GLOMERULAR PERMEABILITY

I. Ferritin Transfer Across the Normal Glomerular Capillary Wall*

By MARILYN G. FARQUHAR, Ph.D., STEVEN L. WISSIG,‡ Ph.D., and GEORGE E. PALADE, M.D.

(From The Rockefeller Institute)

Plates 9 to 19

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Although the structure of renal glomerular capillaries is now well established, little is known about the mechanisms involved in the transfer of substances across their wall from the capillary lumina to the urinary spaces. According to prevailing concepts in renal physiology, the formation of glomerular fluid takes place by passive ultrafiltration of blood plasma through a sieve-like capillary wall provided with rigid pores (1–3). An alternative view assumes that the process involved is one of diffusion across a capillary wall taken to be a gel composed of fibrils of molecular dimensions (4, 5). Neither of these hypotheses, based primarily on permeability studies, takes into account morphological information already available: namely, that the glomerular capillary wall is not composed of a single, homogeneous membrane, but is a multilayered structure.

Several suggestions as to the probable glomerular structure which constitutes the filtration barrier have also been proposed as a result of morphological observations: the majority of electron microscopists (*e.g.*, references 6–9) have assumed that the glomerular basement membrane acts as the main filter, because it is the only continuous layer found in the capillary wall. According to another hypothesis (10), the spaces between the foot processes of the visceral epithelial cells act as "slit pores" which allow free passage of water, small molecules, and electrolytes, but restrict the passage of proteins. Up to the present, however, there has been no direct experimental evidence to support either of these assumptions.

Our approach to this problem was to attempt to visualize directly the pathways across the glomerular capillary wall by introducing into the blood a par-

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‡ National Research Fellow in the Medical Sciences. Present address: Department of Anatomy, University of California, San Francisco.

ticulate tracer which is large and dense enough to be seen in the electron microscope. By following the progression of the tracer across the various layers, we hoped to define the role of each component of the glomerular capillary in the filtration process.

With these intentions, we tested a number of tracers—among them thorotrast, hemoglobin, colloidal gold, colloidal mercuric sulfide, and ferritin. We found ferritin to be superior as a marker to all other substances tried because of its size, recognizable structure, and the fact that it remained in the circulation at high concentration for periods of up to 2 hours. In this paper we will report in detail our findings on ferritin transport in the normal glomerulus, and in a subsequent paper we will give an account of our findings, briefly outlined elsewhere (11, 12), on ferritin transport in nephrotic animals in which glomerular permeability is greatly increased.

Materials and Methods

The observations reported in this study were made on kidney tissue from young adult rats given ferritin. The animals were all Sprague-Dawley males, with a body weight of 150

TABLE I

Time interval,* *min.*	2	5	7	15	60	75	120
No. of animals	1	4	1	5	2	1	1

* Length of time elapsing between intravenous ferritin and fixation of kidney.

to 400 gm. Two ferritin solutions were utilized: one was prepared from horse spleen by the method of Laufberger (13) and contained approximately 20 mg. protein per ml.; the other was a commercial preparation of horse spleen ferritin containing approximately 50 mg. protein per ml., (obtained from Nutritional Biochemicals, Cleveland).

Experimental Procedures.—Ferritin was given to anesthetized animals *via* the saphenous or femoral vein; the dose injected corresponded roughly to 1 cc. ferritin solution per 100 gm. body weight. Kidney tissue was fixed at intervals of 2 minutes to 2 hours following the injection and subsequently prepared for electron microscopy. A total of 15 rats were used: for 11 animals the time period between ferritin administration and fixation of tissue was 2 to 15 minutes, and for the other 4 this interval was between 1 and 2 hours. A detailed list of the time points investigated and the number of animals for each interval are given in Table I.

Fixation.—In order to prevent loss of the vascular contents with their ferritin marker, it was necessary to initiate fixation *in situ* in the living animal. Accordingly 1 to 2 ml. of the fixative was introduced through a fine hypodermic needle into the superficial renal cortex of an anesthetized animal. The renal pedicle was clamped a few seconds after beginning the injection. During the injection a good part of the fixative usually found its way under the kidney capsule. Selected cortical areas which appeared blackened following this procedure were excised and cut into pieces of 1 $mm.^3$ while immersed in a fresh drop of fixative. These small pieces were then transferred to vials containing fresh, cold fixative and placed at 0°C. for 1½ to 2 hours. The fixative utilized was 1 per cent osmium tetroxide in acetate-veronal buffer (pH 7.5–7.7) (14) containing 0.14 M sucrose (15). When fixation was initiated by injection the glomeruli appeared well preserved and the contents of the capillary lumina and capsular

space were retained in most instances. It is assumed that the fixative penetrated chiefly along the peritubular spaces, for they appeared somewhat distended.

Dehydration and Embedding.—Following fixation the tissue was transferred directly to 70 per cent ethanol, dehydrated in graded alcohols, and embedded in a 4:1 mixture of butyl and methyl methacrylates.

Preparation of Sections.—The tissue blocks were cut on a Porter-Blum microtome equipped with a diamond knife (Sorvall). Thin sections were picked up on formvar-coated specimen grids and stained either with lead hydroxide (16), uranyl acetate (17), or phosphotungstic acid (PTA) (17). Following staining the sections were "sandwiched" (18) by evaporation of a thin carbon film onto their exposed surface (19). This latter technique proved very helpful for the preservation of fine structural detail.

Electron Microscopy.—Sections were examined in an RCA 2-B electron microscope equipped with an externally centerable objective aperture and an electrostatic stigmator (Canalco). Selected fields were micrographed at magnifications of 3,600 to 15,000 and enlarged photographically as desired.

OBSERVATIONS

Organization of the Glomerular Capillary Wall

In all mammalian species thus far examined, the glomerular capillary wall is composed of three distinct and successive layers: the endothelium, the basement membrane, and the visceral epithelium. At present there is general agreement about the disposition and principal features of these layers, but considerable controversy persists concerning the presence or absence of a third type of glomerular cell, variously called "mesangial," "intercapillary," or most recently, "intraluminal" (20).

Our observations confirm available information about the stratification of the capillary wall (*cf.* reference 21) and in addition reveal a number of structural features not previously recorded in the literature. For this reason, the organization of the glomerular capillary wall will be described in some detail.

Endothelium.—This layer limits the capillary lumen and consists of relatively large flat cells whose nuclei are usually located in the axial[1] regions of the capillary loops (Fig. 7). Immediately around the nucleus there is a moderate amount of cytoplasm containing the usual cytoplasmic components, including mitochondria, elements of the endoplasmic reticulum with associated ribonucleoprotein (RNP) particles, small piles of Golgi cisternae and vesicles, some free RNP particles, and two centrioles. A very thin layer (down to 200 A) of endothelial cytoplasm extends away from the nucleus around the capillary lumen (Fig. 1). This peripheral cytoplasmic layer is provided with numerous fenestrae which in normal sections are seen as repeated interruptions, giving the endothelium a beaded appearance (Figs. 1 and 15). In grazing sections (Figs. 9, 19, and 21) the attenuated layer appears as a sheet with circular openings, 500 to

[1] "Axial" refers to those portions of the capillary loops which are located deep within the glomerular tuft, in the vicinity of the afferent and efferent arterioles. "Peripheral" refers to those portions which have access to the surface of the tuft and face the glomerular capsule.

1000 A in diameter separated from one another by cytoplasmic strands 400 to 1000 A in width. The fenestrae are estimated collectively to account for about 30 per cent of the total surface of the endothelial sheets. In the peripheral parts of the loops where two endothelial cells come together they typically overlap to some degree (Fig. 8). Along their line of contact there are areas of increased density of the apposed membranes together with increased density of the immediately adjacent cytoplasmic matrix. These specialized regions are similar to the so called "adhesion plates" (22, 23) which are present along the surfaces of contact of epithelial cells and identical with the "attachment belts" described in vascular endothelia (8, 24).

It was previously mentioned that the nuclei and cell bodies of endothelial cells are usually located in the axial or deeper parts of the loops. In these regions the cells are frequently grouped and show some piling or stratification. The superficial cells can be clearly seen to line the capillary lumen, but the exact relationship of the deeper cells to the lumen is not always evident. Sometimes these deeper cells show rounded pseudopodia which push through the cytoplasm of the superficial cells to establish contact with the lumen. The surface of the basement membrane is irregular in the axial regions and usually appears to be provided with "spurs" or processes of varied form which penetrate between the endothelial cells. In addition, spongy-appearing areas are often seen between the basement membrane and the deeper cells or penetrating between cells of the endothelium. The spongy areas resemble the basement membrane in density but appear less compact and more distinctly fibrillar (Figs. 7, 20, 22).

Basement Membrane.—The basement membrane is found between the endothelium on one side and the epithelium on the other. It consists of a continuous layer of moderately dense material which appears relatively homogeneous in unstained preparations. After staining with heavy metals (*i.e.*, lead hydroxide, uranyl acetate, or PTA) it shows a faintly fibrillar structure produced by the presence of fine fibrils (30 to 40 A in diameter) which appear to be embedded in an amorphous matrix (Figs. 1, 9, and 19). Thin strata of low density are sometimes seen on either side of the basement membrane (Figs. 14, 15, and 23). The width of these lighter areas varies noticeably with the amount of extraction incurred during fixation and embedding; in optimal preparations the lighter areas are extremely thin, the basement membrane nearly filling the space between the endothelium and epithelium (Figs. 1 and 10). The thickness of the basement membrane measures 1200 to 1500 A (from the outer endothelial cell membrane to the epithelial cell membrane limiting the foot processes) but varies appreciably from loop to loop, apparently with the degree of dilation of the capillaries.

In addition to the fine, poorly outlined 30 to 40 A fibrils seen throughout the basement membrane, bundles of distinct fibrils are sometimes encountered in the narrow space between the basement membrane and the endothelium (Figs. 1 and 9). The bundles appear frequently disposed parallel to the long axis of the

capillary and often are closely associated with the endothelial cell membrane. These fibrils measure about 110 A in diameter, do not show any periodicity, and appear sometimes in sections examined at high magnifications as pairs of fine lines of medium density. Each of these lines is about 30 A thick and is separated from its companion by a light interval of about 50 A.

Epithelium.—The epithelium forms the third and outermost component of the glomerular capillary wall. It consists of very large cells with abundant cytoplasm. The nucleus is typically found near the cell surface facing the urinary space. The cytoplasm extending away from the nucleus forms a number of branches or "trabeculae" which often exhibit highly ruffled external contours. Each trabecula in turn is elaborately organized into a number of secondary branches, the so called "foot processes" which interdigitate with similar foot processes of an adjacent cell (or those from another trabecula of the same cell) to cover the entire outer surface of the basement membrane (Figs. 1, 20, and 23). Each foot process typically has a narrow stalk at the point of origin from its trabecula but expands into a broader base in contact with the basement membrane. As a result of this arrangement, the space between the foot processes forms narrow slits (2500 to 3000 A) near the basement membrane and rapidly increases in width towards the urinary spaces.

The internal organization of these cells is fully as complex and intriguing as their external features. Throughout the cytoplasm there is a fine feltwork of about 70 A filaments which are occasionally visible in unstained preparations but are much more clearly seen after staining with heavy metals (Figs. 1 and 19). Numerous organelles are characteristically present in the cytoplasm, and in discussing their distribution, it is convenient to divide the cell into three zones: (*a*) the cell body or perikaryon near the nucleus; (*b*) the intermediate cytoplasmic zones constituting the trabeculae; (*c*) the peripheral foot processes.

In the perikaryon, near one pole of the nucleus there is usually a large centrosphere region (Fig. 10) with numerous piles of parallel, closely packed, smooth-surfaced Golgi cisternae, surrounded by swarms of small (600 to 800 A) vesicles, and occasional larger vacuoles. Both smooth surfaced (*i.e.*, without attached RNP particles) and rough surfaced (associated with RNP particles) elements of the endoplasmic reticulum are present in abundance and are frequently seen in continuity with Golgi elements. Mitochondria and multivesicular bodies (rounded, membrane-limited bodies filled with small vesicles)[2] are also typically found in this region.

The intermediate cytoplasmic zones, located between the nucleus and the

[2] Multivesicular bodies were first described in the renal glomerulus by Yamada (8) under the name of "glomerular epithelial vesicular conglomerates." He considered them as characteristic structures of the glomerular epithelium but it has since been shown that they occur in a wide variety of cell types. At present, the multivesicular bodies are assumed to be a constant component of animal cells (cf. reference 25).

peripheral foot processes, contain a well developed endoplasmic reticulum composed of vesicular and tubular elements of both the smooth and rough surfaced varieties. There are also variable numbers of free RNP particles. In these intermediate regions one often encounters rather remarkable structures which represent large distended cisternae of the endoplasmic reticulum, measuring up to 1.5 mμ in diameter (Figs. 11 to 13). They are limited by rough surfaced membranes and are in direct continuity with the more common flattened cisternae. The content of these large local dilations of the endoplasmic reticulum is not of the uniformly light density, characteristic of the content of the remainder of the system in these cells, but shows some denser material which is disposed in unusual and intriguing forms (*e.g.*, spherical shells or plicated sheets and bars, usually of more or less even thickness). It may be significant that this dense material closely resembles, in density and texture, the background matrix of the basement membrane. The intermediate cytoplasmic areas also contain numerous small vesicles and occasional multivesicular bodies, (Fig. 21) together with a few vacuoles and dense bodies (Fig. 23). The vacuoles are membrane-limited and have a content of low density; they range in size from 0.2 to 1.0 μ. The dense bodies are also limited by a membrane but are characterized, as the name implies, by the great density of their content. They range in size from 0.1 to 0.2 μ.

In the foot process the cytoplasmic matrix is frequently denser than in the rest of the epithelial cell (Fig. 1). As a rule these processes do not contain significant numbers of formed elements except for numerous small vesicles (600 to 800 A diameter) of the type present in large numbers throughout the epithelial cytoplasm. Frequently, fine about 30 to 40 A filaments, similar to those making up the fibrillar framework of the basement membrane, are seen extending from the cell membrane limiting the base of the foot processes into the basement membrane (Figs. 9, 14, 15, 16, 19, and 20).

Several features relating to the structure of the epithelial cell membrane are worthy of special comment. The membrane of these cells, like other cell membranes (26), is composed of two dense layers (about 40 A) separated by a space of lesser density (about 30 A) (Figs. 9, 20, 21(a), and 23), but its total thickness (about 110 A) is greater than that of other cell membranes. In addition, its density, particularly after lead staining, is conspicuously higher than that of the endothelial cell membranes present in the same field (Figs. 9 and 19). A mottled texture is sometimes also discernible in the membrane when the latter is seen in full faced view in grazing sections (Fig. 19). It is noteworthy that with prolonged exposure of sections to lead hydroxide or phosphotungstic acid, other cell components are progressively extracted while the contrast and apparent thickness of the epithelial cell membrane is selectively enhanced. For example, after staining in 5 per cent phosphotungstic acid for ½ hour, the whole substance of the basement membrane may be removed, along with many cyto-

plasmic components of both the epithelium and endothelium, while the epithelial cell membrane stands out in striking contrast to the remaining pale background.

A further specialization of the epithelial cells occurs in the slits[3] formed by adjoining foot processes. In sections normal to the capillary wall and perpendicular to the base of the foot processes, a thin line with ill defined limits is frequently seen bridging the narrowest point of the gap between foot processes. This line, which has been called the "filtration slit membrane" (8), is thinner (about 40 A) and more tenuous than the cell membrane (Figs. 2, 16, and 20) and sometimes shows a dense dot halfway along its path between the two foot processes it connects (Figs. 3 and 4). More precise information on the material found in the slits can be gained from sections passing parallel to the basement membrane at the level of the slit membrane. When sectioned in such a plane (Fig. 9), the apposed cell membranes of the two adjacent foot processes show a greater density than that along the remaining perimeter of the epithelial cells, and the cytoplasm in the immediately subjacent areas also appears to be of increased density. Furthermore, under favorable conditions, a fine line (about 40 A) can frequently be seen in the slits equidistant between the two apposed cell membranes and running parallel to them (Figs. 5, 6, 9, 14 and 21). This fine intermediate line is lighter in density than the adjacent cell membranes and is separated from the latter by thin strata of low density. The lighter interspaces do not appear to be empty, for their density is greater than that of the open urinary spaces, and in some places strands of finely filamentous material can be seen extending between the intermediate line and the apposing cell membranes (Figs. 9 (a) and (b). The structural features encountered at this level of the foot processes (*i.e.*, the increased density of the apposed cell membranes, the adjacent areas of increased cytoplasmic density, and the fine intermediate intercellular line) are remarkably similar to those that characterize the "desmosomes" (27) and other so called "adhesion plates" which occur along the surfaces of contact between epithelial cells (22). The possible implications of this finding will be discussed in a section to follow.

It is to be emphasized that in these experiments and in our previous work on normal glomeruli, ferritin of endogenous origin has not been seen, as a rule, in any of the layers of the glomerular capillaries.

Glomeruli of Animals Given Ferritin

Since ferritin molecules are large and dense enough to be seen individually in the electron microscope, their distribution and relationship to glomerular components can be readily determined. Each molecule is composed of an inner

[3] We consider the slits as extending in depth from the level of the basement membrane to that of the slit membrane (see below).

dense core of ferric hydroxide micelles surrounded by an outer protein (apoferritin) shell. Although the total diameter of the molecule is approximately 100 A (28), only the inner dense iron core of the molecule, measuring approximately 55 to 60 A, is visible in our preparations (*cf.* reference 29).

As shown in Table I, animals were examined at successive intervals between 2 minutes and 2 hours following ferritin administration. Since significant differences in ferritin distribution were seen only between animals sacrificed before and after 15 minutes, in describing the results the experimental animals will be divided into two groups representing short (2 to 15 minute) and long (1 to 2 hour) time intervals.

Animals Sacrificed 2 to 15 Minutes Following Ferritin Administration.—Numerous ferritin molecules were present within the glomerular capillary lumina at all early time points following the injection (Figs. 14 and 15). The particles were evenly distributed throughout the lumen and did not show aggregation or areas of preferential concentration. A number of molecules were also seen within the endothelial fenestrae which they appeared to traverse freely thus gaining direct access to the basement membrane. At these short intervals, relatively few tracer molecules were seen within the endothelial cytoplasm or between endothelial cells in the axial regions of the loops.

A few ferritin molecules were found embedded in the basement membrane throughout its entire depth. It should be emphasized, however, that the concentration of the marker in this layer was considerably lower than in the lumen or within the endothelial fenestrae. The actual number of particles within the basement membrane also showed considerable local variations from loop to loop. In experiments in which low concentrations of ferritin were involved, the marker seemed to be randomly distributed in the depth of the basement membrane. With higher concentrations, there was some evidence of ferritin accumulation in the inner (luminal) part of the membrane (Figs. 14 and 15). In all cases there was no detectable evidence of preformed pathways or channels across the basement membrane: there were no lighter or darker tracks ahead or behind the molecules of the tracer, and there was no preferred relationship of the ferritin embedded in the basement membrane to the epithelial slits.

A few tracer molecules were also seen within the epithelium at short time intervals. They were found either within small invaginations of the cell membrane limiting the base of the foot processes (Figs. 16 to 18), or enclosed within small cytoplasmic vesicles located within the foot processes (Fig. 16) or deeper in the cytoplasm. At short intervals after ferritin injection, the total number of tracer molecules seen within the epithelium was small and the distribution quite variable: in a given field a single foot process might show several vesicles containing ferritin molecules, while the vesicles of the other foot processes present in the field were free of marker. It is noteworthy that membrane invaginations containing ferritin occurred as a rule along the portion of the cell

membrane limiting the base of the foot processes and facing the basement membrane, most often in the very center of the base. Membrane invaginations were also commonly present along the stalk of the foot processes above the level of the slit membrane (Fig. 22) or along the membrane limiting the trabeculae (Figs. 11, 20, 21, and 23), but these rarely contained ferritin. In these sites there is frequently a layer of condensed cytoplasmic material immediately against the invaginating membrane (Figs. 11 and 22). It may be significant that pinocytic invaginations were not seen in the portions of the cell membrane that face the slits; *i.e.*, between the base of the foot processes and the slit membranes. No ferritin was seen within the slits and no accumulation of the marker occurred at their introit.

Animals Sacrificed 1 to 2 Hours Following Ferritin Administration.—In glomeruli from rats examined at longer time intervals, numerous ferritin molecules were still present within the capillary lumen and within the endothelial fenestrae (Figs. 19 and 21). Their concentration in these sites appeared to decrease only slightly with elapsed time. A few molecules were seen within the peripheral endothelial cytoplasm enclosed within small cytoplasmic vesicles (Fig. 19). At these later time points there was a discernible accumulation of the tracer against the basement membrane (Figs. 19 and 21); ferritin molecules were found preferentially concentrated either in the subendothelial spaces, *i.e.* between the endothelium and the basement membrane, or within the inner (luminal) part of the latter.

A noteworthy difference also appeared between the peripheral and the axial regions of the capillary loops. While the subendothelial accumulation in the former parts remained small and patchy, the marker reached remarkable concentrations in the axial regions where it practically filled the spongy areas between the deeper endothelial cells and the basement membrane (Fig. 20) Furthermore, the endothelial cells, particularly those located away from the lumina, showed elaborate infoldings of their cell membranes resulting in complicated plications and interdigitations between cells. Numerous marker molecules were often present within these complicated intercellular spaces. Finally, membrane-limited structures packed with ferritin were commonly seen within the cytoplasm of some of the deeper endothelial cells (Figs. 20 and 22). These vacuoles containing the marker were much less frequent within the superficial endothelial cells of the axial regions and especially in the attenuated endothelium of the peripheral part of the capillary loops.

At the later time points examined, the amount of ferritin found in the epithelium also increased. Some tracer molecules were seen within the membrane invaginations of foot processes (Fig. 21) as described for shorter intervals, but many more had accumulated within cytoplasmic vesicles located primarily within the foot processes (Figs. 21 to 23). Some additional ferritin molecules were present within multivesicular bodies (Figs. 10 and 21), large vacuoles of

various sizes (Fig. 23) or dense bodies (Figs. 23 and 24). By 2 hours following ferritin administration, nearly every multivesicular body, vacuole, or dense body contained a few ferritin particles. No ferritin was seen free in the cytoplasmic matrix at any time point investigated. The molecules were always restricted to membrane-limited compartments (*i.e.*, vesicles, multivesicular bodies, vacuoles, and dense bodies) and thus separated from the rest of the cytoplasm.

DISCUSSION

New Morphological Findings

Our findings confirm the well known structural features of the glomerular capillary wall: namely the existence of a continuous basement membrane, lined on the luminal side by an extensively fenestrated endothelium and covered towards the urinary spaces by a continuous array of interdigitating epithelial foot processes. In addition, our observations provide some morphologic information not previously available. The bundles of fibrils between the endothelial cell membrane and the basement membrane, for instance, represents a new finding. The location suggests that they are produced by the endothelium and may be ultimately incorporated into the basement membrane. These fibrils are quite distinctive elements from those which produce the fine fibrous texture visible throughout the basement membrane and which have already been described by others (8, 20, 30, 31). The evidence therefore suggests that, in addition to a still amorphous matrix, two fibrillar components exist in the basement membrane: (*a*) a fibrillar framework composed of 30 to 40 A fibrils visible throughout this layer, particularly after staining with heavy metals,[4] and (*b*) discrete fibrils approximately 110 A in diameter found primarily in the subendothelial spaces. It should be reiterated that both of these fibrillar components are quite distinct from mature collagen or reticulin fibrils which are well known from histochemical (*cf.* reference 32) and electron microscopic evidence to be absent from the normal mammalian glomerulus.

Our evidence indicates that the visceral epithelium is composed of highly active cells. Their cytoplasm contains a large number of membrane-limited structures including vesicles, multivesicular bodies, vacuoles, and dense bodies—all apparently connected with the ability of these cells to incorporate substances in bulk (by "pinocytosis") from the surrounding medium. The presence of a well developed Golgi complex in these cells, together with the evidence (see below) that membranous material is undergoing constant relocation (from capillary surface to intracytoplasmic organelles to urinary surface)

[4] Some reservations should be maintained about the existence of these fibrils *in vivo*. They might be formed or coarsened during fixation which, for instance, precipitates the blood plasma in a fine fibrillar felt "stainable" by heavy metals (see Figs. 7 and 19).

(11), is in keeping with the suggestion (33) that the Golgi region serves the function, among others, of a depot of membranous material within the cell. The epithelial cells also appear to be active in protein synthesis, as suggested by their rich content of RNP particles and well developed endoplasmic reticulum. The presence of an elaborate apparatus for protein synthesis and the frequent occurrence of material resembling basement membrane within the distended cisternae of the endoplasmic reticulum suggest that the epithelium may contribute some components (proteins? mucoproteins?) to the basement membrane and that their RNP particles and endoplasmic reticulum may be concerned with the synthesis, intracellular transport, and storage of these components. Thus our evidence suggests that both the endothelium and epithelium contribute components to the basement membrane.

Finally, we have observed a more complex organization in the epithelial slits than previously assumed[5]: in these locations there is a thickening of the apposed cell membranes of the foot processes, backed by an increased density of the immediately subjacent cytoplasmic matrix. In addition we have detected, in grazing sections, a linear accumulation of dense material bisecting the intercellular spaces. We assume that this intermediate line corresponds in three dimensions to a filament (not a lamella), for in normal sections it appears as a dot. We further assume that the fine wisps that extend between the intermediate line and the adjacent foot processes produce by superimposition in the thickness of a normal section the appearance of a continuous "slit membrane." We recognize, however, that under present conditions of preparation and resolution it is not possible to ascertain whether the slit membrane is a continuous or discontinuous structure.

The structural pattern described in the slits (*i.e.*, the increased density of the apposed cell membranes, the subjacent areas of increased cytoplasmic density, and the fine intermediate, intercellular line) is strikingly similar to that encountered in terminal bars and desmosomes (22, 23). The latter two elements are generally considered to represent "adhesion plates" or areas of particularly firm attachment between cells. Moreover, when continuous (as in terminal bars) such structures are presumed by some to act as effective seals for the corresponding lumina. There is, however, a notable distinction between the structural complex found in the slits and usual desmosomes or terminal bars. The former is very limited in depth (about 40A), whereas the latter

[5] Both Pease (34) and Yamada (8) have described a line bridging the slits in sections passing normally through the capillary wall, but they have interpreted it as being continuous across the base of the foot processes. Pease has concluded that the line represents the free margin of a "cement substance," whereas Yamada has considered it as the outer layer of the cell membrane. They have not detected the intermediate line along the slits and the desmosome-like structure of the latter.

represent plaques or bands 1000 to 5000 A in depth (23). For the moment the significance of the structures found in the slits remains unknown, but the fact that pinocytic activity (*i.e.* membrane invaginations) occurs along the entire perimeter of the epithelial cells except at the level of the slits suggest that these sites are functionally as well as morphologically specialized.

Physiologic Data Obtained

The main result of our study is the finding that the basement membrane functions as the principal filtration barrier. This conclusion is based primarily on the fact that the concentration of ferritin molecules falls off sharply at the level of the basement membrane. Indeed, most of the tracer molecules are retained in the capillary lumen and relatively few are found in the basement membrane and epithelium. This finding amounts to a direct demonstration that the filtration barrier is not freely permeable to molecules of about 100 A diameter. The fact that within 1 hour the tracer gradually piles against the basement membrane provides further proof that this structure acts as a barrier restricting the passage of ferritin molecules.

The basement membrane appears to function as the main filter in spite of the fact that no "pores" or preformed channels are detectable in it with present techniques; furthermore, there are apparently no preferred pathways as indicated by the random distribution of ferritin molecules in its outer portions. We are led to conclude, therefore, either that the assumed channels are extremely tortuous and as such are quite difficult to demonstrate in sections, or that the tracer molecules move through a yielding substrate in the absence of permanent pores or channels.

A considerable accumulation of ferritin molecules was found after longer time intervals in the spongy areas of the basement membrane in the axial segments of the capillary loops. In experiments with colloidal gold (35), as in the case of the ferritin experiments just described, at early time points the tracer particles accumulated against the luminal surface of the basement membrane in peripheral regions of the capillary loops. After longer intervals, relatively few particles were found in this location whereas large numbers were present in the spongy afeas of axial regions. It is therefore tempting to assume that the accumulation of particles in these regions is the result of a "sweeping" of the luminal surface of the basement membrane by the endothelium. The concentration of filtration residues in the axial regions may facilitate their subsequent incorporation by endothelial cells, primarily by the deeper cells of the axial regions. Further studies may indicate how significantly these latter cells differ from the rest of the endothelium and what relationship they may have to the so called "mesangial," "intercapillary," or "interluminal" cells.

The finding that some—albeit a small percentage—of ferritin molecules do

penetrate the basement membrane and are subsequently picked up by the epithelium demonstrates that the basement membrane is not a *perfect* filter. It apparently allows continuous passage of protein molecules (diameter less than 100 A) in relatively small amounts which, at least in part, are subsequently removed from the filtrate by the pinocytic activity of the epithelium. It appears, therefore, that one of the functions of the epithelium is to "check" the glomerular filtrate, a view strongly supported by the finding that this activity is greatly enhanced when appropriately challenged; *i.e.*, when increased quantities of protein appear in the glomerular filtrate, such as in the nephrotic syndrome (11).

Even though we have found a desmosome-like structure (by implication a seal) in the slits, we assume for the time being that normally the bulk of the glomerular filtrate reaches the urinary spaces through the slits of the epithelium. If this be the case, the existing structural arrangements could provide maximal opportunity for protein which leaks through the basement membrane to come into contact with the epithelial cell surface and be incorporated by pinocytosis. The narrow space between foot processes and basement membrane probably allows passage of only a thin film of filtrate at any one time, and the elaborate foot process arrangement greatly increases the area of contact between the epithelial cell membrane and the filtrate. The special morphologic features which we have observed in the epithelial cell membrane (its thickness, density, mottled texture, and staining properties) may reflect special properties connected with the trapping and subsequent incorporation of protein by these cells. After its incorporation into the epithelium, the ingested protein is apparently segregated into multivesicular bodies and vacuoles. The fate of the ferritin within the multivesicular bodies cannot be ascertained at the moment, but the vacuoles appear to undergo condensation into dense bodies. The content of the dense bodies is then partially digested and finally extruded into the urinary spaces. This series of operations suggests that membranous material can be relocated from the cell surface to the interior and then back to the cell surface (*cf.* reference 11). Although the over-all process in normal animals is similar to that already described in nephrotic rats (11), it occurs on a much smaller scale, presumably because much less protein permeates the basement membrane in normal glomeruli. One can speculate that the partial digestion of proteins in visceral epithelial cells might deliver to the urinary spaces small peptides or amino acids which could be more effectively reabsorbed by the tubular epithelia.

Implications of findings

It is generally assumed that the glomerular fluid is formed by a process of passive ultrafiltration of blood plasma (1). The original concept of an essentially protein-free filtrate, formulated by Ludwig in 1844 (36) and elaborated by

Cushny (37), has been modified in recent years to take into account the fact that small quantities of protein normally leak into the capsular space and are reabsorbed by the tubules. Available evidence (*e.g.* 38–42) suggests that the normal glomerulus is to some degree permeable to molecules with a mean diameter in the range of 50 to 100 A, but there is apparently progressive restriction to passage with increasing molecular weight and average diameter. As a result of extensive permeability studies, Pappenheimer and his associates (2, 3) have postulated that the wall of glomerular capillaries, like that of muscle capillaries, consists of a membrane containing pores with an effective diameter of about 60 to 90 A.[6] They have explained the higher filtration rate of glomerular capillaries by assuming that the pores represent collectively 1 to 2 per cent of the total surface of these vessels as opposed to 0.2 per cent in the case of muscle capillaries. Recent morphological evidence indicates, however, that the two types of capillaries differ significantly in structure[7]—and that pores of the expected dimensions are not present in the wall of either type. In some early electron microscopic studies, the newly discovered fenestrae in the endothelium were identified as the pores postulated by the filtration hypothesis, but this interpretation was quickly discarded when it became apparent that the fenestrae are considerably larger than the hypothetical pores and interrupt only one of the three layers of the capillary wall. Since both the endothelial and epithelial layers are discontinuous, the majority of electron microscopists (6–9, 20, 30, 31, 47) have assumed that the basement membrane represents the actual filter, and our results on ferritin and gold[8] transfer provide direct evidence to support this assumption. It should be pointed out, however, that attempts to demonstrate regular porosity in the basement membrane

[6] It should be stressed that Pappenheimer (43) also considered the possibility of a more complex geometry for the filtration barrier as shown by the following quotation: "Perhaps these channels are formed by a gel structure or a fibrous structure. We do not know. We do know that it presents the same barrier to hydrodynamic flow and diffusion of solutes as would uniform cylindrical pores of radius 30 A and area per unit path length 1.3 x 105 cm. per 100 grams of muscle."

[7] It has been established that the walls of blood capillaries throughout the body are stratified structures composed of several layers, and that the exact arrangement of these layers varies considerably and characteristically from one capillary type to another (24). In the case of muscle capillaries (44–46), the endothelium forms a continuous layer without fenestrations, and the outer surface of the basement membrane is in contact with the connective tissue elements. Furthermore, the basement membrane is much thinner (200 to 250 A in heart muscle capillaries and 500 to 550 A in capillaries of limb muscles) and is frequently closely associated on its outer surface with collagen fibrils.

[8] Experiments reported in part elsewhere (35), using colloidal gold particles (40-200 A) as a tracer, indicate that most of these particles are retained by the basement membrane and relatively few reach the epithelium. Preliminary observations suggest that those retained are of larger size than those found in the vesicles and vacuoles of the epithelium.

have met with general failure.[9] Moreover, in our case ferritin molecules were capable of advancing through the basement membrane in the absence of visible pores of appropriate dimensions. The molecules did not follow performed or preferred pathways, and there were neither tracks ahead nor trails behind them.

It was in part the repeated failure to establish the presence of any regular porous structure in the basement membrane that led Hall (10) to formulate his concept of filtration through epithelial "slit-pores." According to this hypothesis the size-limiting structure is the "slit-pore" formed by the close approximation of adjacent foot processes; these slits are presumed to impose a more precise restriction than does the basement membrane. Against the slit-pore hypothesis is the fact that the slits occupy a significantly greater percentage of the total area of the capillary wall than the pores postulated by Pappenheimer *et al.* (about 20 per cent as compared to 2 per cent). In addition our evidence indicates that most of the circulating ferritin molecules are retained in the lumen by the basement membrane before reaching the level of the slits. Finally, in the case of ferritin, we found no direct evidence for any size limitation introduced by the slit-pores (*e.g.* there was no piling of the marker at the slit introit) although molecules of this size are expected to be extensively retained by the glomerular filter.

Our results indicate that the formation of glomerular fluid is a more complicated process than passive filtration through a membrane provided with rigid pores or slit-pores. The findings also suggest a definite role for each of the components of the glomerular capillary wall in the filtration process: the basement membrane as the principal filter; the epithelium as its monitor; and the endothelium as a possible valve, which by varying the number and distribution of its fenestrae, controls the area of the filter directly exposed to the blood plasma. The principal filter (the basement membrane) does not appear to be a simple sieve, but presumably is a gel-like structure with fine fibrillar components embedded in a matrix still amorphous at the present level of resolution. It thus comes close to Chinard's representation (4, 5) of the structure of the capillary wall. As already mentioned, the role of the basement membrane as the principal glomerular filter has been frequently postulated in the past. Likewise, it was previously suggested that the presence of a highly fenestrated endothelium might serve to increase access to the basement membrane (6–8, 24). As far as we know, the view that the epithelium acts as a monitor is a new concept. A complete discussion of the previously expressed opinions concerning the function of the various structural elements of the glomerular capillary can be found in a recent review (21).

[9] Initially Hall (6) described pores (50 to 150 A) in the basement membrane. More recently he has stated (10) that pores are not discernible in the basement membrane and has attributed his early findings to artefacts of fixation and dehydration.

It is obvious that the traditional approaches to the study of glomerular permeability (*e.g.* micropuncture and renal clearance) measure the *effective* permeability of the basic filter (the basement membrane) functioning together with its monitor (the epithelium). Our findings do not allow us to quantitate the amount of protein the visceral glomerular epithelium can recover in a given time. Likewise, we cannot assess the relative importance of this function in comparison to the similar well known activity of the epithelium of the proximal tubule in the reabsorption of protein. The unique and elaborate arrangement of the visceral epithelium might allow more intimate contact with the filtrate in the glomerulus than is possible at the tubular level. Accordingly, it is conceivable that the recovery of protein from the filtrate might be more efficient in the glomerulus than in the tubule and hence sufficient under normal conditions to create a virtually protein-free (less than 30 mg./100 cc.) capsular fluid.

Since each of the layers of the glomerular wall appears to play a part in creating the functional characteristics observed, it follows that filtration through glomerular capillaries could be affected by a change in the arrangement of any one of its components. A multiplicity of agents involved in physiological and pathologic processes could selectively affect, singly or in combination, (*a*) the number and distribution of endothelial fenstrations, (*b*) the composition and integrity of the basement membrane, or (*c*) the arrangement of the epithelial foot processes and slits. Furthermore, since the basement membrane is acellular and probably dependent on both the endothelium and epithelium for its synthesis and maintenance, any agent which would interfere with the metabolism of either of these cells could conceivably affect the chemical composition and permeability characteristics of the basement membrane.

SUMMARY

Ferritin was used as a tracer to investigate pathways and mechanisms for transfer across the various layers of the glomerular capillary wall. Kidney tissue, fixed at intervals of 2 minutes to 2 hours following an intravenous injection of ferritin, was examined by electron microscopy.

The observations confirmed the existence of three distinct and successive layers in the glomerular capillary wall (the endothelium, the basement membrane, and the visceral epithelium). In addition, they demonstrated a number of new structural features: namely (*a*) discrete fibrils in the subendothelial spaces; (*b*) a characteristic, highly elaborate, cytoplasmic organization in the visceral epithelium; and (*c*) special structures resembling "desmosomes" in the slits between foot processes.

In animals sacrificed at short time intervals (2 to 15 minutes) following ferritin administration, ferritin molecules were found at high concentration in the lumen and endothelial fenestrae, at low concentration in the basement membrane, and in very small numbers within the epithelium.

Later (1 to 2 hours), the tracer particles were still present in the lumen and within endothelial fenestrae, and, in addition, had accumulated on the luminal side of the basement membrane, especially in the axial regions of the vessels. Larger numbers of ferritin molecules were also found in the epithelium—in invaginations of the cell membrane at the base of the foot processes, and in various membrane-limited bodies (vesicles, multivesicular bodies, vacuoles, and dense bodies) present within the cytoplasm.

These observations suggest that the endothelial fenestrae are patent and that the basement membrane is the main filtration barrier. Since the basement membrane has no demonstrable pores, it is probably not a simple sieve but presumably is a gel-like structure with two fine fibrillar components embedded in an amorphous matrix. Both the epithelium and endothelium may be concerned with building and maintaining this structure. Finally, the intracellular accumulation of particles in the epithelium suggests that the latter acts as a monitor that recovers, at least in part, the small amounts of protein which normally leak through the filter.

We gratefully acknowledge the technical assistance of Miss Marie Doelger and Miss Sally Westerhoff.

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EXPLANATION OF PLATES

Abbreviations for Figures

B, basement membrane
CP, capillary lumen
EN, endothelium
EP, epithelium
S, spongy areas of the basement membrane
US, urinary space
cm, cell membrane
er, endoplasmic reticulum
f, endothelial fenestra
g, Golgi membranes
in, membrane invagination
m, mitochondria
mv, multivesicular body
n, nucleus
ne, nuclear envelope
p, foot process of the epithelium
sl, slit between foot processes
v, vacuole
ve, vesicle

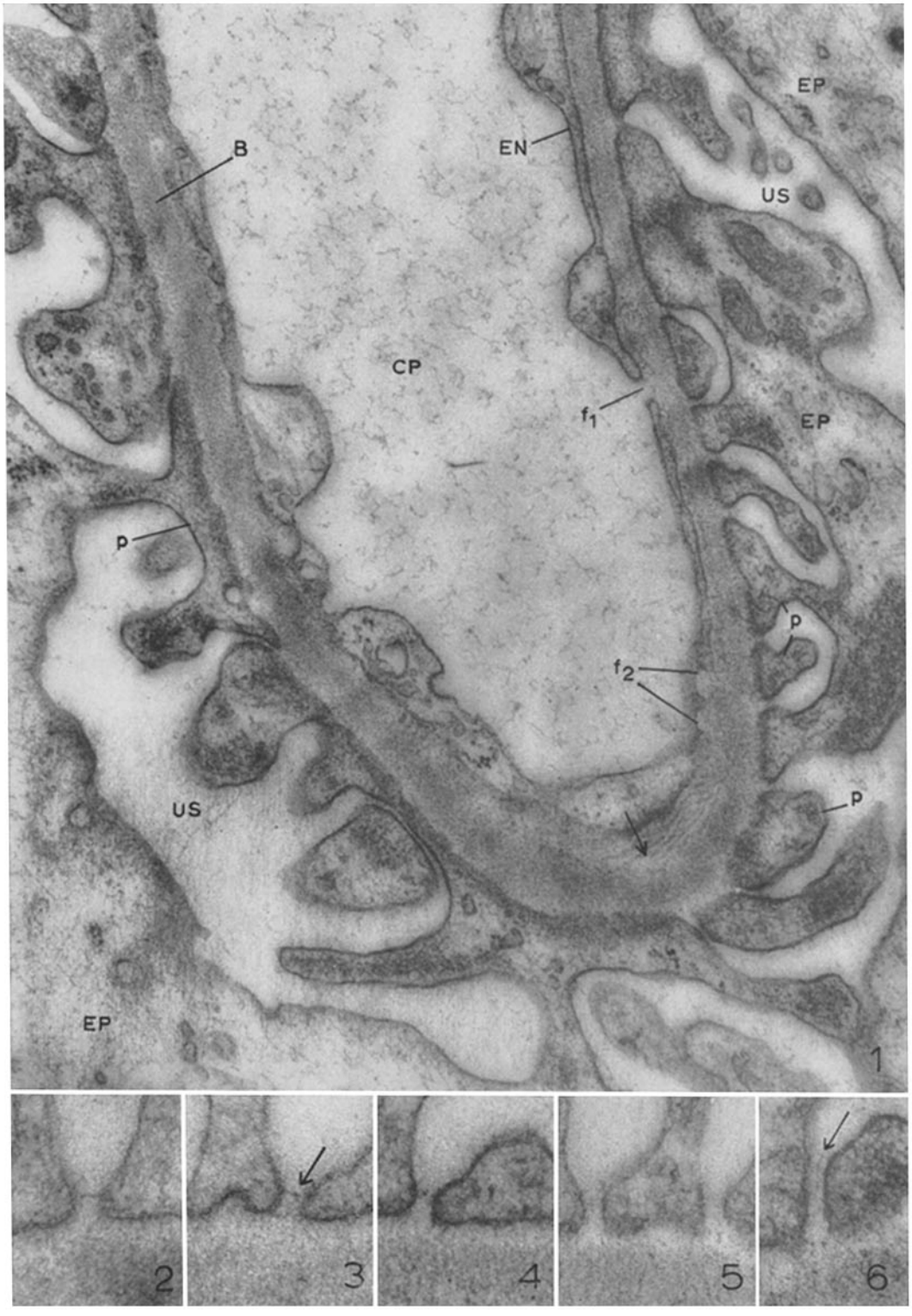
All the figures represent electron micrographs of rat kidney tissues which were fixed in osmium tetroxide and embedded in methacrylate. Sections were stained 3 to 15 minutes in lead hydroxide (16) and subsequently "sandwiched" (18).

PLATE 9

FIG. 1. Glomerular loop seen at relatively low magnification. Three layers are evident in the capillary wall: the endothelium (*EN*) with its fenestrae, the basement membrane (*B*), and the epithelium (*EP*) with its foot processes (p). When the plane of section passes normally through the endothelium, the fenestrae are seen as interruptions in the thin, peripheral, cytoplasmic layer, as at f_1. In oblique section they appear as circular openings 500 to 1000 A in diameter as at f_2. (See also Figs. 9 and 19). The basement membrane (*B*) is a continuous layer of moderate density which shows a faintly filamentous and punctate texture. It virtually fills the space between the endothelium and epithelium and measures 1200 to 1500 A in thickness. In one place (arrow) fine distinct fibrils are present in the narrow space between the endothelium and basement membrane. Such fibrils are seen to better advantage in Fig. 9.

Several large cytoplasmic branches or trabeculae of the visceral epithelium are shown on the right and lower left. The division of the trabeculae into interdigitating foot processes (p) is also depicted. The foot processes typically show a narrow stalk at their point of origin from a trabecula and expand into a broader base in contact with the basement membrane. As a result of this arrangement the space between the foot processes is very narrow (2500 to 3000 A) near the basement membrane but gradually increases in width away from it. In several of the slits a thin line or "slit membrane" is seen bridging the space between adjoining foot processes. Several slit membranes are shown at higher magnification in Figs. 2 to 6. The foot processes do not contain significant numbers of formed elements (e.g. mitochondria and endoplasmic reticulum) except for numerous small 600 to 800 A vesicles of the type present in large numbers throughout the epithelial cytoplasm. The cytoplasmic matrix is somewhat denser in the foot processes than in the rest of the epithelial cell. In some places, particularly in the upper right corner, fine filaments (about 70 A) can be made out in the cytoplasm of the trabeculae. Magnification 39,000.

Figs. 2–6. These figures illustrate varying aspects of the slit membrane. In each picture the foot processes are present above and part of the basement membrane is visible below. In Figs. 2 to 4 a fine line (about 40 A) is seen bridging the slits between foot processes. This "slit membrane" is thinner and more tenuous than the cell membrane. In some cases, as in Figs. 3 and 4, it contains a central dense dot midway along its path between foot processes (arrow in Fig. 3). If the plane of section is oblique the material in the slits is seen in broader aspect and has ill defined boundaries, as shown in Figs. 5 and 6. In such sections a fine grey line can frequently be seen in the slits equidistant between the two apposed cell membranes and running parallel to them (arrow in Fig. 6). It is assumed that the dense dot seen in normal sections corresponds to the intermediate line visible in oblique sections. Magnification 120,000.



(Farquhar *et al.*: Glomerular permeability. I)

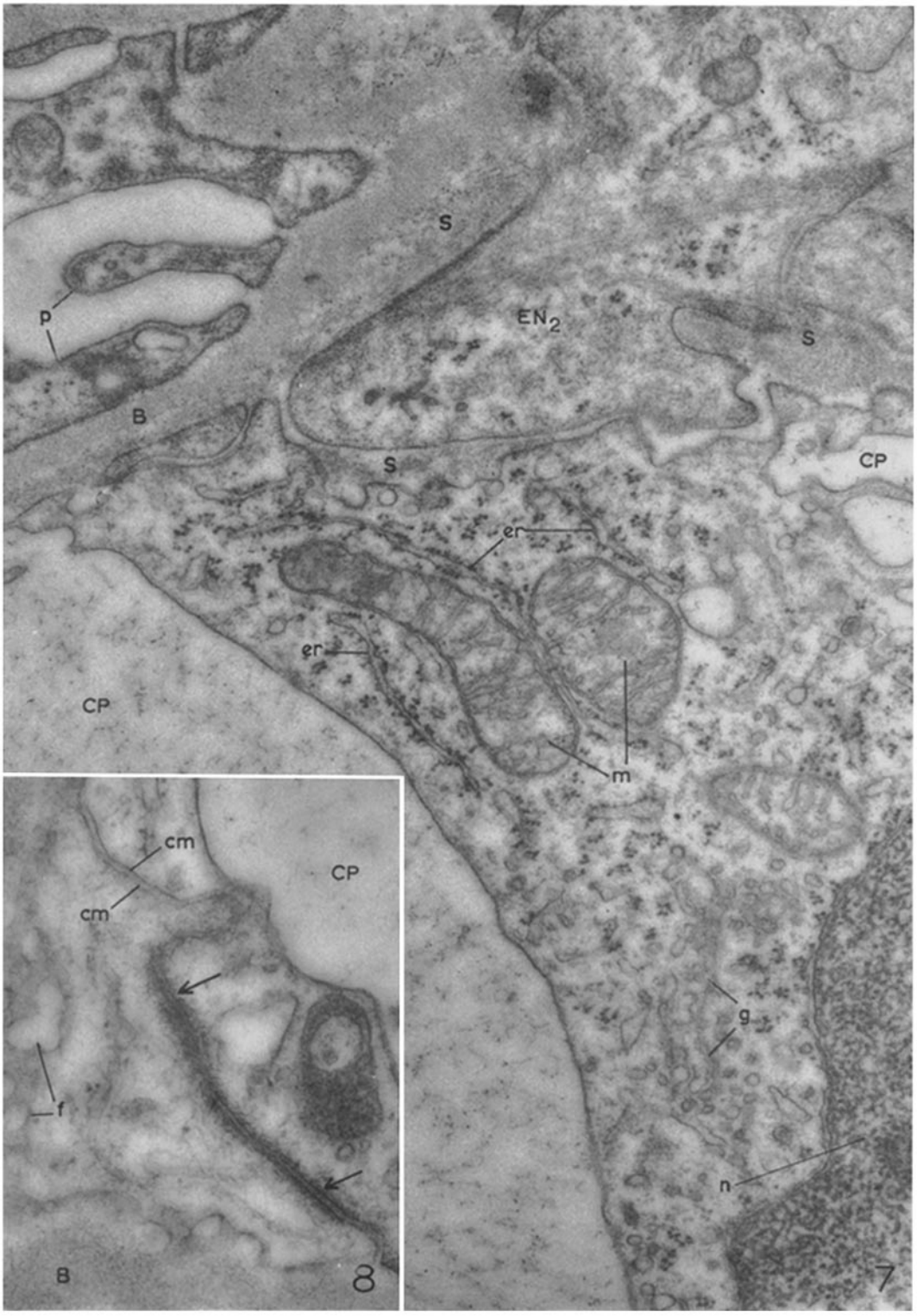
PLATE 10

FIG. 7. Axial region of a glomerular capillary loop illustrating an endothelial cell body and its relationships. Because of the curvature of the loop, the section passes through the lumen in two places (*CP*) with a bridge of endothelial cytoplasm in between. The basement membrane (*B*) and several epithelial foot processes (*p*) are present in the upper left corner. The endothelial cytoplasm, extending between the nucleus and the basement membrane, contains the usual cytoplasmic components including elongated profiles of the endoplasmic reticulum (*er*) with associated RNP particles, some free (unattached) RNP particles, and randomly distributed vesicles.

A portion of the cytoplasm of a second endothelial cell (EN_2) is also present above. The cytoplasm of this deeper cell shows a fibrillar matrix disposed in bundles or fascicles. Spongy-appearing areas (*S*) are seen between the basement membrane and the deeper endothelial cell and penetrating between the two endothelial cells. These spongy areas resemble the basement membrane in density but appear less compact and are more distinctly fibrillar.

Ferritin molecules are present in the lumen and some are also concentrated in the spongy areas, but the particles do not show very well at this low magnification. Magnification 44,000.

FIG. 8. Peripheral region of a glomerular capillary showing a junction line between two overlapping endothelial cells. The apposed cell membranes (*cm*) cross the field more or less diagonally. In the center and lower parts of the field the cell membranes show an increased density along their line of contact (in between arrows). There is also an increase in the density of the immediately adjacent cytoplasm. These specialized regions represent so called "adhesion plates" or "attachment belts" which are present along the surfaces of contact of endothelial cells. Magnification 57,000.



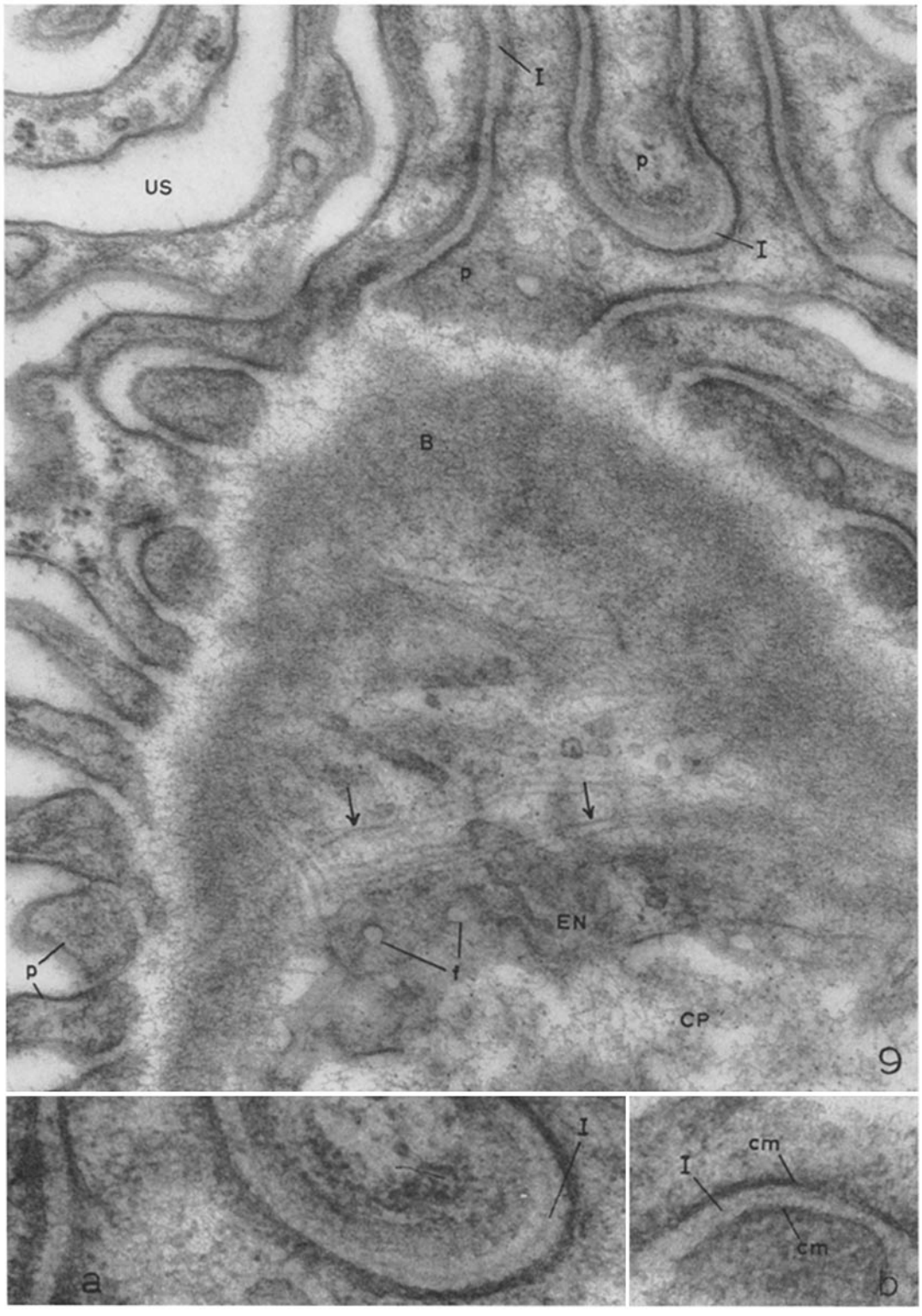
(Farquhar *et al.*: Glomerular permeability. I)

PLATE 11

FIG. 9. Section through a glomerular capillary. Because of the curvature of the vessel, the section cuts obliquely through the endothelium (*EN*), more obliquely through the basement membrane (*B*), and then—in the upper right corner of the micrograph—grazes through the foot processes (*p*) of the epithelium at the level of the slit membranes. The foot processes are cut less obliquely along the left and right side of the capillary. Adjoining the lumen on the lower right is an endothelial layer with several fenestrae (*f*). Fine fibrils (about 30–40A) can be seen throughout the basement membrane, and some extend from the cell membrane limiting the base of the foot processes into the basement membrane. In addition to these fine, poorly outlined elements, there are bundles of larger, more distinct fibrils (about 110 A) in the subendothelial space (arrows). In some areas they appear as pairs of fine lines separated by a lighter interval.

In the upper right corner where the plane of section passes parallel to the basement membrane at the level of the slit membranes, some specialized structural details are evident within and along the slits: the cell membranes are denser here than around the rest of the epithelial cell, and the density of the immediately adjacent cytoplasmic matrix also appears to be increased. In addition, in many places a fine grey line (*I*) can be seen within the slits, equidistant between the apposed cell membranes and running parallel to them. This fine intermediate line is lighter in density than the adjacent cell membrane and is separated from the latter by thin strata of low density. These lighter areas do not appear to be "empty," for their density is greater than that of the open urinary spaces (*US*), and strands of finely filamentous material are frequently seen extending between the intermediate line and the cell membranes. The intermediate line (*I*) and material intervening between it and the cell membranes can be seen to better advantage in the inset (a). Inset (b) shows the various layers of the structural complex found in the slits: the stratified cell membranes (*cm*) of the adjoining foot processes with their associated dense cytoplasmic material, and the intercellular line (*I*), separated from the cell membranes on either side by thin strata of low density.

Except for its shallow depth (about 40 A), the structural pattern found in the slits at this level is quite similar to the organization of desmosomes and other so called "adhesion plates." Magnification × 62,000; insets × 124,000.

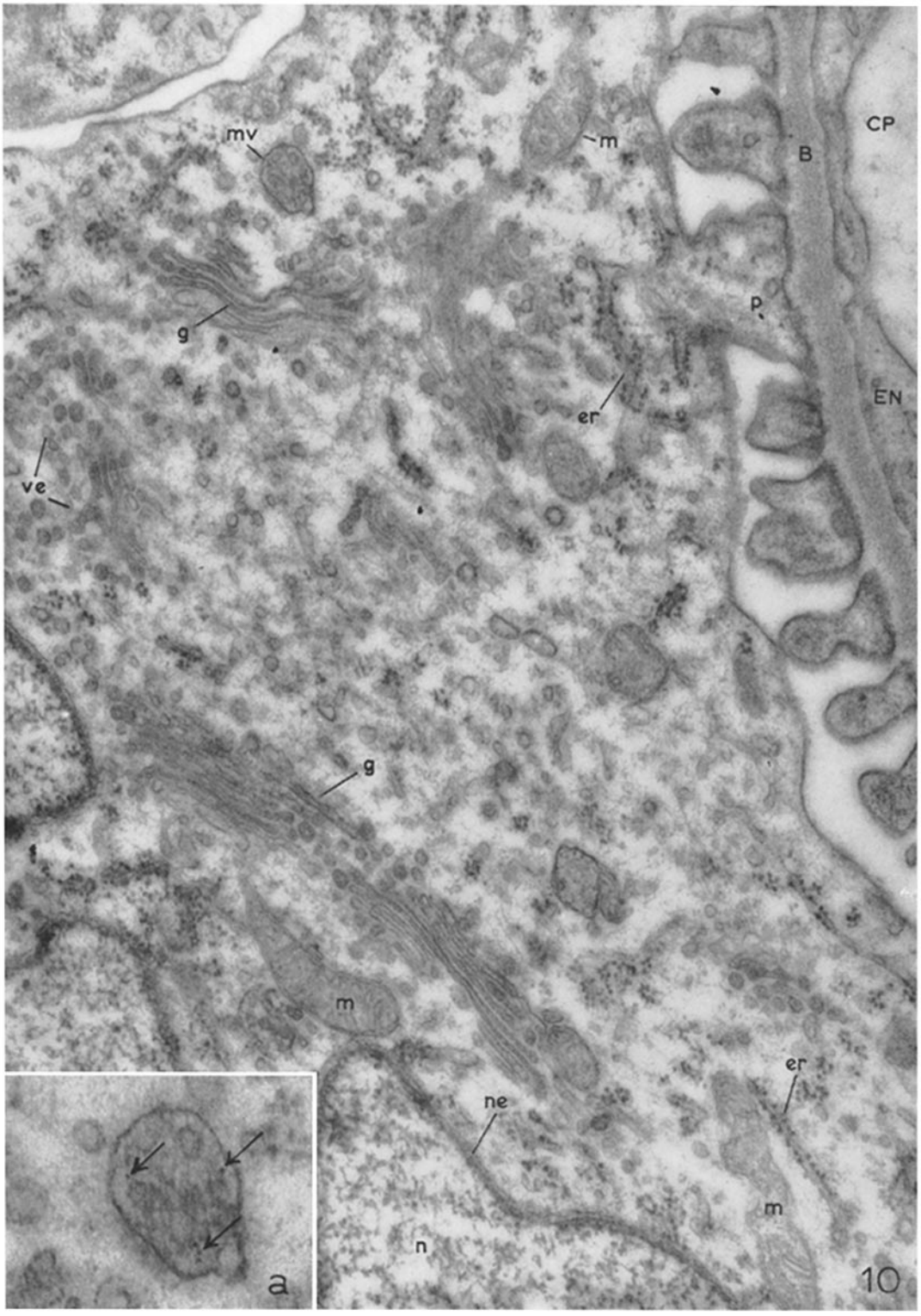


(Farquhar *et al.*: Glomerular permeability. I)

PLATE 12

FIG. 10. Cell body of a visceral epithelial cell showing a large centrosphere region from an animal sacrificed 1 hour following ferritin injection. The nucleus (*n*) and nuclear envelope (*ne*) are visible on the lower left, and foot processes (*p*) extend to contact a basement membrane (*B*) at the upper right of the micrograph. In the cytoplasm there are a number of groups of parallel, closely packed, smooth surfaced Golgi cisternae (*g*) which are surrounded by swarms of small vesicles (*ve*). Also present are elements of the endoplasmic reticulum (*er*) with attached RNP particles, some free (unattached) RNP particles, a few mitochondria (*m*), and a single multivesicular body (*mv*). The latter, which is shown at higher magnification in the inset, is limited by a single membrane and contains, in addition to its characteristic small vesicles, a few ferritin molecules (arrows). (See also Fig. 21.)

The presence of a well developed Golgi complex in these cells and the evidence that membranous material is undergoing constant relocation (from capillary surface to intracytoplasmic vesicles and vacuoles to urinary surface (11)) is in keeping with the suggestion that the Golgi region serves the function, among others, of a depot for membranous material within the cell. Magnification 52,000; inset × 135,000.



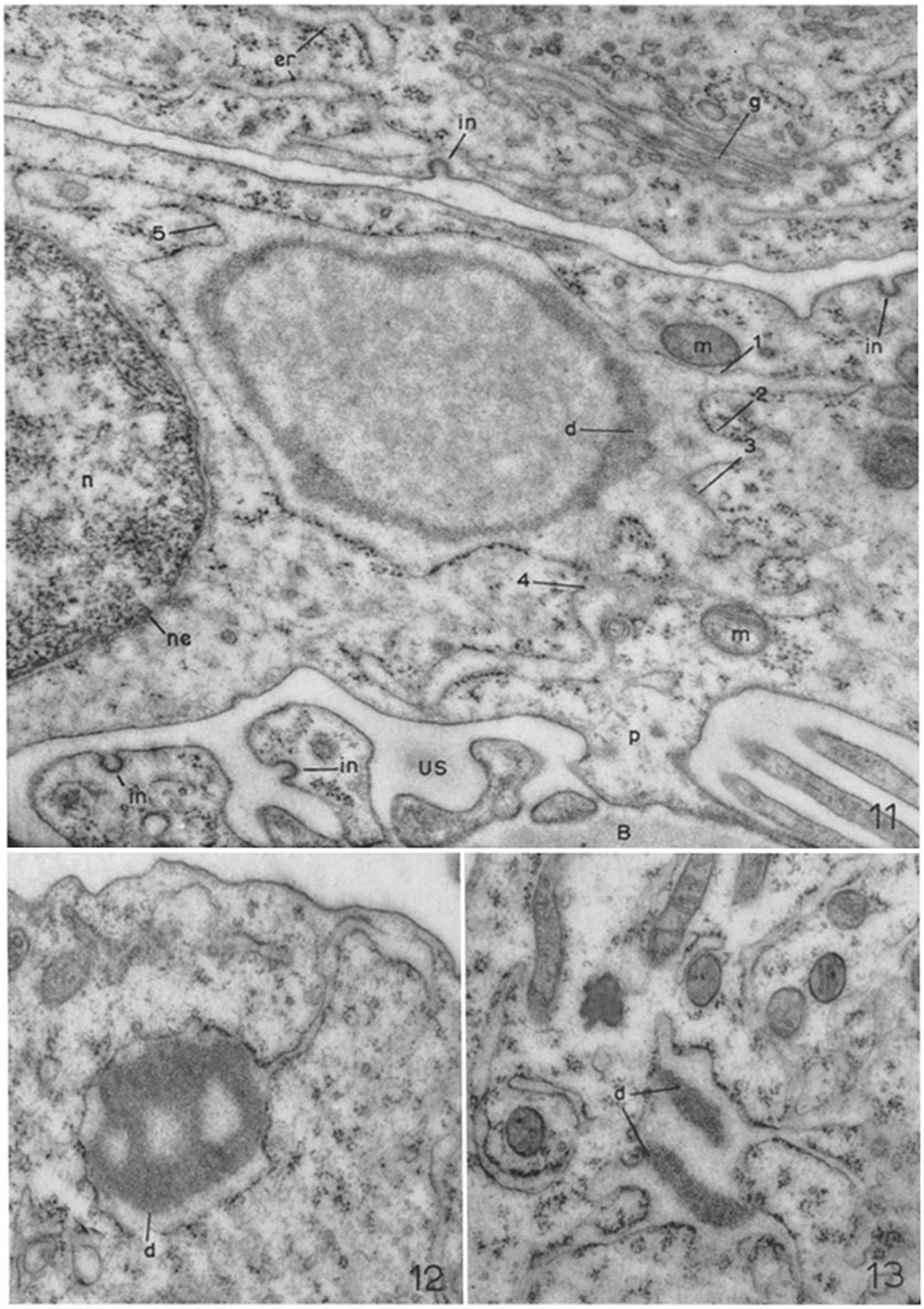
(Farquhar *et al.*: Glomerular permeability. I)

PLATE 13

FIG. 11. Visceral epithelial cell showing the special features of the endoplasmic reticulum found in these cells. The nucleus (*n*) and nuclear envelope (*ne*) are seen on the left; the basement membrane (*B*) and a row of foot processes (*p*) are present below. The center of the micrograph is occupied by a large distended cisterna of the endoplasmic reticulum. It is limited by a rough surfaced membrane (*i.e.*, with attached RNP particles) and is in direct continuity with several of the more common elongated profiles at the points numbered 1 to 5. An aggregate of denser, finely filamentous material (*d*), appearing as a ring profile, forms part of the content of the large cisterna. Large saccular dilitations of the endoplasmic reticulum, such as this, are commonly encountered in the visceral epithelium.

Another common finding in these cells, which is well shown in this field, is the presence of numerous small pinocytic invaginations (*in*) of the cell membrane. Several can be seen along the urinary space. The invaginating membrane is backed on the cytoplasmic side by dense cytoplasmic material sometimes organized in a distinct layer. Magnification 43,000.

FIGS. 12 and 13. These figures also illustrate distended cisternae of the endoplasmic reticulum in the visceral epithelium. As in Fig. 11, these dilated sacs are limited by rough surfaced membranes, are in direct continuity with the more familiar elongated profiles and contain in their cavities a dense component (*d*) disposed in interesting and unusual forms. The large dilated sac in Fig. 12 is in continuity with a single flat cisterna, while that in Fig. 13 communicates with at least 5 cisternae which radiate out from it like spokes from the hub of a wheel. The density and filamentous texture of the denser component of the large sacs resemble those of the basement membrane. Magnification 30,000.



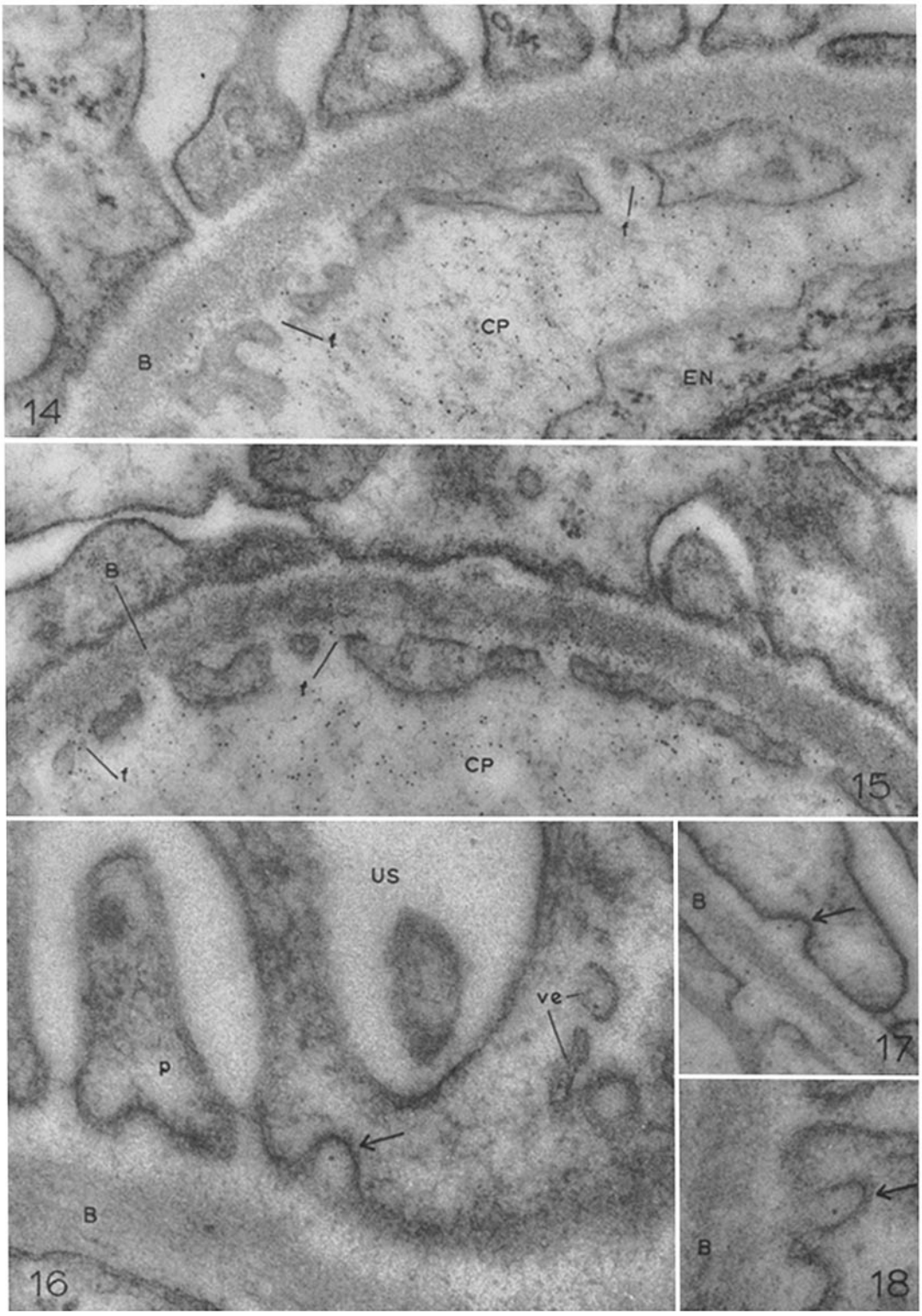
(Farquhar *et al.*: Glomerular permeability. I)

PLATE 14

FIGS. 14 to 18. These figures show the distribution of ferritin in peripheral portions of the glomerular loops at early time points following ferritin administration (10 to 15 minutes). In Figs. 14 and 15, numerous ferritin molecules are seen as fine dense particles in high concentration throughout the capillary lumen (*CP*). A number of particles are also present within the endothelial fenestrae (*f*) which they appear to penetrate freely, thus gaining access to the basement membrane (*B*). In both cases the concentration of the marker appears to be considerably lower in the basement membrane than in the lumen of the vessels. Some accumulation of ferritin is evident in the inner or luminal part of the basement membrane while the rest contains fewer molecules of the tracer. In Fig. 14, for instance, there are 30 molecules in the basement membrane, and of these 25 are located in the inner or luminal half. In a comparable area of the lumen there are 110 ferritin molecules. It is to be noted that the distribution of the marker in the two halves of the basement membrane appears to be random. In Fig. 15, there are 7 molecules in the basement membrane proper, 13 in the subendothelial space, and 9 in the fenestrae. A luminal area equal in size to the basement membrane plus the subendothelial space contains about 140 ferritin molecules.

Figs. 16 to 18 illustrate the distribution of ferritin within the epithelium at early time intervals. In Fig. 16, a single ferritin molecule is present within a tiny invagination of the cell membrane at the base of a foot process (arrow). Other molecules are seen within a foot process, enclosed in cytoplasmic vesicles which have apparently pinched off from the cell membrane. In Figs. 17 and 18, two more membrane invaginations containing ferritin are shown at the base of foot processes. Thus most of the ferritin molecules are retained in the capillary lumen; a few penetrate the basement membrane and at least part of those that traverse it are incorporated into the epithelium by pinocytosis.

Magnifications: Fig. 14, × 75,000; Fig. 15 × 75,000; Fig. 16, × 123,000; Fig. 17, × 67,000; Fig. 18, × 130,000.



(Farquhar *et al.*: Glomerular permeability. I)

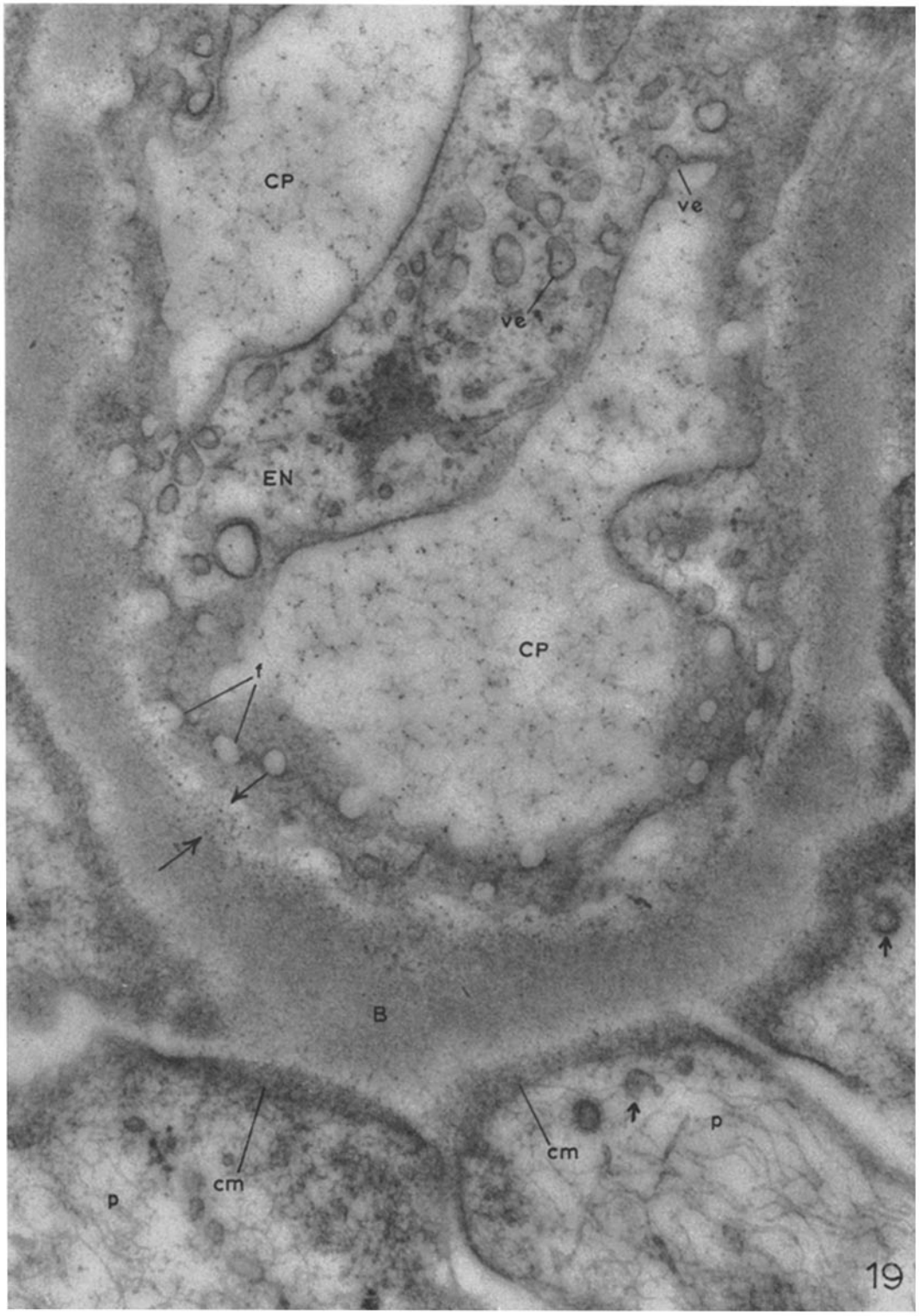
PLATE 15

FIG. 19. Oblique section through a glomerular capillary. Specimen fixed 1 hour after ferritin administration.

Because of the curvature of the vessel and the obliquity of the section, the endothelium appears as a broad band presenting a full faced view of the fenestrae (*f*) which interrupt its attenuated portions. Part of an endothelial cell body (*EN*) projects into the capillary lumen which consequently appears divided into two recesses (*CP*). Numerous ferritin molecules occur in the lumen and a few are seen in small invaginations of the cell membrane (upper *ve*) or isolated vesicles (lower *ve*) in the endothelium. A few tracer molecules are also present within the fenestrae (left arrow of *f*).

The basement membrane (*B*), also obliquely sectioned, appears as a broad band of moderate density and fine fibrillar texture. It contains a relatively large number of ferritin molecules, most of them concentrated in the luminal part of the structure within a relatively thin layer indicated by two opposed arrows. Because of the obliquity of the section, it is not possible to differentiate between molecules in the subendothelial space, and those embedded in the basement membrane.

A few foot processes (*p*) appear in the lower part of the figure and show their obliquely sectioned dense membrane (*cm*), small vesicles—some of which contain ferritin molecules (small arrows)—and fine cytoplasmic filaments (lower right corner). Magnification 67,000.

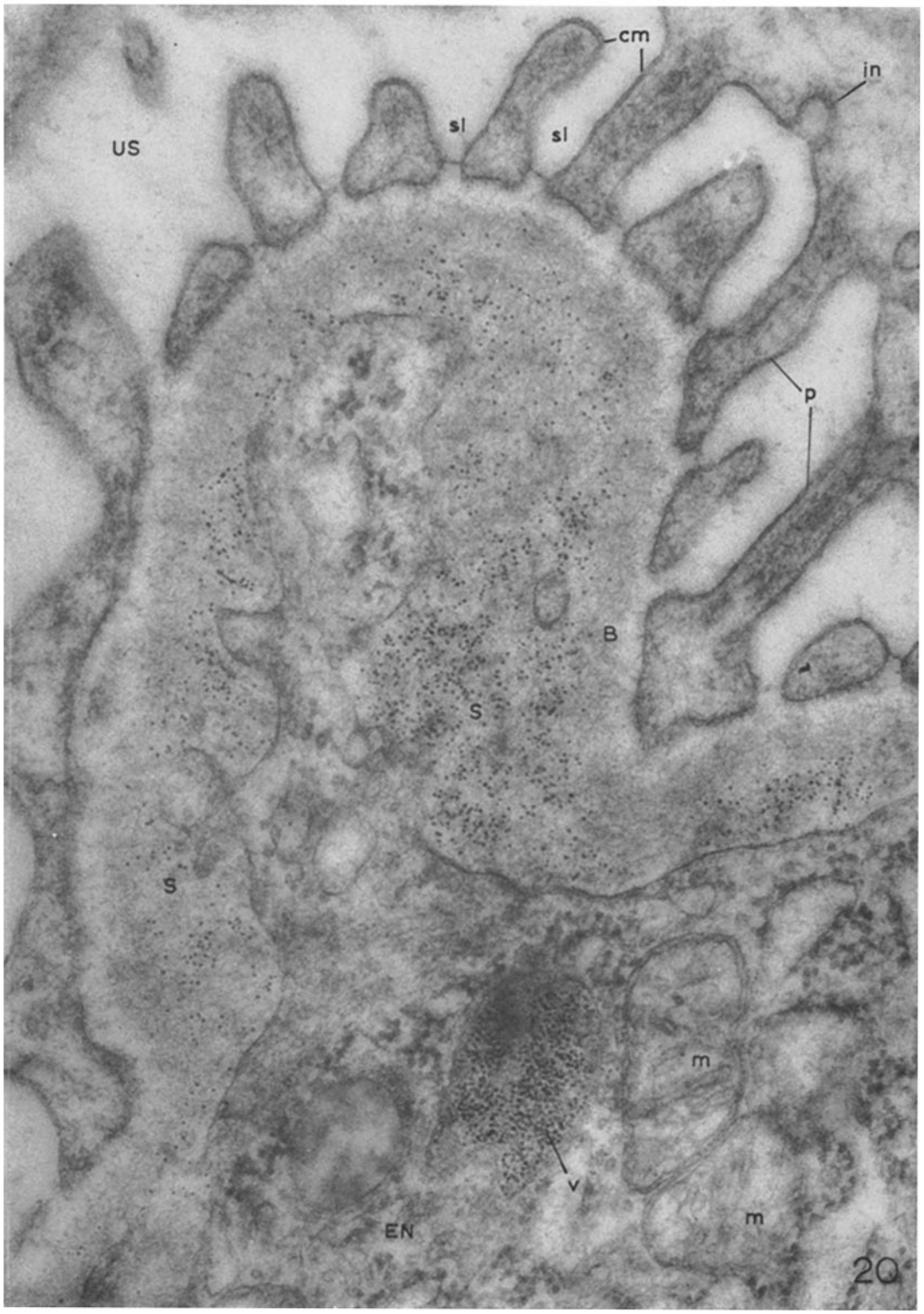


(Farquhar *et al.*: Glomerular permeability. I)

PLATE 16

FIG. 20. Portion of the glomerular capillary wall from an animal sacrificed 1 hour following ferritin injection to illustrate the pronounced accumulation of ferritin which occurs in axial (deeper) regions after longer time intervals. Large numbers of ferritin molecules are concentrated in the spongy areas (*S*) located between the endothelial cell (*EN*) and the basement membrane (*B*). The endothelial cell has several cytoplasmic processes which project into the spongy areas. A membrane-limited body (*v*) packed with ferritin molecules is seen within the endothelial cytoplasm. The accumulation of ferritin in the axial regions of the capillary loops is thought to occur as a result of a "sweeping" of the luminal surface of the basement membrane by the endothelium. The masses of particles retained by the filter appear to be incorporated into the deeper endothelial cells of this region. In this manner filtration residues are apparently removed from the surface and inner layer of the basement membrane.

The two dense layers and the lighter intervening one of the epithelial cell membrane (*cm*) can be seen on the foot processes above. The thinner and more tenuous "slit membrane" bridging the gap between the foot processes shows clearly in several slits (*sl*) marked above. Magnification 94,000.

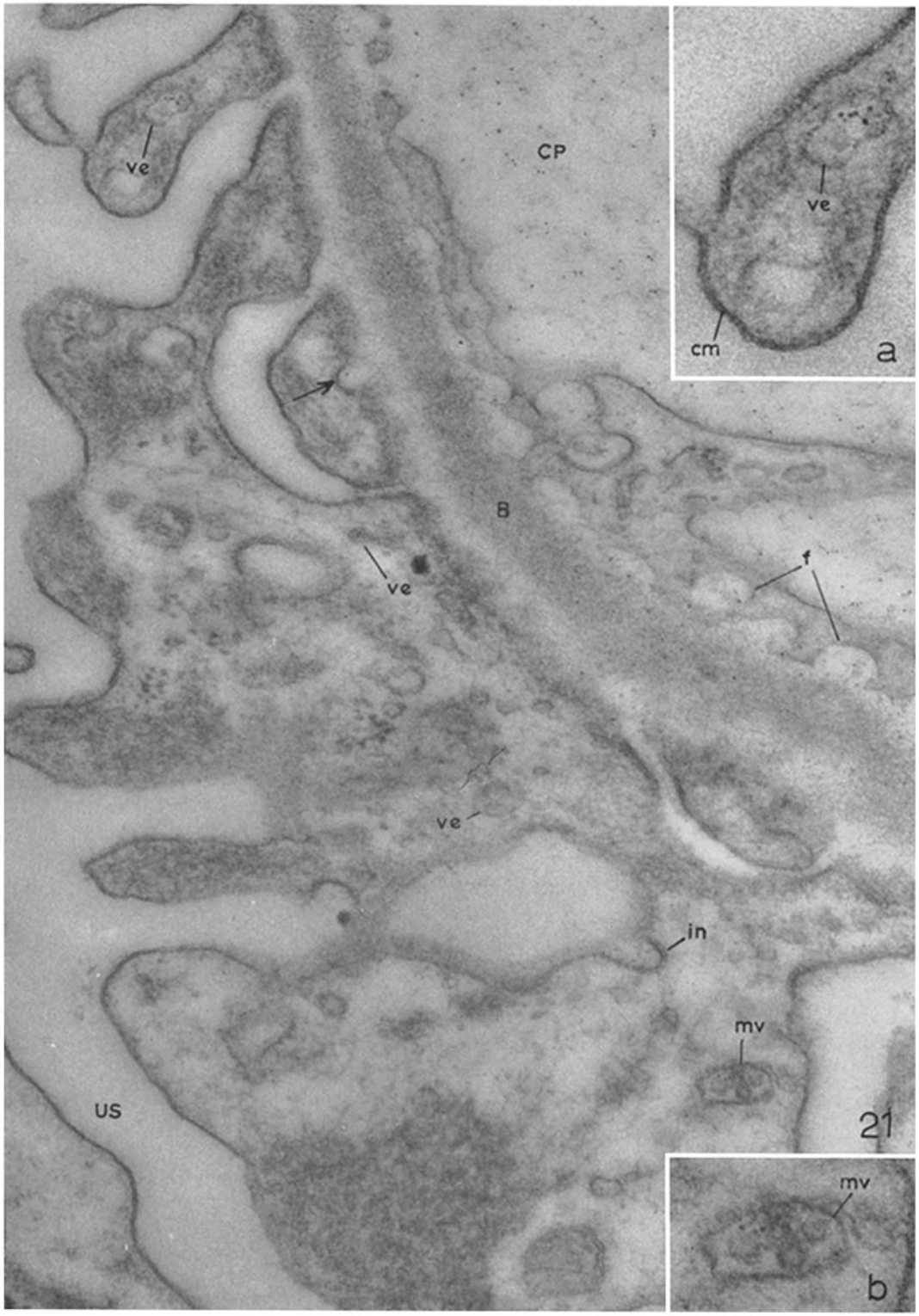


(Farquhar *et al.*: Glomerular permeability. I)

PLATE 17

FIG. 21. Section through the peripheral part of a glomerular capillary loop from an animal sacrificed 1 hour following ferritin injection. Ferritin molecules are seen as fine dense particles within the lumen and the endothelial fenestrae (*f*). A few are randomly distributed throughout the basement membrane. Some are also found in the epithelium: a single molecule is located within a small invagination (arrow) of the cell membrane at the base of a foot process. Apparently this molecule is in the process of being incorporated into the cell by pinocytosis. Other molecules are located in the epithelial cytoplasm enclosed within several small vesicles (*ve*) and within a multivesicular body (*mv*). Inset (a) shows an enlargement of one of the vesicles contaning a group of 5 ferritin molecules. The stratification of the epithelial cell membrane (*cm*) surrounding the foot process can also be seen. The membrane of these cells, like other cell membranes, is composed of two dense layers separated by a space of lesser density, but the total thickness of the layers (about 110 A) is greater than that ascribed to other cell membranes. Inset (b) depicts at a higher magnification the multivesicular body (*mv*) and the ferritin particles present within. Note that the molecules are located within the cavity of the body and not within the small vesicles.

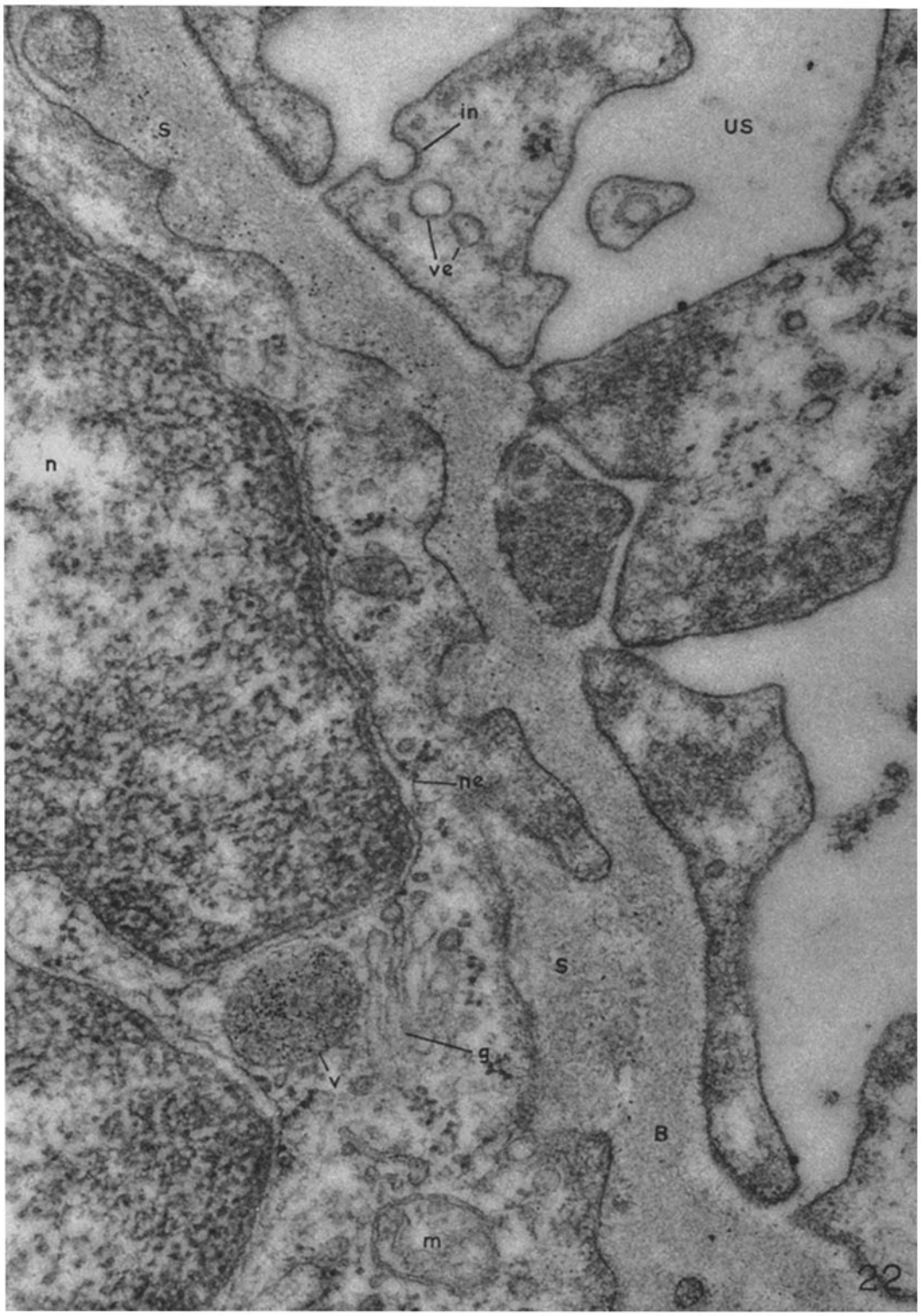
Magnification 58,000; insets × 115,000.



(Farquhar *et al.*: Glomerular permeability. I)

PLATE 18

FIG. 22. Axial region of a glomerular capillary loop 1 hour following ferritin administration. Part of a deep endothelial cell is present on the left. The spongy areas (S) between the endothelial cell and the basement membrane are shown. The endothelial cells have small cytoplasmic processes which project into these spongy areas. Some accumulation of ferritin against the basement membrane is seen in the upper part of the figure. A rounded, membrane-limited body (v) is present within the endothelial cytoplasm. It is presumed that this body is the result of ferritin incorporation and concentration by this deep endothelial cell (See Fig. 20). A small pinocytic invagination (*in*) of the epithelial cell membrane can be seen off the side of a foot process above, and two vesicles (*ve*) are present in the cytoplasm of the same foot process. A dense layer of cytoplasmic material surrounds the invaginating membrane. One of the vesicles contains a single ferritin molecule. Magnification 94,000.

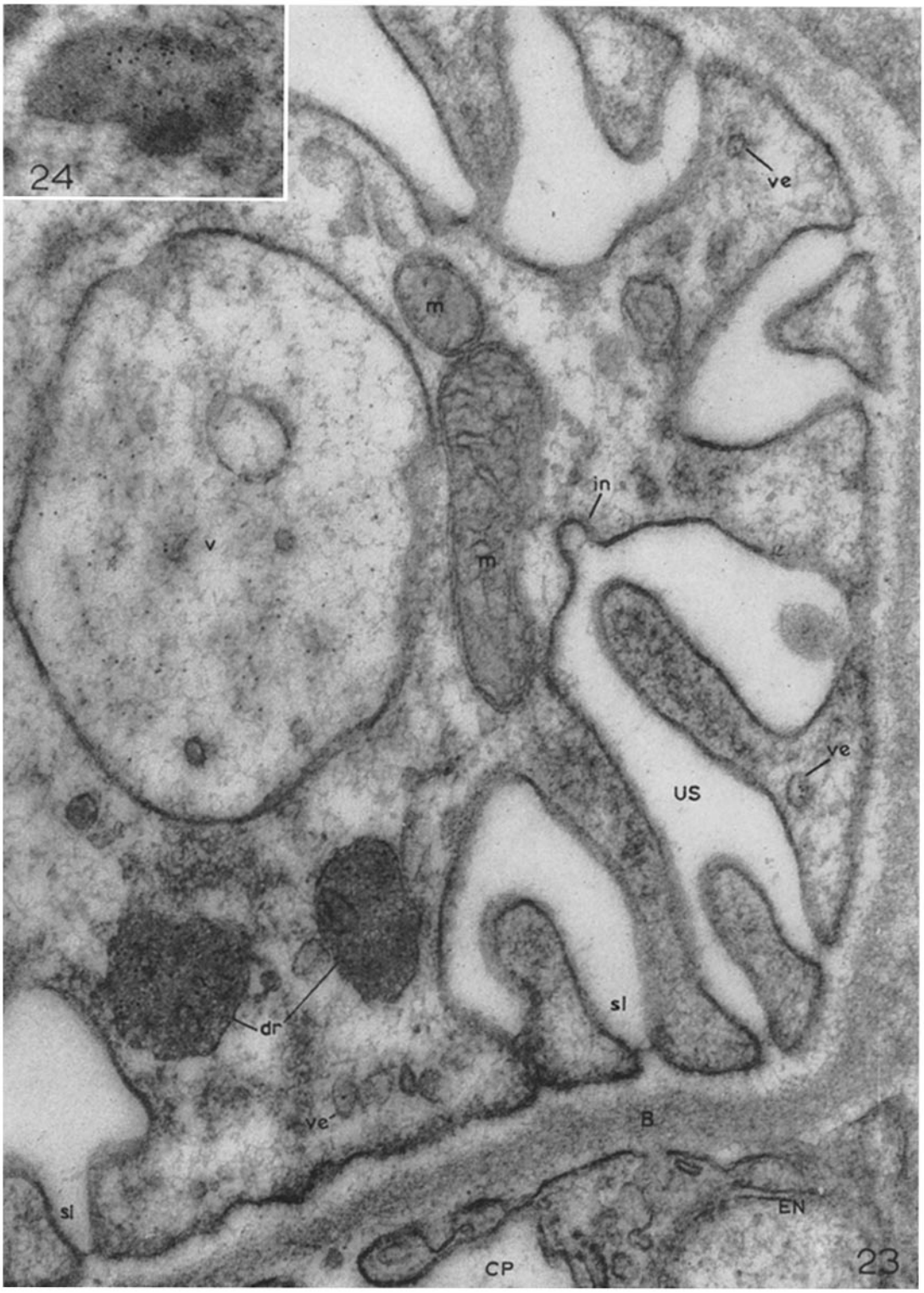


(Farquhar *et al.*: Glomerular permeability. I)

PLATE 19

FIGS. 23 and 24. Visceral epithelial cell in the peripheral region of a capillary loop. Animal sacrificed 1 hour following ferritin administration. The micrograph demonstrates the segregation of ferritin within: (1) small vesicles (*ve*) (about 600 to 800 A); (2) a single large vacuole (*v*); and (3) two dense bodies (*dr*). In Fig. 24, another dense body is enlarged so that the molecules of ferritin it contains can be clearly seen.

The large vacuole is limited by a stratified membrane and has a content of low density. In addition to ferritin molecules, it contains a few vesicles and a fine flocculent precipitate similar to that present in the capillary lumen. The dense bodies or droplets are also limited by a membrane but are characterized by the great density of their content. It is assumed that ferritin molecules—and presumably other proteins which penetrate the basement membrane—are picked up by the epithelium in pinocytic vesicles and transported *via* these small vesicles to vacuoles and multivesicular bodies. The fate of the ferritin within multivesicular bodies is not clear, but the vacuoles appear to undergo condensation into dense bodies. The content of the dense bodies may subsequently undergo partial digestion followed by extrusion into the urinary spaces (11). Magnifications: Fig. 23, × 96,000; Fig. 24, × 120,000.



(Farquhar *et al.*: Glomerular permeability. I)