Segregation of Ferritin in Glomerular Protein Absorption Droplets*

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PLATES 139 TO 145

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ABSTRACT

Ferritin was used as a tracer to study the mechanism by which proteins are segregated into droplets by the visceral epithelium of glomerular capillaries.

In glomeruli from both normal and aminonucleoside-nephrotic rats ferritin molecules introduced into the general circulation penetrated the endothelial openings and were seen at various levels in the basement membrane. Striking differences between nephrotic and controls were seen only in the amount of ferritin incorporated into the epithelium. In normal animals, a few ferritin molecules were seen in small invaginations of the cell membrane limiting the foot processes, within minute vesicles in the epithelium, or within occasional large vacuoles and dense bodies. In nephrotics, epithelial pinocytosis was marked, and numerous ferritin molecules were seen within membrane invaginations and in small cytoplasmic vesicles at all time points. After longer intervals, the concentration of ferritin increased in vacuoles and particularly within the dense bodies or within structures with a morphology intermediate between that of vacuoles and dense bodies. In nephrotic animals cleft-like cavities or sinuses were frequently encountered along the epithelial cell surface facing the urinary spaces. Some of these sinuses contained material resembling that filling the dense bodies except that it appeared less compact.

The findings suggest that ferritin molecules—and presumably other proteins which penetrate the basement membrane—are picked up by the epithelium in pinocytotic vesicles and transported *via* the small vesicles to larger vacuoles which are subsequently transformed into dense bodies by progressive condensation. The content of the dense bodies may then undergo partial digestion and be extruded into the urinary spaces where it disperses.

The activity of the glomerular epithelium in the incorporation and segregation of protein is similar in normal and nephrotic animals, except that the rate is considerably higher in nephrosis where the permeability of the glomerular basement membrane is greatly increased.

A convincing body of histological, cytochemical, and immunological evidence indicates that the nephron epithelium is able to segregate, within distinct cytoplasmic bodies, proteins which pass the glomerular filter. These cytoplasmic bodies have appropriately been named "protein absorption droplets" (1, 2) and are undoubtedly analogous to the so called "hyaline droplets" of pathologists. The ability of the nephron epithelium to segregate in droplets a variety of proteins, such as serum albumins and globulins (3, 4), hemoglobin (5, 1), egg white (4, 6, 7), as well as colloidal dyes (8 to 10), and colloidal particles (11), has been repeatedly demonstrated. The mechanisms involved in the incorporation and segregation of these substances, although the subject of considerable speculation, have remained practically unknown.

Protein absorption droplets are normally present in limited numbers in the epithelium of the proximal tubules of the rat kidney. Their number and average size increases when the nephron is challenged to absorb proteins in amounts greater

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than normal. Studies by Oliver and his associates (1, 2, 4) have shown that when abnormal quantities of protein appear in the glomerular filtrate (such as following intraperitoneal injection of egg white) droplet formation begins in the middle third of the proximal tubule, may extend to the entire proximal convolution, and in extreme cases may even occur in the parietal epithelium of the glomerulus. Occasionally droplets have also been described in the glomerular tuft (12, 13, 1). While numerous studies have dealt with the characterization of tubular droplets, relatively little attention has been paid to the protein absorption droplets (or hyaline droplets) of the glomerulus.

Several recent studies (14–16) have called attention to the presence of numerous PAS-positive bodies, presumed to be "hyaline droplets," in glomeruli of rats with a nephrotic syndrome induced by repeated injections of the aminonucleoside of Puromycin. These droplets can be readily identified in electron micrographs by virtue of their great density and characteristic spherical or ovoid shape. They are definitely located in the cytoplasm of the visceral epithelial cells (16).

We are currently investigating the transport of ferritin molecules across the wall of glomerular capillaries in normal and aminonucleoside-nephrotic rats. In the course of these studies we have made a number of observations on the incorporation of ferritin by the visceral glomerular epithelium and its segregation into cytoplasmic droplets. We consider these observations to be of sufficient interest and significance to be presented separately in this paper. A more detailed account of our findings on ferritin transport under normal and pathologic conditions will be reported subsequently.

Materials and Methods

It has been shown (17, 18) that the classic features of the nephrotic syndrome, namely massive proteinuria, hypoalbuminemia, hyperlipemia, and edema, can be induced in rats by repeated daily injections of the aminonucleoside derivative of the antibiotic Puromycin (6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl) purine). In our experiments, nephrosis was induced in young rats (100 to 150 gms.) by 9 daily injections of 0.3 ml. of a 0.5 per cent aminonucleoside[1] solution per 100 gm. body weight.[2] On the day following the last injection, 2 cc. of a solution of ferritin containing ~20 mg. protein per ml. was administered *via* the saphenous vein to nephrotic animals and to normal controls. The ferritin was prepared from horse spleen by the procedure of Laufberger (20). At intervals of 2 minutes to 3 hours following ferritin administration, fixation of kidney tissue was initiated *in situ* by injecting 1 to 2 ml. of a 1 per cent solution of osmium tetroxide in acetate-veronal buffer (pH 7.6 to 7.4) (21) and 0.14 M sucrose (22) into the renal cortex with a fine hypodermic needle. Subsequently, blocks of less than 1 mm.[3] were cut from the injected kidney, placed in glass vials containing fixative for periods of 1½ to 2 hours at 0°C., dehydrated in graded alcohols, and finally embedded in methacrylate. Some of the thin sections prepared for electron microscopy were picked up on to carbon-coated specimen grids, stained for 1½ to 3 minutes with lead hydroxide (23, 24), and examined without further treatment. Other sections were placed on formvar-coated specimen grids, stained with lead hydroxide, and "sandwiched" (25) by evaporation of a thin carbon film onto their exposed surface (26) before examination in the electron microscope.

RESULTS

Normal Animals.—The wall of the normal glomerular capillary consists of three successive layers (Fig. 1): (*a*) the endothelium with numerous openings or fenestrae in its cytoplasm, (*b*) the basement membrane, and (*c*) the visceral epithelium, with its characteristic "foot processes" which interdigitate to cover the outer surface of the basement membrane. For this study it is pertinent to add that besides the usual cytoplasmic components (*i.e.* mitochondria, Golgi components, RNP particles, and endoplasmic reticulum), the visceral epithelial cells of the normal glomerulus contain many small vesicles about 600 to 800 A, and a few larger vacuoles and dense bodies. These additional structures will be described in detail in the following paragraphs pertaining to the nephrotic glomerulus.

Nephrotic Animals.—Glomerular capillaries of nephrotic rats show striking and characteristic changes which affect primarily the visceral epithelium (Fig. 4). The foot processes of the epithelial cells are lost or greatly distorted, and in their place the outer surface of the basement membrane is covered by broad masses of epithelial cytoplasm

[1] Generously supplied by Lederle Laboratories, American Cyanamid Company, Pearl River, New York.

[2] This protocol was selected because previous studies have consistently established the presence of proteinuria by the 9th day (14, 19).

[3] Distortion of epithelial foot processes has not only been a uniform finding in all aminonucleoside-nephrotic rats (16, 19) but is consistently seen to some degree in all patients with the nephrotic syndrome (27, 28) and has therefore been associated with the presence of *massive* proteinuria (29).

interrupted by relatively few "slits."[3] In addition, the cytoplasm of the epithelial cells contains large vacuoles and dense bodies of varying sizes and distribution: one cell may be literally packed with multiple bodies whereas an adjacent cell may contain none in the plane of section. In spite of this variation most of the epithelial cells in a given glomerulus contain several vacuoles or dense bodies per section.

The vacuoles are membrane-limited bodies of spherical or irregular shape which range in diameter from approximately 0.25 up to 2 μ and are characterized by an internum of low density; some appear virtually "empty" whereas others contain a fine flocculent precipitate similar to that present in the capillary lumina (Fig. 7). Frequently dense cytoplasmic material with a strand-like or particulate texture is found aggregated in the form of a more or less complete halo around the vacuoles. (Fig. 7).

The dense bodies or droplets also range in size from approximately 0.5 up to 2 μ and are recognized, as the name implies, by the great density of their content. They are also bounded by a membrane which, because of the density of the content, is visible only when cut in a plane normal to its surface (Figs. 4, 8, and 12). The content of some of the droplets is uniformly dense with a finely particulate texture (Figs. 8 and 9). Other droplets show areas of lesser density and frequently contain vesicular elements (Figs. 10 and 12). In addition to structures which would be clearly classified as vacuoles or dense bodies, there are many transitional forms of intermediate density (*e.g.*, V_2 in Fig. 8). Finally, one frequently encounters appearances which suggest that several small round droplets, sometimes of different densities, have merged to form a single large droplet of irregular shape (Figs. 4, 9, and 12). It is to be emphasized that no ferritin is seen in the droplets or vacuoles of the epithelium from nephrotic animals not given ferritin. It is also noteworthy that mitochondria can be distinguished clearly from the droplets, and that under our experimental conditions no structures with intermediate morphology were observed (Figs. 4 and 8).

Normal and Nephrotic Animals Given Ferritin.— In glomeruli from both normal (Figs. 1 to 3) and nephrotic (Figs. 5 and 6) animals given ferritin, ferritin molecules[4] were seen within (*a*) the capillary lumina, (*b*) throughout the entire depth of the basement membrane, and (*c*) within the visceral epithelium at all time intervals investigated. Striking differences between normal and nephrotic rats were seen only at the level of the epithelium. In normal animals ferritin molecules were found in small numbers in: (*a*) small invaginations of the cell membrane at the base of the foot processes (Fig. 2); (*b*) in the epithelium enclosed within small vesicles either in the foot processes themselves (Fig. 3) or deeper in the cytoplasm; or (*c*) incorporated into occasional vacuoles and dense bodies.

In nephrotic animals, ferritin molecules were seen in the epithelium in far greater numbers than in normal rats. The total amount seen was somewhat variable but increased with time following ferritin administration: many more ferritin molecules were present within the epithelium at 1 to 3 hours following the injection than at intervals of 5 to 15 minutes. Furthermore, the distribution also varied with time; *i.e.*, after shorter time intervals the ferritin was found predominantly along the epithelial cell membrane facing the basement membrane of the glomerular capillaries. The particles were usually located within small invaginations of the cell membrane or within small vesicles present in the cytoplasm close to the cell surface (Fig. 5). Some particles were also seen within the larger membrane-bounded vacuoles (Fig. 7). Relatively few of the dense bodies contained ferritin molecules.

At intervals of 1 to 3 hours following ferritin administration, large numbers of ferritin molecules were still seen in the lumina, within invaginations of the epithelial cell membrane, or in small cytoplasmic vesicles (Fig. 6), as described for earlier time periods in nephrotic animals; however, many more particles appeared within large cytoplasmic vacuoles and especially within dense bodies (Fig. 8). Although the amount of ferritin in individual cells varied considerably, by 1 hour after administration nearly every cell examined showed ferritin molecules within some of its vacuoles and dense bodies. In most of the cells, multiple ferritin-containing vacuoles or dense bodies were encountered.

It is noteworthy that in both normal and neph-

[4] Ferritin molecules have been shown (30) to be composed of a highly electron-opaque central core of ferric hydroxide surrounded by a protein shell. The complete molecule (ferric hydroxide micelles plus protein) measures approximately 100 A across (30). Since the apoferritin or protein shell is not opaque to electrons in osmium-fixed, methacrylate-embedded tissues (31), it is only the ferric hydroxide core, measuring approximately 55 to 60 A, which is visible in our preparations.

rotic animals and at all time points investigated, no ferritin was found free in the cytoplasmic matrix of the epithelial cells. Ferritin molecules were always restricted to membrane-limited compartments, *i.e.* vesicles, vacuoles, and dense bodies, and thus separated from the rest of the cytoplasm.

It is also noteworthy that ferritin-containing invaginations of the plasma membrane were common only on the cell front facing the capillary. Similar invaginations were also seen on the cell front facing the urinary space, but they rarely contained ferritin. Instead, larger sinuses or cleft-like cavities were often seen along the cell surface facing the urinary spaces. Some of these sinuses contained finely particulate material resembling that filling the dense bodies of the epithelial cells, except that it appeared less well packed (Fig. 11). Other masses of material similar in density and texture to that filling the droplets were also found within the urinary spaces (Fig. 10). It is assumed that the sinuses represent vacuoles or dense bodies in the process of extruding their content into the urinary space (Fig. 10). It should be added that no ferritin was seen in these partially dispersed masses apparently undergoing extrusion from the cell.

DISCUSSION

In these experiments we have taken advantage of the fact that ferritin molecules are dense and large enough to be seen individually in the electron microscope (30, 31), and we have traced their pathway from the lumen to the urinary space across the various layers that form the wall of the glomerular capillary. It can reasonably be assumed that the same pathway is followed by other molecules, especially by proteins of comparable dimensions.

Our observations show that in nephrotic rats, ferritin molecules permeate the basement membrane in large numbers and reach the visceral layer of the epithelium. At this level we have found them located in invaginations of the cell membrane, enclosed within vesicles at the periphery of the cytoplasm, and finally, accumulated within centrally located vacuoles and dense bodies. On the strength of these findings, we assume that epithelial cells incorporate ferritin molecules in bulk by membrane invaginations which subsequently become pinched off to form intracellular vesicles. These vesicles transport the tracer to the large vacuoles of the cell which, by extensive condensation of their content, transform into "dense bodies" or "droplets." Several small ovoid droplets may then merge to form a single larger droplet of irregular shape. The content of at least some of the droplets appears to undergo a partial "digestion" and is finally extruded from the cell into the urinary spaces.

It is evident that the membrane pockets formed by the invginating membrane of the epithelial cell can contain, in addition to ferritin, many other molecules, including the plasma proteins that leak through the defective basement membranes of the nephrotic glomerulus. It is probable that these substances are processed by the cell in the same way as the ferritin, and we assume that upon condensation they form the content of the dense bodies found in the cytoplasm. A similar derivation by dehydration of the vacuole content has been postulated for droplets or "granules" in the epithelium of the proximal convolution by Miller (32).

The interpretation of our results is facilitated by the fact that in glomerular capillaries the general direction in which ferritin molecules move is known, and by the finding that the pattern of intracellular location of the marker shows consistent variations within the time interval explored. For example, ferritin molecules are seen within vesicles and vacuoles at early time intervals but only after 1 hour are they found in appreciable numbers in dense bodies.

Some of the appearances we have encountered suggest that the content of certain large vacuoles and dense bodies is eventually discharged into the urinary space. We should point out, however, that with the evidence at hand we cannot decide whether the discharge is a normal event in the final disposal of incorporated material, or a sign that the amount of material with which the cell is confronted exceeds its capacity for disposal, or, finally, an indication that the ability of the cell to deal with its ingesta is impaired. The fact that ferritin molecules were not found in the discharged residues is for the moment unexplained. It might be due to inadequate timing in our experiments (the dense bodies may be discharged at an "age" greater than the duration of our longest experiments) or, alternatively, to changes undergone by the ferric hydroxide-phosphate micelles of the ferritin molecule after its segregation. The micelles can become soluble if their iron is reduced to the ferrous state in the environment of the droplets, thereby leaving the residual apoferritin molecule which is invisible in our preparations (31).

Ferritin molecules also traverse the basement

membrane of normal glomerular capillaries (33) though in much smaller numbers than in nephrotic animals, and here again the emerging molecules are incorporated by the visceral epithelium which occasionally contains large vacuoles and dense bodies. These findings indicate that a similar process of incorporation in bulk, followed by segregation, occurs in both normal and nephrotic epithelium, only the rate is considerably higher in nephrotic animals. It may be tentatively assumed that "checking" the glomerular filtrate is one of the normal activities of the visceral epithelium. This function is greatly stimulated when increased quantities of proteins permeate the basement membrane and reach the urinary spaces, as in the nephrotic state.

The activity we detected in the glomerular epithelial cells is highly reminiscent of the pinocytotic activity originally discovered in cultured cells by Lewis (34), subsequently described in amoebae by Mast and Doyle (35), and recently studied in more detail in the same material by Holter, Chapman-Andresen, and their collaborators (36–40). As such it could be called "micropinocytosis," since the membrane pockets involved are considerably smaller than those previously observed by light microscopy. As recently pointed out by Holter (40), there is no sharp line of demarcation between micropinocytosis and pinocytosis on one hand, and pinocytosis and phagocytosis on the other. Our findings support the view that these two last processes have many basic mechanisms in common and that the distinction between them is to a large extent arbitrary. In our case, incorporation into the cell takes place by the formation of small, fluid containing membrane pockets, but the fate of the ingested materials, once they have entered the cell, is identical to that seen in the phagocytic process—namely, migration to a more central location in the cell (Golgi region), formation of large vacuoles which become condensed into dense bodies, followed by a digestion of the droplet content and discharge of the residues from the cell.

Recent studies by Straus (41–44) may have considerable bearing on the same problem. Under the name of "phagosomes" he described a special type of cytoplasmic body characterized by high content of acid hydrolytic enzymes (acid phosphatase, ribonuclease, deoxyribonuclease, cathepsin, β-glucuronidase) and by their ability to segregate a foreign protein—horseradish peroxidase. He found phagosomes present in large numbers in nephron epithelia, hepatic parenchymal cells, and in cells of the reticulo-endothelial system (macrophages). They were also present in smaller numbers in a large variety of cell types. Available evidence suggests, as already pointed out by others (45, 46) that Straus' phagosomes are identical with (*a*) the "lysosomes" isolated from liver homogenates (47); (*b*) the "dense bodies" (48) and the "dense peribiliary bodies" (49, 50) described in liver cells *in situ*; (*c*) the "VP granules" of HeLa cells (51), and (*d*) various other inclusion bodies (52). All these bodies are apparently connected with the ability of the corresponding cells to "digest" various materials incorporated from their surrounding medium either in solution or as formed bodies.

In our case, as in the case of Straus' peroxidase, the segregation of a specific protein, ferritin, into certain cytoplasmic bodies has been demonstrated. Moreover the mechanisms involved in its incorporation and intracellular transport have been at least in part elucidated. The presence of digestive enzymes in these bodies has not been ascertained, and the ultimate fate of the ingested protein has not been studied. We can, however, reasonably assume that these droplets contain, like phagosomes and lysosomes, acid hydrolytic enzymes which will digest, and thus dispose of the proteins and other materials picked up by the visceral epithelium from the glomerular filtrate.

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Explanation of Plates

Abbreviations for Figures

BM = basement membrane
CP = capillary lumen
EN = endothelium
EP = epithelium
FP = foot processes of epithelium
US = urinary spaces

All the figures represent electron micrographs taken from tissues fixed in osmium tetroxide and embedded in methacrylate. Sections were stained 1½ to 3 minutes with lead hydroxide (23, 24). Figs. 1 to 8, 11 and 12 are from "sandwiched" preparations (25) whereas Figs. 9 and 10 are not sandwiched.

Plate 139

Figs. 1 to 3. Portions of the glomerular capillary wall from a normal rat given ferritin 10 minutes prior to sacrifice. In Fig. 1, a number of ferritin molecules can be seen in the capillary lumen; some molecules are also present within the endothelial fenestrae (*f*) or are found at various levels in the basement membrane (arrows). In Fig. 2, in addition to the ferritin found in the lumen and in the basement membrane, a single ferritin molecule is present within a small invagination (*in*) of the epithelial cell membrane facing the basement membrane. In Fig. 3, other ferritin molecules are found enclosed within small vesicles (ve_1 to ve_3) located in the cytoplasm of the epithelial foot processes.

In the normal glomerulus, circulating ferritin molecules apparently gain access to the basement membrane by traversing the endothelial fenestrae. A few molecules then penetrate the basement membrane and are incorporated into the epithelium by micropinocytosis. Figs. 1, 2, and 3 × 82,000.

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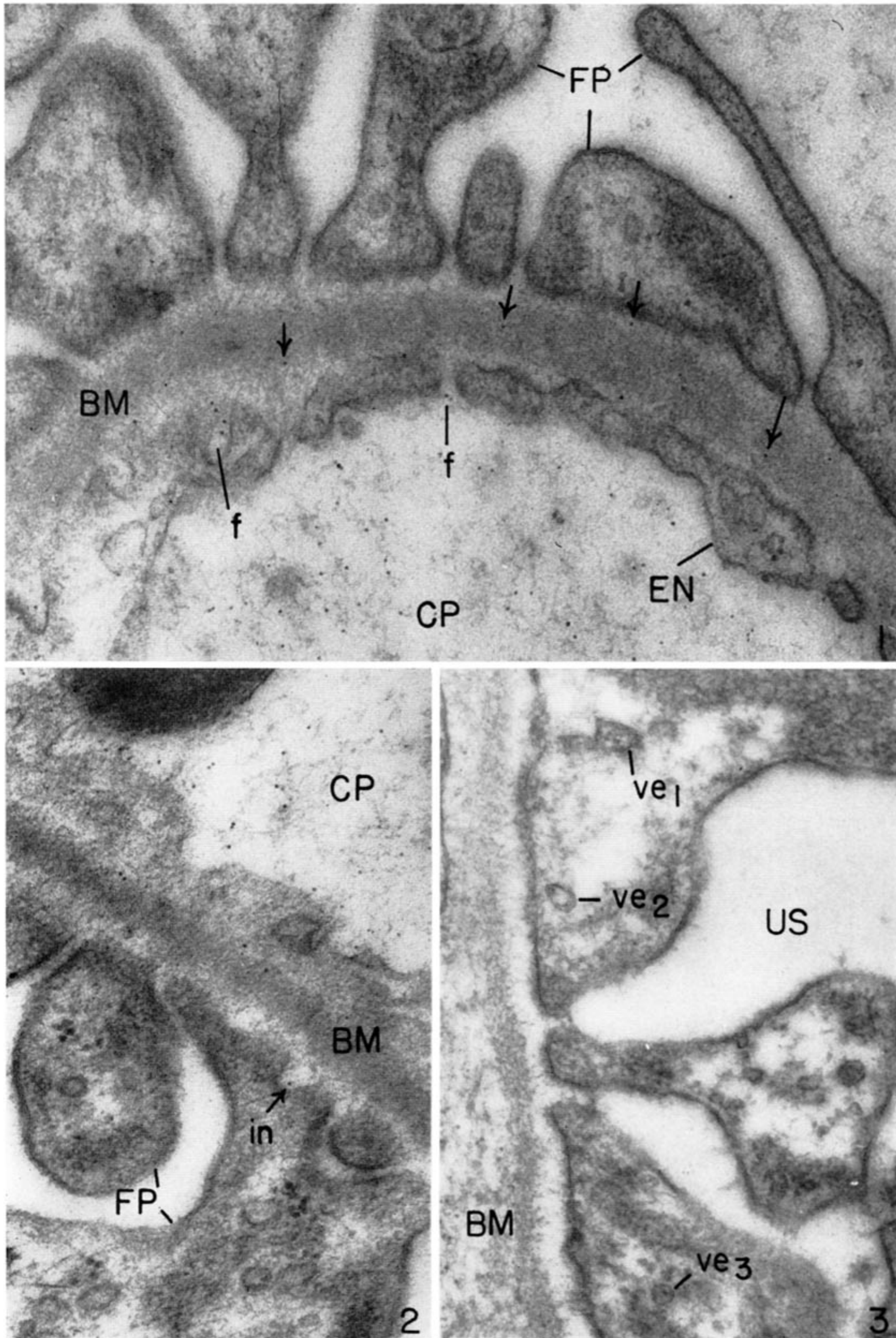
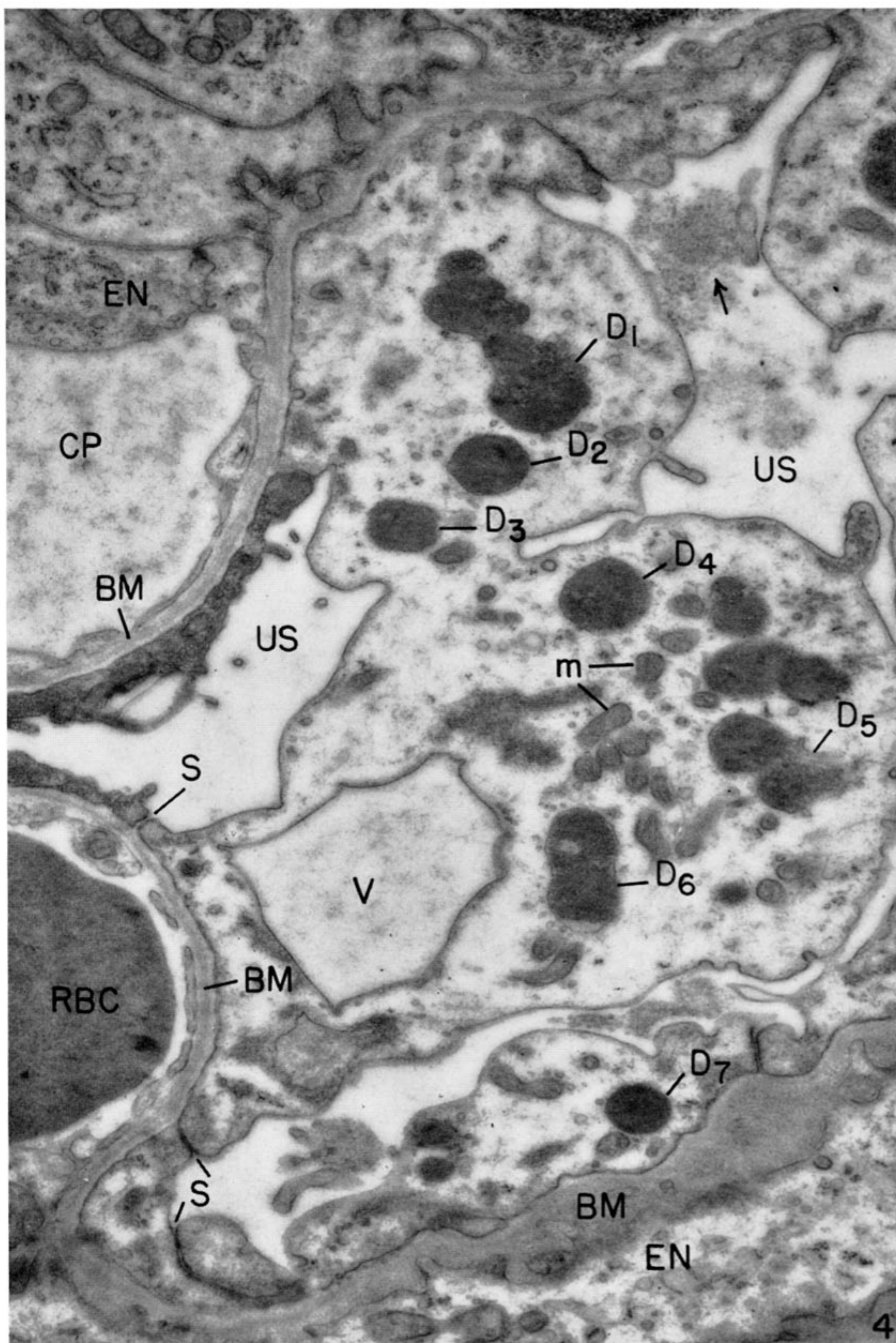


PLATE 140

FIG. 4. A relatively low power field of a glomerulus from a nephrotic animal, showing the striking changes, which are present primarily in the epithelium and which are associated with nephrosis. The lumina of two capillaries are visible on the left; one contains a red blood cell (*RBC*). The foot processes of the epithelial cells are lost and in their place a broad mass of epithelial cytoplasm interrupted by relatively few slits (*S*) covers the outer surface of the basement membrane. The epithelial cytoplasm contains a single large vacuole (*V*) and multiple dense bodies (D_1 to D_7) or "protein absorption droplets." The vacuole is limited by a membrane and shows a relatively low internal density. Dense bodies or droplets are also membrane-limited and are characterized by the great density of their content. The droplets D_2, D_3, D_4, and D_7, are ovoid in shape and have a uniformly dense content. The droplets marked D_1, D_5, and D_6 have an irregularly festooned outline and an inhomogeneous content. These features suggest that several smaller ovoid droplets have merged to form a single larger droplet of irregular shape. Dense bodies can be readily distinguished from mitochondria (*m*) which are smaller and less dense. In the urinary space above (arrow) is a mass of material which may represent a droplet residue extruded from the epithelium (See legend for Fig. 10). × 19,000.

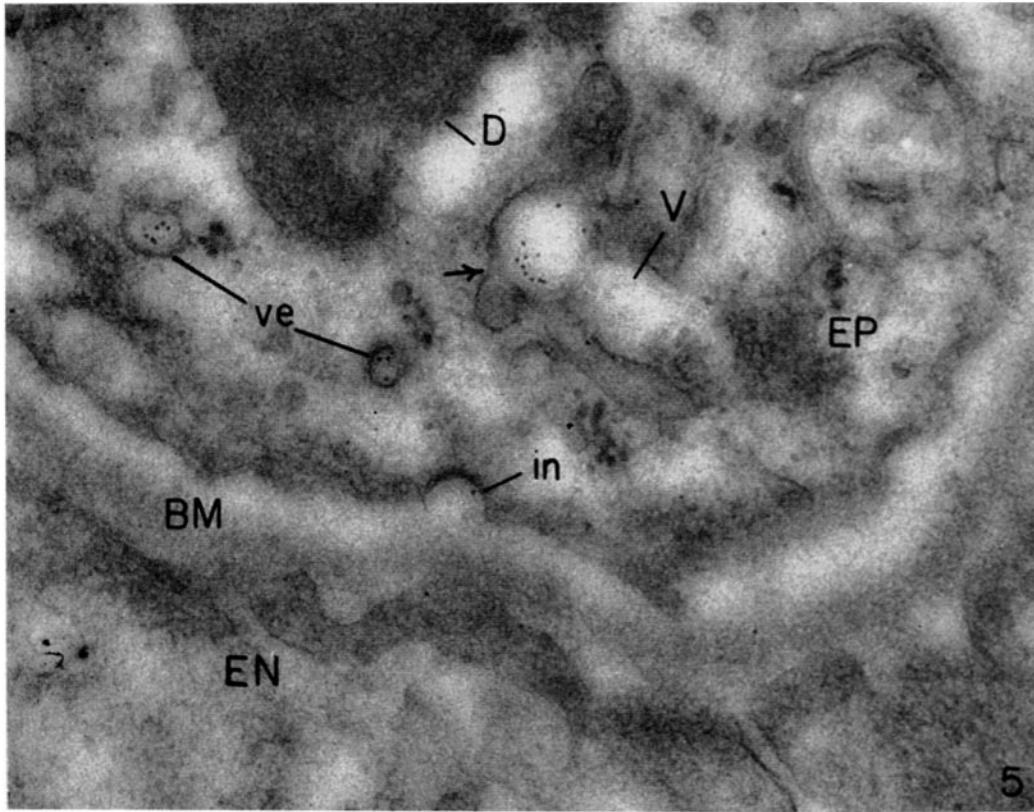


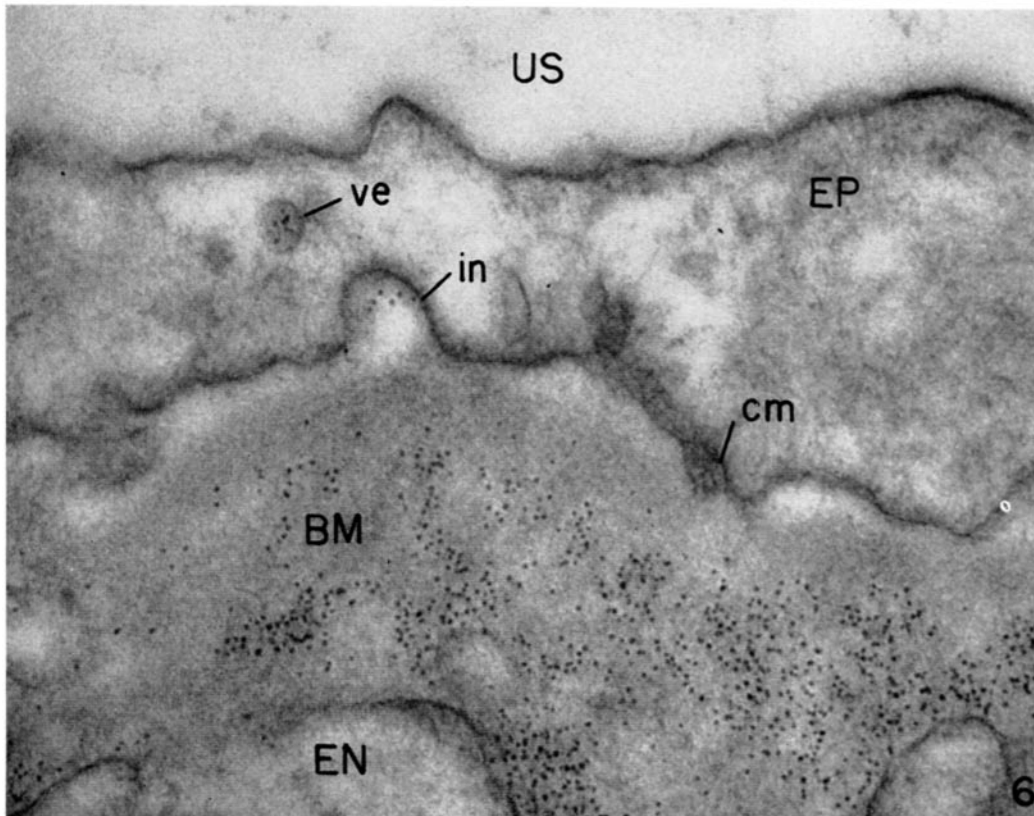
(Farquhar and Palade: Ferritin segregation in protein absorption droplets)

PLATE 141

FIG. 5. Small portion of the glomerular capillary wall from a nephrotic rat given ferritin 5 minutes prior to sacrifice. A single ferritin molecule is present within an invagination (*in*) of the epithelial cell membrane facing the basement membrane. Other ferritin molecules are seen within two membrane-limited vesicles (*ve*) (~600 to 800 A in diameter) located in the epithelial cytoplasm close to the cell surface. Ferritin molecules are also present within a larger, membrane-bounded vacuole (*V*) characterized by its relatively low internal density, but none are seen within the dense droplet (*D*). At the arrow a small vesicle is apparently merging with the larger vacuole, for their limiting membranes are in continuity. × 88,000.

FIG. 6. Small portion of a glomerular capillary wall from a nephrotic rat given ferritin 1 hour prior to sacrifice. Many ferritin molecules are seen within the basement membrane, preferentially concentrated in the inner portion. A group of ferritin molecules are also located within a small invagination (*in*) of the epithelial cell membrane (*cm*), and others are seen within a small vesicle (*ve*) in the epithelial cytoplasm. The local invaginations of the epithelial cell membrane apparently become pinched off from the membrane to form intryactoplasmic vesicles. Thus ferritin molecules, which penetrate the basement membrane of nephrotic animals in large numbers, are incorporated into the epithelium by micropinocytosis. × 123,000.



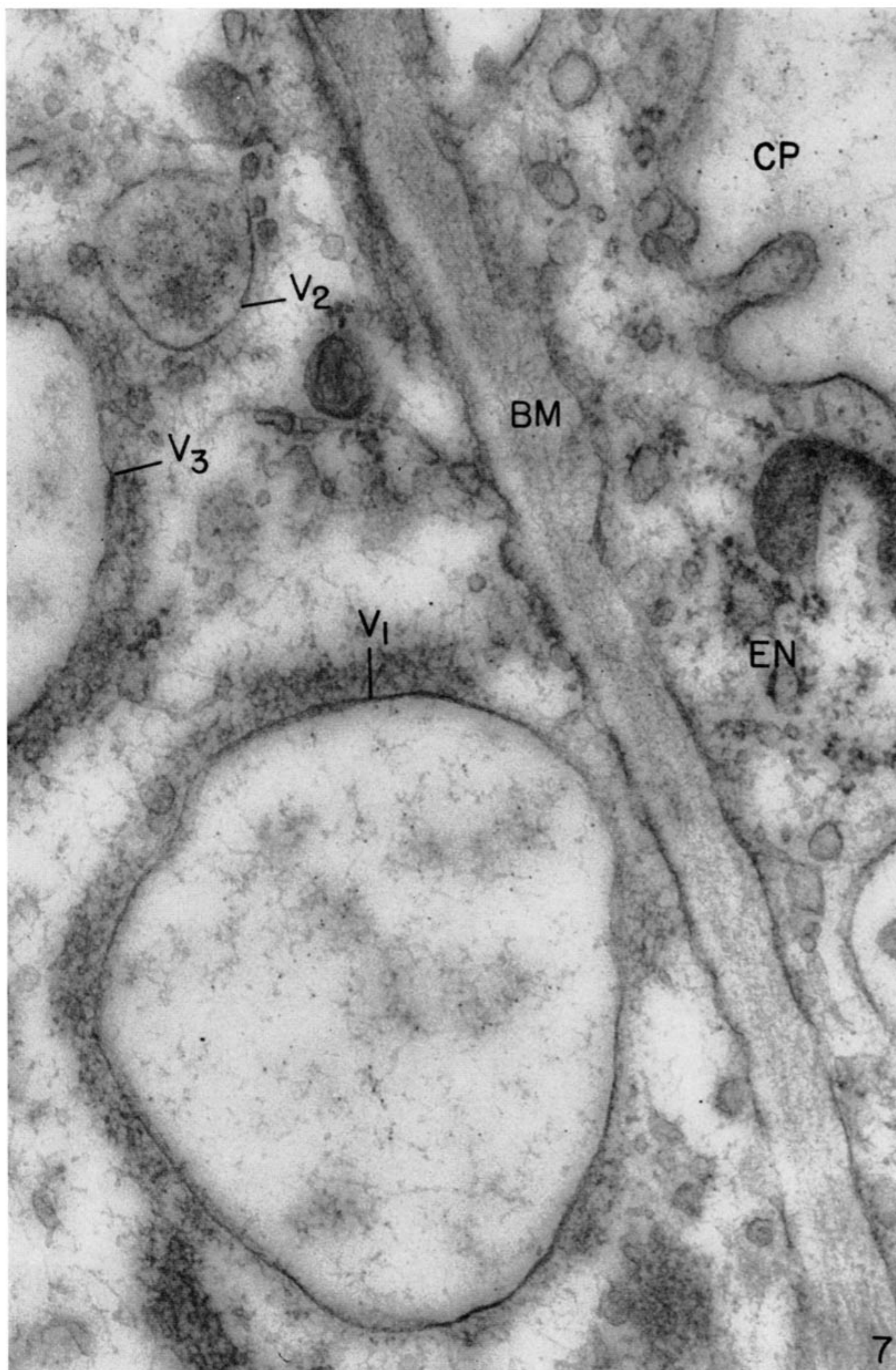


(Farquhar and Palade: Ferritin segregation in protein absorption droplets)

PLATE 142

FIG. 7. Portion of the glomerular capillary wall from a nephrotic rat (15 minutes following ferritin administration) demonstrating the segregation of ferritin within epithelial vacuoles. A portion of the capillary lumen is present in the upper right corner (*CP*), and the basement membrane crosses the field almost diagonally. A fine fibrillar structure is evident in the basement membrane. Ferritin molecules can be seen within the capillary lumen, within a large vacuole occupying the lower half of the field (V_1) and within a smaller vacuole above (V_2). No ferritin is present within the portion of another vacuole included on the left (V_3).

The vacuoles are limited by a membrane and show a low internal density. The large vacuole (V_1) contains, in addition to ferritin, only a fine flocculent precipitate similar to that present in the capillary lumen, whereas the smaller vacuole (V_2) has a somewhat greater background density. In addition, a layer of dense material with finely filamentous and particulate texture is seen around V_1 and V_3. Dense halos of this nature, closely applied to the outer surface of the limiting membrane, are commonly seen around epithelial vacuoles. $\times$ 76,000.



(Farquhar and Palade: Ferritin segregation in protein absorption droplets)

PLATE 143

FIG. 8. Cytoplasm of a visceral epithelial cell from a nephrotic rat (2 hours after ferritin administration), showing segregation of ferritin into a number of vacuoles and dense bodies or droplets. The basement membrane is found in the upper left corner and urinary spaces are shown on the upper left and lower right. Ferritin molecules are present within a large vacuole of relatively low internal density (V_1), and an adjacent vacuole of intermediate density (V_2); they are also seen concentrated within a number of dense bodies (D_1 to D_5). The mitochondria (m) do not contain ferritin and may be distinguished from droplets by their smaller size, lesser internal density and presence of cristae.

The presence of numerous forms of intermediate density (*e.g.* V_2) between vacuoles and dense bodies, together with the fact that ferritin molecules are seen within vacuoles at short time intervals and appear within dense bodies only after long time intervals, suggest that the vacuoles transform into dense bodies by progressive condensation. × 100,000.

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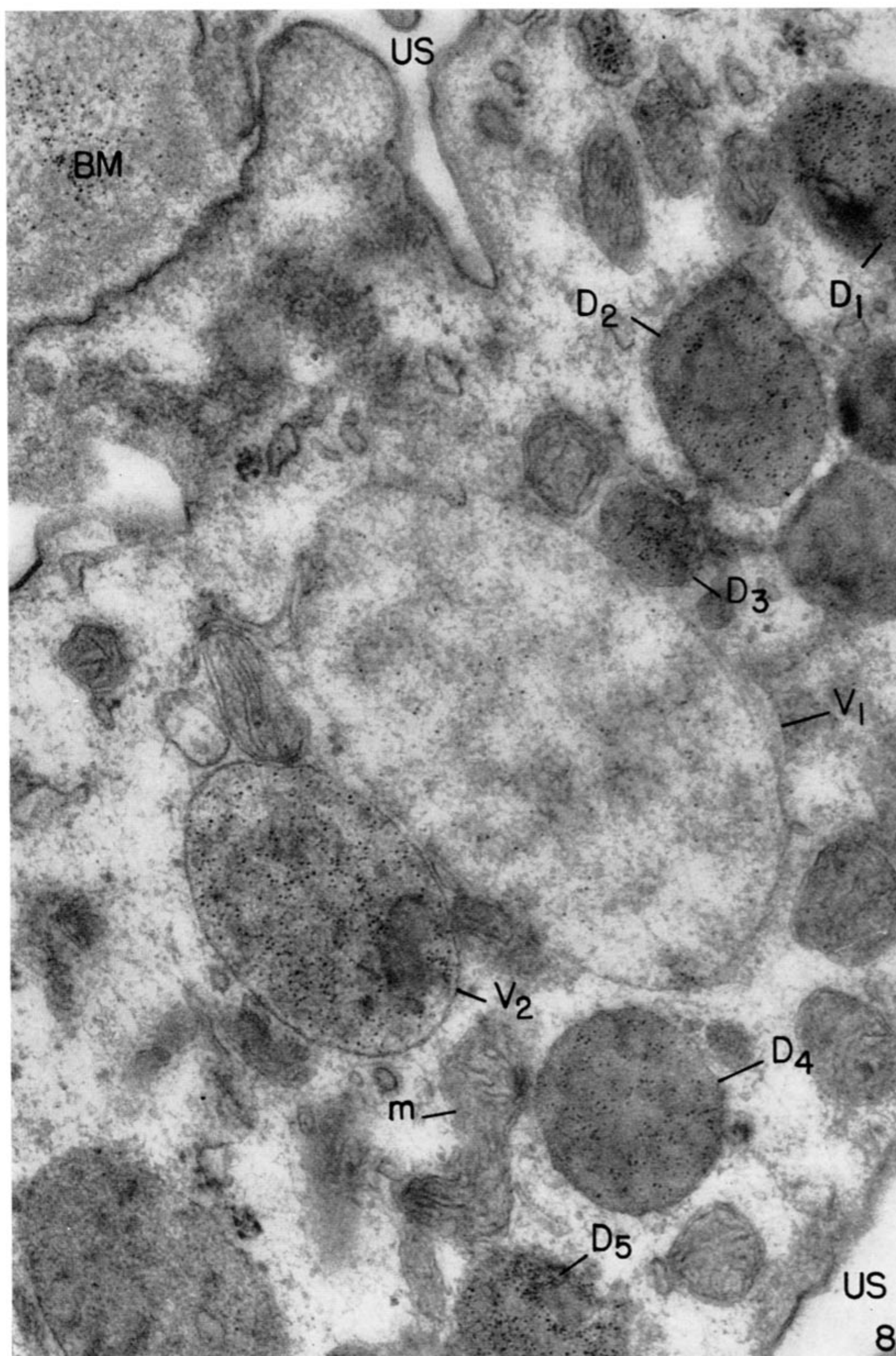
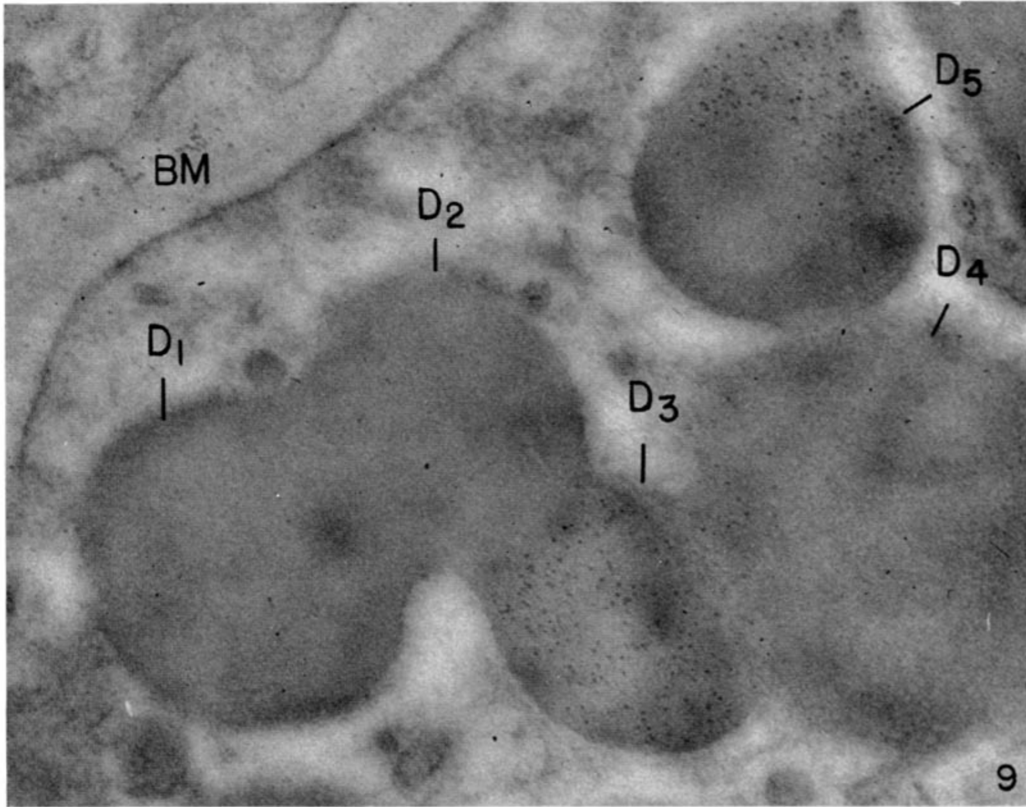


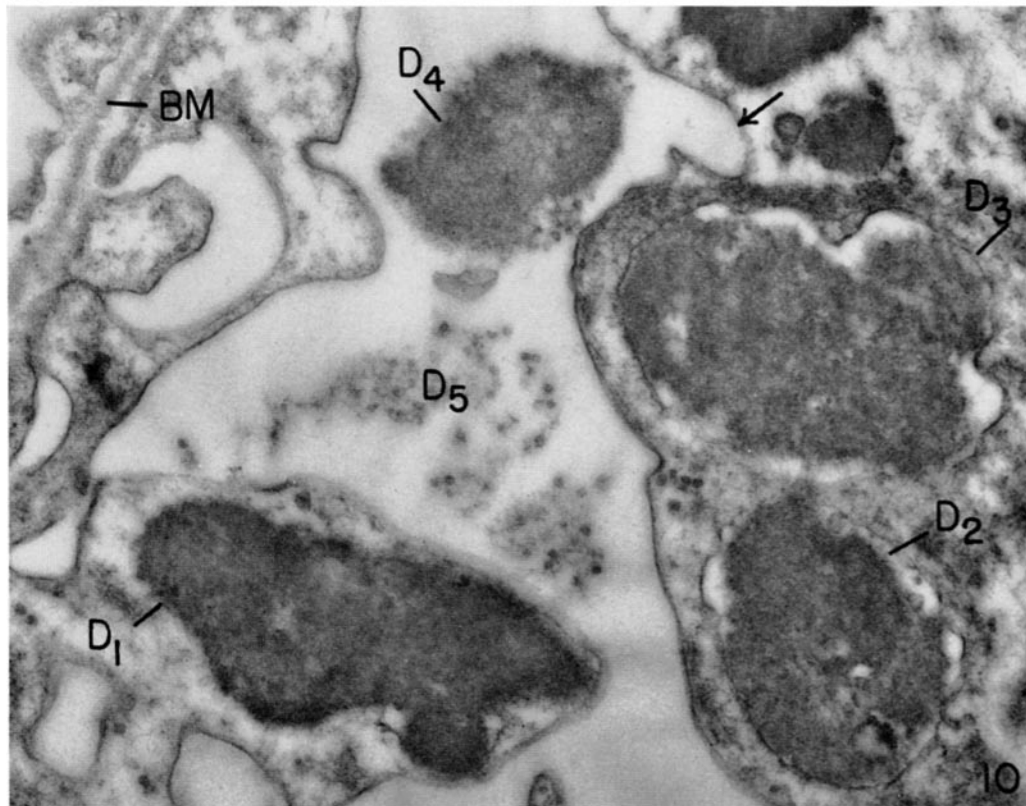
(Farquhar and Palade: Ferritin segregation in protein absorption droplets)

PLATE 144

FIG. 9. Small field from an epithelial cell of a nephrotic rat (1 hour following ferritin administration). The basement membrane is present on the upper left. A large multilobular dense body occupies most of the field. Its shape and content suggest that four ovoid dense bodies (D_1 to D_4) have merged to form a single droplet of irregular shape. D_3 contains numerous ferritin molecules whereas D_1, D_2, and D_4 do not. Another spherical dense body containing ferritin only in its upper half is present at D_5. $\times$ 87,000.

FIG. 10. Portion of a glomerulus from a nephrotic rat showing a series of dense bodies or droplets which seem to be in various stages of digestion and extrusion from the epithelium. At D_1 is a relatively intact droplet of fairly uniform internal density. At D_2 and D_3 are two droplets with peripheral areas of lesser density, suggesting they have undergone partial digestion. At D_4 is a mass of material, similar in density and texture to that filling the dense bodies, located within a urinary space. This mass is thought to represent the content of a dense body extruded from the cell into the urinary space through the neighboring sinus or cleft-like cavity (arrow). The material present within the urinary space at D_5 may represent a further stage in the disintegration of droplet residues. $\times$ 33,000.





(Farquhar and Palade: Ferritin segregation in protein absorption droplets)

PLATE 145

FIG. 11. Glomerular capillary wall from a nephrotic rat sacrificed 15 minutes following ferritin injection. A large cleft-like cavity or sinus is present along a portion of an epithelial cell facing a urinary space. The sinus contains finely particulate material which is similar in texture to that filling the droplets, except it appears less well packed or partially dispersed. No ferritin is present.

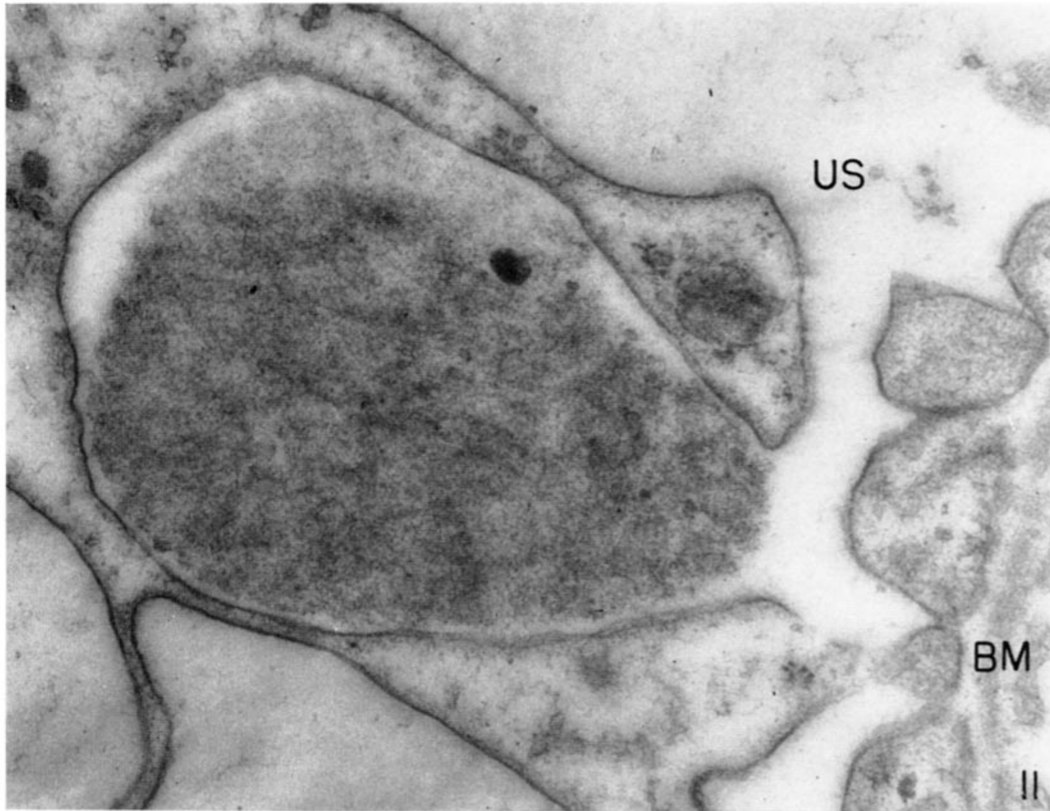
It is assumed that this sinus represents a dense body in the process of extruding its content into the urinary space. The droplets' former membrane is presumed to form part of the cell membrane lining the sinus. × 46,000.

FIG. 12. Portion of the cytoplasm of an epithelial cell from the glomerulus of a nephrotic rat (15 minutes after ferritin injection). The basement membrane is present on the left and a urinary space is shown below.

Two large dense bodies (D_1 and D_2) with an irregular shape are present in the center of the field. In some areas the content of these droplets appears relatively dense and compact, but other areas are much lighter in density. Several small rounded structures are also seen within the droplets (arrows), but no ferritin is present.

Irregularly shaped masses such as these with an inhomogeneous content are believed to represent conglomerates of dense bodies which have undergone partial digestion.

Mitochondria (*m*) can be readily distinguished from droplets by their characteristic smaller size, lesser density and presence of cristae. × 36,000.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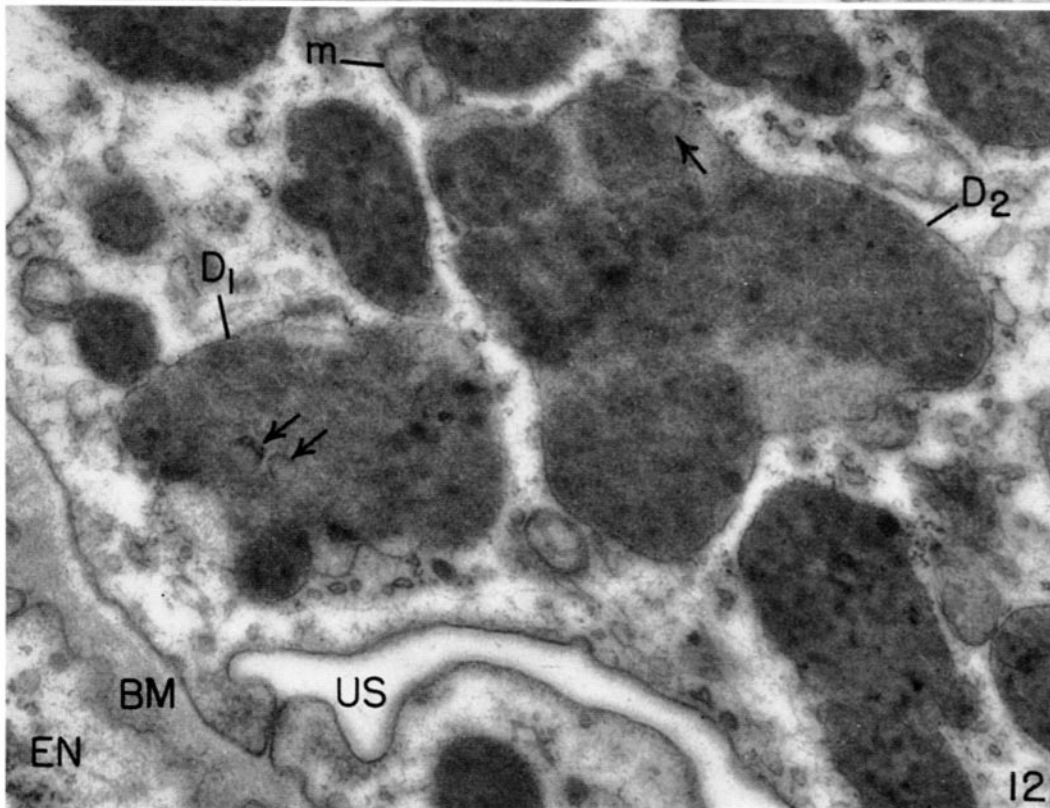
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