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The duodenal mucosa in patients with renal failure: Response to $1,25(OH)_2D_3$

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The duodenal mucosa in patients with renal failure: Response to $1,25(OH)_2D_3$. The structure of the duodenal mucosa was evaluated in duodenal biopsy samples obtained from patients with moderate renal failure (MRF) and in dialysis patients (HD) in an effort to examine the possibility that changes in duodenal mucosa may contribute to the impaired calcium absorption in renal failure (RF). The effect of therapy with $1,25(OH)_2D_3$ on the duodenal mucosa in the HD patients was also studied. The results show that both MRF and HD patients have reduction in calcium reabsorption and in the length of their intestinal villi and crypts of Lieberkuhn. In the HD patients, these structural changes were more severe. Treatment with $1,25(OH)_2D_3$ produced significant improvement in calcium reabsorption ($P < 0.01$) as well as in length of villus and crypt ($P < 0.02$) and increased mitotic activity in the crypts ($P < 0.02$). Electron microscopy revealed the microvilli to be shorter, irregularly distributed, moth-eaten, and grainy, with these abnormalities disappearing after treatment. The data show that duodenal mucosa in RF exhibits structural abnormalities, which were normalized after $1,25(OH)_2D_3$ therapy, and suggest that these derangements may play a role in the defective calcium reabsorption in RF.

La muqueuse duodénale chez les malades en insuffisance rénale: Réponse au $1,25(OH)_2D_3$. La structure de la muqueuse duodénale a été évaluée sur des biopsies duodénales de malades atteints d'insuffisance rénale modérée (MFR) et de malades en hémodialyse (HD) afin d'étudier l'hypothèse selon laquelle des modifications de la muqueuse duodénale pourraient contribuer à l'altération de l'absorption du calcium au cours de l'insuffisance rénale. L'effet du traitement par $1,25(OH)_2D_3$ sur la muqueuse duodénale a été étudié chez les malades HD. Les résultats montrent que les malades MRF et HD ont une diminution de l'absorption du calcium et de la longueur de leurs villosités intestinales et de leurs cryptes de Lieberkuhn. Chez les malades HD ces modifications de structure sont encore plus sévères. Le traitement par $1,25(OH)_2D_3$ détermine une amélioration significative de l'absorption du calcium ($P < 0,01$) de même qu'une augmentation de la longueur des villosités et des cryptes ($P < 0,02$) et une augmentation de l'activité mitotique dans les cryptes ($P < 0,02$). La microscopie électronique montre que les microvillosités sont raccourcies, irrégulièrement distribuées et d'aspect mité et granuleux, anomalies qui disparaissent après le traitement. Les résultats montrent que la muqueuse duodénale des malades RF a des anomalies de structure qui sont normalisées au cours du traitement par $1,25(OH)_2D_3$ et suggèrent que ces modifications peuvent jouer un rôle dans le déficit de au cours de RF.

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It is common to find impaired intestinal absorption of calcium in patients with chronic renal failure [1, 2]. This abnormality has been shown to improve after therapy with 1,25-dihydroxycholecalciferol ($1,25[OH]_2D_3$) [3]. Results of in vitro studies have attributed the improvement of the intestinal absorption of calcium with $1,25(OH)_2D_3$ to enhanced production of an intestinal calcium binding protein [4–10].

It is also possible that either uremia itself or the deficiency of $1,25(OH)_2D_3$ in uremia [11, 12] may alter the structure of the intestinal mucosa and, as such, may affect intestinal absorption of calcium. Indeed, animal studies demonstrated that vitamin D [13, 14] does affect the structure of the intestinal mucosa. Data on the effect of uremia or vitamin D status on the structure of the mucosa of the human intestine are lacking.

The present study was undertaken to examine the structure of the duodenal mucosa in patients with renal failure and to evaluate the effect of $1,25(OH)_2D_3$.

Methods

Studies were carried out on eight patients. Three of them had moderate renal insufficiency, and five patients were treated with chronic hemodialysis from 12 to 78 months (mean, 49 ± 11 months). Those patients with moderate renal failure were not receiving any medication, and their dietary intake of calcium was 300, 457, and 700 mg/day. The pa-

tients on dialysis treatment were receiving folic acid (1 mg/day) and ferrous sulfate (900 mg/day), and their dietary intake of calcium ranged between 460 and 600 (544 ± 25) mg/day. The clinical data describing our patients are shown in Table 1. Patients with moderate renal failure had only baseline evaluation, which included blood chemistry, intestinal calcium absorption, and duodenal biopsy. The patients on dialysis underwent similar evaluation both before and after 6 months of treatment with $1,25(OH)_2D_3$. Prior to the initiation of therapy, blood samples were obtained on three different occasions over a 2-week period and served as the baseline values for total and ionized calcium, phosphorus, parathyroid hormone, alkaline phosphatase, and creatinine. Three 24-hour urine collections were obtained in the three patients who were not treated with hemodialysis in order to measure endogenous creatinine clearance. During therapy with $1,25(OH)_2D_3$, all of these parameters were measured weekly except for alkaline phosphatase, which was measured monthly. The concentrations of alkaline phosphatase, phosphorus, and creatinine were determined by standard laboratory techniques, and those of calcium and magnesium by a atomic absorption spectrophotometer (Perkin-Elmer, model 503). Ionized calcium was measured with an Orion electrode (SS-20, Orion Biomedical, Cambridge, Massachusetts). The levels of PTH in serum were determined by radioimmunoassay with sheep antisera 478 (supplied by Dr. C. Arnaud), bovine PTH labeled with iodine 125, and pooled sera from patients with renal failure as a standard. This assay recognizes predominantly carboxy terminal fragments of PTH. The normal values for this assay in our laboratory are up to 15 μlEq/ml. The intestinal absorption of calcium was determined by the method of Curtis, Fellows, and Rich [15] modified in our laboratory [16]. We used oral and i.v. administration of calcium 47. The oral isotope was delivered with 250 mg of calcium gluconate as a carrier. Forearm radioactivity was measured by a large-volume scintillation counter (Armac, Packard Instrument Co.) 23 to 25 hours after both the oral and i.v. doses of calcium 47 were administered, and the fractional intestinal absorption of the ingested calcium 47 was calculated in a manner previously described [16]. The validity of this arm-counting method for calcium absorption was previously ascertained in our laboratory in 10 subjects by the simultaneous determination of fecal recovery of calcium 47. The results showed that the values for calcium measured by arm counting and fecal recovery of calcium 47 fell near the line of identify [16]. Furthermore, measurement of calcium 47 absorption in the same subjects on two occasions separated by 1 to 25 months demonstrated good reproducibility [16].

Duodenal biopsies were performed with a flexible endoscopy during the baseline period of all patients and after 6 months of therapy in the patients undergoing long-term dialysis. Each biopsy sample was taken on the medial wall in the second portion of the duodenum between the valvulae conniventes. We chose this site because the effect of vitamin D on calcium transport is most pronounced in the proximal duodenum [17]. All studies were performed after their nature and risks were explained to the patients and informed consent was obtained.

Often, a suction instrument is considered a more suitable technique for obtaining a small-bowel biopsy sample because this method allows for proper orientation under a dissection microscope for histologic evaluation. But, the punch biopsy method that we used, although somewhat more difficult to orient microscopically, was proven to provide adequate orientation when the slides were examined.

Table 1. Baseline clinical data on patients

Patient status and no.	Age *yr*	Sex	Creatinine clearance *ml/min*	Duration of renal disease or dialysis therapy *yr*	Diagnosis of renal disease
With moderate renal failure					
1	42	F	51	3	Polycystic kidneys
2	63	F	49	7	Nephrosclerosis
3	31	F	32	6	Proliferative glomerulonephritis
On long-term hemodialysis					
1	27	M		3.5	Chronic glomerulonephritis
2	53	M		5	Nephrosclerosis
3	27	F		4.3	Chronic interstitial nephritis
4	36	M		6.5	Acute poststreptococcal glomerulonephritis
5	26	M		1	Focal sclerosis

The biopsy specimen was fixed in formaldehyde, processed through paraffin, cut at 6 μ, and stained with hematoxylin-eosin and by the periodic-acid Schiff and Schmorl techniques. All available material was embedded and examined. The entire specimen was cut. Depending on its size, it provided 6 to 18 sections. Examination of the histologic preparation in the permanent slide indicated adequate orientation. All sections were examined, and the following parameters were evaluated: *(1)* the length of villi and crypts (only the longest, complete, right-angle oriented, nontangential structures were measured in each section), using a micrometer *(2)* the number of columnar, goblet, Paneth, and argentaffin cells (expressed as percent of total mucosal cells in the measured villi and crypts), *(3)* and the numbers and types of cells in the lamina propria. Tissue for electron microscopy was fixed in glutaraldehyde, processed through epon, cut at 600 to 700 Å and examined on an electron microscope (Hitachi HV-11B). The entire specimen was examined in each case, and the following parameters were evaluated: *(1)* length, shape, and uniformity of microvilli, *(2)* nature of the glycocalyx, and *(3)* number and condition of mitochondria, lysosomes, endoplasmic reticulum, Golgi apparatus, and other organelles. Representative photographs were taken from the tip and middle of a villus from a crypt in each case. The light and electron microscope evaluations were performed by the same pathologist. Dr. Horowitz (Director of Pathology at St. Joseph Medical Center) examined all sections twice, with a 6-week interval between the two readings. Dr. Horowitz, as evaluator, was not aware of the name, diagnosis, or treatment status of the patients from whom the biopsy samples were obtained. The measurements were made of those villi and crypts that were longest and showed no effect of tangential cut, and the mitotic counts were made on the best (the most complete or least tangential) crypts in the specimen.

Therapy with $1,25(OH)_2D_3$ was begun at a dose of 0.5 μg/day. The dose was increased by 0.5 μg/day at 2-month intervals only if the serum calcium concentration did not increase by 1.00 mg/dl. After 6 months of therapy, four of the five dialysis patients were taking 1.5 μg of $1,25(OH)_2D_3$ per day. Statistical analyses were made by the Student's paired *t* test.

Results

The results of the biochemical parameters, intestinal absorption of calcium, and light and electron microscopy of the duodenal mucosa are shown in Tables 2 and 3 and Figs. 1 through 3. Treatment of the five patients undergoing dialysis was associated with a rise in the serum concentrations of total and ionized calcium, a fall in serum alkaline phosphatase, and a decrease in blood levels of PTH. We emphasize, however, that the serum PTH levels after therapy were still markedly elevated. The concentration of serum phosphorus showed no consistent trend.

Table 2. Biochemical data on patients with moderate renal failure and patients on dialysis before and after 6 months of treatment with $1,25(OH)_2D_3$[a]

Patient status and no.	Total calcium *mg/dl*		Ionized calcium *mg/dl*		Phosphorus *mg/dl*		Alkaline phosphatase *BLB units/dl*		Parathyroid hormone *mlEq/ml*		Dose of $1,25(OH)_2D_3$ *μg/day*
	Base	After D_3	Base	After D_3	Base	After D_3	Base	After D_3	Base	After D_3	
With moderate renal failure											
1	9.50		3.90		3.3		1.1		7		
2	9.25		3.84		3.1		2.3		22		
3	9.80		4.04		3.4		4.0		31		
Mean	9.52		3.93		3.3		2.5		20		
± SEM	±0.16		±0.06		±0.1		±0.8		±5		
On long-term chronic dialysis											
1	9.63	10.24	3.90	4.09	3.2	2.4	4.0	3.2	260	120	0.5
2	9.10	9.80	4.20	4.07	4.1	2.8	14.7	10.5	800	485	1.5
3	8.24	9.16	3.74	3.77	3.9	4.4	77.6	52.0	310	215	1.5
4	8.45	9.31	3.54	4.02	4.3	5.0	33.2	14.4	800	498	1.5
5	6.90	9.79	2.98	4.22	5.4	5.2	4.8	3.2	540	147	1.5
Mean	8.46	9.66	3.67	4.03	4.2	4.0	26.9	16.7	542	293	
± SEM	±0.46	±0.19	±0.20	±0.07	±0.3	±0.6	±13.7	±9.1	±115	±83	

[a] "Base" refers to baseline value, and "After D_3" refers to after 6 months' treatment with $1,25(OH)_2D_3$. Normal values in our laboratory are: total calcium, 9.0 to 10.5 mg/dl; ionized calcium, 3.90 to 4.50 mg/dl; inorganic phosphorus, 3.0 to 4.5 mg/dl; alkaline phosphatase, 1 to 3 Bessey-Lowry-Brock units/dl; PTH, <15 μlEq/ml.

Table 3. Effect of moderate renal insufficiency and end-stage renal disease on the fractional intestinal absorption of calcium and the morphology of the duodenal mucosa and the response to treatment with $1,25(OH)_2D_3$ in end-stage renal disease[a]

Patient status and no.	Intestinal calcium absorption		Length of villus *mm*		Length of crypt *mm*		Mitotic activity *no. mitoses/crypt*	
	Base	After D_3	Base	After D_3	Base	After D_3	Base	After D_3
With moderate renal failure								
1	0.19		0.50		0.20		0.60	
2	0.20		0.65		0.30		0.40	
3	0.17		0.55		0.22		0.10	
On long-term hemodialysis								
1	0.18	0.28	0.63	0.75	0.28	0.27	0.15	0.75
2	0.13	0.57	0.43	0.65	0.17	0.30	0.55	2.00
3	0.17	0.75	0.37	0.55	0.15	0.25	0.20	1.50
4	0.12	0.64	0.47	0.62	0.22	0.30	0.10	1.30
5	0.28	0.63	0.40	0.85	0.30	0.35	0.60	0.70

[a] "Base" refers to baseline value, and "After D_3" refers to after 6 months' treatment with $1,25(OH)_2D_3$. Normal values for fraction of ingested calcium absorbed are 0.17 to 0.37 (0.27 ± 0.03). Normal values for length of villus are 0.5 to 1.5 mm, and for length of crypt are 0.3 to 0.5 mm [38].

The baseline determination of the fractional absorption of calcium was below normal or at the lower range of normal values in all patients with moderate renal failure and in four of the five patients on dialysis. All patients on dialysis showed marked increases in the fraction of intestinal calcium absorption (from 0.18 ± 0.03 to 0.57 ± 0.08, $P < 0.01$).

In the three patients with moderate renal failure, the length of intestinal villi was in the lower range of

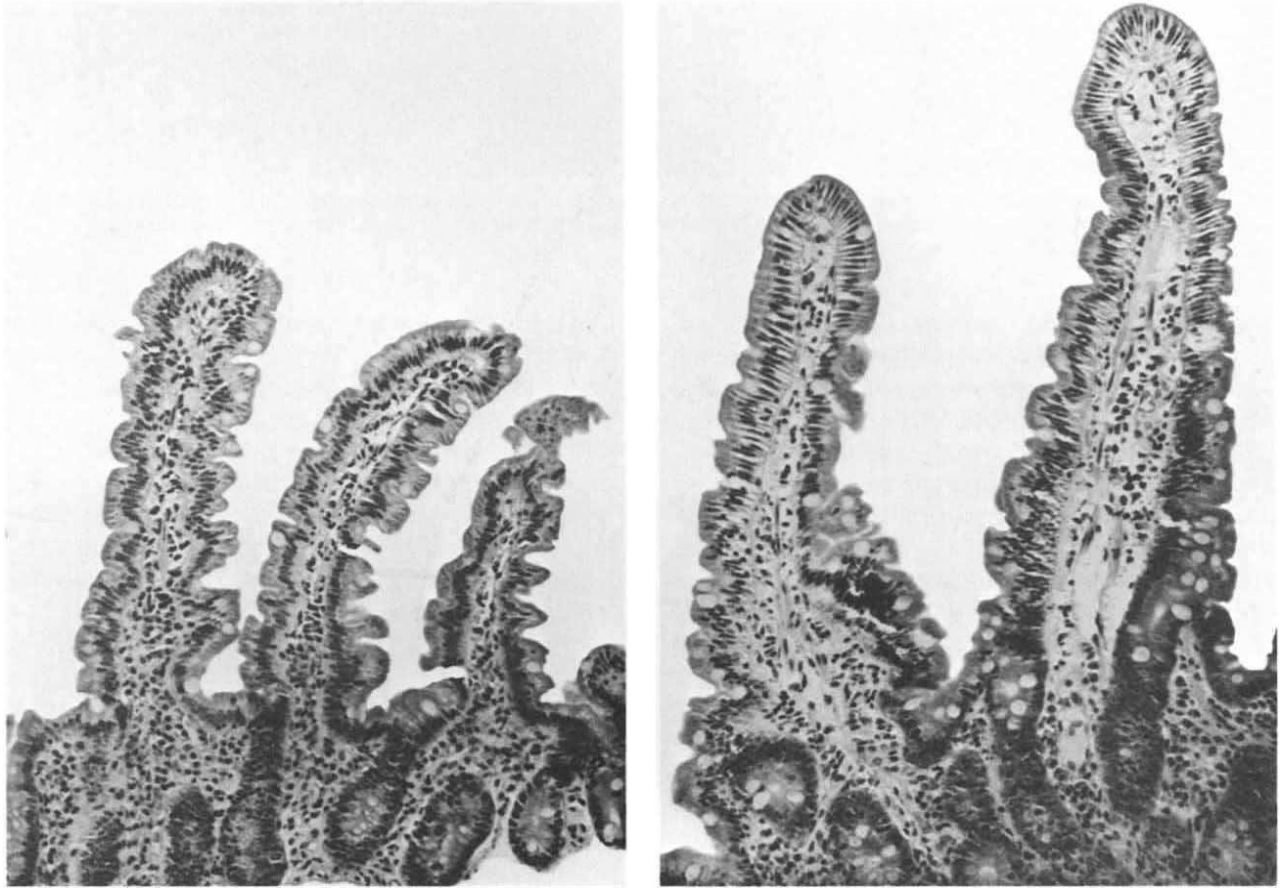

Fig. 1. *Duodenal villi of dialysis patient no. 3 (Table 1) before* (left) *and after* (right) *6 months $1,25(OH)_2D_3$ treatment.* (Hematoxylin-eosin stain; magnification ×450)

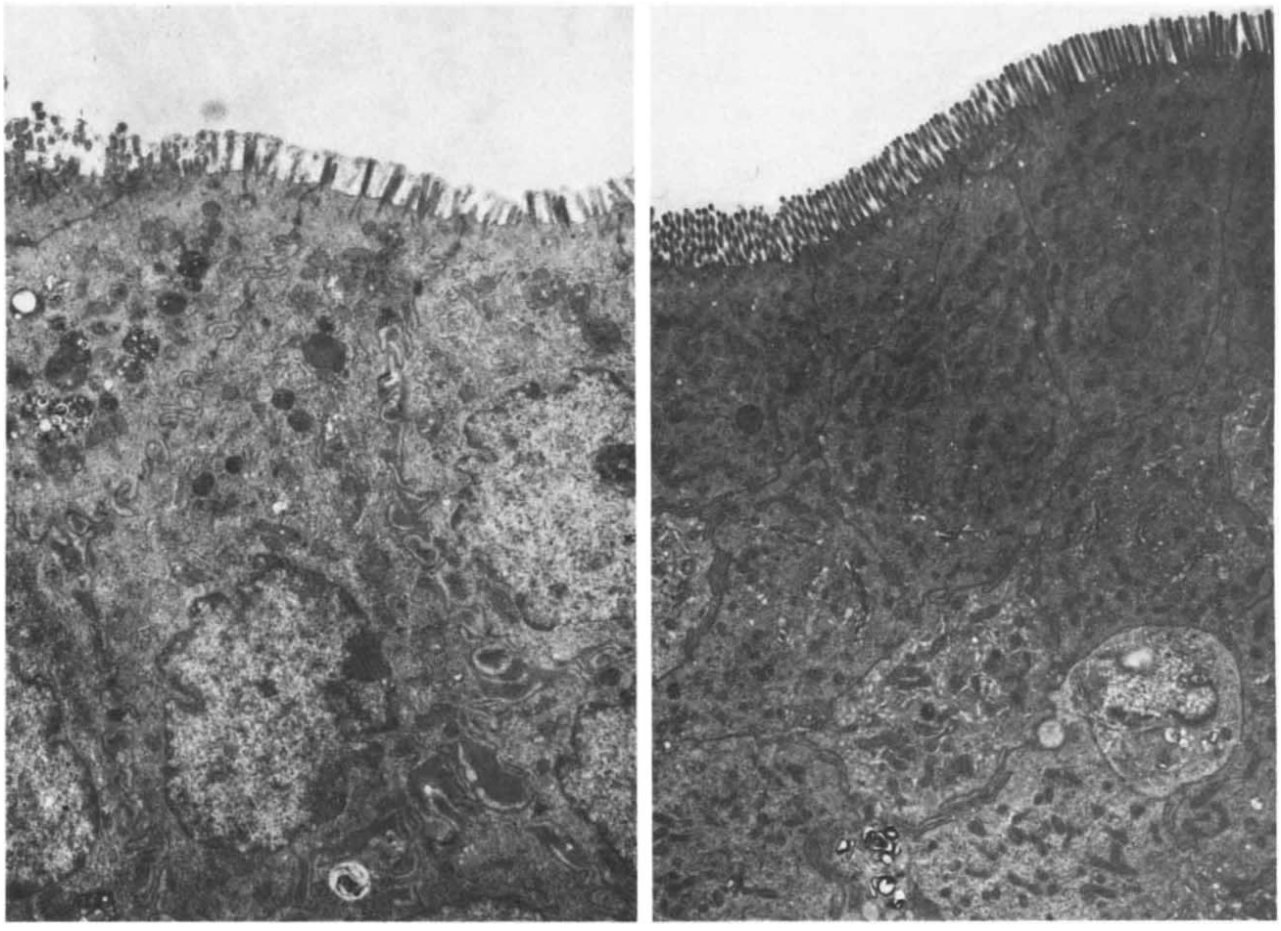

Fig. 2. *Electron photomicrographs of the surface of mucosal cells of duodenal villi of dialysis patient no. 3 before* (left) *and after* (right) *6 months of 1,25(OH)$_2$D$_3$ treatment.* The microvilli before treatment are relatively short and irregular and have a granular glycocalyx compared with microvilli after treatment. (×6000)

normal, the length of the crypts of Lieberkuhn was abnormally low in two of these patients, and the mitotic activity in the crypts was comparable with that observed in patients on dialysis prior to their treatment with 1,25(OH)$_2$D$_3$. In the patients on dialysis, the length of the crypts and villi were less than normal in four of five patients (Table 3, Fig. 1). Electron microscopy of the villi of these patients revealed the microvilli, both at the tip and in the mid zone of the villus, to be short and irregular in shape and spacing, with a "moth eaten" appearance and an irregular granularity of the glycocalyx (Fig. 2).

After 6 months of therapy with 1,25(OH)$_2$D$_3$, there was a notable increase in the mitotic activity in the crypts (+0.93 ± 0.25 mitoses/crypt, $P < 0.02$; Fig. 3) accompanied by a distinct and significant lengthening of the crypts (+0.07 ± 0.02 mm, $P < 0.02$; Fig. 1) and of the intestinal villi (+0.22 ± 0.06 mm, $P < 0.02$; Fig. 1). In addition, there was a marked improvement in the electron microscope appearance of the intestinal mucosa (Fig. 2)—after treatment, the microvilli were taller, more uniform and with a more homogeneous glycocalyx.

There were no differences among all the patients in the numbers and types of cells of the villus surface, in the numbers and types of cells of the lamina propria, or in the numbers and condition of the various cell organelles as examined by electron microscopy. Treatment with 1,25(OH)$_2$D$_3$ did not have an effect on these parameters.

Discussion

The intestine and those components vital to its absorptive abilities are major factors maintaining the homeostasis of many ions. Limited investigations have been reported that suggest that the intestinal absorption of phosphate [18, 19], iron [20, 21] and zinc [22] is impaired in uremia, but considerable data have accumulated to indicate that the intestinal absorption of calcium is clearly defective

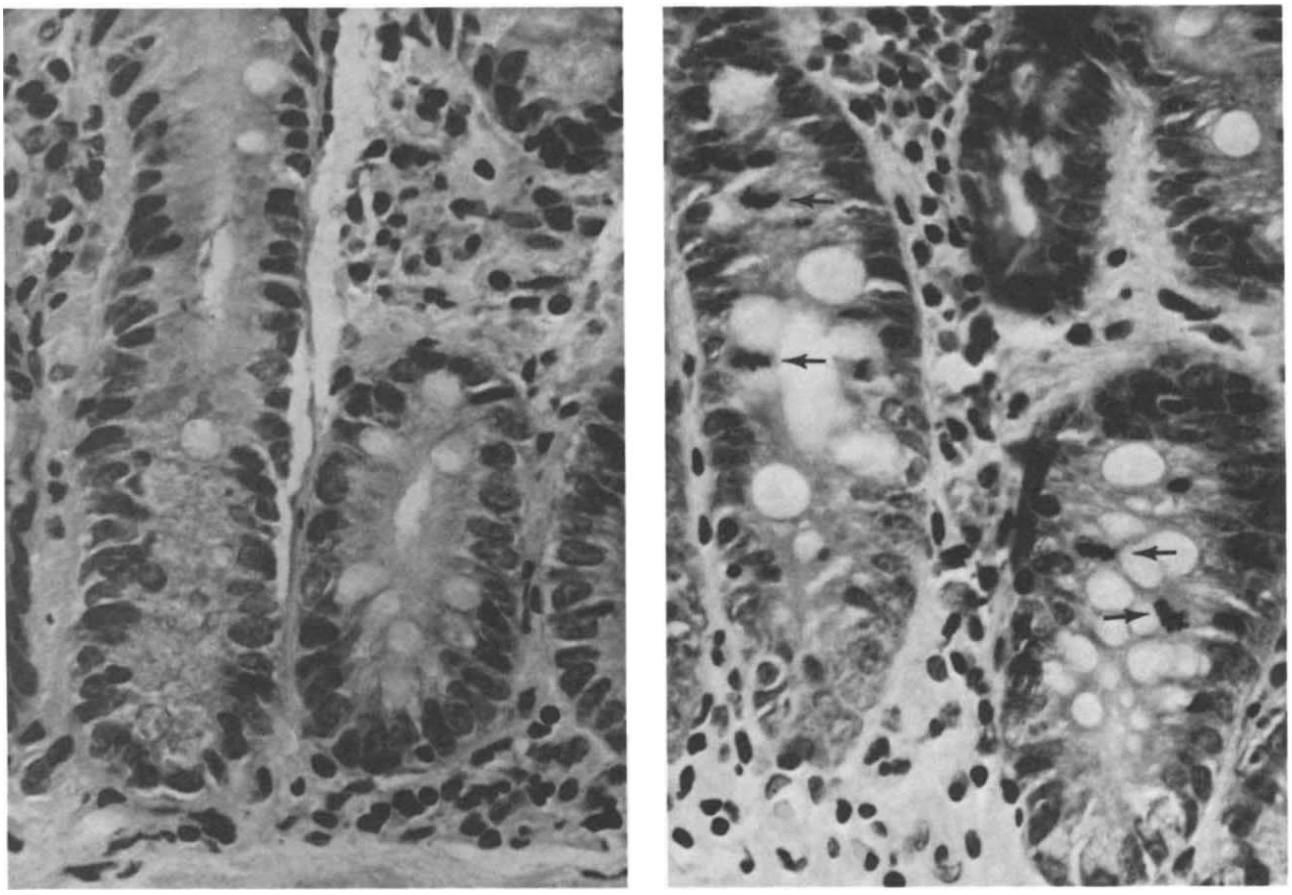

Fig. 3. *Duodenal crypts of dialysis patient no. 3 before* (left) *and after* (right) *6 months of 1,25(OH)$_2$D$_3$ treatment.* Note the increased mitotic activity (→) in the posttreatment specimen. (Hematoxylin-eosin stain; magnification ×450)

in chronic renal failure [1–3], most probably due to deficiency of 1,25(OH)$_2$D$_3$ [11, 12]. Several authors [3, 23, 24] have demonstrated that various vitamin D metabolites enhance the intestinal absorption of calcium in uremia, but the mechanisms of both the impairment and the improvement of calcium absorption following vitamin D administration are complex and not yet clearly understood. Wasserman et al [25–27] have identified an intestinal calcium-binding protein (CaBP), which exists in the glycocalyx of the microvilli, whose secretion is stimulated by vitamin D. The role of CaBP in augmenting calcium entry into the cell remains, however, unclear. The improvement in calcium absorption in response to vitamin D therapy is accompanied also by an increase in the activity of brushborder alkaline phosphatase and a calcium-dependent ATPase [28, 29]. Recent reports [30–32] have shown that 1,25(OH)$_2$D$_3$ enters mucosal cells, binds to a protein receptor, and perhaps stimulates messenger RNA activity. Birge and Alpers [13] and Spielvogel, Farley, and Norman [14] have also shown in rachitic rats and chicks that vitamin D administration stimulates mucosal cell proliferation and an increase in villus length.

These data provide strong evidence that the action of vitamin D appears to involve protein synthesis by, and cellular proliferation of, the intestinal mucosa. Because the bulk absorption of substances across membranes is partially dependent on surface area, it is indeed possible that reduction in number or length of intestinal villi in the uremic, and hence, vitamin-D-deficient patient, may contribute to the impaired intestinal calcium absorption observed in end-stage renal disease.

The results of our study demonstrate that structural abnormalities in intestinal mucosa manifested by reduction in the lengths of intestinal villi and crypts of Lieberkuhn may be apparent in patients with moderate renal failure and become more marked in patients with chronic uremia. In addition, the latter group of patients displayed disruption of the normal structure of the microvilli. These abnormalities may be due to deficiency of 1,25(OH)$_2$D$_3$,

because therapy with this metabolite restored these abnormalities to normal. It was also associated with a significant increment in the mitotic activity of the crypt of Lieberkuhn, suggesting an effect of $1,25(OH)_2D_3$ on the proliferating mucosal cells in the crypts of Lieberkuhn before the cells migrate up the villus. It is theoretically possible that other factors such as hyperacidity in uremia could contribute to the abnormalities in intestinal mucosa seen in our patients. We do not have data on gastric acid secretion. If the latter is important, however, in the pathogensis of the structural changes in intestinal mucosa, one may speculate that the improvement noted with $1,25(OH)_2D_3$ is partially mediated through the effect of the sterol and gastric acid secretion.

The observation that $1,25(OH)_2D_3$ therapy was associated with reversal of the abnormalities in intestinal mucosa and with an increase in intestinal calcium absorption suggests that a reduction in the absorptive surface area and the structural disruption of the microvilli contribute to the impairment in intestinal absorption of calcium. Thus, it appears that $1,25(OH)_2D_3$ stimulation of intestinal calcium absorption is the consequence of not only its action on the calcium transport mechanism but also of its effect on the rate of mucosal proliferation and an increase in the effective absorptive area. The augmentation of intestinal absorption of other substances such as phosphorus [18, 19] and zinc [23] during therapy with $1,25(OH)_2D_3$ or vitamin D for patients with uremia provide further support for the role of the structural derangement of the intestinal mucosa on the overall absorptive function. Finally, abnormalities in intestinal absorption occur in other diseases in which the length of the villus is markedly reduced. For example, Madangopolan, Shiner, and Rowe [33] found that the average length of the intestinal villi in patients with celiac disease is 0.13 mm and in those with untreated idiopathic steatorrhea is 0.18 mm. Following therapy, the length of the villi increased.

The finding that patients with moderate renal failure did display mild abnormalities in their intestinal mucosa suggests that they may also have a deficiency of $1,25(OH)_2D_3$. Although we did not have measurements of blood levels of $1,25(OH)_2D_3$ in the three patients with moderate renal failure, Slatopolsky et al [34] have reported that the blood levels of $1,25(OH)_2D_3$ are usually normal in patients with renal failure who have GFR's greater than 30 ml/min. It is, therefore, reasonable to assume that our patients with creatinine clearances of 32 to 51 ml/min did not have low blood levels of $1,25(OH)_2D_3$. Available data indicate, however, that such patients have evidence of target organ disease secondary to vitamin D deficiency, including impaired intestinal absorption [35], defective mineralization of osteoid [36], and impaired calcemic response to PTH [37]. To reconcile such evidence of a vitamin-D-deficient state and normal blood levels of $1,25(OH)_2D_3$, one must consider the possibilities that either the requirements of the target organs for $1,25(OH)_2D_3$ are increased or that there may be a state of vitamin D resistance in patients with moderate renal failure. Indeed, recent studies from our laboratory are consistent with these notions. Under such circumstances, a state of relative vitamin D deficiency could exist despite normal blood levels of $1,25(OH)_2D_3$. Thus, it appears that $1,25(OH)_2D_3$ stimulation of intestinal calcium absorption is the consequence of not only its action on the calcium transport mechanism but also of its effect on the rate of mucosal proliferation and an increase in the effective absorptive area.

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