GLOMERULAR EXCRETION OF MACROMOLECULAR SUBSTANCES

Electron Microscopic Study of Rat Kidney After Administration of Human Serum Albumin

C. T. Ashworth, M.D., and J. A. James, M.D.

From the Departments of Pathology and Pediatrics, The University of Texas Southwestern Medical School, Dallas, Texas

It was shown earlier that dextran injected intraperitoneally in rats was excreted across the glomerular capillary wall, forming aggregates in the glomerular epithelial cells. The substance appeared in the proximal convoluted tubular epithelial cells, and some was taken up by histiocytes in the renal interstitial tissue.[1] For further study of the mechanism of excretion of macromolecular substances in glomerular filtrate, and of the effects of this phenomenon upon the kidney, human serum albumin was given intraperitoneally to rats, producing a state of hyperproteinemia and albuminuria. The kidneys were subsequently examined with electron and light microscopy.

Methods

Six young Sprague-Dawley rats, weighing from 100 to 190 gm., were given intraperitoneal injections of human serum albumin.* Albumin solution diluted to 3 per cent in 0.45 per cent saline was administered in amounts of 1 cc. per 10 gm. of body weight twice daily for 14 days. Four control rats of similar size were given equal amounts of 0.45 per cent saline intraperitoneally. Animals received no other fluids, and were kept on a stock pellet diet.

Body weight and 12-hour urinary protein output[2] were determined every third day. Terminally, total serum proteins were evaluated by a biuret method, and kidney weights were obtained. Kidney tissue was fixed in neutral buffered formalin for histologic sections. Hematoxylin and eosin and the periodic acid-Schiff-Alcian blue stains[3] were used. Blocks of kidney cortex (1 cu. mm.) were fixed in a chrome-osmium fixative[4] for electron microscopy. Tissue was dehydrated in alcohols, embedded in methacrylate, sectioned with a diamond knife, and examined with an RCA EMU-3 electron microscope.

Results

Determinations of body weight, kidney weight, urinary protein output, and terminal serum protein levels are given in Table I. Body weights

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* Albumisol, ® Merck, Sharp and Dohme, Philadelphia, Pa.

changed only slightly in both control and experimental groups. The kidney wet weights, expressed as gm. per hundred gm. of body weight, were considerably increased in the rats receiving serum albumin injections. Urinary proteins were markedly increased in the experimental group after the third day of the experiment.

TABLE I

EFFECT OF INTRAPERITONAL SERUM ALBUMIN INJECTIONS * IN RATS

	Control group	Albumin-injected group
Number of animals	4	6
Average wt. at start (gm.)	173	122
Average 12 hr. urine protein output on sixth day (mg.)	1.9	72
Average terminal serum protein (gm./100 cc.)	6.3	8.3
Average terminal wt. of kidneys (gm./100 gm. of body wt.)	0.899	1.207

* Daily injections for 2 weeks.

With light microscopy, hematoxylin and eosin stains of the kidneys showed a distinctive thickening of the glomerular capillary walls and the presence of intraglomerular hyaline droplets. PAS-stained preparations revealed a normally thin glomerular basement membrane, but PAS-positive hyaline droplets were identified in the glomeruli (Fig. 1). The exact localization of these droplets within the capillary wall could not be clearly defined, however.

Electron microscopy revealed the glomerular alterations in greater detail. The glomerular epithelial cells were moderately swollen (Fig. 2). The patency of capillary lumens did not appear significantly affected even though the pericapillary epithelial cytoplasm was increased in amount. The net result was a comparatively greater filling of the glomerular space by the capillary loops and associated epithelial cells. In most instances the swollen epithelium contained a light, loose-appearing cytoplasm, in contrast with the compact and dense cytoplasm of epithelium in normal glomeruli. Within the light cytoplasmic areas were fine granules about 80 to 120 Å in diameter.These were similar in size and density to the fine granules seen within capillary lumens and apparently represented plasma protein or lipoprotein macromolecules (Fig. 3). These granules were about one-half the diameter of ribonucleprotein (RNP) granules and not as dense. RNP granules could be identified in all portions of the epithelial cytoplasm and not only in the less dense portions. Usually the pedicels retained their characteristically compact and dense structure, but in several instances this was replaced entirely or partially by looser and less dense cytoplasm. For the most part, the

pedicels remained discrete. In some areas, however, where pervaded by the lighter cytoplasm, the pedicels had become swollen and confluent (Fig. 4). Study of various stages of foot process fusion indicated that this had resulted from flattening out of the interpedicellar spaces due to the increased amount of cytoplasm; as a result the cell membrane was pushed down against the basement membrane.

The most striking alteration appeared in the cytoplasm of the glomerular epithelium where localized, dense aggregates of fine particles or granules occurred. These bodies measured up to 1 μ in diameter and at all stages of development appeared to be independent of mitochondria (Figs. 5 and 6). What appeared to be their earliest stages consisted of poorly demarcated densities in the glomerular epithelial cytoplasm. The individual granules measured about 100 Å. As the aggregates became larger, they became more distinctly rounded or oval, were more compact, and gradually developed a sharper demarcation from the surrounding cytoplasm, although no limiting membrane was seen. In some cells the bodies occurred in such numbers that they almost replaced the remaining cytoplasm. The densities corresponded to the hyaline droplets and PAS-positive bodies observed in the glomeruli by light microscopy.

Neither the endothelium nor the basement membranes of the glomerular capillaries manifested any definite structural alterations. In the cytoplasm of some proximal convoluted tubular cells, prominent vacuoles measuring up to 1 μ were observed. These usually contained fine granules resembling those seen in the loose cytoplasmic portions of the glomerular epithelium (Fig. 7). Also, many of the epithelial cells in the proximal convoluted tubules contained oval or round, dense bodies similar in size and appearance to those encountered in the glomerular epithelium (Fig. 8). Evidence of neither the granular material nor the dense bodies was found in the interstitial tissue or in histiocytes of the peritubular stroma.

Discussion

Baxter and Cotzias[5] have reported hyperproteinemia and marked albuminuria in rats after intraperitoneal injection of human serum albumin. The kidneys in their animals became enlarged, and the glomerular capillary walls underwent thickening. In the present study, these observations were confirmed. The alterations in the glomeruli consisted of a distinctive watery swelling of the epithelium and the accumulation of oval, dense bodies in their cytoplasm. The rapid occurrence of albuminuria, and the absence of any evidence of cell injury, histologically or electron microscopically, favor the hypothesis that foreign protein reaction is not the cause of the changes observed. Instead, the hyperproteinemia is believed to be the basis for the glomerular alterations.

The accretion of very fine granules, the density and size of which were

similar to those of the plasma protein, and the formation of dense oval bodies in the glomerular epithelium suggested that albumin molecules were taken up by the glomerular epithelium from the glomerular filtrate. This proposition has been substantiated by the demonstration that dextran accumulated in the glomerular epithelium in a similar manner.[1] This suggests that some plasma macromolecules with molecular weight ranging from 20 to 60 thousand may transgress the glomerular capillary wall in the normal rat and that some of these macromolecules may then be incorporated into the cytoplasm of the glomerular epithelium. It can be hypothesized that so long as the capacity of the cell for uptake and elimination of the imbibed macromolecules is not exceeded, no accretion of these substances would occur. If, however, the mechanisms for elimination of imbibed material are overloaded by increased concentration of macromolecules in the glomerular filtrate, then accumulation of these substances in the epithelium would become evident. This hypothesis is supported by the work of Farquhar and Palade,[6] who demonstrated the appearance of small amounts of ferritin in glomerular epithelium in normal rats and much larger amounts in nephrotic rats. Vernier and associates[7] showed that silver-tagged protein accumulated in the glomerular epithelium of nephrotic rats but not in normal rats. In our experiments it should be emphasized that no glomerular injury had been produced and that the serum albumin injected had not been altered from its natural state. The accumulation of macromolecules in glomerular epithelium may be of significance as a possible explanation for the localization of antibody[8] and gamma globulin[9] in this region. Whether the deposition of proteins here could in itself result in glomerular injury is yet to be determined.

Although macromolecules of the blood are able to transgress the glomerular capillary wall and form prominent aggregates in the glomerular epithelium, the rate of their escape across the capillary wall has not been established. Gradual accretion of the macromolecular substances could result in relatively large intracellular deposits if the rate of cellular uptake from the glomerular filtrate exceeds the rate of secretion by the glomerular epithelium. Likewise, the mechanism by which macromolecules gain entrance into the cells is a matter for speculation. Pinocytosis, as suggested by Farquhar and Palade,[6] seems to be a likely possibility. We also observed pinocytotic vesicles in the interpedicellar spaces relatively frequently in the swollen epithelial cells of rats after albumin injections. They occurred in control animals also, but to a much lesser degree. Subsequent to the presumed pinocytotic uptake, some of the imbibed protein and fluid could then be released into the glomerular space.

It has been suggested[6] that protein molecules taken up by epithelium

are incorporated into existing cytoplasmic bodies, the lysosomes.[10] However, our observations that the earliest stages of accretion of proteinoid granules were independent of recognizable cytoplasmic organelles do not substantiate this proposal. Instead, it appeared that the macromolecules were incorporated as fine particles and gradually coalesced to form the dense bodies observed by electron microscopy or the hyaline droplets noted with light microscopy.

The fusion of foot processes has been emphasized in the descriptions of glomerular lesions in nephrosis.[11,12] Some features in the development of this lesion seemed to be revealed in the present study. It was noted that fusion of foot processes occurred only in limited areas and, when found, was associated with a watery, swollen epithelial cytoplasm and an "ironing out" of the interpedicellar spaces. This resulted in the displacement of the cell membrane against the basement membrane over a segment of the outer surface of the capillary wall. Thus, the fusion of foot processes may be viewed as the result of cytoplasmic swelling subsequent to the increased uptake or storage of glomerular filtrate fluid and protein.

The alterations induced in these experiments were thought to be of a reversible nature, since no evidence of severe cell injury, proliferative reaction, exudation, or capillary obliteration occurred. All of the structural changes were considered to be the result rather than the cause of albuminuria. The similarity of the glomerular alterations to those seen in pure nephrosis of human subjects indicates that these also may be due to albuminuria.

Summary

Following the intraperitoneal injection of human serum albumin in rats, hyperproteinemia and marked proteinuria occurred. Light microscopy revealed thickened glomerular capillary walls and PAS-positive droplets in both glomeruli and proximal convoluted tubules. Electron microscopy showed dense oval bodies believed to be protein aggregates in the glomerular epithelium with pale cytoplasmic swelling and focal fusion or obliteration of foot processes. These changes were considered to indicate an increased glomerular excretion of some of the injected albumin, followed by pinocytotic uptake of the macromolecules and fluid by the glomerular epithelium. This was associated with widespread cytoplasmic swelling and with focal "ironing out" of foot processes. The glomerular alterations were therefore interpreted as the result rather than the cause of albuminuria. Moderate increase in the cytoplasmic content of oval dense bodies and of droplet-like formations of fine granules in proximal convoluted tubular epithelium were considered indicative of tubular reabsorption of protein from the glomerular filtrate.

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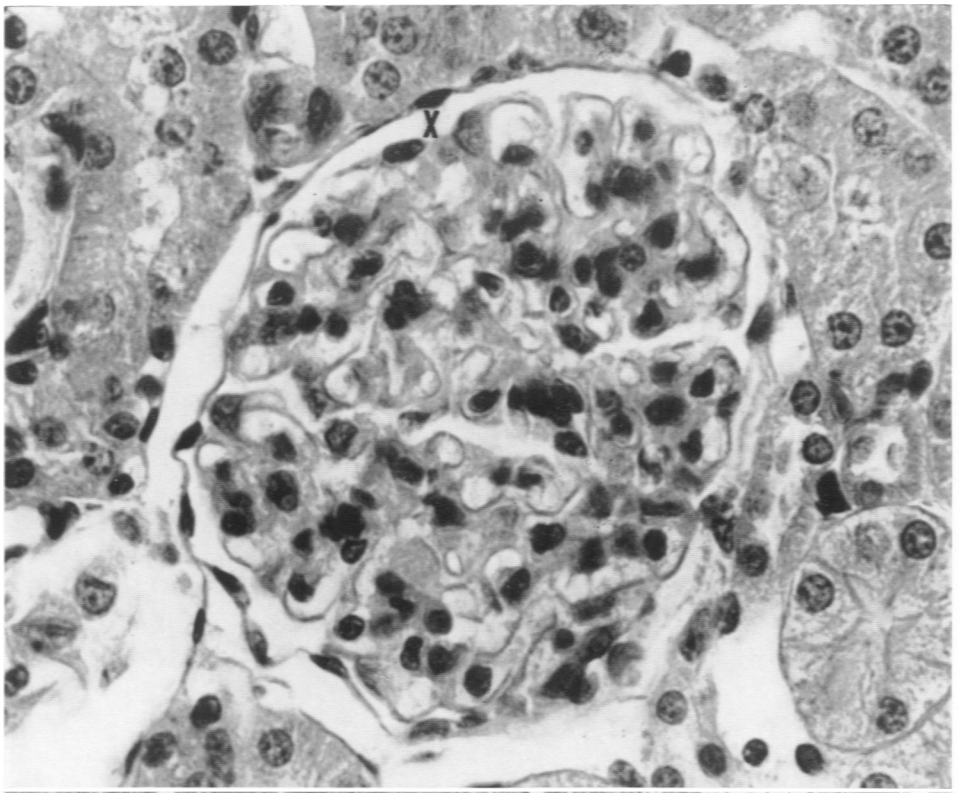
LEGENDS FOR FIGURES

FIG. 1. Renal glomerulus in a rat receiving 3 per cent albumin intraperitoneally for two weeks. Swelling of the glomerular epithelium between capillary loops can be seen, and PAS-positive aggregates (X) are visible in the capillary walls. Periodic acid-Schiff stain. × 760.

FIG. 2. A glomerulus in an animal receiving albumin. Fine particulate material is noted in the glomerular space (X), and in the cytoplasm of glomerular epithelium (O). This is similar in particle size and density to the fine granules of plasma proteins in capillary lumens (C). At F a small area of fused foot processes is seen. The cytoplasm of glomerular epithelium also contains dense proteinoid aggregates (D). × 13,000.



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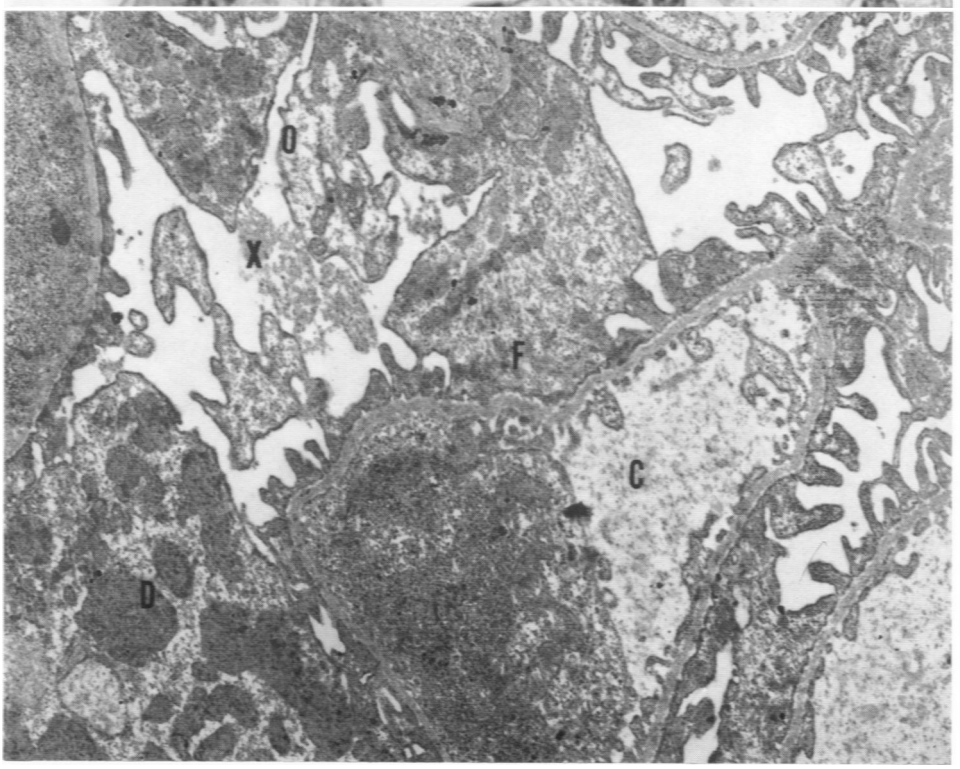

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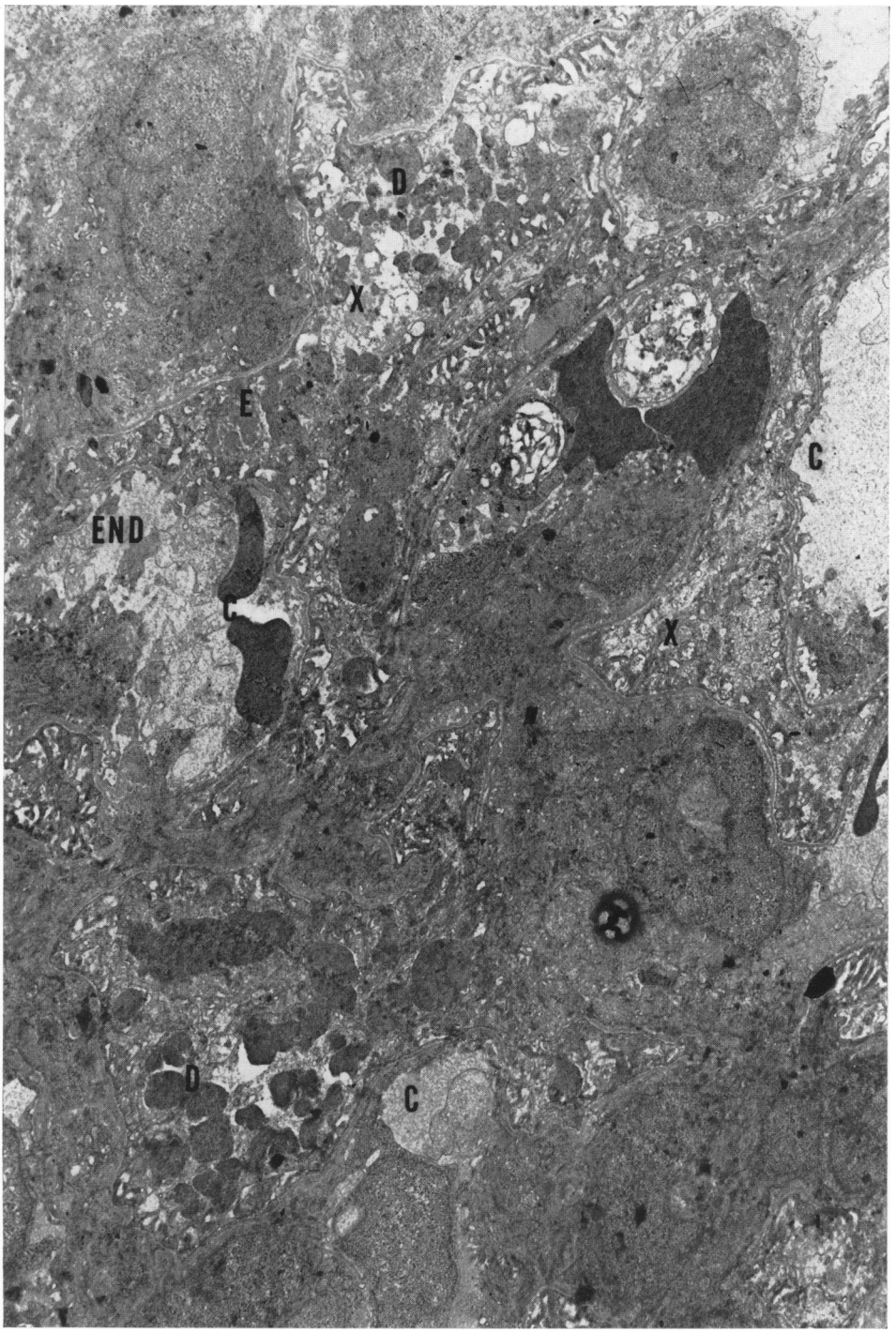

FIG. 3. A portion of a glomerulus in an animal receiving albumin injections. The capillary loops (C) are patent, but glomerular epithelium (E) is swollen and contains dense intracytoplasmic aggregates (D), and the cytoplasm contains swollen, pale areas (X). The endothelium (END) is also swollen in some capillary loops. × 5,000.

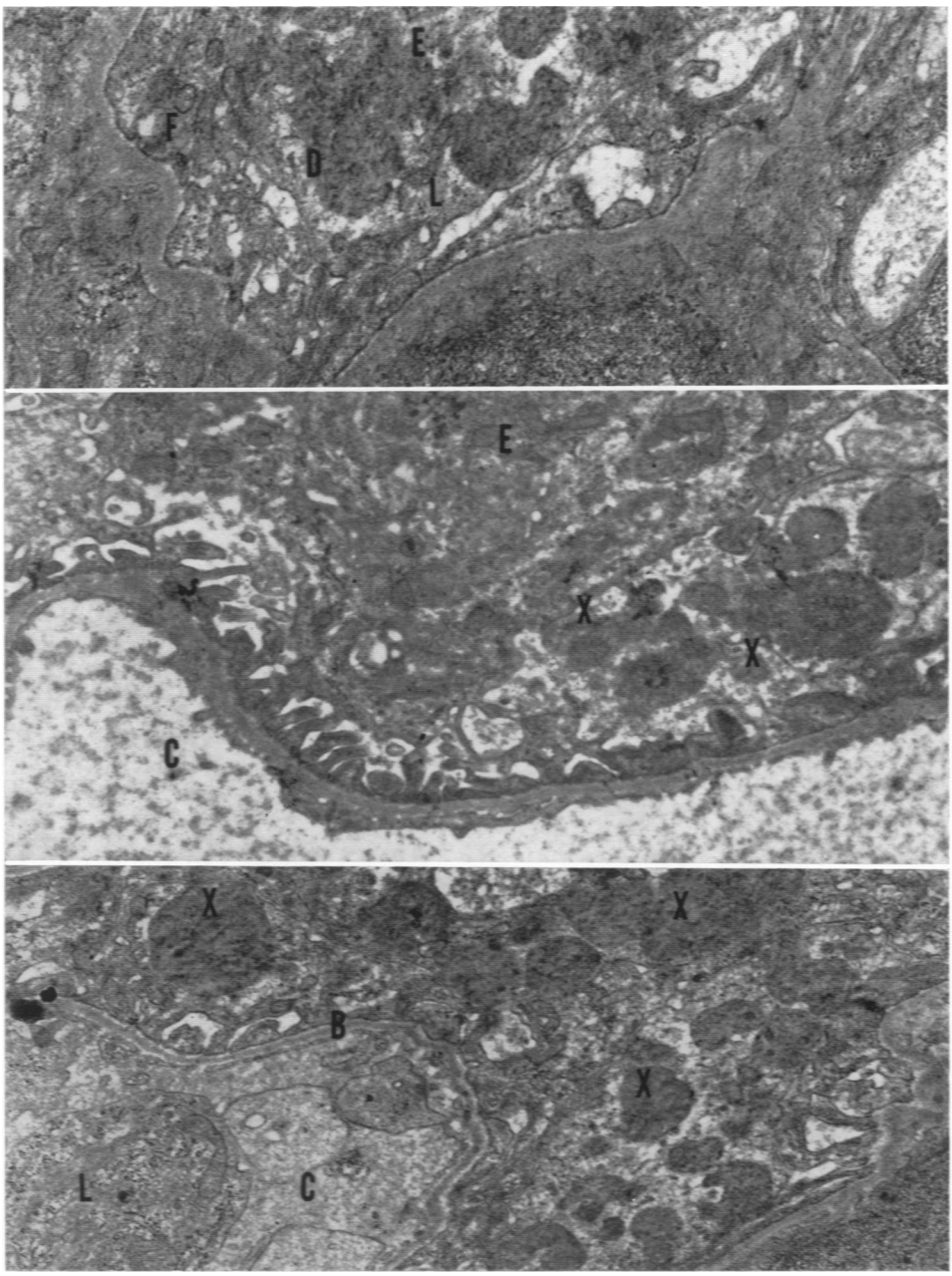

FIG. 4. Focal fusion of foot processes (F) is evident. The cytoplasm of the glomerular epithelium (E) is swollen, containing light, loose areas of cytoplasm (L) and dense proteinoid aggregates (D). × 18,000.

FIG. 5. Early stages in formation of dense aggregates are noted at X in the glomerular epithelial cell (E). A part of the lumen of a capillary (C) is manifest. × 13,000.

FIG. 6. A more advanced stage of dense aggregate formation is evident in the glomerular epithelium (X). A glomerular capillary (C) and basement membrane (B) are shown in part, and a leukocyte (L) is present in the capillary. × 13,000.

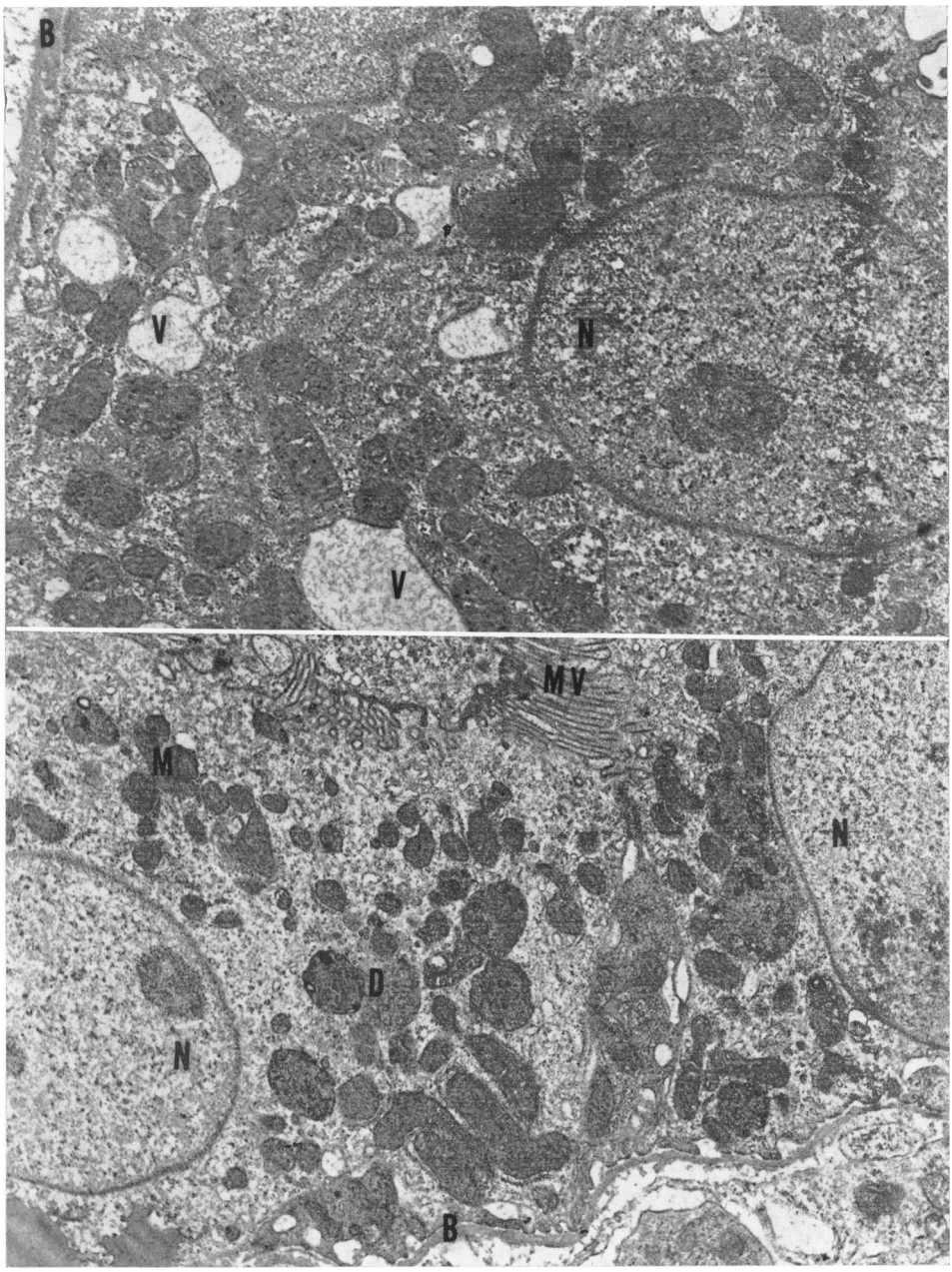

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FIG. 7. A portion of proximal convoluted tubular epithelium in an animal receiving albumin injections. Several vesicles (V) are observed in the cytoplasm and between adjacent cells. These contain a fine granular substance. The basement membrane (B) and a nucleus (N) of the epithelial cell are shown for orientation. × 13,000.

FIG. 8. Portion of a proximal convoluted tubule. Dense oval bodies (D) are found in the cytoplasm. These are similar to the bodies encountered in the glomerular epithelium. They are readily differentiated from mitochondria (M), and there are no intermediate stages to indicate any relationship. Nucleus (N), basement membrane (B) and microvilli (MV) of the renal tubule are also shown. × 13,000.