THE KIDNEY IN EXPERIMENTAL MAGNESIUM DEPRIVATION

A Morphologic and Biochemical Study

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Experimental magnesium deprivation has been vigorously studied in recent years. Among the manifestations of the hypomagnesemic state is extensive calcification superficially resembling that found in hypercalcemic states with principal involvement of cardiac muscle, skeletal muscle and kidney. Modern techniques have been used increasingly to investigate the renal calcification induced by hypervitaminosis D,[1] hyperparathyroidism and calcium gluconate injections.[2] These studies have emphasized the role of local factors in calcification, particularly the role of the cell, its organelles and its regional matrices. Significant differences have been shown to exist, among the different forms of calcification, particularly from the ultrastructural standpoint. The present report is concerned with the nephrocalcinosis of magnesium deficiency as studied by several techniques. It emphasizes and amplifies the differences by comparing the lesions with descriptions of other forms of nephrocalcinosis and with the myocardiopathy of magnesium deprivation.[3]

Material and Methods

Male Wistar rats, weighing 100 to 150 gm, were fed a synthetic diet containing 0.5 to 0.7 mg magnesium per 100 gm. The composition of the diet is shown in Table I. Control pair-fed animals received an identical diet to which 65 mg magnesium per 100 gm was added. Magnesium-free distilled water was provided to both groups *ad libitum*. Four experimental and 2 control animals were sacrificed each week for 6 weeks and blood removed by aortic puncture. Tissues taken from each major organ were fixed in formalin or buffered 5 per cent glutaraldehyde for histologic examination. One mm cubes of renal cortex and medulla were fixed in cold veronal-buffered 1 per cent osmium tetroxide or chilled 5 per cent cacodylate-buffered glutaraldehyde at pH 7.3 and embedded in Epon. Aldehyde-fixed material was post-osmicated before

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embedding. Thin sections stained with toluidine blue were examined by light microscopy to select fields for electron microscopy. Grids stained with lead hydroxide were examined with an RCA EMU 3F electron microscope. Glutaraldehyde-fixed slices of kidney were sectioned in a cryostat and stained for acid phosphatase (AcPase)

TABLE I

Composition of the magnesium-deficient diet	
Casein, acid-washed	30.0 gm %
Dextrose	48.0 gm %
Sodium bicarbonate	1.2 gm %
Potassium bicarbonate	1.5 gm %
Corn oil	15.0 gm %
Vitamin diet fortification mixture	2.15 gm %
Salt mixture	2.15 gm %
Composition of the salt mixture	
Calcium phosphate, monobasic	66.0 gm %
Calcium chloride	27.2 gm %
Ferrous citrate	5.82 gm %
Copper sulfate.$5H_2O$	0.06 gm %
Zinc sulfate.$7H_2O$	0.12 gm %
Manganese sulfate.H_2O	0.80 gm %

according to the method of Gomori.[4] AcPase staining for electron microscopy was accomplished by the Sabatini, Bensch and Barrnett method [5] in selected animals of the experimental and control groups. Cryostat sections of fresh and fixed kidney were stained for ionic calcium by the glyoxal method [6] and the ash was examined after micro-incineration.

In a second group of rats mitochondria were isolated from kidney homogenates by differential centrifugation in sucrose.[7] Total AcPase activity, was assayed by the method of Huggins and Talalay [8] in portions of the homogenate after repeated freezing and thawing to release lysosomal enzymes. Total calcium and magnesium were determined in sera and in isolated mitochondria with a Perkin-Elmer atomic absorption spectrophotometer, Model 214.

RESULTS

Typical signs of magnesium deficiency in the rat appeared after 4 to 6 days on the deficient diet. These included pronounced transient hyperemia of the ears, snout and foot pads and increasing irritability. After 3 weeks patchy alopecia, particularly at the base of the ears and neck, was noted. During this period occasional animals developed fatal audiogenic convulsions. At the end of the 5-week period most of the experimental animals had lost weight and had loose diarrheal stools. Because it was clear that this would cause imbalance in electrolytes other than calcium and magnesium, no attempt was made to carry the experiment beyond the sixth week.

Light Microscopy. No microscopic abnormalities were noted in the parathyroid glands. The earliest alteration in the kidney was swelling

of tubular epithelium, most severe in the pars recta of the proximal convoluted tubules (Fig. 1). In the early stages it was limited mainly to the apical pole of the cell and later involved most of the cytoplasm. Coincidental with the swelling, increased amounts of intracellular calcium were demonstrated by the glyoxyl reaction and by micro-incineration. Foci of renal calcification appeared in all magnesium-deficient animals in the first week, but were particularly prominent in the third and fourth weeks. The deposits were located near the cortico-medullary junction (Fig. 2). Most concretions appeared to be intraluminal in location (Fig. 3) although small intracellular and interstitial deposits were occasionally visible.

Cellular degeneration, proliferation and atrophy, as well as mononuclear cell infiltration were conspicuous around the calcium casts and at this time enlargement and clumping of lysosomes could be seen in focal areas of the nephron in sections stained for AcPase (Fig. 8a). No abnormalities were observed in the glomeruli, blood vessels or medulla.

Electron Microscopy. The earliest lesion was characterized by cellular swelling presumably the result of hyperhydration. All segments of the nephron were affected, although the pars recta of the proximal convoluted tubules were more severely involved. Even in severely swollen cells, the preservation of organelles, notably mitochondria, was remarkable (Figs. 4 and 5). Very early lesions were focal within the cell and in the proximal tubular epithelium were characterized by focal effacement of brush borders. The membranous canaliculi that prolong the brush border into the apical cytoplasm often showed disorganization and clumping (Fig. 6).

Particularly noticeable after 3 weeks on the deficient diet were focal increases in the size and number of lysosome-like bodies within the proximal tubular epithelium (Fig. 7). Many of these structures appeared to be forming at the apex of the cell (phagosomes) and were pale. Those deeper within the cell had contents that varied from flocculent deposits to irregular membranous arrays. Electron microscopic histochemical stains for AcPase showed that most of the lead phosphate deposits were located in the deeper lysosomes (Fig. 8). Cytosomes of the autophagic type were found occasionally in the vicinity of the calcium casts.

The intracellular calcium deposits were needle-like and have been shown by Schneeberger and Morrison by electron diffraction to be apatite.[9] The deposits were located in the straight portions of the proximal convoluted tubules (Fig. 9), in the cells of Henle's loop and in the collecting tubules. Small, early calcium deposits were infrequently found and seemed to originate in or about lysosomes (Fig. 10). Apatite crystals also were seen within the cytoplasm with no apparent relation to any

particular organelle (Fig. 11). No mitochondrial calcification was found.

Interstitial calcification did not involve basement membranes and was formed by similar but smaller needle-like crystals. (Fig. 12) As noted by light microscopy the calcium casts were lamellated and produced marked attenuation of the tubular epithelium, at times reducing them to mere cytoplasmic rims (Fig. 13).

In two instances helicoidal intramitochondrial bodies were found. These were oriented parallel to the long axis of the organelle accompanied by stacks of cristae (Fig. 14). Although similar structures were not noted in control animals, they could have been missed because of their apparent rarity.

Biochemical Data. During the first week of the dietary regime the average serum magnesium levels in the experimental group declined to less than 50 per cent of the control values and remained low for the rest of the experiment (Table II). No significant change was found in the serum calcium (Table II). AcPase determination in whole kidney homogenates showed no significant increase in activity in the magnesium deficient animals (Table II). The calcium and magnesium content of isolated mitochondria was not altered (Table II).

TABLE II

BIOCHEMICAL DATA ANALYSIS *

	Experimental (E)			Control (C)			
	Mean	Standard deviation	No. of assays	Mean	Standard deviation	No. of assays	P value
Serum Mg values in mg %	0.91	0.2909	18	2.43	0.2865	7	$P < 0.001$
Serum Ca values in mg %	10.81	0.8611	18	10.20	0.8445	7	$0.05 < P < 0.10$
Acid phosphatase in kidney homogenate †	58.20	3.242	11	56.88	8.485	6	$0.30 < P < 0.40$
Calcium in isolated mitochondria §	7.98	1.993	9	7.14	2.0434	5	$0.20 < P < 0.25$
Magnesium in isolated mitochondria §	2.34	0.6710	9	2.92	0.7861	5	$0.05 < P < 0.10$

* Standard "t" test analysis.

† Expressed in units as defined by Huggins and Talalay[8] in 100 ml of a solution containing 1 mg nitrogen per ml.

§ Values represent pooled kidneys from two rats. Expressed in gammas per milligram dry weight.

DISCUSSION

The morphologic abnormalities observed in the kidney during magnesium deficiency had, in common, increased amounts of intracellular calcium. In the early phase, characterized by an increase of ionic calcium, cellular swelling was the predominant finding. Later, crystal

formation began and the subsequent changes were probably due to the effect of the calcium deposits themselves.

Early swelling of tubular epithelium concomitant with increased ionic calcium has been found in nephrocalcinosis produced by vitamin D and parathyroid hormone.[1,2] There were, however, significant differences between these forms of renal calcification and that resulting from magnesium deficiency. By light microscopy the renal calcium deposits induced by excess vitamin D or parathyroid hormone were more widespread. In magnesium deficiency, they were restricted to the area of the cortico-medullary junction. Ultrastructurally, the most conspicuous difference was the absence of mitochondrial calcification in magnesium deprivation nephrocalcinosis and its prominence in nephrocalcinosis due to excessive dosage of vitamin D or parathyroid hormone.[1,2] Our mitochondrial isolation chemical data confirmed the absence of calcification of this organelle.

The data thus do not support the suggestion that hyperparathyroidism is a causative factor in the nephrocalcinosis of magnesium deficiency.[10] Simple hypercalcemia was probably not responsible for the renal calcification. Serum calcium levels were normal in our animals. Indeed, nephrocalcinosis has even been described in combined calcium and magnesium deficiency states in which hypocalcemia was present.[10] It is to be noted also that elevation of serum calcium by excess calcium gluconate results in basement membrane deposits, apparently composed of calcium carbonate.[2]

Another explanation for the nephrocalcinosis in magnesium deficiency has been proposed by Alcock and MacIntyre.[11] They have postulated an increase in the tubular reabsorption of calcium facilitated by low magnesium levels in the tubular fluid. Experimental data have shown antagonism between these two ions for sites of membrane attachment.[12,13] The peculiar distribution of the calcium deposits in magnesium deficiency is not well explained by this hypothesis and it is evident that more knowledge of the renal handling of ions, particularly at the cellular and subcellular levels, is needed.

It is interesting to compare the cardiac lesions produced by magnesium deprivation with those which occurred in the kidney. In the myocardium an early and apparently primary sarcosomal calcification preceded more severe cellular changes.[3] This is in contrast with the absence of mitochondrial calcification in the kidney. The apparent difference in mitochondrial behavior is perhaps attributable to structural and functional differences among mitochondria in different organs. Mitochondria isolated from the heart, but not from liver or kidney, in magnesium-deficient rats display uncoupling of oxidative phosphorylation.[14,15] A

decreased capacity of sarcosomes to synthesize phospholipids has also been found in magnesium deficiency.[16] These differences may be related to the fact that myocardial mitochondria are particularly rich in magnesium.[15]

The increase in number and the enlargement of lysosomes were focal and most severe at the time the calcific casts were more numerous. An obstruction of the tubular lumen as suggested by microdissection studies may have been responsible for this phenomenon.[17] The normal AcPase activity in kidney homogenates may reflect the focal nature of the lysosomal changes, although it must be kept in mind that, even in conditions causing diffuse and severe lysosomal hypertrophy, net enzyme changes have often been absent[18] or restricted to only one or two lysosomal hydrolases.[19]

We offer no explanation for the intramitochondrial helical bodies. To the best of our knowledge these have not, heretofore, been described in the kidney. Identical structures, however, have been seen within mitochondria in astrocytes of the corpus striatum in the cat and in normal hepatocytes.[20]

In a recent study, Schneeberger and Morrison[9] described changes somewhat different from those we observed. In particular, they did not note the early cellular swelling. Calcification was largely restricted to the thick segment of Henle's loop. Lysosomal enlargement and calcification were more prominent and focal degenerative changes in uncalcified tubules were seen. The latter finding was considered to be secondary to tubular obstruction. The differences may relate either to the initial age of the experimental animals or to the composition of the diet used. Schneeberger and Morrison used animals younger than ours and a diet richer in calcium. Their animals exhibited hypercalcemia; ours did not. It seems from the foregoing and other data that the hypothesis attributing cellular damage in renal tubules of a type that promotes calcification to hypercalcemia or hypercalcinuria no longer appears tenable. Hypervitaminosis D and hyperparathyroidism, neither of which necessarily induce hypercalcemia in man or animals, results in morphologic changes quite different from those following nephrocalcinosis due to calcium gluconate injections or magnesium deficiency.

Calcification induced by magnesium deprivation is characterized by apatite crystal formation which begins in the mitochondria when it affects cardiac muscle but spares the mitochondria in renal tubular epithelium. If calcification is defined as a change of state[21] with the formation of mineral crystals, this is ultimately a simple physico-chemical process. The studies reported, however, re-emphasize what pathologists have long suspected; namely, that the roads leading to this change of

state may be many and that cellular mechanisms play a vital role. A given cell may calcify in different locations in response to different types of insults and different types of cells may calcify in different locations, or not at all, in response to the same metabolic insult. Further, the response to calcification may also vary, depending on the cell affected, the type of insult, and the state of the cell or its environment.

Summary

The earliest renal lesion in magnesium deprivation was edema of tubular epithelium accompanied by increased amounts of ionic calcium in the cytoplasm. Subsequently, apatite crystal deposition occurred in the pars recta of proximal convoluted tubules, loops of Henle and collecting tubules. These deposits were both extracellular and intracellular and occasionally formed casts. Early intracellular calcium deposits arose in the hyaloplasm or in lysosomes. Focal increase in numbers and enlargement of lysosomes possibly represented a response to damage to the nephron by the calcium deposits. Calcification of mitochondria was not observed and suspensions of isolated mitochondria showed no change in their calcium content. Mitochondrial calcification occurs in magnesium deficient rat sarcosomes and in renal mitochondria in vitamin D and parathyroid induced nephrocalcinosis. Only basement membranes calcify when calcium gluconate is given intravenously.

This is in contrast to the distribution of calcium in the cardiac lesion of magnesium deficiency and in other types of nephrocalcinosis. These morphologic and biochemical differences among the various types of abnormal calcification re-emphasize the role of the cell and its organelles in this pathologic process.

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[*Illustrations follow*]

Legends for Figures

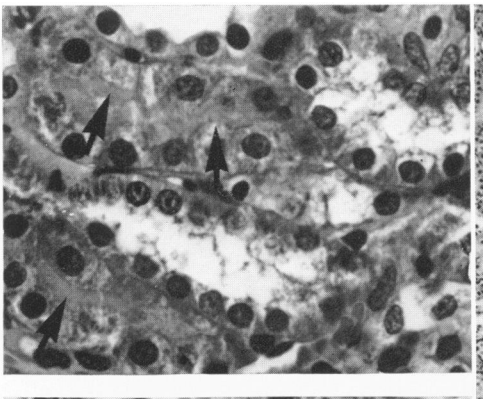
Fig. 1. Kidney, magnesium-deficient rat 1 week on the dietary regime. There is marked swelling involving all cells but with varying degrees of severity. The brush border, although markedly affected, can be seen in some areas (arrows). Glutaraldehyde-fixed, hematoxylin and eosin (H&E) stain. × 200.

Fig. 2. Calcareous deposits appear at the cortico-medullary junction in the kidney of a rat with magnesium-deficiency. Most deposits are intraluminal and cast-like but even at this low magnification interstitial and possibly intracellular deposits can be detected (arrows). Glutaraldehyde-fixed, H&E stain. × 100.

Fig. 3. Large intraluminal deposits are evident within the renal tubules of a magnesium-deficient rat. Concentric lamination is apparent. Atrophy can be seen in the cells surrounding the casts. Glutaraldehyde-fixed, H&E stain. × 400.

Fig. 4. Portion of a distal tubular epithelial cell. The apical pole (upper portion) shows severe hydropic swelling with scattering of cytoplasmic structures. Mitochondria, however, retain a normal appearance. × 4,500.

Fig. 5. An unidentified tubule, probably a collecting tubule, exhibits severe cytoplasmic swelling. Scattered portions of rough-surfaced endoplasmic reticulum (thin arrows) and the Golgi apparatus (thick arrows) lie within the loose-textured hydropic cytoplasm. The nucleus (N) and mitochondria are unaffected. × 4,500.



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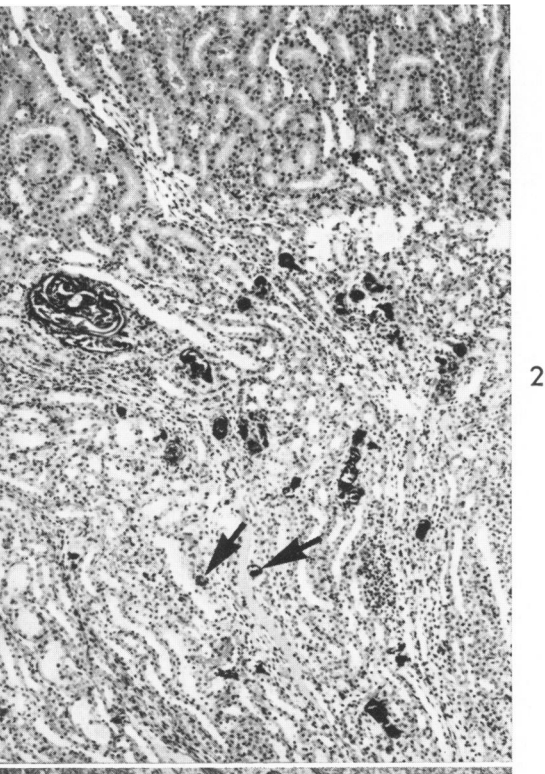

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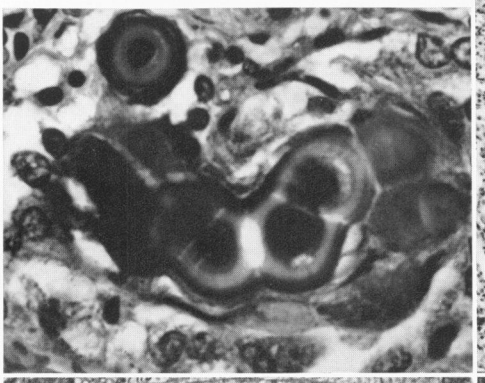

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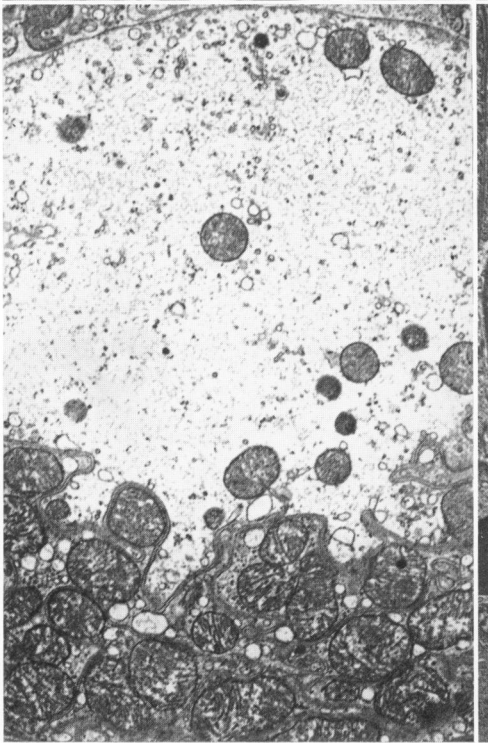

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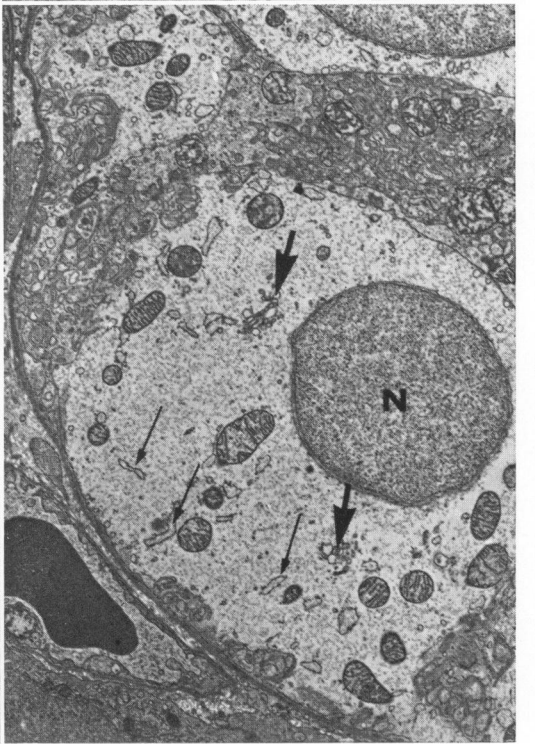

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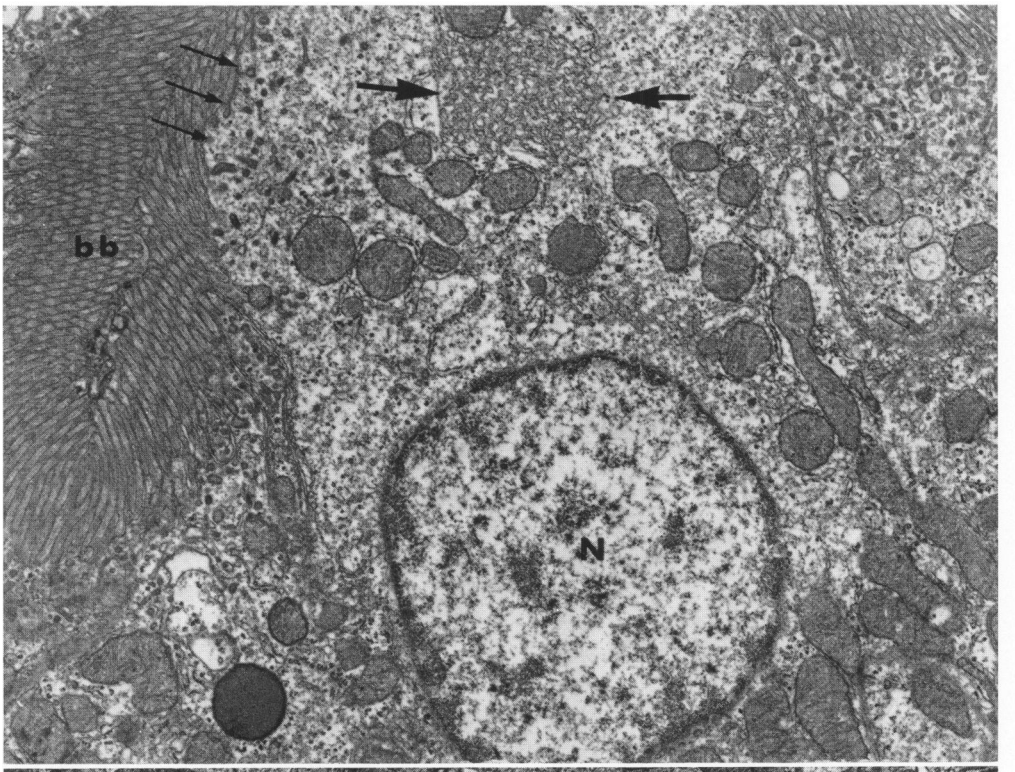
FIG. 6. Proximal convoluted renal tubule in a magnesium-deprived rat. The brush border (bb) exhibits focal areas of effacement (thin arrows). Clustering of the cytoplasmic tubules which continue the brush border into the apical cytoplasm is seen between thick arrows. Mitochondria are normal. N = nucleus. × 8,500.

FIG. 7. Proximal convoluted tubule. Abundant electron-clear phagosomes (arrows) are present near the brush border (bb). Large lysosomes (ly) are seen toward the basal area of the cells. The nuclei (N) and mitochondria show no abnormalities. × 4,500.



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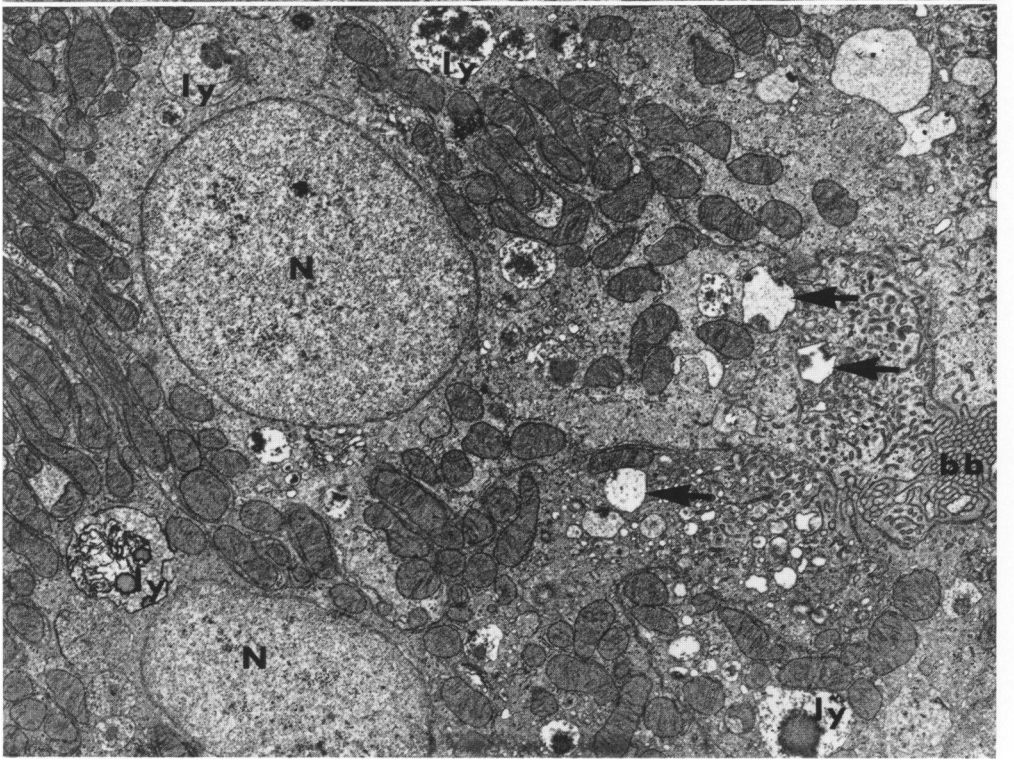

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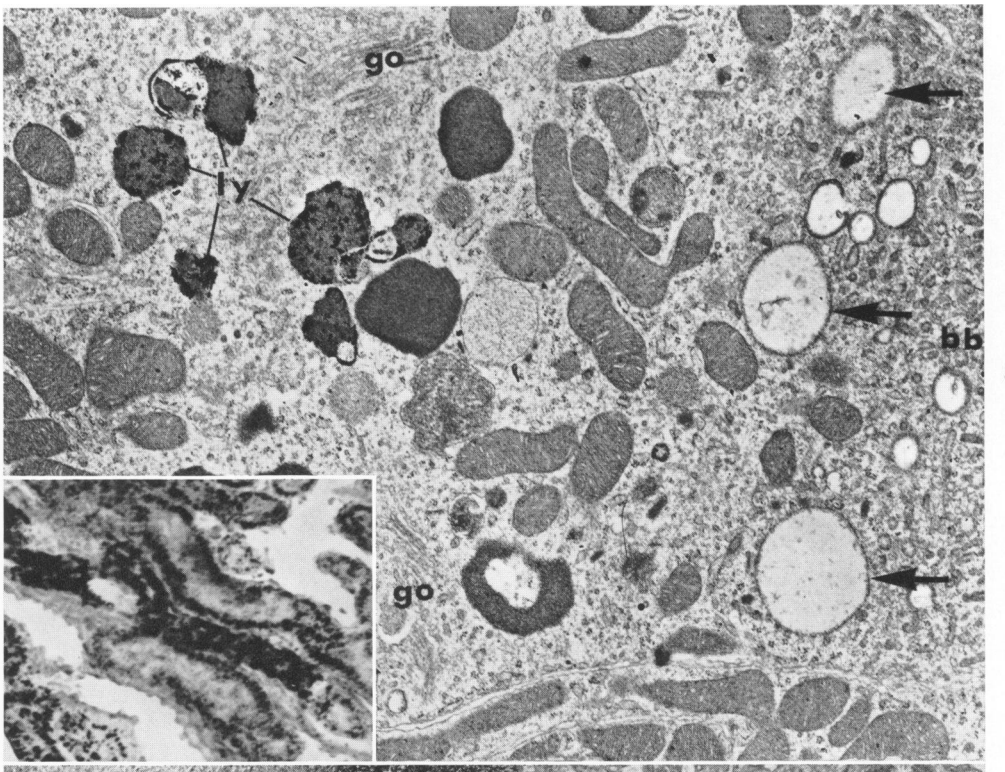
FIG. 8a. Glutaraldehyde-fixed kidney from a magnesium-deprived rat incubated for 15 minutes in Gomori's acid phosphatase medium. The phagosomes (arrows) located in proximity to the brush border (bb) show no enzymatic activity. The Golgi apparatus (go) likewise exhibits no activity. Heavy lead phosphate deposits, indicating acid phosphatase activity are seen over the deeper lying lysosomes and their membranes. × 11,500.

FIG. 8b. Glutaraldehyde-fixed, frozen section of a magnesium-deprived rat kidney incubated for 30 minutes in Gomori's medium for acid phosphatase. Counterstained with eosin. There is focal increase in the number of lysosomes in segments of tubules. The lead sulfide positive granules are enlarged and clustered. Adjacent tubules, however, are normal. × 75.

FIG. 9. A part of a calcified proximal convoluted tubule at its pars recta. A large calcium deposit (upper left corner) is present. The brush border (bb) serves to localize the cell to the proximal tubule. There is mitochondrial swelling of moderate degree but no mitochondrial calcification. A large lipid droplet appears within one of the tubular cells (li). N = nucleus. × 4,600.



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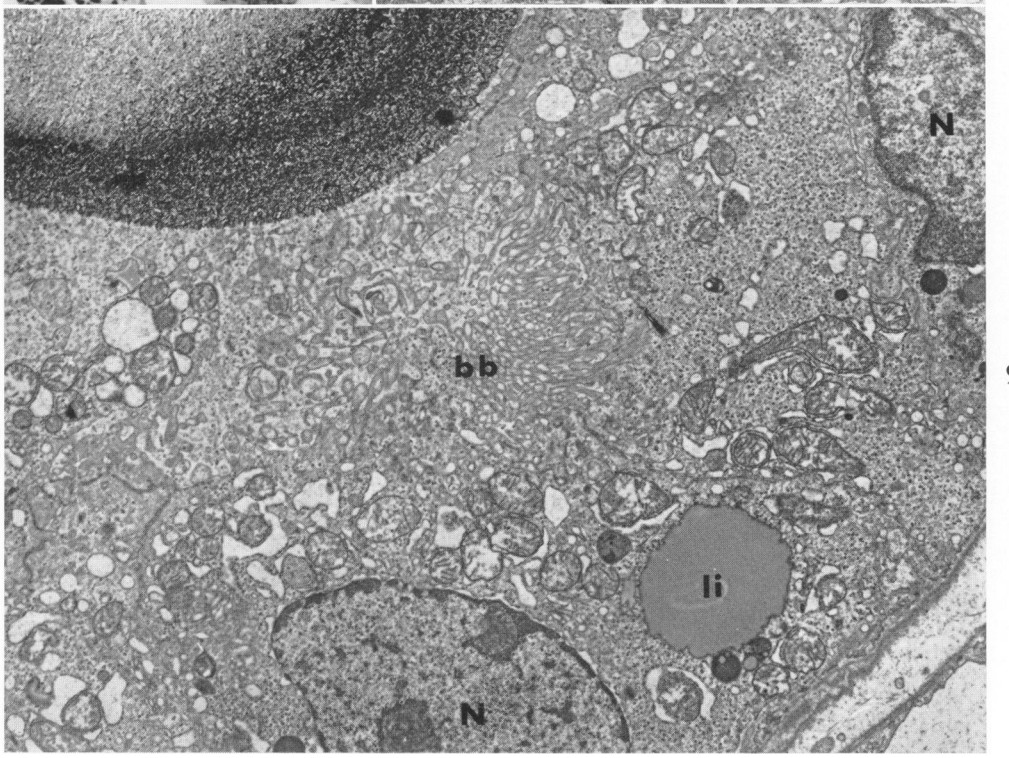

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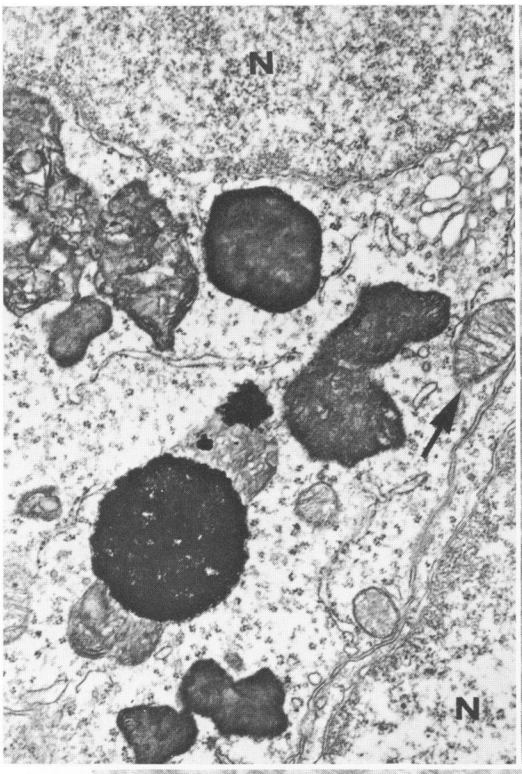

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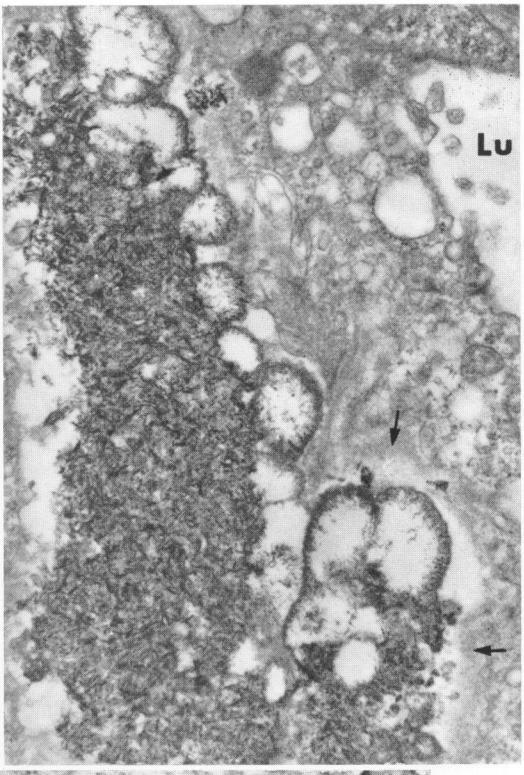

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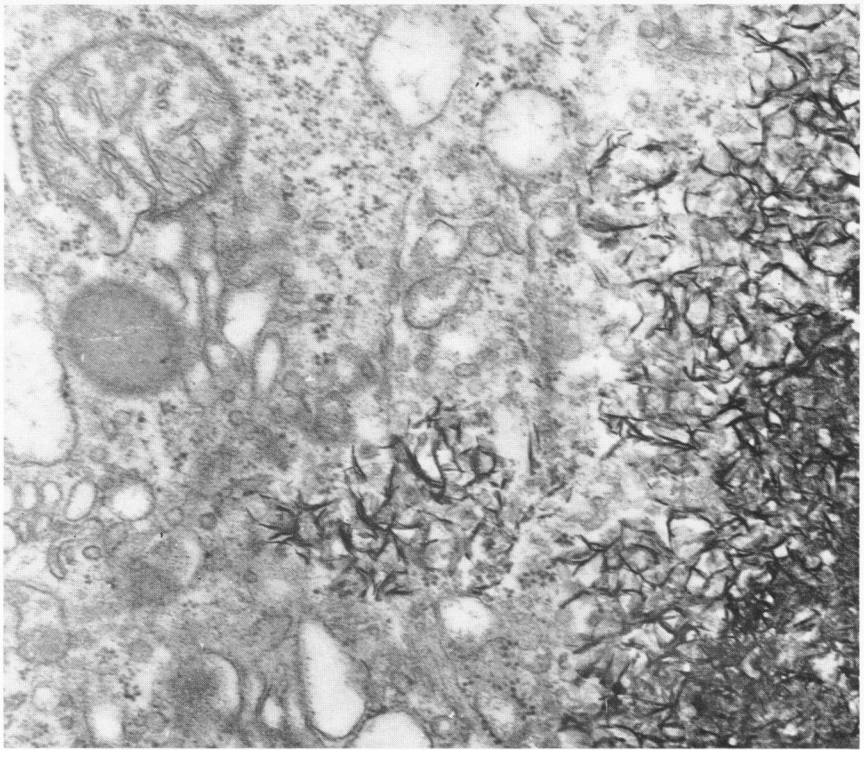

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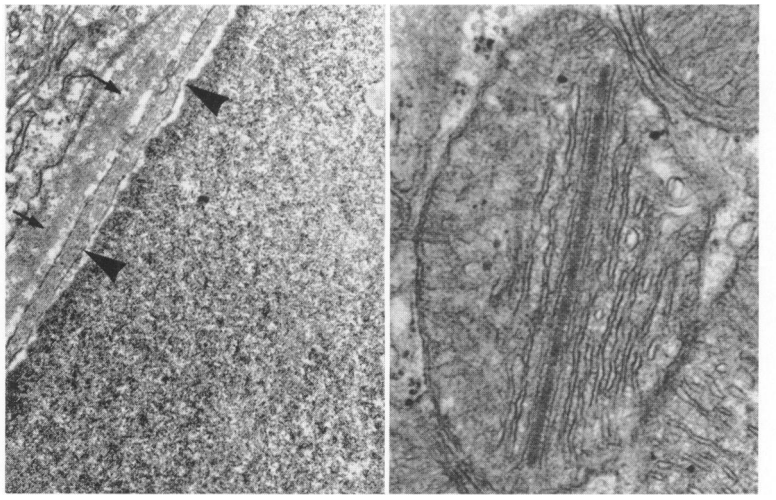

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FIG. 10. Early calcium deposits apparently originated within lysosomes. Several uncalcified, as well as calcified, lysosomes are seen in this (probably collecting) portion of a tubule. A mitochondrion (arrow) shows no swelling or calcification. There is a moderate degree of hydropic change in the cytoplasm. The nuclei (N) are normal. × 15,500.

FIG. 11. Needle-like crystals, presumably apatite, lie within a proximal tubular epithelial cell. A mitochondrion appears in the upper left corner. No particular organelle seems to be acting as a nidus for the deposition of calcium salts. There is little evidence of cell damage. × 33,000.

FIG. 12. Interstitial deposits of calcium salts are evident next to a portion of Henle's loop. The deposits show ring-like formations. The basement membrane (arrows), despite its proximity to the calcium salts, is not calcified. Lu = lumen. × 21,500.

FIG. 13. Portion of a large calcified cast. The tubular epithelium (arrowheads) has been reduced to a thin rim of cytoplasm. The tubular basement membrane is not calcified. × 10,000.

FIG. 14. An intramitochondrial helicoidal body. The structure runs parallel to stacks of cristae and has a uniform periodicity. × 30,000.