The Fine Structure of Endothelium of Large Arteries*

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ABSTRACT

Endothelium of large arteries from several species was studied in thin sections with the electron microscope. Before sacrifice, some animals received an intravenous injection of colloidal thorium dioxide which was visualized in the sections. Surface replicas were prepared by carbon evaporation on either frozen-dried endothelium or on endothelium dried by sublimation of naphthalene with which the tissue had been impregnated. Cell boundaries, stained with silver, were observed in sections and also from the surface by stripping off the inner part of the endothelium.

In addition to the usual cytoplasmic organelles, the endothelial cells showed certain characteristic features, namely, large invaginated pockets communicating with the arterial lumen, numerous much smaller vesicular structures immediately under the plasma membrane and apparently also communicating with the lumen, and inclusions, into which injected thorium particles were incorporated. Intercellular boundaries appeared as regular double membranes in thin sections, and they were outlined by a double row of silver granules after silver staining. No evidence was obtained of permeation of intracellular spaces by colloidal thorium.

The structure of the endothelium of capillaries has been studied with the electron microscope (1, 2), and the finding of tiny invaginations of the plasma membrane has suggested a mechanism to provide for the transmural movement of blood-borne substances. The same features were found in endothelial cells of small arteries of heart muscle (2). That transmural movement of certain lipides may take place in large arteries has been established with histological and chemical methods (3, 4), and that trypan blue may pass into the media of arteries devoid of vasa vasorum in their inner two-thirds has been observed (5, 6). The present work was undertaken to study the fine structure of the endothelium of large arteries in the light of its known permeability to such substances.

Methods

Arteries were obtained from 30 rats, 10 rabbits, 3 puppies, 1 cat, and 3 ferrets. Usually only the thoracic aorta was studied but sometimes the femoral and splenic arteries as well. The most satisfactory method of fixation was as follows. Under nembutal anaesthesia the desired segment of artery (usually ½ to 1 cm. in length) was quickly removed to a small Petri dish containing ice cold fixative (1 per cent osmium tetroxide buffered to pH 7.3, as recommended by Palade, 7). A Pasteur pipette was quickly introduced into one cut end, and fixative sucked back and forth for several minutes through the lumen. Fixation was then continued by immersion for 1 hour. The tissue was washed in water briefly and placed in 70 per cent alcohol, usually overnight, cut into small blocks, dehydrated in graded alcohols, infiltrated with and embedded at 45°C. in *n*-butyl methacrylate, containing 5 per cent methyl methacrylate and 0.5 per cent benzoyl peroxide. Sections were cut on a Porter-Blum microtome and examined with a Philips EM 100A electron microscope.

In order to study the uptake of particulate matter in the endothelium, many of the animals were given an intravenous injection of colloidal thorium dioxide (thorotrast[1]) ½ to 24 hours before sacrifice. This material was diluted with about 5 volumes of 6 per cent dextran in saline (intradex[2]), and the dose was equivalent to 5 cc./kg. of the original thorotrast.

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[1] Testagar & Co., Detroit.

[2] Glaxo Laboratories, Toronto.

To study the intercellular boundaries, a modification of the classical vital silvering technique was used, in which a segment of aorta from a heparinized animal was opened and pinned to a piece of cardboard, washed for 30 seconds with 5 per cent glucose, immersed 30 seconds in 0.4 per cent silver nitrate, washed 60 seconds in 5 per cent glucose, then fixed in the sunlight in slightly alkaline 10 per cent formalin for several hours. Some tissue so treated was embedded in methacrylate and sectioned in the usual manner. Other pieces were used to prepare thin films believed to contain only the innermost part of the endothelium, including the silver lines. The films were made by dehydrating the fixed and silvered tissue to 70 per cent alcohol, then placing for only a minute or so in absolute alcohol, after which the endothelial surface was painted with a 0.5 per cent solution of celloidin in amyl acetate. When this had dried very slightly the surface was coated with glyptal[3] cement, and the preparation allowed to dry until the cement was of rubbery consistency. The coating was then gently stripped from the surface, carrying the silver lines with it. The film so obtained was placed on a formvar-coated grid, the glyptal cement removed with benzene, and the preparation examined in the electron microscope.

Surface replicas of the endothelium were prepared by the following method. A segment of aorta fixed either with osmium by the usual method or by perfusion with 10 per cent formalin at a pressure of 100 mm. Hg, was dried either by freeze-drying at −40°C., or, more conveniently, by infiltrating it with naphthalene, which was then sublimed under vacuum. In the latter method the fixed tissue was dehydrated in graded alcohols, passed through benzene, then benzene saturated with naphthalene, and finally placed for a minute or so in melted naphthalene at 80°C. It was quickly picked out on the liquid naphthalene and placed under a vacuum of 0.5 mm. Hg for about an hour. Having obtained the dried tissue by either method, a carbon replica was made (8), the tissue digested for a few hours with 10 N sodium hydroxide at 45°C., the carbon replica washed by transfer to distilled water, picked up on unfilmed copper grids, dried, and shadowed with palladium.

OBSERVATIONS

The endothelial cells, particularly in the large muscular arteries such as the femoral, were ballooned out into the lumen, and their outer margins extended into the accordion-like folds of the internal elastic lamina. The aortic endothelial cells showed these features to a lesser degree, presumably because of the relatively smaller amount of contraction of the aortic wall on removal from the animal (Fig. 1). Perfusion of fixative at 100 mm. Hg via the aorta rendered the endothelium extremely thin (0.5 to 1.5 μ), with very much more flattened nuclei. However, although this might possibly represent a more life-like state of these cells, preservation of cellular details was not satisfactory.

In the 5 species studied, the endothelial cells showed essentially the same characteristics. The endothelium lay either directly (Fig. 2) on the internal elastic lamina or on a fine network of unit fibrils of collagen (Figs. 1 and 6).

The major part of the cytoplasm of the endothelial cells (Fig. 2) was rather light and contained finely distributed granular material, relatively few mitochondria, numerous small oval or circular, smooth surfaced profiles, more irregular granular profiles of the endoplasmic reticulum, occasional inclusions or vacuoles, and the membranes of a Golgi apparatus. The plasma membrane was prominent, with a thickness of 50 to 100 A. Associated with its surfaces, luminal, outer, and intercellular, were numerous small vesicular structures, frequently appearing as invaginations of the plasma membrane. They measured 300 to 500 A across. Their very great number was best appreciated in replicas (Fig. 10) and in sections cut almost parallel to the surface (Fig. 3). Such sections also showed the relatively greater density of the cytoplasm immediately adjacent to the plasma membrane. In this dense type of cytoplasm most of the vesicles were situated. Much larger invaginations of the plasma membrane, better described as pockets rather than vesicles, were also observed in sections (Figs. 1, 2, 4, and 5), and like the main part of the plasma membrane, these were often studded with the small vesicles. They were in open communication with the arterial lumen. Because they may extend well into the cytoplasm, they must effectively reduce the path of materials being transported across these cells.

Also in the cytoplasm were a number of relatively dense bodies, limited by a single membrane and about the size of mitochondria, but having no such distinctive internal structure. They were found to contain particles of thorium dioxide when this was given 3 to 24 hours before sacrifice (Figs. 1, 2, and 6). They were thus demonstrated to be inclusions and concerned with phagocytosis, but the nature of the dense material they contained is not known. The mode of entrance of thorium

[3] Canadian General Electric Co. Ltd., Toronto.

particles into the inclusions and their fate are still under study. Particles were not observed in the small vesicles at the surface, although they were frequently seen in the larger pockets.

Intercellular boundaries were studied in sections, with and without silvering, and in spreads of the inner part of the silvered endothelium. In sections cut normally (Figs. 4 and 6), the intercellular boundaries consisted of a light space enclosed by the dense plasma membrane of each cell. The space measured 70 to 200 A across. Frequently the bordering margins of adjacent cells appeared to overlie each other (Fig. 1), but this was a consequence of contraction of the artery. When fixed by perfusion, the cells had straight margins running perpendicular to the internal elastic lamina. Treatment with silver revealed a deposition apparently on each plasma membrane almost entirely confined to the points of contact between the cells (Fig. 9). When viewed from the luminal surface in the spreads of silvered preparations, a double line of silver was also sometimes seen (Fig. 8). The silver appeared as coarse granules outlining the cells, but in addition, showed ring-shaped deposits on the surface of the cells (Fig. 7). These may possibly represent deposition around the mouths of the large pockets projecting into the cytoplasm from the plasma membrane. The intercellular boundaries were also seen in replicas (Fig. 10), especially after fixation by perfusion, when they appeared as wavy, depressed lines.

DISCUSSION

The structure of the endothelial cells of the large arteries corresponds closely to that described by Palade (1, 9) and Moore and Ruska (2) for capillary endothelium. Both contain the numerous small vesicles or invaginations of the plasma membrane and the same general cellular structures. The endothelial cells of capillaries seem to possess no structure not shared by those of arteries. Presumably, if the capillaries utilize the small vesicles for the transport of fluids (pinocytosis) as suggested, the arterial endothelium does also.

Blood vessels such as the normal aorta of man have a capillary blood supply only in the outer one-third of the media (10, 11). Casual observation of the aortic media of rats and rabbits suggests that this tissue is largely devoid of capillaries in these species as well. The muscle and connective tissue cells of part of the media must, therefore, effect their metabolic exchanges through the arterial endothelium. Direct evidence that this function is concerned with the small vesicles of either capillary or large arterial endothelium is still lacking.

Unfortunately, colloidal thorium can provide no information on the possible function of the small vesicles, because the particles of hydrophobic colloids, including thorium, tend to become agglutinated in the blood. Thus, although the size of the individual particles in the original suspension is less than that of the openings of the small vesicles, particles do not enter them because of aggregation.

Lipoprotein may, perhaps, enter the cells in a molecular form through the small vesicles. However, in the case of cholesterol-fed rabbits, the lipoprotein induces such a reaction that the endothelial cells can no longer be considered normal. The reaction to cholesterol feeding is the subject of a paper to be published elsewhere.

That colloidal particles or aggregates of colloidal particles can pass into the cytoplasm of endothelial cells is shown by the incorporation of thorium dioxide less than 3 hours after its intravenous administration. The particles are confined to membrane-bounded vacuoles, so that the process appears to be one of phagocytosis. The amount of thorium taken up is quite small, and it is not surprising that Duff, McMillan, and Lautsch (12), studying histological sections, failed to find an uptake of thorotrast in the aortic endothelium of normal rabbits. It was detected, however, in atheromatous lesions of cholesterol-fed rabbits.

Under the conditions of the present experiments, particles of thorium dioxide were not found in intercellular spaces. The intercellular boundaries must be regarded as non-permeable, at least to the aggregated particles. The view supported by Chambers and Zweifach (13) that capillaries are permeable to particulate matter, partly on account of the intercellular cement, apparently cannot be applied to normal arterial endothelium.

It has been observed that in sections stained with silver nitrate, the silver produced a deposit throughout the entire extent of the cells' interfaces. Thus the intercellular cement lines exist as bands with two dimensions. The plasma membrane on the luminal surface shows a negligible amount of silvering. The localized nature of the silver deposit suggests either that a relatively freer pathway is offered for the silver along the intercellular boundary or that there is present some material which has a specific affinity for it. The latter view is the

one generally held (14). There is assumed to be a reaction between silver and intercellular cement substance. The silver deposits consist, usually, of a double band, each half of which corresponds to the entire interface between the cells. It is possible that if a single band of silver were deposited initially between the cells, it might have been pulled apart during fixation to create the appearance of a double band. However, the double line might also suggest that the silver reacts with the plasma membranes, rather than with the material contained in the light space between them, assumed to be intercellular cement. It may be that the silver combines loosely and non-specifically with the plasma membrane of the luminal surface as well, but is removed from this more exposed part in the subsequent washing step. Another possibility is that the silver reacts, not with plasma membranes or intercellular cement, but with a part of the cytoplasm in the form of terminal bars or desmosomes. The relatively wide nature of the silver deposits (by comparison with the plasma membranes) lends some support to this suggestion. Obviously, it would be unwise to be dogmatic about the precise location of the silver deposit. Precise localization is made difficult by the coarse nature of the silver granules and by the necessity of using formalin rather than osmium tetroxide, as a fixative.

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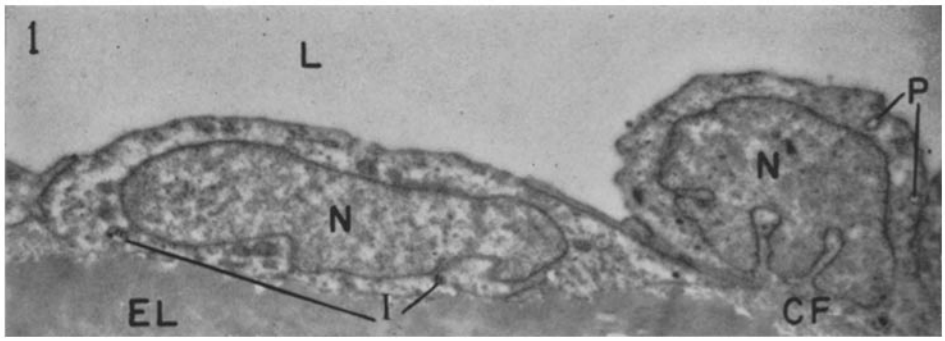
Explanation of Plates

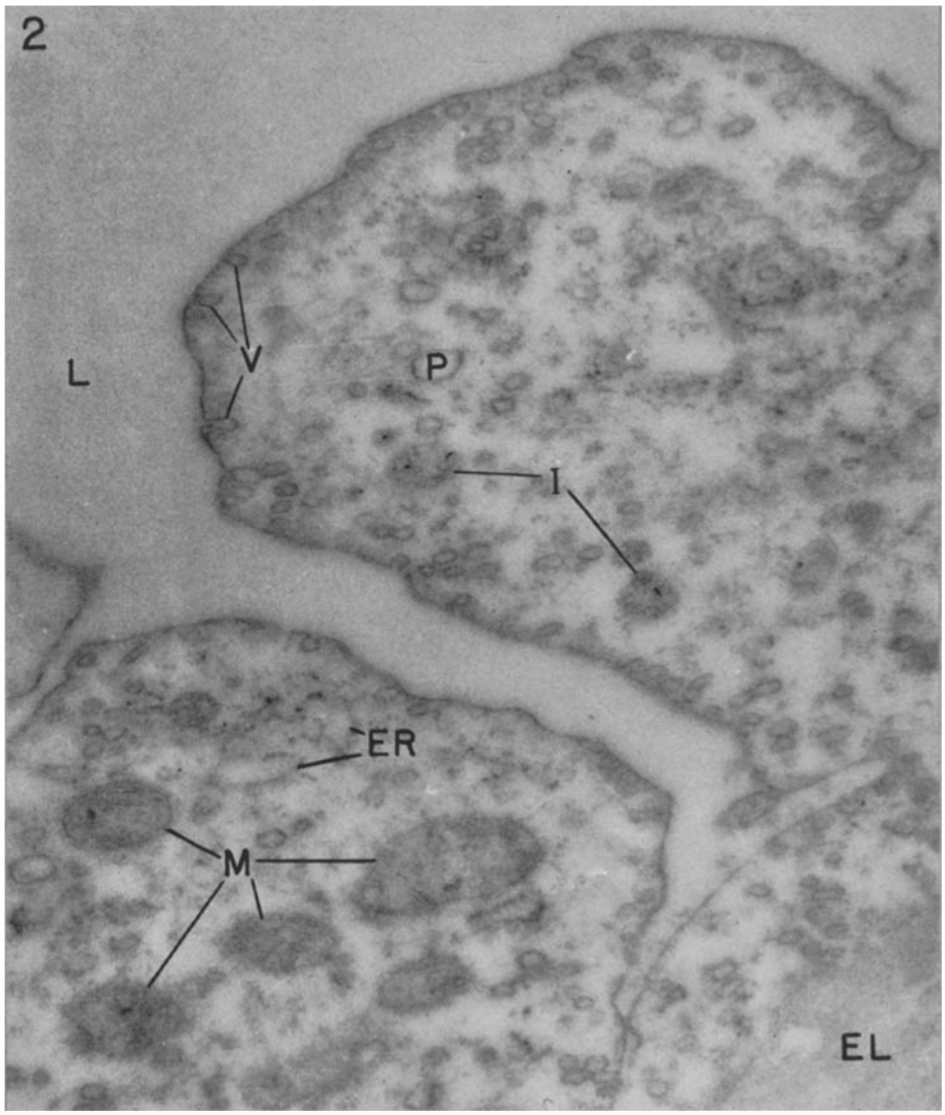
L, lumen of artery.
N, nucleus.
EL, internal elastic lamina.
CF, collagen fibrils.
I, inclusion.
P, pocket or large invagination of plasma membrane.
V, vesicle, or small invagination of plasma membrane.
ER, endoplasmic reticulum.
M, mitochondrion.

Plate 95

Fig. 1. Endothelium of rabbit aorta. When fixed by immersion, the endothelial cells bulge into the lumen and tend to overlie each other as shown here. The cells rest apparently directly on the internal elastic lamina (*EL*), or on some fine collagen fibrils (*CF*). Two large invaginations of the plasma membrane, or pockets, are shown at *P*. Thorotrast (12 cc.), administered intravenously 3 hours before death, is seen in inclusions (*I*). × 11,000.

Fig. 2. Endothelial cell cytoplasm of rabbit aorta. The plasma membrane shows many small invaginations or vesicles (*V*). A single pocket (*P*) of the plasma membrane is seen. The endoplasmic reticulum (*ER*) includes fine granules, sometimes arranged in irregular ring profiles. Mitochondria (*M*) contain a dense matrix and few cristae. Inclusions (*I*) are indicated by their incorporation of thorotrast administered intravenously 24 hours before death. × 47,000.





(Buck: Fine structure of endothelium)

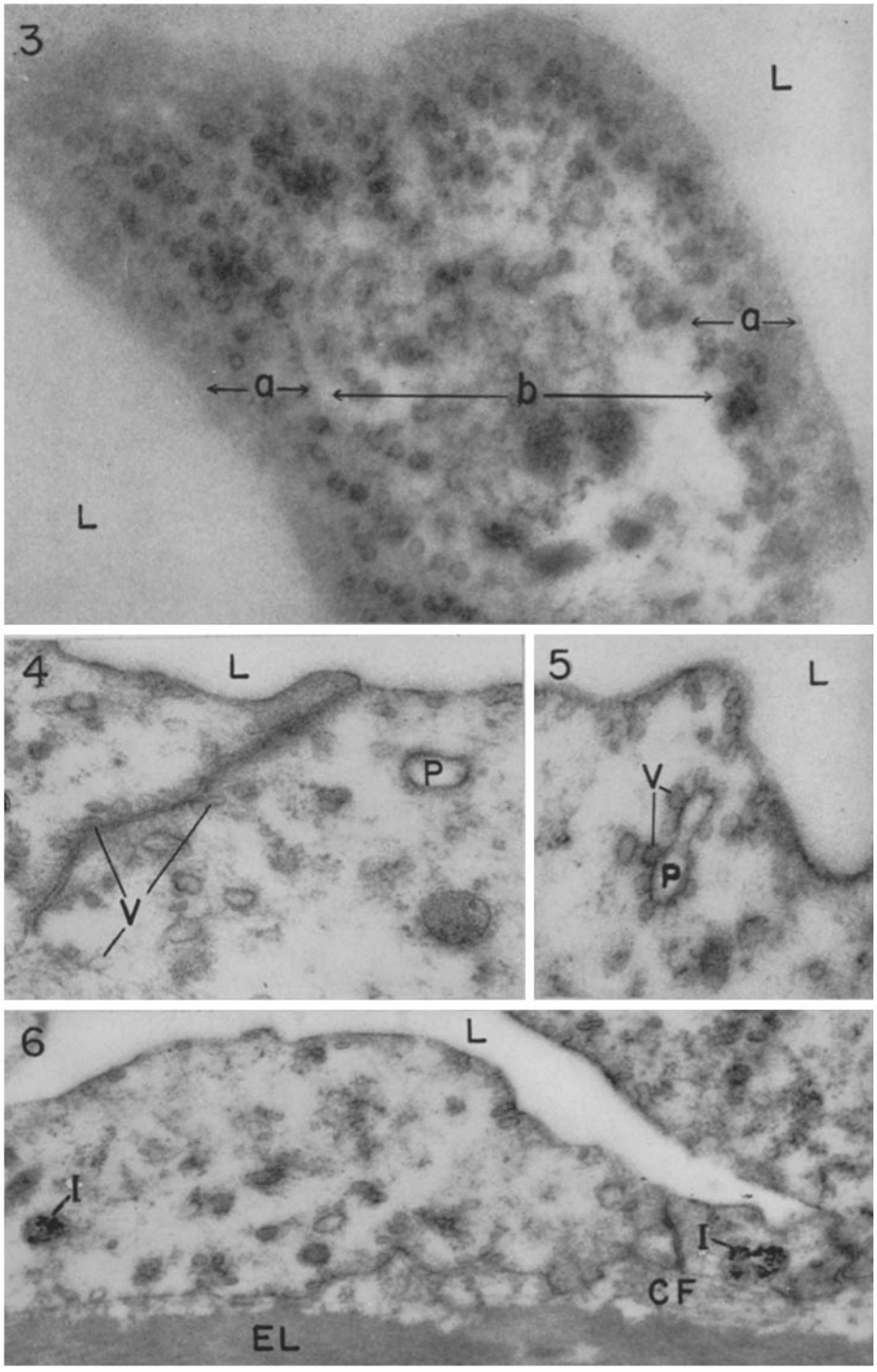
PLATE 96

FIG. 3. Section of rat femoral artery cut almost parallel to the endothelial surface. When the cell is cut at this angle, the great number of vesicles under the plasma membrane is appreciated. They are numerous in a cortical layer of dense cytoplasm (*a*), and relatively sparsely scattered in the interior of the cell (*b*). × 36,000.

FIG. 4. Endothelium of rat aorta, showing boundary between two endothelial cells. Vesicles (*V*) are seen along this boundary. × 42,000.

FIG. 5. Endothelium of rat aorta, showing a large invagination or pocket (*P*) of the surface plasma membrane, having vesicles (*V*) arising from it. × 53,000.

FIG. 6. Endothelium of rabbit aorta 3 hours after intravenous administration of 15 cc. of thorotrast. Aggregations of thorotrast particles are seen in inclusions (*I*). × 37,000.



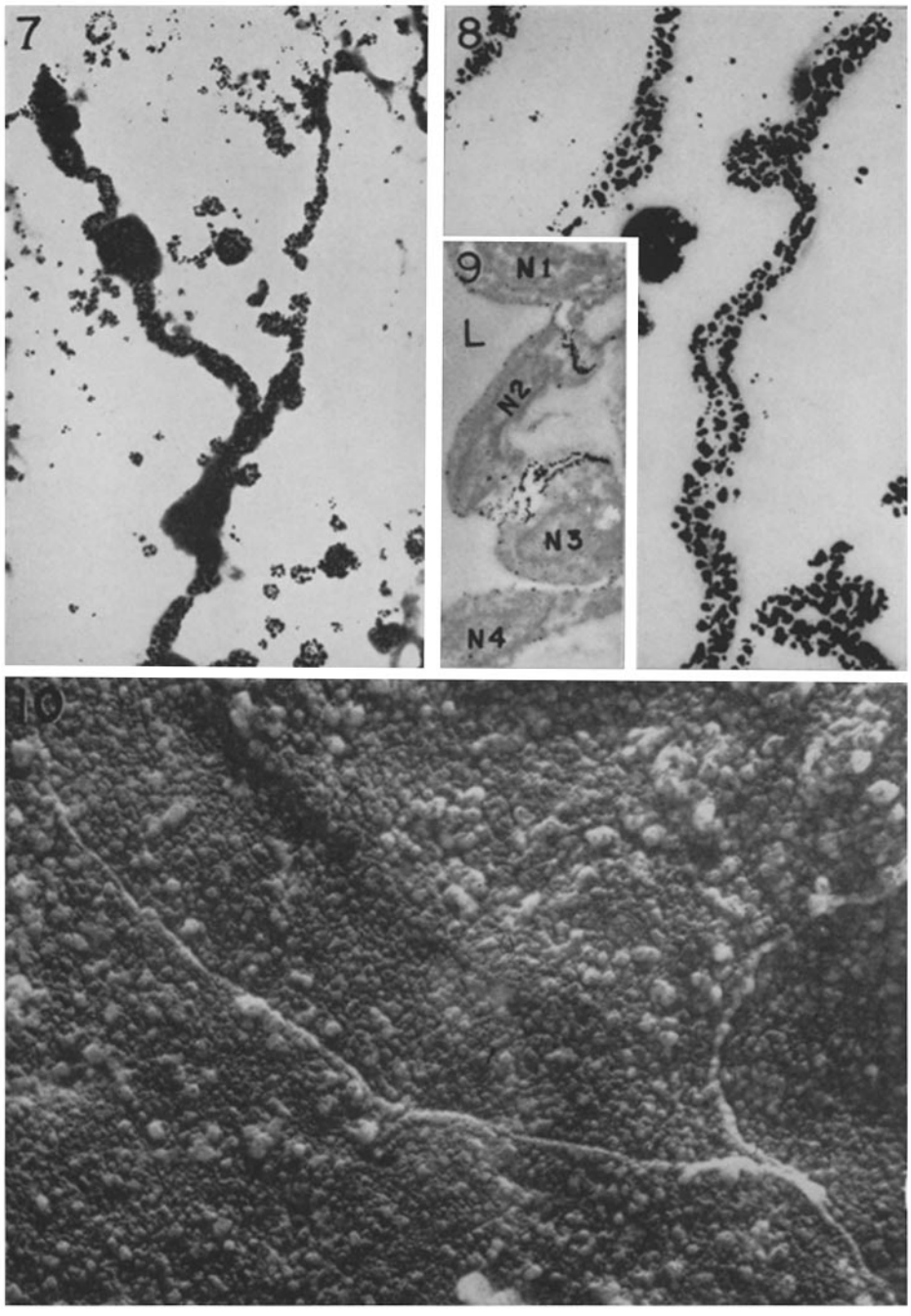
(Buck: Fine structure of endothelium)

PLATE 97

FIGS. 7 and 8. Surface spreads of rabbit aortic endothelium, showing silver staining at intercellular boundaries (stained supravitally with silver nitrate). Fig. 7 shows that intercellular boundaries are visualized by the deposit of silver. In addition, a number of ring-shaped deposits are seen on the cell surface. × 5,000. Fig. 8 shows that the intercellular deposits may appear as double rows of granules. × 9,000.

FIG. 9. Section of rabbit aortic endothelium after silver nitrate treatment. Fixation with 10 per cent formalin. Four endothelial cell nuclei are shown and two intercellular boundaries. The latter appear as a double line of silver granules extending from the surface to the outer margin of the cells. × 3,000.

FIG. 10. Palladium-shadowed carbon replica of the surface plasma membrane of aortic endothelium from a young dog. Boundaries between three cells are shown. The surface of the cells is highly nodular and appears to reflect the great number of vesicular invaginations of the plasma membrane. Fixation by perfusion with formalin, drying by sublimation of naphthalene. Negative print. × 24,000.



(Buck: Fine structure of endothelium)