FINE STRUCTURAL ALTERATIONS IN THE RAT KIDNEY FOLLOWING INTRAPERITONEAL BOVINE ALBUMIN

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The structure of the normal mammalian kidney has been well established by electron microscopy,[1-6] and recently attempts have been made to determine the alterations attributable to proteinuria alone. These attempts have been mainly concerned with ultrastructural changes in the glomerulus. Vernier and colleagues[7,8] have reported fusion of the glomerular foot processes following infusion of canine serum protein in dogs. The areas of fusion could not be identified after proteinuria had ceased, i.e., following recovery. In 1954 Davies[9] presented a light microscopic study of hyaline droplet formation in the renal tubules of several species after experimentally induced proteinuria. These workers and others[10-16] have shown that certain morphologic derangements accompany the egress of excess protein through the glomeruli and that the glomerular and tubular changes occur invariably. The present report extends these initial observations and attempts to elucidate their significance.

MATERIAL AND METHODS

Ten male albino rats weighing 100 gm. each received intraperitoneal injections of 10 to 20 per cent crystalline bovine albumin in physiologic saline solution. One and one-half gm. of albumin prepared in this manner were administered in divided doses to each rat per day. The injections were continued for either 3 or 7 days. Proteinuria developed within 6 to 24 hours in all animals and ranged from 0.4 to 0.8 gm. per 24 hours. Some animals were killed at the end of the 3 or the 7 day periods, while others were allowed to recover for varying intervals up to 10 days. Significant proteinuria ceased within 24 hours following the last injection.

The animals were killed by decapitation, and blood was obtained for serum electrophoresis and chemical determinations. The kidneys were rapidly removed. One kidney was utilized for enzymatic study; the other was bisected and one half fixed in Bouin's solution. Hematoxylin and eosin, oil red O, and periodic acid-Schiff stains were employed for light microscopy. The remaining half of the kidney was cut into blocks less than 1 mm. in diameter and fixed for electron microscopy in Dalton's solution[17] for one hour. Blocks were dehydrated in graded concentrations of ethanol, infiltrated in 7:1 butyl-methyl methacrylate, and embedded in a partially prepoly-

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merized mixture of methacrylate to which benzoyl peroxide had been added as a catalyst. When embedding was not immediately feasible, the dehydrated blocks were stored in tertiary butyl alcohol, and later the embedding process was completed. Thin sections were cut on glass knives on a Serval Porter-Blum microtome. Sections were examined in either an RCA EMU-2E or 3B electron microscope. Some sections were stained with lead acetate [18] and then "sandwiched." [19] Original micrographs were taken on Cramer plates at magnifications of 1,000 to 10,000 diameters and enlarged photographically as desired. The present paper is concerned with the morphologic alterations occurring in the renal glomeruli and tubules. The enzymatic and chemical results will be reported at a later date.

Observations

The most striking change in the kidneys of animals killed at the 3 or the 7 day periods, as observed by phase microscopy, was the presence of numerous round or oval dense droplets in both the proximal convoluted tubules and the glomerular epithelium. The droplets were positive when stained with the periodic acid-Schiff method (Fig. 1). The distribution and quantity of the droplets could be even better appreciated by staining the phase section with toluidine blue. In frozen sections the droplets did not stain with oil red O.

Low power electron microscopy showed a similar distribution of droplets and, in addition, two features that were not readily apparent by light microscopy. The first of these was that in addition to the dense oval droplets, there was an almost equal population of similar structures with intermediate and light density (Fig. 2). The droplets varied in size from those slightly larger than mitochondria to others almost one-half the size of a nucleus. In general, the more dense droplets or bodies were the smallest while the paler ones were the largest. There was a spectrum from the dense to the pale droplets; those of intermediate density frequently had condensations of more electron-dense material at their peripheries or exhibited an over-all mottled appearance. The droplets were surrounded by a single smooth membrane with no attached ribonucleoprotein granules.

The second additional feature was that in ⅔ to ¾ of the glomeruli examined, areas of fusion or smudging of the foot processes were present (Fig. 2). The droplets had a unique relation to these lesions. When either light or dark bodies were observed close to the foot processes, there was a tendency for the latter to be broad and in some instances actually fused (Fig. 3).

In the proximal convoluted tubule the droplets were more numerous on the lumen side of the cell; however, they were sparsely distributed in relation to the palisade arrangement of basal mitochondria (Fig. 4). Patchy edema in the apexes of the proximal tubular epithelium was a usual finding, with concomitant obliteration of the normal brush border

(Fig. 5). In the ballooned-out portion of the tubular cells, apical microvesicles were still present (Figs. 6 and 7), even though the brush border was indistinct. No droplets were observed in the distal convoluted or collecting tubules. Blood vessels uniformly appeared normal.

When proteinuria had ceased for 24 hours, in animals given albumin for either 3 or 7 days, the glomerular epithelium was free of droplets, but a few could still be identified in the proximal tubular region. Swelling and fusion of the foot processes regressed until the pattern appeared normal (Fig. 8); after several days of recovery, both the glomeruli and tubules were normal. A rare droplet could be identified in the proximal convoluted tubular epithelium, but this was not considered abnormal.

Discussion

Numerous attempts have been made to correlate the structural alterations accompanying proteinuria with concomitant functional derangements. These attempts have met with variable degrees of success. Oliver, MacDowell and Lee [15] have reported that the droplets observed in the cells of the proximal convoluted tubules were the result of absorption or, in some instances, secretion of excess protein filtered at the glomerulus. Moreover, they related the droplets to mitochondria on the basis of staining property and reaction. Rhodin [20] also concluded that the droplets were altered mitochondria. Others,[9,11,12] however, were unable to implicate mitochondria directly in droplet formation.

In our observations, mitochondria did not appear to be involved in the formation of pale, intermediate or dark droplets. In some instances mitochondria were located at the peripheral margins of droplets but were structurally separate. Myelin figure formation was observed in a few of the smaller, more dense bodies, which would indicate a lipid component.[21] Since they were neither large nor numerous, the participation of lipid was probably not great.

The density spectrum in the droplets was interesting in that it could represent either accrual of additional protein in an oval structure or the metabolic degradation of this protein. From our morphologic data alone, it is difficult to determine which type of droplet appeared first; however, it seems reasonable to postulate that the light and, generally, larger ones were the forerunners of the smaller dense droplets. The intermediate variety with peripheral clumped densities or mottled appearance would represent evolutionary stages. Since the initial entrance of protein into a cell would be diffuse and scant in amount, it would follow that as the amount increased, the droplet density also increased. This is supported by the observation that immediately following recovery, when some droplets were still present, they were of the dense type.

When droplets were in close association with foot processes, there was a tendency toward spreading or fusion of these. Vernier and coworkers,[7,8] using dogs, reported that the "smudging" of foot processes was reversible and suggested that this lesion might be the result rather than the cause of proteinuria. Our own morphologic data support this view, suggesting that the alteration in the foot processes was a mechanical one due to the large droplets in the adjacent epithelial cytoplasm. Furthermore, in animals allowed to recover for even a brief period, neither droplets nor altered foot processes were evident. Some support for the mechanical thesis is gained from the changes in the proximal convoluted tubule. When edema was present in the apexes of these epithelial cells, there often was also an accumulation of droplets. The brush border was stretched, and the numerous delicate infoldings of the plasma membrane were no longer evident. Osmotic imbalance and resultant edema in association with the droplets might well account for the smoothing of the plasma membrane. The droplets were similar to the dense bodies described by Feldman and Fisher[22] and by Harkin and Recant[23] in glomerular epithelium in aminonucleoside nephrosis where fusion of foot processes was invariable.

Clark[24] showed that droplets and vacuoles filled with amorphous material were characteristic of epithelial elements in the newborn mouse kidney. The droplets were correlated with functional immaturity of the newborn kidney in which albumin was inadequately retained by the glomerulus. In the mature mammalian kidney, droplets are found only rarely.

Farquhar, Vernier and Good[25] demonstrated the accumulation of ferritin particles within cytoplasmic vacuoles and dense bodies in glomerular epithelium and suggested that the epithelial cells acted to recover the small amounts of protein normally filtered. Ashworth and James[26] also observed dense oval bodies in the cytoplasm of glomerular and tubular epithelium following administration of human serum albumin to rats. They considered the fusion and obliteration of foot processes to be an indication of increased glomerular protein excretion with resultant pinocytotic uptake by glomerular epithelium.

It seems clear that droplet formation appears when proteinuria is present and that the associated foot process and the tubular epithelial alterations are not the factors causing proteinuria but rather the result of filtration of excess protein.

Summary

Proteinuria was induced in rats for either 3 or 7 days with a solution of bovine albumin injected intraperitoneally. Some animals were killed

at the end of either of these periods while others were allowed to recover. Characteristic droplets were observed in both the glomerular and proximal convoluted tubular epithelium. A density spectrum in these droplets and their possible mechanical relation to alterations in the foot processes has been discussed. The droplets were not derived from mitochondria. The glomeruli and tubules reverted to their normal structure in the recovery period.

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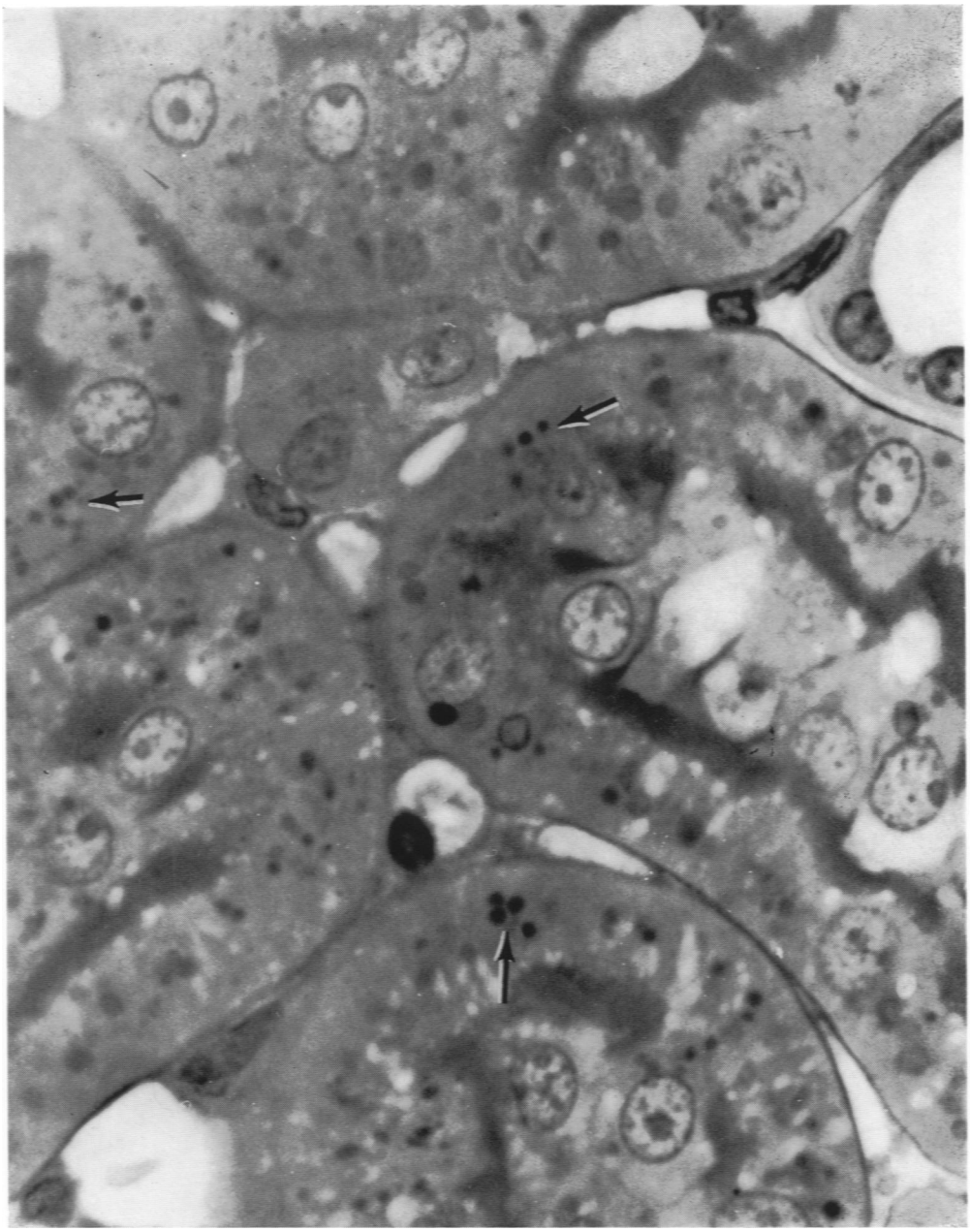
LEGENDS FOR FIGURES

Key:

B = basement membrane
CP = capillary lumen
EN = endothelium
EP = epithelium
US = urinary space
MV = microvesicle
N = nucleus
P = epithelial foot process
RBC = red blood cell
M = mitochondria
L = tubular lumen
F = foot process
C, D, I and H = droplets of different densities (see description of figures)

FIG. 1. Proximal convoluted tubules in a rat receiving 10 per cent bovine serum albumin intraperitoneally for 3 days. Clear vacuoles are present beneath the brush border, and PAS-positive droplets are distributed throughout the epithelial cytoplasm (arrows). Periodic acid-Schiff stain. × 900.



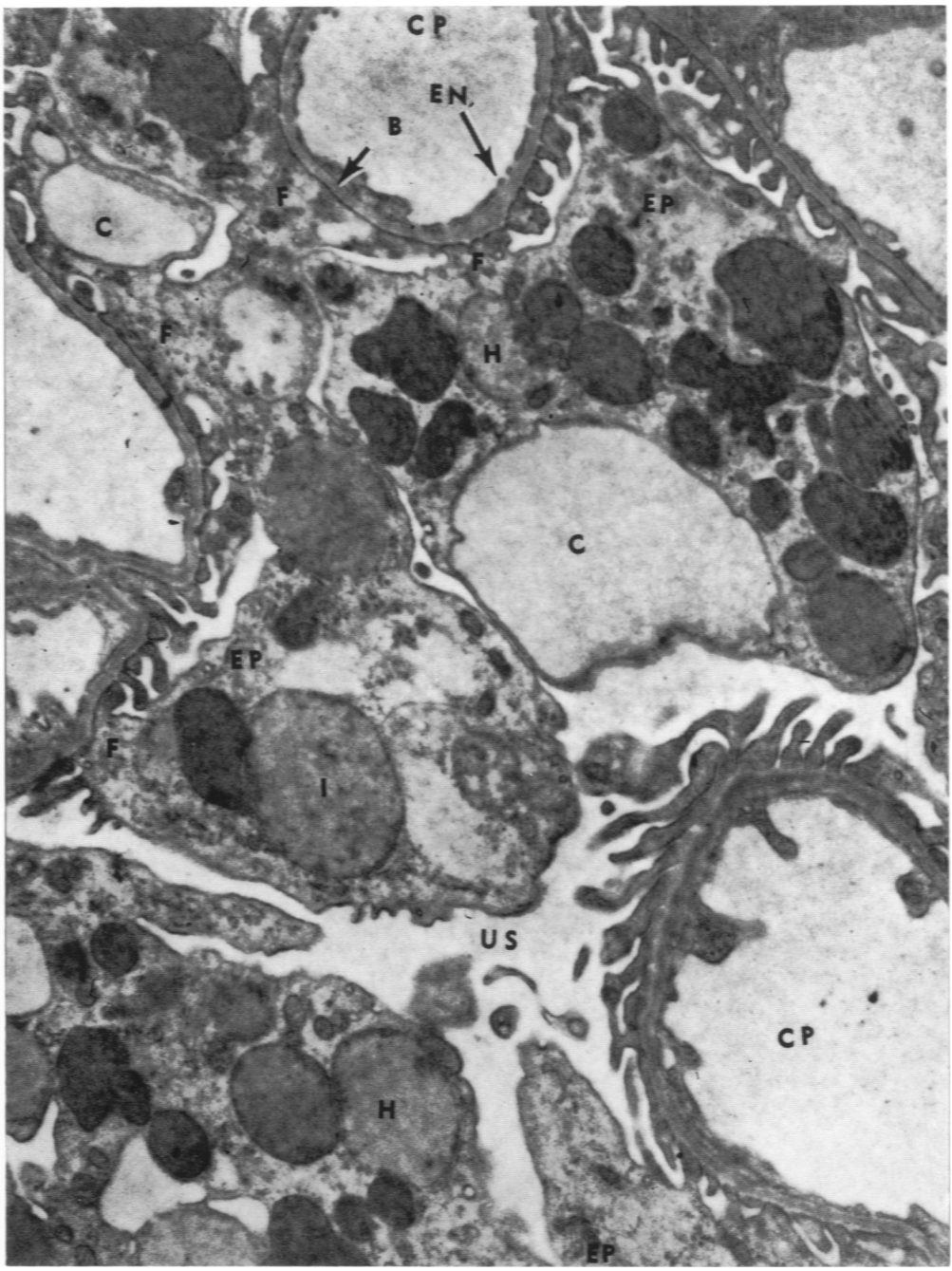


FIG. 2. A glomerulus from an animal receiving albumin. The cytoplasm of the epithelial cells contains numerous droplets of different electron densities. The lighter ones appear to represent large distended cytoplasmic cisternas containing an amorphous protein material (C). Intermediate forms are present, some with peripheral clumped densities (H) and others with an over-all mottled appearance (I). Many smaller dense droplets are seen. In several areas there is fusion and swelling of foot processes (F). The endothelium and basement membranes appear normal. × 11,500.

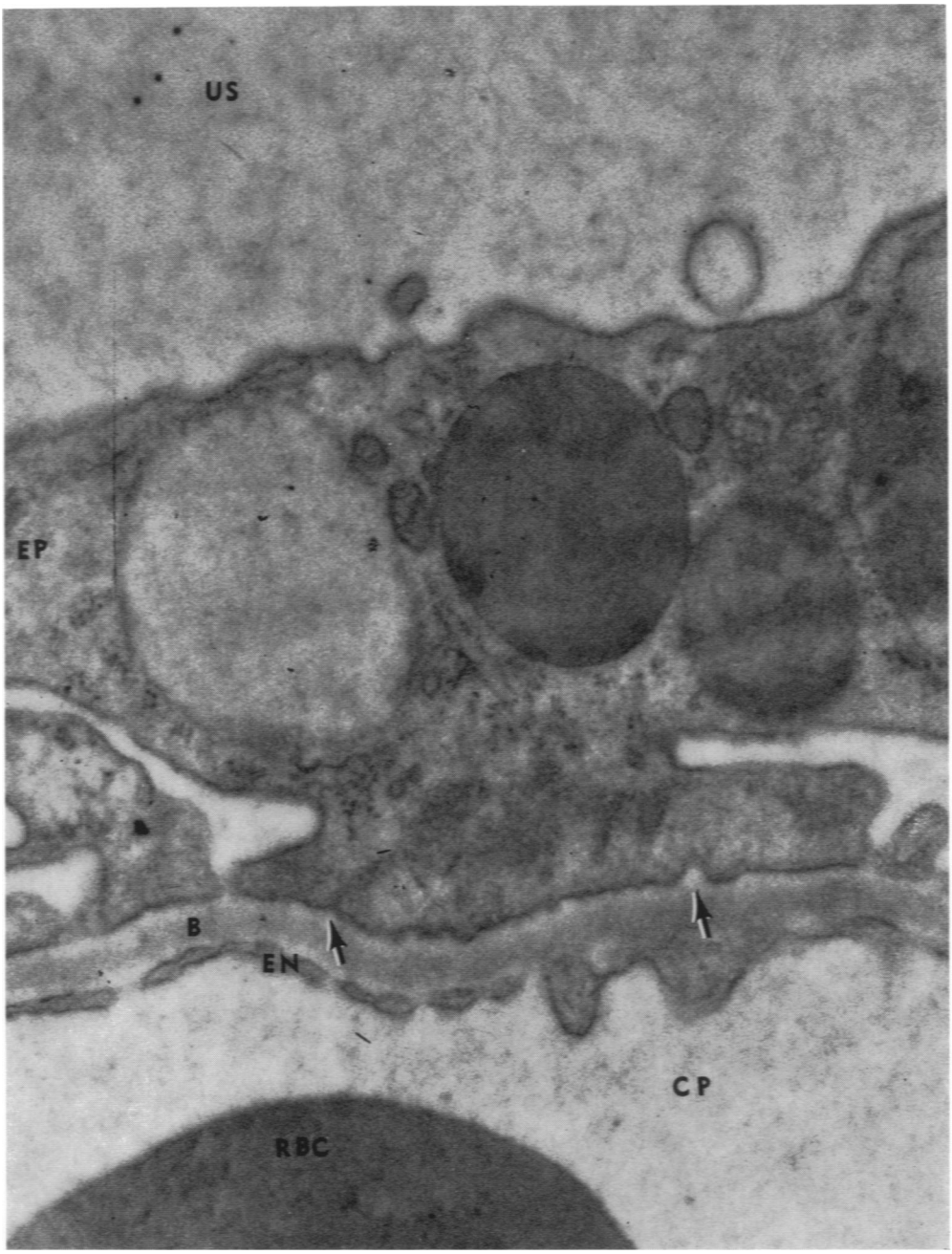


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FIG. 3. Portion of a glomerular loop in a rat that received albumin for 7 days. The basement membrane and endothelium appear normal. The epithelial cytoplasm contains several droplets of varying density. The foot process immediately adjacent to the group of droplets is swollen and broad, and along its junction with the basement membrane there are small invaginations (arrows), suggesting that separate and distinct foot processes were formerly present in these locations. The proximity of the droplets to the altered foot process suggests that mechanical factors may have been related to its alteration. × 40,000.

FIG. 4. Portion of a proximal convoluted tubule in a rat that had received bovine albumin. The brush border is present at the lower right. Mitochondria are numerous and have a characteristic elongated shape and orientation. Droplets of varying density are scattered throughout the cytoplasm. Relatively clear vacuoles contain an amorphous substance (C). Intermediate (H) and dense forms (arrows) are also present. The droplets are round to oval and are surrounded by a single dense membrane. × 4,500.

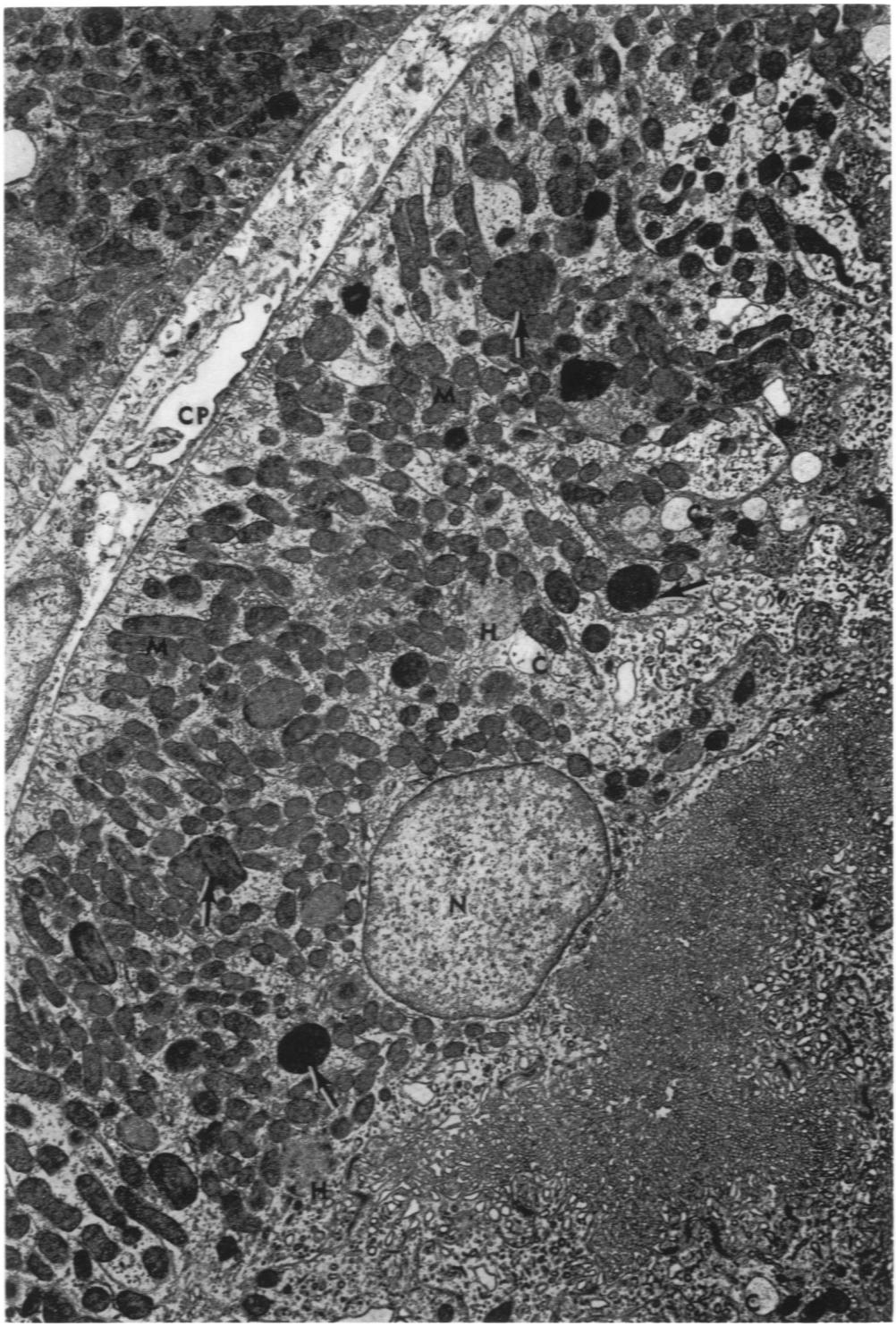

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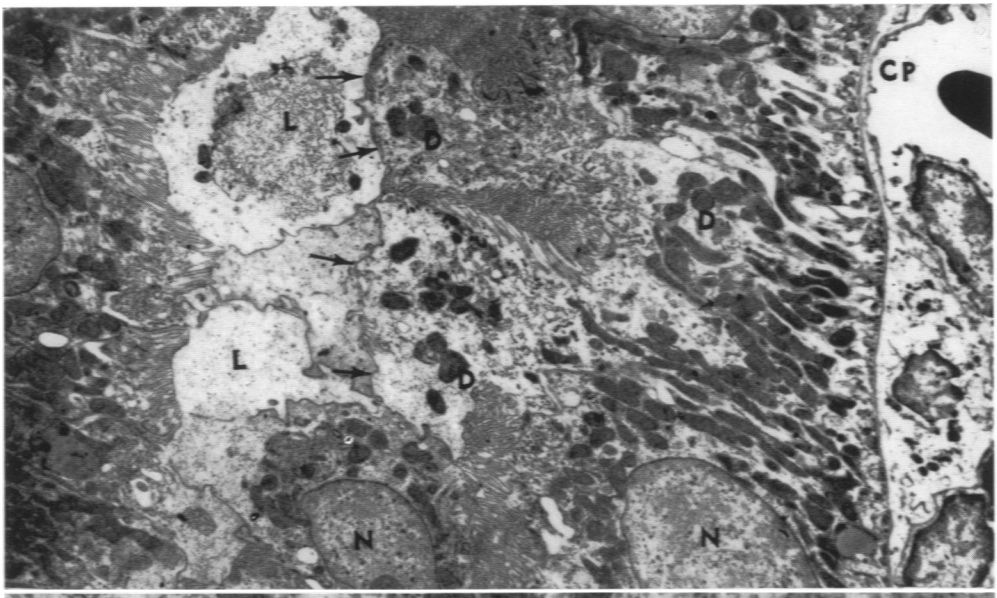
FIG. 5. Proximal convoluted tubule from a rat receiving albumin for 3 days. The base of the tubule is to the right while part of the lumen (L) is to the left. The apexes of two epithelial cells are edematous and have ballooned into the lumen. The arrows indicate the stretched plasma membrane at the lumen surface of these cells. The brush border between adjacent epithelial cells is intact. The cytoplasm of the edematous apexes is less dense, and the cytoplasmic particles are more separated than in adjacent cells. Several dense bodies are present (D). Lead acetate stain. × 4,500.

FIG. 6. Proximal convoluted tubular epithelium in a rat that received albumin for 7 days. The tubule lumen is evident at L, and this area is surrounded by the lumen borders of 3 cells. The brush borders of the 2 cells in the lower portion of the micrograph appear normal whereas this is almost lacking in the upper cell, only a small remnant remaining at "X." Apical microvesicles (MV) are retained in both the upper and lower cells. Droplets are present in the cytoplasm (D), and myelin figure formation has occurred in several of these (arrows). Lead acetate stain. × 14,000.



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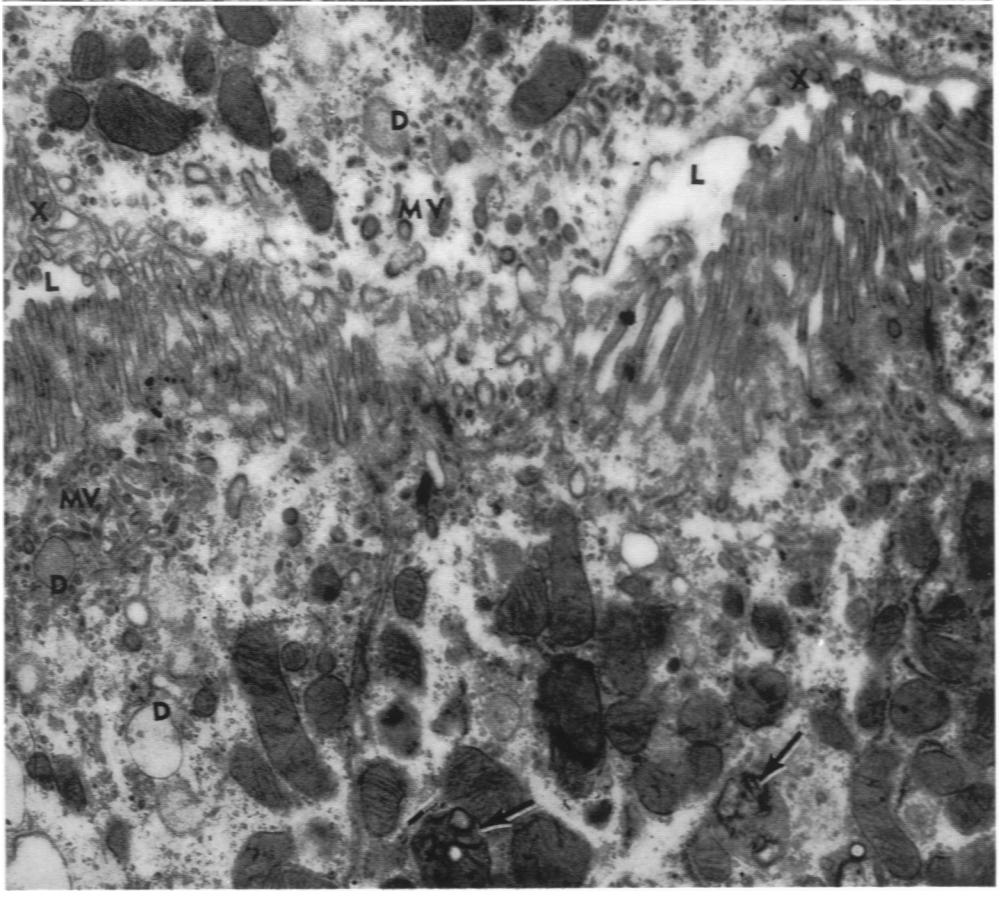

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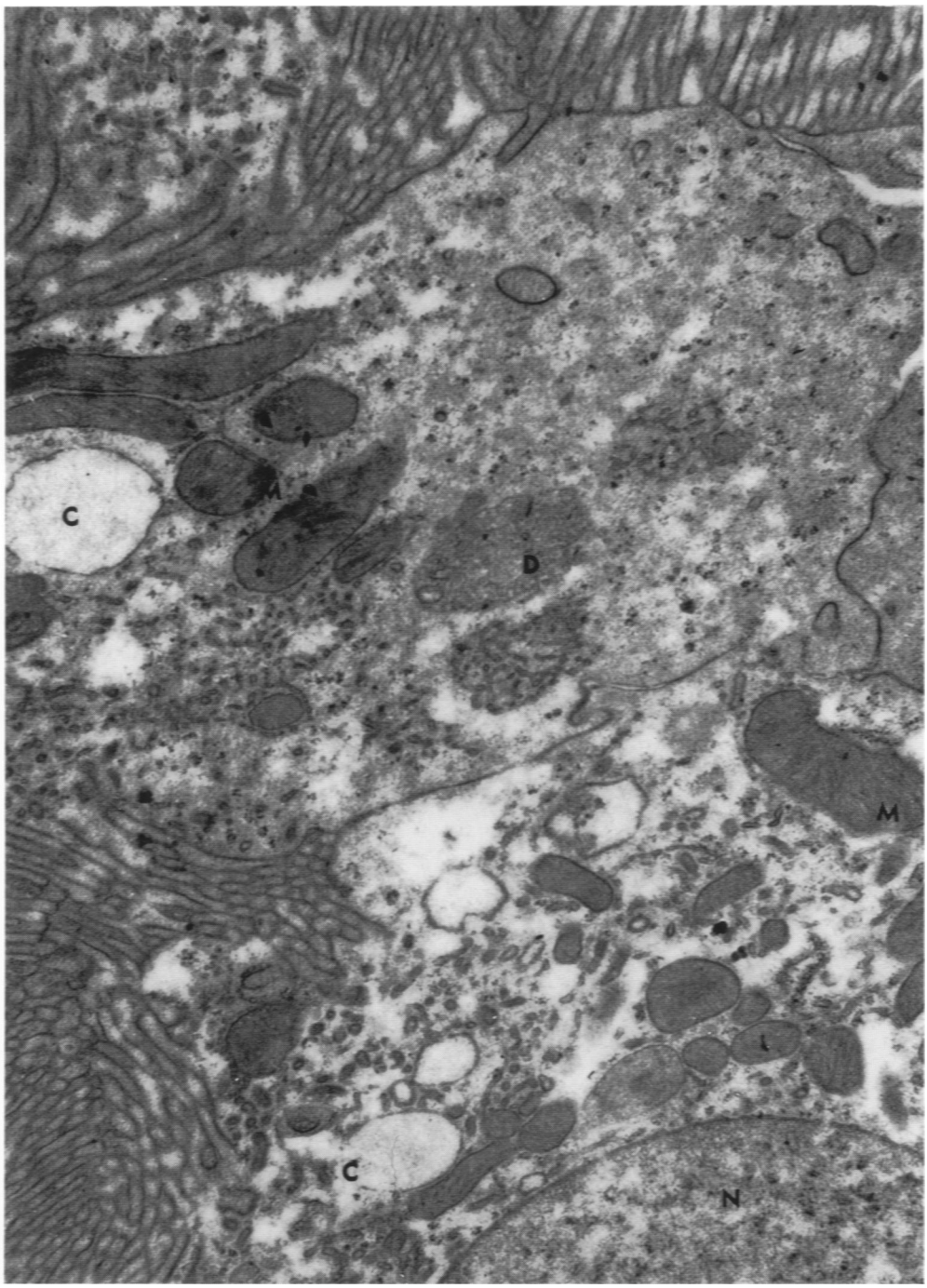

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FIG. 7. A proximal convoluted tubule in a rat that received albumin for 7 days. The central part of the micrograph is occupied by a ballooned portion of apical cytoplasm. The plasma membrane is smooth, and a brush border is not evident in this area. Normal brush border is present at the upper right and the lower left. Several droplets (D) and vacuoles (C) appear in the cytoplasm. Lead acetate stain. × 18,000.

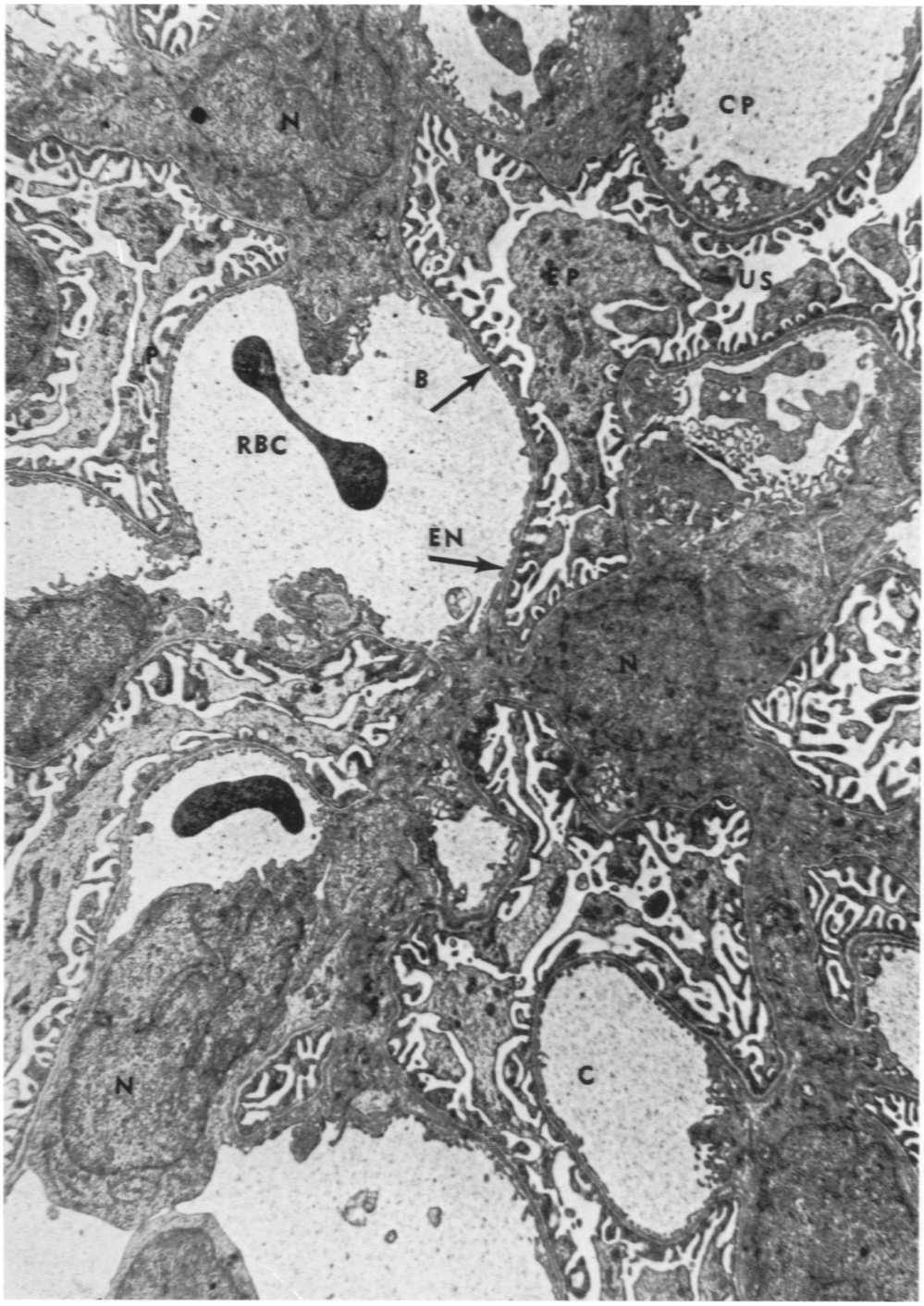

FIG. 8. A glomerulus in a rat that received bovine albumin intraperitoneally for 7 days and then was allowed to recover for 4 days. Proteinuria ceased within the first 24 hours. Droplets are no longer visible in the epithelial cytoplasm and the foot processes are now normal, without evidence of fusion or swelling. × 4,500.