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Disparate Effects of Vitamin D Treatment upon Mitochondrial Granulation in Proximal and Distal Renal Tubule (41581)

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Abstract. The distribution of calcium phosphate granules in mitochondria of proximal and distal renal tubules of nonuremic and uremic children was analyzed by electron microscopy of material obtained by percutaneous kidney biopsy. Although distal tubule had fewer granules/mitochondrion than proximal tubule, uremia induced a significant drop (50%) in both, related to an increase in mitochondria containing 0 granules and a decrease in mitochondria with 2+ granules. The decrease observed in uremic children was reproduced experimentally by partial nephrectomy in rats. Uremia resulted in a 58% decrease of calcium phosphate granules in rat proximal tubule while a smaller but significant decrease (36%) occurred in distal tubule. Vitamin D deficiency in rats was associated with greatly decreased granulation in proximal tubule (80%) whereas distal tubule was less severely affected (36%). Supplementation of vitamin D to uremic rats restored mitochondrial granulation to normal in proximal tubule in 24 hr, but had no effect in distal tubule since the number of granules/mitochondrion, 0.5 ± 0.1, remained statistically similar to that of untreated animals. Granulation in both proximal and distal tubule of uremic rats was unaffected by parathyroid hormone administration. Since restoration of granulation occurred only in proximal tubule, the defect in uremia which can be overcome by vitamin D treatment appears localized at the level of the proximal tubular cell membrane, indicating an action of vitamin D on calcium and/or phosphorus translocation into the proximal tubule.

Electron-dense granules, partly composed of an amorphous form of calcium phosphate (1, 2), are prominent in mitochondria of tissues involved in calcium transport (3, 4). Both their size and density are affected by parathyroid hormone (5) and vitamin D (6) and appear to be related to intracellular calcium and phosphate concentration (3).

We have previously reported (7) that uremia significantly reduced the number of mitochondrial granules in the proximal renal tubule of children and rats and that normal granulation was restored in uremic rats 24 hr after administration of vitamin D, but was unaffected by exogenous parathyroid hormone. Micropuncture studies indicate that parathyroid hormone has distinct effects upon calcium reabsorption in proximal and distal renal tubule, inhibiting reabsorption in the proximal tubule but enhancing it in the distal region of the nephron (8, 9). When parathyroid hormone and serum calcium are controlled, the net effect of vitamin D treatment is enhancement of reabsorption, the site of which is unclear (10).

Since changes in mitochondrial granulation may indicate hormonal action influencing reabsorption by a target organ, we have extended our studies to the distal tubule in order to assess not only the effect of uremia upon granulation but also to determine whether distal tubule granulation in the uremic state is responsive to parathyroid hormone or vitamin D.

Materials and Methods. *Children.* Proximal and distal renal tubule tissue was obtained from nonuremic children (aged 5–18 years) and from uremic children (aged 10–17 years) coincident with diagnostic percutaneous kidney biopsy. None was receiving vitamin D therapy and all were on a general diet. Calcium, phosphorus, alkaline phosphatase, creatinine, blood urea nitrogen (BUN), total protein, and albumin were determined within 24 hr of biopsy, and are summarized in Table I. Renal abnormalities in nonuremic children were nephrotic syndrome (8), hematuria (3), and proteinuria (3). Uremic chil-

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TABLE I. SERUM CHEMISTRY OF NONUREMIC AND UREMIC CHILDREN

	Nonuremic children (n = 13)	Uremic children (n = 13)
Calcium (mg/dl)	9.2 ± 0.4	8.6 ± 0.3
Phosphorus (mg/dl)	4.8 ± 0.2	5.3 ± 0.2
Ca × P product (mg/dl)	43.6 ± 6.8	47.2 ± 11.5
Alkaline phosphatase (IU)	111 ± 16	58 ± 10
Creatinine (mg/dl)	0.7 ± 0.1	3.3 ± 0.8
BUN (mg/dl)	13 ± 1	53 ± 9
Total protein (g/dl)	5.6 ± 0.4	5.8 ± 0.3
Albumin (g/dl)	3.2 ± 0.4	2.7 ± 0.2

dren were diagnosed as having Goodpasture's syndrome (2), hemolytic uremic syndrome (4), acute glomerulonephritis (4), sclerosing glomerulonephritis (1), membranoproliferative glomerulonephritis (1), and familial nephritis (1). Average serum creatinine level in uremic children was 3.3 ± 0.8 (Table I).

Rats. Control and experimental male Sprague–Dawley rats were 4 weeks old and weighed between 90 and 115 g. They were given standard rat chow and water *ad libitum.* Uremia was induced by removal of the left kidney, followed by removal of 3/4 of the right kidney 24 hr later. A BUN level of 100 mg/dl 7 days after the initial operation, assayed by the diffusion method (11), was used to define uremia. Weanling vitamin D deficient rats were fed a vitamin D deficient diet (ICN Pharmaceuticals) containing 0.47% calcium and 0.3% phosphorus, starting at day 18 after birth and continuing for 41 days. Vitamin D deficiency was established on the basis of a low serum calcium (5.5–6.0 mg/dl) and the ability of isolated kidney to convert 75% or more of administered ^{3H}25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3. Vitamin D_2 (1600 U, Drisdol) was administered to uremic rats by gavage 24 hr before sacrifice. Parathyroid extract (PTE, 100 USP U, Lilly) was given intraperitoneally 5 hr before sacrifice.

Fresh tissue was fixed immediately in 1% glutaraldehyde buffered with 0.1 *M* cacodylate (pH 7.4). Postfixation was in 1% osmium tetroxide in the same buffer at the same pH. Tissue was dehydrated in ethanol and embedded in Epon–Araldite. Following ultramicrotome sectioning (0.05 μm), tissue was stained in uranyl acetate and lead citrate, and examined on an RCA Emu-3F transmission electron microscope. Proximal tubule was identified by the presence of brush borders; distal tubule by a luminal surface with only vestigial microvilli.

Twenty electron micrographs were printed on standard photographic paper at ×32,000 for each sample and coded to prevent bias in the analysis. Granules per mitochondrion were counted by three independent investigators and the results were averaged. Mitochondria were sorted into categories containing 0, 1, and 2+ granules. Results are expressed as the mean ± 1 SD. Significance was established by unpaired Student's t test.

Results. *Children.* A signifcant decrease ($P < 0.001$) in mitochondrial calcium phosphate granulation induced by uremia in both proximal and distal renal tubule is shown in Fig. 1. Although distal tubule had fewer granules per mitochondrion than proximal tubule, uremia resulted in an approximately 50% decrease in granulation in both.

Figure 2 illustrates that the decrease in granulation in uremia in both proximal and distal renal tubule was related to an increase in the percentage of mitochondria containing 0 granules and a decrease in the number of mitochondria containing 0 granules and a decrease in the number of mitochondria containing 2+ granules ($P < 0.001$ in both cases).

Rats. The decrease in mitochondrial granulation observed in uremic children was reproduced experimentally by partial nephrec-

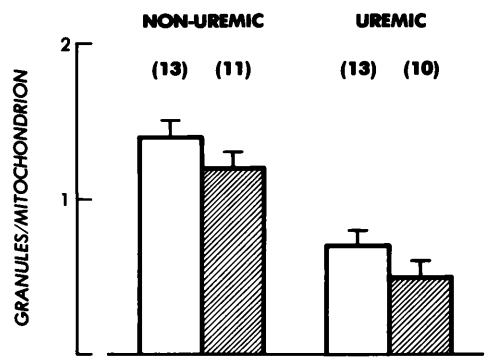

FIG. 1. Number of electron-dense, calcium phosphate granules per mitochondrion in the proximal (open bars) and distal (closed) renal tubule of nonuremic and uremic children. (*N*), total number of samples.

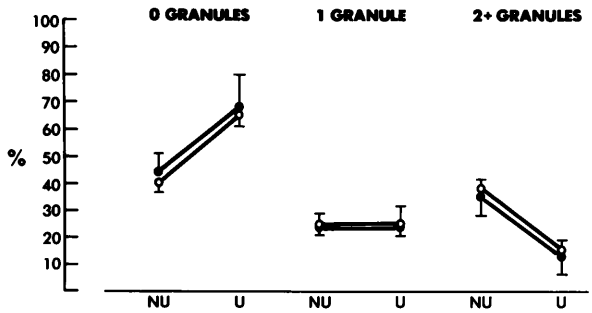

FIG. 2. Percentage of mitochondria containing 0, 1, or 2+ granules from proximal (open circles) and distal (closed circles) renal tubule in nonuremic (NU) and uremic (U) children. Number of samples is the same as in Fig. 1.

tomy in rats (Fig. 3). As in children, there were more granules per mitochondrion in the proximal tubule of nonuremic rats compared to the distal tubule (1.2 ± 0.1 versus 0.86 ± 0.1, respectively). Uremia resulted in a decrease of 58% in proximal tubule (0.5 ± 0.1 granules/mitochondrion) ($P < 0.001$) whereas a smaller (35%) but equally significant ($P < 0.001$) decrease was found for distal tubule. Vitamin D deficiency greatly decreased mitochondrial granulation in proximal tubule with an approximately 80% decrease. Granulation in distal tubule was less severely affected but fell to levels similar to those observed in uremia (35% decrease). Supplementation of vitamin D to uremic rats restored mitochondrial granulation to normal in the proximal tubule in 24 hr. Vitamin D treatment had no effect upon granulation in the distal tubule of uremic rats with the number of granules/mitochondrion, 0.45 ± 0.1, remaining essentially similar to that of untreated animals. Parathyroid hormone administration to uremic rats did not affect granulation in either the proximal or distal renal tubule.

The distribution of mitochondrial granulation in the proximal and distal renal tubule of rats is illustrated in Fig. 4. As occurred in children, the decrease in granulation induced by uremia in rats was associated with a significant increase ($P < 0.001$) in the number of mitochondria containing 0 granules and a significant decrease ($P < 0.001$) in the number containing 2+ granules in the proximal tubule. The number of mitochondria contain-

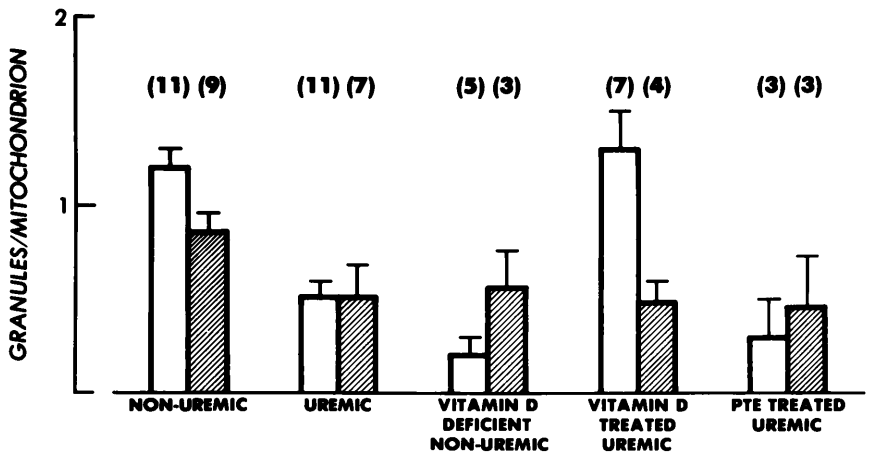

FIG. 3. Number of granules per mitochondrion in the proximal (open bars) and distal (closed bars) renal tubule of nonuremic, uremic, vitamin D-deficient nonuremic, vitamin D-treated uremic, and PTE-treated uremic rats. (N), total number of samples.

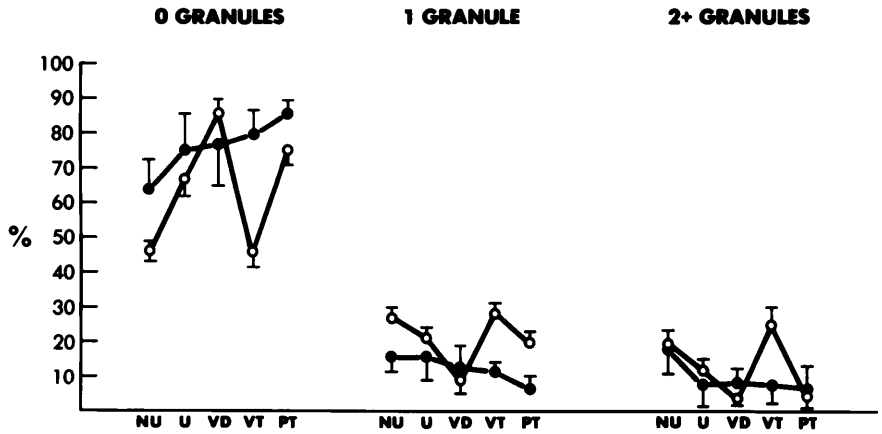

FIG. 4. Percentage mitochondria containing 0, 1, or 2+ granules from proximal (open circles) and distal (closed circles) renal tubule in nonuremic (NU), uremic (U), vitamin D-deficient nonuremic (VD), vitamin D-treated uremic (VT), and PTE-treated uremic rats (PT). Total number of samples is the same as in Fig. 3.

ing 0 granules was greater and more variable in nonuremic distal tubules and although uremia resulted in an increased number of mitochondria with 0 granules and decreased number with 2+ granules, the differences due to the variability were not significant at the same level ($P \simeq 0.001$). Vitamin D deficiency in proximal tubule was characterized by a large increase in the number of mitochondria containing 0 granules and by significant decreases in the number with 1 and 2+ granules ($P < 0.001$). The distal tubule results were again variable, but showed the same trend. Vitamin D treatment of uremic rats restored the granulation pattern observed in control, nonuremic rats for the proximal tubule ($P < 0.001$) but had no effect on the granulation pattern in distal tubule, which remained statistically similar to that of control uremic rats. Parathyroid hormone was also without effect.

Discussion. These studies clearly demonstrate that uremia induces a significant drop in the number of calcium phosphate granules in mitochondria in both the proximal and distal renal tubule of children and rats. Chronic renal failure is associated with high serum phosphorus and secondary hyperparathyroidism (12), conditions which generally stimulate granulation in such transport tissues as osteoblasts (13), osteoclasts (14), intestine (15), and normal kidney (5). Other factors appear to be present in uremia which modify the high serum phosphorus and parathyroid hormone effect.

Average serum calcium was lower and average serum phosphate was higher in uremic than in nonuremic children. Our previous studies have established that serum calcium and phosphate solubility product is unrelated to mitochondrial granulation in proximal tubule (7). It is possible that decreased granulation in both the proximal and distal renal tubule was related to an effect of uremia upon calcium and/or phosphate translocation in the tubule, either at the level of the cell membrane, mitochondrial membrane or directly upon formation of the granules.

None of the uremic children in these studies was receiving vitamin D therapy. Impaired uptake and release of calcium from intestinal mitochondria, unresponsive to 25-hydroxyvitamin D_3 treatment, have been reported in uremic rats (16). Portale *et al.* (17) have correlated a decrease in the level of the most active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3, with the severity of renal failure, and it is generally agreed that very low concentrations of this metabolite are present in patients with end stage renal failure (12). In the present experiments, a pharmacological dose of vitamin D_2 was utilized to overcome the characteristic vitamin D resistance associated with the uremic state, obviating the necessity for normal formation of physiolog-

ically active metabolites such as 1α,25-dihydroxyvitamin D_3 to achieve a vitamin D effect. Mitochondrial granulation was restored to normal levels in the proximal tubule of rats by administration of pharmacological amounts of vitamin D_2 but was unaffected in the distal tubule. Parathyroid hormone treatment failed to restore granulation in either region. These findings suggest a direct effect of vitamin D upon calcium and/or phosphate translocation, localized in the proximal renal tubule. If the restoration of granulation in uremia was mediated by a general effect of vitamin D upon uptake of calcium by mitochondria or upon granule formation within the mitochondrial matrix both the proximal and distal tubule should have responded. Since restoration of granulation occurred only within the proximal region, it seems likely that the defect in uremia which can be overcome by vitamin D treatment is at the level of the proximal tubular cell membrane, indicating an action of vitamin D on calcium and/or phosphorus translocation into the proximal tubule. The cause of unresponsiveness of the distal tubule to vitamin D treatment as well as the fall in mitochondrial granulation in both children and rats in this region cannot be ascertained from these experiments, but may be related to conditions which prevail in uremia other than relative deficiency of physiologically active vitamin D metabolites.

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