STUDIES ON BLOOD CAPILLARIES

I. General Organization of Blood Capillaries in Muscle

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ABSTRACT

The wall of the blood capillaries of skeletal muscles (diaphragm, tongue, hind legs) and myocardium of the rat, guinea pig, and hamster consists of three consecutive layers or tunics: the endothelium (inner layer), the basement membrane with its associated pericytes (middle layer), and the adventitia (outer layer). The flattened cells of the endothelium have a characteristic, large population of cytoplasmic vesicles which, within the attenuated periphery of the cells, may attain a maximum frequency of $120/\mu^2$ of cell front and occupy $\sim$18% of the cytoplasmic volume; these values decrease as the cells thicken toward the perikaryon. The vesicles are 650–750 A in over-all diameter and are bounded by typical unit membranes. They occur as single units or are fused to form short chains of two to three vesicles. Each configuration may lie entirely within the cytoplasm or open onto the cell surface. In the latter case, the unit membrane of the vesicle is continuous, layer by layer, with the plasmalemma. Chains of vesicles opening simultaneously on both the blood and tissue fronts of the endothelial tunic have not been observed either in sections or in a tridimensional reconstruction of a sector of endothelial cell cytoplasm. Adjacent endothelial cells are closely apposed to one another and appear to be joined over a large part of their margins, possibly over their entire perimeter, by narrow belts of membrane fusion (*zonulae occludentes*). Except for tongue capillaries, patent intercellular gaps are rare or absent. The middle layer is formed by a continuous basement membrane ($\sim$500 A thick) and by pericytes which lie in between leaflets of this membrane. The tips of the pericyte pseudopodia penetrate through the inner leaflet of the basement membrane and join the endothelium in *maculae occludentes*. The adventitia is a discontinuous layer comprising cellular (macrophages, fibroblasts, mast cells) and extracellular (fibrils, amorphous matrix) elements. The same general type of construction appears to be used along the entire length of the capillary.

INTRODUCTION

It is now well established that the walls of blood capillaries are comprised of concentric layers of cellular and extracellular elements and that several types of capillary vessels, distinguished by structural differences within these layers, occur in the mammalian vasculature (1–5).

In the present investigation, the blood capillaries of the diaphragm and myocardium of rats were examined by electron microscopy to establish: the general organization of the concentric layers of their walls; the size, frequency and disposition of endothelial cell vesicles; the type of intercellular junctions in the endothelial tunic; and the structural characteristics of pericytes and perivascular cells. This investigation forms the morphological basis for a subsequent study on

transport of ferritin molecules across the capillary wall (6).

MATERIALS AND METHODS

Materials

Most of the work was carried out on the diaphragm and heart of Sprague-Dawley rats weighing 100–200 g. These muscles were selected because of their extensive vascularization and accessibility for fixation in situ. The findings were confirmed and extended by a survey of other skeletal muscles (leg, tongue, abdominal wall) of the rat and of the myocardium of other species (mouse, guinea pig, hamster).

Methods

The diaphragm was fixed in situ with 6.25%, cold (~4°C) glutaraldehyde (Union Carbide Co., New York) in 0.1 M Na-phosphate buffer, pH 7.4 (7) by injecting, under ether anesthesia, about 5 ml of the fixative into the pleural—and about 15 ml into the peritoneal cavity. After ~10 min, the diaphragm was excised and cut into blocks of less than 1 $mm.^3$ Such blocks, taken at random from the part of the muscle bordering the central tendon, were further fixed in buffered glutaraldehyde for 2–4 hr at ~4°, then washed with four to five changes of phosphate buffer (2–24 hr) for the purpose of removing excess glutaraldehyde, and finally "postfixed" in 1% OsO_4 in 0.1 M phosphate buffer, pH 7.4, for 1–1.5 hr at ~4°. Hereafter, this method of fixation will be referred to as glutaraldehyde-OsO_4 fixation.

To fix the myocardium in situ, the thorax was opened under ether anesthesia, the two cavae were clamped, the right cavities were flushed by injection of phosphate- or acetate Veronal-buffered OsO_4(pH 7.4) (8, 9) into the right ventricle, and then distended by continuing the fixative injection after a clamp was placed on the pulmonary artery. The surface of the heart was also flooded with fixative. 5–10 min after the injection, pieces of the right atrial and ventricular wall were excised, cut into small pieces, and fixed in buffered OsO_4 at 4° ~ for 1–1.5 hr. As far as the structure of the capillary wall is concerned, fixation in situ of the diaphragm by OsO_4 and of the myocardium by glutaraldehyde–OsO_4 gave results comparable to those to be described.

Specimens of rat tongue were fixed in acetate Veronal-buffered OsO_4, pH 7.4, for 1–2 hr at ~4° and then treated with uranyl acetate in Michaelis buffer at pH 5.0 for 2 hr before dehydration, as described by Farquhar and Palade (10).

All tissues were dehydrated in 70, 95, and 100% ethanol or acetone and embedded in Epon according to Luft (11).

Preparation of Sections and Microscopy

Sections showing silver-to-grey interference colors were cut with diamond knives (R. E. Sugg, Rondkin Corp., Wilmington, Del.; E. I. duPont de Nemours & Co., Wilmington, Del.) on an LKB Ultrotome; mounted on grids covered with a carbon film (12); stained with "lead hydroxide" according to Karnovsky (13) or with 2 or 6% aqueous uranyl acetate (14) for 30 min–2 hr followed by "lead hydroxide" for 10 min; and examined in a Siemens Elmiskop I electron microscope operated with a double condenser, a 400-μ aperture in condenser II, and a 50-μ aperture in the objective lens. Electronic magnifications ranged from 10,000–40,000. Most micrographs were taken around × 20,000.

Quantitative Measurements

The microscope magnifications were determined and repeatedly checked on the carbon replica of a diffraction grating with 2160 lines/mm (E. F. Fullam, Inc., Schenectady, N. Y.). Various cell structures were measured on electron micrographs with a vernier caliper or with a calibrated 7x magnifying glass both of which were accurate to 0.1 mm. Measurements of area were made on electron micrographs with a Keuffel & Esser planimeter. Estimates of volume were based on the assumption that the sections studied were 500 A thick. Since ~5% shrinkage occurs in cells and tissues during processing for electron microscopy (15, 16) and the magnification of the microscope may vary 5–10% during normal use (17), we may reasonably assume that our measurements are accurate within 10–15%.

Tridimensional Reconstruction of a Sector of an Endothelial Cell

The spatial relationships of endothelial cell vesicles to one another, to the cell surface, and to intracellular organs were examined in a tridimensional, lucite model (Figs. 9, 10) which was constructed as follows. A sector of the attenuated periphery of an endothelial cell, present in each of seven serial sections, was micrographed at a magnification of 40,000 and enlarged photographically to a magnification of 200,000. Profiles of the plasmalemma, plasmalemmal vesicles, and various cell organs were traced on transparent paper and the seven tracings superimposed in their correct sequence to give best over-all fit. Two straight pins were pushed through the seven superimposed sheets at two arbitrary points

within the cell boundaries so that we could establish reference points. The profiles on each of the seven sheets were then transferred to lucite plates whose thickness (10 mm) was chosen to correspond to an estimated section thickness of 500 A magnified 200,000 times.[1] The cell outlines on the lucite sheets were cut out with a jigsaw, and ⅛-in. holes were drilled through each lucite sheet at the reference points. The sheets were stacked in sequence and held in correct relationship to one another by two ⅛-inch brass rods placed in the reference holes. Thus, a rough, three-dimensional model having the outline of a sector of an endothelial cell was obtained in which each section could be removed for further work and replaced in its correct relationship to the other sections.

The original seven tracings on transparent paper were again superimposed in correct sequence, oriented by the reference points, and stapled together along one edge. By examining the superimposed transparent paper tracings, the position of each vesicle could be determined by noting its location on a given section and its extension or lack of extension into the preceding and following ones (in the two surface sections the extension of a vesicle could be determined only in one adjacent section). After the position of a single vesicle was determined from the transparent paper tracings, its position was established on the lucite plates and the corresponding cavity was cut out of one or more plates, as the case required, with a motor-driven hand drill. After all vesicles were cut out of the model, its surfaces were polished so that the vesicles inside could be visualized.

[1] Actually, two plates of lucite, each 5 mm thick, were used to represent one section. This arrangement facilitated cutting out vesicles located mainly in one section.

OBSERVATIONS

Applicability of the Findings

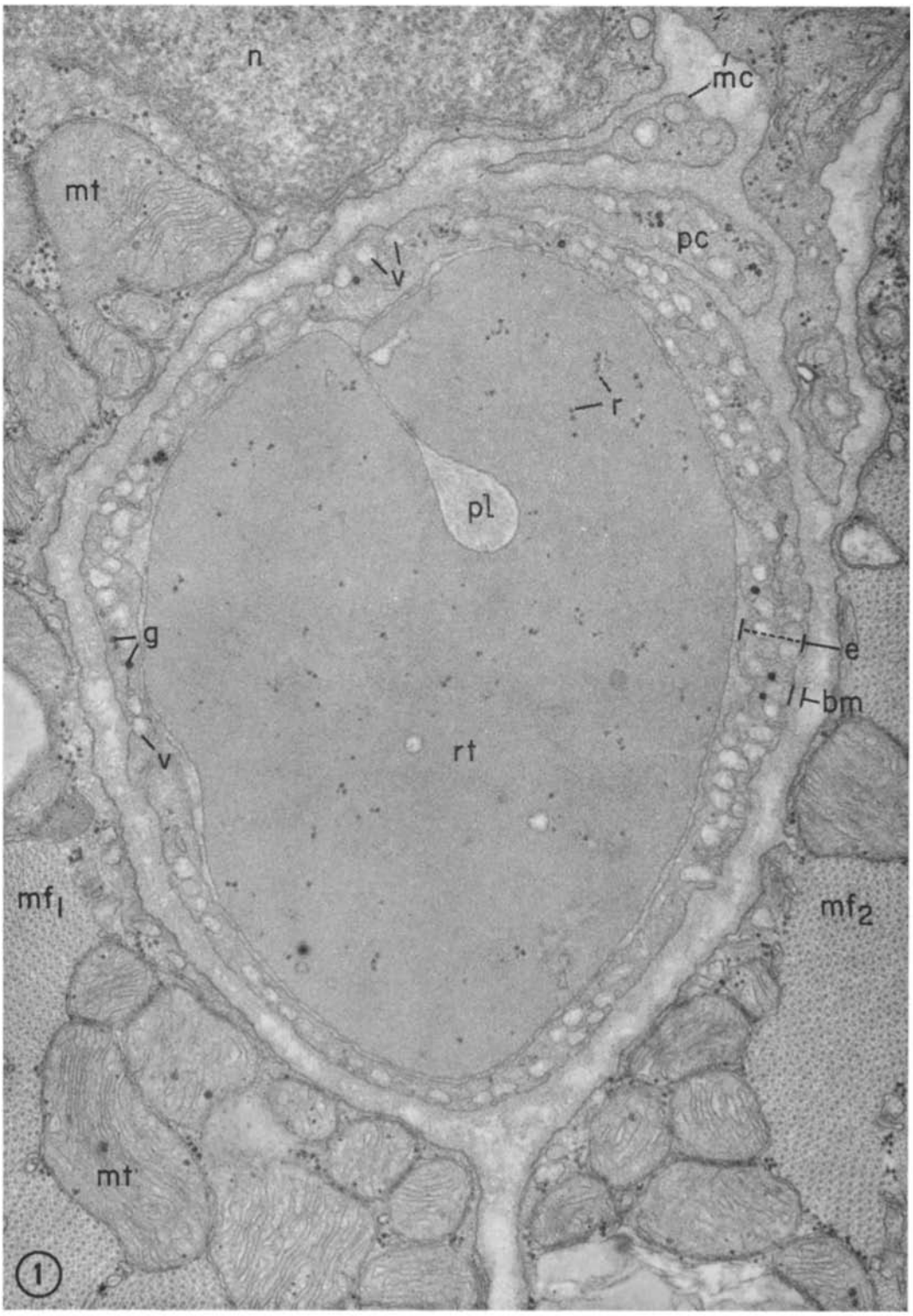
The following description is based on extensive studies carried out on the capillary vessels of the diaphragm and myocardium of the rat, as well as on more limited observations made on other mammalian species (hamster, mouse, guinea pig) and other striated muscles (leg, tongue, abdominal wall). The work was generally restricted to blood vessels with a diameter $\leq 7\ \mu$; hence, it does not cover small arteries and venules immediately connected with the capillary bed. Within these limits, no consistent structural variations were recognized among the numerous capillary profiles examined, although undoubtedly the latter represented random sections at various levels from the arterial to the venous "ends" of the vessels. Accordingly, the description applies, at least tentatively, to blood capillaries of mammalian striated

List of Abbreviations

bm, Basement membrane
cf, Collagen fibrils
db, Dense body
e, Endothelium
fb, Fibroblast
ff, Fine fibrils
g, Glycogen
j, Intercellular junction
l, Capillary lumen
mf, Muscle fiber
mt, Mitochondrion
n, Nucleus
pc, Pericyte
r, Ribosomes
rc, Erythrocyte
rer, Rough-surfaced endoplasmic reticulum
ser, Smooth-surfaced endoplasmic reticulum
v, Plasmalemmal vesicle

FIGURE 1 Cross-section of a blood capillary and adjacent skeletal muscle fibers in rat diaphragm. The capillary lumen is almost completely filled with a reticulocyte (*rt*) which leaves little space for plasma (*pl*). The thin endothelium (*e*) contains numerous vesicles (*v*) and scattered dense particles (*g*) ~350 A in diameter thought to be glycogen. The capillary basement membrane (*bm*) is visible in some regions and encloses a portion of a pericyte (*pc*), which also contains glycogen and vesicles. The naked pseudopods of a macrophage appear in the pericapillary space at *mc*. Two skeletal muscle fibers are marked mf_1 and mf_2.

Specimen fixed with glutaraldehyde -OsO_4; section stained with uranyl followed by lead. × 40,000.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muscles in general as well as to the full length of these vessels. The last point requires, however, further investigation, since we were unable to identify either the arterial or the venous "ends" of capillaries in electron microscopical preparations.

All capillary profiles examined were characterized by three successive layers in their wall which in three dimensions represent coaxial tunics. The organization of each tunic will be described in detail after a few comments on preparatory techniques.

Comments on Preparatory Techniques

The general organization of the vessels is the same in OsO_4- and in glutaraldehyde OsO_4–fixed specimens, but the latter procedure has some advantages: it gives a fixation of satisfactory and uniform quality throughout the tissue blocks; it fixes evenly the blood plasma and retains it in the capillary lumina; and it does not distend the pericapillary spaces, thereby disrupting their organization. It gives a cytoplasmic matrix of high density in all cells and usually preserves satisfactorily cellular structures, with the exception of the cell membrane which occasionally forms blebs and of mitochondria which frequently show local disruption by swelling or myelin figure production.

Osmium tetroxide–fixation insures a satisfactory preservation of cellular detail; allows a better visualization of membrane-bounded structures because of the low density shown by the cytoplasmic matrix; and also permits better definition of basement membranes. It has the major disadvantage of not fixing and regularly retaining the blood plasma in the vascular lumina and of causing extensive distension and disorganization in the pericapillary spaces.

THE CAPILLARY WALL

The Inner Tunic—the Endothelium

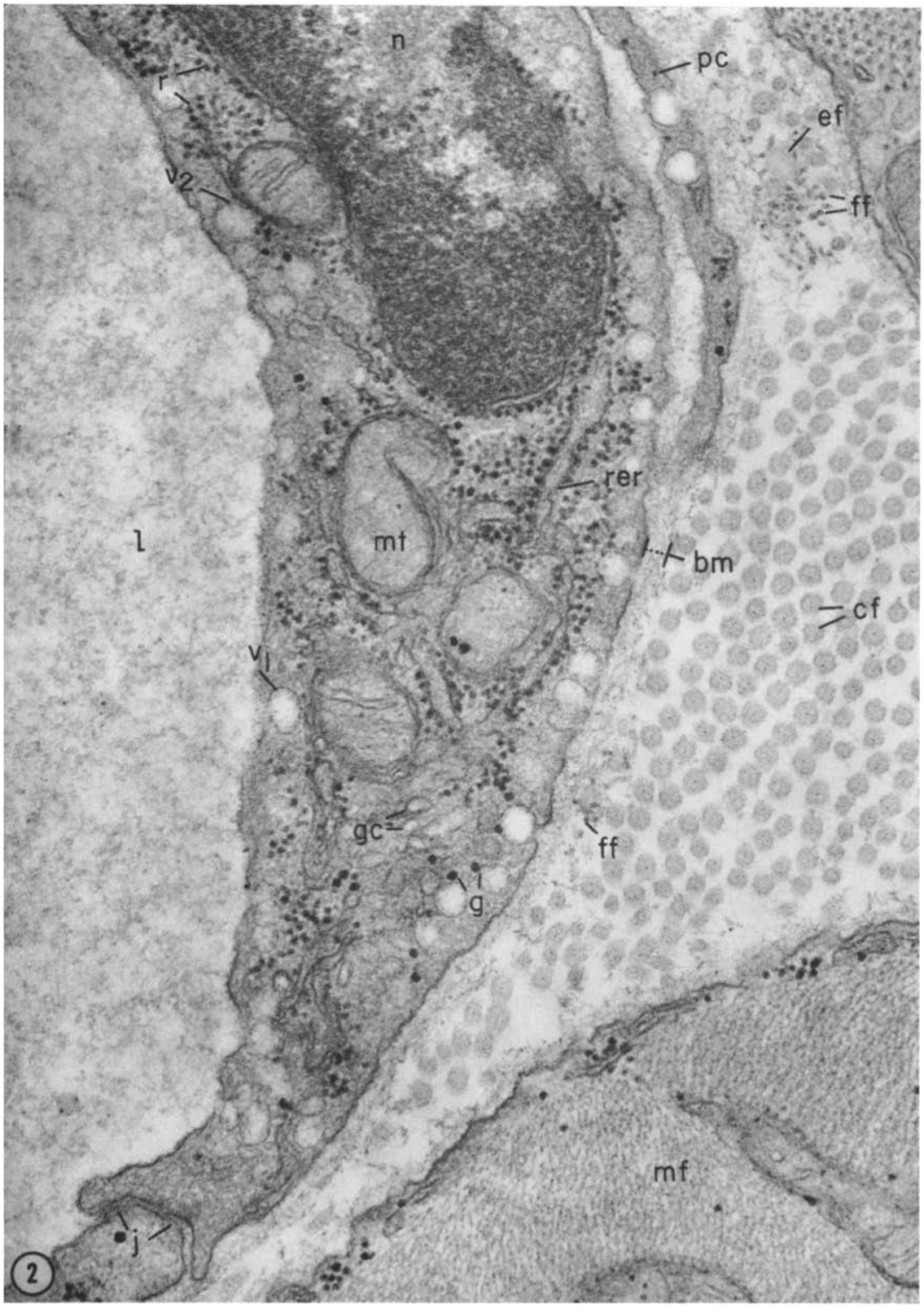
The inner tunic consists of a single, continuous layer of extremely flattened cells whose inner or luminal surface (blood front) is in contact with the blood, and whose outer surface (tissue front) is covered by the basement membrane and through it faces the interstitia of the tissue (Figs. 1, 2). The cells make contact and join with one another along their narrow rims. Single endothelial cells in full-face view have an irregular outline and, according to light microscope observations, measure $\sim 10 \times 30\ \mu$ (18, 19). These figures represent the approximate size of endothelial cells in large blood vessels, as the size of capillary endothelial cells is unknown. In cross-section, they are $\sim 3\ \mu$ thick in the nuclear region and flatten towards the periphery to $0.2\ \mu$ or less (Fig. 2). The nucleus and perikaryon usually protrude into the capillary lumen and occupy ~30% of the area of the cell viewed in full face[2]; hence, most of the inner tunic consists of the thin periphery of the endothelial cells and measures on the average 0.2–0.3 μ in thickness (Fig. 1).

The Endothelial Cells

CELL MEMBRANE: Each endothelial cell is bounded by a cell membrane or plasmalemma which has the usual unit-membrane structure (Fig. 3) as already described by Robertson (21)

[2] As photographs of capillary endothelial cells in full-face view are unavailable, this value was obtained by measuring with a planimeter, on photographs of endothelial cells of rat aorta (Florey, Poole, and Meek (20); Figs. 5 and 12), the approximate fractional area occupied by the nucleus and perikaryon.

FIGURE 2 Perikaryon of a capillary endothelial cell in rat diaphragm. The cell is $\sim 1\ \mu$ thick in the nuclear region and tapers to $\sim 0.3\ \mu$ near the intercellular junction (*j*). It contains mitochondria (*mt*), rough endoplasmic reticulum (*rer*), smooth-surfaced vesicles, and cisternae which apparently belong to a Golgi complex (*gc*), free ribosomes (*r*), dense glycogen particles (*g*), and numerous plasmalemmal vesicles whose content varies from as light as the embedding matrix (v_1) to as dense as the blood plasma (v_2). The plasma is retained in the capillary lumen (*l*). The pericapillary region beyond the basement membrane (*bm*) contains numerous transversely sectioned collagen fibrils (*cf*) and clusters of fine fibrils (*ff*), some of which are associated with the periphery of an elastic fiber (*ef*). Fixation and staining as for Fig. 1. × 63,000.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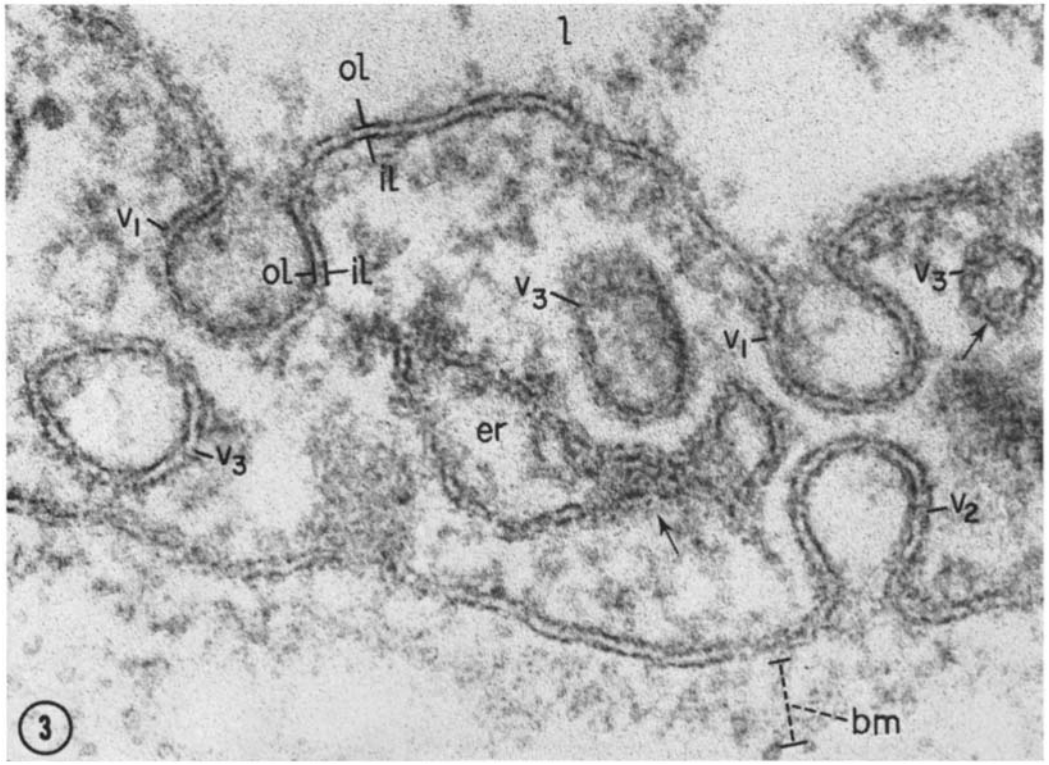


FIGURE 3 Small sector in the wall of a blood capillary of the tongue (rat). The micrograph shows layer-by-layer continuity of the plasmalemma with the limiting membrane of plasmalemmal vesicles. Two of these vesicles (v_1) open on the blood front of the endothelium, one (v_2) opens on the tissue front, and three (v_3) seem to be closed and completely surrounded by cytoplasmic matrix. *ol* marks the outer leaflet of the membranes, and *il* the inner leaflet. An element of the endoplasmic reticulum appears at *er*. Some detail of surface structure is visible where vesicle or endoplasmic reticulum membranes are grazingly sectioned (arrows).

Specimen fixed with OsO_4 and stained in block with uranyl acetate. Section stained with uranyl followed by lead. × 280,000.

and by Muir and Peters (22). The staining characteristics of the membrane leaflets depend upon the types of fixation and staining used. When tissue is fixed in glutaraldehyde-OsO_4 or OsO_4 and the sections are stained with uranyl acetate or lead hydroxide, the membrane is highly asymmetrical with a dense inner leaflet of ~35 A, a light intermediate layer of 20–25 A, and an outer leaflet of similar thickness whose density is noticeably lower than that of the inner leaflet and which frequently appears discontinuous (Fig. 12). The asymmetry is less pronounced after $KMnO_4$ staining in block (23) or in Vestopal-embedded specimens. The inner and outer leaflets of the plasma membrane are of similar thickness in tissues fixed with glutaraldehyde-OsO_4 or OsO_4 and stained in block with uranyl acetate at pH 5.0. The structure of the membrane always appears to be unifom along the entire perimeter of the cell except at the level of cell junctions (see below), and is not affected by local differentiations such as cell processes or plasmalemmal invagniations (Fig. 3).

CYTOPLASM: The cytoplasm contains the usual complement of cell organs of which some, i.e., a Golgi complex and two centrioles, are generally restricted to the perikaryon; whereas others, i.e., multivesicular bodies, dense bodies, rough and smooth elements of the endoplasmic reticulum, ribosomes, and mitochondria, are con-

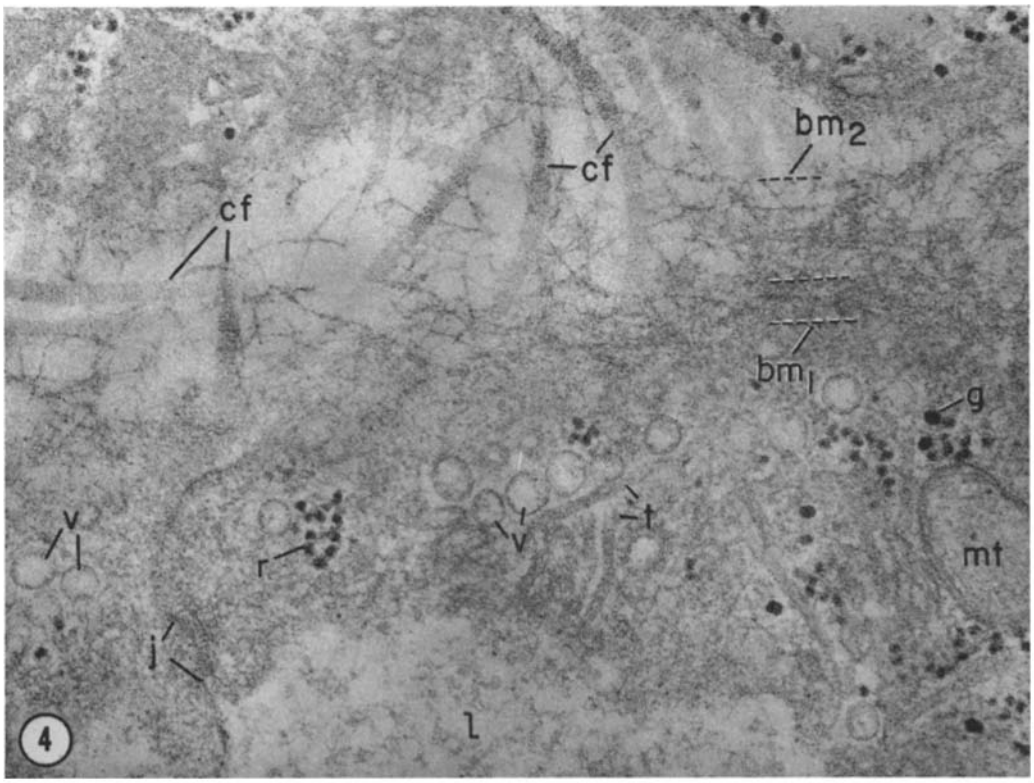


FIGURE 4 This oblique section of a capillary wall shows the fibrillar character of the basement membrane (bm_1) and the coarsening of its fibrils towards the periphery of this layer (bm_2). Microtubules (t) lie among the plasmalemmal vesicles in the cytoplasmic matrix of an endothelial cell. Rat diaphragm; fixation and staining as for Fig. 1. × 70,000.

centrated around the nucleus (Fig. 2) and, in addition, occur scattered in smaller numbers in the attenuated periphery of the cell (Fig. 23). The Golgi complex consists, as usual, of stacked, smooth-surfaced cisternae and clusters of small vesicles of varied size; large Golgi vacuoles are rare or absent. The piles of cisternae are frequently disposed in a discontinuous spherical shell whose center is usually occupied by a finely fibrillar material in which the centrioles are located (Fig. 10 in reference 6). The complex is often accompanied by a few multivesicular bodies and dense bodies of polymorphic content (conglomerates of dense particles and lamellar structures). Occasionally, such bodies also occur in the attenuated peripheral cytoplasm (Fig. 9 in reference 6).

The rough-surfaced endoplasmic reticulum is represented by a few randomly disposed cisternae whose attached ribosomes preferentially form rosettes or spiral patterns (Fig. 17) and whose content is moderate in density and amorphous or finely fibrillar in texture. The endoplasmic reticulum also has smooth-surfaced elements scattered throughout the cytoplasm (Fig. 6) in addition to those grouped in the Golgi complex. The elements of the endoplasmic reticulum have a layered membrane which measures ~60 A in thickness and is less asymmetrical than the cell membrane. Free ribosomes, usually grouped in clusters, are more frequent than attached particles and are distributed more evenly throughout the cytoplasm (Fig. 2). The mitochondria are few in number, small in dimensions, and poor in cristae, but otherwise typical in organization (Fig. 2). In lead-stained specimens, the cytoplasm contains scattered, dense particles which measure 250–300 A in diameter and have a somewhat rectangular profile (Figs. 1, 2). Similar particles occur in the sarcoplasm of adjacent muscle fibers. They

are tentatively identified as glycogen particles because of their similarity to the β-glycogen particles isolated and studied by Drochmans (24). Usually, there are no lipid droplets in the endothelial cytoplasm.

The cytoplasmic matrix contains a small number of "fibrils" of ~240 A diameter (Figs. 4, 5, 25) originally mentioned in reference 25. They are hollow cylindrical structures of undetermined length which appear straight over relatively long distances, presumably because of their rigidity, and occasionally occur in bundles. Morphologically, they are similar to the microtubules described in a number of cell types of animal (26, 27) and plant (28) origin. Finally, fine wavy fibrils of ~70 A diameter are sometimes encountered in the cytoplasm either isolated or in small bundles. They are reminiscent of the fibrils found in pericytes (see below) and smooth muscle cells, but they are far from representing a regular, volumetrically important component of the cytoplasm. Such fibrils are more numerous in the endothelia of larger vessels (arterioles, venules) in mammals, and particularly numerous in all vascular endothelia in amphibians (29).

PLASMALEMMAL VESICLES: A characteristic feature of the endothelial cell, originally pointed out in reference 25 and repeatedly confirmed (1–5, 30, 31), is a large population of small vesicles scattered throughout the cytoplasm (Fig. 1), but normally concentrated in more or less distinct layers along the two fronts of the cell in the immediate vicinity of the plasmalemma (Figs. 5, 6). Some of these vesicles lie free within the cytoplasm, whereas others open onto the blood or tissue front of the cell. Each vesicle is bounded by a membrane identical in thickness and in asymmetry to the plasmalemma; moreover, the unit membrane of "opened" vesicles is continuous, layer by layer, with the cell membrane (Figs. 3, 8). The vesicular content varies in density from as light as the content of the pericapillary spaces to as dense as the blood plasma.

In capillaries of the diaphragm, the mean outer diameter of the plasmalemmal vesicles is 700 A, but this value varies slightly with the fixation procedure as shown in Fig. 7. Table I gives the diameter of vesicles, measured with less precision, of leg muscle capillaries.

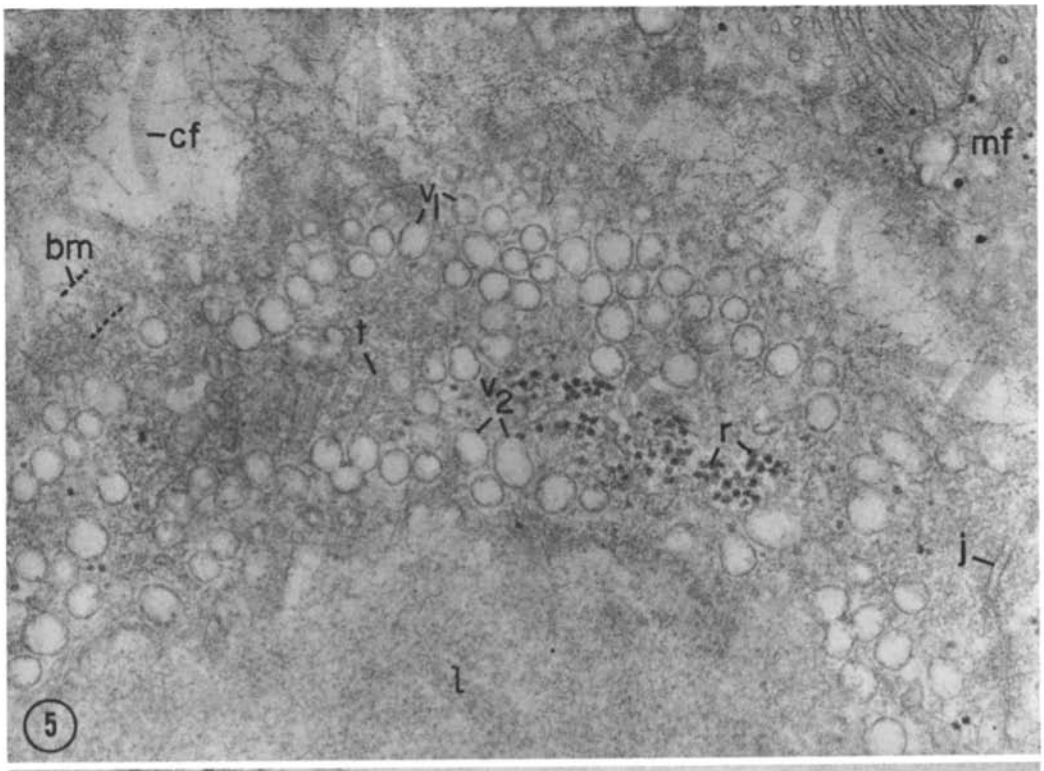
Vesicles on both cell fronts often open onto the surface of the cell through a stoma which is connected to the main cavity of the vesicle by a narrow neck having a diameter of ~300 A (range: 100–500 A). In most cases, the content of the vesicle communicates freely with the external medium, but in some instances, especially in rat tongue, a band ~50 A thick having a small central density spans the stoma of the vesicle (Fig. 8). Grazing sections reveal that the "bands" are apertures or diaphragms containing a central "knob" or thickening ~100 A in diameter, which appears similar to those reported in fenestrated capillaries of mouse kidney (32) and intestine (33) and in the adrenal medulla of the rat (34).

The frequency of vesicles varies with the cell region. They reach their highest density (~120/μ^2 of cell front) in the attenuated periphery of the cell and are least numerous in the perikaryon. Table II gives the results obtained by counting vesicles in grazing sections of heart muscle capillaries. If endothelial cell volume, rather than surface area of cell front is considered, the number of vesicles varies again with the thickness of the endothelium; it reaches ~883 vesicles/μ^3 of cytoplasm (~18% of the volume) in the peripheral regions of the cell 0.1–0.2 μ thick, and falls to ~190 vesicles/μ^3 (~4% of the volume) in the perikaryon (Table III).

The spatial relationships of plasmalemmal vesicles to one another and to the cell surface are not completely apparent in sections. Theoretically, the circular profiles seen therein could represent: (1) single spherical vesicles, (2) chains of vesicles, or (3) tortuous channels of circular cross-section. Each configuration could be: (1) isolated in the

FIGURE 5 Oblique section of the endothelium in a blood capillary of rat diaphragm showing the large population of vesicles and their concentration in two layers along the blood (v_2) and tissue (v_1) fronts of the cell. Fixation and staining as for Fig. 1. × 70,000.

FIGURE 6 Grazing section of the endothelium in a blood capillary of rat myocardium showing the large accumulation of plasmalemmal vesicles (v) on the tissue front. The small, circular profiles (s) probably represent the necks or stomata of vesicles opening onto the cell surface. Specimen fixed in OsO_4; section stained with lead hydroxide. × 48,000.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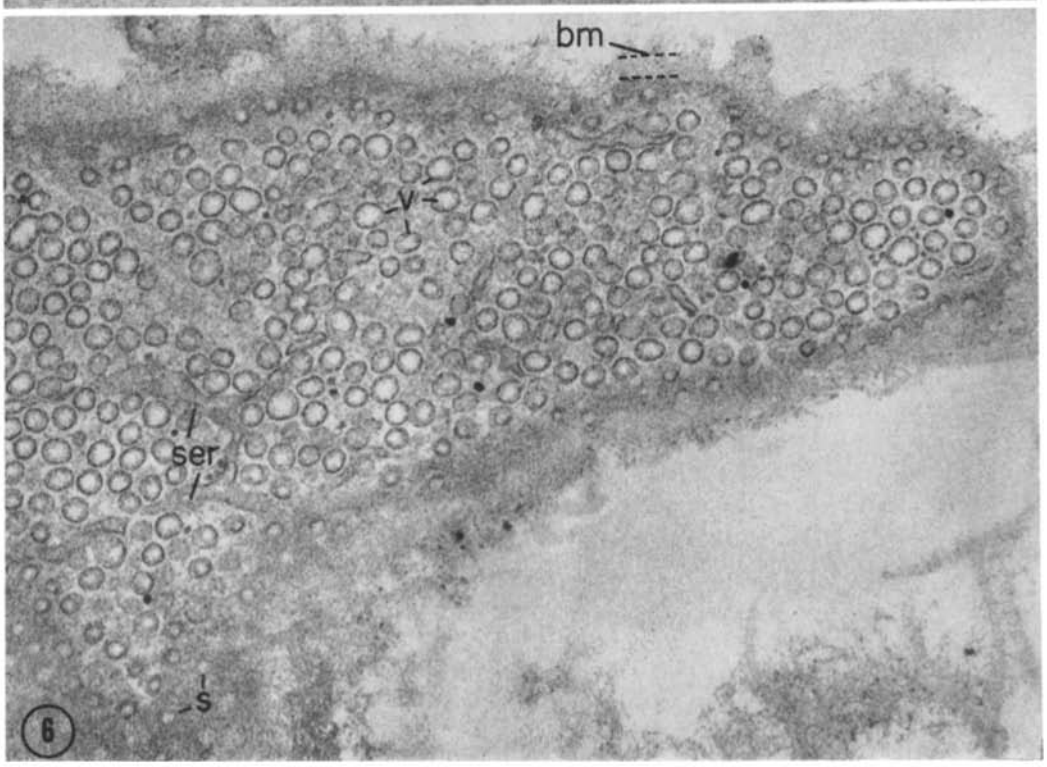
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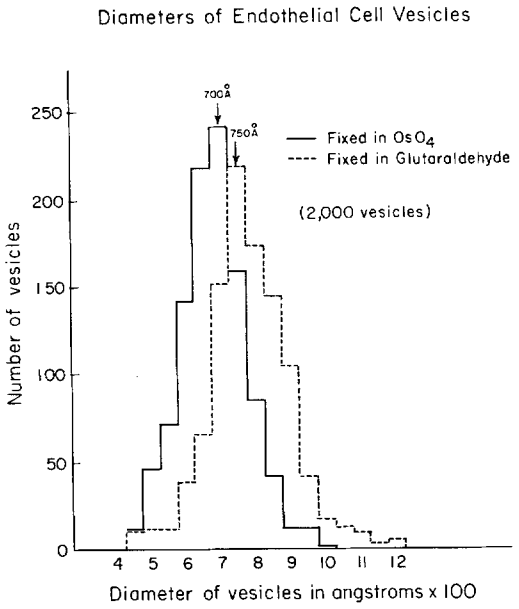


FIGURE 7 Diameter of endothelial cell vesicles in blood capillaries of rat diaphragm.

cytoplasm, (2) open on one front, or (3) open on both fronts of the cell. The tridimensional model in Figs. 9 and 10, constructed for solving such problems, shows that the circular profiles seen in the corresponding sections represent isolated spherical vesicles or short rows of vesicles, some completely isolated in the cytoplasm and some open either on the blood or tissue front of the endothelial cell. Within the limited endothelial volume reconstructed ($\sim$0.135 μ^3), there are no patent channels or rows of vesicles opening simultaneously on both the blood and tissue front of the endothelium.

Plasmalemmal vesicles are still present in regions where the endothelium thins out to a layer of 0.1 μ or less. In this case, the vesicles are disposed in a single layer and open more or less alternatively on one front or the other. Vesicles are usually rare along the narrow rim of the cell and absent at the level of cell junctions and of luminal cell processes. So far, no simultaneous opening of a plasmalemmal vesicle on both fronts of a cell has been detected in diaphragm and myocardium capillaries, although a large number of preparations have been examined; but such vesicles have occasionally been encountered in the capillaries of the tongue. No continuity of the vesicles with elements of the endoplasmic reticulum or Golgi complex has been recorded, and no special relationship with other subcellular components has been noted.

Vesicles of similar size and shape occur usually in the vicinity of the cell membrane in many cell types such as pericytes, smooth muscle fibers (31), striated muscle fibers (35), and epithelial cells (36) in which they have been described by Yamada as caveolae intracellulares, but, in no case, do they reach the population density encountered in the endothelium.

In addition to the prevalent type already described, endothelial cells occasionally contain slightly larger and structurally different vesicles; they are flask-shaped, appear either free in the cytoplasm or connected with the cell surface by a narrow neck or a solid strand, and are typically provided with a corona of condensed fibrillar material on the cytoplasmic side of the inner leaflet of their limiting membrane. The corona often shows a suggestion of radial orientation. A dense, more or less continuous coating usually covers the leaflet of the membrane facing the center of the vesicle. Such vesicles are common in macrophages, have been noted in glomerular epithelial cells (37), and described as "alveolate vesicles" in Purkinje cells (38) and as "coated vesicles" in oocytes (39) and spinal ganglia (40) and in other cells actively engaged in pinocytosis (41).

Along their luminal front, the endothelial cells are occasionally provided with processes which vary considerably in length (up to a few microns) and measure $\sim$750 A in thickness where normally sectioned. In three dimensions, they appear to be flaps or ruffles. They are preferentially located on one or both sides of the intercellular junctions which they may overlap. Their general morphology fully justifies the term "marginal flap" (or fold) already used in the literature for their designation (4). It should be noted that these marginal flaps vary in frequency and are often absent. Moreover, their frequency seems to be influenced by a number of preparative factors; they are more frequent in OsO_4 than in glutaraldehyde-OsO_4-fixed specimens; they are more frequent in the center than at the periphery of myocardium blocks; and have been reported as unusually large and numerous in the capillaries of human myocardium fixed postmortem (42).

The tissue front of the mature endothelium is generally free of cell processes. The ones occasionally encountered are short, blunt, and do not

TABLE I

Diameter of Endothelial Cell Vesicles in Leg Muscle of Rat*

Diameter‡ of vesicles in A	450–500	500–550	550–600	600–650	650–700	700–750	750–800
No. of vesicles counted	10	26	58	70	70	42	18

Total number of vesicles measured: 294.

* OsO_4-fixed specimens.

‡ Outer diameter.

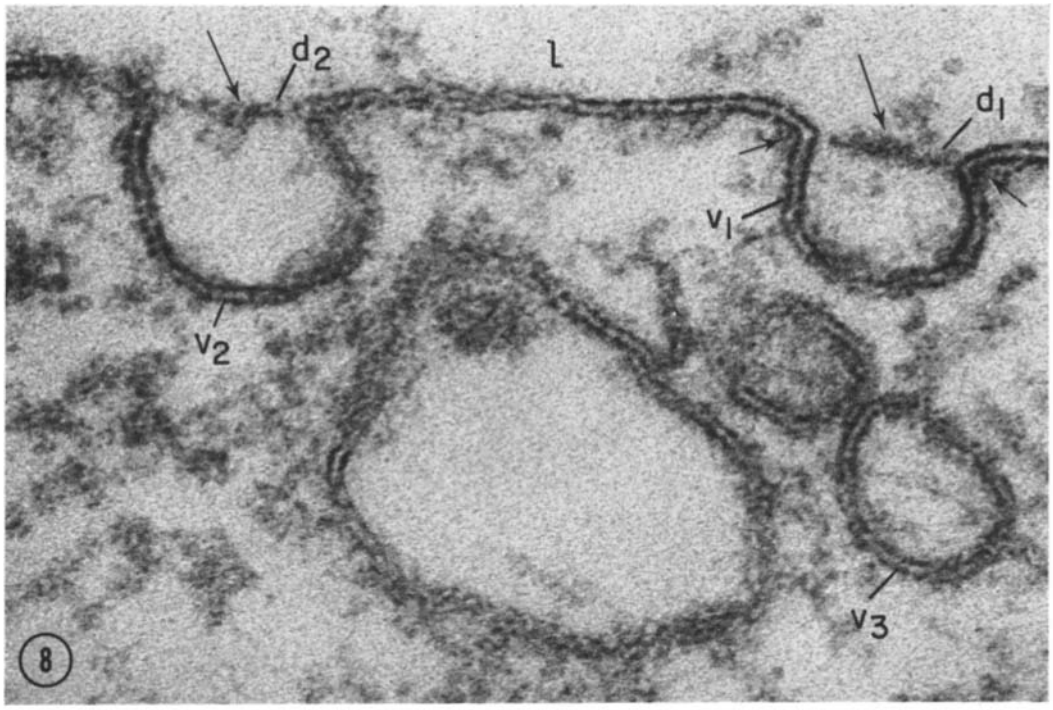


FIGURE 8 Small field in the endothelium of a capillary showing plasmalemmal vesicles (v_1, v_2) whose opening (stoma) is subtended by a diaphragm which consists of a single dense layer at d_1 and possibly more than one layer at d_2. Each diaphragm has a central thickening or knob (long arrows). A closed vesicle is marked v_3. Note the layer by layer continuity of the plasma membrane and vesicle membrane in the case of v_1. Note also the sharp rims of the vesicular stoma (short arrows).

Specimen fixed in OsO_4, and stained with uranyl in block. Section stained with uranyl and lead. × 300,000.

disturb the usual construction of the vessel. The situation is quite different in growing capillaries (43) and in the endothelium of small arteries (44).

NUCLEUS: As clearly established by light microscopic studies (cf. 45), the nucleus of the endothelial cell is ovoid and flattened. It normally protrudes in the lumen and is often bent to follow the curvature of the vessel. It is frequently indented and occasionally provided with a "hof" in which the centrioles and the centrosphere are located. Like the nucleus of other animals cells, it is surrounded by a nuclear envelope provided with pores and attached ribosomes; and contains one or two nucleoli, round bodies (46), irregular masses of chromatin, large perichromatin granules (47), and fibrillar or granular nucleoplasm. The chromatin

is usually concentrated at the periphery of the nucleus as in early prophase.

INTERCELLULAR JUNCTIONS: The margins of adjoining endothelial cells either abut bluntly, overlap, or interdigitate. Accordingly, the geometry of the intercellular spaces, and the path length along them vary considerably from place to place.

Within the luminal one-third of the junction, the intercellular gap is obliterated by a zone of fusion of the adjacent cell membranes which usually measures only ~200 A in depth, but can be clearly recognized by its typical "quintuple-layered structure" (22). Wherever normally sectioned, the whole structure measures ~150 A across with ~30 A for each layer, including the central one (the line of fusion) which represents the fused outer leaflets of the two cell membranes involved in the junction (Figs. 11, 12). This finding, together with the recognition of fusion lines continuous over relatively long distances in grazing sections, suggests that the cells of the endothelium are joined to one another by occluding zonules of the type described in other epithelia (48). As in these cases, continuity from one zonule to the next is occasionally seen in sections grazing the zone of contact of three endothelial cells (Fig. 13). Other types of junctions less frequently encountered than occluding zonules are: regions of more advanced fusion resulting in spotlike elimination of the fusion line (Fig. 14), and regions of simple apposition apparently without elimination of outer leaflet material (Fig. 15). Patent intercel-

TABLE II

Frequency of Vesicles on Endothelial Cell Fronts in Heart Muscle Capillaries (Rat)

Endothelial front	No. fields measured	Total area measured	Total no. vesicles	Vesicles/μ^2
				(range)
Blood	2	2.27 μ^2	242	106 (102–108)
Tissue	8	12.29 μ^2	1,442	117 (114–123)

TABLE III

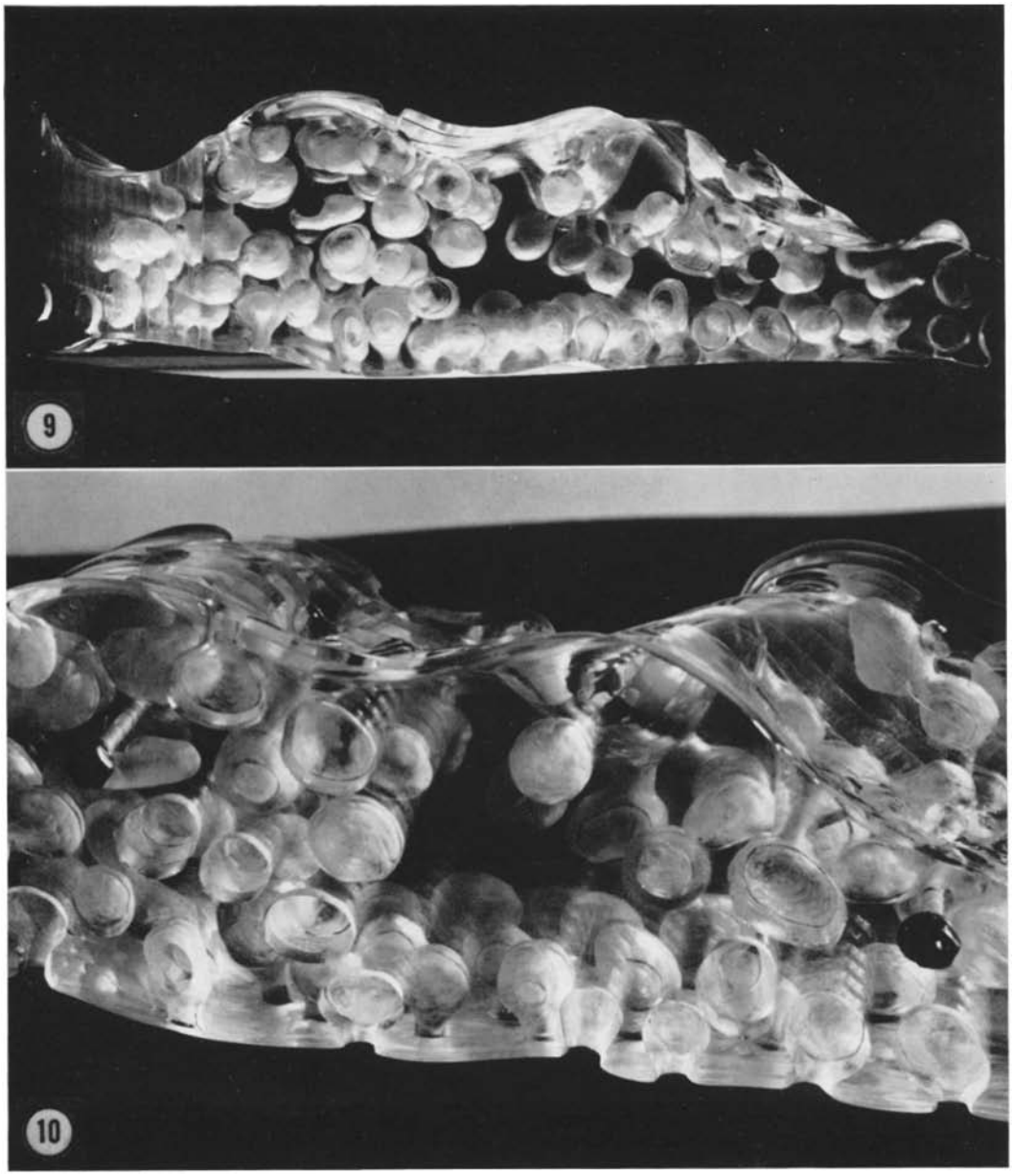
Frequency and Fractional Volume of Plasmalemmal Vesicles in Relation to Endothelium Thickness

(Rat diaphragm capillaries)

Thickness of endothelium	No. of samples	Aggregated volume of endothelium*	Total no. of vesicles counted	No. vesicles/μ^3 of cytoplasm	Fractional volume of cytoplasm occupied by vesicles‡
μ		μ^3		*range*	%
0.1	1	0.01	21	—	42
0.1–0.2	22	0.30	249	883 (421–2,000)	18
0.2–0.3	14	0.35	290	827 (422–1,160)	17
0.3–0.4	5	0.16	107	617 (406–1,000)	12
0.4–0.9 (perikaryon)	1	0.04	8	—	4

* For these measurements, normal sections of the endothelium were used. Areas within which vesicles were counted were measured with a planimeter and the corresponding volumes were calculated by assuming an average thickness of 500 A for each section.

‡ Fractional volume was calculated by assuming that the vesicles have an over-all diameter of 700 A. A correction of 0.5 must be applied to obtain the fractional volume of vesicular contents.



FIGURES 9 and 10 Tridimensional lucite model of a segment of endothelial cell cytoplasm. The model represents a segment of endothelium ~1.33 μ long, ~0.33 μ wide, and ~0.30 μ thick. It contains 98 vesicles of which 23 open onto the blood front of the cell, 35 open on the tissue front, and 24 are completely enclosed within the cytoplasm. Sixteen vesicles occur in the cut edges of the model so that their relationship to the cell surface or to other vesicles cannot be determined. All vesicles exist as single units except in five instances where adjacent vesicles appear to be fused in pairs. Two of these open through one of the pair onto the plasma front of the cell; similarly, one vesicle pair opens onto the tissue front and two pairs are completely enclosed within the cytoplasm. If we assume that these vesicles have a mean diameter of 700 A, 90 vesicles (vesicles on the edge of the model are each counted as one-half vesicle) occupy ~14% of the volume of the model.

Note in Fig. 10 the necks of varied length and diameter through which many vesicles open to the surface. In the original model, this sector of endothelium was magnified × 200,000. In Fig. 9, the magnification is ~ 87,000; in Fig. 10, ~ 162,000.

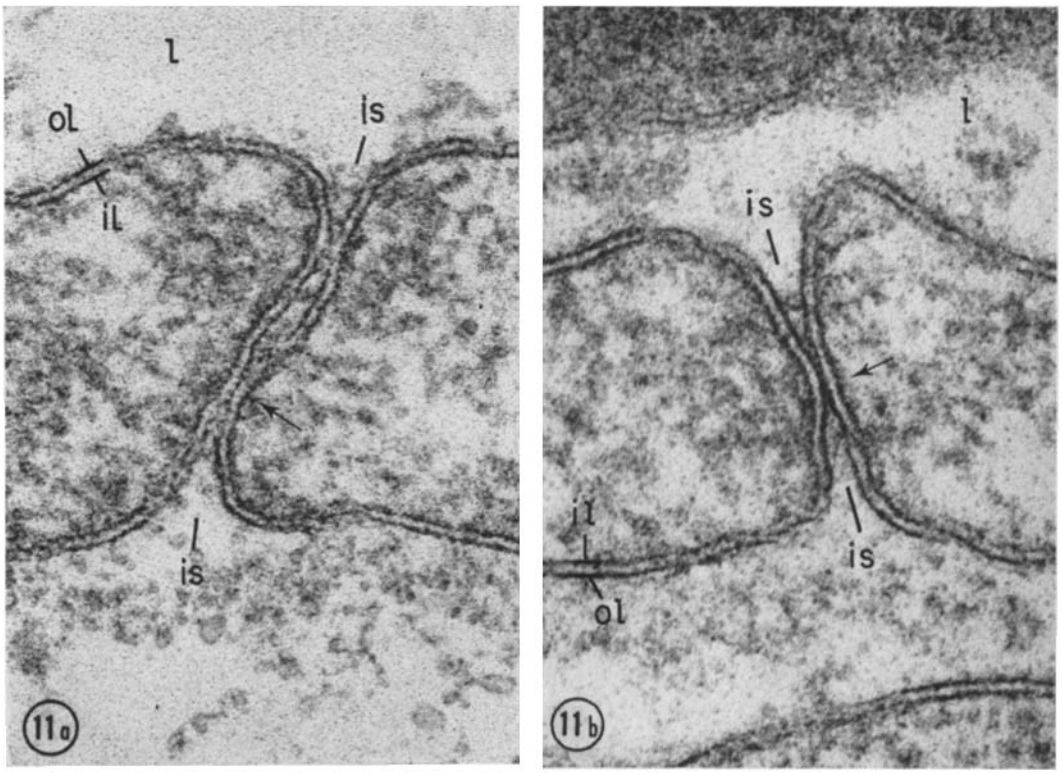


FIGURE 11 Cell junctions in the endothelium of tongue capillaries (rat). The arrows mark narrow regions along each intercellular space (*is*) where the two adjacent membranes are fused. In Fig. 11 *a*, the fused layer (~20 A) is thinner than the single outer leaflet (~30 A) beyond the junction; it also appears uneven, possibly discontinuous. In Fig. 11 *b*, the fusion layer is slightly thicker (~40 A) than a single outer leaflet (~30 A), but considerably thinner than two apposed ones (~60 A).

Specimens prepared as for Fig. 8. *a*, × 260,000; *b*, × 275,000.

lular gaps up to 200 A wide have been observed so far only occasionally in the endothelium of tongue capillaries (Fig. 16).

A band of dense, finely fibrillar material is usually found in the cytoplasmic matrix immediately under the cell membranes fused in an occluding zonule (Fig. 15). The band shows increased density after phosphotungstic acid staining and often extends beyond the occluding zonule toward the basement membrane. A rudimentary adhering zonule may occur at this level, with an intercellular gap of ~200 A. Grazing sections show that such condensations of subjacent cytoplasmic matrix extend without interruption over indefinite distances. Typical desmosomes or adhering maculae were never encountered in our material. They are known to occur, however, in the vascular endothelium of lower vertebrates (49, 50).

The Middle Tunic

The principal and in many regions the only component of the middle tunic is the basement membrane, a continuous, relatively thin sheet of extracellular material. A less important component, in terms of area covered, is a special cellular element, the pericyte, which in previous reports (3) was described as part of the adventitia. Inclusion of the pericytes in the middle tunic seems justified by the observation that they are located entirely within the basement membrane (4) and are topographically and, in part, morphologically similar to smooth muscle cells of arterioles and venules. The latter cells are generally considered

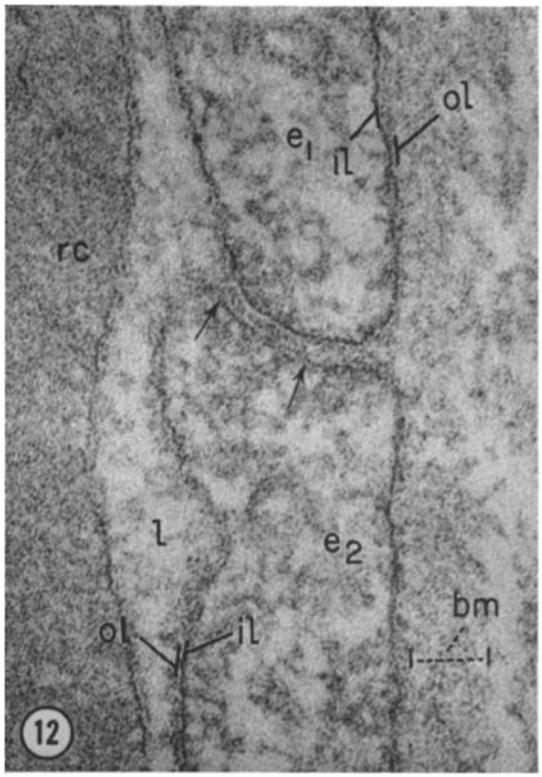


FIGURE 12 Junction between two endothelial cells (e_1, e_2) in a blood capillary of rat diaphragm. The plasma membranes show a dense inner leaflet (*il*) and a less dense, thinner, and apparently discontinuous outer leaflet (*ol*). The outer leaflets of the two adjacent membranes are fused (in between arrows) into a quintuple-layered structure of alternating dense and light strata which has an over-all thickness of ~170 A.

Specimen fixed in OsO_4; section stained with uranyl acetate followed by lead hydroxide. × 180,000.

as part of the middle tunic of the corresponding vessels (45).

BASEMENT MEMBRANE: The basement membrane is 300–500 A in thickness. Its inner margin is relatively well defined and usually separated from the endothelium by a narrow, though expandable subendothelial space; its outer margin which faces the adventitia is less distinct. Most of the basement membrane consists of a single layer; in certain regions, however, it splits into two or more leaflets of unequal thickness between which pericytes and their foot processes are usually located. The innermost leaflet is often attenuated to less than 200 A and is frequently interrupted by relatively large gaps, through which the tips of the foot processes of the pericytes reach the endothelium (Figs. 22, 23). Since the outer leaflets are generally continuous, it follows that the gaps do not lead through the basement membrane into the pericapillary spaces, but into closed pockets occupied by pericytes.

In OsO_4-fixed specimens, the basement membrane has the appearance of a distinct layer of moderately dense, usually amorphous material (Figs. 6 and 17). It has, in places, a faintly fibrillar texture which is greatly enhanced and rendered generally visible after staining with heavy metals (lead or uranyl salts and phosphotungstic acid). In such preparations, and at high magnifications, the basement membrane appears as a tight felt of randomly oriented fibrils ~50 A in diameter, which seem to be embedded in an amorphous matrix. Rarely is some preferred orientation of the fibrils observed; when present, it is usually perpendicular to the long axis of the vessels. In glutaraldehyde-OsO_4-fixed specimens, the basement membrane appears as a poorly defined layer of low density which acquires a faint fibrillar texture after heavy metal staining (Figs. 1 and 2). Towards the adventitia, the basement membrane feltwork acquires a coarser texture and its fibrils often appear thicker and denser (Figs. 4 and 5).

At the periphery of the basement membrane, fibrils of a special type are encountered. They have a diameter of ~100 A, a faint periodicity of ~200 A, and cross-sections that appear circular and "hollow" (Fig. 2). They are unevenly distributed, occur either singly or in bundles of varied sizes (Fig. 17), and their appearance is little influenced by the fixation procedures. Fibrils of similar general appearance (Fig. 18), profile (Fig. 19), and periodicity (Fig. 20) regularly occur in more or less continuous envelopes around elastic fibers (Figs. 18 and 19).

PERICYTES: These cells are distinguished by their characteristic location between leaflets of the basement membrane, a feature that makes their topography comparable to that of the smooth muscle cells of larger vessels. In terminal arterioles (44), the relationship is similar to that found in capillaries: in most places, a single leaflet of basement membrane is interposed between the endothelium and the subjacent cells; in larger arterioles, diameter $\geq 50\ \mu$ (44), the endothelium and the smooth muscle cells have distinct basement membranes separated by elastic fibers. The general form of the pericytes, difficult to visualize from

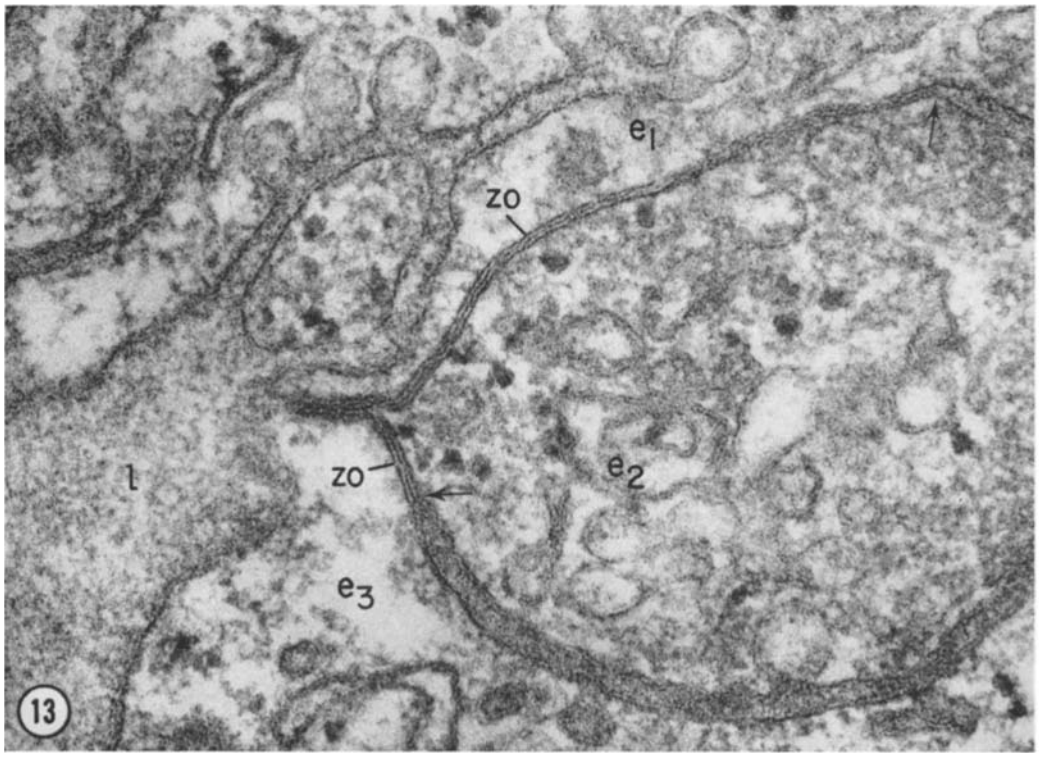


FIGURE 13 Continuity of occluding zonules (*zo*) among three adjacent endothelial cells (e_1, e_2, e_3) in blood capillary of rat diaphragm. The capillary lumen is at *l*. Beyond the arrows, where the occluding zonules leave the plane of section, the cells are separated by a space of ~200 A which contains a dense material and resembles a rudimentary adhering zonule.

Specimen fixed in OsO_4. Section stained with lead hydroxide. × 133,000.

electron micrographs, has been well established by the extensive light microscope studies of Rouget (51) and Zimmermann (52). Each cell has a perikaryon from which arises an elaborate system of processes usually disposed perpendicular to the long axis of the vessels. Electron micrographs of the perikaryon show that it contains a large nucleus which protrudes towards the pericapillary spaces (Fig. 23), a usual centrosphere, an endoplasmic reticulum which varies markedly in the extent of its development, a small population of free ribosomes, and a large number of mitochondria (Fig. 21). The cytoplasm is distinguished by its relatively high content of dense cytoplasmic particles, tentatively identified as glycogen (Figs. 21 and 23). They are generally more numerous than in endothelial cells, are frequently disposed in clusters and occasionally aligned between smooth-surfaced cisternae of the endoplasmic reticulum.

Finger-like or spinous processes extend in large numbers from the perikaryon. They have a circular or oval profile in cross-section and measure from 0.05 to 0.4 μ in diameter. Their cytoplasm contains a high concentration of fine fibrils preferentially oriented parallel to the long axis of the pseudopodium. Other subcellular components are rare or absent in these processes. The bundles of fine cytoplasmic fibrils frequently extend from one process to another, thus forming a continuous concave "sole" applied against the curvature of the endothelium (Figs. 21, 23). Many processes penetrate the subendothelial space through gaps in the inner leaflet of the basement membrane and run for a while in contact with the endothelial cells, which sometimes appear grooved or deeply depressed at their level (Figs. 21 and 22). At the tip of the process, the cell membrane of the pericyte is fused in an occluding macula (10) to the cell membrane of an endothelial cell (Figs. 22 and 24).

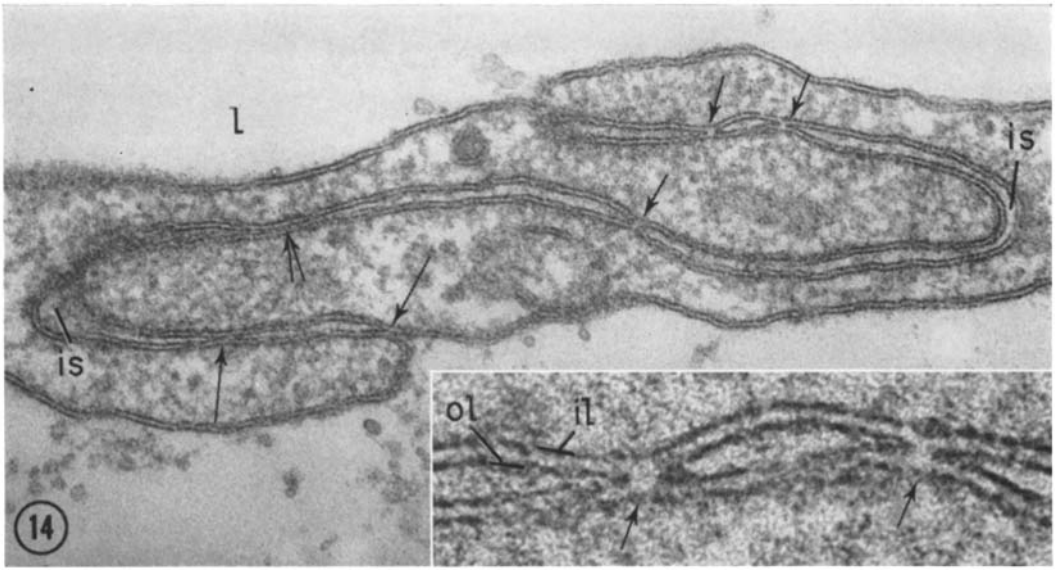


FIGURE 14 Cell junction in the endothelium of a tongue capillary (rat). Along the s-shaped intercellular space (*is*), there are three areas in which the fusion of the adjacent cell membranes results in the elimination of the outer leaflets (short arrows), two areas where a usual dense fusion layer is visible (long arrows), and one region in which the membranes appear to be apposed but not fused (double arrow). The inset shows at a higher magnification two of the areas in which the fusion layer is focally eliminated.
Specimen preparation as for Fig. 8. × 120,000; inset, × 480,000.

The two membranes have the same thickness and the same asymmetric stratification (Fig. 24). The pericytes are provided with a relatively large number of plasmalemmal vesicles, morphologically similar to, but usually less numerous than the vesicles described in endothelial cells. They appear restricted to the perikaryon and the roots of the processes. The tips of the latter, as well as their connecting "soles," are generally free of vesicles (Figs. 21 and 23).

The Adventitia, or Outer Tunic

The adventitia, or outer tunic, is a highly discontinuous layer comprising cellular (fibroblasts, macrophages, mast cells) and extracellular (collagen and elastic fibers) elements. Inward, it is relatively well outlined by the basement membrane, but outward it has no distinct limits; its cellular and extracellular components are similar to, and continuous with the elements which populate the pericapillary spaces.

Cellular Elements

Macrophages occur more or less regularly along muscle capillaries at intervals of 40–50 μ (6). As elsewhere in the body (53, 54), they are large cells which, in addition to the usual cell organs (i.e., mitochondria, rough-surfaced endoplasmic reticulum, a Golgi complex, free ribosomes, etc.; some of these components are shown in Fig. 25) contain a whole spectrum of structures connected with uptake of matter in bulk. The spectrum includes: large ruffles (veils) and deep infoldings of the cell membrane; few plasmalemmal vesicles and numerous vesicles with a fibrillar corona (Fig. 26); multivesicular bodies; vacuoles of different sizes and varied content; and residual bodies which contain packed membranous and granular remnants of previous lytic activities. There is among these macrophages a certain amount of variation in size and cytoplasmic organization; the smaller ones have a larger population of free ribosomes (Figs. 25 and 27), a relatively small endoplasmic reticulum, and fewer structures connected with endocytosis. Their appearance suggests that they are young cells at the beginning of their differentiation into full-fledged macrophages.

Fibroblasts with the usual organization already described in other parts of the body (55, 56) occur rather frequently in the adventitia (Fig. 27).

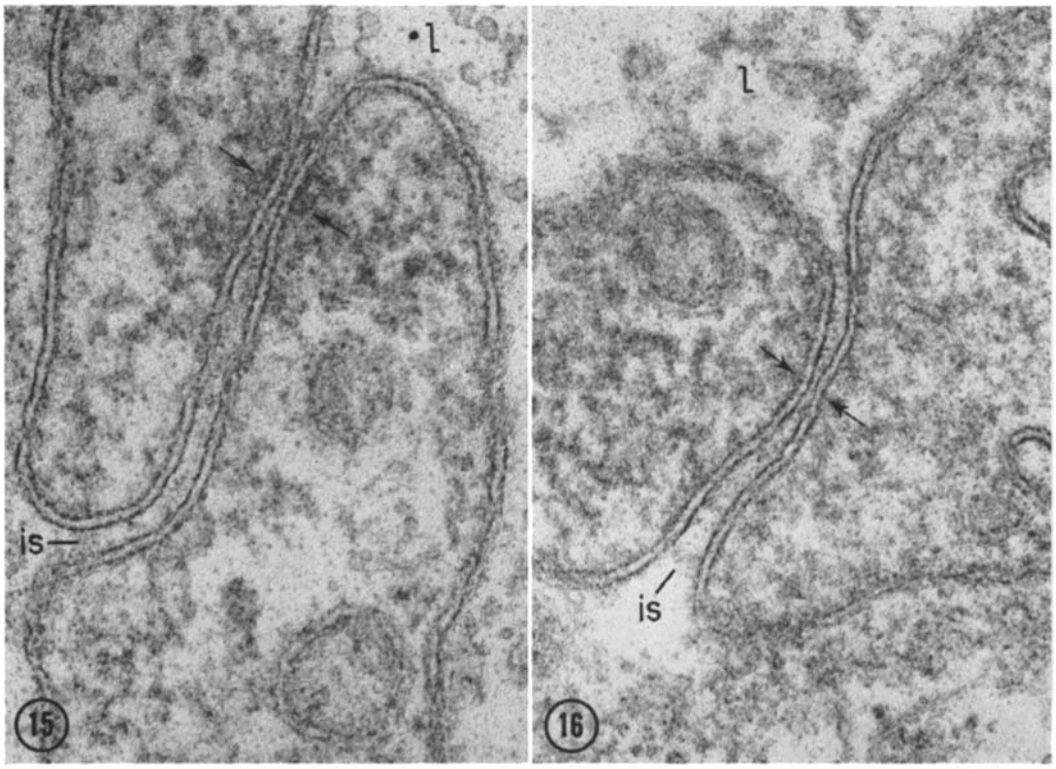


FIGURE 15 Cell junction in the endothelium of a tongue capillary (rat). Along the intercellular space (*is*) there is a single region in which the cell membranes come in close contact, but do not apparently fuse (arrows). × 240,000.

FIGURE 16 Cell junction in the endothelium of a tongue capillary (rat). Along the intercellular space (*is*), narrowed at arrows to less than ~40 A, there is no evidence of membrane contact or fusion.
Specimens prepared as for Fig. 8. × 225,000.

Mast cells are fewer in number than other adventitial cells. As elsewhere (57), they are characterized by their numerous, large (0.5–1.0 μ), dense granules, their small number of usual cell organs, and their frequent, short, laminar, and apparently rigid processes.

Extracellular Elements

COLLAGEN AND OTHER FIBRILS: The adventitia contains a large number of fibrils which range in diameter from 500 to 600 A (Figs. 17 and 18). Those thicker than ~450 A show the typical periodicity (~600 A) of collagen fibrils. In general, the fibrils form large bundles in which they are regularly packed parallel to one another. The small fibrils (~100 A in diameter) are relatively sparse, when compared with well characterized collagen fibrils. They occur either singly or in poorly ordered small bundles and are morphologically similar to the fibrils described at the periphery of the basement membrane and to those usually encountered in the envelope of elastic fibers (see below).

Elastic fibers of small diameter (<0.1 μ) occur irregularly in small numbers in the adventitia of the capillaries. They appear homogeneous and moderately dense, have irregular profiles in cross-section, and are typically surrounded by a more or less continuous envelope of fine (~100 A) fibrils of the type already described (Fig. 18). These fibrils are usually disposed parallel to the long axis of the elastic fiber. Similar envelopes are seen around the large elastic fibers found in endocardium and in the walls of larger vessels (Fig. 19).

MATRIX: The cells and fibrils of the adventitia are surrounded by a matrix of low density and no

visible structure which, as a rule, cannot be distinguished from the embedding resin. In certain cases, however, which involve water removal from the intercellular spaces (by a hypertonic fixative or by experimental increase of the protein content of the plasma[3]), these spaces are found shrunken and filled by a continuous pervasive material of moderate density against which individual collagen fibrils appear in negative image. It is difficult to differentiate this condensed matrix from adjacent basement membranes.

DISCUSSION

General Construction and Distribution of Muscle Capillaries

Our observations show that the wall of the blood capillaries of the diaphragm and myocardium in rats comprises three coaxial tunics: an *inner tunic* represented by a continuous endothelium; a *middle tunic* which includes a continuous basement membrane and associated pericytes; and a discontinuous *outer tunic* or adventitia consisting of cellular and extracellular elements. The same construction was found in the capillaries of other skeletal muscles (abdominal wall, limbs) in rats; in those of the myocardium of other species (guinea pig, hamster, rabbit); and in those of human skeletal muscle (58). Since similar vessels occur in the skin (59), hypodermis and tendons (60), it appears that this type of capillary is generally used in the vascularization of the soma of mammals, with two exceptions so far recorded: the capillaries of the bone marrow which appear to be true sinusoids (61, 62) and those of the fat pads of synovial membranes which have a fenestrated endothelium (63). Occasional endothelial fenestrations also are seen in the capillaries of the tongue.[4] In addition to the myocardium, capillaries with the same general construction occur, however, in many viscera, e.g., the mesentery (65), and the smooth muscle of the digestive and reproductive tracts. Finally, variants of the same type of construction (continuous endothelium and continuous basement membrane) are encountered in the lung (16, 66) and the central nervous system (64, 67). In view of this wide distribution and of the enormous extent of the vascular bed of striated muscles (68), it is clear that such vessels represent by far the predominant type of blood capillary in the mammalian vasculature.

[3] R. R. Bruns and G. E. Palade. Unpublished observations.

[4] Maynard et al. (64) noted similar fenestrations in the capillaries of loose connective tissue in unspecified regions of the body.

Systematization of the Structural Elements Constituting the Capillary Wall

On the basis of our observations and those of others, we propose that the main structural elements of the blood capillary wall, which are now well recognized, be grouped into three layers or tunics (inner, middle, outer) instead of the one (68) or two layers (4, 45) previously described. This systematization is justified by the following considerations:

(1) It recognizes the continuity of the vascular wall layer by layer from arterioles through capillaries to venules and it points out the similarity in relationship between basement membranes on the one hand, and pericytes or smooth muscle cells on the other hand, at different levels in the vascular bed.

(2) It allows the consideration of distinct functional roles for each tunic or layer within a larger functional unit which is represented by the entire capillary wall (see below and reference 6).

(3) It facilitates a general systematization of blood capillaries as shown by Bennett, Luft, and Hampton (1).

There are, of course, differences in the structure of the vascular wall from capillaries to large vessels, e.g., the appearance in the latter of two separate basement membranes, one for the endothelium and the other for the cells of the media, and the interposition of a large amount of connective and elastic tissue in between these two membranes; but we feel that these differences do not basically conflict with the systematization proposed.

Since the main function of the capillary wall is the control of exchanges between the blood plasma and the interstitial fluid, the various structures known, or likely to be involved in these exchanges, will be considered in each of the three tunics of the vessel.

The Inner Tunic—Endothelium

PLASMALEMMAL VESICLES: Our data permit an evaluation of the surface area and capacity of the vesicular population. They show that behind 1 μ^2 of cell front there are $\sim$2 μ^2 of vesicular

membrane, as calculated for 100–120 vesicles of 700 A outer diameter. A large reserve of membrane is, therefore, present in endothelial cells.

The volume of a plasmalemmal vesicle, calculated for an inner diameter of 550 A is $\sim 10^{-4}$ μ^3; hance, there are ~ 0.01 μ^3 of vesicular volume (calculated for 120 vesicles) immediately behind 1 μ^2 of cell front. The fraction of endothelial volume accounted for by plasmalemmal vesicles is more difficult to assess, because their frequency varies with the thickness of the endothelium. Table III shows that this value decreases from 42 to 12% as endothelial thickness increases from 0.1 to 0.4 μ. (The corresponding value in the model in Figs. 9 and 10 is ~14%, in agreement with the data in Table III.) In the much thicker perikaryon, vesicular volume is expected to drop further, possibly to less than 5%. If we assume that blood $\rightleftarrows$ tissue exchanges are negligible at the level of the perikaryon, and estimate that the latter takes ~30% of the cell surface, then in the remaining 70%, which represents the attenuated (0.2–0.3 μ) "functional" periphery of the cell, the plasmalemmal vesicles account for 15–20%[5] of the endothelial volume. For a capillary of 7 μ inner diameter, this figure represents ~6% of the plasma volume (corrected for hematocrit).

Our observations, and especially our tridimensional reconstruction, indicate that plasmalemmal vesicles do not establish continuous channels from one cell front to another, although they frequently form chains of two to three units. In other cell types, chains of vesicles appear in OsO_4–fixed specimens in locations in which continuous membrane infoldings are found in glutaraldehyde- (70) or $KMnO_4$- (71) fixed material. In the endothelium, the general appearance of the vesicles is the same in OsO_4- and in glutaraldehyde-OsO_4 fixed specimens.[6] Hence, it seems unlikely that we are dealing with an artifact.

Transport by vesicles appears to be a discontinuous process carried out in quanta (3, 25) or droplet by droplet. Table II suggests that the frequency of plasmalemmal vesicles is slightly higher on the tissue front than on the blood front of the endothelium, and in the model (Figs. 9, 10) there are 35 vesicles opened onto the tissue front, for 24 isolated in the cytoplasm, and 23 opened onto the blood front. If we assume that they represent successive stages in a common cycle, it follows that a vesicle probably spends ~40% of its cycle-time on the tissue front, ~30% transversing the cytoplasm, and ~30% on the blood front.

CELL JUNCTIONS: The cells of the endothelium appear to be joined to one another by simplified junctional complexes which consist of occluding zonules followed by more or less well defined adhering zonules. The bands of membrane fusion are generally narrow (200–400 A), a situation reminiscent of that encountered in the proximal convolution of rat nephrons (48). In other epithelia, occluding zonules were shown to be impermeable to concentrated protein solutions (48), and more recent findings suggest that they are also impermeable to water and small, water-soluble molecules (cf. 10, 72). By analogy, the occluding zonules of the endothelium may represent sites where diffusion across the capillary wall is prevented, a view which is contrary to current assumptions in the field (33, 73, 74). Since it is difficult, if not impossible, to demonstrate that the occluding zonules run uninterrupted around the entire perimeter of their respective cells, and since types of junctions other than occluding zonules are occasionally encountered in the endothelium,

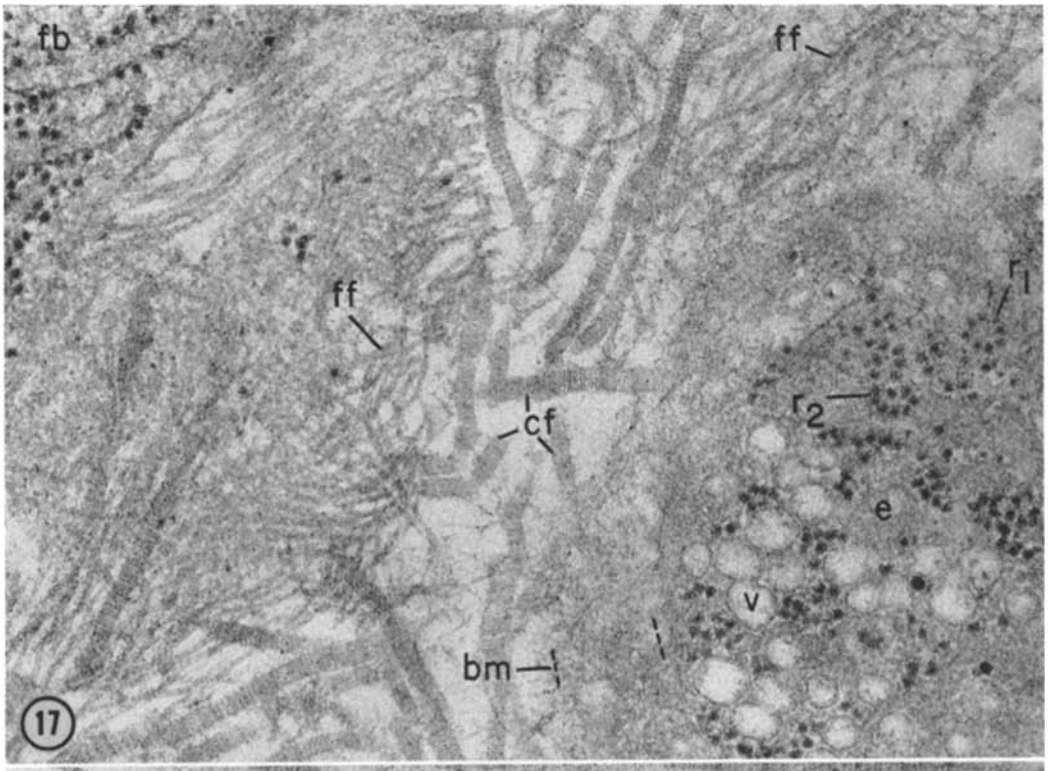
[5] A comparable fractional volume (20%) is estimated by Renkin (69) for the whole endothelium.

[6] The same applies for $KMnO_4$-fixed specimens according to available, but much more limited observations.

FIGURE 17 Fine fibrils (*ff*), in unusually large bundles, and collagen fibrils (*cf*) lying between a blood capillary (*e*) and a fibroblast (*fb*) in a rat diaphragm. Ribosomes grouped in a circle (r_1) and a rosette (r_2) appear in the endothelial cell. × 67,000.

FIGURE 18 Collagen fibrils (*cf*) and elastic fibers (*ef*) with their characteristic envelope of fine fibrils (ff_1), in the pericapillary spaces of a rat diaphragm. Some fine fibrils (ff_2) appear to be independently scattered in the connective tissue spaces.

Specimen prepared as for Fig. 1. × 115,000.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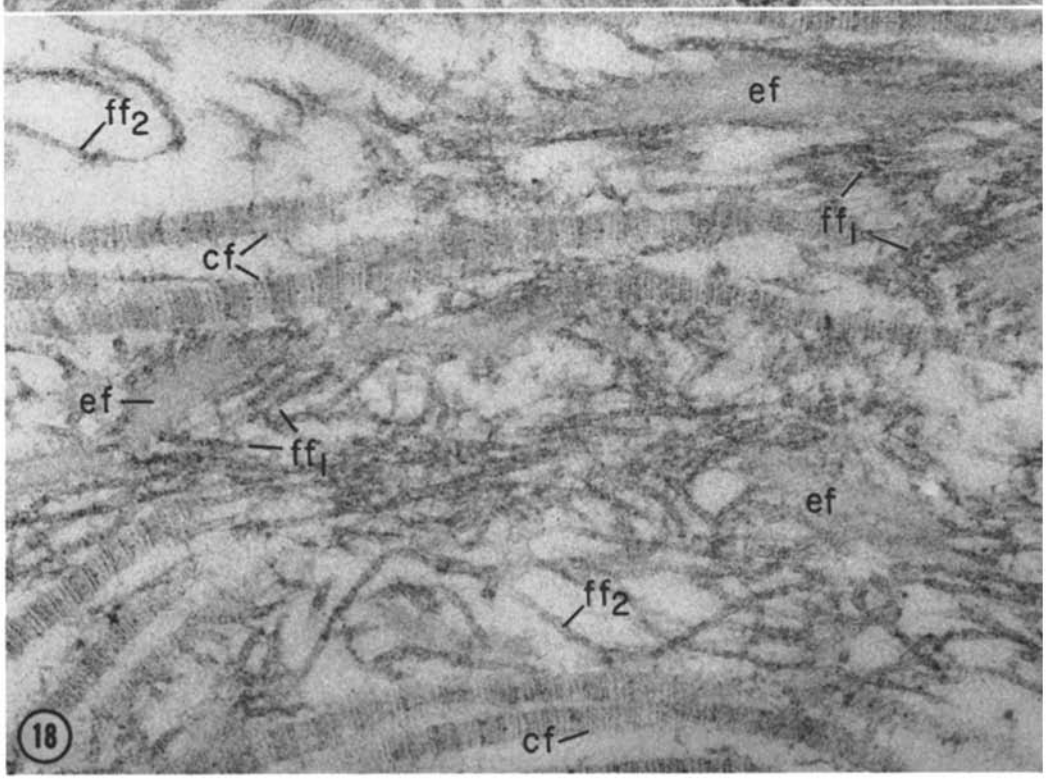
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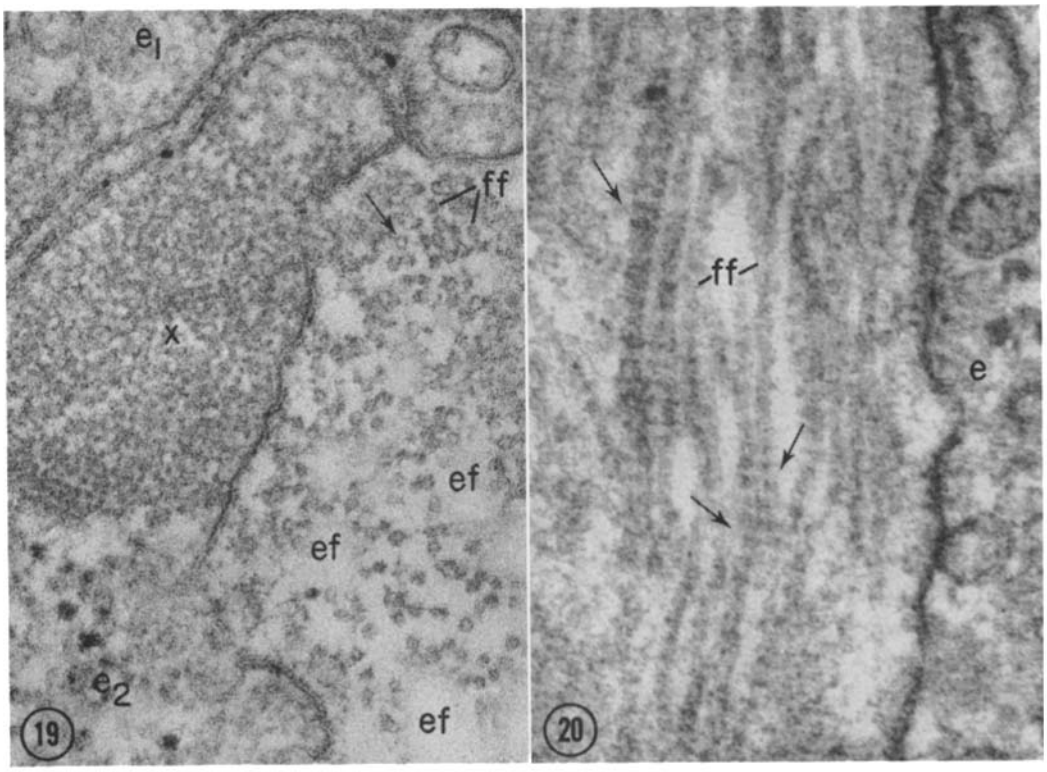


FIGURE 19 Part of the internal elastic membrane and of two endothelial cells (e_1, e_2) in a small arteriole in a rat diaphragm. Fine fibers (*ff*), ~100 A in diameter, which appear "hollow" (arrow), surround a group of small elastic fibers (*ef*). *x* marks the center of a large bundle of cytoplasmic fibrils in e_2.

Specimen prepared as in Fig. 1. × 122,000.

FIGURE 20 Bundle of fine fibrils in the internal elastic membrane of a rat arteriole. The bundle is part of the envelope of a large elastic fiber whose profile is out of this field. Note the periodicity visible in a number of places (arrows) in the bundle where fibrils appear to form lateral aggregates.

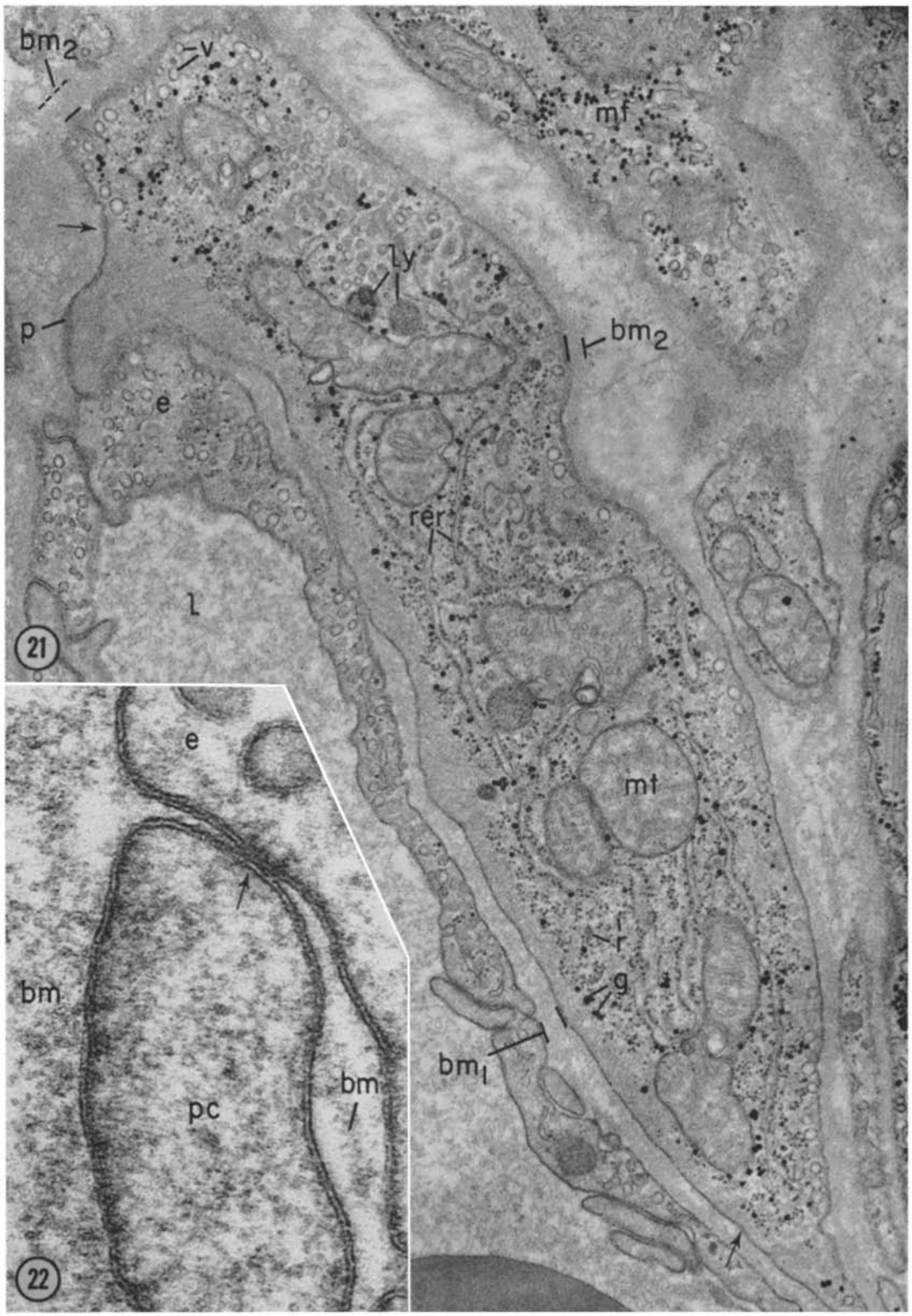
OsO_4–fixed specimen, stained with lead hydroxide. × 140,000.

FIGURE 21 Pericyte in a blood capillary of the myocardium (rat). The cytoplasm contains many mitochondria (*mt*), a relatively large number of cisternae of the rough endoplasmic reticulum (*rer*), lysosome-like bodies (*ly*), free ribosomes (*r*), glycogen particles (*g*), and plasmalemmal vesicles (*v*). Note the "sole" (between arrows) of finely fibrillar material and the lack of plasmalemmal vesicles along the front of the cell facing the endothelium. A pseudopodium (*p*) makes contact with the endothelium after penetrating through the basement membrane. The latter is split into two leaflets (bm_1 and bm_2) which surround the pericyte.

Specimen prepared as for Fig. 6. × 30,000.

FIGURE 22 Macula occludens (arrow) between the membranes of an endothelial cell (*e*) and that of a pericyte pseudopodium (*pc*) in a tongue capillary (rat). Leaflets of basement membrane (*bm*) appear on both sides of the pericyte.

Specimen prepared as for Fig. 8. × 146,000.


bm2
v
mf
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bm2
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bm1
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bm
bm
pc
22

the existence of open intercellular channels cannot be denied, but their frequency must be low.

Areas of membrane fusion have already been noted and described under various names in the capillary endothelium (22, 21) especially in the central nervous system (22). Our observations extend these findings to other capillaries, and show that such zonules are of widespread occurrence in the endothelium of these vessels.

Negative Evidence on Pores

Isolated, perfused legs of small mammals, i.e., specimens most probably provided with the type of capillary described in this paper, have been extensively used in studies of capillary permeability. To explain permeability data obtained on such preparations, Pappenheimer et al. (75) have postulated that the capillary wall is provided with patent pores (water-filled channels) which have an effective radius of ~30 A, an aggregated area of 0.1 to 0.2% of the luminal surface, and a frequency of 10–20/μ^2. Following an earlier suggestion by Chambers and Zweifach (76), the pores were assumed to be the intercellular spaces of the endothelium. More recently, Landis and Pappenheimer (73) have revised the effective pore radius upwards to 45 A, and assumed that the pores are actually represented by the intercellular spaces because the width (100 A) and the aggregated area of these spaces, estimated from available electron micrographs (2), were found to correspond to those expected for pores. Since the pore theory had had considerable influence on current views on capillary permeability, the bearing of our findings on this theory will be discussed in some detail.

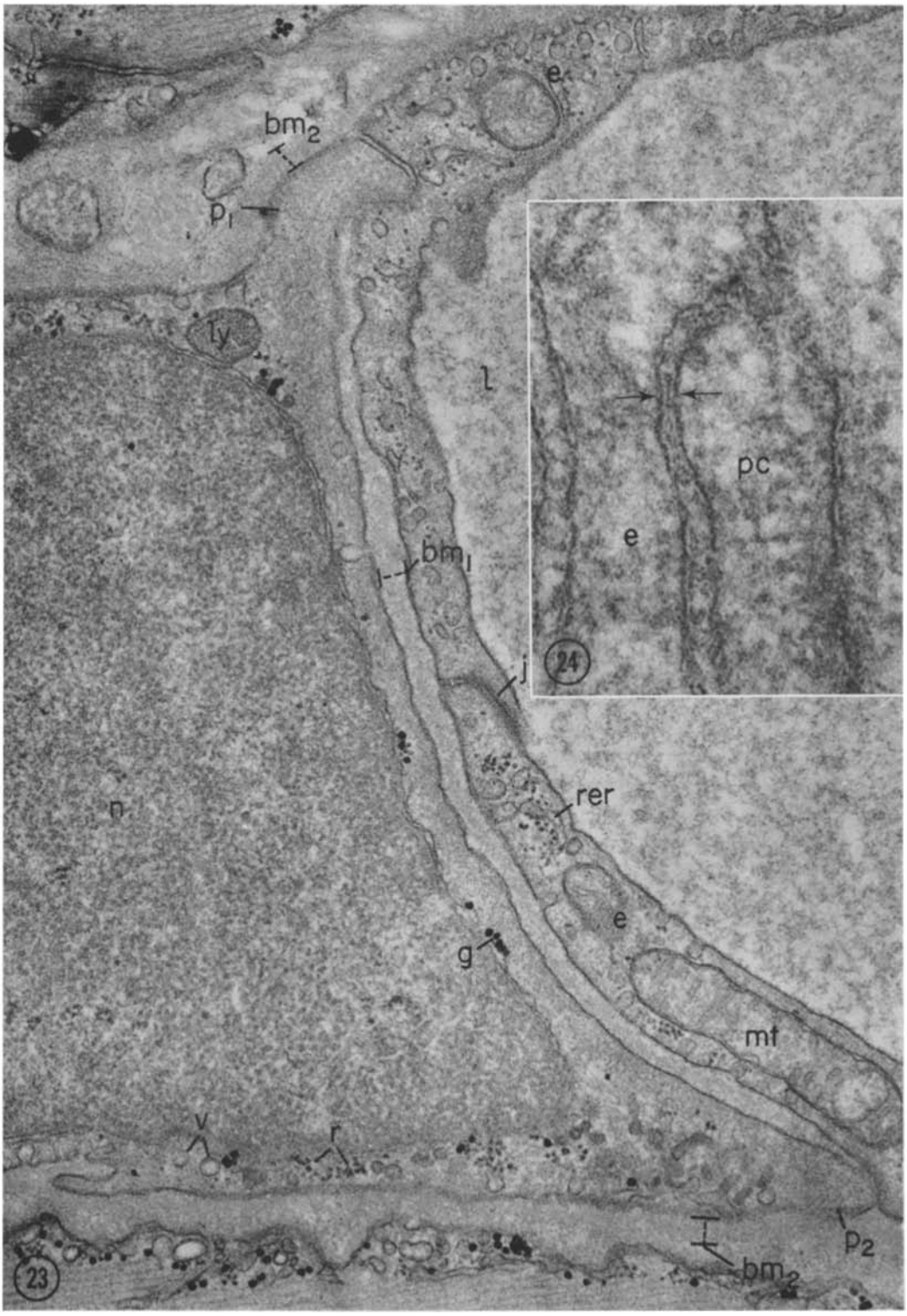
In the material examined, we did not find pores of the dimensions, distribution, and location postulated. The fractional surface of the network of intercellular spaces in a population of flattened cells, in which each cell measures 10 × 30 μ, and is separated from its neighbors by a gap of 100 A, calculates at ~0.1%. If this network is equated with a system of slit pores, all intercellular spaces of the endothelium should be open to a gap of ~100 A. If the cell boundaries follow a meandering course, part of the intercellular spaces could be occluded and part open. Yet even in cases in which meandering would increase the length of the boundaries by a factor of three or four, the proportion of open spaces should remain high enough (33–25%) to be easily detectable. In any case, the open gaps should measure ~90 A to satisfy current physiological interpretations (73). In cases in which gaps have been mentioned in electron microscopical studies (33, 74), considerably smaller values have been considered.

Pores penetrating directly the attenuated periphery of the cell should be easily visible on account of their large dimensions (I.D. ≃ 90 A; O.D. ≃ 240 A) and favorable contrast conditions due to the continuous sleeve of plasmalemma which is expected to bound their lumen. If we assume an even distribution of pores along the capillary, four to eight such structures should be present in our model (Figs. 9 and 10), and eight to 16 in a 500-A-thick cross-section of a capillary 5 μ in diameter. None has been found.

A possibility still to be considered is that the pores are represented by chains of vesicles simultaneously opened on both cell fronts. Such chains would be more difficult to detect if long and of complicated geometry. Although we found none in our model, this possibility and its implications deserve further discussion, which will fit better in the context of the following paper.

FIGURE 23 A cross-section of the capillary wall in rat atrial muscle, showing the relationship of a pericyte to the endothelium (*e*). The pericyte, with its large nucleus (*n*), bulges toward the adventitia. It contains ribosomes (*r*), glycogen particles (*g*), and plasmalemmal vesicles (*v*). The cell border facing the endothelium is occupied by a layer of fine fibrillar material which forms a sole extending in between two foot processes (p_1, p_2). The latter penetrate the basement membrane to make contact with the endothelium. Note that the sole is relatively free of other subcellular components. The two leaflets of basement membrane which envelope the pericyte are marked bm_1 and bm_2. × 38,000.

FIGURE 24 Occluding macula (arrows) between an endothelial cell (*e*) and the tip of pericyte (*pc*) pseudopodium. Note the asymmetry of all cell membranes which is similar to that in Fig. 12. Rat diaphragm prepared as in Fig. 6. × 210,000.


e
bm2
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bm2
p2

All these considerations concern the small pore system. The large pore system (73) which is assumed to consist of opened channels of 400–700 A diameter presumably occupies such a small fractional area ($\sim 3 \times 10^{-5}$) that its detection by simple morphological analysis is impractical. The following paper identifies the structural equivalent of this system by using a tracer molecule of appropriate dimensions.

Middle Tunic

BASEMENT MEMBRANE: For capillary permeability, the important component of this tunic is the basement membrane which appears as a continuous sheet of tightly matted fibrils (each ~50 A thick) embedded in an amorphous matrix. It is not known to what extent this appearance is affected by our preparation procedures; they may coarsen an in vivo finer gel-like structure. The degree of porosity of the basement membrane is not clearly apparent in sections. It is known, however, from previous experimental work that it varies with the type of capillary. It retains rather efficiently ferritin (diam. ~110 A) in glomerular capillaries (37), but it does not retain particles smaller than ~200 A in venous capillaries and venules of skeletal muscle in which the endothelium is rendered discontinuous by topical application of histamine or serotonin (77, 78).

The fine fibrils of ~100 A diameter seen at the periphery of the basement membrane and throughout the adventitia were noted before in the connective tissue of the trachea (79), lung (80), aorta (81), myocardium (82) where Low described them under the name of "microfibrils," and more recently in the basement membrane of glomerular capillaries (37). Such fibrils have been studied in more detail in elastic tissues where they form bundles at the beginning of elastogenesis and "mantles" around elastic fibers at the end of the process (83). Evidence so far available suggests that they do not consist of collagen (83, 84). Our observations point out a number of characteristic features of these fibrils, namely their hollow profile (85), their ~200 A periodicity, and their frequent, but not obligatory association with elastic fibers. Structurally, they represent a common fibrillar constituent of capillary basement membrane and internal limiting elastica of arterioles and venules.

Morphologically, the basement membrane appears heterogeneous; biochemically, it consists, according to recent evidence, primarily of collagen or collagen-like protein (86, 87) with a relatively small glycoprotein admixture (88). The collagen-like protein has linked glucose-galactose chains (88). All these components are asymmetrical macromolecules expected to form fibrils. It is tempting to assume that the two types of fibrils described represent different molecular species. They could, however, together with the full-fledged collagen fibrils of the adventitia, represent different forms of aggregation of tropocollagen (see, however, reference 84).

PERICYTES: The pericytes, the other compo-

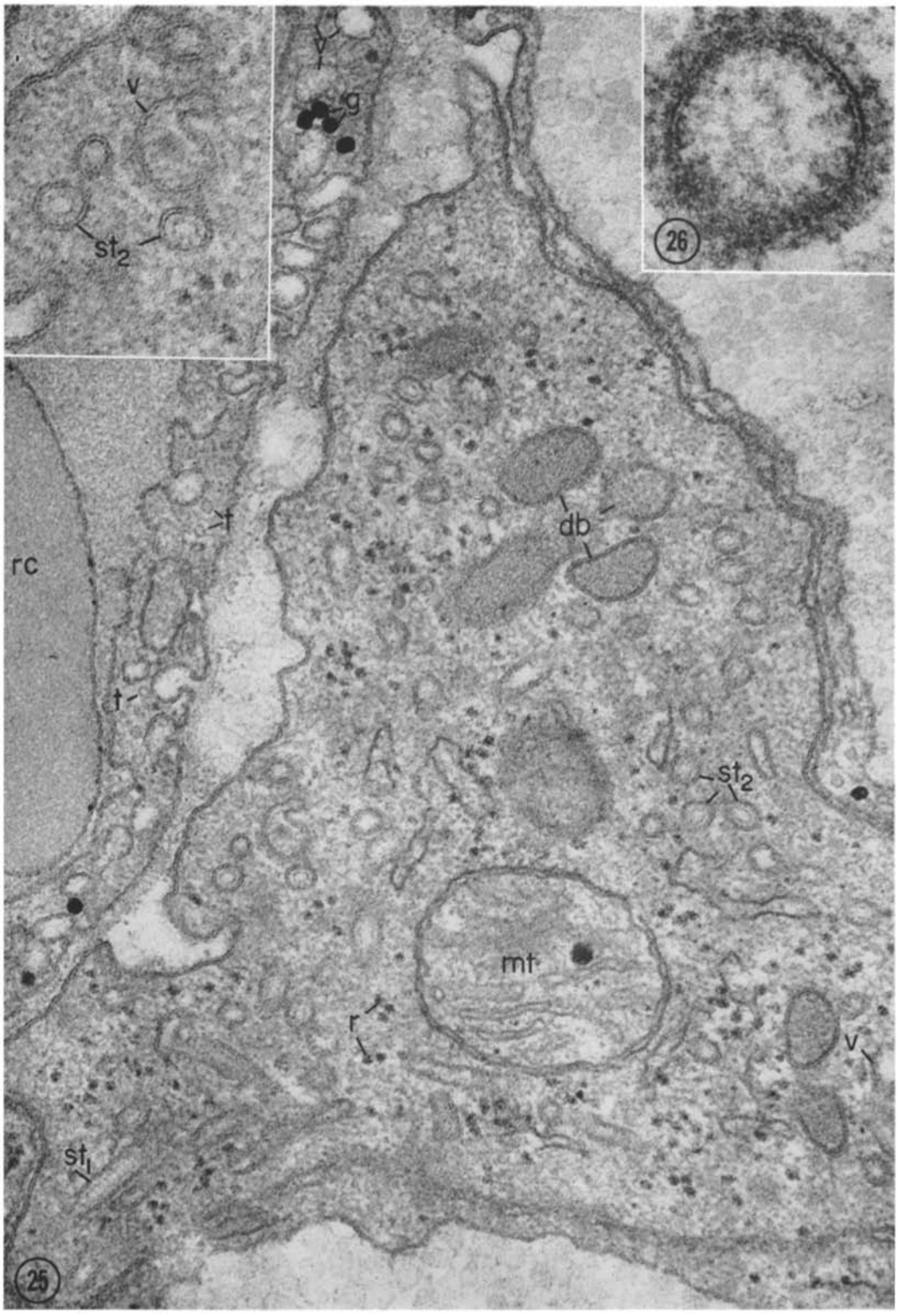
FIGURE 25 Macrophage in the adventitia of a blood capillary (rat diaphragm). The macrophage contains numerous membrane-bounded dense bodies (*db*)—probably lysosomes, mitochondria (*mt*), and numerous small vesicles (*v*) and tubules which appear in longitudinal (st_1) and transverse (st_2) section. The endothelial cell contains, as usual, numerous vesicles (*v*) and a few glycogen particles (*g*). It also contains a number of microtubules (*t*) which appear in cross-section.

Specimen prepared as for Fig. 1. × 74,000.

Inset: Higher magnification of a group of membrane-bounded profiles from the same cell. Some of them represent vesicles (*v*), others, cross-sections of tubules (st_2) which appear to have a regular inner coating. Such coated tubules are not frequently encountered in the macrophages of higher animals. They have been seen, however, in phagocytic cells in insects (41). × 133,000.

FIGURE 26 "Coated" vesicle of a type frequently found in macrophages. The outer (or cytoplasmic) surface of the vesicle is covered with a ~200-A-thick corona consisting of moderately dense "spikes." Dense material of unknown nature is attached to the inner surface of the vesicle.

Specimen prepared as in Fig. 1. × 198,000.


v
g
st2
26
rc
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25

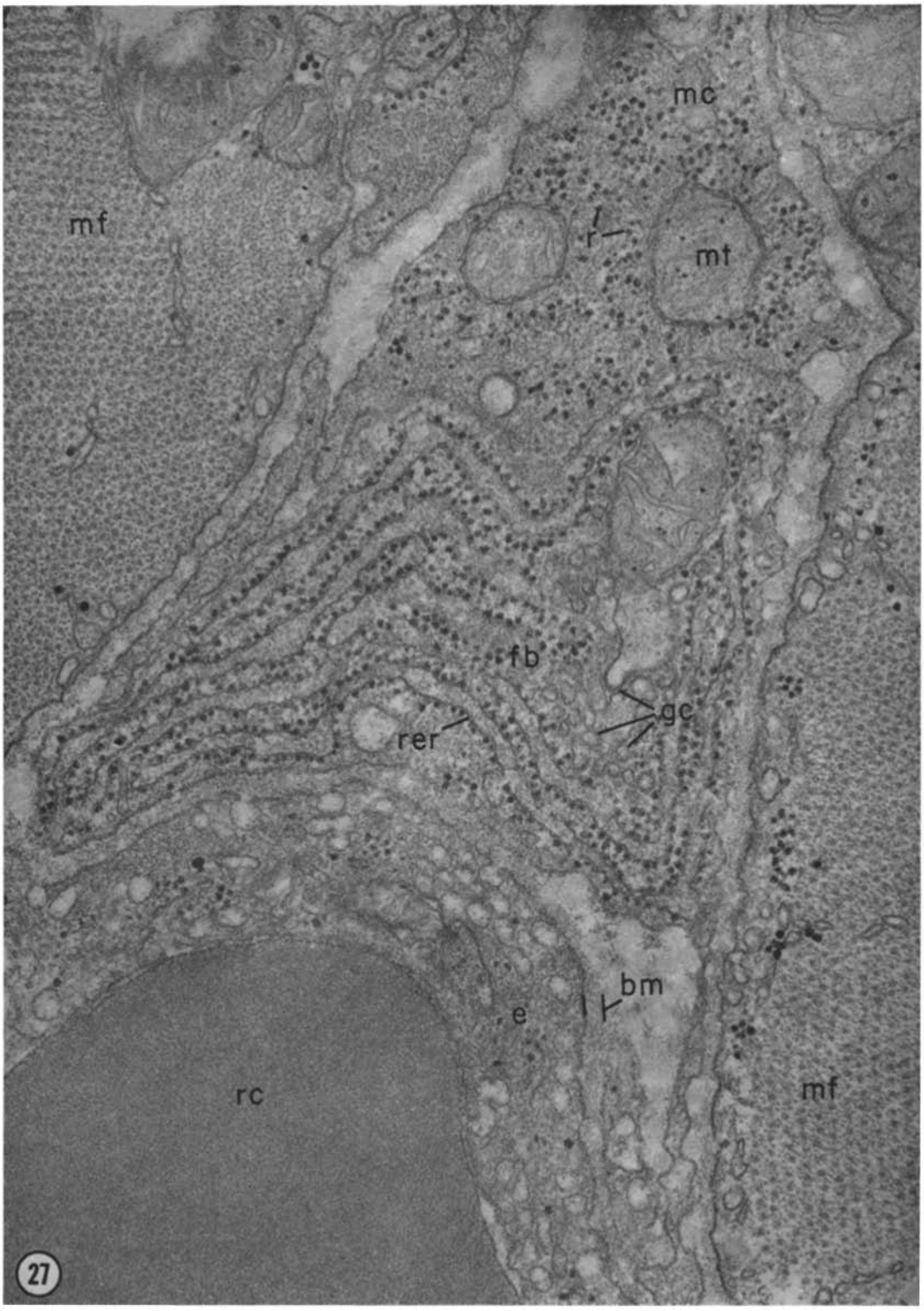

FIGURE 27 A fibroblast (*fb*) and a macrophage (*mc*) in the adventitia of a blood capillary of the diaphragm (rat). The fibroblast characteristically contains a large number of rough-surfaced cisternae (*rer*) and a cluster of smooth-surfaced vesicles probably marking the periphery of a Golgi complex (*gc*). The macrophage has a large number of free ribosomes (*r*) which suggest that the cell is not yet fully differentiated. Specimens prepared as in Fig. 1. × 74,000.

nent of the middle tunic, have been considered contractile elements by Bensley and Vimtrup (89) who showed that these cells contract when electrically stimulated in the nictitating membrane of the frog. Zweifach (90), however, did not obtain a similar response by mechanical stimulation (micromanipulation) in the mesentery of the frog. Some structural features of the pericyte (i.e., the fibrils of their matrix, the glycogen reserves, and the relatively numerous mitochondria) suggest that these cells rather than the endothelia are contractile, but experimental proof is evidently needed to solve this problem. The location of these cells in between leaflets of the basement membrane has been suggested already by light microscope studies (91) and confirmed by electron microscopy (92, 93). It has also been shown that pericyte pseudopodia penetrate in places the basement membrane, to come into direct contact with the endothelium in dermal (92) and retinal (93) blood capillaries. Our observations extend these findings to muscle capillaries and show that the membrane of the penetrating pseudopodia is frequently fused to the endothelial cell membrane in an occluding macula. As elsewhere in the body (94–96, 72), such maculae may represent sites of low resistance coupling, in this case, between pericytes and endothelia. Recently, areas of membrane fusion between endothelial and smooth muscle cells have been described by Rhodin (44) in arterioles.

Adventitia

Because of the gross discontinuity of the outer tunic, its cellular and fibrillar elements cannot play a direct role in capillary permeability. Yet the function of most adventitial cells appears to be related to the vessel: macrophages are active in monitoring the fluid that diffuses or filters through the capillary wall, and in removing therefrom foreign particles, including exogenous proteins (see reference 6). Mast cells presumably participate in the control of capillary permeability by the vasoactive amines they can release (97, 98). Finally, fibroblasts are probably involved in morphogenetic processes, like the renewal of basement membranes and that of the collagen fibrils which form the outer support of the vessel. When these aspects are taken into consideration, all these elements form a functional unit together with the inner and middle tunic of the capillary.

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