ELECTRON MICROSCOPIC STUDY OF RENAL CALCIFICATION

J. B. CAULFIELD, M.D., AND P. E. SCHRAG, B.A.

From the Edwin S. Webster Memorial Laboratory, Department of Pathology, Massachusetts General Hospital and Harvard Medical School, Boston, Mass.

The role cells play in calcification has been poorly defined. Much has been written about metastatic calcification since Virchow first described calcified deposits in the kidney and called them calcium metastases.[1] This concept ascribed a passive role to the precalcified tissue. Other traditional concepts allowed cells only a limited role in calcification; either the cells were dead or they existed in an environment of lowered pH.[2,3] Interest in parathyroid hormone (PTH) has resulted in additional theories concerning the production of calcified deposits in kidneys.[4–8] It has been shown by light microscopy that PTH-induced calcification has a different localization in the mouse kidney from that resulting from calcium gluconate administration.[9] Electron microscopy has given the matter a new impetus and has made possible greater correlation of cellular structure with physiologic activity.

The present investigation describes the changes induced in mouse kidney cells by PTH and calcium gluconate. Kidneys of mice treated for varying lengths of time with these two agents were examined by electron microscopy. Sequential changes and cellular recovery after the induction of calcification are described. Electron diffraction was used to characterize the calcific deposits produced. The structure of the alterations as well as the nature of the calcified material produced by these two agents was distinctly different.

MATERIAL AND METHODS

Young virgin female Swiss type A mice, weighing 15 to 18 gm., were used. Food was withheld from the mice 16 hours prior to removal and fixation of tissue. This established a morphologic base line as described by Oliver.[10]

Mice were treated with daily intraperitoneal injections (0.5 cc.) of a 6 per cent aqueous solution of calcium gluconate, U.S.P., at 24-hour intervals for 3, 5, 8, 11, 15 and 18 days. Some animals treated for 10, 11 and 13 days were allowed to recover for 3, 8 and 12 days before being sacrificed. Groups of 3 animals were given either 10, 20, 40, 50 or 80 units of parathyroid hormone, U.S.P., daily for 2 to 5 days. The animals given 10 and 20 unit doses failed to show calcification at 5 days. Those receiving 40 units showed occasional calcification at 4 days and slightly more at 5 days, but

This work supported by United States Public Health Service Grant #CA 04955.
Presented at the 57th annual meeting of the International Academy of Pathology.
Accepted for publication, September 17, 1963.

insufficient amounts to overcome the sampling problem inherent in electron microscopy. Three injections of 50 or 80 units produced calcification at 72 hours and a single subsequent injection resulted in 50 per cent mortality with 50 units and 100 per cent mortality with 80 units. No calcification was visible 48 hours after the first of 2 injections of 50 units, and all of the animals exhibited extensive calcification 72 hours after the first of 3 injections. Groups of 4 animals were examined at 48, 51, 54, 57, 60, 63 and 72 hours after the first of 3 daily injections of 50 units. Some animals were allowed to live 3, 8 and 12 days after their last injection. Mice treated simultaneously with calcium gluconate and diamox, U.S.P., were given intraperitoneal injections of the latter (0.2 cc., 1 per cent aqueous solution) for 7 and 12 days.

A total of 130 mice were sacrificed, and multiple sections with a minimum of 4 blocks from each animal were examined with the electron microscope. Paraffin sections were also prepared and examined. The animals were lightly anesthetized with ether. First the left kidney was removed by clamping and then severing the renal pedicle. The right kidney was removed 3 minutes later. Tissues for electron microscopy were immediately immersed for 1½ hours in cold 1 per cent osmium tetroxide buffered at pH 7.4 and containing 4.5 gm. per cent sucrose.[11,12] The tissues were dehydrated by passage through graded alcohols and were embedded in N butyl methacrylate. Sections were cut with glass knives on a Porter-Blum microtome,[13,14] collected on carbon-coated formvar-covered copper grids and examined with an RCA EMU-3B electron microscope.[15] Electron micrographs were taken at magnifications of 3,000 to 15,000 times. Selected area diffraction was carried out using a Siemens Elmiskop with a cold stage attachment. Gold, shadow cast on carbon-coated grids, was used to calibrate the microscope for diffraction.

Results

The cortex of the mouse kidney consists of two regions. The outer, more cortical area contains glomeruli, the convoluted portion of the proximal tubule and the distal convoluted tubule. The inner zone contains the distal or straight portion of the proximal tubule, collecting tubules and ascending limb of Henle's loop. Various histochemical and functional differences between the two zones have been described.[17,18] Rhodin[16] and Caulfield and Trump,[19] using the electron microscope, have described differences between proximal tubules of zone 1 and proximal tubules of zone 1A.

Parathyroid Hormone

Proximal Tubules, Zone 1. With the electron microscope, cells in zone 1 were identified by the deep basilar infoldings and abundant elongated mitochondria which occupy a large portion of the cell. This portion of the proximal tubule was unaffected by the PTH.

Zone 1A. The earliest changes visible with the electron microscope were found exclusively in the epithelium of the distal portion of the proximal tubule (Figs. 1 and 2). These cells showed a pale cytoplasm with increased separation of the formed elements. The over-all appearance of excess cellular hydration was associated in frozen sections with a positive glyoxyl reaction for ionic calcium.[20]

The first evidence of precipitated calcium appeared at 51 and 54 hours. When present in a given cell, the calcific deposits were generally quite large and occupied extensive portions of the cytoplasm (Fig. 7). It was difficult to find fields with small deposits of the salt (Fig. 3). The early deposits were observed in mitochondria and in some of the vacuoles (Figs. 4 and 5). The areas of calcification in mitochondria occurred first as aggregates of small dense spheres with a range of 100 to 200 Å in diameter. As the areas of cytoplasm became more extensively calcified, the deposits were usually evident as crystals. The earliest calcification in the vacuoles appeared as elongated crystals. During this stage many partially calcified mitochondria exhibited extensive architectural distortion; at this early period many cells were free of calcium deposit, and adjacent cells might or might not contain calcified areas.

At 57 and 60 hours it was easier to find fields containing calcium (Figs. 6 and 7). Calcification was manifest intracellularly before any intraluminal crystals appeared. On occasion the mitochondria and vacuoles were densely mineralized, with coalescence resulting in large areas of mineralization. The calcifying process did not involve the mitochondria and vacuoles exclusively at this stage. Rather, needle-shaped deposits were found on many membrane surfaces throughout the cell (Figs. 6 and 7). Surrounding a nidus of calcification, in some, a second wave of mineral was deposited with a laminated appearance but with no clear membranous matrix in the second ring. Large masses of crystalline material were eventually formed through growth and coalescence of the many smaller deposits. These often protruded into the lumen of the tubule, and the border between the cell and the tubule lumen was then no longer well defined. The large calcified masses involved the apical portion of the cell above the nucleus and extended to the mid-nuclear region of the cytoplasm laterally, the nucleus never showing calcification. The cell apparently was capable of sloughing this large calcified portion since many crystalline masses were present in the lumens of the distal convoluted tubules. Portions of normal cytoplasm remained attached to the basement membrane even when the mineralized apical region had sloughed. In the animals given PTH for 3 days and then allowed to recover for 3 days, almost no intracellular crystals remained (Fig. 8), and the needle-shaped deposits were very small. The tubular lumens in some instances contained cellular debris with aggregates of crystal. At this stage the entire basement membrane in the proximal tubules in zone 1A was covered by mature-appearing cells. Some of the cells were somewhat overhydrated in appearance, but basilar infoldings were present and normal. The mitochondria appeared in usual numbers and the brush border was intact (Fig. 8). When recovery

was allowed to continue for 8 days, no intracellular crystalline material was evident and tubular cells were mature and normal, except for very slight cellular swelling.

Selected area electron diffraction produces patterns that are analogous to x-ray diffraction patterns. In the case of electron diffraction much smaller samples can be analyzed.[21] Crystalline masses of about 2 μ diameter and 200 Å thickness in tissue sections are sufficiently large to produce diffraction patterns that permit identification of crystals by comparison with x-ray diffraction patterns.[22] Crystalline masses produced by the PTH gave an electron diffraction pattern consistent with apatite patterns obtained by x-ray diffraction of bone.[23,24]

Calcium Gluconate

Proximal Convoluted Tubule, Zone I. The earliest changes in this region were present at 3 days. Occasionally there was focal thickening of the basement membrane. The area of thickening was usually related to a lamellar body. Several such round bodies might lie adjacent to each other and be enveloped by a common outer lamella (Fig. 9). At 5 days the bodies were more abundant, and at 12 days they often extended along and replaced the entire basement membrane of the cell (Fig. 10). The number of concentric lamellas constituting each body also increased. At 3 days there were 3 or 4 lamellas per body; at 12 days these increased to 6 to 8. The lamellas had a membranous appearance and small black dots appeared in association with the laminations. These dot-like areas of extreme electron density increased in number as the treatment period was extended (Fig. 11). The laminated bodies were always extracellular and did not migrate from the basement membrane. Very occasionally they appeared as a cluster and were not arranged in a straight line along the base of the cell. Such areas, however, probably resulted from oblique sectioning of the basement membrane.

Another change at 12 days was a separation of the infolded membranes at the base of the cell. The separation accentuated the basilar cell processes, the tips of which were only slightly separated from the basement membrane.

At 18 days the laminated bodies were much more numerous (Fig. 12). The small bodies were very electron opaque and were deposited in sufficient numbers to obscure the concentric lamellas. At times the entire basement membrane appeared as a dense mass of mineral deposit (Fig. 11). The disposition of the mineral was limited to a portion of zone 1 proximal tubules, since in all specimens with calcification many regions of convoluted tubules were free of deposit. No needle forms suggestive of crystals were observed in the basement membranes or in

the lamellated bodies. Intracellular structures were well defined, and no changes appeared in the nuclei or intracellular membranes. An occasional small cluster of needle-shaped crystals similar to the apatite seen with PTH was found within mitochondria. At 18 days there was further separation of basilar cell processes. Occasionally these were enormously enlarged so that they constituted saccular cavities which separated the cell from the underlying basement membrane. The cells with large saccular extracellular spaces usually lay above a basement membrane containing many dense mineralized laminated bodies.

Animals treated simultaneously with diamox and calcium gluconate also developed laminated bodies (Fig. 13). However, the small areas of extreme electron opacity were either absent or minimal in quantity at 12 days. In animals treated with calcium gluconate and allowed to recover for 8 days laminated bodies were present but free of dense mineralized deposits. Selected area electron diffraction techniques to characterize the intralaminar deposits were unsuccessful.

Distal Tubules. At 12 days laminated bodies were observed in the distal convoluted tubules in zone 1. These were less well developed and less extensive than in the proximal tubule. The space between basilar cell processes was accentuated, as in the proximal tubule. No laminated bodies were encountered in the basement membrane of other portions of the tubule, the glomerulus or the renal vessels.

Discussion

The alterations described indicated that the calcification in renal tubules was dependent upon cellular mechanisms. Parathyroid hormone has been shown to have a direct effect on renal tubular epithelium, both proximal and distal portions.[25–27] Differences in enzyme activity in proximal tubular cells of zones 1 and 1A have been known to exist for some time [17,18]; it is reasonable to assume that these are associated with functional differences. The sharp localization of the hormone effect is in keeping with the morphologic and enzymatic differences previously shown to exist between these two proximal tubule zones. Localization of PTH activity by stop-flow analysis has not been sensitive enough to delineate its site of activity, but the results are compatible with localization in the *pars recta* of the proximal tubule.[26]

An initial effect of PTH is to produce an abundance of intracellular calcium ions. These are accompanied by an increase in intracellular water apparent as cellular swelling. From our data there was no way to determine whether the PTH administration resulted primarily in active concentration of Ca^{++} or $PO_4{\equiv}$. However, shortly before intracellular calcification commences there is an increase in ionic calcium.

Precipitation of the calcium begins intracellularly in the form of an apatite.

The presence of the increased calcium and water in a very limited portion of the tubule, although calcium is reabsorbable in other areas, implies a highly selected locus of action for the parathyroid hormone. Biochemical evidence of altered respiration in kidney and bone tissue but normal respiration in liver and heart treated with PTH suggests an action of this hormone on enzymes located within mitochondria.[28] Whether this effect on oxidative enzymes is solely responsible for the increased calcium uptake has not been made apparent. The earliest intracellular calcium deposition is within mitochondria and vacuoles; this is difficult to explain. It is known that some vacuoles contain acid phosphatase, and the increased phosphate associated with enzyme activity may be responsible for the early precipitation.[29] This type of vacuole is thought to contain a variety of enzymes, and the calcification may be due to other effects. Certainly there are many facets of mitochondrial activity that might explain the presence of early calcification. These organelles contain active ATPase and have, as well, the ability to bind calcium ions.[30] Since renal mitochondria are stimulated by PTH, this in itself might alter their internal environment sufficiently to produce calcification. There are no morphologic criteria to indicate one mechanism over another.

The fact that there were few cells containing minimal calcification and many with large masses would indicate that once the process of intracellular calcification started, it proceeded quite rapidly and affected large areas of the cell. The frequently observed concentric rings of calcified material could be related to the Liesegang phenomenon of crystal growth in gels.[31]

Though large quantities of crystal material and cytoplasmic debris appeared in tubule lumens, nuclei were not manifest here. The areas of calcification extended fairly deeply into the cells on occasion although mineral deposit never involved the nucleus. A zone of morphologically healthy cytoplasm appeared adjacent to the basement membrane in most cells that could be adequately evaluated. The fact that the nucleus was never affected in the process of calcification, and that some normal cytoplasm persisted, coupled with the observation of relatively normal structure 3 days after treatment would indicate that this dosage schedule produced a rather extensive intracellular calcification with little complete cellular destruction.

Alterations produced by calcium gluconate, including calcification of tubule basement membrane, have been observed by others.[8,9] These are highlighted by localization of early calcification in the tubular base-

ment membrane of zone 1, an organized structure of laminated bodies, the increase in their size and number in a highly characteristic way and retention of the normal intracellular content in tubular epithelium. These features, in the presence of extensive basement membrane changes, are all indicative of a specific response to excess calcium. The membranes which formed the laminated bodies probably derived from the tubular epithelium near or at the plasma membrane since they were never observed within the cytoplasm. Whether these changes were the result of interference of calcium with the normal formation of membranes or whether the formation of laminated bodies at the basement membrane constituted a pathologic alteration is uncertain.

The opaque mineral deposits in the lamellas were not hydroxyapatite. It is possible that the calcium deposited with an organic matrix. This would explain the failure to obtain a diffraction pattern which is only obtained when a crystalline state is present.[32] The laminated bodies were well localized in the tubules of zone 1 and did not occur in glomeruli. This localization would not be well explained by assuming that calcium was bound to mucoprotein material in basement membranes. Another possibility was that calcium deposited as calcium carbonate; this in itself is difficult to diffract with electrons and forms a nondiffractable, noncrystalline precipitate in the presence of phosphate ions. Calcium carbonate was identified in kidney tubules by H. Gideon Wells, who reported that calcium phosphates were present in the epithelium and that calcium carbonate was present in the renal interstitium.[33]

Carbonic anhydrase, known to be present in the kidney,[34] is associated with acidification of the urine. Recent work indicates that the major reduction of tubular fluid pH occurs in the proximal tubule with a subsequent fall of lesser degree in the distal tubule.[35] The localization of the calcific deposits was clearly greatest in the proximal tubule; fewer aggregates deposited later in the course of the experiments appeared in the distal tubule. Since carbonic anhydrase activity might well result in the production of carbonate, one would expect calcium carbonate precipitation in the regions where this enzyme activity prevailed.[35,36]

Eggshells consist of calcium carbonate, and there is a high carbonic anhydrase activity in the hen oviduct. If diamox is given to a hen, the eggshell matrix is formed but fails to calcify.[37] Simultaneous administration of diamox and calcium gluconate in mice did not prevent formation of laminated bodies. However, significantly less mineral was deposited in the lamellas than when calcium was administered alone. This might have been the result of carbonic anhydrase inhibition by diamox although a nonspecific removal of the mineral by the diuresis induced by the diamox could not be excluded.

The localization of calcific deposits to limited areas in zone 1 proximal tubules and a portion of the distal tubule could represent localization of one of two activities or both. The areas of precipitate might correspond to local areas of carbonic anhydrase activity, the calcium actually being reabsorbed over rather broad areas of the nephron. Conversely, the precipitation might be associated only with sites of high calcium concentration and be independent of carbonic anhydrase activity, or the two activities, carbonic anhydrase and calcium reabsorption, could coincide. On the basis of all evidence, it would seem that the precipitates probably occurred in the region of both carbonic anhydrase activity and calcium concentration.

Separation of basilar cell processes and the formation of large saccular cavities between the epithelium and the underlying basement membrane have been previously described.[19] These changes were noted to be reversible when occurring in rats given intravenous infusions of saline and examined 2 to 30 minutes later. The spaces reflected an accumulation of fluid secondary to ion transport. The saccular cavities were encountered after prolonged administration of calcium gluconate in instances where the basement membrane was densely mineralized. It is reasonable to assume that reabsorbed ions and water could not readily penetrate the severely altered basement membrane and therefore accumulated, forming the large extracellular spaces.

Summary

Changes produced by calcium gluconate and parathyroid extract (PTH) in the proximal tubules of the mouse kidney were examined by light and electron microscopy.

The proximal tubular epithelium in the mouse kidney consists of two morphologically different zones. Cells in zone 1 and zone 1A responded differently to the calcifying agents. This would appear to indicate that the cells in these zones had different functions.

The administration of PTH resulted in intracellular apatite formation first in mitochondria and vacuoles and later in large areas of the cytoplasm. This was restricted to zone 1A of the proximal tubule. The changes induced by calcium gluconate were manifest in the basement membranes of zone 1 proximal tubules and to a lesser extent in the basement membrane of the distal convoluted tubules. These areas correspond to the sites of acidification of intratubular fluid.

Two different types of calcific deposit were encountered. Parathyroid extract produced masses of needle-shaped crystals with the diffraction pattern of apatite. Calcium gluconate caused electron-opaque deposits of confluent dots; in these no diffraction pattern was obtainable. These deposits were thought to be calcium carbonate, and it is suggested that

they were related to carbonic anhydrase activity. It is emphasized that tubular epithelium plays an active and predominant role in renal calcification.

REFERENCES

1. VIRCHOW, R. Kalk metastasen. *Virchows Arch. path. Anat.*, 1855, **8**, 103–113.
2. BARR, D. P. Pathological calcification. *Physiol. Rev.*, 1932, **12**, 593–624.
3. COHNHEIM, J. Lectures on General Pathology. McKEE, A. B. (transl.). The New Sydenham Society, London, 1889, Vol. II, pp. 640–652.
4. ENGEL, M. B. Mobilization of mucoprotein by parathyroid extract. *Arch. Path.*, 1952, **53**, 339–351.
5. BAKER, R.; REAVEN, F., and SAWYER, J. Ground substance and calcification: the influence of dye binding on experimental nephrocalcinosis. *J. Urol.*, 1954, **71**, 511–522.
6. BAKER, R., and SISON, F. Demonstration of altered tissue mucopolysaccharides in renal calculous disease by selective staining techniques. *J. Urol.*, 1954, **72**, 1032–1045.
7. BOYCE, W. H.; GARVEY, F. K., and NORFLEET, C. M., JR. Proteins and other biocolloids of urine in health and in calculous disease; electrophoretic studies at pH 4.5 and 8.6 of those components soluble in molar sodium chloride. *J. Clin. Invest.*, 1954, **33**, 1287–1297.
8. GRIMES, W. A. A phase contrast study of the mechanisms of renal calcification. *J. Urol.*, 1957, **78**, 553–574.
9. SCHNEIDER, A. F.; REAVEN, E. P., and REAVEN, G. A comparison of renal calcification produced by parathyroid extract or calcium gluconate. *Endocrinology*, 1960, **67**, 733–743.
10. OLIVER, J. R., and LUND, E. M. Cellular mechanisms of renal secretion. A study by the extra vital method. II. The functional phase of the secretory mechanism. *J. Exper. Med.*, 1933, **57**, 459–483.
11. PALADE, G. E. A study of fixation for electron microscopy. *J. Exper. Med.*, 1952, **95**, 285–298.
12. CAULFIELD, J. B. Effects of varying the vehicle for OsO_4 in tissue fixation. *J. Biophys. & Biochem. Cytol.*, 1957, **3**, 827–830.
13. LATTA, H., and HARTMANN, G. F. Use of a glass edge in thin sectioning for electron microscopy. *Proc. Soc. Exper. Biol. & Med.*, 1950, **74**, 436–439.
14. PORTER, K. R., and BLUM, J. A study in microtomy for electron microscopy. *Anat. Rec.*, 1953, **117**, 685–710.
15. WATSON, M. L. The use of carbon films to support tissue sections for electron microscopy. (Brief note). *J. Biophys. & Biochem. Cytol.*, 1955, **1**, 183–184.
16. RHODIN, J. Anatomy of kidney tubules. *Internat. Rev. Cytol.*, 1958, **7**, 485–533.
17. STERNBERG, W. H.; FARBER, E., and DUNLAP, C. E. Histochemical localization of specific oxidative enzymes. II. Localization of diphosphopyridine nucleotide and triphosphopyridine nucleotide diaphorases, and succindehydrogenase system in the kidney. *J. Histochem.*, 1956, **4**, 266–283.
18. WACHSTEIN, M. Histochemical staining reactions of the normally functioning and abnormal kidney. *J. Histochem.*, 1955, **3**, 246–270.
19. CAULFIELD, J. B., and TRUMP, B. F. Correlation of ultrastructure with function in the rat kidney. *Am. J. Path.*, 1962, **40**, 199–218.
20. KASHIWA, H. K., and ATKINSON, W. B. A sensitive and specific method for demonstrating intracellular calcium with glyoxal bis (2-hydroxy-anil). (Abstract) *Anat. Rec.*, **1962, 142, 246.**

21. BIGELOW, W. C. Electron Diffraction. In: Physical Methods in Chemical Analysis. BERL, W. G. (ed.). Academic Press, Inc., New York, 1960, ed. 2, Vol. 1, p. 620.

22. RASMUSSEN, P., and CAULFIELD, J. B. The ultrastructure of Schaumann bodies in the golden hamster. *Lab. Invest.*, 1960, 9, 330–338.

23. ENGSTRÖM, A., and AMPRINO, R. X-ray diffraction and x-ray absorption studies on immobilized bones. *Experientia*, 1950, 6, 267–269.

24. ENGSTRÖM, A., and ZETTERSTRÖM, R. Studies on the ultrastructure of bone. *Exper. Cell Res.*, 1951, 2, 268–274.

25. NICHOLSON, T. F. The mode and site of the renal action of parathyroid extract in the dog. *Canad. J. Biochem. & Physiol.*, 1959, 37, 113–117.

26. SAMIY, A. H.; HIRSCH, P. F.; RAMSAY, A. G.; GIORDANO, C., and MERRILL, J. P. Localization of the renal tubular action of the parathyroid hormone. *Endocrinology*, 1960, 67, 266–270.

27. LEVINSKY, N. G., and DAVIDSON, D. G. Renal action of parathyroid extract in the chicken. *Am. J. Physiol.*, 1957, 191, 530–536.

28. COHN, D. V., and FORSCHER, B. K. Effect of parathyroid extract on the oxidation *in vitro* of glucose and the production of $^{14}CO_2$ by bone and kidney. *Biochim. et biophys. acta.*, 1962, 65, 20–26.

29. 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.

30. DELUCA, H. F., and ENGSTROM, G. W. Calcium uptake by rat kidney mitochondria. *Proc. Nat. Acad. Sc.*, 1961, 47, 1744–1750.

31. ALEXANDER, A. E., and JOHNSON, P. Colloid Science. Oxford Univ. Press., 1949, p. 603.

32. TRAVIS, D. Structural features of mineralization from tissue to macromolecular levels of organization in the decapod crustacea. Comparative biology of calcified tissues. *Ann. New York Acad. Sc.* (In press)

33. WELLS, H. G.; HOLMES, H. F., and HENRY, G. R. Studies of calcification and ossification. *J. Med. Res.*, 1911, 25, 373–392.

34. ROUGHTON, F. J. W., and CLARK, A. M. Carbonic Anhydrase. In: The Enzymes. Chemistry and Mechanism of Action. SUMNER, J. B., and MYRBÄCK, K. (eds.). Academic Press, Inc., New York, 1951, Vol. 1, part 2, pp. 1250–1265.

35. BANK, N., and AYNEDJIAN, H. S. Measurements of tubular fluid pH *in vivo* in rats. (Letter to the editor) *Nature, London*, 1963, 197, 185–186.

36. GOTTSCHALK, C. W.; LASSITER, W. E., and MYLLE, M. Localization of urine acidification in the mammalian kidney. *Am. J. Physiol.*, 1960, 198, 581–585.

37. BENESCH, R.; BARRON, N. S., and MAWSON, C. A. Carbonic anhydrase sulphonamides and shell formation in the domestic fowl. (Letter to the editor) *Nature, London*, 1944, 153, 138–139.

LEGENDS FOR FIGURES

FIG. 1. Calcium deposits induced by 3 injections of PTH appear in zone 1A. Zone 1, containing the proximal portions of the proximal convoluted tubules, is free of mineral whereas all of the mineral is evident in the distal portion of the proximal convoluted tubule. Von Kossa stain. × 32.

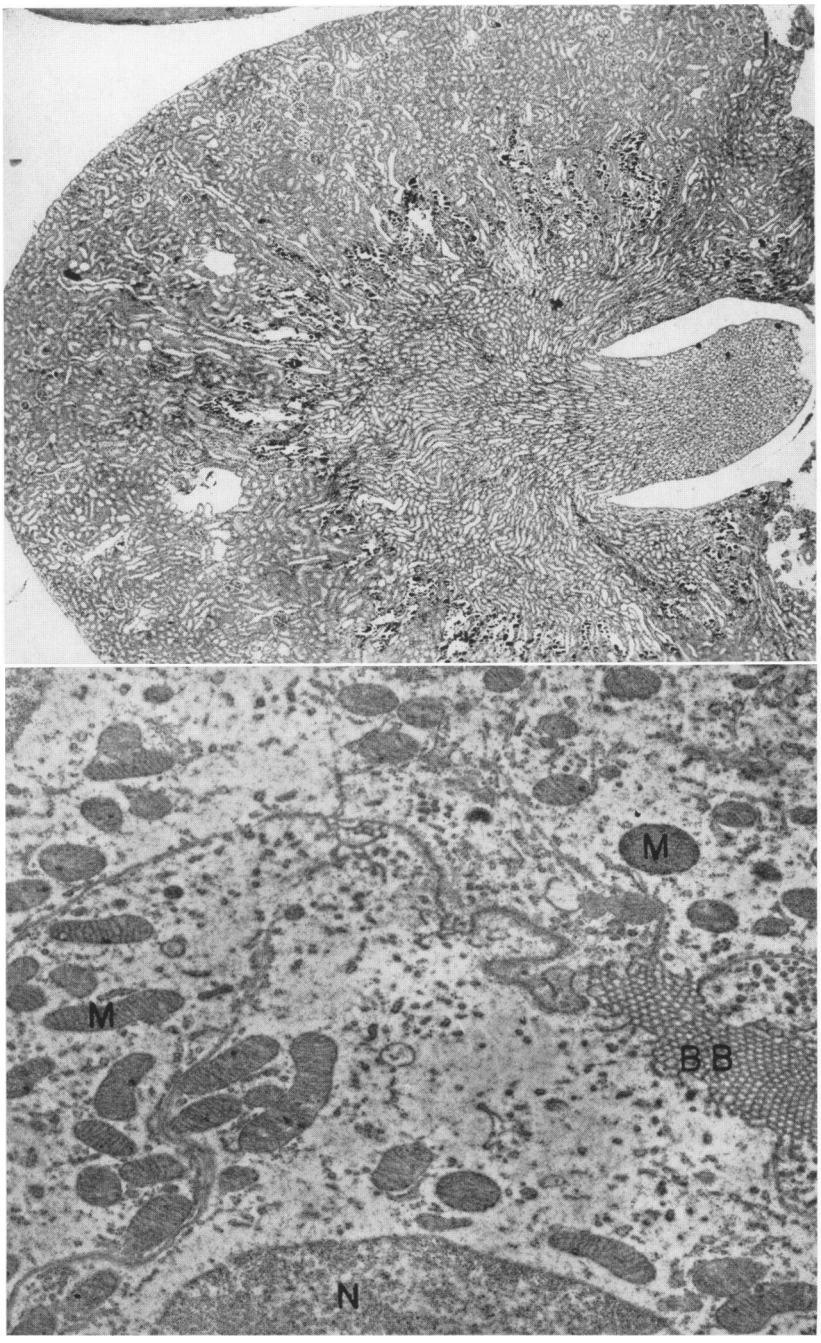


FIG. 2. Distal portion of the proximal convoluted tubule after two injections of PTH. The cell is overhydrated, and at this stage a positive reaction for ionic calcium can be obtained. Though the cytoplasm appears pale and swollen, the nuclei (N), mitochondria (M) and brush border (BB) are normal. × 9,000.

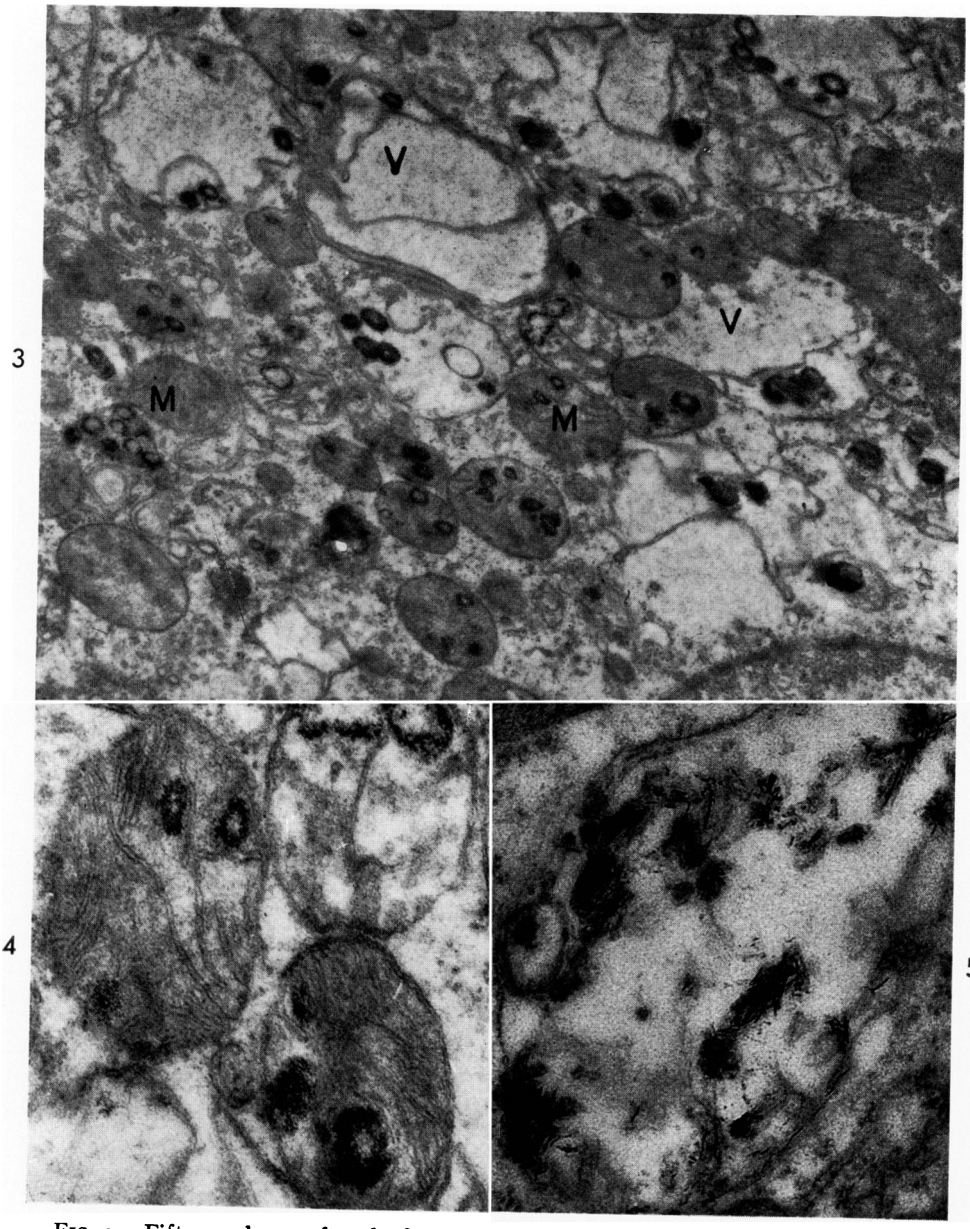
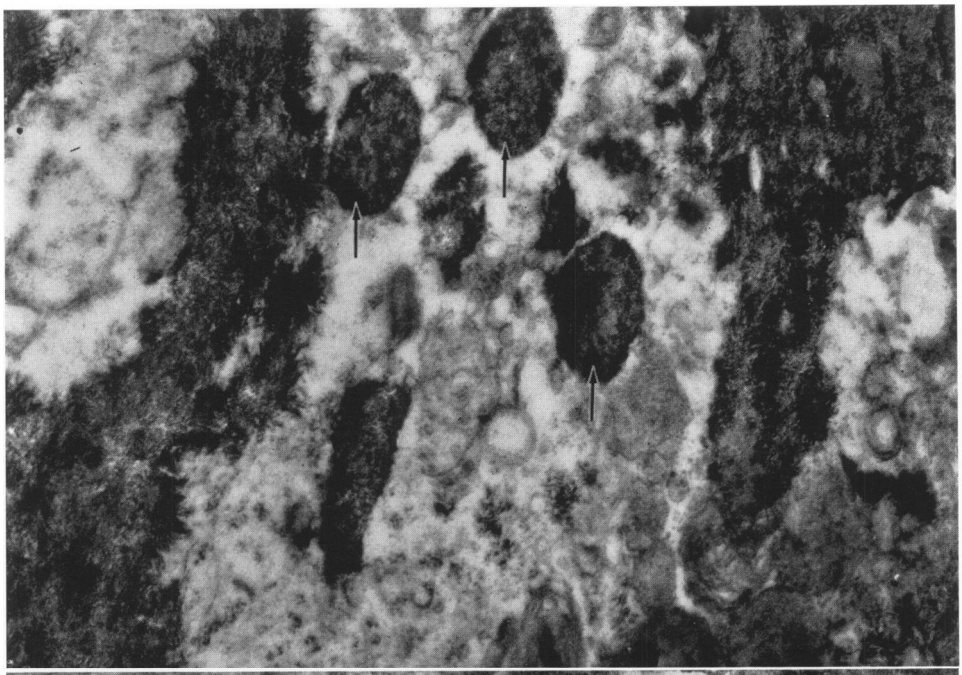


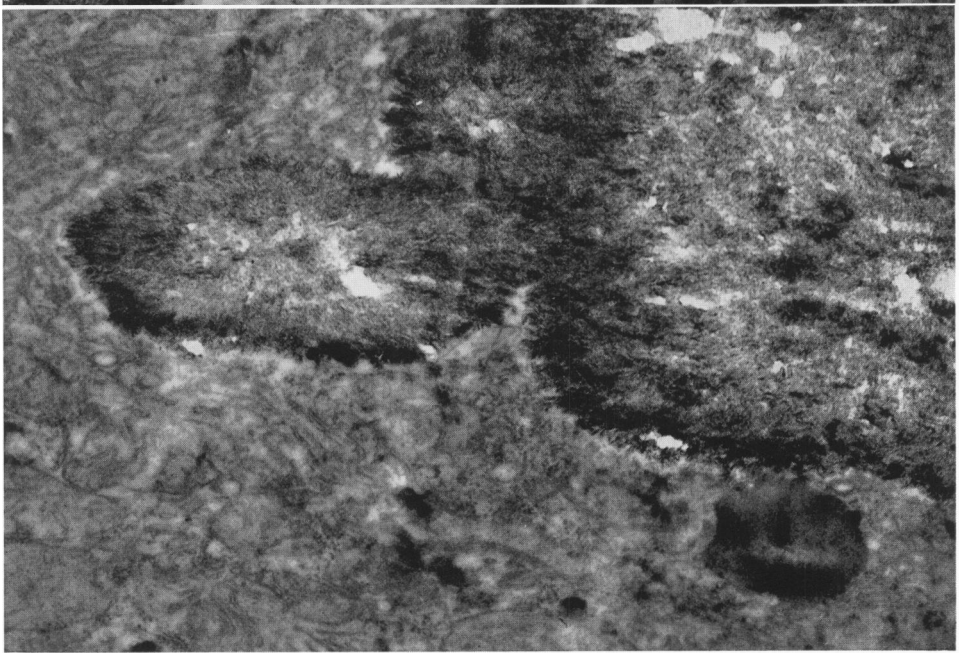
FIG. 3. Fifty-one hours after the first of 3 PTH injections. Dense deposits composed of aggregates of small granules measuring 100 to 200 Å in diameter are visible in mitochondria (M) and vacuoles (V). Many of the vacuoles may be distended mitochondria since membranous remnants are present. × 10,000.

FIG. 4. Higher magnification of the area shown in Figure 3. The small dense spheres have an intramitochondrial location. × 60,000.

FIG. 5. An area from the same kidney illustrated in Figures 3 and 4. The elongated forms are consistent in size and shape with apatite crystals observed in bone. × 60,000.



6



7

FIG. 6. At 57 hours there is more extensive mineralization. Coalescence of mineralized material and intervening non-mineralized cytoplasm are shown. Bodies (arrows) resembling mitochondria in size and shape appear separated from the main mass of mineral. × 9,000.

FIG. 7. At 60 hours large areas of cytoplasm are completely calcified. There is no recognizable structure within the dense mass. However, noncalcified cytoplasm exhibits its usual content of cellular organelles. × 12,000.

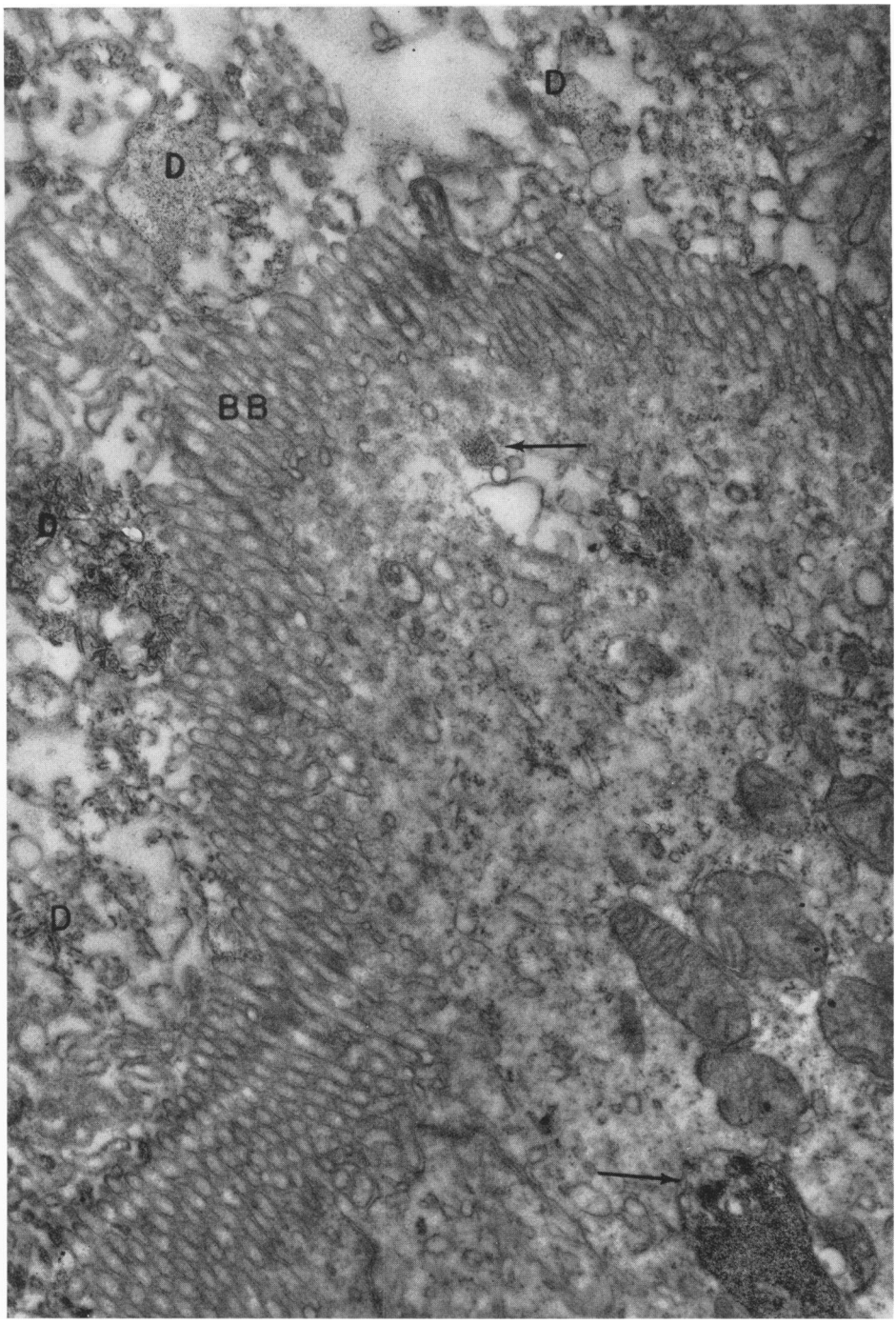
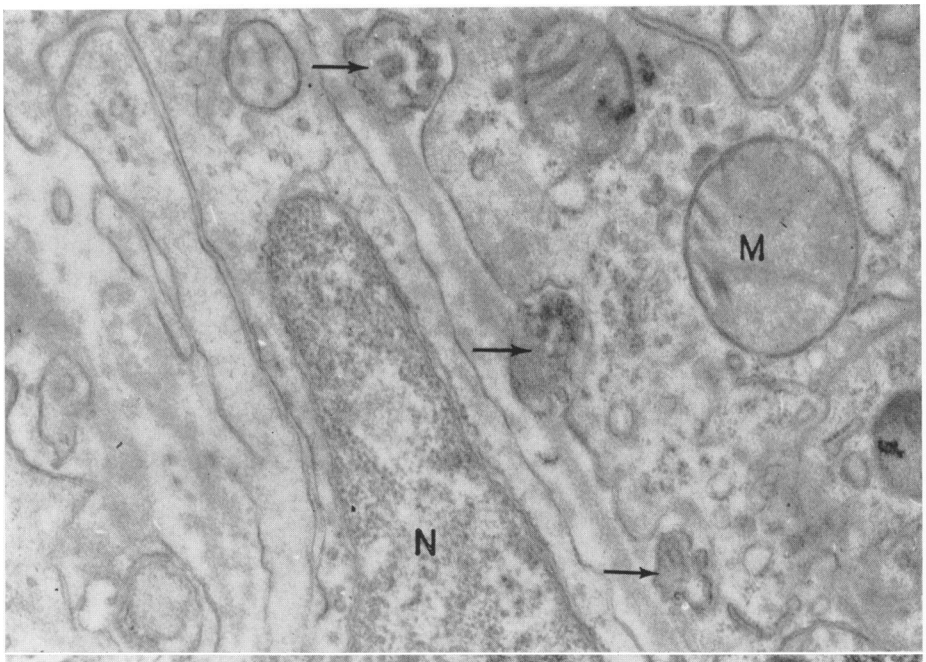
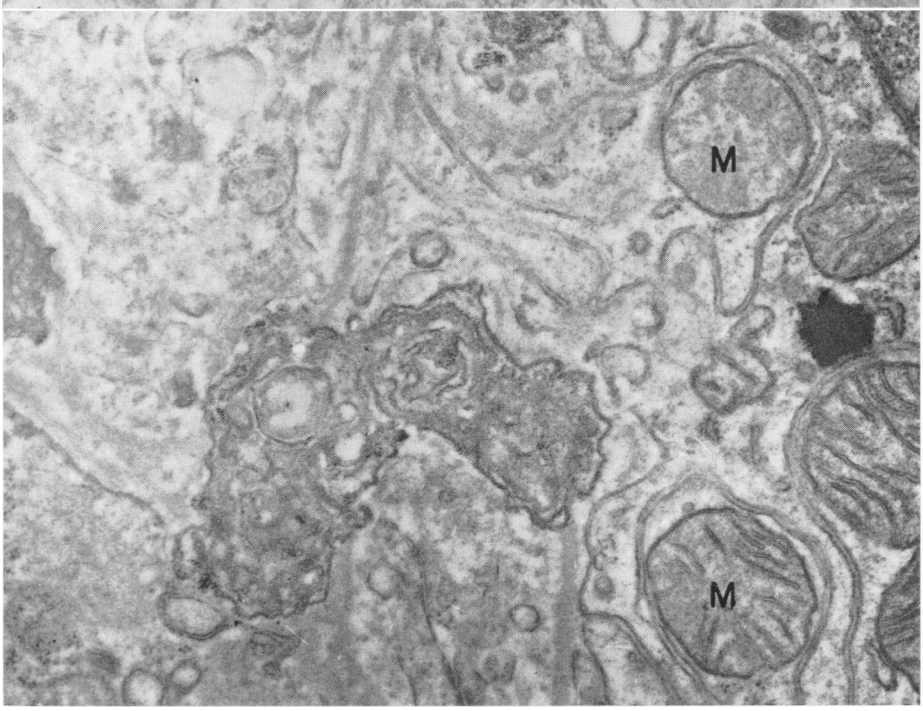


FIG. 8. Three days after the third injection of PTH. Some calcified material remains within cells of zone 1A. (arrows). The tubule lumen contains calcified cellular debris. At this stage the tubular epithelium has a relatively normal appearance, as do its organelles. There has been almost complete recovery from the extensive calcification shown in Figure 7. × 10,000.



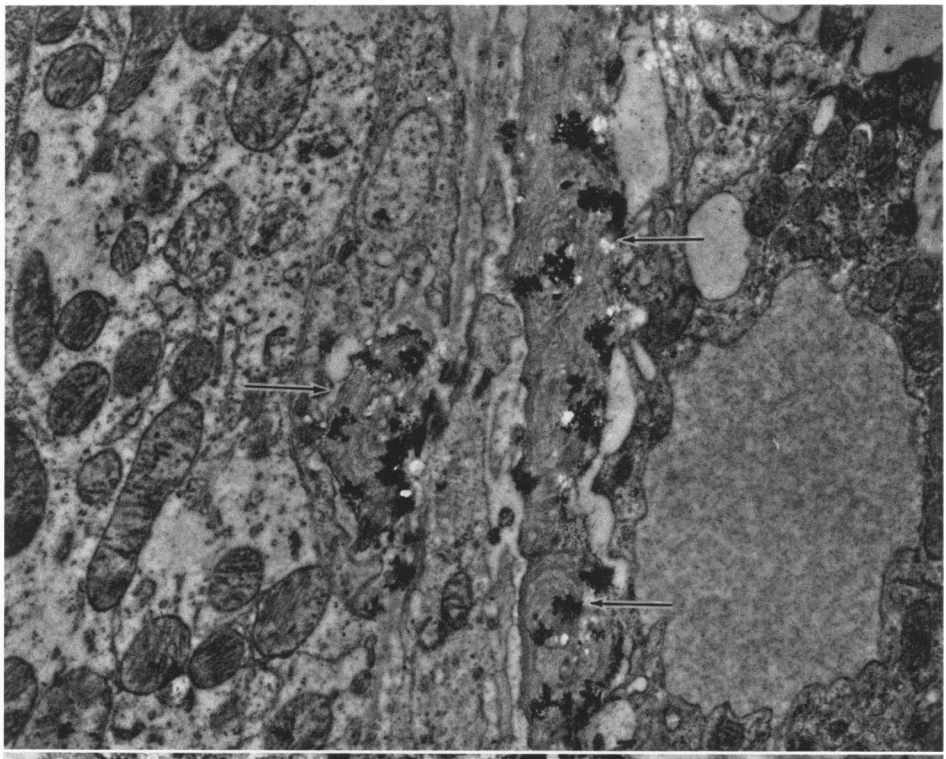
9



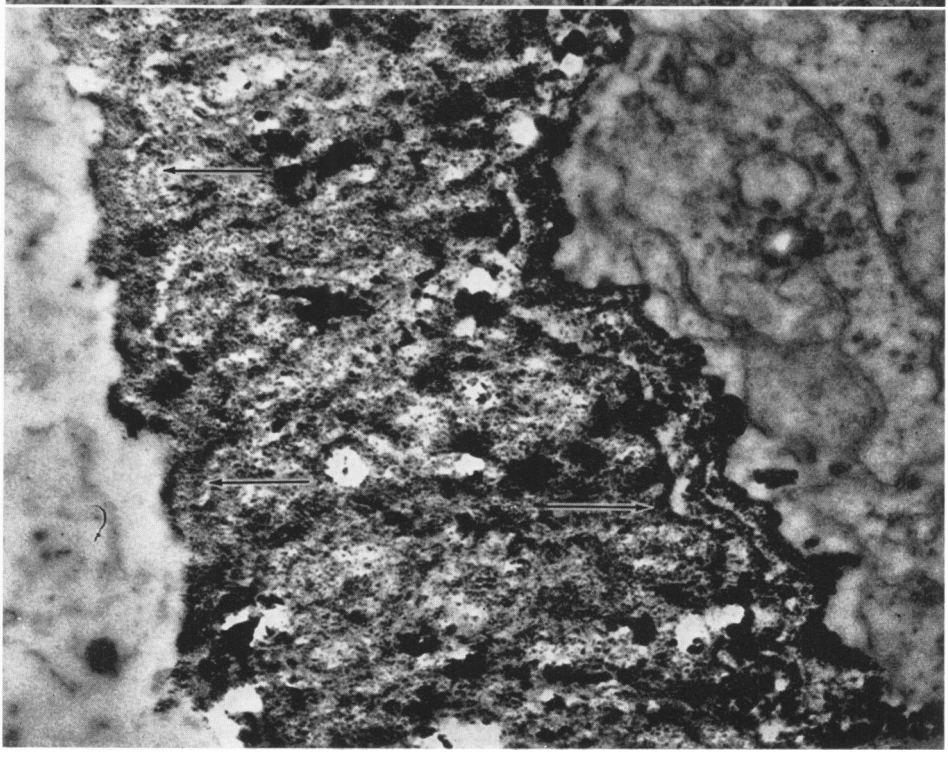
10

FIG. 9. After 3 days of calcium gluconate injection, small ovoid structures composed of concentric lamellas (arrows) appear at the base of proximal tubule epithelium in zone 1. The basement membrane itself is also involved. ×25,000.

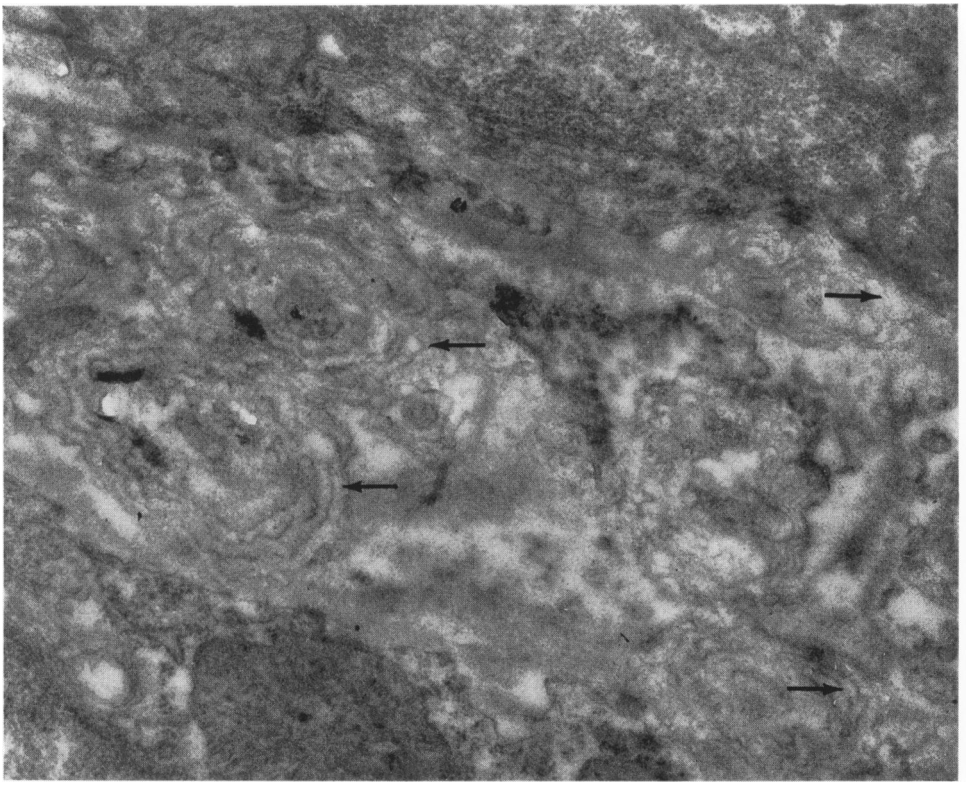
FIG. 10. Tubular epithelium from an animal that received calcium gluconate for 5 days. The laminated bodies are larger and more dense. × 15,000.



11



12



13

FIG. 11. After 12 days of calcium gluconate administration, the laminated bodies are larger and masses of mineral appear within them. × 12,000.

FIG. 12. Eighteen days of calcium gluconate injection. The basement membranes of the proximal tubules in zone 1 contain densely mineralized areas. Remnants of the lamellar structures are still visible (arrows). The mineral deposits involve only portions of the *pars convoluta* but, where present, are of this degree of severity. × 24,000.

FIG. 13. Simultaneous injections of diamox and calcium gluconate result in the production of laminated bodies. However, after 13 days of this treatment the laminated bodies remain uncalcified. × 22,000.