GLOMERULAR PERMEABILITY*

II. Ferritin Transfer Across the Glomerular Capillary Wall in Nephrotic Rats

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In a previous article (1) we reported results obtained by using ferritin molecules as a tracer to investigate glomerular capillary permeability in the normal rat. Up to 1 hour after intravenous administration, the tracer was present at high concentration in the capillary lumina, at considerably lower concentration in the basement membrane, and in very small numbers in the epithelium, apparently incorporated therein by pinocytosis. In view of these findings and of the fact that there was a marked accumulation of the tracer against the basement membrane at later time points, we concluded that the basement membrane is the main filter of the glomerulus and postulated that the epithelium acts as a monitor which compensates, at least in part, for the imperfections of the filter. We also suggested that the endothelium may function as a valve controlling the area of the filter directly exposed to the blood plasma by varying the number and distribution of its fenestrae.

To check the validity of these conclusions we decided to carry out similar experiments on nephrotic animals in which glomerular permeability is greatly increased. Indeed the various manifestations of the nephrotic syndrome (proteinuria, hypoproteinemia, hypercholesterolemia, and edema) are all believed to result, either directly or indirectly, from a glomerular defect which allows excessive leakage of plasma proteins into the glomerular filtrate. It has been shown that various structural alterations accompany this increased permeability but their significance and relationship to the permeability defect have remained obscure.

The nephrotic syndrome occurs spontaneously in man or can be produced experimentally in animals. It can be induced in rats, for example, by prolonged treatment with the aminonucleoside derivative of the antibiotic puromycin (PA). The sequence of events which develops as a result of this treatment is characteristic and highly

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reproducible (3–8). During the first 5 to 6 days of the standard treatment (daily injections of 1.67 mg PA/100 gm body weight), the chemistry of the blood and urine and the organization of the glomerular capillaries remain virtually normal. From about the 6th to the 12th day, there is increasing proteinuria, followed by hypoproteinemia, hypercholesterolemia, and generalized edema. At the same time morphological changes develop in the glomerular capillaries and affect primarily the epithelium which shows loss of its foot processes and becomes loaded with vacuoles and absorption droplets. After 12 days there is evidence of progressive renal failure leading to azotemia and eventual death of most animals by the 16th day. During this phase, additional alterations appear in the glomeruli and consist of basement membrane thickening, hypercellularity, and partial or complete hyalinization.

In the present article we report the results obtained by using ferritin as a tracer to investigate glomerular permeability in rats with proteinuria induced by PA treatment. The findings confirm the conclusions drawn and support the assumptions formulated in our previous paper. In addition, they provide new information about certain morphological and physiological aspects of the nephrotic syndrome.

Materials and Methods

Ferritin.—Several ferritin solutions were used: one was prepared from horse spleen (9) and contained approximately 20 mg protein/ml. Two other preparations of horse spleen ferritin containing approximately 100 mg protein/ml were obtained commercially from Nutritional Biochemicals, Cleveland, Ohio, and from Pentex, Inc., Kankakee, Illinois. In each case the ferritin was dialyzed for 24 to 48 hours against 3 changes 0.07 M phosphate buffer (pH 7.2) just prior to injection. When animals were sacrificed within 1 hour following ferritin administration the injection was relatively well tolerated and no further modification of the ferritin was found necessary; however, with the commercial preparations, when the time interval was extended over 2 hours, the animals developed dyspnea and cyanosis, terminating in death by 3 to 4 hours after the injection. Reducing the dosage, diluting the ferritin, and prolonging the injection time had no effect on the mortality. Both commercial ferritin preparations were subsequently found to contain cadmium detectable in dilutions as low as 10^{-5} (see below). Hence the ferritin was dialyzed for 36 to 48 hours against 3 changes of 0.1 M EDTA[1] in 0.07 M phosphate buffer (pH 7.2) followed by dialysis against 2 to 3 changes of phosphate buffer alone for 24 hours. After this treatment the ferritin solutions were cadmium-free in dilutions of 10^{-1}. Animals given the EDTA-treated, cadmium-free ferritin showed no ill effects and lived apparently undisturbed up to 10 days—the limit of the time interval explored. Short term experiments were carried out with both EDTA-treated and non-EDTA-treated ferritin. Only the EDTA-treated tracer was used in long term experiments.

Test for Cadmium.—A modification of a method previously utilized by Granick (10) was used to test for the presence of cadmium in the ferritin solutions. Serial dilutions of the ferritin (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) in 0.07 M phosphate buffer (pH 7.2) were prepared in test tubes. Two or three drops of dithizone solution (0.3 mg in 100 ml chloroform) were then added to each test tube and the latter agitated. A positive test for cadmium was indicated by a change in the color of the dithizone solution from blue to pink.

Animals.—Thirty young Sprague Dawley male rats, with an initial body weight of 100 to 150 gm and weighing 150 to 200 gm at the time of sacrifice were used in these experiments.

[1] Disodium ethylenediaminetetraacetate.

Experimental Procedures.—Each animal was given 9 daily subcutaneous injections of a 0.5 per cent solution of the aminonucleoside of puromycin at a dose of 1.67 mg/100 gm body weight. 6 separate groups of animals were started at different times; in 3 groups the daily dosage of aminonucleoside was calculated to the nearest 100 gm body weight while in the others the dosage was adjusted upward for each 15 gm increase in body weight. On the day following the last injection (*i.e.*, the 10th day after beginning the aminonucleoside treatment) the animals were anesthetized and 0.5 to 2 cc of ferritin administered via the saphenous or femoral vein. Kidney tissue was fixed at selected intervals from 5 minutes to 44 hours following ferritin injection and prepared for electron microscopy. Table I gives a list of the time points investigated and the number of animals for each interval.

Animals given 9 daily injections of aminonucleoside were selected for these experiments because previous studies (6–8) had established that at this time proteinuria is nearly maximal while glomerular changes are minimal, involving primarily the visceral epithelium. At the time of sacrifice all animals had ascites and edema of the loose connective tissue within the peritoneal cavity, but both of these signs varied considerably in degree from one animal to another.

Processing of Tissue.—Methods for initiating fixation of the kidney *in situ*, collecting the specimens, dehydration and embedding of tissues, and preparation of thin sections for electron microscopy were described in a previous paper (1).

TABLE I

Time interval*	5 min.	15 min.	1 hr.	3 hrs.	6 hrs.	12 hrs.	24 hrs.	44 hrs.
No. of animals	5	4	8	5	2	2	2	2

* Time elapsed between intravenous ferritin injection and fixation of the kidney.

OBSERVATIONS

Glomerular Structure in Nephrotic Rats

Our observations confirm that at the time investigated, *i.e.*, 10 days after the onset of the treatment, typical lesions are found in all glomeruli, their extent and severity being roughly correlated with the dosage of PA. There is, however, considerable variability in the severity of the lesions from one animal to another, among glomeruli of the same kidney and even among loops of the same glomerulus. As repeatedly reported, the lesions at this period affect primarily the epithelium, but since we have also noted alterations in the endothelium and the basement membrane, and since some of the epithelial changes we encountered have not been previously reported, we shall describe the morphology of the nephrotic glomerulus in some detail, layer by layer.

Endothelium.[2]—The change most frequently encountered in the superficial endothelial cells is a thickening of the peripheral cytoplasmic layer and a reduction in the number and collective area of its fenestrae (Figs. 1, 10, 12, and 15). Although the alteration does not affect all capillary loops and is spotty in its distribution along the same loop, the aggregated area of the

[2] All cells located on the luminal side of the basement membrane have been referred to as "endothelial." "Deep" refers to those which are not clearly in contact with the lumen while "superficial" refers to those which directly line the capillary lumen (1). In addition to this difference in topography, the deep cells can be distinguished from the superficial by their extensive phagocytic activity (14).

fenestrae, and therefore presumably the surface of the basement membrane directly exposed to the blood plasma, appears to be definitely reduced in comparison to normal animals (1). The second change encountered is an increase in the number of cells, primarily of "deep" cells, in the axial regions of the loops. These cellular elements (Figs. 1, 18, and 20) resemble macrophages (histiocytes) by virtue of their pseudopodia and the polymorphism of their inclusions. Many of them have however, a fine fibrillar cytoplasmic matrix reminiscent of that of smooth muscle cells. Sometimes a pseudopod projects through the cytoplasm of the superficial endothelium to contact the capillary lumen (Fig. 18).

It is to be noted that by comparison with the epithelium, the endothelium showed less extensive lesions and retained an apparently normal morphology in many capillary loops.

Basement Membrane.—No uniform or generalized changes are visible in the basement membrane. Its general morphology appears normal in many loops (Figs. 1, 3, 5, and 6) but in others the total thickness of this layer is reduced and its luminal strata appear less compact than the rest (Figs. 10 and 17). This defect is particularly visible in the axial regions where it is clearly demonstrated by the pattern of deposition or piling of ferritin (Figs. 5, 17, and 20) (see page 704). Furthermore, the fibrillar texture of the basement membrane as a whole is more frequently evident than in normals (Figs. 9 and 11). In addition, the spongy areas in the axial regions are more numerous, larger and more complicated in outline (Figs. 1, 5, 17, and 20), complementing the complex disposition of the endothelial cells.

It is to be emphasized that no discontinuities or interruptions of the basement membrane have been detected in any of the many glomeruli examined. Other types of defects (*e.g.*, local convolutions, foci of thickening accompanied by decreased density, "laminar" splittings into two distinct layers separated by a clear interspace) are occasionally encountered, but they are limited in extent and diverse in appearance.

Epithelium. The most striking and constant changes occur in the epithelium. The epithelial foot processes are lost or greatly distorted and, as a result, the outer surface of the basement membrane is covered by broad, continuous sheets of epithelial cytoplasm interrupted by rare intercellular spaces (*e.g.*, Figs. 1, 4, 5, 6, and 17). In addition, the contours of the epithelial cells are highly irregular, and consequently the geometry of the urinary spaces appears more complicated and tortuous than in normal animals, showing many sinuses and recesses (Figs. 4 and 5). These spaces are usually bordered by one or more cells and are frequently occupied by formed or partially dispersed residues apparently derived from discharged absorption droplets (Fig. 8) (see below).

Definite alterations are also evident in the internal organization of the epithelial cells, the most noticeable being a general increase in formed cytoplasmic components. The greatest increases are seen in the number of vacuoles and dense bodies of the cytoplasm (Figs. 1, 5, 17, and 19), but other components are also involved, *e.g.*, mitochondria, free ribonucleoprotein (RNP) particles, Golgi elements, and smooth and rough surfaced elements of the endoplasmic reticulum (Figs. 6 and 16). The latter frequently showed distended cisternae partially filled with plicated sheets of a material comparable in density and texture to the basement membrane (Fig. 16). Such images, suggesting the intracisternal segregation of a cell product, were encountered previously in normal animals (1).

There is considerable variation in size, density, and internal structure among the dense bodies of the epithelial cells: many appear uniformly dense (Figs. 1, 5, and 17); others have a more pale content with a fine granular texture; and still others contain small vesicles similar to those found in multivesicular bodies (Fig. 7). Structures intermediate in morphology between the large vacuoles and the dense bodies are frequently seen. A few of the vacuoles, intermediate forms, and dense bodies are regularly found in the centrosphere region, but most of them are concentrated in the cytoplasmic branches or trabeculae. Some of the latter appear to

be almost exclusively occupied by vacuoles and dense bodies. The cytoplasmic sheets or "soles" of the cell, which cover the basement membrane in lieu of foot processes, have a fibrillar matrix and contain numerous small vesicles ∽600 A in diameter, but are otherwise virtually free of formed cytoplasmic components (Fig. 6). The cell membrane facing the basement membrane is finely ruffled owing to the presence of numerous small pinocytic invaginations (Figs. 4, 5, 10, 12, and 17). The cell membrane facing the urinary spaces shows similar but much less frequent invaginations (Figs. 4, 5, and 15). In addition, large sinuses or cleft-like cavities with a content similar to that of some dense bodies frequently occur along the same cell surface. Such sinuses presumably represent dense bodies in the process of discharging their content into the urinary spaces (Figs. 7, 8) (see reference 11).

As already indicated, the epithelial sheet surrounding the capillary loops is interrupted by considerably fewer intercellular spaces than in normal animals. Actually, the aggregated area of these spaces represents less than 5 per cent of the outer capillary surface as compared to ∽20 per cent in the normal (see reference 1). In the few areas where remnants of foot processes persist (Figs. 5 and 8), the intercellular spaces have an organization approaching that of normal epithelial slits. In a previous paper (1), we defined the slits as extending to a depth of approximately 300 A from the basement membrane to the slit membrane. At the level of the latter the gap is narrowed to 250 to 300 A.[3] In the nephrotic animal, the majority of the junctions appear tighter, for the intercellular space is reduced to as little as 80 A (Figs. 2, 3, 5, 11, and 17). Moreover, the junctions are considerably increased in depth (Figs. 2, 5, and 11), as indicated by the close association of the apposed cell membranes over considerable distances. In many instances, especially in oblique sections, this type of "tight" junction found close to the basement membrane is followed towards the urinary spaces by a broader (approximately 300 A) gap bisected by an intermediate line (Figs. 2 and 3), reminiscent of that described in normal slits. Sometimes a similar, but less pronounced intermediate line can be made out in the tight part of the junction (Fig. 3 *a*).

Glomeruli of Nephrotic Animals Given Ferritin

Table I shows the distribution of the time points examined from 5 minutes to 44 hours following ferritin administration. Significant differences in ferritin distribution were seen only with large differences in time intervals. Hence, the description of the results will be simplified by dividing all the time points into 3 distinct groups:

Animals Sacrificed 5 to 15 Minutes Following Ferritin Administration.—At all early time points, large numbers of ferritin molecules were seen in the capillary lumina and within the residual endothelial fenestrae (Fig. 9). A few molecules were present within the endothelial cytoplasm enclosed within small vesicles or within multivesicular bodies (Fig. 12). Many molecules were also found embedded in the basement membrane throughout its entire depth (Figs. 9, 10, and 12), although the concentration of the marker in this layer was considerably lower than in the lumen. Even after these relatively short intervals some evidence of accumulation of ferritin was already found in the spongy regions between the endothelial cells and the basement membrane (Figs. 13 and 14). In general there were some local variations in the number of tracer molecules found in the basement membrane and in the spongy areas of axial regions, but usually the ferritin concentration in these sites roughly paralleled luminal concentrations. In all cases there was no detectable evidence of preformed pathways or channels across the basement membrane: there were no lighter tracks ahead or behind the molecules of

[3] In our previous paper (1) the slit width was incorrectly given as 2500 to 3000 A.

tracer and there was no preferred relationship of the ferritin embedded in the basement membrane to the residual epithelial slits or other cell junctions.

At these short time intervals some ferritin molecules were also seen within the epithelium. They were found primarily within small invaginations of the plasmalemma (Figs. 10 and 12), or within small cytoplasmic vesicles located close to the basement membrane (Figs. 10, 12, and 14). Two or more ferritin molecules were commonly seen within a single invagination or vesicle. The extent of the pinocytic activity along the cell front facing the basement membrane was so marked that within relatively small segments of this front (*e.g.*, Fig. 12) molecules were frequently caught in all the stages of the process: *i.e.*, located in membrane invaginations, in vesicles nearly detached from the membrane, and, finally, in isolated intracytoplasmic vesicles. Some ferritin molecules were also seen within multivesicular bodies (Fig. 13) and large vacuoles in the epithelial cytoplasm. Relatively few of the dense bodies contained ferritin after these short intervals (Fig. 13).

In a few instances ferritin was found within the residual epithelial slits, but this was not a frequent finding, and no accumulation of the marker occurred at the introit of either the slits or other cell junctions. More frequently molecules of the tracer were seen within pockets of varied sizes formed by the epithelium (Fig. 15). Such pockets were recognized as extracellular recesses, rather than intracellular vacuoles, by virtue of their continuity with either the slits or the urinary spaces. Usually the continuity was established through an intercellular junction (Fig. 4). More rarely the epithelial cell membrane appeared to have retracted away from the basement membrane, thus forming a blind pouch limited by the basement membrane over a rather broad area (Figs. 15 and 18). Sometimes, also, ferritin was found in what appeared to be an "open" urinary space; however, because of the complex arrangement of these cells it was frequently impossible to determine what was an "open" urinary space and what was an intercellular pocket. For the same reason, unless a connection *via* an intercellular space was evident in the plane of section, it was frequently impossible to distinguish between extracellular recesses and intracellular vacuoles.

Animals Sacrificed 1 to 3 Hours Following Ferritin Administration.—At these later time points, numerous ferritin molecules were still present within the capillary lumina and endothelial fenestrae. Many molecules were also seen at all levels randomly distributed throughout the basement membrane (Fig. 17). In the peripheral regions of the capillary loops there was no discernible piling of the tracer against the basement membrane, but in the axial regions massive accumulations of the marker were found in the subendothelial spaces, especially in the spongy areas where the ferritin masses penetrated deeply into the luminal strata of the basement membrane (Figs. 5, 17, and 20). Both the superficial and especially the deep cells showed numerous pseudopodia (Figs. 17, 18, and 20) and elaborate infoldings of their cell membranes; numerous marker molecules were often present in the complicated intercellular spaces thus formed. Furthermore, membrane-limited vacuoles packed with ferritin were commonly seen in the cytoplasm of both cell types, but were more frequent in the deep cells (Figs. 18 and 20). Frequently, a whole spectrum of phagocytic vacuoles, from some with a content comparable to the extracellular deposits in the spongy areas to others with a condensed content of high density, could be found in the same cell (Fig. 20).

Many more molecules of ferritin were found in the epithelium after these intervals. Some were present within membrane invaginations or within small vesicles located close to the basement membrane as described for shorter intervals (Fig. 17). Some were also seen within extracellular pockets as previously mentioned. However, many more molecules appeared within large cytoplasmic vacuoles, multivesicular bodies, and dense bodies than before (Figs. 17 and 19). Some of the dense bodies had a homogeneous, others a heterogeneous content. Between them and the large vacuoles a whole spectrum of possible intermediate forms was encountered, all of them marked by the tracer. No ferritin was seen within droplets which appeared to be in

the process of discharging their content into the urinary spaces (Fig. 7) or within the droplet residues lying free in the urinary spaces (Fig. 8). It is to be emphasized that at these time points within the epithelium the marker was, as a rule, restricted to membrane-limited compartments (*i.e.*, vesicles, multivesicular bodies, vacuoles, dense bodies, and intermediate forms); no ferritin was found free in the cytoplasmic matrix.

Animals Sacrificed 6 to 44 Hours Following Ferritin Administration.—Ferritin molecules were found in the circulation up to 44 hours following injection, although their concentration in the capillary lumina appeared to decrease with elapsed time. The ferritin masses found in the spongy areas of axial regions became larger and more extensive with passage of time. In addition, many more membrane-limited bodies containing ferritin were found in the cytoplasm of both the superficial and deep endothelial cells, but were especially numerous in the latter. At 6 hours and thereafter, the ferritin in some of these cytoplasmic bodies appeared more concentrated than at earlier intervals.

After these long intervals increased quantities of ferritin were also found within the epithelium. Many molecules were located within membrane invaginations, vacuoles, multivesicular bodies, and dense bodies as before. However, others were found in droplets in the process of discharging their content into the urinary spaces and, particularly, distributed throughout the cytoplasmic matrix (Fig. 21). Hence the principal new finding within the epithelium at these intervals was the fact that the ferritin was not confined exclusively to membrane-limited compartments but was "free" within the cytoplasmic matrix as well.

DISCUSSION

Morphological Findings

Our observations show that after 9 days of aminonucleoside treatment, when massive proteinuria is already established (4, 6, 7), marked alterations are found in the visceral epithelium of the renal glomeruli. There is extensive loss of the foot processes of the epithelial cells and concomitant decrease in the total number and collective area of intercellular junctions. In this respect and to this extent, we have confirmed observations already made by others (6–8). In addition, we have detected less striking and less generalized, yet undoubtedly important changes in the other layers of the glomerular capillaries: the reduced frequency of the endothelial fenestrae, the increased number of deep endothelial cells in the axial regions of the capillary loops, and the "loose" texture of the basement membrane—particularly its luminal strata—can be listed as such changes.

Our observations further indicate that at least some of the normal activities of the visceral epithelium are greatly enhanced in nephrosis. There is, for example, a striking increase in the pinocytic activity of these cells as evidenced by the high frequency of small membrane invaginations, small cytoplasmic vesicles, multivesicular bodies, large vacuoles, and dense bodies—in various stages of condensation or discharge. Our tracer experiments show that all these elements are connected with the ability of the epithelial cells to incorporate substances in bulk from the surrounding medium and dispose of them completely or in part (11). From a general cytological point of view, it is noteworthy that the tracer, although present in the vesicles and vacuoles mentioned, does not gain access to the entire system of membrane-limited spaces of the cytoplasm. It is not found, for instance, within the rough surfaced cisternae of the system, or within the piled cisternae and small vesicles of the Golgi complex.

Cytoplasmic components usually associated with protein synthesis, *i.e.*, RNP

particles and rough surfaced elements of the endoplasmic reticulum, are present in higher concentrations than in normal visceral epithelia, and the occurrence of dense laminated material resembling basement membrane within dilated cisternae of the endoplasmic reticulum is a frequent finding. It therefore appears that the protein-synthesizing equipment of the cell is augmented under the influence of aminonucleoside. The increase could be related either to the synthesis of basement membrane components or to the production of hydrolytic enzymes needed for the digestion of incorporated substances (11).

As far as the epithelial intercellular junctions are concerned, it should be stressed that not only are they reduced in number, but also most of those still present are tightened and increased in depth. As a result, direct access from the basement membrane to the urinary space is rendered more difficult. Actually, the "tight" junctions found in nephrotic animals are comparable to those recently described by Peachey and Rasmussen (12) in the epithelium of the toad bladder and assumed by them to "serve a function in reducing the leakage path through the bladder wall between the cell."[4] The normal slits as well as the tight junctions have structural features reminiscent of usual epithelial desmosomes (*i.e.*, strict parallelism and increased density of the apposed cell membranes and presence of an intermediate line), but they lack the supposedly characteristic layering of dense material along the cell membrane and the elaborate fibrillar differentiation of the subjacent cytoplasmic matrix (13). The transition along a given junction from a normal to a narrowed intercellular gap presumably represents an obliteration of the intercellular space and may correspond to the replacement of usual urinary slits by "watertight seals." The process seems to proceed progressively from the basement membrane toward the urinary spaces.

Physiologic Data Obtained

The retention of large amounts of ferritin in the capillary lumina, the marked difference in the concentration of the marker in the blood plasma and in the basement membrane at the early time points, and especially the piling of ferritin against the basement membrane after long time intervals, indicate that in the nephrotic glomerulus, as in the normal, the basement membrane is the main filtration barrier. In the nephrotic glomerulus, however, the filter (*i.e.*, the basement membrane) is defective and allows leakage of increased amounts of ferritin, as evidenced by the fact many more tracer molecules are found embedded in the basement membrane and incorported into the visceral epithelium than is the case in normal animals (1). The random distribution of large numbers of ferritin molecules throughout the basement membrane and the absence of visible channels or pathways suggests that the increased permeability of this layer in the nephrotic animal is due to a fine, generalized defect.

[4] A similar junction has been described by Karrer in cervical epithelium (47) and in the cardiac muscle of thoracic veins (48), and by Rosenbluth and Palay (49) in the contact areas between layers in loose myelin. Karrer called it a "quintuple-layered cell interconnection," and all authors mentioned recognized its similarity to the "external compound membrane" of Robertson (50). In fact a similar structure appears to be of widespread occurrence around lumina which are supposed to be watertight.

The accumulation of ferritin against the basement membrane is greater in the axial than in the peripheral regions of the loops and becomes more extensive with time. Furthermore, evidence of incorporation in bulk of these filtration residues is found in all endothelial cells, especially in those described as "deep" cells, which show elaborate pseudopodia and increasing phagocytosis with time. On the basis of these findings it can be tentatively assumed that "sweeping" of filtration residues to axial regions and their subsequent incorporation by the deep cells found in these sites may represent the mechanism by which the filter is declogged or reconditioned. Since along with the ferritin residues the deep cells also ingest some portions of the basement membrane, particularly its spongy layers, one can speculate that these cells may also function at the removal end of the turnover process of basement membrane by ingestion of its worn out layers. Further studies are needed, however, to establish whether or not such a mechanism operates in the normal glomerulus. Our findings, which indicate that a functional distinction based on extent of phagocytosis can be made between the superficial and deep cells, and our views on the relationship of these findings to the "mesangial" concept will be fully treated in a separate paper (14).

The fact that increased quantities of ferritin are found within the epithelium demonstrates that the recovery and segregation of ferritin by the epithelium is greatly stepped up in the nephrotic animal. This process is apparently facilitated by the diversion of a substantial part of the filtrate flow through the epithelial cells. The suggestion that in nephrosis much of the filtrate must pass through the epithelial cells was originally advanced as a result of a study of children with the nephrotic syndrome, in which normal and occasionally supernormal glomerular filtration rates were found in association with a decrease in slit area and with signs of increased epithelial activity (15). This view is strengthened by the present results which show that most intercellular junctions in the nephrotic epithelium are "tight"; that many of the remaining slits lead into blind recesses; and that there is no accumulation of ferritin at the introit of the few slits which remain apparently normal, while there is massive accumulation of the marker within the epithelial cells themselves. Although the preponderance of the intracellular route appears strongly supported by the new findings, there is also evidence that smaller amounts of the tracer may reach the urinary spaces via intercellular channels: occasionally ferritin molecules are found within some slits, and more frequently the marker occurs in appreciable concentration in some extracellular diverticula of what appear to be "open" urinary spaces. Its persistence in such places throughout the preparation procedure, may be due to poor drainage of the diverticulae or to a high protein content in the filtrate.

The disappearance of the foot processes and slits and the concomitant diversion of a substantial part of the filtrate flow through the epithelial cells can be interpreted as an attempt to reduce the protein loss caused by the increased

permeability of the basement membrane. The high concentration in the epithelium of "absorption droplets," containing ferritin and probably plasma proteins, supports the view that this diversion apparently increases the chances of protein recovery from the filtrate. The first part of the recovery operation is carried out by pinocytosis of the "micro" variety but is not preceded, as in amoebae (16–19), by visible protein or tracer adsorption onto the cell membrane. It is of interest that in amoebae, at least, proteins and salts are the most efficient inducers of pinocytosis (20). Hence one can speculate that the presence of protein in the glomerular filtrate may act as a stimulus for pinocytosis by the epithelium.

Up to 3 hours the ferritin remains confined almost exclusively within membrane-limited bodies (vesicles, vacuoles, multivesicular bodies, and droplets), suggesting that during this period ferritin enters the cell only by transport "in bulk" (*i.e.*, membrane invaginations) and indicating that neither the cell membrane nor the derived membranes of the various cytoplasmic bodies are directly permeable to ferritin. The fact that ferritin *is* found in the cytoplasmic matrix after 3 hours suggests that after long intervals the cell membrane or droplet membrane becomes disrupted or in some manner more permeable to ferritin, and indeed this possibility cannot be excluded as a late event (21, 22). However, on the basis of available information (21–25) it seems likely that much of the "free" ferritin is newly synthesized or endogenous in origin. The liberation of iron following partial digestion of the ferritin segregated within the droplets could act as a stimulus to new ferritin production. Such a sequence of events would be comparable to that which apparently occurs in cells of the proximal convolution (21, 25) and visceral glomerular epithelium (22), where ferritin is found within and around droplets following the degradation of absorbed hemoglobin. This interpretation is also in keeping with Richter's recent finding (24) that recognizable, presumably newly synthesized ferritin appears in cultured cells 4 hours after the introduction of ferric sulfate into the culture medium.

Comments on the Pathology and Physiology of the Nephrotic Syndrome

The nephrotic syndrome, whether occurring spontaneously in man or produced experimentally in animals, is characterized by massive loss of blood proteins—primarily albumin—in the urine. The other derangements found in this condition, *i.e.*, hypoproteinemia, hypercholesterolemia, edema, etc., are generally considered to be consequences of the proteinuria. Although it is still debated whether or not diminished tubular reabsorption contributes to the protein loss (26–29), increased glomerular permeability is now widely accepted as the basic functional defect involved in the syndrome. The view is clearly supported by the finding that the glomerular clearance for albumin (26–29), other serum proteins (27, 30), and sized dextran molecules (31) is greatly increased in nephrotics. Available permeability data indicate that the

glomerular capillary wall behaves like a sieve in the normal (see reference 1) as well as in the nephrotic (26–31) condition. In both cases there is progressive restriction to filtration with increasing molecular weight and average diameter of the circulating molecules, but in the nephrotic glomerulus the "pores" of the sieve, having a slightly greater effective diameter, allow the plasma proteins, especially albumin, to escape more readily into the urinary spaces.

Electron microscopical studies have shown that the massive proteinuria of the "pure" or "uncomplicated" nephrotic syndrome is regularly associated with glomerular changes which affect the visceral epithelium and characteristically consist of a reduction or disappearance of the epithelial foot processes and of the intervening slits. This association, described originally in nephrotic children (15, 32), has been repeatedly confirmed in humans (*e.g.*, 33–39) as well as in aminonucleoside nephrotic rats (6–8).

It was originally suggested (15, 32) that the epithelial changes mentioned might indicate either direct injury to the epithelium or reflect damage to the basement membrane which was too fine to be resolved by electron microscopy. Our findings support the second alternative by showing that the basement membrane is partially altered in structure and especially by demonstrating directly that it is more permeable to ferritin. Accordingly, the changes mentioned in the epithelium could be considered as a secondary reaction to the increased permeability of the filter proper. This view derives considerable support from the recent demonstration (40, 41) that comparable changes (*i.e.*, reduction in the number of epithelial foot processes and slits) can be experimentally induced in animals by massive intravenous infusions of plasma proteins. The alterations are accompanied by proteinuria and are easily reversible: upon cessation of protein infusion the normal arrangement of the foot processes is restored as the proteinuria diminishes. It appears, therefore, that the epithelial changes are the consequence rather than the cause of proteinuria, but this conclusion does not preclude direct involvement of the epithelium as a whole in the nephrotic process. The basement membrane is an acellular layer probably produced and maintained by the adjoining epithelium and endothelium. Defects in its structure and function could be the ultimate consequence of disorders in the biosynthetic activities of either of the two adjacent cellular layers.

As far as the nephrotic basement membrane itself is concerned, our results indicate that its permeability defects are fine and widespread; moreover, they suggest that these defects occur at or near the molecular level of organization of the layer. Indeed, our tracer molecules were found randomly distributed in large numbers throughout the compact strata of the basement membrane which otherwise appeared "normal" at the level of resolution attained. The texture of luminal strata of the filter was frequently loosened, as already mentioned, but no uniform defect penetrating the whole thickness of the basement membrane could be resolved. Especially no gaps or holes of the type described by

Spiro (37, 38) in nephrotic humans were encountered. Spiro has assumed that such gross discontinuities (1000 to 1500 A in diameter) are responsible for proteinuria, but his findings have not been confirmed by other workers, and, furthermore, defects of such order cannot explain clearance differences which still exist among the various plasma components in the nephrotic syndrome (27–30). Such differences are, however, compatible with the fine, generalized increase in permeability demonstrated by our experiments.

Regarding the mechanism responsible for the increased basement membrane permeability, it should be pointed out that puromycin and its aminonucleoside derivative used to produce the experimental nephrosis, are structurally similar to adenosine (42). Moreover, puromycin, at least, has been demonstrated to inhibit RNA and protein synthesis (43). It is also known that concomitant administration of adenine attenuates the nephrotic syndrome induced by aminonucleoside whereas, curiously enough, ATP worsens the disease (44, 45). The long lag between the initiation of PA treatment and the emergence of the nephrotic syndrome renders a direct effect on the basement membrane unlikely. Interference with the biosynthetic activities of the epithelium, resulting in the production of a defective constituent (protein? mucoprotein?) of the filter which shows its effects only after sufficient accumulation seems more likely. Future studies on the chemistry of the basement membrane and on the turnover of its constituents may elucidate not only the mechanisms involved in the experimental production of the nephrotic syndrome but those involved in the "spontaneous" nephrotic syndrome occurring in humans as well.

Comments on the Physiology of the Glomerulus

In general our findings on ferritin transfer in nephrotic animals are compatible with the hypothesis formulated in our previous paper (1) concerning the functional role of each layer of the glomerular capillary wall. According to this hypothesis the basement membrane is the principal filter while the endothelium acts as a valve before it and the epithelium functions as a monitor behind it. In the nephrotic syndrome the filter is leaky and, as might be expected, the monitoring activities of the epithelium are greatly enhanced. At the same time, access to the urinary space is limited by the extensive replacement of the normal slits by "tight" junctions and by a decrease in the collective area occupied by intercellular spaces. The changes in the endothelium could also be interpreted as an attempt to compensate for the leakiness of the basement membrane, for decreasing the total area of the endothelial fenestrae may lead to a decrease in the amount of blood plasma that directly reaches the filter.

Finally, the drastic reduction in slit area and indications that in nephrotic animals a large part of the glomerular filtrate traverses the epithelial cells to reach the urinary spaces imply that in normal glomerular capillaries the

situation is reversed: most of the filtrate flows through the slits and only part of it is directed through the epithelial cells. It should be emphasized, however, that this interpretation is tentative, for there is no direct evidence concerning the flow of filtrate through the slits.

SUMMARY

Ferritin was used as a tracer to investigate glomerular permeability in the nephrotic rat. The results were compared with those previously obtained in normal animals. A nephrotic syndrome was induced by 9 daily injections of the aminonucleoside of puromycin. Ferritin was administered intravenously on the 10th day, and kidney tissue was fixed at intervals of 5 minutes to 44 hours after injection of the tracer and examined by electron microscopy.

The observations confirmed that at this stage of the experimental nephrotic syndrome the changes affect predominantly the visceral epithelium (loss of foot processes, reduction and modification of urinary slits, and intracellular accumulation of vacuoles and protein absorption droplets). Less extensive changes were found in other layers (reduction of endothelial fenestrae, an increase in the population of "deep" cells, and a thinning and "loosening" of the basement membrane.)

At short intervals (5 to 15 minutes) after ferritin administration, the tracer was found at high concentration in the lumen and endothelial fenestrae, and at decreasing concentrations embedded throughout the basement membrane and incorporated into the epithelium (within cytoplasmic vesicles and within invaginations of the plasmalemma facing the basement membrane).

After longer intervals (1 to 3 hours) the distribution of the tracer within the capillary wall was similar except that its concentration in the epithelium was higher, and, in addition to plasma membrane invaginations and small vesicles, ferritin also marked larger vacuoles, dense bodies, and intermediate forms. Large accumulations of tracer typically occurred in the spongy areas of the basement membrane, especially in the axial regions. Ferritin also appeared in the endothelium within membrane-limited vacuoles and dense bodies, particularly in the deep cells.

After 6 to 44 hours the tracer still occurred in the lumen and throughout the basement membrane. The ferritin deposits in the spongy areas as well as the ferritin-containing vacuoles of the deep endothelium were larger and more numerous. In the epithelium ferritin was found not only within various membrane-limited bodies, but also "free" within the cytoplasmic matrix.

These observations indicate that in the nephrotic glomerulus, as in the normal, the basement membrane functions as the main filtration barrier; however, in nephrosis, the basement membrane is defective and allows leakage of increased quantitites of ferritin and presumably plasma proteins. The basement membrane defect appears to be fine and widespread, occurring at or near

the molecular level of organization of the filter. The accumulation of unfiltered ferritin in axial regions together with the demonstration of its subsequent phagocytosis by the "deep" endothelial cells suggest that the latter may function in the removal of filtration residues. Finally, the findings indicate that in the nephrotic, as in the normal animal, the epithelium acts as a monitor that recovers, at least in part, the protein which leaks through the filter, and that in nephrosis, the recovering activities of the epithelium are greatly enhanced because of the increased permeability of the basement membrane.

We gratefully acknowledge the technical assistance of Mrs. Jacqueline Mach and Miss Sally Westerhoff.

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EXPLANATION OF PLATES

Abbreviations for Figures

B, basement membrane
CP, capillary lumen
DE, deep endothelial cell
EN, superficial endothelial cell
EP, epithelium
RB, red blood cell
S, spongy areas of the basement membrane
US, urinary space
cm, cell membrane
dr, dense body or "protein absorption droplet"
er, endoplasmic reticulum
f, endothelial fenestra
g, Golgi membranes
in, membrane invagination
j, epithelial intercellular junction
m, mitochondria
mv, multivesicular body
n, nucleus
ne, nuclear envelope
p, foot process of epithelium
ps, pseudopodia of deep endothelial cell
v, vacuole
ve, vesicle

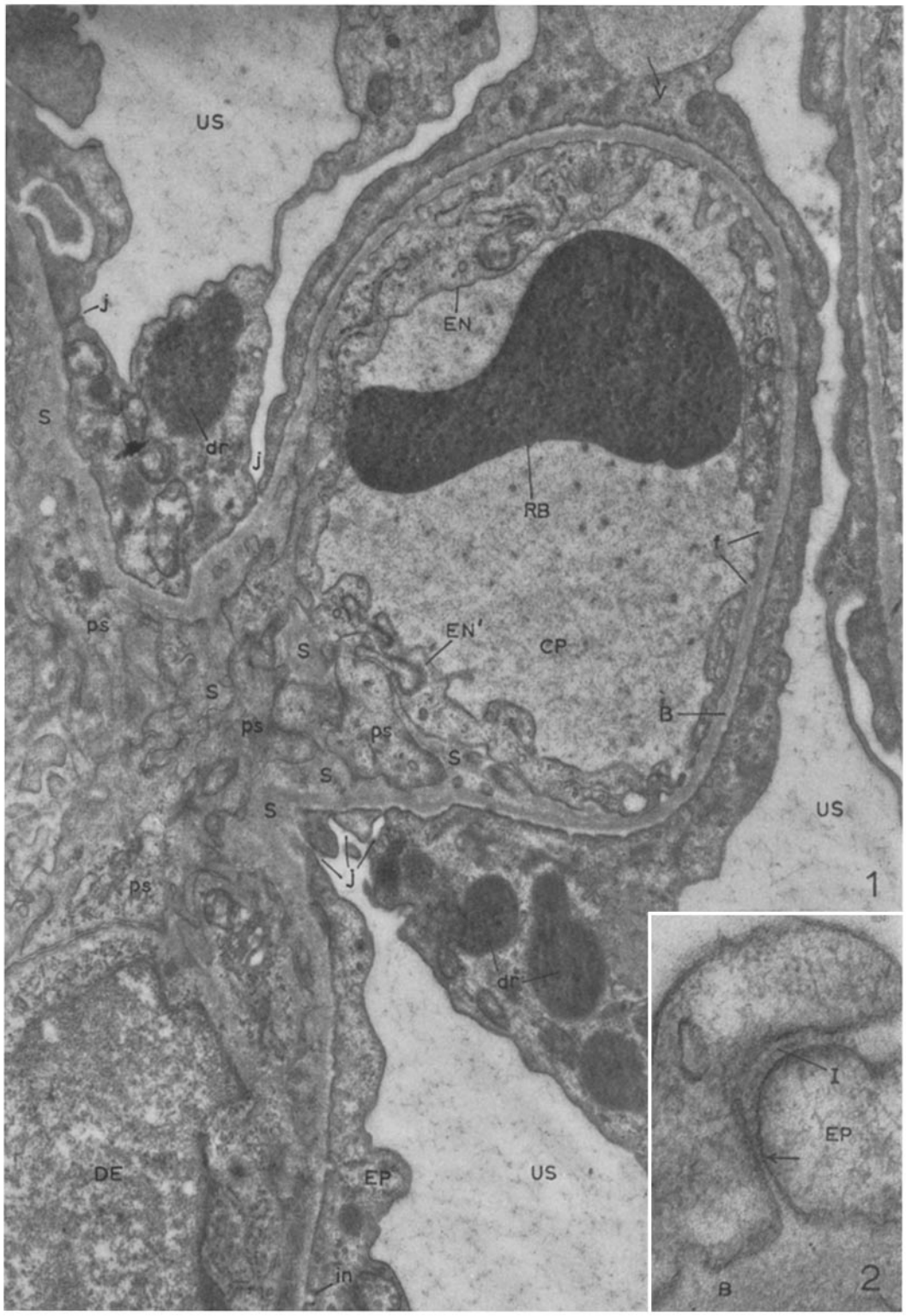
All figures are electron micrographs of renal glomeruli from rats rendered nephrotic by 9 daily injections of aminonucleoside. All except Fig. 21 show tissues fixed in osmium tetroxide and embedded in methacrylate. The corresponding sections were stained 15 to 30 minutes in lead hydroxide and subsequently "sandwiched" with carbon (see reference 1). Fig. 21 is from kidney tissue embedded in epon (46) and stained with lead hydroxide. The micrographs were taken with either an RCA, EMU 2-B or a Siemens Elmiskop I electron microscope.

PLATE 61

FIG. 1. Glomerular loop at relatively low magnification, showing the characteristic changes which occur in the organization of the glomerular capillary wall after 9 days of aminonucleoside treatment. The most striking changes are found in the epithelium: the foot processes are lost, and the outer surface of the basement membrane is covered by a broad, continuous layer of epithelial cytoplasm interrupted by rare intercellular spaces (*j*). In addition, the epithelial cytoplasm is loaded with vacuoles (*v*) and dense bodies (*dr*). There is also a thickening of the peripheral cytoplasmic layer of the superficial endothelium (*e.g.*, at *EN*), and, although some endothelial fenestrae (*f*) are seen, their frequency is definitely reduced. The general morphology of the basement membrane appears normal.

Part of a "deep" endothelial cell (*DE*) is present in the axial region on the lower left. It shows numerous pseudopodia (*ps*) separated by extensive spongy areas (*S*) of complicated outline. This cell is not in contact with the lumen at this level, for a thin layer of cytoplasm from a superficial endothelial cell (*EN'*) is present between it and the lumen. The lumen contains a red blood cell (*RB*) and fine fibrillar and globular elements of precipitated plasma. Magnification 18,000.

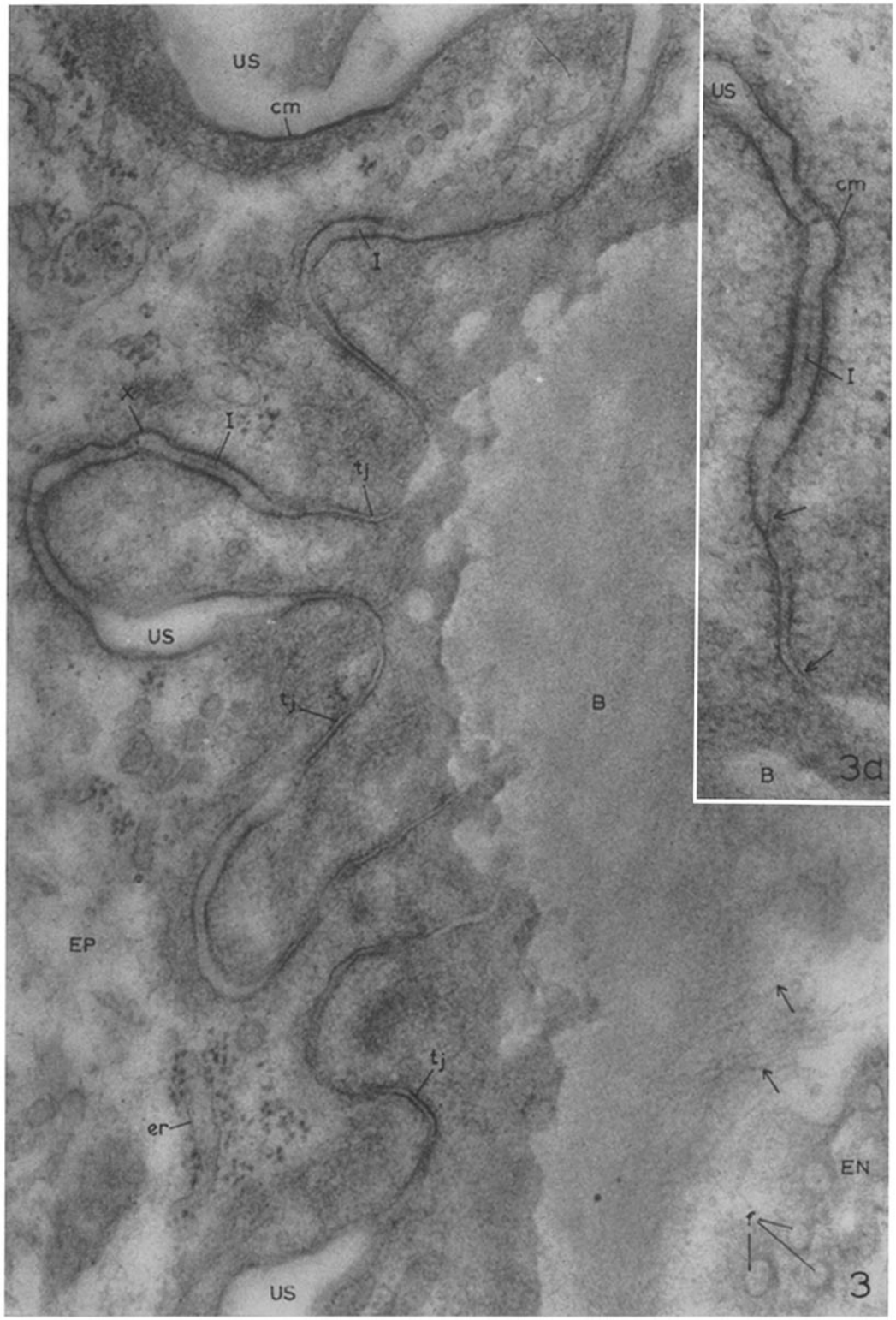
FIG. 2. Higher magnification of a small portion of the glomerular capillary wall to illustrate the type of epithelial intercellular junction commonly encountered in nephrotic animals. The junction line is quite deep, as indicated by the increase in the density of the apposed epithelial cell membranes and subjacent cytoplasmic matrix over a considerable distance (approximately ½ μ). Nearer the basement membrane the intercellular space is narrowed to about 80 A (arrow), but towards the urinary space the gap is broader (approximately 300 A) and is bisected by a fine intermediate line (*I*). (See also Fig. 3). Magnification 67,000.



(Farquhar and Palade: Glomerular permeability)

PLATE 62

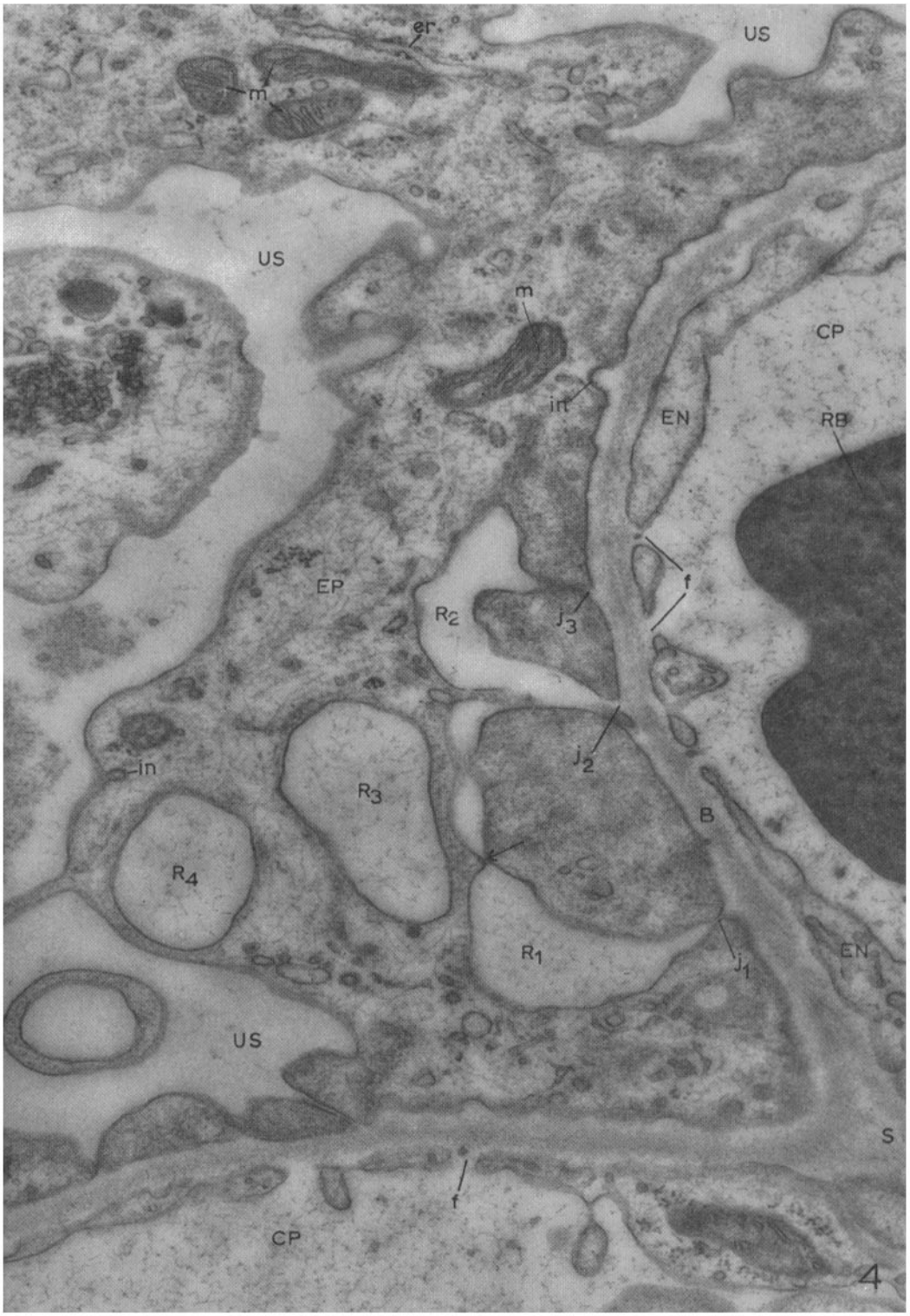
FIG. 3. Oblique section through the glomerular capillary wall of a nephrotic animal, illustrating the arrangement of the epithelial junctions. Part of the endothelium, showing several fenestrae (*f*) cut in full faced view, is included in the lower right corner. Distinct fibrils are also visible in the subendothelial space (arrows). The section cuts broadly through the basement membrane and epithelium to the left, and a number of special details are evident within and along the epithelial intercellular junctions. There is an increase in the density of the apposed cell membranes associated with increased density of the immediately subjacent cytoplasm over relatively large distances. Near the basement membrane the junctions (*tj*) are "tighter," for the intercellular space is reduced to as little as 80 A. Towards the urinary spaces the gap is broader (about 300 A) and is bisected by a distinct intermediate line (*I*). One of these junctions (*X*) is enlarged in the inset 3A where a "tight" junction (marked by arrows) is seen nearer the basement membrane below, and a broader gap is shown nearer the urinary space above. In several places (*cm*) the stratification of the epithelial cell membrane can be seen. A distinct intermediate line (*I*) is seen in the wider gap, and a fainter line bisects the tight junction. Magnification 73,000; inset, × 126,000.



(Farquhar and Palade: Glomerular permeability)

PLATE 63

FIG. 4. Part of a bent glomerular capillary with the epithelium on the left and the lumen to the right and below. The thickness of the basement membrane is within normal limits but its texture appears "loosened" or spongy, particularly in the upper part of the micrograph. Fine filaments are visible in the epithelial cytoplasm (*EP*) near the center of the field. The contours of the epithelial cell are very irregular and, as a result, the complementary urinary spaces show many sinuses and recesses (R_1 to R_4). Several of these recesses (R_1, R_3, and R_4) have a finely flocculent content similar to the blood plasma in the lumen of the capillary. R_1 is in continuity with the cell surface facing the basement membrane through a tight intercellular junction (j_1), and with the urinary space through a similar gap (arrow). Another recess (R_2) also reaches the basement membrane through the junctions marked j_2 and j_3, but, because of its light and homogeneous content appears virtually "empty". R_3 and R_4, which have a content similar to that of R_1, are thought to represent sections through recesses whose relations to the basement membrane and urinary spaces *via* intercellular channels are not evident in this plane of section. Unless such a connection is present in the field of view, it is frequently impossible to distinguish between extracellular recesses and intracellular vacuoles. Magnification 31,000.

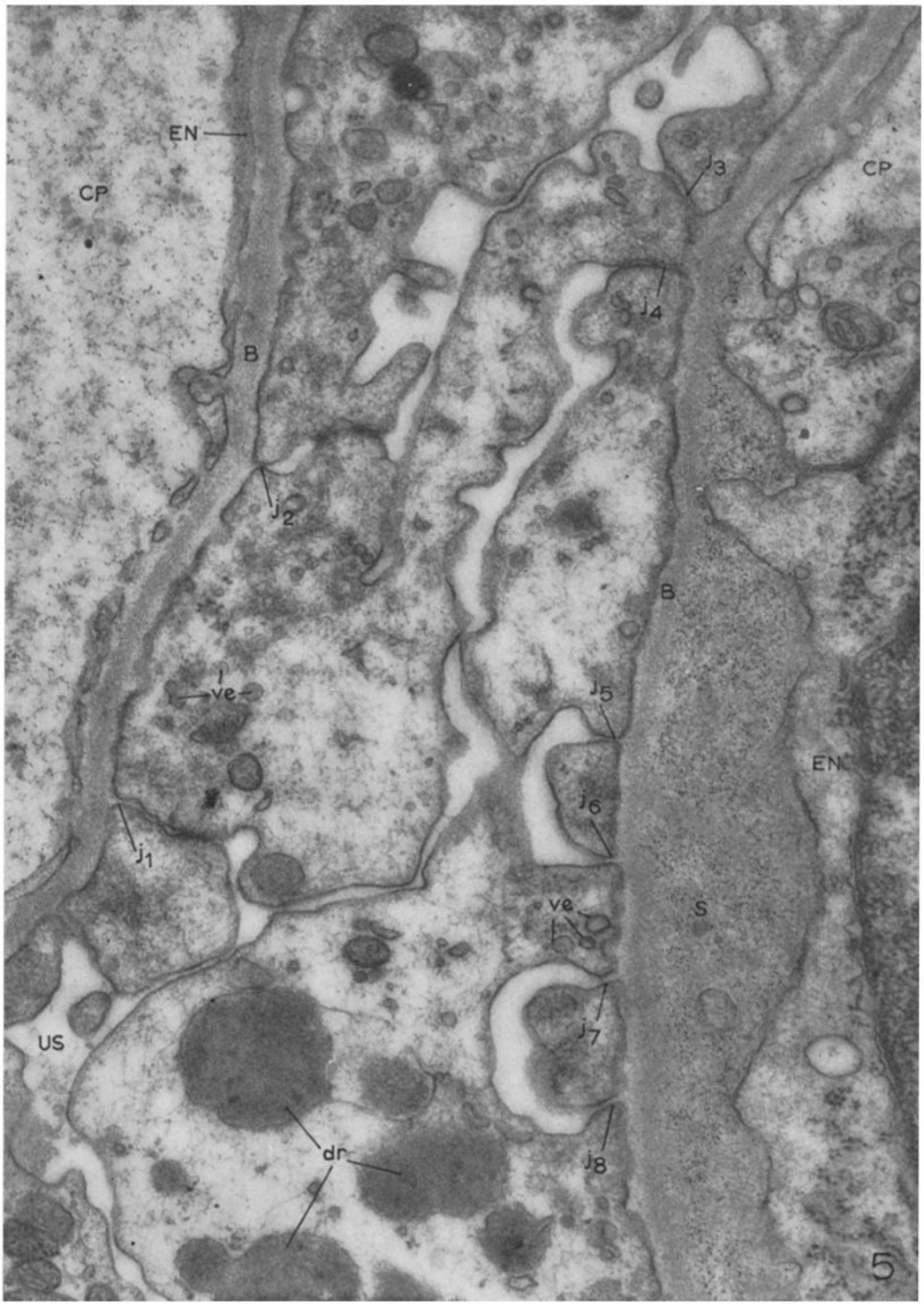


(Farquhar and Palade: Glomerular permeability)

PLATE 64

FIG. 5. Low power view, showing parts of two glomerular capillaries from a nephrotic animal sacrificed 10 minutes following ferritin administration. Even at this low magnification, ferritin molecules can be recognized distributed throughout the capillary lumen which also contains globular elements (larger lipoproteins?) and a fine fibrillar precipitate (plasma proteins?). (See also Figs. 1 and 18). To the right there is a large accumulation of ferritin in a spongy area (*S*) between the endothelium (*EN*) and basement membrane (*B*).

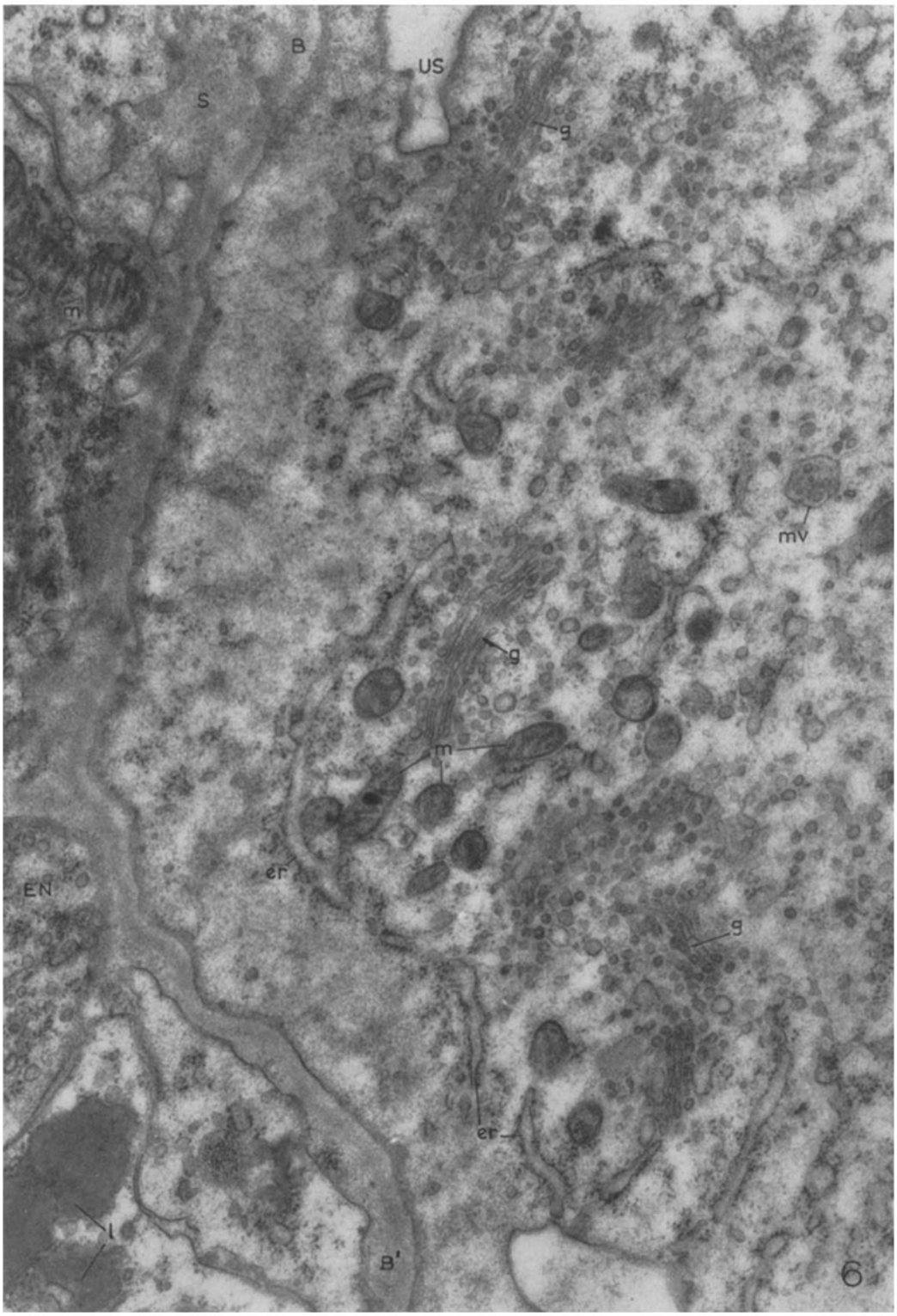
The complex disposition of the epithelium, the irregular configuration of the urinary spaces, and the arrangement of the intercellular junctions is well illustrated. Most of the intercellular junctions (j_1 to j_5) are characterized by an intercellular gap narrowed to approximately 80 A over a considerable depth. Opposite the spongy area, however, the foot process organization is nearly normal and the intercellular junctions (j_6 to j_8) more limited in depth, approaching the morphology of normal epithelial slits. Numerous small vesicles (*ve*), a few droplets (*dr*), and fine filaments are present in the epithelial cytoplasm. Magnification 38,000.



(Farquhar and Palade: Glomerular permeability)

PLATE 65

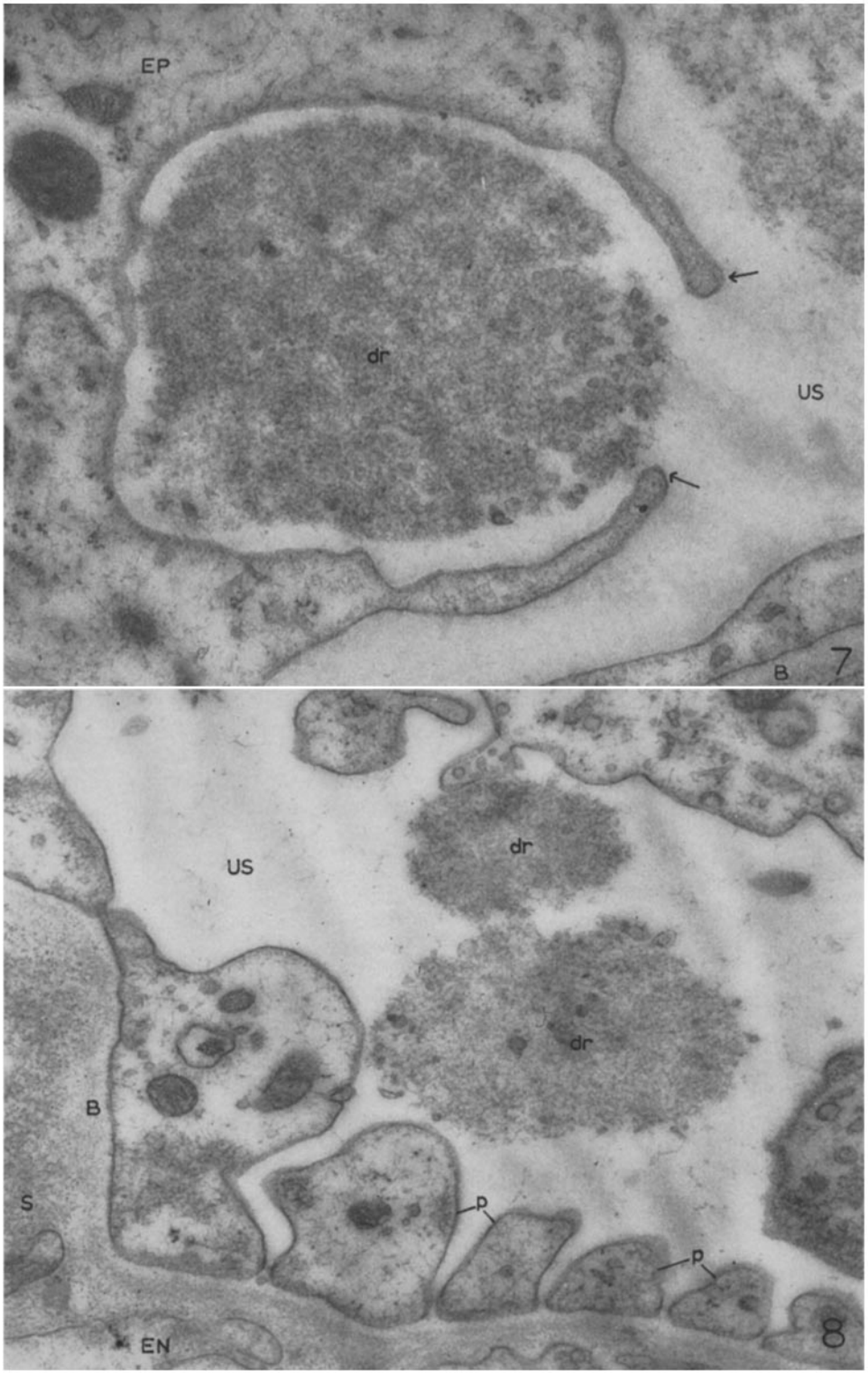
FIG. 6. Portion of a visceral epithelial cell from a nephrotic animal sacrificed 1 hour following ferritin administration. The basement membrane and endothelium are seen to the left. The latter contains a few lipid inclusions (*l*). The cytoplasmic sheet or "sole" of an epithelial cell completely covers the outer surface of the basement membrane from *B* to *B'*—in lieu of foot processes; it has a moderately dense, finely fibrillar matrix, contains a few scattered vesicles and RNP particles, but is otherwise strikingly free of formed cytoplasmic elements. In contrast, the remaining epithelial cytoplasm is packed with cytoplasmic organelles which include numerous groups of closely packed, smooth surfaced Golgi cisternae (*g*) surrounded by swarms of small vesicles, both rough (*er*) and smooth surfaced elements of the endoplasmic reticulum, some free RNP particles, mitochondria (*m*), and a multivesicular body (*mv*). Magnification 32,000.



(Farquhar and Palade: Glomerular permeability)

PLATE 66

FIGS. 7 and 8. Small fields from nephrotic glomeruli (3 hours after ferritin). Fig. 7 shows a dense body or protein absorption droplet which appears to be in the process of discharging its content into the urinary spaces. The droplet membrane has apparently merged with the cell membrane at the points marked by arrows, thus creating a sinus-like cavity which is open to the urinary spaces. The content of the sinus is similar to that of the droplets (with finely fibrillar and vesicular components), only it is partially dispersed. Fig. 8 shows two masses of material, presumed to represent droplet residues, lying free in the urinary space. These residues retain the ovoid shape, the finely filamentous texture, and the included vesicles characteristic of that of some droplets. No ferritin is seen in either the discharging droplet above or the residues below. Magnification 40,000.



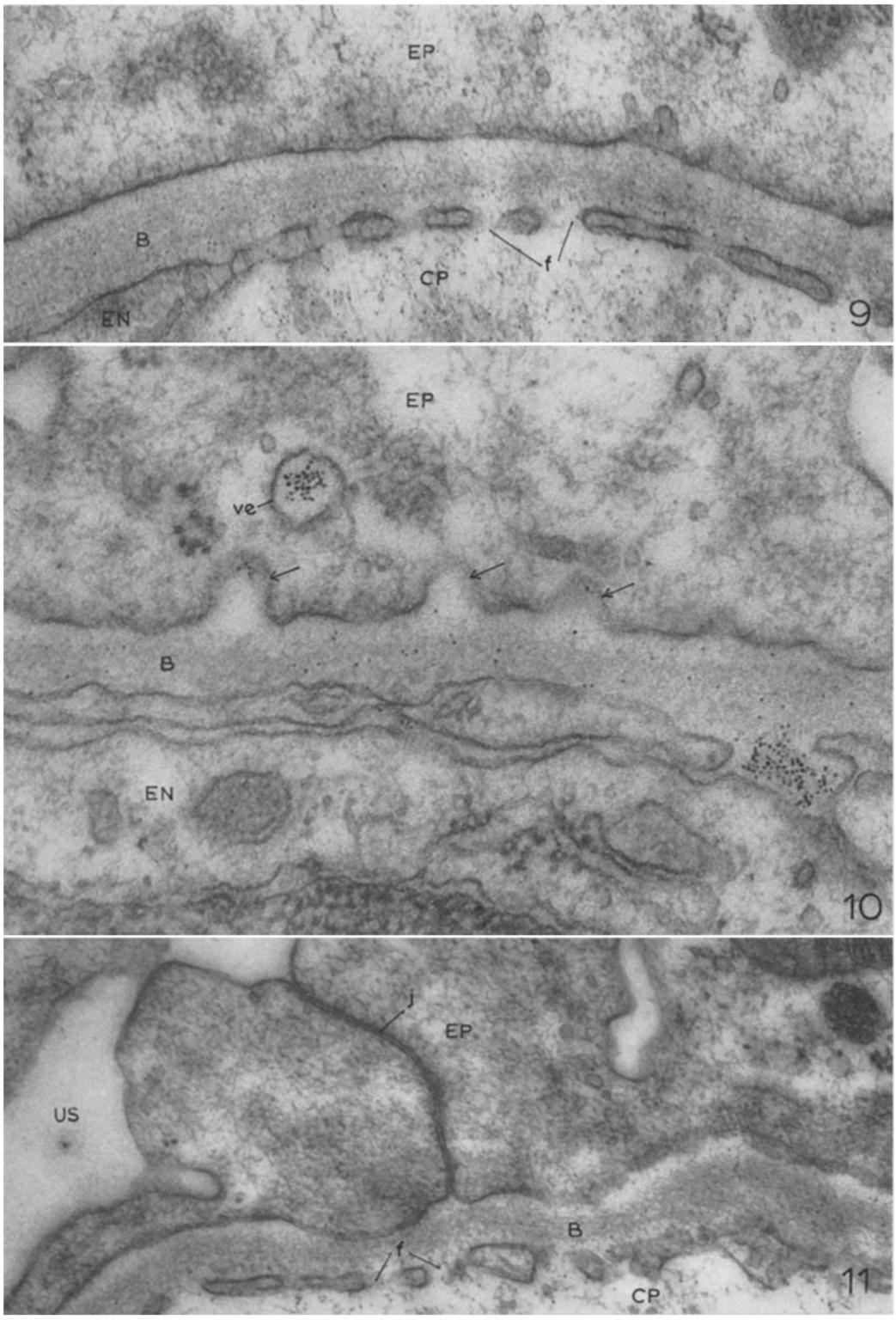
(Farquhar and Palade: Glomerular permeability)

PLATE 67

FIGS. 9 and 10. These figures show the distribution of ferritin in the wall of nephrotic glomerular capillaries 10 to 15 minutes following ferritin administration. In Fig. 9, ferritin molecules (seen as fine dense particles) occur at high concentration in the capillary lumen (*CP*) and in the endothelial fenestrae (*f*) which they appear to penetrate freely. Many molecules are also found embedded in the basement membrane, randomly distributed throughout its entire depth.

In Fig. 10, ferritin molecules are present in relatively large numbers throughout the basement membrane which appears slightly thinner than normal and rather "loose" in texture. Other ferritin molecules are present within tiny invaginations (arrows) of the epithelial plasmalemma facing the basement membrane, and at higher concentration within a vesicle (*ve*) deeper within the epithelial cytoplasm. Magnifications: Fig. 9, 70,000; Fig. 10, 84,000.

FIG. 11. Section from the glomerular capillary wall of a nephrotic rat, showing a conspicuous junction line (*j*) between two epithelial cells. The zone of close contact between the adjoining cells is quite extensive, measuring nearly a micron from the basement membrane to the urinary space above. Along it the intercellular space is narrowed to approximately 70 A. Magnification 51,000.

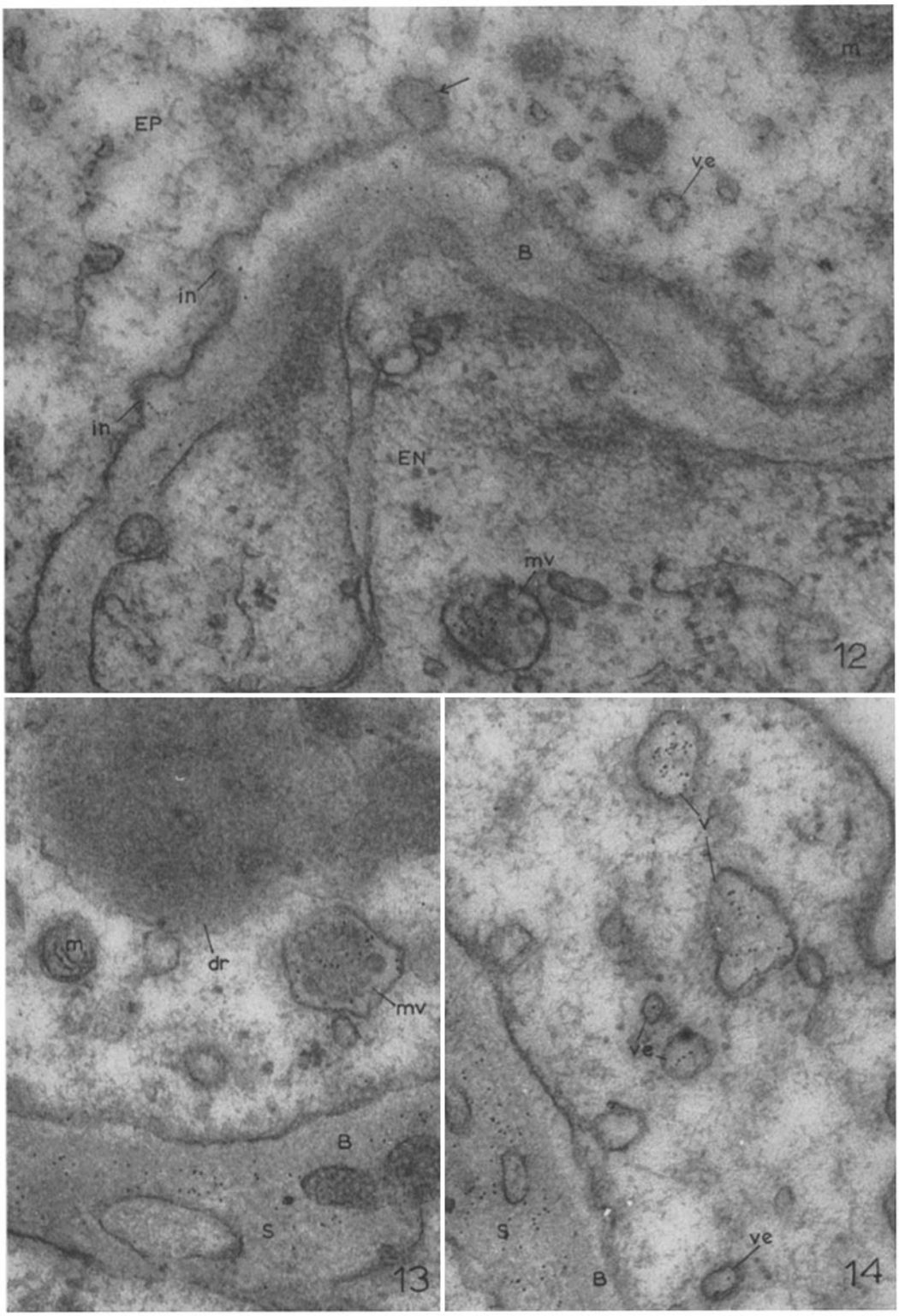


(Farquhar and Palade: Glomerular permeability)

PLATE 68

FIGS. 12 to 14. Portions of glomeruli from nephrotic animals sacrificed 10 to 15 minutes following ferritin administration. In Fig. 12, ferritin molecules are seen throughout the basement membrane (*B*), and also apparently captured in all stages of the process of incorporation into the epithelium—located within invaginations of the epithelial cell membrane (*in*), within a vesicle nearly detached from the cell membrane (arrow), and within an isolated cytoplasmic vesicle (*ve*). Several molecules of the tracer are also present within a multivesicular body (*mv*) in the endothelium.

FIG. 13 shows a few ferritin molecules in an axial spongy area (*S*) and others in the epithelium segregated within a multivesicular body (*mv*). No ferritin is seen within the dense body above (*dr*). Figure 14 shows ferritin within several membrane-limited vesicles and small vacuoles in the epithelium. Magnifications: Fig. 12, 76,000; Figs. 13 and 14, 72,000.

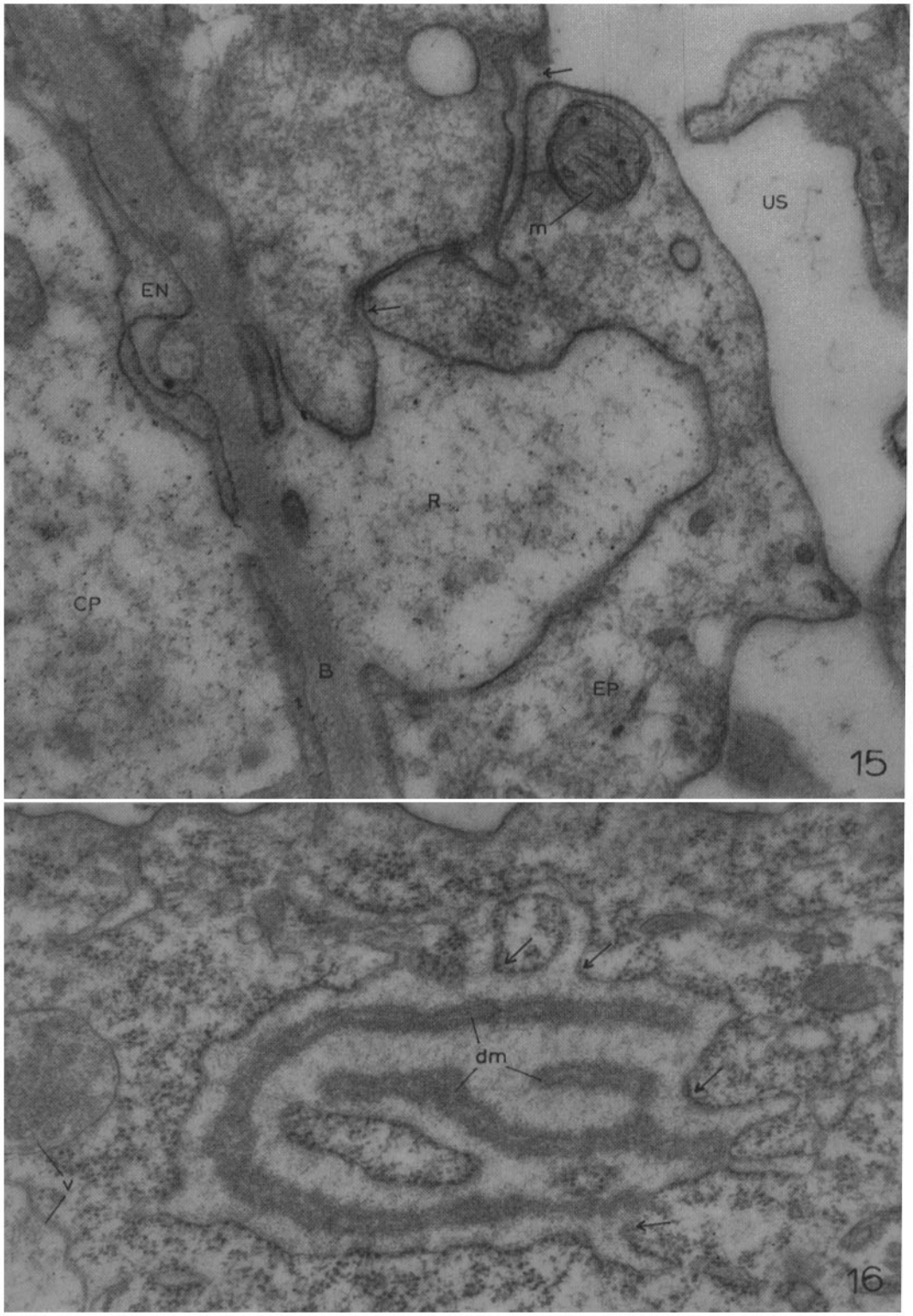


(Farquhar and Palade: Glomerular permeability)

PLATE 69

FIG. 15. An intercellular recess or pocket formed by the epithelium (nephrotic rat, 10 minutes after ferritin administration). The capillary lumen (*CP*) is to the left and the "open" urinary space (*US*) to the right. The epithelial cell membrane appears to have retracted away from the basement membrane over a relatively broad area forming a blind pouch which is in continuity with the urinary spaces through a narrow intercellular space (marked by arrows). The ferritin concentration within the pouch is approximately the same or only slightly lower than in the lumen, but definitely less than in the apparently "empty" urinary space. No ferritin is seen within the connecting intercellular space. Magnification 60,000.

FIG. 16. Portion of a visceral epithelial cell of a nephrotic rat, depicting a large distended cisterna of the endoplasmic reticulum of the type commonly seen in these cells. The cisterna is limited by a rough surfaced membrane and is in direct continuity with several of the more common elongated profiles of the system at the points marked by arrows. The surrounding cytoplasm contains numerous "free" RNP particles. A folded sheet of dense, finely filamentous material (*dm*) forms part of the content of the large sac. The general appearance of the dense material resembles closely that of the background matrix of the basement membrane. Magnification 21,000.



(Farquhar and Palade: Glomerular permeability)

PLATE 70

FIG. 17. Glomerular capillary wall from a nephrotic rat (1 hour after ferritin). The basement membrane crosses the field almost diagonally with the endothelium to the right and the epithelium to the left. A massive accumulation of ferritin is present in the spongy area (*S*) between the endothelium and the basement membrane. The neighboring endothelial cell shows slender pseudopodia (*ps*) and a membrane-limited vacuole (*v*) packed with ferritin, suggesting that part of the subendothelial deposit has been phagocytized by the endothelium.

Ferritin molecules are also found scattered throughout the basement membrane which appears slightly thinner than normal (approximately 1000 A in places) and somewhat "loose" in texture. The tracer is also seen within small pinocytic invaginations (*in*) of the epithelial plasmalemma which faces the basement membrane. Within the epithelium the ferritin molecules are seen segregated within small cytoplasmic vesicles (*ve*), larger vacuoles (*v*), or dense bodies (*dr*) (protein absorption droplets). Magnification 60,000.

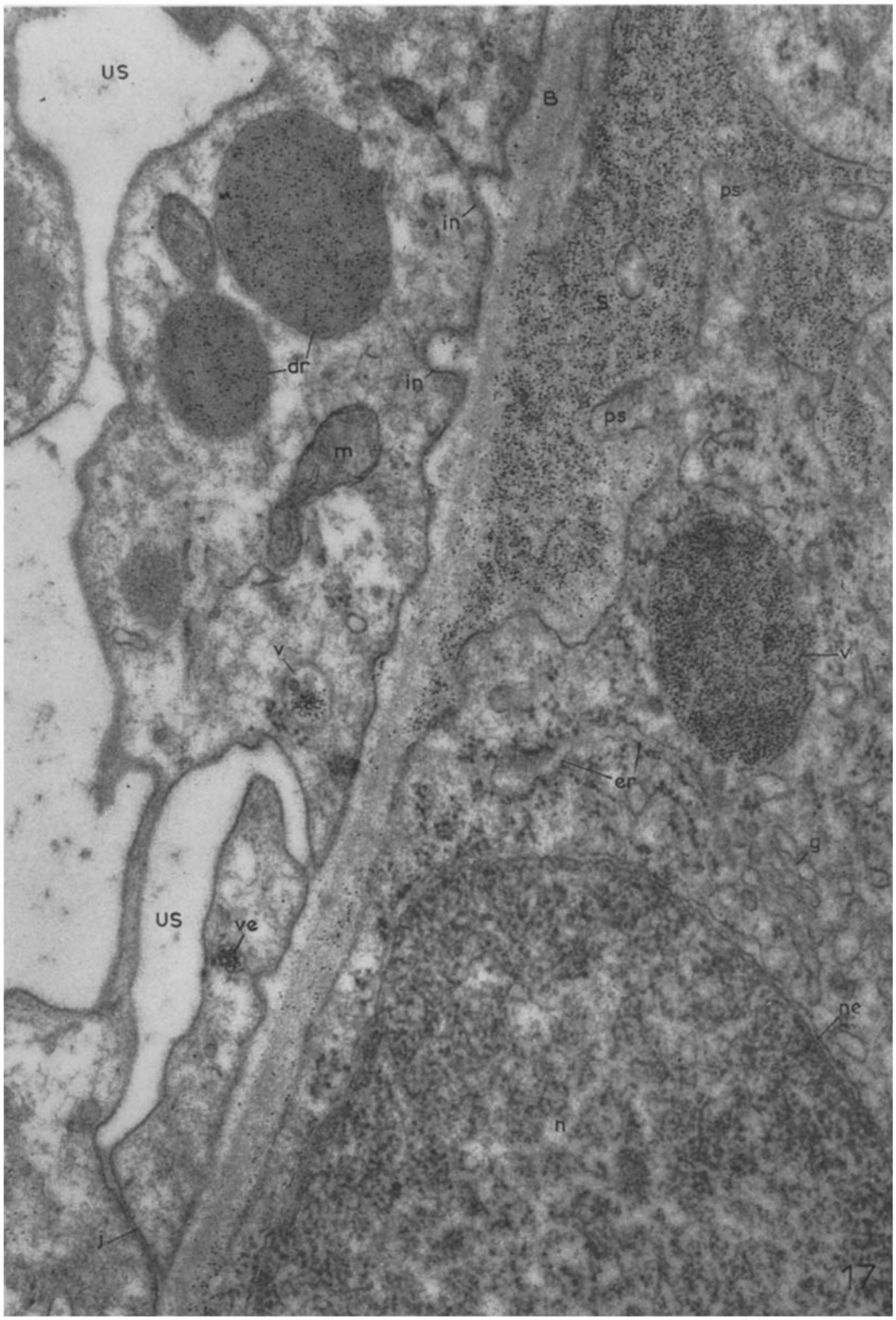


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PLATE 71

FIG. 18. Axial region of a glomerulus from a nephrotic rat sacrificed 3 hours after ferritin administration, demonstrating the typical arrangement and relationships of a "deep" endothelial cell. A portion of the nucleus is present in the upper left corner and a broad cytoplasmic pseudopod projects into the capillary lumen on the right. Except for this large pseudopod, the cell is separated from the lumen by a thin layer of fenestrated cytoplasm belonging to a superficial endothelial cell, and by layers of spongy material (*S*) which penetrate between the superficial and the deep cell. The intraluminal pseudopod is notably devoid of organized elements while the remainder of the cytoplasm has a finely filamentous matrix and is filled with cytoplasmic organelles including: elements of the endoplasmic reticulum (*er*), mostly of the rough surfaced variety; free RNP particles; mitochondria (*m*); and dense bodies (*d*) packed with ferritin.

Spongy areas containing large ferritin accumulations are also present in the subendothelial areas on the lower left where the deep cell faces the basement membrane. Slender pseudopodia (*ps*) project into these areas. The ferritin masses of the spongy areas are apparently phagocytized by the deep cells and concentrated into cytoplasmic dense bodies.

In addition to the usual finely flocculent material (presumed to represent the proteins of the plasma), large numbers of globules (400 to 600 A in diameter) are also seen in the capillary lumen. The latter are thought to represent circulating lipoproteins which are known to be elevated in the nephrotic syndrome.

Also shown in this figure is an epithelial pocket or recess (*R*) which is open to the basement membrane through a larger gap (about 1000 A) than is usually the case and which, in the original micrograph, could be seen to contain a number of ferritin molecules. Magnification 37,000.

FIG. 19. Portion of a visceral epithelial cell from the glomerulus of a nephrotic rat, 3 hours after ferritin injection, showing the segregation of a large amount of ferritin within cytoplasmic dense bodies. This finding together with the frequent occurrence of multilobulated dense bodies (see Fig. 5) suggest that the absorption droplets grow by the progressive coalescence of small dense bodies. Magnification 61,000.

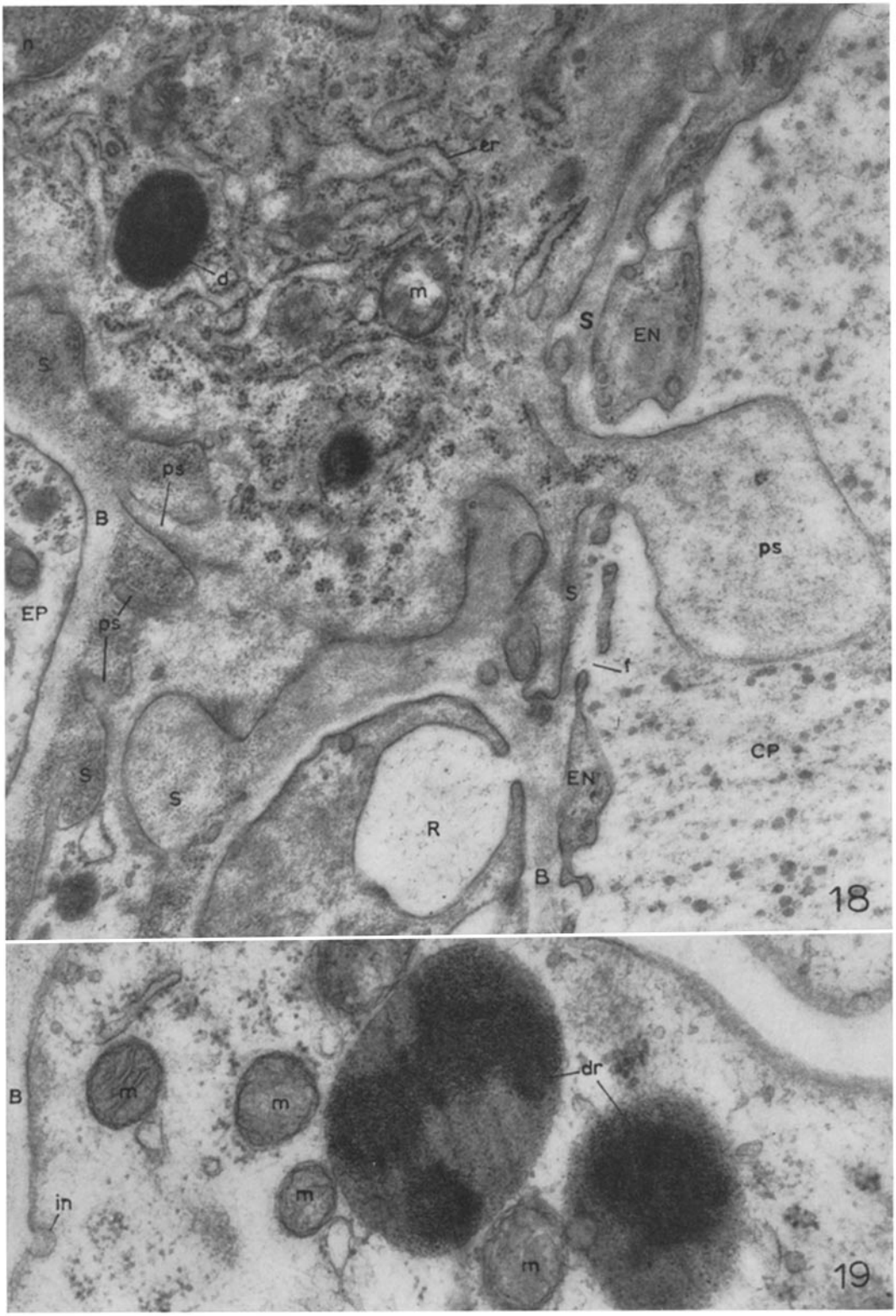

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PLATE 72

FIG. 20. Axial region of a nephrotic glomerulus 3 hours after ferritin administration. A deep endothelial cell occupies most of the field. Part of the lumen, containing a white blood cell (*W*), is seen on the upper right. A thin layer of cytoplasm belonging to a superficial endothelial cell (*EN*) separates the deep cell from the lumen. Spongy areas (*S*) with a finely fibrillar texture partly separate the two cells. Large masses of ferritin are seen in the subendothelial spongy areas between the deep cell and the basement membrane; one of these is enlarged in the inset. The ferritin deposits are penetrated and divided by numerous pseudopodia (*ps*) of the deep cell. The cytoplasm of the latter contains many vacuoles marked by ferritin. Some of these vacuoles (v_1 and v_2) have a ferritin concentration comparable to that of the extracellular deposits in the spongy areas, but in others (d_1 to d_3) the concentration of the marker is noticeably increased, suggesting that the ferritin residues are incorporated into the deep cells and undergo progressive condensation with time. Magnification 24,000; inset, × 50,000.

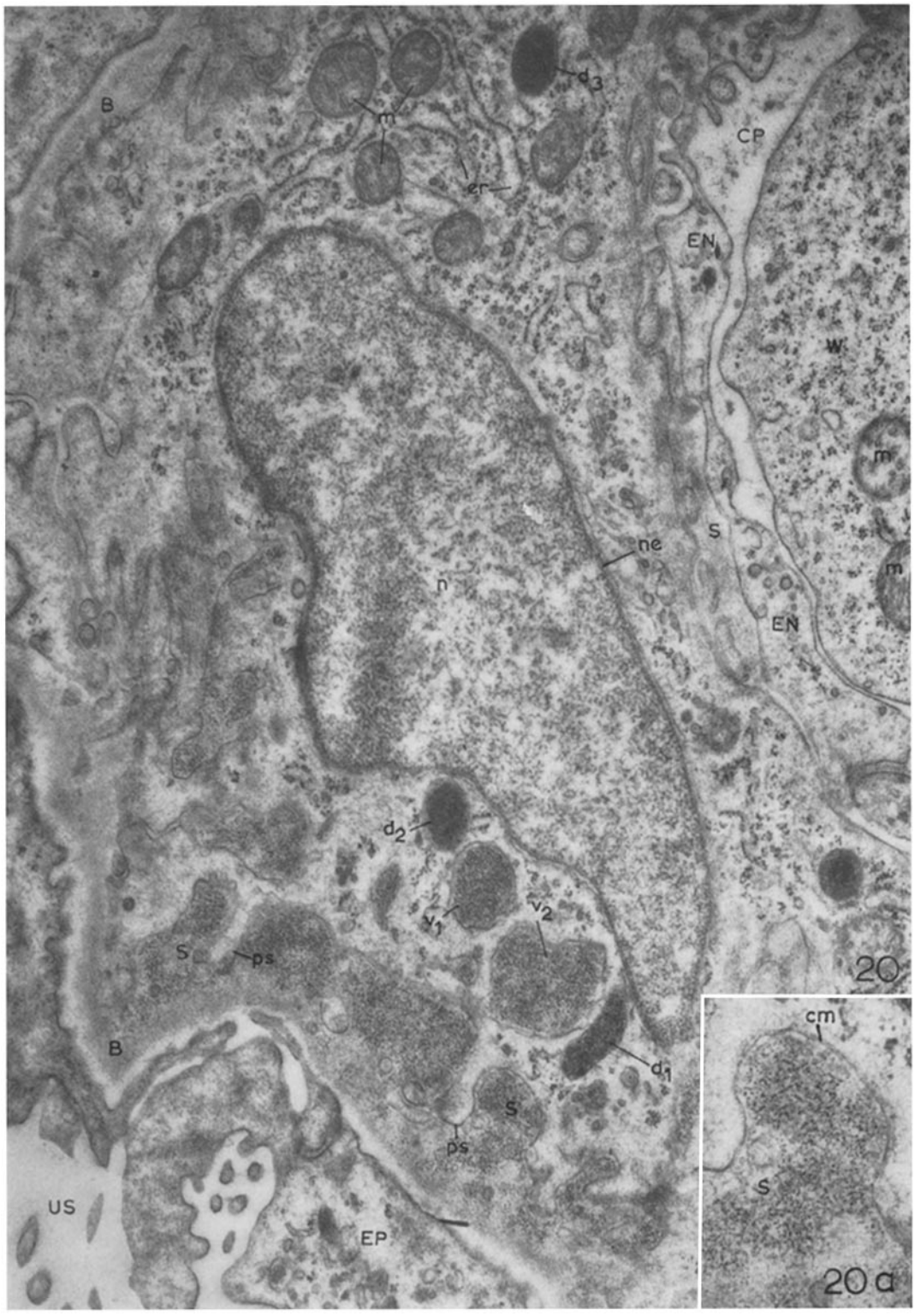

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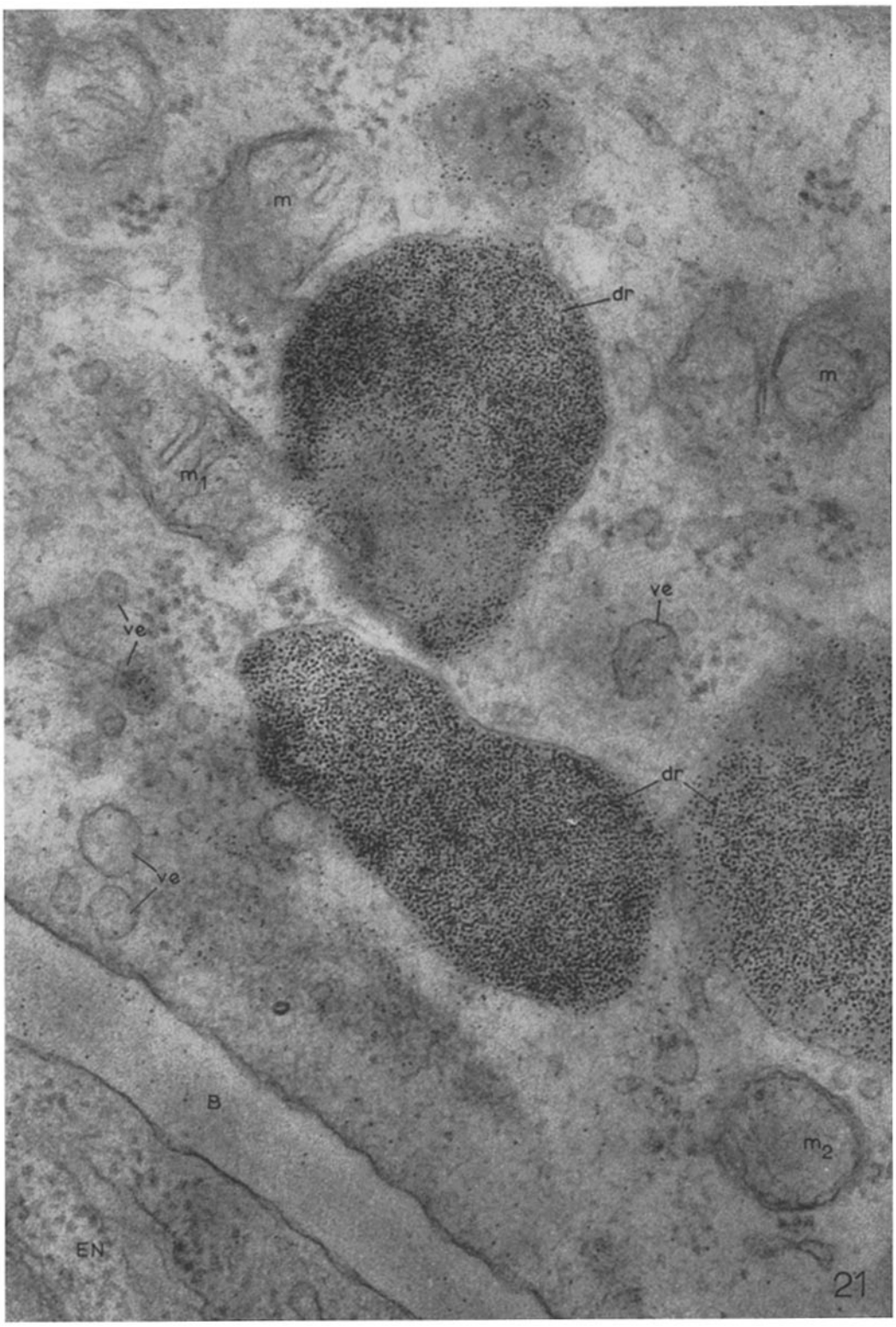
PLATE 73

FIG. 21. Portion of a visceral epithelial cell, 12 hours after ferritin administration. Large numbers of ferritin molecules are seen within various membrane-limited bodies (vesicles and dense bodies) as at earlier intervals. In addition, ferritin is found within several mitochondria (m_1 and m_2) and freely distributed throughout the cytoplasmic matrix, particularly in the zone between the droplets and the basement membrane on the lower left. For reasons discussed in the text, this "free" ferritin, seen only at very long intervals after ferritin administration, is thought to be a newly synthesized or endogenous protein rather than the exogenous tracer. Magnification 80,000.



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