Acute Hyperuricemic Nephropathy in Rats

An Electron Microscopic Study

Jerry Waisman, MD, Lawrence M. Mwasi, MSc, Rodney Bluestone, MD, MRCP, and James R. Klinenberg, MD

Hyperuricemia and uricosuria were induced in rats fed uric acid and oxonic acid. Kidneys then were studied by light and electron microscopy. After 1 day of hyperuricemia, animals had deposits of uric acid and urate crystals within collecting tubules of the renal papillae, and tubular cells were altered. By 10 days, there was an exudative response with further injury to epithelium. Clear spaces within lumens, epithelium, and neutrophils suggested the presence of crystals; however, there was no direct ultrastructural evidence that neutrophils or epithelial cells ingested crystals and suffered injury. Presumably, crystals readily seen in frozen, unfixed tissue were lost during preparation for electron microscopy. Nonetheless, the ultrastructural findings indicated that hyperuricemic nephropathy was initiated in a fashion analogous to urate arthropathy. Urate crystals formed within collecting tubules, epithelial cells were altered, and most likely there was chemotaxis of neutrophils which underwent degranulation and vacuolation followed by lysis freeing any ingested urate. Release of ingested crystals plus precipitation of new crystals both might serve to sustain the nephritis. (Am J Pathol 81:367–378, 1975)

A MODEL FOR HYPERURICEMIC NEPHROPATHY originally was described by Stavric, Johnson, and Brice,[1] and we enlarged the observations.[2] In these experiments young rats were fed a standard laboratory chow supplemented by uric acid and a related compound, oxonic acid. The latter inhibits hepatic uricase, thus preventing the degradation of uric acid,[3] and rats fed the diet for 3 or 4 weeks became hyperuricemic. They had extreme uricosuria and developed nephropathy with acute and chronic inflammation and tophi. Amorphous deposits and acicular crystals in the kidney corresponded to uric acid and monosodium urate, respectively.[4] The renal lesion was maximal in the papilla, where the collecting

From the Departments of Pathology and Medicine, University of California, Los Angeles, School of Medicine; The Medical and Research Services, Veterans Administration, Wadsworth Hospital Center; and Cedar-Sinai Medical Center, Los Angeles, California.

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Address reprint requests to Dr. Jerry Waisman, Department of Pathology, UCLA Center for the Health Sciences, Los Angeles, CA 90024.

tubules appeared to be the primary site of uric acid and urate deposits. After intervals of 9 to 12 months on the diet, almost all animals had uric acid stones in the urinary tract along with a chronic interstitial nephritis.[5,6]

This series of experiments demonstrated a practical model for hyperuricemic nephropathy and for uric acid stone disease with an obvious potential for therapeutic studies on the related conditions. However, the experiments provided little information about the initiation of renal disease in hyperuricemia. For this purpose, morphologic studies (including electron microscopy) were performed after 1 to 10 days of experimental hyperuricemia, and the results are reported herein.

Materials and Methods

Twenty-two male Wistar rats weighing 150 g or more were allowed to eat freely of a daily dietary mixture consisting of 19 g of standard laboratory chow, 0.4 g oxonic acid, and 0.6 g uric acid. The daily intake of food was determined while the animals were allowed water *ad libitum*. Groups of 2 to 4 from these 22 animals were killed after 1, 2, 4, 7, 8, and 10 days. Seven comparable control rats that were daily offered 20 g of the standard laboratory chow were killed 2, 4, 7, or 10 days after initiation of the experiment. Table 1 indicates the exact number of rats in each group along with the diet and interval before death.

In all animals the body weight was recorded before death, and venous blood was obtained for an enzymatic determination of the plasma level of uric acid. The right kidney, taken post mortem, was processed in the manner described in a prior publication to reveal the amount of uric acid per gram of wet tissue.[7] The left kidney was perfused with a 1.5% phosphate-buffered solution of glutaraldehyde, weighed, and processed for routine light microscopy as well as electron microscopy. For the latter, 1-mm cubes of renal papillae were postfixed in osmium tetroxide, dehydrated in graded alcohol solutions and passed through propylene oxide into an epoxy resin. One-half-micron-thick sections of the plastic-

Table 1—Data From Acutely Hyperuricemic Rats

Days on diet	No. of rats	Mean body weight (g)	Mean kidney weight (g)	Mean uric acid		Mean tubular exudate	Mean tubular deposits
				Plasma (mg%)	Kidney (mg/g)		
Oxonic acid and uric acid							
1	3	158	0.6	11.0	1.4	0	1
2	5	188	0.9	5.8	2.5	0.4	1.6
3	1	228	1.1	8.0	8.7	0	1
4	5	194	1.0	4.1	3.0	0	1.4
7	2	238	1.3	3.3	4.6	1.5	2.5
8	4	205	1.1	6.5	6.5	1	1.5
10	2	233	1.4	8.5	4.8	2	2
Chow							
2	1	222	0.9	1.2	0.2	0	0
4	1	228	1.0	0.8	0.4	0	0
7	1	242	0.9	0.6	0.9	0	0
8	3	221	0.6	1.6	0.5	0	0
10	1	236	0.8	0.2	0.2	0	0

embedded tissue were stained with a modified toluidine blue solution; ultrathin sections were stained with uranyl acetate and lead citrate. For routine microscopy, sections of paraffin-embedded tissue were stained with hematoxylin and eosin. In these preparations the amount of exudate and other deposits in collecting tubules were estimated separately; zero represented the absence of a finding; one, minimal changes; two, intermediate changes; and three, the maximal abnormality.[2]

Results

The results of biochemical studies and postmortem examination are summarized in Table 1. There was no difference in the body weight of the rats in any group regardless of diet or length of the experiment, nor did kidney weights differ between the groups. The plasma uric acid level was elevated significantly ($P < 0.05$) in the rats fed oxonic acid and uric acid, while the amount of urate eluted from the right kidneys of hyperuricemic rats was increased ($P < 0.05$). Both plasma and renal elevations of uric acid were observed in rats killed after 1 day of oxonic acid and uric acid and at all subsequent intervals through 10 days.

Table 1 also summarizes the light microscopic findings. Amorphous and laminated deposits were observed within the collecting tubules of the renal papillae of hyperuricemic rats throughout the experiment (Figure 1). These appeared basophilic and corresponded to the site of uric acid and urate deposits as determined in prior experiments by the examination of frozen, unfixed tissue or tissue fixed in alcohol.[2] Many collecting tubules were dilated and lined with a flat epithelium. With the exception of 1 animal, an exudative response was not observed by routine microscopy until the seventh day of hyperuricemia, when neutrophils were present within blood vessels of the renal papillae, the adjacent interstitium, and the collecting tubules (Figure 2). The inflammatory response was associated with destruction of epithelial cells, and regeneration of the epithelium was suggested by infrequent mitotic figures. Mitotic figures also were observed in tubular lining cells prior to the appearance of exudate. Tophi occasionally were seen in renal papillae after 2 days of hyperuricemia; however, no interstitial fibrosis was detected at any time through 10 days. Arteries, arterioles, and glomeruli appeared normal in all hyperuricemic rats, and no renal lesions were observed in the rats fed the standard laboratory chow.

The observations by electron microscopy were similar to those of light microscopy. After 1 or 2 days of hyperuricemia, collecting tubules were dilated (Figure 3). Two common findings in tubules at this interval were expanded intercellular and intracellular spaces and intraluminal membranous remnants (Figure 4). Basilar structures filled with membranes were also present. Most of the cellular spaces contained fine

granules with few clear areas suggesting a dissolved crystal. Although inconspicuous, neutrophils could be found within blood vessels of the papillae. Some neutrophils were caught in the process of diapedesis (Figure 5), while others showed margination within small blood vessels.

Many collecting tubules were filled with cellular debris and other matter corresponding to the basophilic deposits evident by light microscopy (Figure 6), and intraluminal spaces between the debris vaguely outlined shapes suggesting dissolved crystals. In dilated tubules, the lining cells were compressed and had scant microvilli. Collecting tubules with this appearance were common after 2, 3, or 4 days of hyperuricemia.

The exudative response was prevalent after intervals of 7, 8, or 10 days of hyperuricemia with neutrophils present in blood vessels, interstitium, and collecting tubules. Migration of neutrophils through the epithelium of collecting tubules was indicated by the presence of the leukocytes in this layer (Figure 7). Within collecting tubules there were neutrophils in all stages of degranulation and degeneration (Figure 8). Some were vaculoted, and partly lysed neutrophils contained angular clear spaces indicating prior crystalline deposits. In tubules there was considerable distortion of the epithelial cells with large spaces within or between them. Many lysosomes, large and small, were present (Figure 9); however, phagolysosomes with angular clear spaces were not found.

As seen in the electron microscope, the renal papillary interstitium was expanded in hyperuricemic rats, suggesting edema. Interstitial cells were conspicuous, with extensive processes, many complex lipid bodies, and an abundant endoplasmic reticulum.

Discussion

Hyperuricemic nephropathy in human subjects is thought to result from an excess of uric acid in the glomerular filtrate with the precipitation of amorphous uric acid deposits in collecting tubules. The precipitation is caused by supersaturation of the urine with uric acid and is exaggerated by acidification of the urine within the medulla of the kidney. The intraluminal deposits lead to obstructive nephropathy which may be complicated by acute pyelonephritis. Often, the tubules are destroyed by deposits, leading to an interstitial reaction with urate crystals and giant cells creating a tophus, and urinary tract stones of uric acid are common. The ultimate result of hyperuricemia and uricosuria may be a chronic lesion characterized by small scarred kidneys with pyelonephritis, vascular sclerosis, and extensive ischemic injury; for example, up to 40% of patients with gout may have significant chronic renal disease.

Renal lesions similar to those of hyperuricemic nephropathy in human

patients have been observed in experimental circumstances. In addition to nephropathy produced by oxonic acid [1,2] and avian models of hyperuricemia,[8] acute urate nephropathy has been induced in several species (including dogs) by the infusion of lithium urate.[9] In these experiments, the primary lesion is the precipitation of uric acid deposits and urate crystals within collecting tubules. Epithelial cells are injured, and exhibit signs of necrosis and repair. In circumstances such as the oxonic acid model where hyperuricemia is sustained, the animals develop an acute and chronic nephritis comparable in morphology to human gouty nephropathy.[5]

The mechanism whereby the precipitation of uric acid and urates within collecting tubules leads to an acute and chronic nephritis remains to be clarified. In part the injury may be mechanical; however, there is considerable evidence that urate crystals mediate cell injury and inflammation by other means. In human joint spaces, urate crystals are ingested by neutrophils and by mononuclear cells, including some resembling synovial lining cells.[10,11] *In vivo* experimental studies in dogs reveal that synovial lining cells ingest urate crystals injected into knee joints.[12] Subsequently, neutrophils localize in the region of the joint and exhibit degranulation and lysis; some neutrophils from the joint fluid contain urate crystals. Many of the phagocytic neutrophils from joint fluids lack granules, and some have a vacuolated (honeycomb) appearance. Recent *in vitro* electron microscopic studies indicate that human neutrophils and dogfish leukocytes rapidly ingest monosodium urate crystals.[13-16] Within neutrophils the crystals are enclosed by a single membrane constituting a phagosome, and eventually the phagosome is converted into a phagolysosome containing at least one hydrolase, acid phosphatase. There is dissolution of the membrane about the phagolysosome and release of contents into the cytoplasmic matrix leading to lysis of the neutrophil itself. The urate crystal is thereby free to initiate injury in other cells.

Our observations upon the early renal changes associated with hyperuricemia seem to agree with the findings in urate-induced arthritis and in urate-treated neutrophil suspensions. Thus, rats made hyperuricemic by oxonic acid immediately develop uric acid deposits and urate crystals within the collecting tubules of their kidneys. The deposits may be recognized as gross yellow striations in the renal medulla. By light microscopy the crystals may be observed in fresh-frozen tissue or tissue fixed in absolute alcohol; however, routine preparations utilizing aqueous fixatives dissolve most of the crystals, leaving a residue of basophilic material. Routine electron microscopy introduces the same problem. Uric acid and urate are relatively soluble in the various fixatives and buffers re-

quired during the procedure, and all that remains in the collecting tubules is granular and membranous debris. Empty spaces in this debris signify substances removed during the preparation of the tissue, and some empty spaces are angular suggesting prior crystalline deposits.

The presence of urate crystals within the collecting tubules of hyperuricemic rats immediately is associated with alterations in the structure of epithelial cells lining these tubules. By light microscopy, some cells contain mitotic figures and have abundant basophilic cytoplasm indicating repair. An added finding by electron microscopy is the presence of many intercellular and intracellular spaces containing granules and membranous debris. A few such spaces are clear, with contours suggesting prior crystalline deposits. Lacking direct evidence we can only suppose that renal epithelial cells like synovial cells ingest urate crystals, are injured, and thereby contribute to the next phase of the lesion which is acute inflammation. The means by which neutrophils are attracted to the renal papilla are not clear, but neutrophils appear after crystals are observed and epithelial cells altered. Neutrophils are seen first at the margins of blood vessels, then pass through the vessels and into the interstitium. Only after several days are large numbers of neutrophils present in the tissue, filling and distorting collecting tubules. We are unable to demonstrate crystals within neutrophils, but the progressive vacuolation, degranulation, and lysis of neutrophils follows the pattern of urate-induced injury observed in joint fluids or isolated cell suspensions. Furthermore, a few vacuoles in degenerate neutrophils contain clear spaces which vaguely resembly crystalline profiles. We cannot explain why the acute inflammatory response to urate crystals is delayed for several days in kidneys when it is not in joints.

In conclusion: Our ultrastructural findings in acutely hyperuricemic rats indicate that uric acid and urate deposits initiate injury in the lining cells of renal collecting tubules. This is followed by an exudative inflammatory reaction which further injures the renal tissue and contributes to a chronic renal lesion similar to hyperuricemic nephropathy in human subjects.

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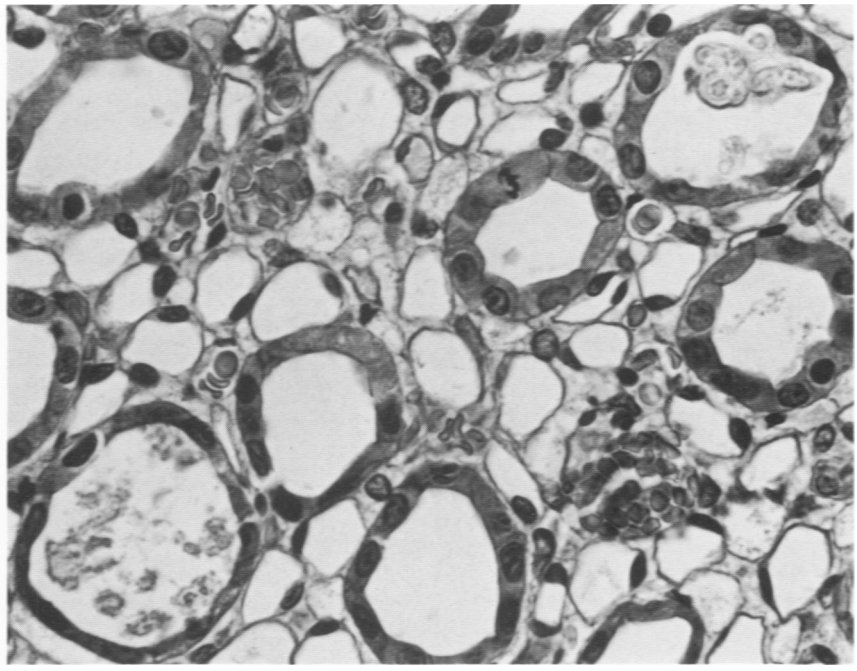


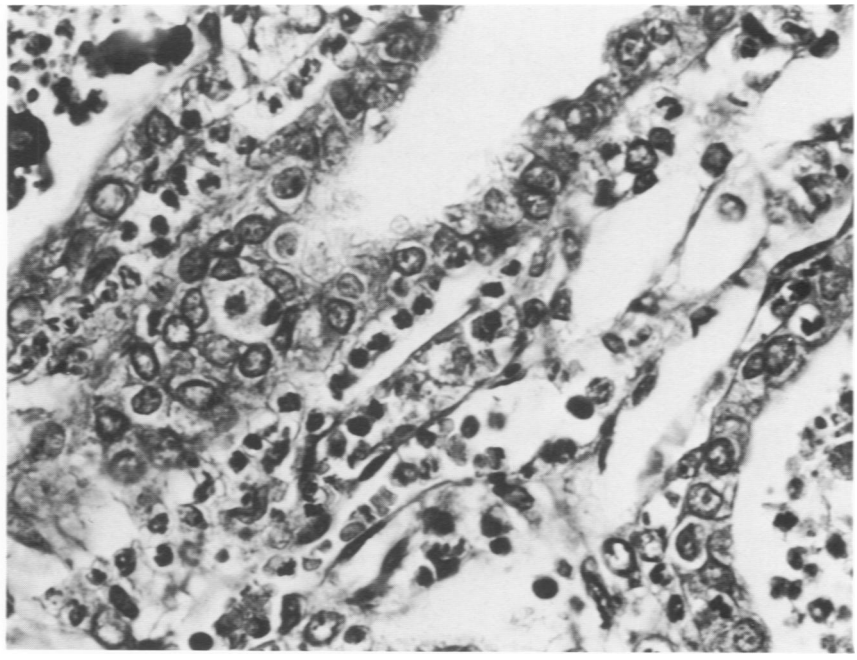
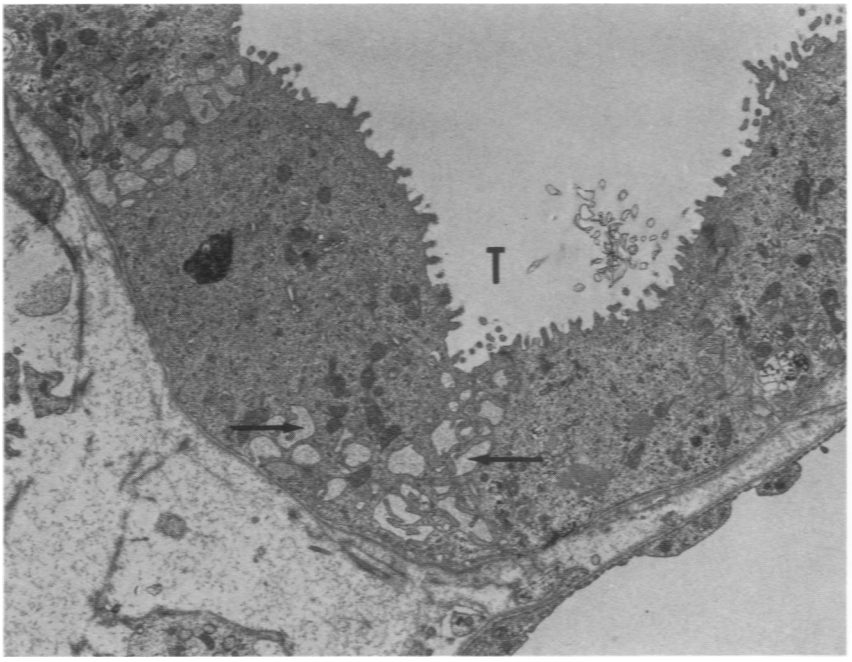


Figure 1—Dilated collecting tubules in the renal papilla from a rat with hyperuricemia for 2 days contain granular debris and laminated concretions. A mitotic figure is present in a collecting tubule. (H&E, × 680) **Figure 2**—After 7 days of hyperuricemia, there is acute inflammation with neutrophils throughout the tissue (H&E, × 680)



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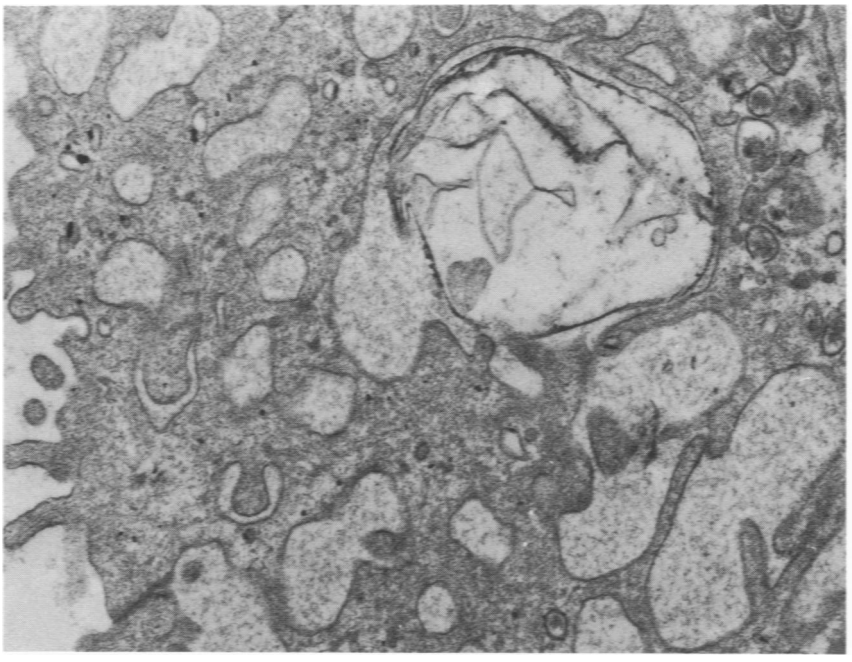

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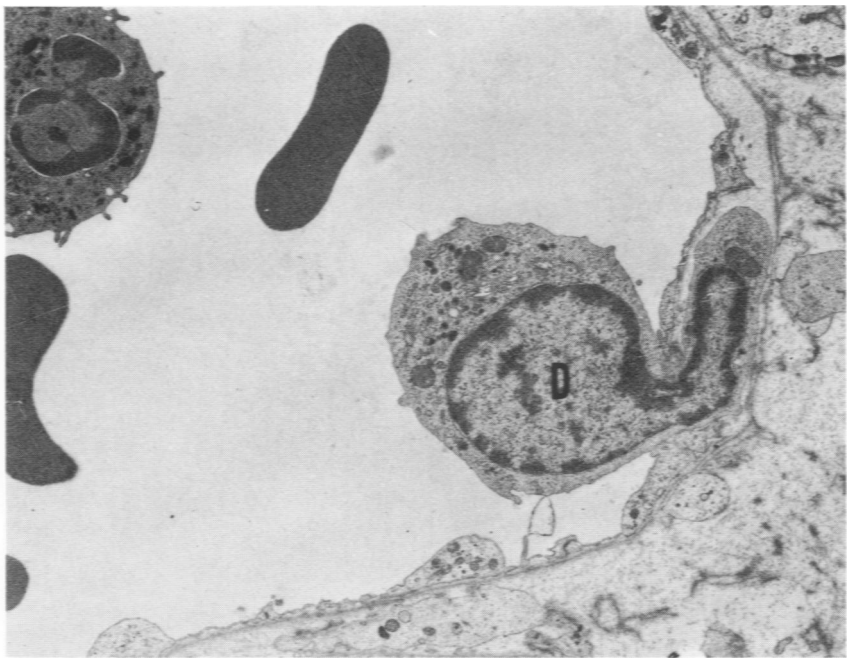
Figure 3—Dilated collecting tubule (*T*) from the same area shown in Figure 1. Expanded intercellular and intracellular spaces are indicated by *arrows*. (Uranyl acetate and lead citrate, × 5700) **Figure 4**—Same animal, same area as Figure 3; membranous swirls are present in a dilated space (Uranyl acetate and lead citrate, × 27,600).



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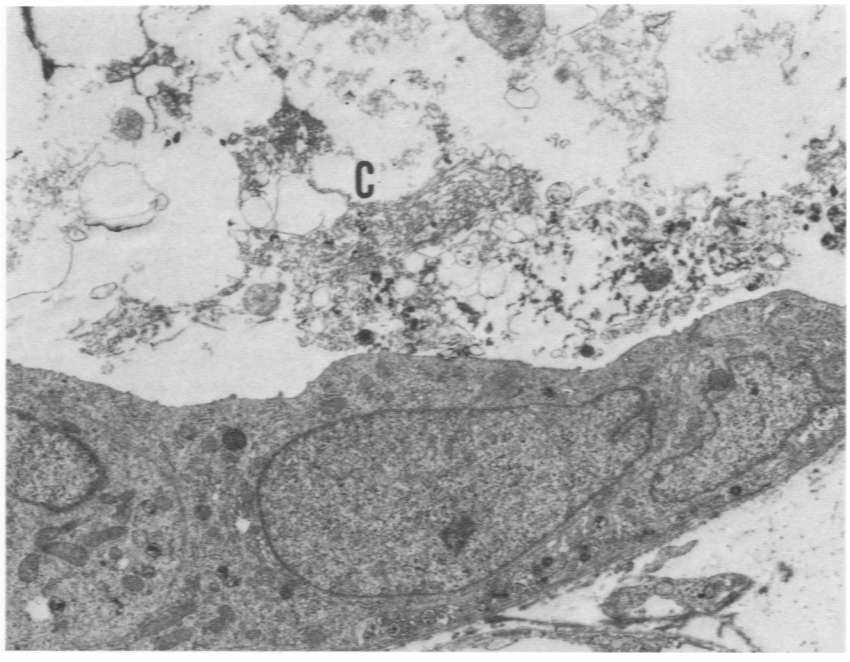

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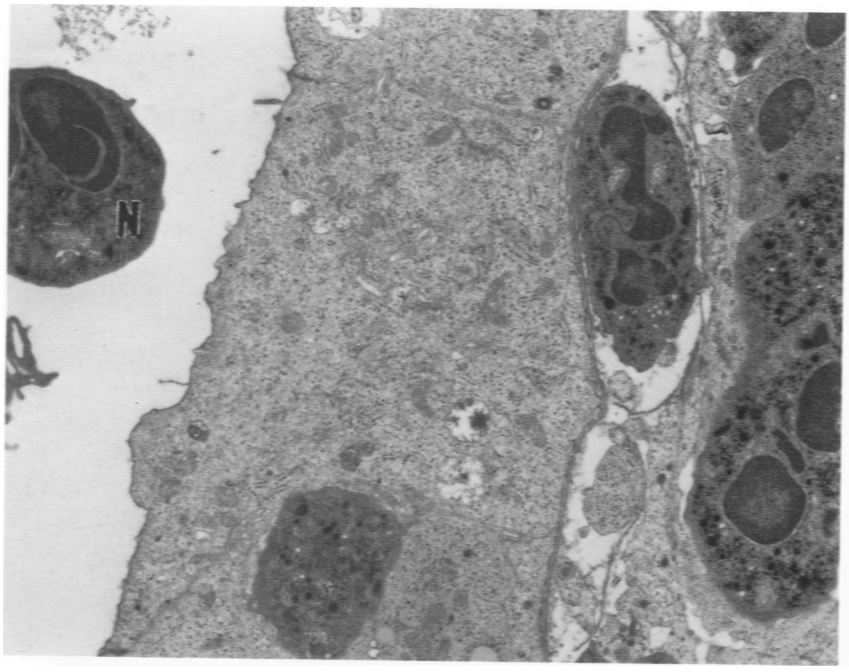
Figure 5—Papillary blood vessel from same area as preceding figure. One leukocyte is trapped in the process of diapedesis (*D*); another lies within the lumen (Uranyl acetate and lead citrate, × 5700). **Figure 6**—Dilated collecting tubule after 4 days of hyperuricemia. Luminal spaces outlined by debris suggest crystals (*C*) (Uranyl acetate and lead citrate, × 5700).



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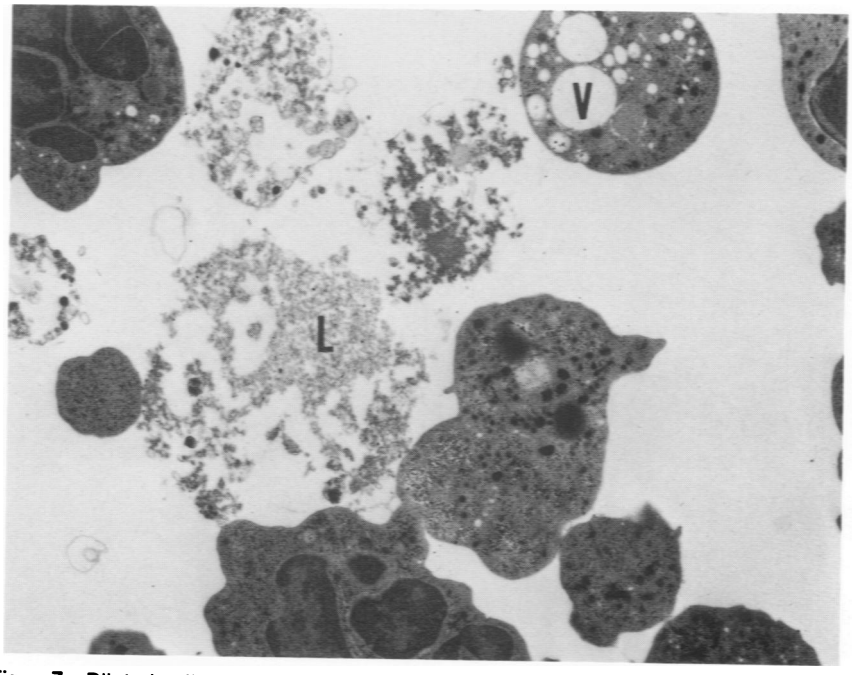

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Figure 7—Dilated collecting tubule after 10 days of hyperuricemia contains a neutrophil (*N*). Leukocytes are present in the epithelium, interstitium, and a blood vessel (Uranyl acetate and lead citrate, × 5700). **Figure 8**—Dilated collecting tubule filled with neutrophils, same animal as Figure 7. One leukocyte is vacuolated (*V*); others lysed (*L*). (Uranyl acetate and lead citrate, × 5700)

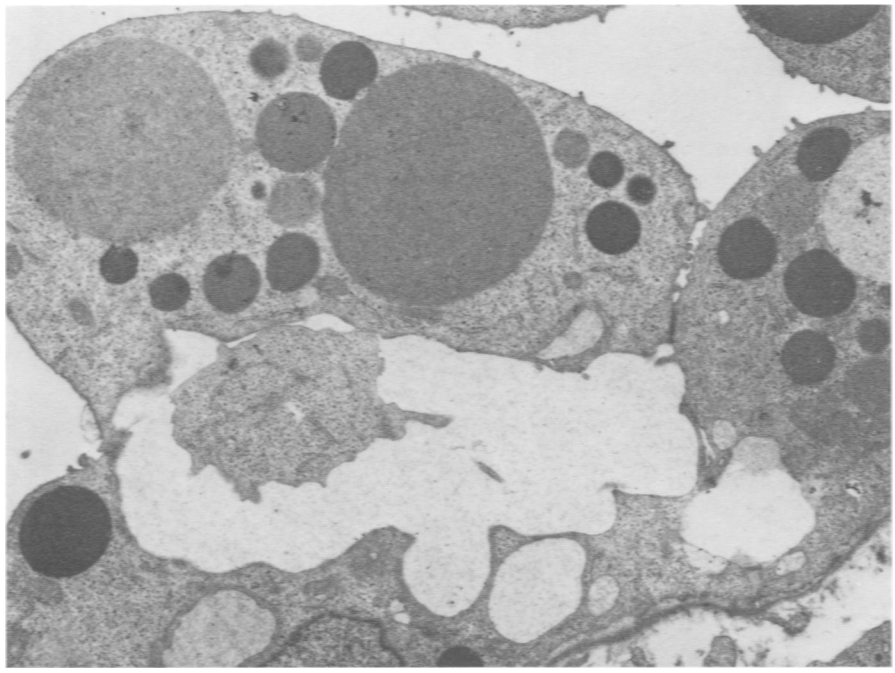

Figure 9—Distorted collecting tubule from same animal and area as Figures 7 and 8. Epithelial cells contain many lysosomes. (Uranyl acetate and lead citrate, × 5700).