evolution of the atherosclerotic plaque

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Evolution of the Atherosclerotic Plaque

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Preface

This volume is a collection of the presentations given at an international symposium held in Chicago, March 28–29, 1963, under the joint sponsorship of the Chicago Heart Association and the Council on Arteriosclerosis of the American Heart Association. This symposium was dedicated to an evaluation of the current concepts of the origin, growth, and final morbid disposition of the atherosclerotic plaque. The reader needs no reminder that it is this lesion of arteriosclerosis which, at the same time that it provides the greatest threat to life, allows the most hope for its reversal. Knowledge of the cause of this pathological entity is essential to its rational treatment or prevention.

Acknowledgments

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The smooth operation of this symposium was due in no small part to the efforts of Dr. Margaret Brookes and her assistants from the Chicago Heart Association staff. The symposium arrangements were handled by Dr. Louis Cohen, Dr. Seymour Glagov, and Dr. Angelo Scanu of the committee. The editor is also indebted to Miss Lilian Roberts, who assisted in handling the correspondence preceding the symposium as well as helping in the preparation of the final manuscript. The discussion periods were promptly edited at the time of the meeting by the contributors, who also facilitated the early publication of the symposium proceedings by their co-operation in the rapid transmission of manuscripts and copy.

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Part I

Fine Structure of the Plaque

Fine Structure of the Vascular Wall

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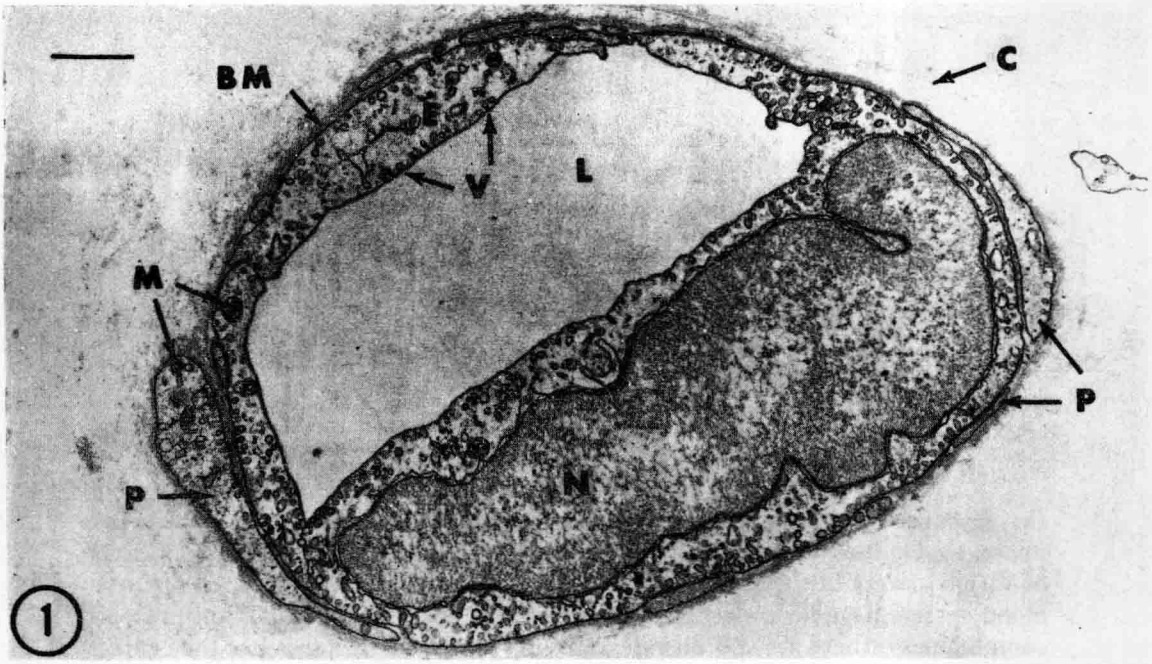
This symposium concerns the normal structure and pathology of the vascular system. As a visual refresher, I shall present a series of electron micrographs illustrating some of the normal structural features of blood vessels of various sizes. Little novelty will appear, since most of the features of these blood vessels have been described already. Nor does this presentation pretend completeness; there are special categories of vessels, such as those of the central nervous system and skin, that are omitted. The tissues represented here come from the rat, mouse, or frog, fixed an hour or two with buffered osmium tetroxide;[1] imbedded in an epoxy resin;[2] stained with lead,[3] phosphotungstic acid, or uranyl acetate; and examined in a Siemens Elmiskop I or RCA-2C electron microscope.

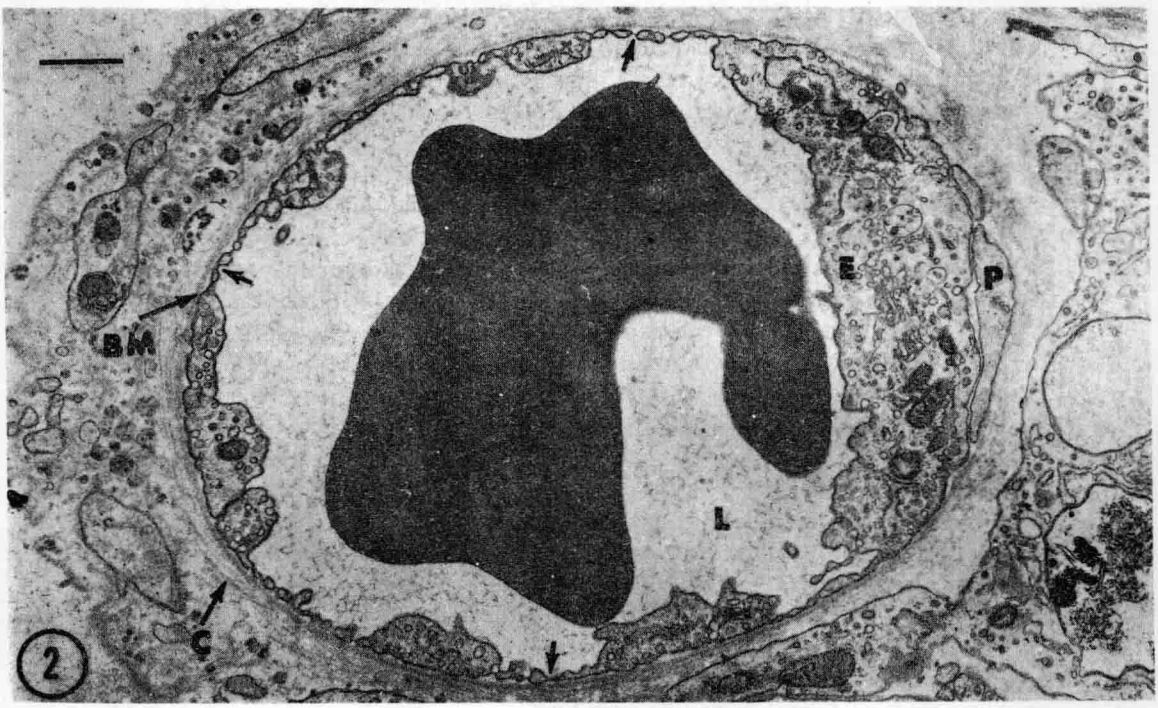
Several years ago Bennett, Luft, and Hampton[4] proposed a classification of capillaries intended to emphasize that three classes of structural elements existed in capillaries, namely, *endothelium*, *basement membrane*, and *pericapillary cells*. Variations in the distribution of these three components served to distinguish, for example, muscle capillaries from brain or intestinal capillaries. Palade[5] recently described the capillary wall as consisting of three concentric layers (endothelium, basement membrane, and adventitia). I propose to expand these categories to accommodate the larger blood vessels of vertebrates. From the vessel lumen outward, and in cumulative sequence with increasing complexity, these are:

1. Endothelium
2. A fibrillar, extracellular component composed of
   a) Fine (~40 A) filaments, such as occur in basement membrane
   b) Coarser fibers, such as reticular fibers (~400 A diam.) or collagen (~800 A diam.)
   c) Thick strands or sheets of elastin

This work was supported in part by grant No. H-2698 from the Public Health Service and by a grant from the Life Insurance Medical Research Fund.

PLATE I





The bar in each figure represents 1 micron.

1, Capillary from frog sartorius muscle. Two endothelial cells (*E*) with vesicles (*V*) enclose the lumen (*L*), one showing a nucleus (*N*). Mitochondria (*M*) are very small. Cytoplasm of pericapillary cells (*P*) surrounds part of the endothelium. The whole is enclosed by a feltlike basement membrane (*BM*). Lead. ×11,000.

2, Capillary containing an erythrocyte from mouse intestinal villus. Endothelium (*E*) of "perforated" type shows diaphragms at arrows. Basement membrane (*BM*) is complete with some reinforcing collagen fibers (*C*). Pericyte (*P*) abuts against thick endothelium. Lead. ×11,000.

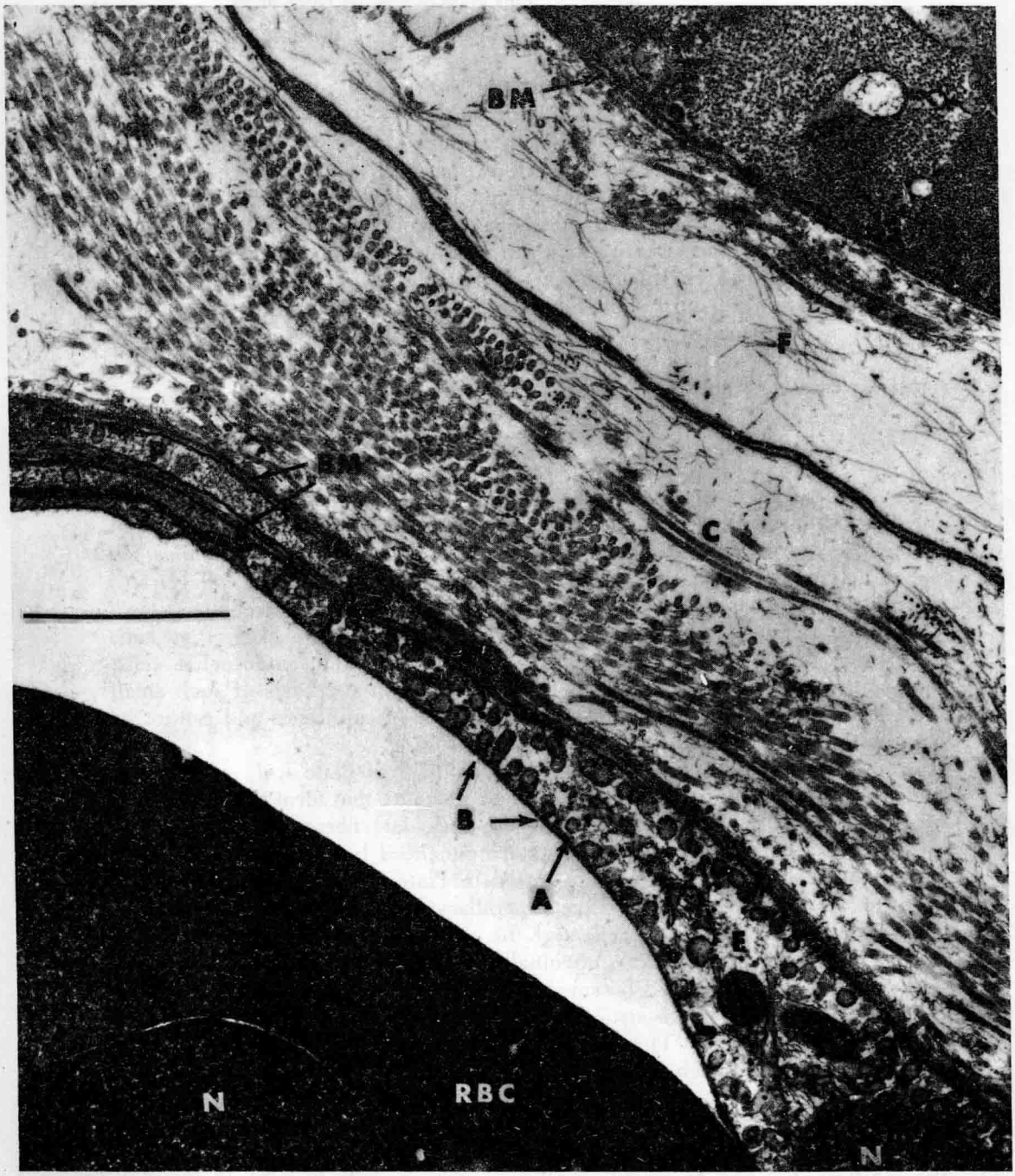
3. Supporting cells of common origin but of varying specialization as
   a) Pericytes around capillaries
   b) Smooth muscle cells
   c) Adventitial cells (epithelioid fibroblasts)

There is redundancy in that 1 and 3 are both cellular. I do not wish to defend the separation into categories. I regard all these cells, including mesothelium, as part of a continuum, and I hope to underline this feature with the illustrations. Also, there probably exist substances of major significance that are either not preserved or not visualized by current electron microscope techniques, such as mucopolysaccharides or mucoproteins. They would best fit in group 2a above. However, until such time as these ghosts materialize, we must be content with the small favors that we have been granted. The distribution given above will be considered for capillaries, arterioles and small veins, muscular arteries, and aortas.

Capillaries

The most frequent capillary on the basis of quantity is represented by that of skeletal muscle illustrated in Plate I, *1*. Minimal elements of the three major categories are present: two endothelial cells (*E*), a rather inconspicuous but nevertheless continuous basement membrane (*BM*) with a little collagen (*C*) of reticular size, and several fragments of cytoplasm, which are interpreted, or, rather, defined, as portions of pericytes (*P*). It is quite likely that some of these cytoplasmic profiles represent tongues of other endothelial cells, but I am unable to distinguish the two uniquely on the basis of such small samples. Pericapillary cells are a constant feature of capillaries and cannot be ignored by designating them all endothelium.[4, 6]

The basement membrane or "basement lamina"[7] of Plate I, *1*, is typical of the appearance after lead staining. The fine filaments that form this dense feltwork are much smaller than the collagen (reticular) fibers (*C*) that lie near them. Both collagen and the filaments are enhanced by double staining with uranyl acetate and lead. This is illustrated in Plate II, again taken from frog sartorius muscle, showing a portion of capillary with a nucleated erythrocyte, an edge of a striated muscle cell, and the connective tissue elements in between. The filaments (*F*) are individually detectable when dispersed but are easily lost in the condensed basement membrane. There is evidence that this basement membrane has a structural integrity of its own, as suggested by Birks, Katz, and Miledi.[8] These authors encountered in atrophic frog skeletal muscle a region in which the muscle and its sarcolemma had shrunk, leaving the basement membrane completely intact but floating by itself in the extracellular space. This same muscular basement membrane is seen at the upper right of Plate II. It appears to be qualitatively no different from that encircling the capillary, but the basement membrane of the capillary is at least twice as thick and probably correspondingly stronger. The thin, diagonal sheet



3, Capillary with portion of nucleated erythrocyte from frog sartorius. Cross section of striated muscle myofibrils appears at upper right. Both muscle and capillary have a basement membrane (*BM*) of filamentous appearance but more condensed than free filaments (*F*) in extracellular space. Two classes of vesicles, free (*A*) and attached to cell membrane (*B*), are illustrated. This capillary, as well as the one in Plate I, *1*, was incubated 2 hours in Ringer as an isolated muscle preparation before fixation. Uranyl acetate and lead. ×31,500.

of cytoplasm between muscle and capillary has no basement membrane and probably is part of a fibroblast.

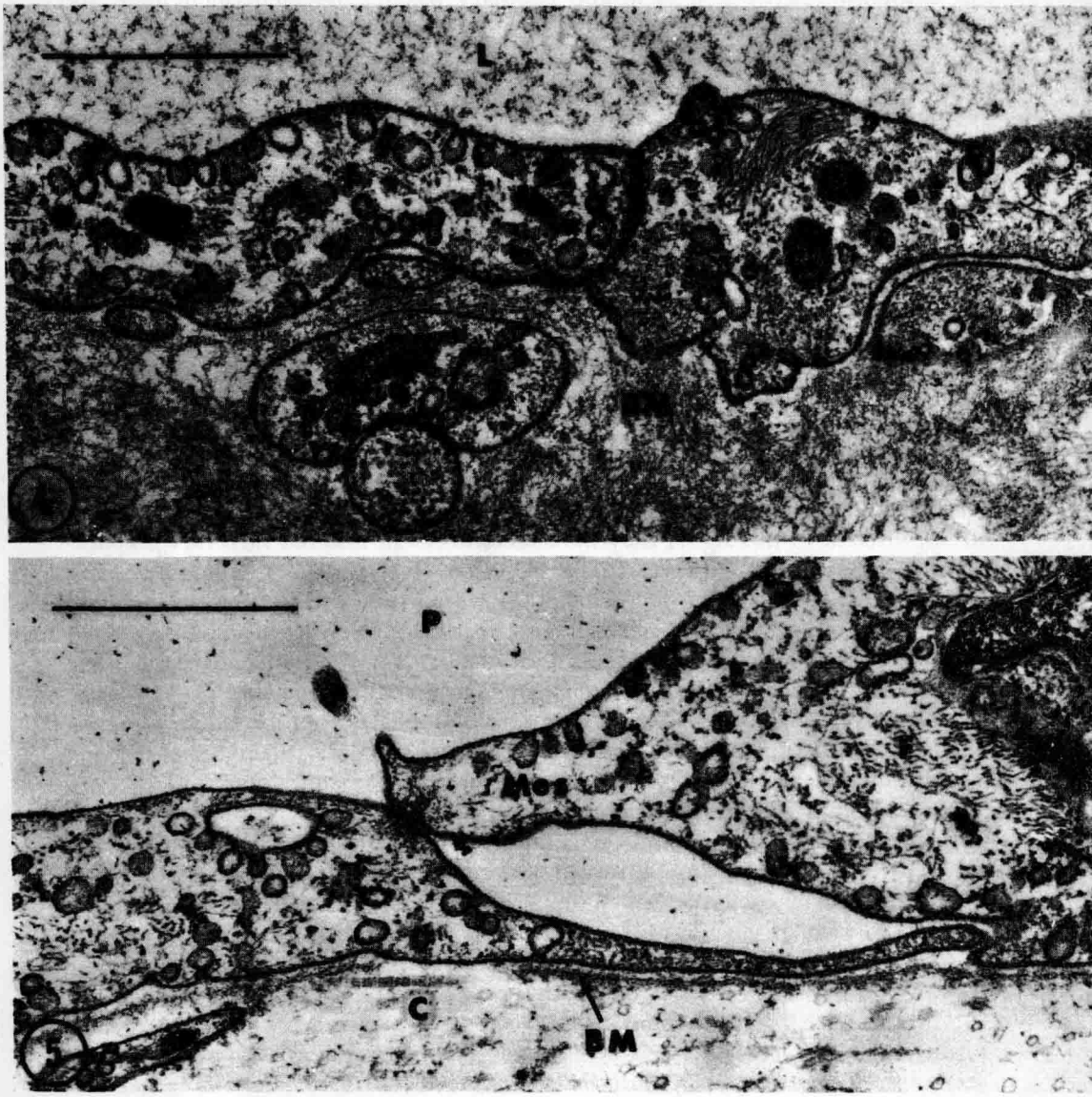
The endothelium has been the subject of numerous papers. Since 1953, when Palade[9] first described the vesicles in the capillary wall, all authors have commented on these cytoplasmic profiles, which are characteristic of, but by no means unique to, endothelium. These vesicles (*V*) are recognizable in Plate I, *1*, and in all subsequent pictures that show endothelium. The term "vesicle" is often applied both to completely membrane-bound outlines (roughly 400–500 A diam.) and to those sac-like invaginations of the plasma membrane of similar dimensions; these two varieties are illustrated by arrows *A* and *B*, respectively, in Plate II. To the latter group of vesicles Yamada[10] gave the name *caveolae intracellulares*. Regardless of the name used, there is no question of their existence. Concerning their function, there is much less certainty. Evidence resulting from experiments using dense colloidal particles as tracers suggests that the vesicles are involved in bulk transport across the endothelial wall.[5, 11, 12]

Some skepticism has been cast on the transport function of these vesicles by Brandt,[13] and in most cases with uninjured capillaries the accumulation of particles seems to be quite long—in the order of hours. Plate I, *1*, and Plate II are of interest in this light; they were taken from an isolated sartorius preparation maintained in amphibian Ringer solution. These capillaries had been two hours without a blood supply before they were fixed, and yet the complement of vesicles and caveolae is not different from that of a fresh capillary. Such stability of the vesicular component of capillaries does not seem consistent to me with the hypothesis of the vesicles' being engaged actively in a transport process.

There are other interesting details of endothelial structure, such as endothelial cell junctions, endothelial flaps,[14] and extracellular filamentous coats,[13] which must be overlooked for now. However, there is another important type of endothelium in capillaries of various endocrine glands, kidney glomeruli, and intestinal villi that should be mentioned. In these tissues the endothelium frequently appears to be quite thin and perforated in many places, as illustrated in Plate I, *2*, from mouse intestine. Actually, the perforations are only apparent, for a thin diaphragm or membrane is seen to close the pore. This feature can be seen at the arrows in Plate I, *2*, and is discussed in detail by Rhodin;[15] Pappas and Tennyson[12] present evidence of the integrity of the seal to colloidal materials.

The structure of endothelium does not appear to be unique. Vesicles, caveolae, intracellular filaments, intercellular contacts, and basement membrane are all seen in the layer of capillary endothelium (*E*) in Plate III, *4*. These same features are seen in Plate III, *5*, which is mesenteric mesothelium, although admittedly to different degrees. Both tissues are from the frog, and the pictures were taken within 100 microns of each other. Pericytes, smooth muscle cells, and even epithelioid fibroblasts of the adventitia of larger vessels or nerves all partake of the same features, although the fibroblasts usually have little or no basement membrane. I am unable to make a positive structural identification of endothelium out of histological context.

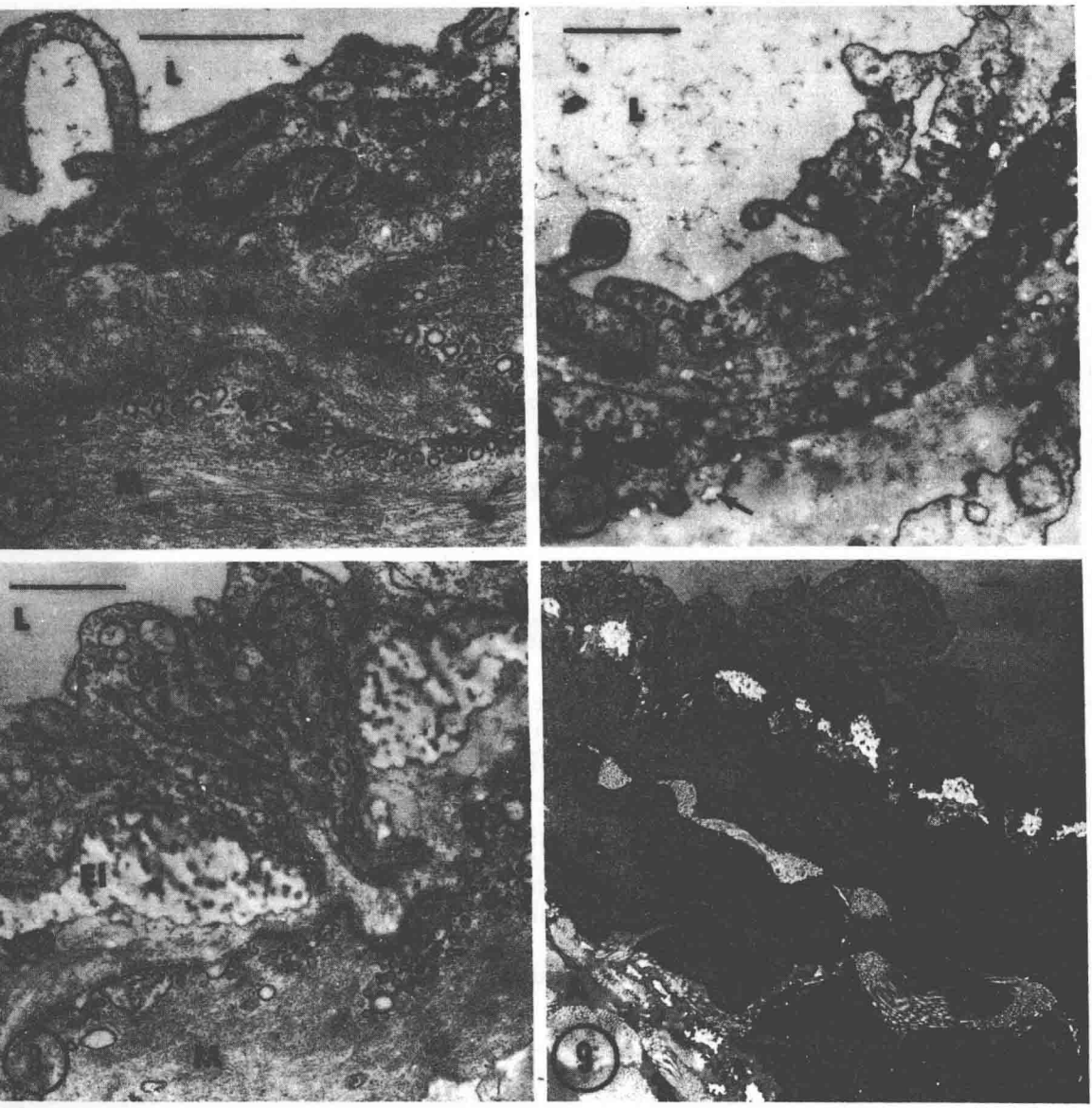
PLATE III



4, Endothelium from capillary in frog mesentery. Lumen (*L*) contains precipitated plasma. Junction between t endothelial cells (*E*) has a dense region near lumen. Filaments (*F*) occur in endothelial cytoplasm along with mi chondria and vesicles. Basement membrane (*BM*) is thick and finely filamentous. Lead. ×34,000.

5, Mesothelium (*Mes*) from frog mesentery faces peritoneal space (*P*). Junction between two mesothelial cells resemb that in *4*. Filaments (*F*) and vesicles are seen in cytoplasm. Basement membrane (*BM*) is condensed but shows def just above collagen fiber (*C*). Lead. ×34,000.

PLATE IV



6, Arteriole from frog mesentery. Two endothelial cells (*E*) make contact with flap developed from one. Basement membrane (*BM*) is diffusely filamentous. Smooth muscle cell (*M*) is cut obliquely, showing various planes of section through vesicles and caveolae. Arrow points to an unusual structure associated with smooth myofilaments. Lead. ×22,500.

7, Small vein from mouse. Arrows indicate small elastic filaments in the region of basement membrane and adventitia. Lead. ×16,000.

8, Cross section of femoral vein, mouse. Two large elastic fibers (*El*) underly the basement membrane. A smooth muscle cell (*M*) is also present. Lead. ×16,000.

9, Cross section of same vein showing vessel lumen (*L*), media with smooth muscle (*M*), and adventitia (*A*). Negative images show collagen as regular threads and elastic tissue white. Lead stained after examination in electron microscope. ×4,000.