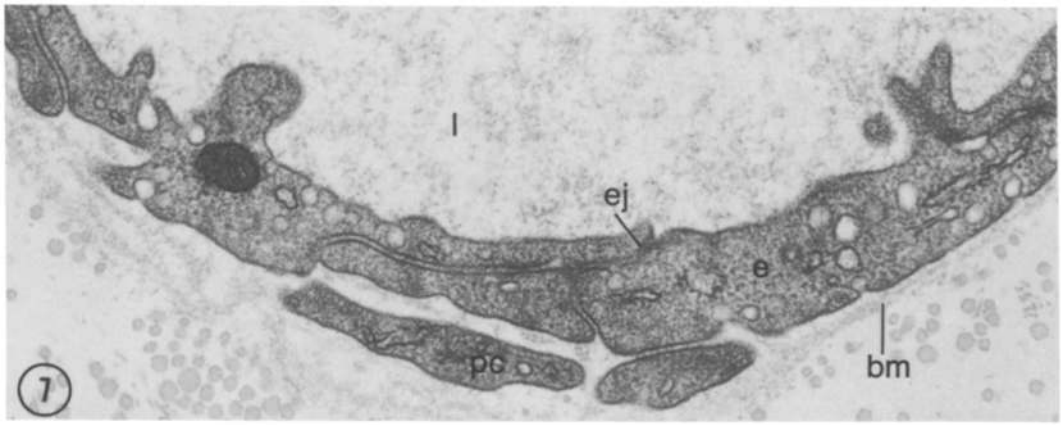


FIGURE 7 Rat omentum. Cross section of an isolated capillary showing the general structure of its wall. × 39,000.

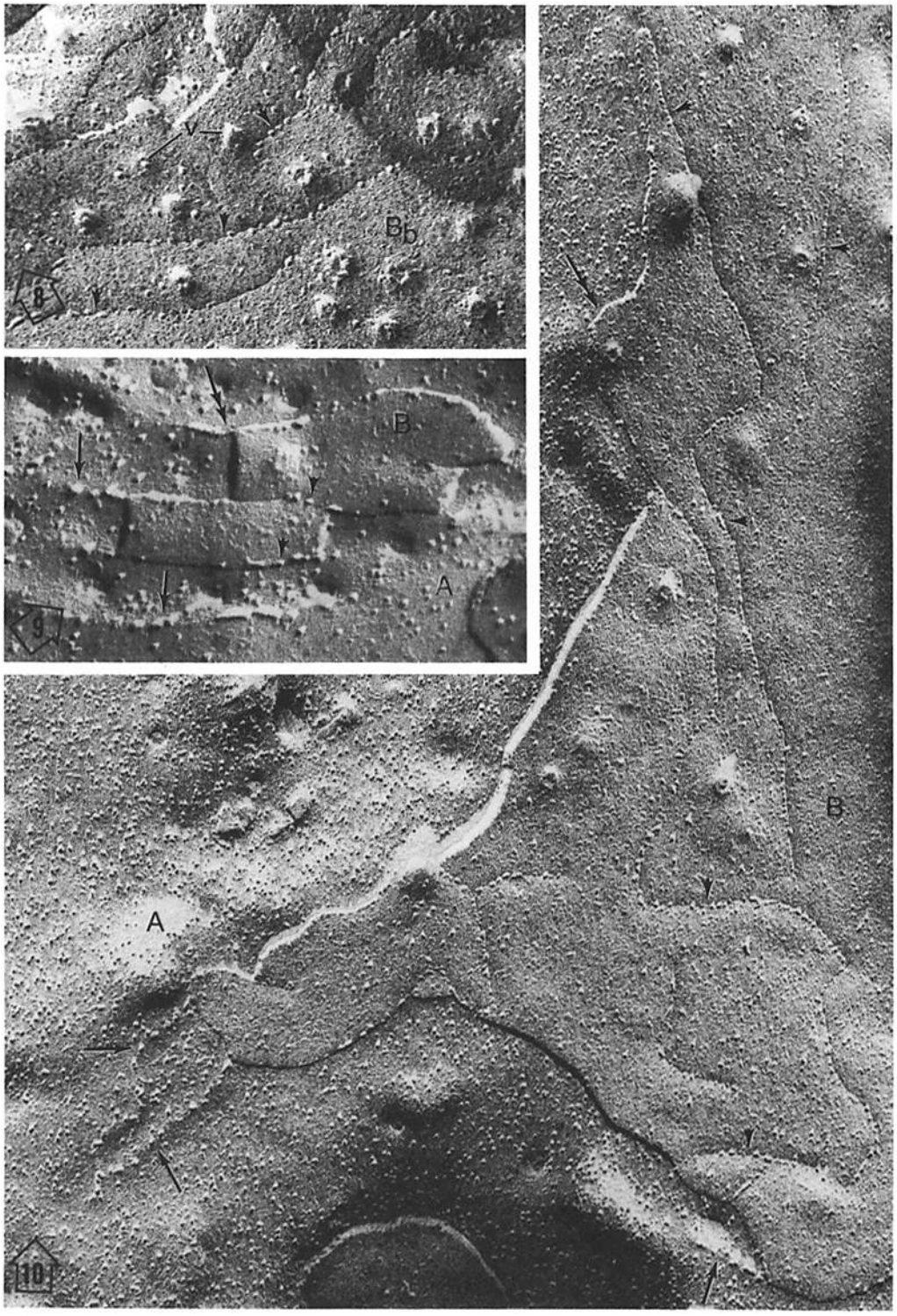
arranged in such a way that there is no uninterrupted lane leading in between the ridges from one side of the junction to the other. Hence, it is probably (but not proven) that these junctions are true occluding zonules.

CAPILLARIES: In these vessels, which measure 5–10 μm in diameter, the cellular elements of the wall are reduced to the endothelium and a few pericytes (Fig. 7). The endothelial cells are linked by tight junctions only. In freeze-cleaved preparations, the junctions consist of 2–5 low ridges or shallow grooves which form either a continuous network with some free ends (Fig. 8) or a maze (Figs. 10, 21) in which the grooves are staggered and generally parallel to one another. In the latter arrangement, uninterrupted lanes are occasionally found which appear to lead from one side of the junction area to the other in between the ridges (Fig. 9). As in arterioles, the ridges or grooves are marked by rows of protruding particles (diam. 80–100 Å). Their frequency within the rows is variable, but generally lower than in arterioles. Occasionally, rows of particles are found to continue uninterrupted from the B face to the A face of the two membranes which form a junction. Since the shadow cast by the row segments on the A face is noticeably longer than that produced by equivalent segments on the B face (Fig. 9), it can be assumed that the particles in the joint membranes behave during cleavage as single units (irrespective of the process involved in their morphogenesis).

FIGURE 8 Rat omentum; isolated blood capillary. The tight junction seen on this B_b face consists of a maze of staggered and branching grooves marked by discontinuous rows of particles (arrowheads). Note the presence of a number of vesicle openings (v) within the maze. × 93,000.

FIGURE 9 Rat diaphragm; small vessel, probably a blood capillary. The cleavage plane reveals an A face of the plasmalemma of an endothelial cell surrounding an island of the B face of the plasmalemma of its neighbor. The staggered ridges and grooves represent the local endothelial tight junction. Note the continuity of a row of protruding particles from a ridge of the A face through a groove of the B face. Note also that the shadow cast by junctional particles and strands is longer on the A face (arrows) than on the B face (arrowheads). As in Fig. 6, note the tendency of the membrane leaflets to break along the ridges or grooves of the tight junction (double arrow). × 101,000.

FIGURE 10 Rat omentum; isolated blood capillary. The cleavage plane reveals the B and A faces of the cell membrane of two adjacent endothelial cells and exposes over a long distance (~3 μm) a tight junction which consists of a maze of branching and staggered grooves (arrowheads) on the B face and ridges (arrows) on the A face. Most of them form a continuous network; a few appear disconnected. Grooves as well as ridges are marked by discontinuous rows of particles. As in Fig. 6, note the tendency of the membrane leaflets to fracture along the grooves or ridges of the junction (double-arrow). × 68,000.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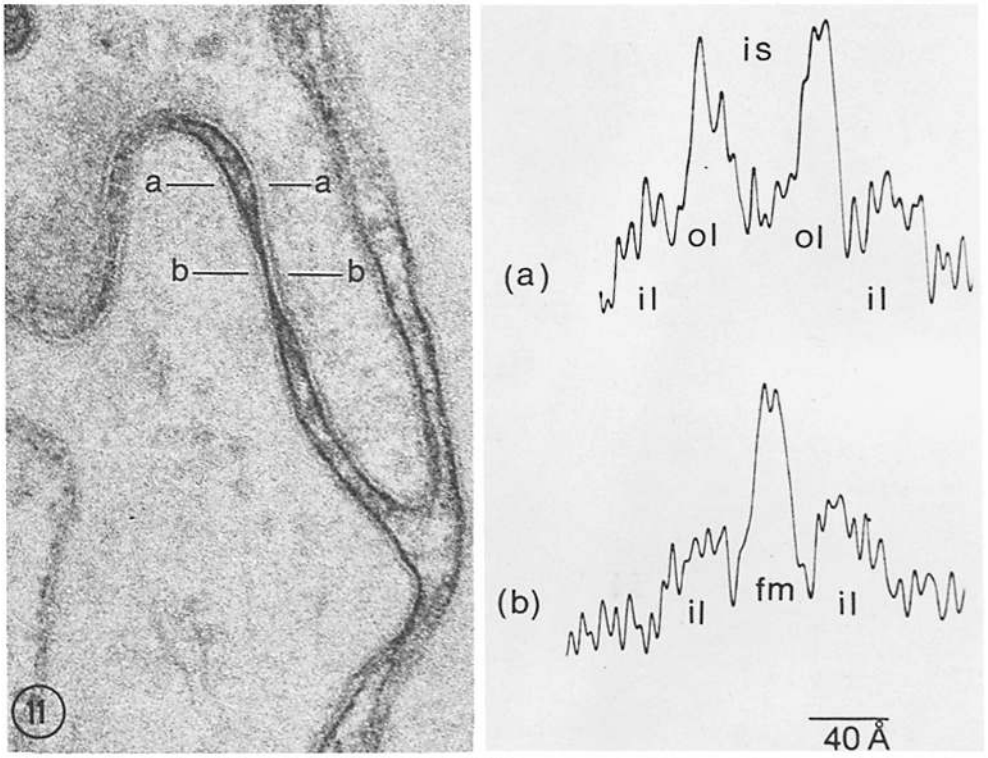
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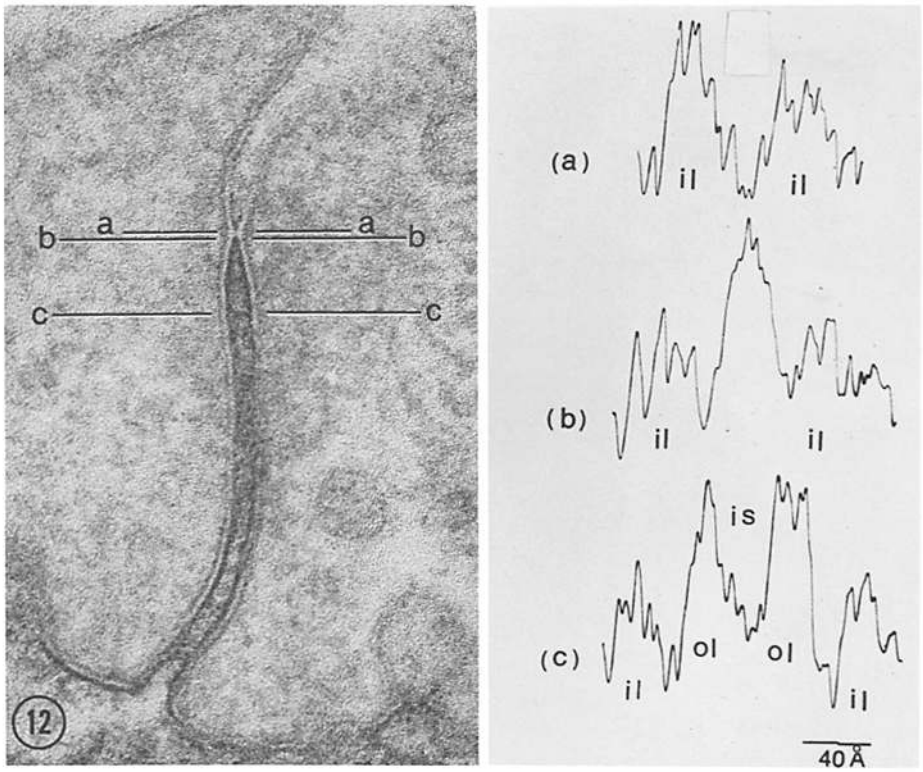
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TABLE III

Average Number and Spacing of Fusion Points (Thin Sections) and Strands (Freeze-Fractures) in the Endothelial Junctions in Different Capillaries

Tissue	Points of fusion (thin-sectioned specimens)		Junctional strands (freeze-cleaved specimens)	
	Mean no.	Mean spacing	Mean no.	Mean spacing
		nm		*nm*
Diaphragm	2.2 (1–3)*	130 (70–240)*	3.8 (2–5)*	70 (40–90)*
Heart	1.7 (1–2)	145 (80–250)	3.2 (2–4)	85 (50–165)
Pancreas	2.3 (1–3)	140 (110–235)	4.3 (2–6)	75 (55–145)
Jejunum	2.1 (1–3)	150 (130–210)	3.9 (2–5)	80 (45–150)

Measurements were made on 60 to 70 endothelial junctions for each tissue (thin sectioned specimens), and on 99 freeze-cleaved junctions (diaphragm, 34; heart, 23; pancreas, 35 and jejunum 7). Points and bands of fusion were counted on sections 60–70 nm thick, whereas the junctional strands on freeze-cleaved specimens were counted on areas ranging from 1.5 μm to 6 μm in width. The discrepancy between frequency of fusion points and frequency of strands can be explained only in part by the staggered arrangement of the strands.

* Range in parentheses.

In sectioned specimens, the appearances encountered vary from membrane fusion with focal but complete elimination of the dense outer membrane leaflets (Fig. 12), to incomplete fusion or close contact in which the intermediate band is narrower than two outer leaflets (Fig. 11). In the sample of 22 junctions examined by densitometry (selected for clear visualization of membrane layers), complete fusion (Fig. 12) was encountered 14 times, and close contact (Fig. 11) eight times; junctions with a detectable gap were not found. Although the general position of junctional structures is the same in the two types of preparations, the number of fusion bands visible in sections is generally lower than the number of ridges seen in replicas of freeze-cleaved junctions (Table III).

VENULES: In thin sections of isolated venules, two distinct types were encountered. (*a*) An immediately postcapillary vessel, ~20–50 μm in diameter, in which the endothelium is covered by an extensive but not continuous layer of pericytes (Fig. 13); this segment, designated "pericytic venule," corresponds to the postcapillary venule and collecting venule in the perimuscular connective tissue (38). (*b*) The second type (which follows the first) has a larger diameter (~50–200 μm) and a media which consists of a continuous layer of smooth muscle cells (Fig. 18). On this account we call this type of vessel a "muscular venule" a term identical to that in (38).

PERICYTIC VENULES: In these vessels, the junctions appear as a collection of scattered ridges (A faces) and grooves (B faces) which frequently show free ends (Figs. 14–16). In general, they take the form of straight lines of a rather constant length which frequently form sharp angles where they join. Their orientation relative to the general direction of the intercellular junction varies from parallel to perpendicular (Fig. 15). The ridges are marked by particles which vary in frequency from quasi-continuous to absent (Figs. 15–17) and appear to be smaller than the usual intramembraneous particles of the A faces (Fig. 17). The grooves on the B faces are generally free of particles (Fig.

FIGURE 11 **Blood capillary (rat diaphragm, specimen treated with digallic acid).** Microdensitometric tracings across the intercellular space (*a*) and intercellular junction (*b*). In (*b*), the width of the two fused membranes (*fm*) equals that of one membrane leaflet. Outer leaflet, *ol;* inner leaflet, *il;* intercellular spaces, *is*. × 175,000.

FIGURE 12 **Blood capillary (rat diaphragm, specimen treated with digallic acid).** Microdensitometric tracings at different levels of the intercellular boundary between two endothelial cells: (*a*) across a fusion point, (*b*) next to the latter, and (*c*) across the intercellular space. In (*a*) the outer leaflets are eliminated over a distance of ~80 Å. In (*b*) the tracing passes through the line of continuity of the two outer leaflets. Outer leaflet, *ol;* inner leaflet, *il;* intercellular space, *is*. × 180,000.

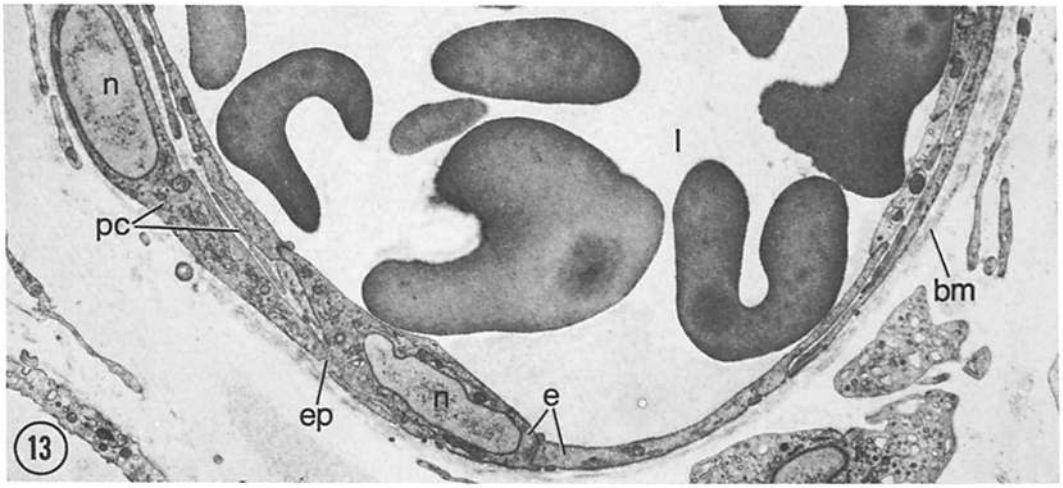

FIGURE 13 Rat omentum; isolated pericytic venule. Cross section showing the components of the vascular wall. Pericytes and their pseudopodia cover most of the outer surface of the endothelium. Endothelial-pericytic junction, *ep*. × 6,300.

15). The general arrangement is such that numerous uninterrupted lanes are found in between the ridges. Assuming that these ridges correspond to the tight junctions seen in sectioned specimens, it can be expected that some thin sections will show patent intercellular spaces because of the reasonably high probability of missing the lines of fusion. Gap junctions are absent at this level.

MUSCULAR VENULES: The organization of the tight junctions in these vessels is very similar to that already described in pericytic venules, except that small and irregular gap junctions occur associated with (but not surrounded by) the system of low-profile ridges and grooves of the tight junctions (Figs. 19, 20).

TRANSITION FORMS: Since the differences in the organization of the junctions in the three different segments of the microvasculature are large, one would expect to find transitional (or intermediary) forms in each group of isolated vessels examined. Such forms have not been recognized so far at the arterial end of capillaries, but seem to occur at the venous end. The low frequency with which they are encountered suggests that they are of rather limited distribution, but this aspect definitely requires further investigations.

DISCUSSION

ACV Units

The present study of intercellular junctions has been carried out on reliably identified segments of the microvasculature in the omentum and mesentery of the rat. As already mentioned, the segments have been obtained from ACV units which consist of an arteriole that branches into a number of capillaries, which in turn converge into a venule that changes its character from pericytic to muscular. The use of such ACV units has allowed us to identify the structural characteristics of endothelial junctions for each segment of the microvas-

FIGURE 14 Rat omentum; isolated pericytic venule. In this specimen the endothelial tight junction is seen as a series of discontinuous ridges (arrows) marked by rows of particles on the A face (A_b) and shallow grooves devoid of particles on the B face (B_b) (arrowheads). Note that the ridges and grooves have free ends, are straight, and tend to be of similar length. × 65,000.

FIGURE 15 Rat omentum; isolated pericytic venule. The endothelial junction appear on an A face (A_t) as discontinuous low-profile ridges with few associated particles (arrows) and on the B face (B_t), as discontinuous shallow grooves free of particles (arrowheads). Some of these grooves are oriented almost perpendicularly to the general direction of the junction. Pericyte, *pc*. × 60,000.

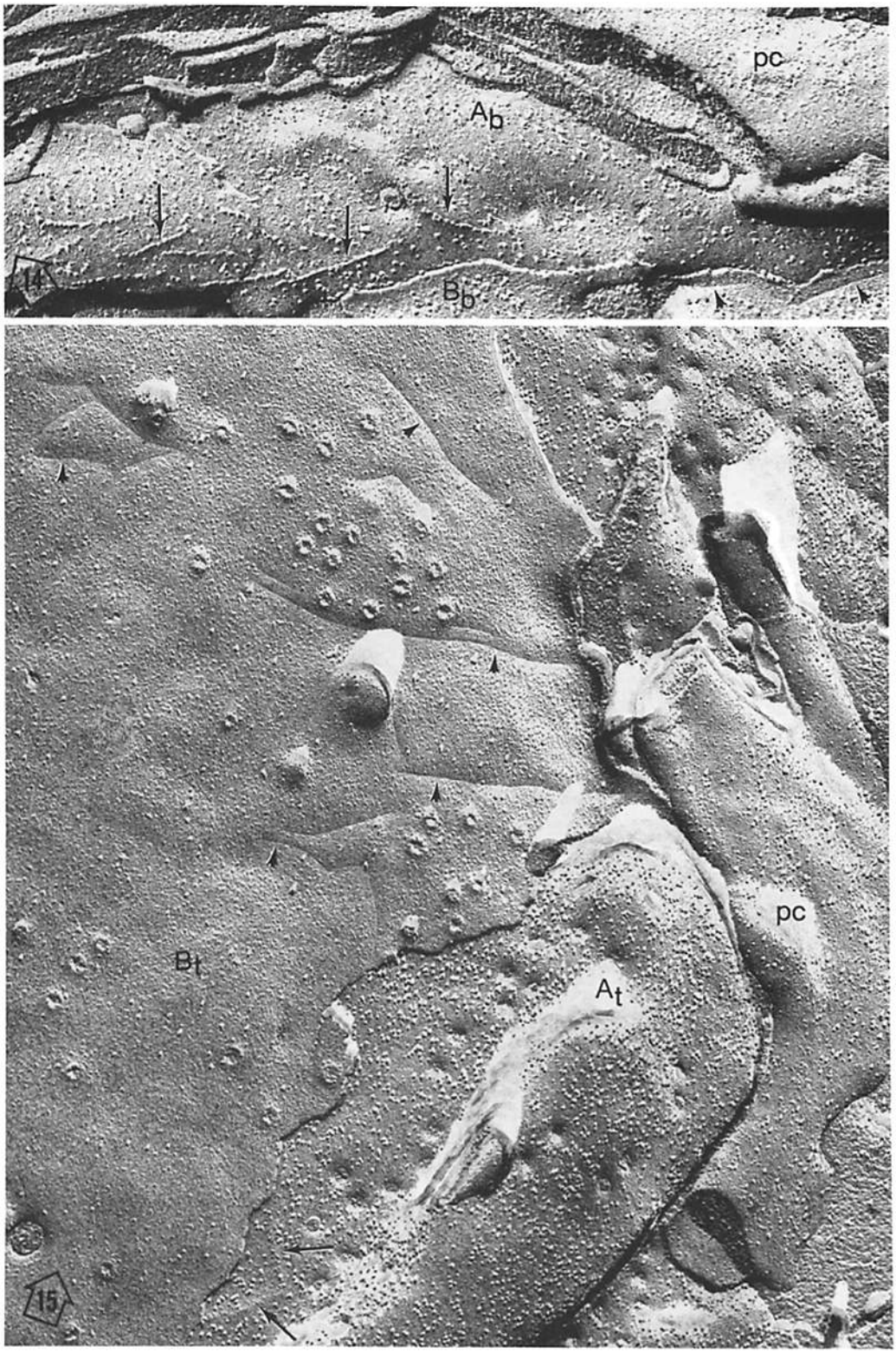
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FIGURE 16 Rat diaphragm; small vessel, probably a pericytic venule. On this A_b face of the endothelial plasmalemma, the tight junction appears as a series of discontinuous low-profile ridges (arrows) which on most of their length are free of associated particles. × 60,000.

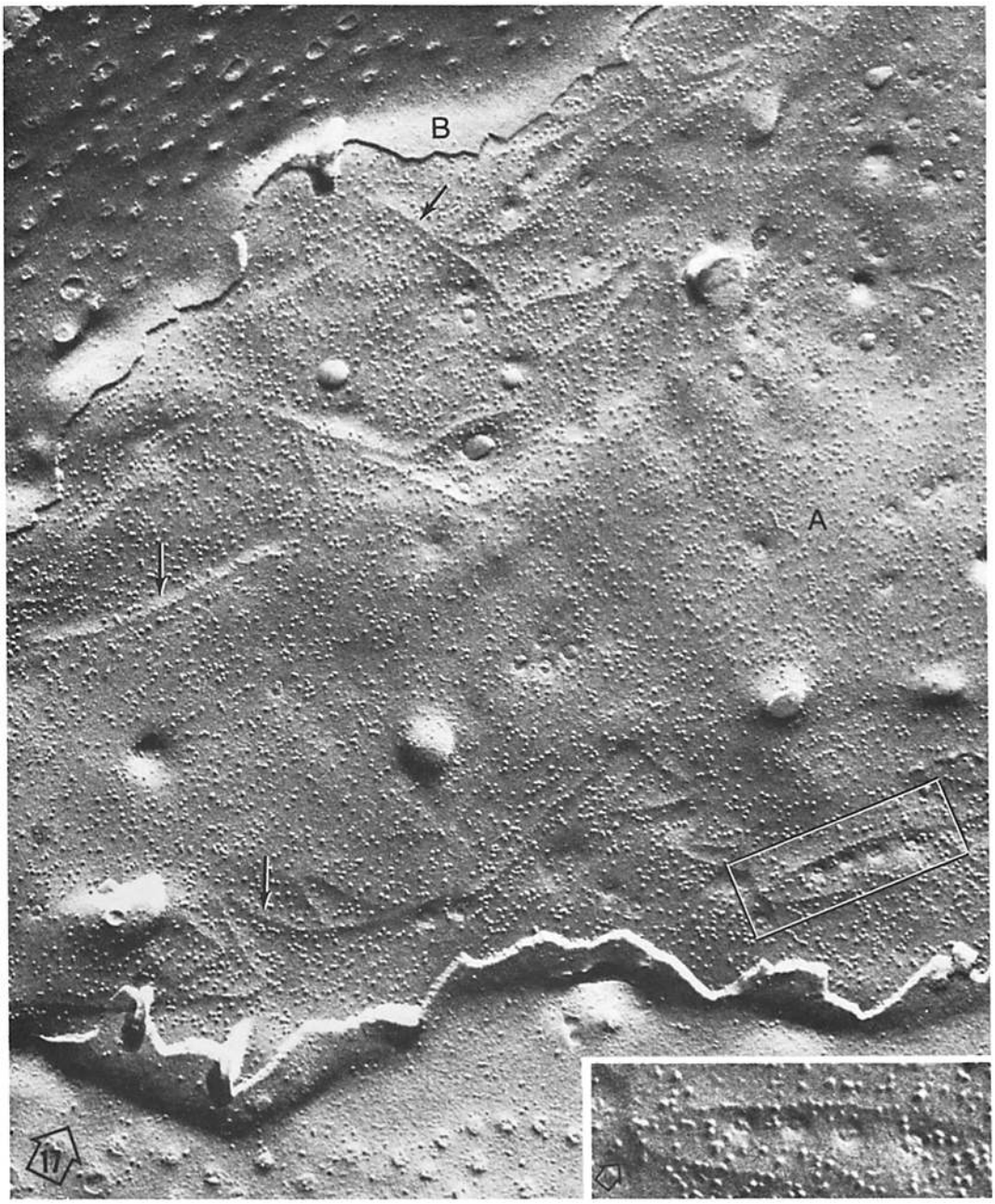


FIGURE 17 Rat pancreas; small vessel, probably pericytic venule. The endothelial junction appears as a system of discontinuous low profile ridges (arrows) which in many places are free of associated particles. In other places, (see inset) the ridges are marked by particles which appear to be smaller and less protruding than the usual intramembranous particles of the A faces. × 58,000; inset, × 20,000.

culature. It is hoped that in the future such units could be used to investigate possible variations in the pathways followed by molecules of graded dimensions across the endothelium of each segment. At that time, it will be possible to integrate the present information on junctions into the general picture of segmental variations in vascular permeability.

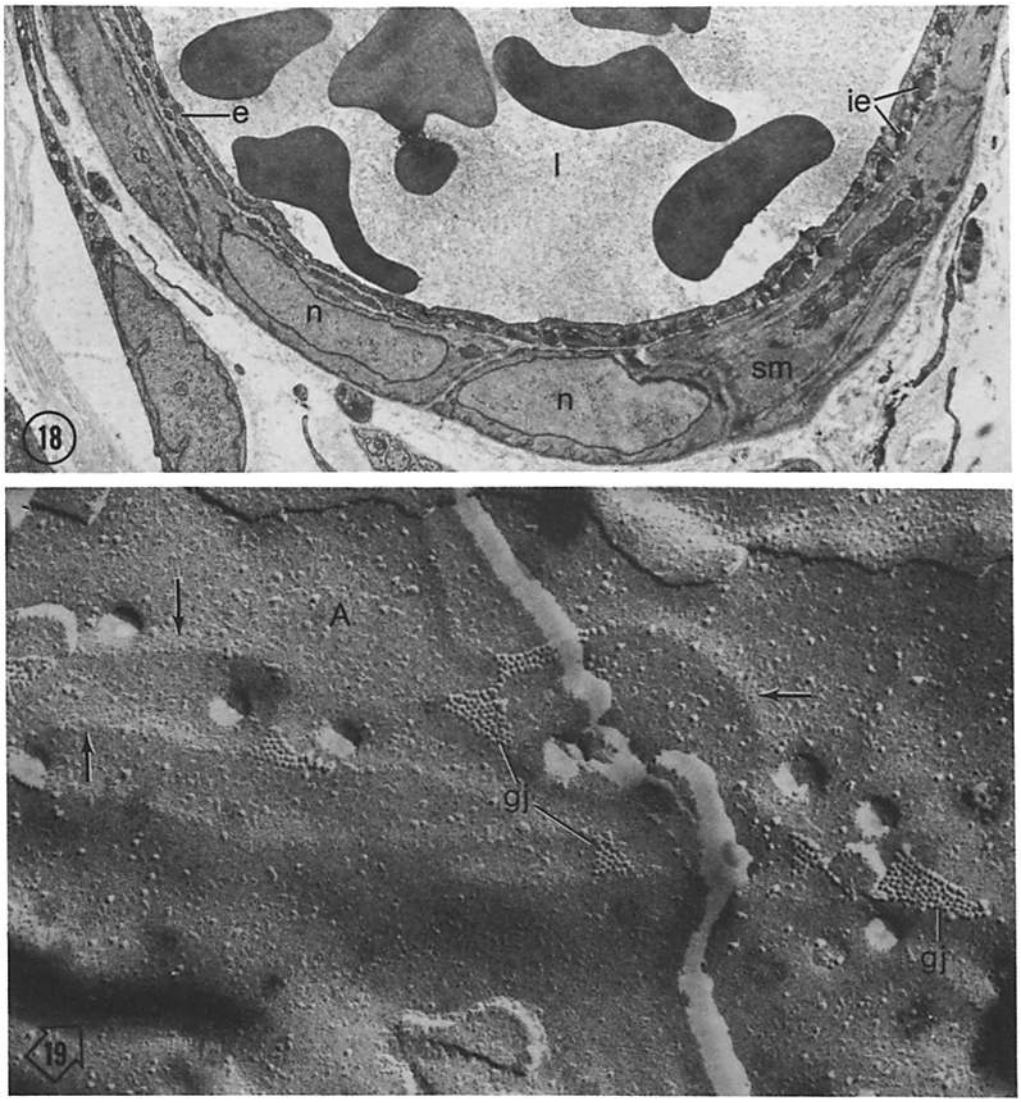


FIGURE 18 Rat omentum. Cross section of an isolated muscular venule exhibiting its wall structure. At *ie* the discontinuous internal elastic membrane. × 5,000.

FIGURE 19 Rat omentum; isolated muscular venule. The endothelial junction on the A face (*A*) appears as a system of low-profile ridges (arrows) largely free of associated particles. Small irregular gap junctions (*gj*) appear scattered among the ridges. Their frequency in this specimen is higher than usual. × 88,000.

Segmental Differentiation of Intercellular Junctions in the Endothelium of the Microvasculature

Our results demonstrate the existence of striking and characteristic variations in the extent and type of organization of intercellular junctions from segment to segment in the vascular beds investigated. The most elaborate system, consisting of a combination of occluding junctions (organized in depth in successive rows) and intercalated gap junctions, has been found in arterioles. Judging by information available from other systems, this combination is expected to insure strong cell to cell adhesion by both types of junctions; to seal off the intercellular spaces by its occluding junctions, and

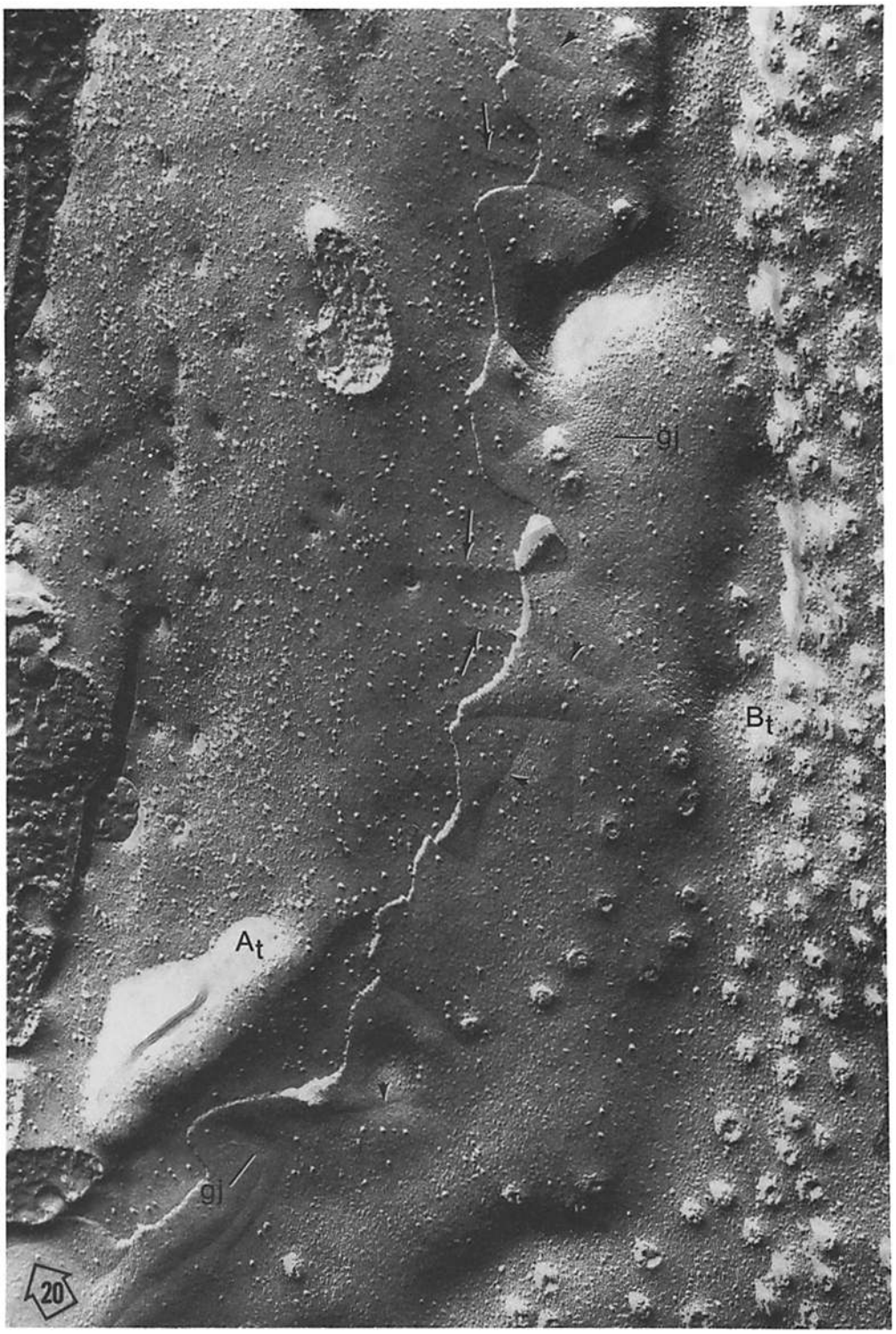


FIGURE 20 Rat diaphragm; small vessel, probably muscular venule. The tight junction appears as a system of discontinuous low-profile ridges on the A_t face and shallow grooves on the B_t face. The grooves (arrowheads) are free of associated particles while the ridges (arrows) are marked in places by discontinuous rows of particles. Two small isolated gap junctions (*gj*) appear in the vicinity of the grooves of the B face. × 62,000.

to establish cell to cell communication by its gap junctions.

The endothelium of capillaries is characterized by continuous or quasi-continuous occluding junctions less elaborately organized in depth. The junctions consist of two–three successive rows either branched and continuous, or staggered and discontinuous. Gap junctions are absent; hence, it can be assumed that the type of intercellular communication they mediate is missing in the endothelium of blood capillaries.

In the venules we have found the simplest endothelial junctions which at present are difficult to classify. They appear as positive (A face) or negative (B face), well defined, linear creases of the membrane. The positive creases or ridges protrude slightly on the cleavage surface; they subtend a large angle (greater than 90°), and generally follow the regions of intercellular contact, but seem to be scattered at random within these regions, so that numerous uninterrupted lanes appear on the cleavage plane in between adjacent ridges. In their simplest version, the positive and negative forms of the creases are completely free of particles, but all intermediates are encountered between a particle-free type and a type in which the whole length of the crease is marked by intramembranous particles.[3]

Figs. 21 and 22 demonstrate (at high magnification) segmental differences in the morphology of B faces in junctional areas: the grooves are provided with rows of particles in the endothelium of arterioles and capillaries (Fig. 21), and are usually devoid of particles in the endothelium of venules (Fig. 22). Fig. 23 shows the peculiar A face ridges, almost free of particles, which characterize the endothelial junctions of most venules.

The same type of junction is found in percytic as well as in muscular venules, the only difference being the presence of small gap junctions in the latter. Gap junctions appear to be present only in those small vessels, the media of which contains smooth muscle cells (i.e., arterioles and muscular venules); the endothelium of vessels with a media formed exclusively by pericytes (capillaries and pericytic venules) displays tight junctions only. This peculiarity could make the endothelium of capillaries and pericytic venules a suitable system for testing the possible role played by occluding junctions in the low-resistance coupling of adjacent cells.

The variety of appearances described for the endothelial junctions in venules could be rationalized in terms of a common infrastructure (the creases) provided to a variable extent with a superstructure (the rows of particles). In fact, all the junctions of the vascular endothelium could be interpreted in such terms, the superstructure becoming progressively more obvious at the level of the capillary and arteriolar endothelium.

The type of junctions found in the venular endothelium are of special interest not only because of their novelty but also because of the implications of their morphology. These relatively sharp creases exist in a membrane which is supposed to be fluid (46), and are maintained in many cases without the participation of intramembranous particles detectable at the level of resolution attained in our preparations. Intramembranous particles appear to function in a number of situations as "stitches" which affix one membrane to another (35, 40), or to some submembranous structures (13–15) and thereby maintain it bent or

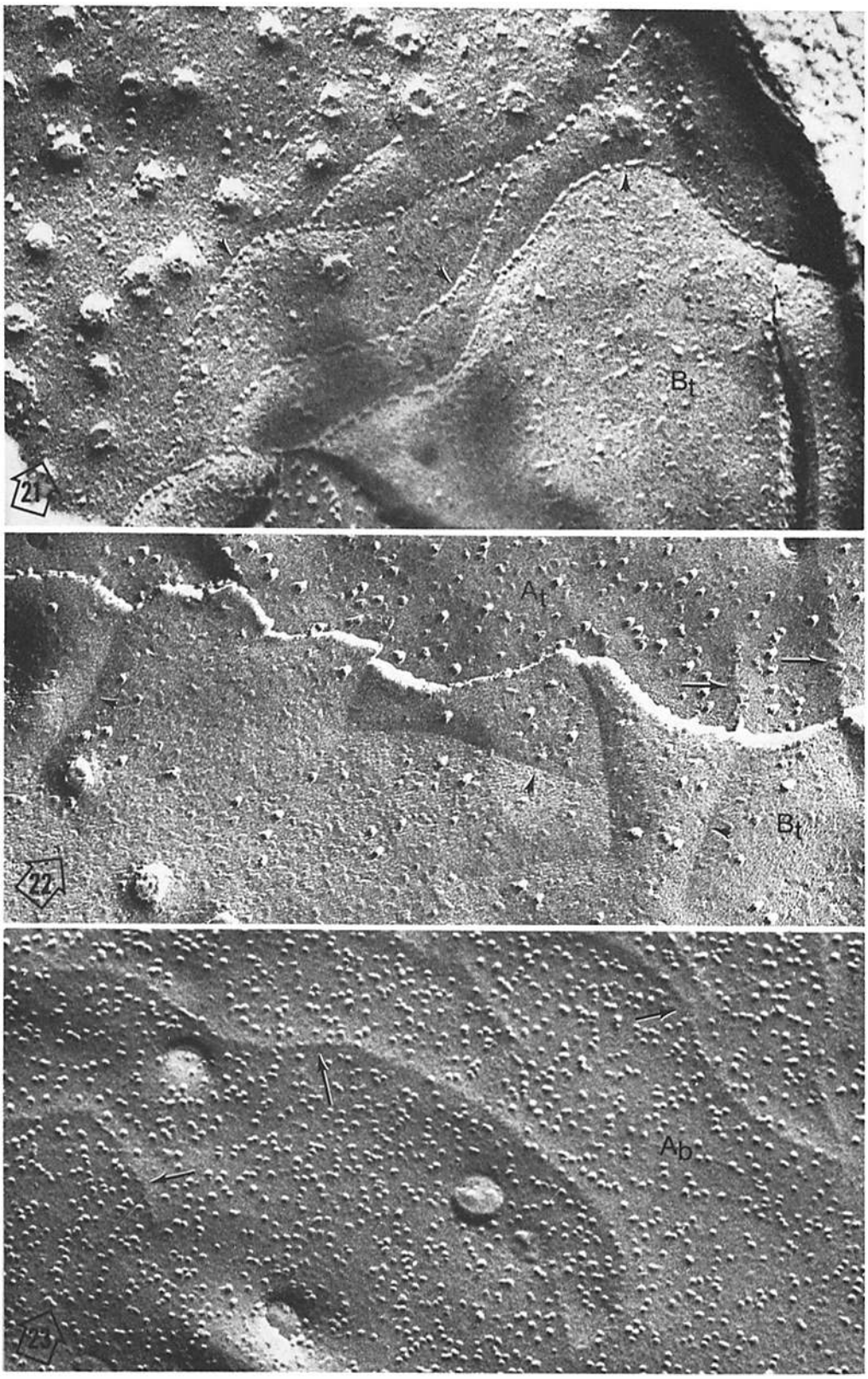
[3] Junctions of similar appearance were recently found in the small vessels of the rat jejunal mucosa by Dr. L. A. Staehelin, Univ. of Colorado, Boulder, Colorado (personal communication).

FIGURES 21, 22, and 23 Composite setting to show the different appearance of the tight junctions in three microvascular segments: Fig. 21, capillary, Fig. 22, muscular venule, Fig. 23, pericytic venule.

FIGURE 21 Small vessel, probably a blood capillary in a rat diaphragm. The fracture plane exhibits a B_t face of the endothelial plasmalemma on which the tight junction is seen as a maze of branching and staggered grooves marked by discontinuous rows of particles (arrowheads). Note that within the small area shown on this replica, the grooves form a continuous network with few free ending spurs (*). × 120,000.

FIGURE 22 Small area enlarged from Fig. 20 (see legend). The cleavage plane reveals a B_t face with discontinuous shallow grooves (arrowheads) devoid of particles. On the A face, the tight junction appears as discontinuous low-profile ridges (arrows) marked by a few particles. × 120,000.

FIGURE 23 Small area enlarged from Fig. 16 (see legend). On this A_b face the tight junction is represented by discontinuous low-profile ridges which on most of their length are devoid of particles (arrows). × 96,000.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folded. The strands or rows of particles revealed by by freeze-cleavage in occluding zonules in various epithelia (6, 30, 48) also behave as intramembranous structures which perform a similar function, except that they attach the membrane of their cell to that of its neighbor. It is possible that, in the special situation of the creases of the venular endothelium, other factors than transmembrane proteins are involved in the deformation of the membrane (e.g., interactions among peripheral proteins on either the extracellular or the intracellular aspect of the cell membrane).

Position of Intramembranous Particles in Relation to the Cleavage Plane

The behavior of the cleavage plane in relation to the intramembranous particles (or strands) of the tight junctions appears to vary characteristically in the endothelium of the different segments of the microvasculature.

In the endothelium of arterioles and capillaries, the cleavage plane leaves these particles preferentially in the grooves of the B faces,[4] a situation rarely encountered in other epithelia (for such rare examples, see 6, 47) in which the particles (fibrils or strands) usually remain on the ridges of the A faces (30, 48). This finding suggests that the particles of the joined membranes interact so strongly at the level of the junction that they behave as "single units" which are torn away from their attachments to the cytoplasmic leaflet when the membrane is cleaved. Their behavior is comparable of that described by Wade and Karnovsky (51) as the "single fibril model" in other epithelia, except that in our case the single unit (i.e. the two joint particles) preferentially remains with the B face. The finding indicates that the interactions of the single unit with the outer leaflet (and beyond it with the membrane of the adjacent cell) are stronger than those with the inner, cytoplasmic leaflet of the cleaved membrane. This behavior probably reflects the adaptation of the endothelium to the stress conditions which prevail in this part of the microvasculature.

In the venular endothelium the cleavage plane follows the outer aspect of the junctional particles so that the latter appear on the A face ridges (when present), while the grooves of the B faces are generally particle-free. The situation is similar to that described by the two fiber model proposed by Chalcroft and Bullivant (4). The finding suggests that in the venular endothelium, the interactions between particles (or strands) in phase at the level of the junctions are weaker than in arterioles and capillaries.

These differences in behavior can be better rationalized in the terms mentioned (strong vs. weaker interactions between two sets of junctional particles or strands in phase) rather than in terms of true structural differences (one vs. two fibers), since in the latter case it would be difficult to explain how two cells can generate a single (common) connecting structure.

Difficulty in Correlations Encountered

The information obtained from freeze-cleaved junctions applies strictly to the situation existing at the level of the cleavage plane. In fact, no direct information can be obtained by this technique re: structural details in the areas of cell to cell contact on the true cell surface. Such information is provided by sectioned specimens which demonstrate the existence of points (supposedly lines) of contact or fusion of adjacent cell membranes.

A correlation of the two types of findings has been attempted in the endothelium of blood capillaries, the only vessels for which we have reasonably extensive documentation. The correlation obtains only in broad terms (general location and geometry), if we assume that the intramembranous ridges seen in freeze-cleaved preparations correspond to the points (or lines) of cell contact or cell fusion seen in sections. It does not apply in detail, however, since the number of ridges is larger than the number of lines of fusion seen in normally sectioned junctions (Table III) selected to minimize possible underestimates due to the obliquity of the plane of section. It follows that the ridges must be considered potential, rather than actual, junctions and that some of them may not be involved in cell to cell contact at any given time. This situation might be related to the variations in the asymmetry of distribution of intramembranous particles mentioned in the previous section. Since information bearing on the situation at the cell surface cannot be obtained from freeze-cleaved preparations, it also follows that the absence or the discontinuity of ridges can not be correlated with

[4] The discontinuities encountered in the rows (or strands) of particles can be true (absence of particles) or apparent (the missing particles are on the complementary A face ridge). This alternative cannot be resolved in the absence of complementary replicas.

the existence of patent intercellular spaces (open junctions) along the area of cell to cell contact.

Another appearance found in sectioned junctions which cannot be satisfactorily correlated with structural details seen on freeze-cleaved specimens is the frequently encountered broad band of cell to cell contact or partial fusion of the type seen in Fig. 11. There is at present no recognized structural equivalent on the cleavage plane of the junctions for such broad bands.

With the information at hand and within the limitations mentioned, we cannot ascribe at present functional correlates to the various types of junctions described. There are in the literature suggestions that the venular endothelium is highly permeable, and that "leaks" may be located along its intercellular junctions (22–24, 31, 39, 49). Moreover, such leaks have been demonstrated on venules of 20–80 μm diameter in the vascular bed of muscles topically treated with histamine or serotonin (27). Similar results have been reported in the venules of salivary glands (50) and on bronchial venules during endotoxin shock (34). Yet, a final correlation of "leaks" with this type of endothelial junction must await more precise information concerning differences in the structural aspects of permeability from one vascular segment to another. As already mentioned, the ACV units represent a favorable object for such studies.

A Change in Nomenclature Proposed

There are at present in use at least six terms [nexus (9), close junction (12), macula or fascia occludens (12), gap junction (36), macular close junction (26), and small subunit gap junction (30)] for the structure originally described as "nexus" by Dewey and Barr (9) and "gap junction" by Revel and Karnovsky (36). To establish a direct connection with the existant literature, we have used throughout this paper the term gap junction, although it is widely recognized as inappropriate (1, 29) to the point that some investigators deliberately avoid it (2, 26). The term stresses the existance of a local intercellular gap which is not compatible with the main function of the junction. Moreover, in the special case of the vascular endothelium, the term is also confusing since junctions open to a gap of 20–40 Å have been described by Karnovsky as occurring instead of tight junctions of the capillary endothelium (18, 19). We would like to propose instead the term "communicating junction" (*macula communicans*, *maculae communicantes*) which has the following advantages: it relates to the main function established so far for this type of junction in other epithelia (1, 25); hence, it is preferable to the strictly morphological term nexus; it describes appropriately the macular geometry of the structure; and it brings its nomenclature in line with that already in use for other junctional elements.

We gratefully acknowledge the excellent technical assistance of Amy Haskell, Pam Stenard, Philippe Male, and Hans Stukenbrok.

This work was supported by Public Health Service grant HL-17808-02.

These results were presented in part at the XIV Annual Meting of the American Society for Cell Biology, 1974, San Diego, California (44).

Received for publication 3 April 1975, and in revised form 24 July 1975.

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