AN ELECTRON MICROSCOPIC STUDY OF TUBULAR LESIONS IN HUMAN KIDNEY BIOPSY SPECIMENS

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Most of the recent studies of the fine structure in renal diseases have emphasized glomerular changes,[1-3] while tubular lesions have received little attention. Practically all clinically significant renal disorders, however, lead to renal tubular degradation which contributes to the biochemical alterations of the associated renal failure. Knowledge of the subcellular basis for this tubular functional impairment is incomplete.

Normal renal tubules have been carefully described,[4,5] and the proximal convoluted tubules have been studied with electron microscopy during obligatory reabsorption of water, glucose, sucrose, dextran and protein.[6-9] Only a few investigations have been directed specifically toward pathologic lesions in renal tubules.[10,11] It was decided, therefore, to survey the changes in renal tubules encountered in a series of kidney biopsy specimens derived from patients with a variety of renal disorders, using electron microscopy.

TABLE I

INCIDENCE OF RENAL DISEASE IN 122 PERCUTANEOUS RENAL BIOPSY SPECIMENS STUDIED BY LIGHT AND ELECTRON MICROSCOPY

Acute glomerulonephritis	22
Chronic glomerulonephritis	16
Nephrotic syndrome	28
Pyelonephritis	4
Idiopathic proteinuria	11
Diabetic nephropathy	5
Lupus nephritis	11
Toxemia of pregnancy	3
Miscellaneous	22
Total	122

MATERIAL AND METHODS

Needle biopsy specimens from 122 patients with a variety of renal disorders were available for light and electron microscopic study (Table I). This material had been treated as follows: One cu. mm. blocks were immediately removed from each of

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the two ends of the specimen, fixed in 2 per cent buffered osmium tetroxide, dehydrated in graded alcohols, and embedded in butyl-methyl methacrylate or in Maraglas.[12] Sections were prepared with glass or diamond knives. Sections cut at 0.5 to 1 μ were stained with hematoxylin and eosin for conventional microscopy; ultrathin sections were examined with an RCA EMU-3 electron microscope. The remainder of the specimen was fixed in neutral buffered formalin and embedded in paraffin. Sections cut at 4 to 6 μ were stained with hematoxylin and eosin and by the periodic acid-Schiff (PAS) method. Other special stains were employed as indicated.

The presentation of data will pertain to the ultrastructural appearance of normal human renal tubules, and to the features of mild acute tubular injury, fatty metamorphosis, proteinuria and hyaline droplets, atrophy, tubular exudation, epithelial desquamation and cast formation.

Observations

Normal Tubules

The proximal convoluted tubules (PCT) frequently contained cellular debris in the lumens. Blebs of cytoplasm which focally obliterated the microvilli were occasionally observed on the lumen surfaces of the tubular epithelium. When such cells ruptured, the remaining cytoplasmic content was loose and partially depleted. All of these alterations were thought to be artifacts of preparation and were not considered to be tubular lesions.

In normal PCT (Fig. 1) the microvilli were compact, measuring about 1 μ in length and 100 mμ in width. Membrane-lined extensions from the intermicrovillous spaces formed a series of narrow cytoplasmic tubules in the subjacent cytoplasm. Some of these were demonstrably continuous with larger channels and vesicles located deeper in the cell. The deeper vesicles measured up to 1 μ in diameter and contained only scanty, finely granular material. Reabsorption and cellular transport have been ascribed to these cytoplasmic vesicles.[6,13] Mitochondria were compact, narrow, and elongated. Convoluted cell membrane infoldings enclosed mitochondria in parallel alignment in the basal portion of the cells. Cytoplasmic transport vesicles occasionally were seen to open onto the surface of the basal membrane infoldings. The cells of the PCT also contained round or oval dense bodies, probably lysosomes,[14] measuring up to 1 μ in size. These had a homogeneous and moderately dense internal matrix, and electron-dense particles were occasionally located inside or were attached to the exterior of an outer, single, limiting membrane. The PCT basement membrane was slightly laminated and averaged 250 mμ in thickness. A thin layer of connective tissue ground substance, scattered collagen fibers, and cytoplasmic strands of fibrocytes surrounded the tubules.

The loop of Henle (HL) was lined by cuboidal or flattened cells (Fig. 2). The distinctive thin portion was lined by squamous cells characterized by pale cytoplasm, few and small dense mitochondria, and

widely separated minute microvillous projections. The nuclei frequently protruded into the lumens. Small Golgi structures were present, and were usually located laterally in the supranuclear portion of the cells. Basal cell membrane infoldings were very sparse. Intercellular junctions were oblique and a terminal bar was frequently present near the lumen. The basement membrane was slightly laminated and comparatively thick, averaging 300 mμ. Capillaries lay close to the tubules, but a very narrow zone of connective tissue or ground substance intervened.

The cells lining the distal convoluted tubules (DCT) had small, widely separated microvilli (Fig. 3). Vesicles were frequently noted in the cytoplasm beneath the microvilli, but were much less prominent than in the PCT. Golgi bodies were numerous near the lateral cell membranes and in the supranuclear area. Numerous dense mitochondria were in parallel arrangement between the basal membrane infoldings. Compact dark cells frequently occurred side-by-side with larger, pale cells in the DCT. The intercellular spaces formed narrow channels about 100 Å wide, and there were numerous interlocking connections between adjacent cells. Along the course of the intercellular spaces between some of the epithelial cells were dilated areas resembling canaliculi. These were lined by cell membranes, from which were numerous small microvillous projections. These occurred at different levels and occasionally opened into a trough between two adjacent cells, thereby communicating with the lumen.

The collecting tubules (CT) were lined by large cuboidal or columnar pale cells (Fig. 4). The cell membrane at the lumen was almost straight, forming only rare microvilli. Mitochondria were sparse, small, dense, and oval in profile. Very few vesicles appeared in the cytoplasm. Golgi bodies were sparse and were usually located close to the lateral cell membrane near the lumen. Basal cell membrane infoldings were rarely present, and intercellular junctions formed straight lines devoid of interlocking connections. These cells frequently contained rounded, dense, osmiophilic, lipid-like bodies (2 to 3 μ) in the supranuclear area.

Acute Renal Tubular Injury

In cases of acute glomerulonephritis, renal injury by drugs, and in severe progressive chronic renal disease, there were found changes in both PCT and DCT believed to represent recent sublethal cell injury. Light microscopy showed that the tubules were the seat of cloudy swelling or hydropic degeneration. Electron microscopically the epithelium was swollen, and the microvilli were often thickened and flattened. Throughout the cytoplasm were numerous distended, intercommunicating vesicles and channels which were mainly responsible for swelling of the entire cell. The vesicles contained only scanty finely granular mate-

rial, and they closely resembled normal transport vesicles except that they were more numerous and larger (Fig. 5). The mitochondria were usually swollen and less dense than normal. In some instances the mitochondrial cristae appeared to have lost their integrity and had undergone fusion, producing osmiophilic deposits of flocculated material within the outer mitochondrial membrane. The basal cell membrane infoldings were diminished or obliterated. Ribonucleoprotein (RNP) granules were decreased in number throughout the cytoplasm, but the nuclei remained normal.

Reabsorption Vesicles and Hyaline Droplets

Hyaline droplets in the PCT cells were observed frequently by light microscopy. They were most abundant in cases of membranous glomerulonephritis with lipoid nephrosis but also occurred in many other renal diseases. In what appeared to be the earliest stages of hyaline droplet formation, electron microscopy showed the cells of the PCT to be strikingly vesiculated and honeycombed (Fig. 6). The vesicles were similar to reabsorption vesicles but were much larger and more numerous. They were lined by a single membrane and were free from internal membranes or cristae. The vesicles were usually round in profile but were sometimes continuous with one another, producing a cavernous network throughout the cytoplasm. Numerous fine, moderately electron-dense granules were present in the vesicles, and larger homogeneous osmiophilic lipid droplets were noted in some. Even with these marked vesicular cytoplasmic changes, the RNP granules and mitochondria usually appeared quite normal.

A later stage in the formation of hyaline droplets was represented by the beginning flocculation of the granular content of the large vesicles, and a still later stage was indicated by larger, compact, and almost homogeneous dense bodies (Fig. 7) still enclosed within membrane-lined vesicles. These dense bodies corresponded in size and distribution to the eosinophilic and PAS-positive hyaline droplets visualized with light microscopy in methacrylate and paraffin sections from the same block.

Abnormal Intracellular Lipid Deposits

Several apparently different forms of lipid accumulation were observed in altered renal tubules. In acute tubular injury, lipid droplets were found in the basal portions of cells of the PCT and DCT. They were homogeneously dense and ranged up to 2 μ in size. The lipid droplets were clearly unrelated to mitochondria. The remainder of the cytoplasm usually showed evidence of acute cellular injury, with swollen mitochondria and distended cytoplasmic vesicles. With light microscopy, these tubules revealed the changes of cloudy swelling and, additionally,

were the seat of vacuolation of the cytoplasm, especially in the subnuclear region, a characteristic of fatty degeneration.

In many atrophic renal tubules the cells contained large droplets of dense osmiophilic material. These droplets measured up to 4 μ in size and sometimes replaced large parts of the cell body. The droplets were usually markedly vacuolated, and occasionally appeared foamy because of an abundance of minute empty vacuoles. Dense, osmiophilic, membranous aggregates were sometimes incorporated within these droplets. The light microscopic appearance of these cells was simply that of marked atrophy with reticulation of the cytoplasm.

A third type of lipid deposition was encountered in the PCT in cases of lipoid nephrosis. In some of the milder examples, the lipid occurred as osmiophilic particles aggregated within cytoplasmic transport vesicles (Fig. 8). Transport vesicles were increased in size and number, but the cells were otherwise unaltered. In some of the more severe lesions, large osmiophilic droplets within vesicles pervaded the cytoplasm (Fig. 9). Usually, these larger droplets were vacuolated and were only partially filled with osmiophilic substance. The remainder of the cytoplasm was reduced in amount, and mitochondria and other organelles were diminished in size and number. Accompanying this marked degree of lipid accumulation in tubular cells in lipoid nephrosis, similar lipid droplets were found in the connective tissue interstices of the surrounding stroma and in the cytoplasm of macrophages (Fig. 10). Interstitial lymphatic capillaries were also increased in prominence and sometimes contained large lipid droplets. Light microscopy in these cases showed swollen foamy cells in the PCT, and foamy macrophages were occasionally visible in the stroma.

Tubular Atrophy

One of the most common and characteristic changes observed in chronic sclerosing glomerulonephritis and other obliterative glomerular diseases with both light and electron microscopy was atrophy of tubular epithelium. This was found in conjunction with both dilated and collapsed tubules (Fig. 11). Several stages of this process were recognized, leading ultimately to the complete disappearance of the tubular epithelium. In earlier stages of atrophy of PCT, microvilli were sparse and shortened and the total amount of cytoplasm was decreased. In DCT and HL, thickened rings of tubular basement membrane enclosed either markedly atrophic cell bodies or scattered remnants of cytoplasm containing lipid particles and RNP granules. Mitochondria were reduced in size and number, and reabsorption vesicles were diminished or absent. RNP granules were compactly arranged in the cytoplasm, while endoplasmic reticulum and Golgi apparatus were rarely recognized in these

atrophic cells. Osmiophilic bodies were increased in prominence, forming clusters of densely stained, finely granular or vacuolated droplets near the lumen surface.

The basement membrane was markedly thickened around atrophic tubules. It measured up to 3 to 4 μ in thickness and was usually laminated. Lipid droplets and remnants of cytoplasm were often incorporated in the thickened basement membrane.

The fibrous tissue surrounding atrophic tubules was increased in amount. It was more compact and contained greater amounts of collagen than normal peritubular connective tissue. These changes resulted in greater than normal separation of the tubular lumen content from peritubular capillaries.

Tubular Epithelitis. With severe tubular epithelial degeneration and atrophy, a common finding was the interposition of neutrophil and eosinophil leukocytes and lymphocytes between the lining epithelial cells of the DCT (Fig. 12). These migratory cells occupied spaces between the basement membrane and the base of displaced epithelium or between adjacent separated epithelial cells. Leukocytes were also frequently observed in the lumens of such affected tubules. At the sites of these wandering cells the normal connections between adjacent epithelial cells were obviously loosened. In some cases the epithelium in the plane of section appeared to have lost its mooring completely, resulting in the formation of large intercellular lakes. Pseudopods of leukocytes protruded between adjacent epithelial cells, indicating active leukocytic migration into or through the epithelial layer.

Tubular Epithelial Detachment and Desquamation. Detached epithelium was observed in the DCT lumens in cases with severe renal injury. This frequently accompanied tubular epithelitis, but it also occurred independently. Epithelium was sometimes attached to the tubular lining only by the tenuous remnant of an intercellular junction. Segments of the basement membrane of some tubules were completely denuded by epithelial detachment. The remaining tubular cells were usually the seat of severe degenerative changes, such as lipid droplet formation, mitochondrial swelling, or cytoplasmic vesiculation. Occasionally, entrapped debris of defunct epithelium remained between the viable lining cells.

The end stage of tubular desquamation was seen in a few severe renal lesions. This consisted of a remnant of partially collapsed tubular basement membrane, without a semblance of lining epithelium. The lumen was usually occupied by osmiophilic debris in which could be recognized cytoplasmic components undergoing degradation.

Tubular Cast Formation. Various stages of cast formation were manifest. In some instances light microscopy showed a loose, granular, eosin-

ophilic debris in dilated segments of the DCT and HL; this was believed to be the earlier stage of cast formation. Electron microscopy of such tubules showed the lumens to contain a mixture of materials, only some of which were identifiable (Fig. 13). Osmiophilic droplets resembling lipid were usually present, and a major component was minute granules about 200 Å in size. Scattered through this granular matrix were cytomembrane fragments and other larger, densely osmiophilic particles. These resembled various cellular organelles undergoing degradation; among these could be recognized nuclei, mitochondria, endoplasmic reticulum and other protoplasmic constituents.

When light microscopy demonstrated the usual appearance of fully developed, homogeneous, eosinophilic, PAS-positive casts, electron microscopy exhibited a degree of resemblance to the earlier stages of cast formation described above. Protoplasmic remnants were, however, usually no longer readily recognizable. Instead, the entire cast was composed of amorphous, finely granular material. Lipid droplets were also usually present, and in some cases fine needle-shaped crystals were observed. In such affected tubules the lining epithelium was markedly atrophic (Fig. 14).

Discussion

Limitations of Morphologic Studies of Renal Tubular Lesions

The fine structural changes of renal tubular injury can be readily defined morphologically, but their effect upon specific tubular and total renal function is highly complex. Tubular function is a composite of the activity in all segments of the renal tubule; in each segment diverse and incompletely known types of activity prevail.[15] Furthermore, the quantitative estimation of the extent of renal tubular fine structural alterations is extremely difficult because of the sampling problem.[16]

Although precise and complete correlation of fine structural changes with total renal function seems impracticable at present, some of the effects of structural alterations upon functional activity of individual cells may be surmised from existing knowledge of cellular dynamics. Renal tubular function can be summarized as conduction, reabsorption, secretion and biosynthesis. The effects produced upon these by intrinsic cellular changes might constitute a favorable direction of inquiry. This approach, however, has certain inherent limitations since the cellular dynamics of normal renal tubular function are only incompletely understood.

Significance of Acute Tubular Epithelial Degeneration and Atrophy

Sublethal cellular injury causes impairment of energy production and work activity in injured cells.[17] In renal tubules this would lead to a re-

duced efficiency of tubular reabsorption, of active transport, and to impaired biosynthesis and secretion, since all of these are energy-requiring activities.[18]

Mitochondrial swelling is known to be an early morphologic expression of acute cell injury.[19,20] While this has been studied in hepatic cells,[21,22] our observations also indicate that mitochondrial swelling in renal tubule cells is an expression of cloudy swelling. Swelling of mitochondria is possibly due to reduction of mitochondrial adenosine triphosphate (ATP), which occurs if oxidative phosphorylation [23] is impaired. Mitochondrial ATP content has been shown to be related to mitochondrial size in other biologic systems.[24]

Distention of cytoplasmic vesicles is another major feature accounting for the swelling of acutely injured cells.[22] The distended vesicles, except for their larger size, are similar to or identical to reabsorption vesicles in normal tubular cells. On the assumption that generated energy is required for the normal transgression of reabsorption vesicles from lumen to base of the cell, an increase in their size and number could be explained by failure of energy production. The relationship of cytoplasmic vesicles in cloudy swelling and reabsorption vesicles in normal tubular cells to the endoplasmic reticulum is not entirely certain. RNP granules were not usually present around the vesicles, nor could we establish a definite continuity of the vesicles with endoplasmic reticulum.

Atrophy of renal tubular cells characteristically occurs in the late and more advanced stages of renal disease. It is an especially important structural lesion because it is, in the main, renal tubular alteration accompanying progressive chronic renal failure. Reduction of the total amount of cytoplasm, mitochondria and cytoplasmic transport vesicles in atrophic cells probably results in decreased intracellular transport and synthesis. These alterations might explain in part the decreased concentrating ability and impaired selective electrolyte conservation which characterize these chronic renal diseases.

In acutely injured cells, as well as in atrophic tubular epithelium, accumulation of lipid droplets characteristically occurs. This may be explained by decreased cellular utilization of lipid normally brought to the cell; or it might be the result of some abnormality of lipid transport, such as that believed to occur in acutely injured hepatic parenchymal cells.[25]

The Pathogenesis of Reabsorption Tubular Lesions

Abnormal accumulation of reabsorbed materials in the PCT has recently been established as the basis for significant pathologic lesions. Reabsorbed sucrose,[18] hemoglobin,[26] protein,[27] and dextran [8] are some of

the substances which have been included in these studies. Glycogen infiltration of renal tubules also is probably based upon increased glucose reabsorption.

These lesions occurred mainly in the PCT, and were characterized by the enlargement and increased number of reabsorption vesicles, in which the reabsorbed material accumulated. Overloading of the reabsorption potential in the PCT appears to be largely responsible,[27] but decreased ability to reabsorb and transport because of intrinsic cell injury and decreased energy production may also play an important role.

In human renal disease the most common tubular reabsorption lesions are those characterized by the formation of hyaline droplets in association with severe proteinuria,[27] and the development of lipid droplets accompanying the lipiduria of nephrosis.[28] The mechanism seems to be the same in these two lesions, namely, overloading of glomerular filtrate with protein, lipid, or both. The stages of development were best illustrated in our material by the formation of hyaline droplets. Early stages consisted of granular debris in the distended reabsorption vesicles, while in later stages the vesicle contents became compact and homogeneous, forming mature hyaline droplets. That this accretion occurred in transport channels was further suggested by the conformity of the dense bodies with circuitous, cavernous, membrane-lined intracellular channels. There now seems to be ample evidence that hyaline droplets form independently of mitochondria.[9,26,29] There was no evidence in our material that they were related to normally occurring dense bodies (probably lysosomes).[26,29]

Lipid droplets in otherwise normal PCT appeared to develop in our cases of nephrotic hyperlipemia in the same way as hyaline droplets. Although histochemical studies for cholesterol were not possible, it is well known that the lipid droplets in the renal tubules in lipoid nephrosis contain abundant cholesterol.[28] This might account for the vacuolation in our cases of lipoid nephrosis, since cholesterol-containing lipid frequently is vacuolated in electron microscopic preparations.[30,31]

The large, pale and yellow gross appearance of the kidney and lipid droplets in tubular epithelium in lipoid nephrosis appear to be the result of a lipid reabsorption phenomenon rather than a form of degeneration signifying cell injury. The presence of lipoid droplets in the interstices of the kidney cortex and in macrophages is a further indication that lipid is being reabsorbed, along with protein, and that this lipid is released first into the connective tissue around tubules before entering the renal capillaries or lymphatics. In some of the more advanced cases of lipid nephrosis, dilated lymphatics were seen in the peritubular tissue. This suggested that in the capillaries of the kidney, as in other parts of

the body,[32] the removal of reabsorbed macromolecules such as albumin and lipoprotein from tissue spaces might depend more upon lymphatics than upon blood capillaries.

Possible Relation of Fine Structural Renal Lesions to Active Transport and Passive Diffusion

Active transport by renal tubular epithelium is only partially understood. The uptake by cell membrane infolding, or pinocytosis, is believed to be one of these mechanisms,[33,34] and to be highly active in the PCT. Impairment of transport resulting from failure of vesicles to be carried across the cell has already been discussed in connection with reabsorption defects.

Another vital cellular mechanism of active transport is that mediated by a molecular carrier system.[35,36] This also represents a form of work activity, and must be susceptible to impairment of energy production by acute and chronic cell injury. At the present time there is no known morphologic counterpart to either the normal or the pathologic activity of the molecular carrier systems.

Passive diffusion is believed to be a significant form of renal tubular transfer of water and solutes.[37] More recently, it has been suggested that the thin segment of HL plays a major role in this type of tubular adjustment,[38,39] and it has been proposed that gradients of osmolar concentration within the peritubular interstitial tissue determine the restoration of isotonic urine in the HL. The normal structure of this part of the tubule is ideally suited for this function. The minutely narrow layer of epithelial cytoplasm comprises a thin protoplasmic membrane, comparable to capillary endothelium and to the pulmonary alveolar epithelial lining, across which passive diffusion of fluid, solutes and gases readily occurs. The simplicity of the cytoplasm, sparsity of mitochondria, and absence of reabsorption vesicles further attest to the probability of a major element of passive diffusion in this part of the tubule. Whether diffusion occurs across the cytoplasm or between the cells is uncertain. Most observers seem to favor the idea that intercellular junctions are water-tight and that diffusion occurs across the cytoplasm.[40,41] This question needs further study.

In the DCT we have observed the presence of small canaliculus-like areas between epithelial cells. These contained microvillous projections and were located at various levels from base to apex of the epithelium. They appeared to open into intercellular troughs at the apical cell level. The significance of the intercellular channels is not known, but their position and configuration strongly suggest that they may represent the open form of interlocking processes of the cell membrane. They could comprise a labile excretory canalicular system resembling bile canaliculi.

Further study of these structures is required before their physiologic and pathologic significance can be determined.

In renal tubular lesions many fine structural abnormalities occur in the epithelium lining the tubules in the HL, DCT, and CT which might affect passive diffusion. In atrophic tubular cells, the thinned cytoplasm might more readily permit passive diffusion. Or the wide intercellular gaps resulting from tubular epithelitis and desquamation of lining cells might increase diffusion, or lead to passive diffusion in portions of the tubules where it normally does not occur. The basement membrane itself may possibly influence diffusion. Basement membranes elsewhere in the body, however, appear to be readily permeable to water and solutes of small molecular size.[42] If this also prevails in renal tubules, even marked thickening of the basement membrane, a common finding in chronically injured and atrophic tubules, may have little effect upon diffusion of water and solutes. On the other hand, the basement membrane does appear to cause the partial retention of macromolecular substances.[43] This might explain the retention of lipid droplets observed in the thickened basement membranes of atrophic tubules.

Another effect of renal disease upon diffusion across tubules may be related to the connective tissue increase around atrophic and otherwise injured tubules. The intimate relationship of the base of the renal tubular epithelium to the adjacent capillary wall is well shown electron microscopically. In diseases where this connective tissue layer is increased in thickness, it is only logical to suppose that diffusion might be delayed or otherwise impaired.

The Concept of Tubular Exudation

The migration of leukocytes across epithelial membranes is by no means a new concept. The presence of lymphocytes and other wandering cells within the intestinal [44] and endometrial epithelium,[45] and in the lumens of these organs is well known. Stages of the migration of leukocytes into and across the epithelial lining in inflammatory diseases of the intestinal mucosa has also been observed.[46] A similar process is relatively common in severe renal tubular injury, and we have referred to this process as tubular epithelitis. It must be concluded that leukocytes are able to migrate through the tubular basement membrane, perhaps in a manner similar to their penetration of the capillary basement membrane in inflammation.[47,48] Once leukocytes gain entrance into the epithelial layer, they extend pseudopods into intercellular spaces, separating the cells from one another. This process is probably related to cellular desquamation into the tubules, but desquamation also occurs in the absence of exudation.

Although leukocytes in the urine in renal disease are derived partly

from glomerular exudation, our studies indicate that another probable source is tubular trans-epithelial leukocytic migration.

Mechanism of Formation of Tubular Casts

The source of the inspissated material comprising tubular casts has not been completely elucidated.[49] The evidence from our study is not conclusive, but it does provide some clues. Mixed cellular debris was frequently seen in the tubular lumens of the PCT, while in lower tubules in the same specimen occasional cytoplasmic remnants were found in fully-formed casts. Thus, tubular casts may be produced by gradual inspissation of lipid, protein, and polysaccharide debris from degenerating, desquamating epithelium and from leukocytes gaining entrance into the lumens. The background of fine granules present in casts is probably derived from unresorbed glomerular filtrate protein.

Mechanisms of Tubular Injury

The wide diversity of types of renal diseases in this series suggests the presence of common denominators in the expression of various types of cell injury. None of the alterations described can be designated as characteristic of a specific disorder. Rather, it appears that a limited number of morphologic responses to cell injury may be called into play by a variety of causes.

Reasoning from the nature of the underlying renal disease, it is surmised that tubular cell injury in most of our cases was due to ischemia resulting from obliterative or obstructive glomerular lesions. This is particularly the case in cloudy swelling, fatty degeneration, atrophy, cellular desquamation and cast formation. Hyaline droplet and some forms of fat droplet accumulation, on the other hand, may be ascribed to increased glomerular permeability. It is apparent also that tubular injury may be induced directly by chemical agents and by infection, as well as by other means.

It can be concluded that, for the most part, the recognition of tubular lesions in the kidney has little bearing on the recognition of the underlying renal disease, but determination of the extent of renal tubular damage may have value in prognosis. Furthermore, an understanding of the fine structural background of tubular lesions should aid considerably in the analysis of the intricate problems of renal insufficiency.

SUMMARY

A survey of the fine structural changes seen in the renal tubular epithelium in 122 specimens from individuals with various types of acute and chronic renal disease has been presented.

Acutely injured cells were marked by a diminution or loss of microvilli, an increase in cytoplasmic transport vesicles, mitochondrial swelling, and by the presence of cytoplasmic lipid droplets. Altered reabsorption of protein and lipid, encountered especially in nephrosis, was manifested by the accumulation of protein-like material and lipid, respectively or jointly, within the cytoplasmic transport vesicles in the proximal convoluted tubules. Accumulation of lipid in macrophages in the interstitial tissue was also observed in nephrosis.

In chronic, severe renal disease atrophic tubules were found to have either collapsed or dilated lumens. The atrophic cells exhibited reduction in the total amount of cytoplasm and organelles; microvilli were reduced in size and number. These changes were accompanied by thickening of the basement membrane, cytoplasmic lipid droplets and an increased amount of peritubular fibrous tissue. Various degrees of detachment and desquamation of the tubular epithelial cells were noted in the loops of Henle and distal convoluted tubules. Migration of leukocytes through the tubular epithelium between adjacent cells occurred in some severely injured tubules.

Early stages of cast formation were evidenced by fine granular debris and degenerating fragments of cellular organelles, leukocytes and epithelial cells filling tubule lumens. In later stages, dilated tubules were lined by atrophic cells and casts were composed almost entirely of granular or amorphous material.

References

1. Farquhar, M. G.; Vernier, R. L., and Good, R. A. An electron microscopic study of the glomerulus in nephrosis, glomerulonephritis, and lupus erythematosus. *J. Exper. Med.*, 1957, **106**, 649–660.
2. Spiro, D. The structural basis of proteinuria in man. Electron microscopic studies of renal biopsy specimens from patients with lipid nephrosis, amyloidosis, and subacute and chronic glomerulonephritis. *Am. J. Path.*, 1959, **35**, 47–73.
3. Churg, J.; Mautner, W.; Grishman, E., and Eisner, G. M. Structure of glomerular capillaries in proteinuria. *Arch. Int. Med.*, 1962, **109**, 97–115.
4. Pease, D. C. Fine structures of the kidney seen by electron microscopy. *J. Histochem.*, 1955, **3**, 295–308.
5. Rhodin, J. Anatomy of kidney tubules. *Internat. Rev. Cytol.*, 1958, **7**, 485–534.
6. Caulfield, J. B., and Trump, B. Correlation of ultrastructure with function in the rat kidney. *Am. J. Path.*, 1962, **40**, 199–218.
7. Maunsbach, A. B.; Madden, S. C., and Latta, H. Light and electron microscopic changes in proximal tubules of rats after administration of glucose, mannitol, sucrose, and dextran. *Lab. Invest.*, 1962, **11**, 421–432.
8. James, J. A., and Ashworth, C. T. Some features of glomerular filtration and permeability revealed by electron microscopy after intraperitoneal injection of dextran in rats. *Am. J. Path.*, 1961, **38**, 515–525.

9. BREWER, D. B., and EGUREN, L. M. The fine structure of protein droplets in proximal convoluted tubule of the mouse kidney. *J. Path. & Bact.*, 1962, **83**, 107–116.

10. JACKSON, J. D.; SMITH, F. G.; LITMAN, N. N.; YUILE, C. L., and LATTA, H. The Fanconi syndrome with cystinosis: electron microscopy of renal biopsy specimens from five patients. *Am. J. Med.*, 1962, **33**, 893–910.

11. DALGAARD, O. L., and PEDERSEN, K. J. Some Observations on the Fine Structure of Human Kidney Biopsies in Acute Anuria and Osmotic Diuresis. Ciba Foundation Symposium on Renal Biopsy. WOLSTENHOLME, G. E. W., and CAMERON, M. P. (eds.). Little, Brown & Co., Boston, 1961, pp. 330–353.

12. FREEMAN, J., and SPURLOCK, B. A new epoxy embedment for electron microscopy. *J. Cell Biol.*, 1962, **13**, 437–443.

13. TRUMP, B. F., and JANIGAN, D. T. The pathogenesis of cytologic vacuolization in sucrose nephrosis. An electron microscopic and histochemical study. *Lab. Invest.*, 1962, **11**, 395–411.

14. DE DUVE, C. Lysosomes, a New Group of Cytoplasmic Particles. In: Subcellular Particles. HAYASHI, T. (ed.). Ronald Press Co., New York, 1959, pp. 128–159.

15. FORSTER, R. P. Kidney Cells. In: The Cell: Biochemistry, Physiology, Morphology. BRACHET, J., and MIRSKY, A. E. (eds.). Academic Press, New York and London, 1961, Vol. 5, Specialized Cells, pp. 132–149.

16. SOMMERS, S. C.; ROBBINS, G. B.; BABIN, D. S., and KNAACK, C. T. Chronic pyelonephritis, renal tubular atrophy and hypertension. *Arch. Int. Med.*, 1962, **110**, 505–510.

17. CHRISTIE, G. S., and JUDAH, J. D. Mechanism of action of carbon tetrachloride on liver cells. *Proc. Roy. Soc., London, s.B*, 1954, **142**, 241–257.

18. LEAF, A., and RENSHAW, A. The anaerobic active ion transport by isolated frog skin. *Biochem. J.*, 1957, **65**, 90–93.

19. GANSLER, H., and ROUILLER, C. Modifications physiologiques et pathologiques du chondriome. Étude au microscope électronique. *Schweiz. Ztschr. allg. Path.*, 1956, **19**, 217–243.

20. STONE, R. S.; BENCOSME, S. A.; LATTA, H., and MADDEN, S. C. Renal tubular fine structure; studied during reaction to acute uranium injury. *Arch. Path.*, 1961, **71**, 160–174.

21. OBERLING, C., and ROUILLER, C. Les effets de l'intoxication aiguë au tétrachlorure de carbone sur le foie de rat; étude au microscope électronique. *Ann. anat. path.*, 1956, **1**, 401–427.

22. ASHWORTH, C. T.; LUIBEL, F. J.; SANDERS, E., and ARNOLD, N. Hepatic cell degeneration: Correlation of fine structural with chemical and histochemical changes in hepatic cell injury produced by carbon tetrachloride in rats. *Arch. Path.*, 1963, **75**, 212–225.

23. FONNESU, A., and SEVERI, C. Oxidative phosphorylation in mitochondria from livers showing cloudy swelling. *J. Biophys. & Biochem. Cytol.*, 1956, **2**, 293–299.

24. PACKER, L. Metabolic and structural states of mitochondria. I. Regulation by adenosine diphosphate. *J. Biol. Chem.*, 1960, **235**, 242–249.

25. RECKNAGEL, R. O.; LOMBARDI, B., and SCHOTZ, M. C. A new insight into pathogenesis of carbon tetrachloride fat infiltration. *Proc. Soc. Exper. Biol. & Med.*, 1960, **104**, 608–610.

26. MILLER, F. Hemoglobin absorption by the cells of the proximal convoluted tubule in mouse kidney. *J. Biophys. & Biochem. Cytol.*, 1960, **8**, 689–718.

27. OLIVER, J.; MACDOWELL, M. C., and LEE, Y. C. Cellular mechanisms of protein metabolism in the nephron. I. The structural aspects of proteinuria, tubular absorption, droplet formation and disposal of proteins. *J. Exper. Med.*, 1954, **99**, 589–604.

28. ALLEN, A. C. The Kidney, Medical and Surgical Diseases. Grune & Stratton, New York, 1962, p. 280.

29. STRAUS, W. Rapid cytochemical identification of phagosomes in various tissues of the rat and their differentiation from mitochondria by the peroxidase method. *J. Biophys. & Biochem. Cytol.*, 1959, **5**, 193–204.

30. GEER, J. C.; MCGILL, H. G., JR., and STRONG, J. P. The fine structure of human atherosclerotic lesions. *Am. J. Path.*, 1961, **38**, 263–287.

31. LEVER, J. D. Electron microscopic observations on the adrenal cortex. *Am. J. Anat.*, 1955, **97**, 409–429.

32. DRINKER, C. K., and FIELD, M. E. Lymphatics, Lymph and Tissue Fluid. Williams & Wilkins Co., Baltimore, 1933, pp. 34–45.

33. LEWIS, W. H. Pinocytosis. *Johns Hopkins Hosp. Bull.*, 1931, **49**, 17–26.

34. PALADE, G. E. Transport *in quanta* across the endothelium of blood capillaries. (Abstract) *Anat. Rec.*, 1960, **136**, 254.

35. HOKIN, M. R., and HOKIN, L. E. The role of phosphatidic acid in transmembrane transport. *Lab. Invest.*, 1961, **10**, 1151–1161.

36. FRANCK, J., and MAYER, J. E. An osmotic diffusion pump. *Arch. Biochem.*, 1947, **14**, 297–313.

37. ROBINSON, J. R. Reflections on Renal Function. Charles C Thomas, Springfield, Ill., 1954, p. 27.

38. LAMDIN, E. Mechanisms of urinary concentration and dilution. *Arch. Int. Med.*, 1959, **103**, 644–671.

39. SMITH, H. W. The Kidney. Structure and Function in Health and Disease. Oxford University Press, New York, 1951, p. 245.

40. PEACHEY, L. D., and RASMUSSEN, H. Structure of the toad's urinary bladder as related to its physiology. *J. Biophys. & Biochem. Cytol.*, 1961, **10**, 529–553.

41. ROBERTSON, J. D. The ultrastructure of cell membranes and their derivatives. *Biochem. Soc. Symposia*, 1959, No. 16, 3–43.

42. BENNETT, H. S. Morphological aspects of extracellular polysaccharides. *J. Histochem.*, 1963, **11**, 14–23.

43. FARQUHAR, M. G., and PALADE, G. E. Segregation of ferritin in glomerular protein absorption droplets. *J. Biophys. & Biochem. Cytol.*, 1960, **7**, 297–304.

44. ANDREW, W., and COLLINGS, C. K. Lymphocytes within the cells of intestinal epithelium in man. *Anat. Rec.*, 1946, **96**, 445–457.

45. BARTELMEZ, G. W., and BENSLEY, C. M. Human Uterine Gland Cells. In: Special Cytology. COWDRY, E. V. (ed.). Paul B. Hoeber, Inc., New York, 1932, ed. 2, vol. 3, pp. 1525–1563.

46. ASHWORTH, C. T., and CHEARS, W. C., JR. Follow-up of intestinal biopsy in nontropical sprue after gluten-free diet and remission. *Fed. Proc.*, 1962, **21**, 880–890.

47. MARCHESI, V. T., and FLOREY, H. W. Electron micrographic observations on the emigration of leukocytes. *Quart. J. Exper. Physiol.*, 1960, **45**, 343–348.

48. MAJNO, G., and PALADE, G. E. Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J. Biophys. & Biochem. Cytol.*, 1961, **11**, 571–605.

49. MCQUEEN, E. G. The nature of urinary casts. *J. Clin. Path.*, 1962, **15**, 367–373.

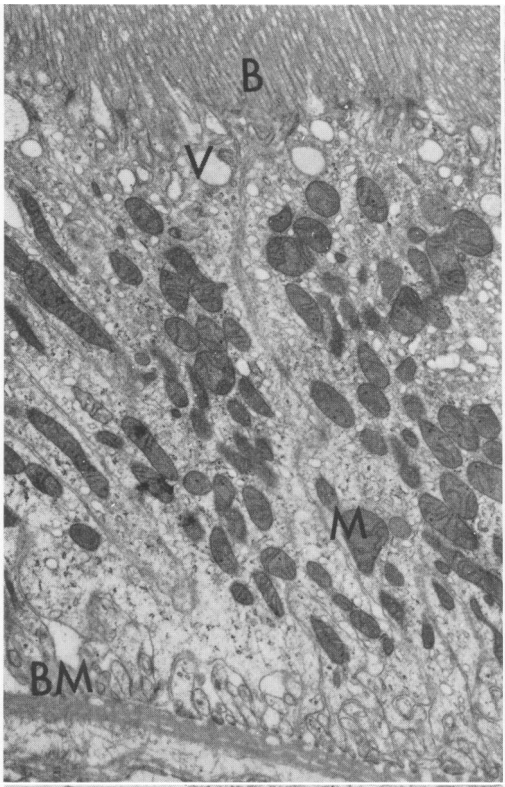
Legends for Figures

Fig. 1. Normal proximal tubular cell with brush border (B), mitochondria (M), transport vesicles (V) and basement membrane (BM). × 6,000.

Fig. 2. Normal cells in the thin portion of Henle's loop with lumen (L), thin unspecialized cytoplasm (C) and thin stroma (S) separating the lumen from a capillary (Cap). × 5,000.

Fig. 3. Junction of two normal cells in the distal convoluted tubule (DCT) with mitochondria (M), nucleus (N). There is a prominent intercellular "lake" (L). × 11,000.

Fig. 4. Normal collecting tubular cells with nucleus (N) and mitochondria (M). × 7,000.



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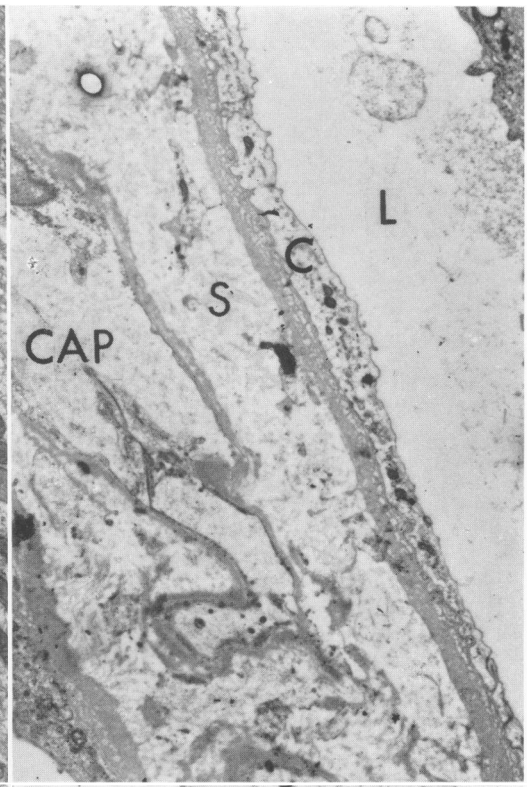

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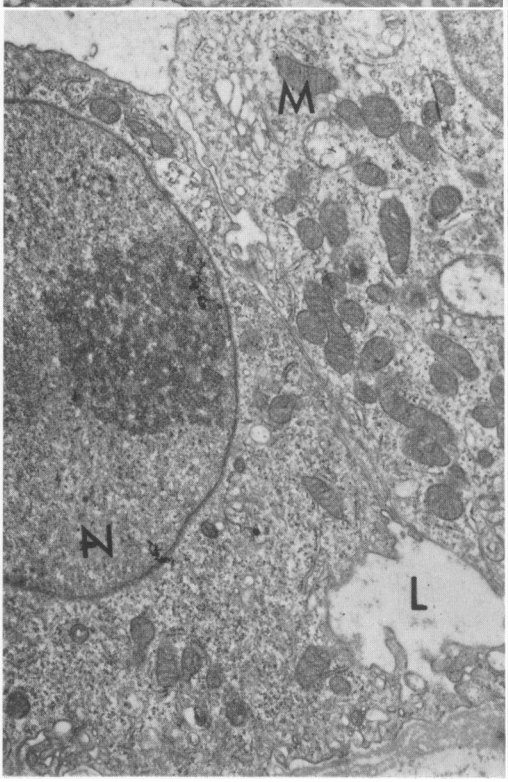

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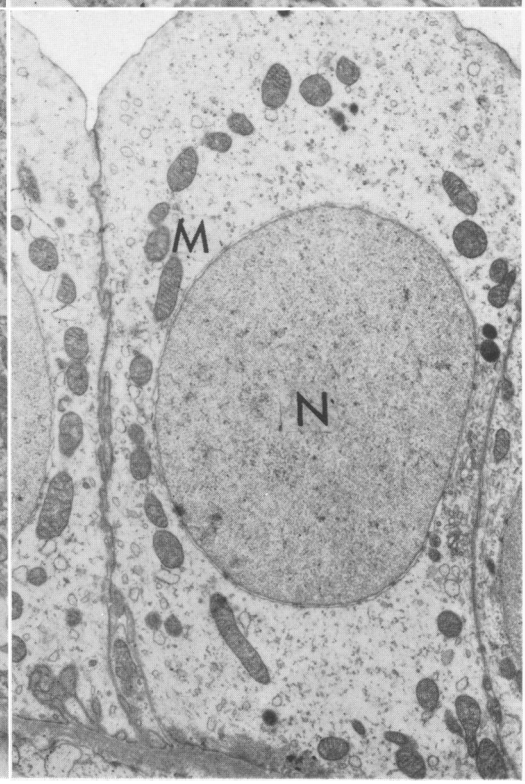

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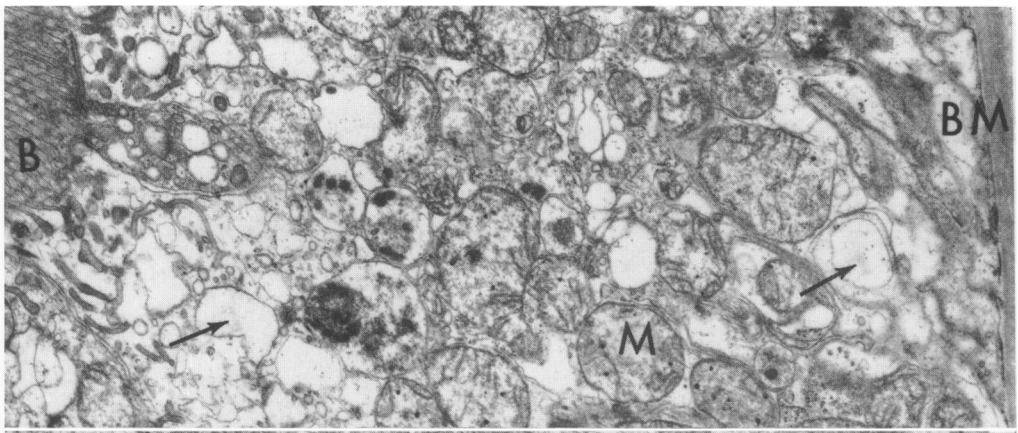
FIG. 5. Proximal tubular cell in acute sublethal injury. Mitochondria (M) are swollen and transport vesicles (arrows) dilated. Brush border (B) is on the left and basement membrane (BM) on right. × 12,000.

FIG. 6. Proximal tubular cell with early hyaline droplet formation. Transport vesicles (V) are filled with dense material. Mitochondria (M) are essentially normal. Basement membrane (BM) is on the right. × 12,000.

FIG. 7. Proximal tubular cell in a late stage of hyaline droplet formation. Brush border (B) is on left. The interconnecting transport vesicles (V) are filled with dense material. Mitochondria (M) are slightly swollen. × 14,500.



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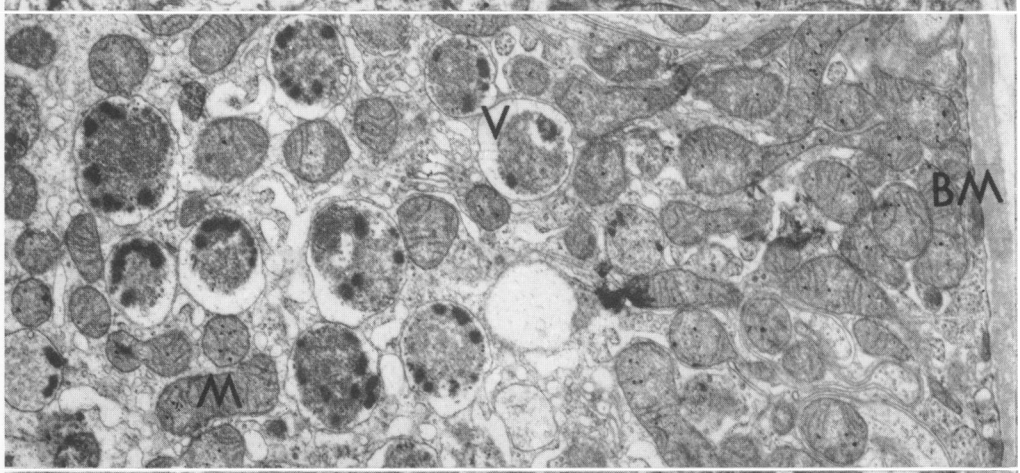

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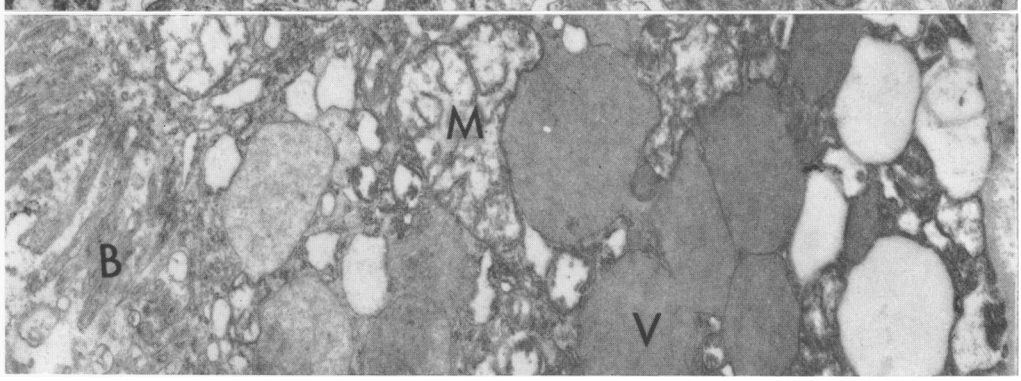

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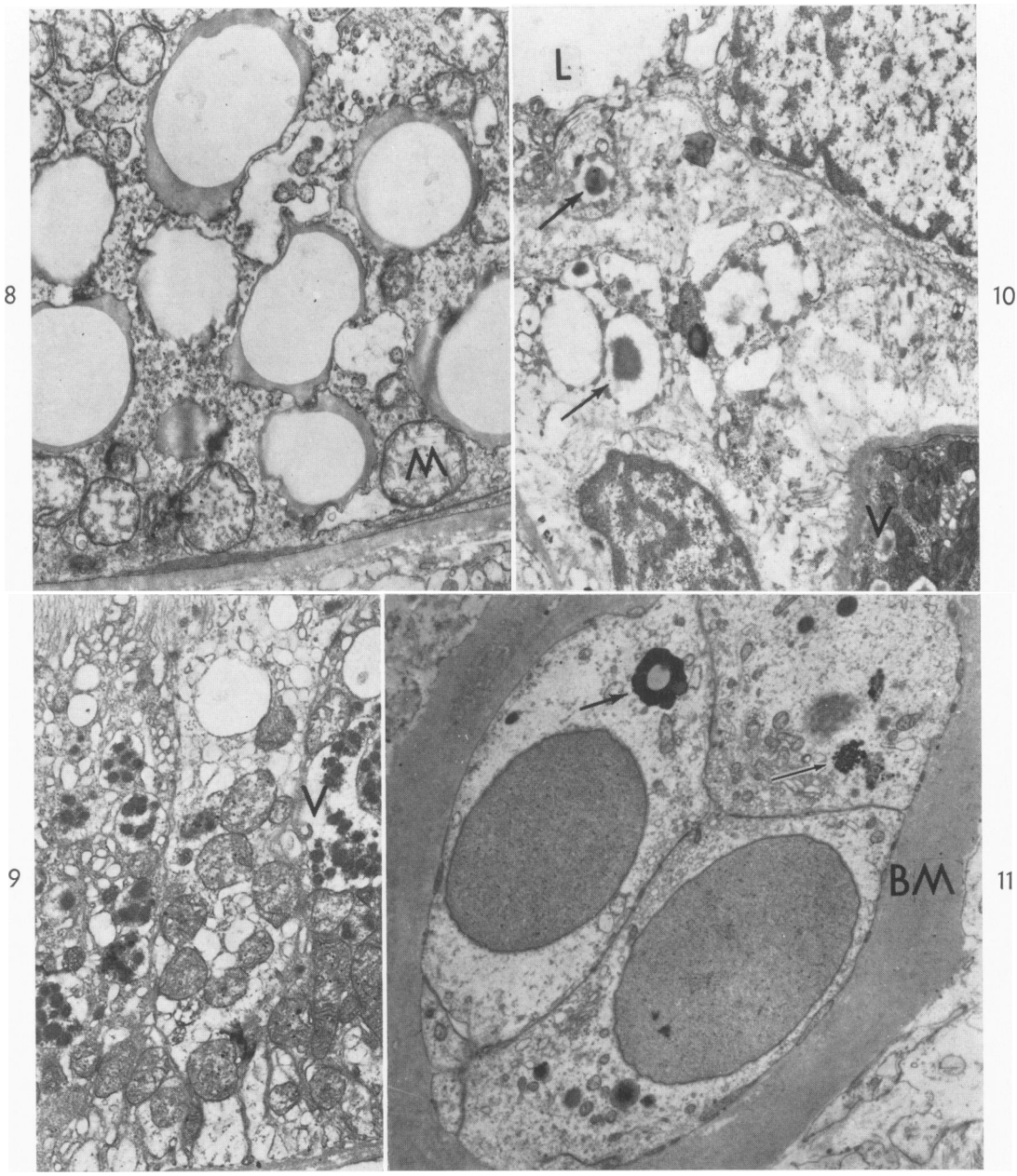

FIG. 8. Proximal tubular cell contains reabsorbed lipid in distended reabsorption vesicles. Mitochondria (M) are swollen. × 10,000.

FIG. 9. Proximal tubular cell with reabsorbed lipid in transport vesicles (V). × 10,000.

FIG. 10. Lipid droplets (arrows) appear in the cytoplasm of a stromal interstitial cell adjacent to a lymphatic (L). Lipid in tubular cytoplasmic vesicles can be seen at the lower right (V). × 9,000.

FIG. 11. Atrophic tubule with markedly thickened basement membrane (BM) and collapsed lumen. Lipid droplets (arrows) can be seen in the cytoplasm of the atrophic cells. × 7,000.

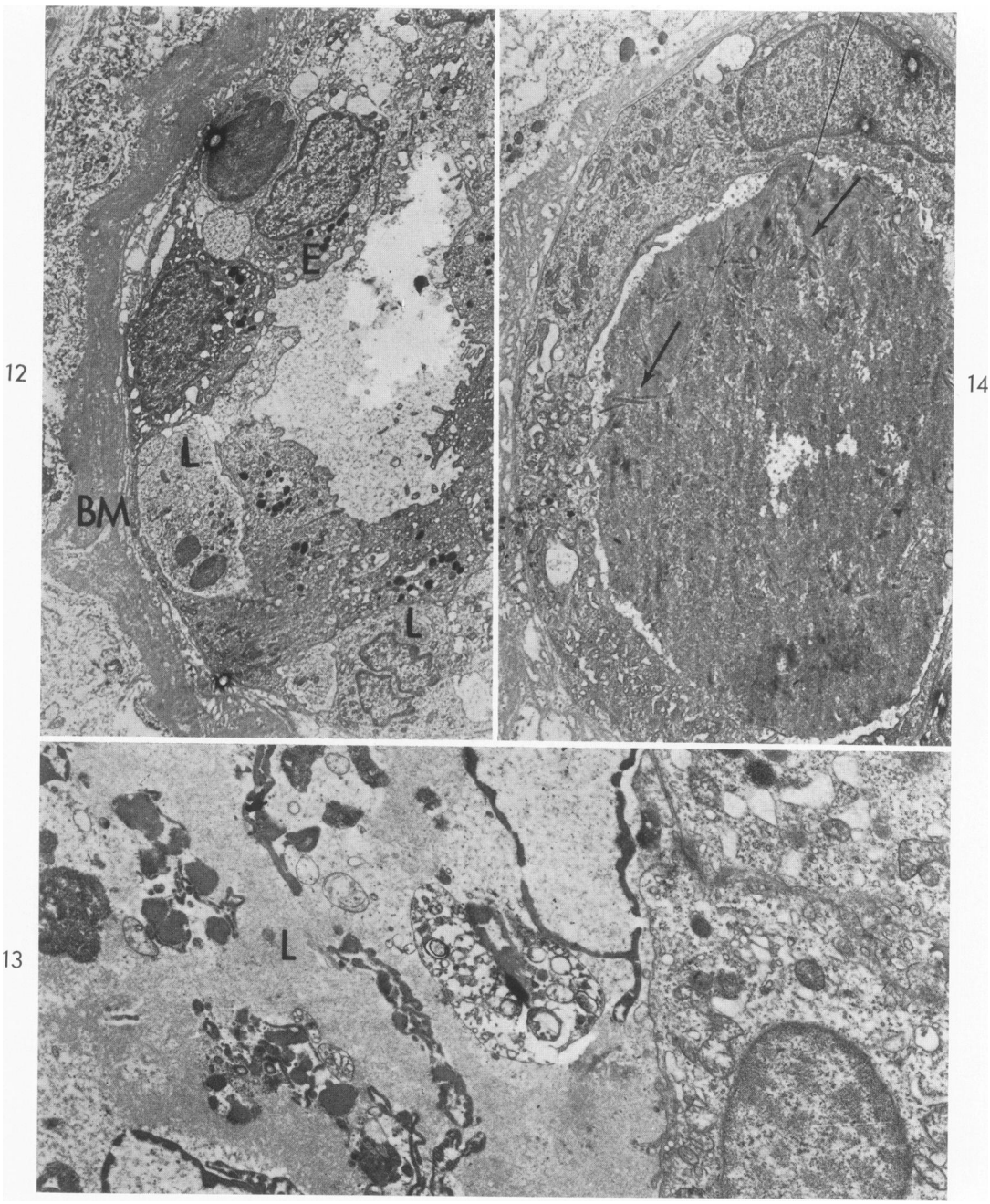

FIG. 12. A tubule contains migrating leukocytes (L). The basement membrane (BM) is thickened and the tubular epithelium (E) is partially detached. × 4,000.

FIG. 13. Wall of a tubule in an early stage of cast formation. Granular debris, densely osmiophilic clumps and organelle remnants appear in the lumen (L). × 10,000.

FIG. 14. Late stage of cast formation. A granular amorphous cast fills a dilated lumen. There are atrophy and increased cytoplasmic density of tubular cells. Needle-shaped crystals (arrows) can be seen in the cast. × 5,000.