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Effects of 25-hydroxycholecalciferol on bone lesions of children with terminal renal failure

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Effects of 25-hydroxycholecalciferol on bone lesions of children with terminal renal failure. Quantitative histology was performed on serial iliac crest biopsies obtained from 14 children with terminal renal failure. A long-term study on the comparative effects of vitamin D_2 and 25-hydroxycholecalciferol [25-(OH)D_3], in five patients with severe lesions of osteomalacia and/or osteitis fibrosa, demonstrated the efficiency of 25 to 200 μg/day of 25-(OH)D_3 and the lack of therapeutic action of 345 to 685 μg/day of vitamin D_2. In nine subjects with normal roentgenograms or minimal skeletal alterations, the first biopsy taken at the beginning of intermittent hemodialysis showed evidence of defective mineralization and/or lesions of resorption. Four of these children were treated with 25-(OH)D_3 (25 to 50 μg/day) and calcium supplementation orally (0.5 to 1.5 g/day); five children received calcium orally (0.5 to 0.75 g/day) alone. Aggravation of bone lesions during intermittent hemodialysis was observed in patients treated with calcium supplements alone. In subjects who were given 25-(OH)D_3, mineralization improved and marrow fibrosis disappeared. However, as the two groups of patients were different in composition and in the manner in which they were treated, it is difficult to state whether the beneficial effects observed were solely attributable to 25-(OH)D_3 administration. 25-(OH)D_3 therapy induced severe intoxication in two patients. A rise in plasma calcium concentration to 11.0 to 11.5 mg/100 ml was observed in two other patients. It is concluded that: *a)* pharmacologic doses of 25-(OH)D_3 are highly effective in healing bone lesions of children with terminal renal failure; *b)* such treatment requires strict clinical surveillance as 25-(OH) D_3 intoxication may occur even in anephric patients.

Effets du 25-hydroxycholecalciferol sur les lesions osseuses dans l'insuffisance renale terminale de l'enfant. Le tissu osseux de quatorze enfants en insuffisance rénale terminale a été étudié par des techniques d'histologie quantitative sur des biopsies itératives de la crête iliaque. Chez cinq malades ayant des lésions sévères d'ostéomalacie et/ou d'ostéite fibreuse la comparaison des effets thérapeutiques à long terme de la vitamine D_2 (345 à 685 mg/jour) et du 25-(OH)D_3 (25 à 200μg/jour) a mis en évidence la grande efficacité du 25-(OH)D_3 et le manque d'action de la vitamine D. Chez neuf enfants devant être traités par hémodialyse chronique et dont les radiographies du squelette étaient normales ou à peine altérées, les biopsies ont montré un défaut de minéralisation et/ou des lésions de résorption. Parmi ces sujets quatre malades ont reçu un traitement associant 25-(OH)D_3 (25 à 50 μg/jour) et une supplémentation orale de calcium (o,5 à 1,5 g/jour), les cinq autres seulement du calcium (o,5 à o,75 g/jour). Chez les malades recevant seulement une supplémentation orale de calcium les lésions osseuses se sont aggravées sous hémodialyse chronique; par contre chez les malades recevant du 25-(OH)D_3 la minéralisation s'est améliorée et la fibrose médullaire a disparu. Cependant, la composition des deux groupes de malades et leur traitement n'ayant pas été identiques, il est diffcile d'affirmer que les améliorations observées ne sont dûes qu'au 25-(OH)D_3. Chez deux malades, le 25-(OH)D_3 a entraîné une intoxication sévère. Chez deux autres la calcémie s'est élevée à 11,0 -11,5 mg/100 ml. En conclusion, ces résultats montrent: *a)* l'activité thérapeutique du 25-(OH)D_3 sur les lésions osseuses d'enfants en insuffisance rénale terminale; *b)* la possibilité d'intoxication par le 25-(OH)D_3 même de sujets anéphriques d'où la nécessité d'une surveillance clinique et biologique constante de tout malade traité par ce dérivé de la vitamine D.

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Osteodystrophy is a disabling complication of renal failure. In 1966 we reported that among 149 cases of renal failure analyzed, 41 patients (27.5%) had severe osseous abnormalities [1]. These results, showing that the incidence of renal osteodystrophy in children and adolescents is higher than that reported in adults, are in agreement with more recent studies by Fine et al [2]. Since 1966, we have observed a marked decrease in the percentage of uremic patients developing skeletal alterations (unpublished data). This was achieved as a result of a systematic therapy designed to prevent hyperparathyroidism. This treatment included administration of vitamin D_2 or D_3 (4,000 to 12,000 IU/day or 100 to 300 μg/day) and calcium supplements orally (0.5 to 1.5 g/day) to all patients with creatinine clearances less than 60 ml/min/1.73 m^2, and the prescription of aluminum gels when plasma phosphorus concentrations were higher than 6.0 mg/100 ml. Vitamin D, calcium supplements, and aluminum gels were stopped or doses modified for each patient according to roentgenographic and biochemical changes observed during follow-up.

However, this therapeutic approach did not eradicate severe bone lesions in all of our patients with renal insufficiency. Therapeutic failures were attri-

buted to late diagnosis, inadequate patient compliance in taking prescribed medication, especially phosphorus-chelating drugs, and possibly other factors undetectable by our current means of investigation. To this small number of patients with overt bone alterations, one has to add a new group of patients prone to skeletal lesions: children and adolescents undergoing long-term hemodialysis. In fact, we have observed in some patients receiving maintenance hemodialysis the appearance, persistance and increase of bone lesions. The use, since 1972, of softened water and calcium-rich dialysate (3 to 3.5 mEq/liter) has not completely abolished the occurrence of skeletal complications, a finding in agreement with the results of several investigators [3–7].

These observations led us to attempt a therapeutic trial with 25-hydroxycholecalciferol [25-(OH)D_3] for these patients. Our reasons for choosing this metabolite, despite the fact that it is generally considered a simple circulating form of vitamin D, were the following: *1)* Results from our laboratory and from other investigators have clearly shown that pharmacologic doses of 25-(OH)D_3 are active even in the absence of kidney tissue [8–11]. *2)* The more potent kidney-made hormonal form of vitamin D_3, 1,25-dihydroxycholecalciferol, or its synthetic analog, 1,α-hydroxyvitamin D_3, was not available for long-term therapy.

The present paper reports: *a)* the comparative effects of 25-(OH)D_3 and vitamin D on severe lesions of renal osteodystrophy, *b)* the evolution of bone lesions during maintenance hemodialysis in patients treated with 25-(OH)D_3 plus calcium orally and in patients treated orally with calcium alone, and *c)* the tolerance of uremic children to long-term administration of pharmacologic doses of this vitamin D metabolite.

Methods

Fourteen patients aged 4 yr, 2 months to 16 yr, 8 months were the subjects of this investigation. They had severe renal failure with endogenous creatinine clearances less that 10 ml/min/1.73 m^2. Table 1 summarizes their main clinical data. Two groups of subjects were studied.

Children of group I were characterized by severe lesions of osteodystrophy (patients 1 to 5). They were the only ones with such lesions among the patients seen in our department between November, 1969, and November, 1970. All of them had a congenital disease. At the time the first bone biopsy was performed, these patients had had symptoms of severe glomerular functional impairment for more than four years. For these subjects, the therapeutic effects of 25-(OH)D_3 and of vitamin D_2 (Stérogyl, Laboratoire Roussel, Paris, France) were compared.

Children of group II (patients 6 to 14) had essentially normal radiograms. Three subjects (patients 7, 10 and 12) had congenital diseases; five subjects (patients 6, 8, 9, 11 and 13) had acquired diseases which had progressed to terminal renal failure within the two years preceding the first biopsy. One patient was anephric due to the removal of a solitary kidney after a traffic accident. These patients had recently been started on a program of intermittent hemodialysis (patients 6 and 8) or such a program was begun during the week(s) following their first bone biopsy. These nine patients were randomly distributed in two subgroups: subjects of group II-A (patients 6, 7, 8 and 9) received 25-(OH)D_3 and calcium supplements orally; subjects of group II-B (patients 10, 11, 12, 13 and 14) received calcium, orally, alone (Calcium "forte", Laboratoires Sandoz, Rueil-Malmaison, France). The doses of elementary calcium prescribed varied from 0.5 to 1.5 g/day. All children were prescribed aluminum hydroxide gel (Lithiagel, Laboratoire Brunet, Boulogne sur Seine, France), i.e., 5 to 90 ml/day; the content of this preparation is expressed by the manufacturer as percent of aluminum (Al_2O_3), i.e., 5.85 g/100 ml.

This second investigation was designed to analyze the preventive action of 25-(OH)D_3 on bone lesions during intermittent hemodialysis (IHD).

Intermittent hemodialysis. Hemodialysis was performed with a disposable multilayer RP5 dialyzer. Patients were dialyzed for eight to ten hours two times weekly. For two patients of group I who required IHD (patients 4 and 5), the dialysate was prepared with softened water and a calcium concentration of 6.0 mg/100 ml (3 mEq/liter). Among the subjects of group II, only one patient treated with 25-(OH)D_3 plus calcium supplements (patient 6) and one treated with calcium supplements alone (patient 11), were dialyzed with this type of fluid. For the others softened water with 7.0 mg/100 ml (3.5 mEq/liter) of calcium was utilized. All patients were prescribed a diet with limited sodium and protein intake; they received hydrosoluble vitamins, iron, and exchange resins.

Radiological evaluation. The severity of bone lesions was evaluated on standard radiograms by three investigators. Roentgenograms were obtained a few days to two months before the first bone biopsy, then every six months and/or immediately prior to or after the second biopsy.

The skeletal lesions were classified as metaphyseal abnormalities and lesions of osteitis fibrosa. Their degree of severity was designated from + to +++.

Table 1. Clinical data of the fourteen patients before the first bone biopsy[a]

Patient				Diagnosis	Plasma			Radiology		Treatment					
	Age						Alkaline				Vitamin D		Ca,		
No.	*yr*	*mo*	Sex		Calcium	Phosphorus	phosphatase (KA)	Metaphyseal lesions	Osteitis fibrosa	CDR	dose *μg/day*	duration	orally adminstered	Al gel	IHD
1	16	8	M	Nephronophtisis	6.9	6.1	22 B	+++	+	0.33	—	—	—	—	—
2	9	8	M	Oligonephronic hypoplasia	6.7	7.1	50	++ R	(+)	0.50	685	1 mo	+	—	—
3	11	4	F	Urinary tract malformation	9.9	6.2	24 B	(+)	+++	0.41	635 345	6 mo 4 mo	+	—	—
4	13	6	F	Urinary tract malformation	9.2	5.8	48	+	+++	0.50	455	2y	+	+	—
5	14	1	F	Nephronophtisis	8.0	5.4	17	+R	+++	0.37	—	—	+	—	—
6	12	7	M	Proliferative GN (diffuse crescents)	9.3	5.0	12	0	(+)	0.44	200	5 mo	+	+	+ (3 mo)
7	15	0	M	Dysplastic solitary kidney	6.9	8.8	11	+	(+)	0.44	—	—	—	—	—
8	7	6	M	Hemolytic uremic syndrome	8.5	6.0	6	0	0	0.31	—	—	—	—	+ (1 wk)
9	8	2	F	Proliferative GN (diffuse crescents)	6.2	7.8	6	0	0	0.37	—	—	—	—	—
10	13	10	F	Nephronophtisis	6.3	7.4	34	(+)	+	0.35	—	—	—	+	—
11	4	2	M	Proliferative GN (diffuse crescents)	6.8	8.2	8	0	(+)	0.39	—	—	—	—	—
12	13	3	M	Urinary tract malformation	6.5	9.8	6	(+)	(+)		—	—	—	—	—
13	10	3	M	Proliferative GN (focal crescents)	8.3	6.2	4	0	0	0.50	—	—	—	—	—
14	11	5	M	Nephrectomy on solitary kidney	9.2	6.8	5	0	0	0.50	—	—	—	—	PD (1 mo)

[a] R = rickets; IHD = intermittent hemodialysis; mo = month(s); yr = year(s); wk = week; GN = glomerulonephritis; PD = peritoneal dialysis; KA = King Armstrong units; B = Bodansky units; Al gel = aluminum hydroxide gel; CDR = cortico-diaphyseal ratio.

When the radiological features were considered as almost normal, (0), by two investigators and as + by the third, the sign (+) was given. The metaphyseal lesions were assessed at the ends of long bones: + and ++ were used to indicate abnormalities of the growth plate's calcification lines, such as small irregularities or blurred and fragmented lines. When frayed metaphyses with varus or valgus deformities of the lower extremities or slipped epiphyses were present, the lesions were designated as +++. The term "rickets" (R) was used only for those metaphyseal lesions showing all the characteristics usually associated with nutritional rickets, i.e., widened metaphyseal-epiphyseal distances, cupped and enlarged metaphyses. The lesions of osteitis fibrosa were looked for on radiograms of the phalanges; the cortical bone of the tibia, femur, and humerus; the vault of the skull; and the lamina dura around the teeth sockets. They were classified as follows: (+) or + = granular rarefaction of the vault of the skull and/or few subperiosteal erosions of phalanges; ++ = evident and numerous subperiosteal erosions of phalanges and loss of lamina dura; +++ = partial dissolution of terminal phalanges, multiple cystic rarefactions, subperiosteal cortical resorption of long bones. Bone density was evaluated by measuring the cortical and the diaphyseal thickness of the left tibia at equal distances from the two growing plates. The values were expressed as the cortico-diaphyseal ratio,

$$\text{CDR} = \frac{\text{diaphyseal thickness} - \text{cortical thickness}}{\text{diaphyseal thickness}}$$

According to Bernard et al [12], normal values for the age group studied range from 0.40 to 0.55.

Bone histology. All patients had two biopsies. Bone specimens were obtained from the anterior iliac crest by the technique of Bordier et al [13]. For the majority of the subjects, the biopsies were performed under general anesthesia during a required surgical procedure, i.e., arteriovenous shunt or fistula for hemodialysis, nephrectomy or transplantation. The interval between the first and the second biopsy varied from two months (patient 4) to sixteen months (patient 6); however, for most children this interval was of five to eight months' duration. The bone samples, fixed in ethyl alcohol, were embedded in methylmetacrylate without previous decalcification. Slices obtained with a diamond wheel sectioning machine (Gillings–Hamco thin sectioning machine, Rochester, N.Y., U.S.A.). were hand-ground to 15μm thickness and stained with basic fuchsine or eosine-azur. Histological measurements were performed with Zeiss integrating eyepieces at magnification × 200 in trabecular bone. For each specimen the mean of 2,000 counts was used for "volume" data and the mean of 1,000 counts for "surface" data.

For quantitative evaluation of bone structure and formation the following parameters were chosen [14, 15]:

bone volume: fraction (%) of spongiosa volume represented by bone matrix;

calcified volume: fraction (%) of spongiosa volume represented by calcified tissue;

osteoid volume: fraction (%) of spongiosa volume represented by osteoid;

osteoid "surface": fraction (%) of trabecular surface lined by osteoid;

osteoblast "surface": fraction (%) of trabecular surface lined by osteoblasts;

relative osteoblastic activity: fraction (%) of osteoid surface covered by osteoblasts;

mean osteoid seam thickness (μm): osteoid volume divided by osteoid surface.

In normal bone, osteoblastic activity and osteoid volume are in close relation, thus reflecting the synchronization between the rate of matrix formation and the rate of mineralization. In pathological conditions this synchronization may be disturbed. In osteomalacia there is too much osteoid compared to the number of osteoblasts; this indicates a delayed mineralization (increased osteoid volume, increased osteoid seam thickness, and decreased relative osteoblastic activity). In contrast, in hyperparathyroidism the increased amount of osteoid goes with an increase in the number of bone-forming osteoblasts; this indicates undisturbed mineralization and increased bone formation (increased osteoid volume, normal osteoid seam thickness, and increased relative osteoblastic activity) [16].

For quantitative evaluation of bone resorption and osteitis fibrosa, the following parameters were chosen [17]: *osteoclast surface* = fraction (%) of trabecular surface covered by osteoclasts, *marrow fibrosis volume* = fraction (%) of spongiosa volume occupied by fibrous tissue.

Plasma chemistry. Plasma calcium and phosphorus concentrations and plasma alkaline phosphatases were checked regularly. In the patients submitted to IHD, blood samples were obtained immediately before dialysis. The following techniques were used: plasma calcium, atomic spectrophotometry (Perkin Elmer); plasma phosphorus, Fiske and Subbarow technique adapted to an autoanalyzer (Technicon, Daumont, France); plasma alkaline phosphatases, King and Armstrong method or Bodansky method.

Table 2. Quantitative bone histology of normal French and American children

	Bone volume	Calcified volume	Osteoid volume	Osteoid surface	Osteoblast surface	Osteoclast surface
French, $N = 9$ (mean ± 1 SD)	24.2 ± 1.6	20.8 ± 2	3.4 ± 1.5	27.9 ± 9.1	12.3 ± 3.7	0.81 ± 0.37
American, $N = 13$ (mean ± 1 SD)	22.0 ± 1.87	21.1 ± 1.73	4.2[a] ± 2.01	22.0 ± 7.60	8.3 ± 4.44	1.8 ± 0.72

[a] Dr. P. Bordier expresses osteoid volume as the percentage of bone volume represented by osteoid tissue. To allow comparison our values were transformed to represent the same percentage.

25-hydroxycholecalciferol. The synthetic 25-(OH)D_3 used in this study was a gift from the Laboratoires Roussel, Paris, France. It was given to the patients once a day each morning, orally, as propylene glycol solution.

Normal controls. Due to the fact that specimens from normal children were not available, we examined samples that were kindly provided by Dr. J. Jowsey. Hence, our normal controls concern children from Minnesota, U.S.A, whose death was caused by accident. However, as this could not be considered an ideal control group for patients from France, we compared our results to those obtained by Dr. P. Bordier in nine French children aged 5 to 14 yr. The results of both groups are given in Table 2. Except for slight but not significant differences in osteoclast surface, all other parameters are comparable in the two groups.

Results

Group I: Patients with severe osteodystrophy (Tables 1 and 3, Fig. 1a and b, Fig. 2a and b, Fig. 3)

Quantitative histologic examinations performed on the first biopsy specimens obtained from the five children with severe osseous abnormalities showed a marked increase in osteoid and osteoblast surfaces, and the presence of marrow fibrosis with increased osteoclast surface.

On the biopsy specimens of the two children (patients 1 and 2) with predominantly metaphyseal lesions on roentgenograms, the mean osteoid seam thickness was greatly increased, and the relative osteoblastic activity was normal in patient 1 and low in patient 2. This indicates delayed mineralization. Osteoid was also found deeply embedded in trabeculae; it contained calcified granules in some areas. The limit between calcified tissue and osteoid seam was irregular; the matrix was mainly formed by regularly oriented collagen fibers. Woven bone was scarce. Osteocytic lacunae, often enlarged, contained a large pale nucleus. The most peculiar osteocytic aspect was the presence of cells deeply buried in large osteoid seams, and surrounded by mineral deposits. Marrow fibrosis was limited and localized under the endosteum and parallel to the trabecular surface.

On the biopsy specimens of the patients with predominantly resorptive lesions on radiograms (patients 3, 4, and 5), the mean osteoid seam thickness was slightly increased; the relative osteoblastic activity was very high. This indicates that the mineralization was near normal while the formation of bone was accelerated. In these cases woven calcified bone and woven osteoid were abundant. The tiny trabeculae of woven bone were surrounded by a thick layer of osteoblasts. Osteocytic lacunae were enlarged, often confluent, and showed a strong metachromatic reaction. Marrow fibrosis was diffuse.

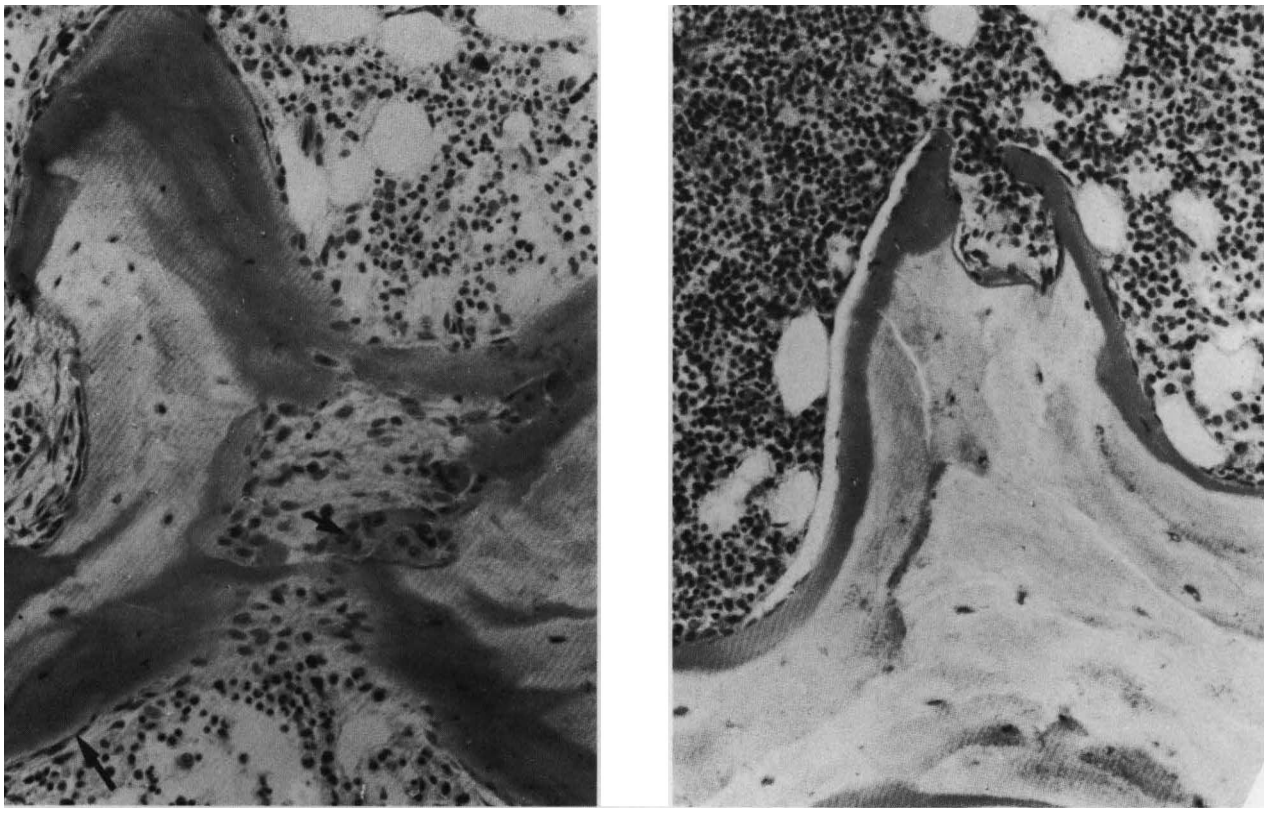
a. Therapeutic effects of vitamin D_2. In patients previously *treated with vitamin D* (patients 2, 3, and 4), no correlation could be found between the histology and vitamin D therapy. In spite of their long-standing treatment with therapeutic doses of vitamin D_2, i.e., 345 to 685μg/day (13,000 to 27,400 IU/day), the histological aspect of the bones of patients 3 and 4 were similar to that of patient 5 who had never been given any vitamin D. For this latter subject a second bone biopsy specimen was obtained five months after the initiation of vitamin D_2 therapy at daily doses varying from 300 to 380μg: no clear-cut effect of vitamin D was observed on bone histology except for a decrease in marrow fibrosis and an increase in osteoclast surface (Table 3, patient 5). Her plasma calcium concentration remained low and was normalized only when she was started on IHD one month after the beginning of vitamin D therapy.

b. Therapeutic effects of 25-(OH)D_3. The bone lesions were improved in the four patients treated with 25-(OH)D_3 (patients 1 through 4). The main effects were an improvement of mineralization, a reduction of osteitis fibrosa and of osteoblasts; osteoclast surface decreased in all but patient 4. The beneficial effect of 25-(OH)D_3 on mineralization was more impressive on the biopsy specimens of the two patients who had prevailing metaphyseal lesions on roentgenograms (patients 1 and 2). Their osteoid volumes as well as their mean osteoid seam thickness were greatly reduced by treatment (Fig. 1, a and b). The

Table 3. Effects of vitamin D or 25-(OH) D_3 on five patients with severe lesions of osteodystrophy[a]

	Therapy			Bone histology									Remarks
	Daily dose		Duration	Bone	Calcified	Osteoid	Osteoid	Mean seam	Osteoblast	Osteobl S / Osteoïd S	Marrow	Osteoclast	
Patient No.	μg	$\mu g/kg$	*months*	volume	volume	volume	surface	thickness (μm)	surface	%	fibrosis	surface	
1 No vitamin D therapy				30.7	20.2	10.5	77.6	25.6	27.0	34.8	9.3	10.2	
After 25-(OH)D_3	200	6.7	12	18.4	17.2	1.2	37.9	10.3	8.8	23.2	0	6.9	
2 After vitamin D_2	685	41.5	1	44.3	25.0	19.5	84.6	46	16.5	19.5	3.8	8.6	
After 25-(OH)D_3	80	4.8	1										
	100	6	1 (3)										
	120	7.3	1	42.5	34.0	8.3	65	21.9	2.7	4.2	0	5.6	Hypercalcemia (15.8 mg/100 ml)
3 After vitamin D_2	635	27.6	6										
	345	15	4 (10)	27.5	23.0	4.6	59.5	17.2	48.5	81.5	36.8	15.6	
After 25-(OH)D_3	40	1.7	3										
	25	1	3 (6)	22.1	19.2	2.9	69.4	13.2	24.4	35.2	3.2	6.0	
4 After vitamin D_2 (1)	435	19.8	24	30.9	23.7	7.2	75.7	13	53.1	70.1	31.6	8.3	
After 25-(OH)D_3	100	4.5	2	16.4	14.9	1.5	34	8.3	3.0	8.7	2.8	10.0	Hypercalcemia (16.8 mg/100 ml)
5 No vitamin D therapy (2)				26.4	16.8	9.6	67.7	18.2	49.2	72.7	56.0	7.5	
After vitamin D_2	380	15.2	2										
	300	12	3 (5)	16.3	8.7	7.6	73.4	18.9	52.0	70.8	38.9	13.6	
Normal controls $n = 13$ (mean ± 1 SD)				22.0 ±1.87	21.1 ±1.73	0.9 ±0.42	22.0 ±7.60	9.3 ±3.74	8.3 ±4.44	36.9 ±14.33	0	1.8 ±0.72	

[a] Osteobl = osteoblast; S = surface. (1) hemodialyzed for two months; (2) hemodialyzed during the last four months of study.



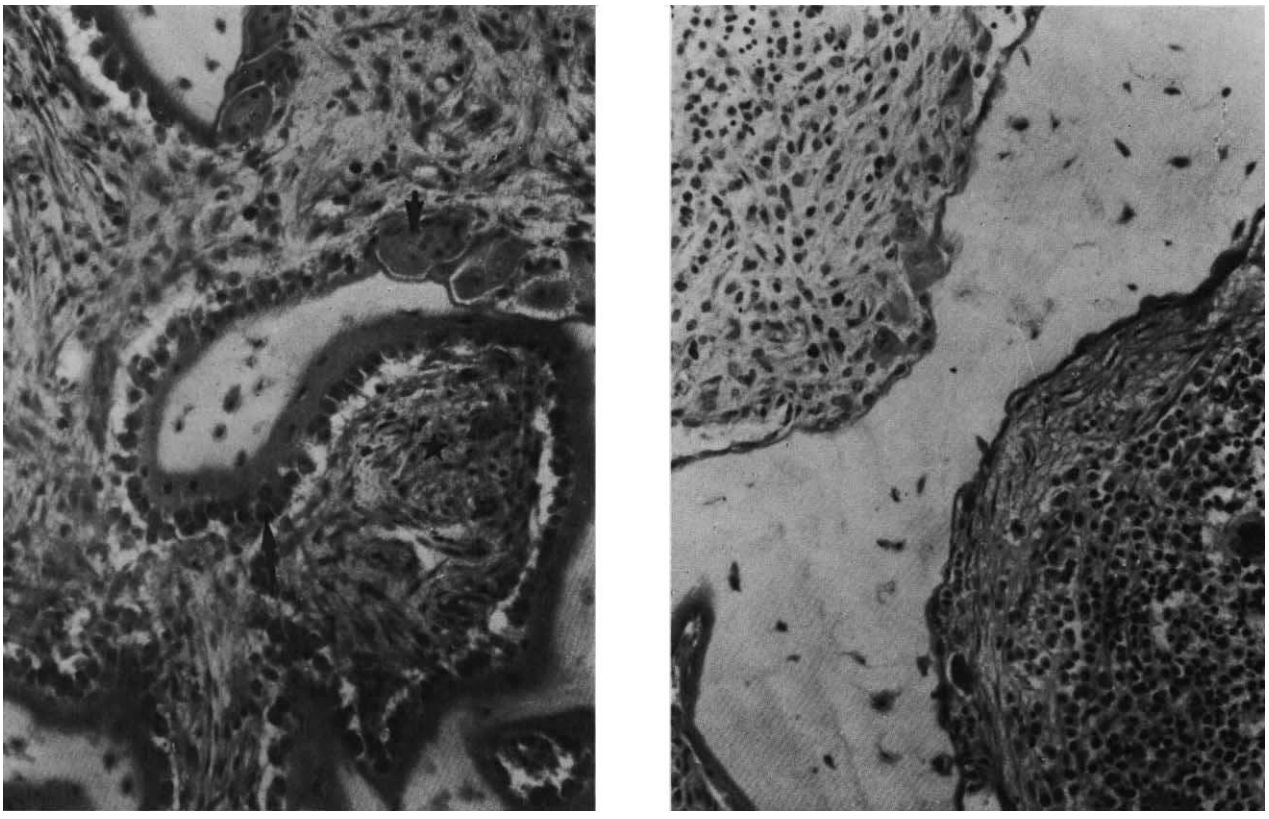
a b

Fig. 1. *Histologic aspect of bone of patient 2: (a) before and (b) after 25-(OH)D_3 a,* Osteoid seams (*grey*) are thick; multinucleated osteoclasts (*short arrow*) erode the calcified tissue (*white*) in the middle of trabecula; osteoblasts are flat (*long arrow*); the marrow fibrosis is scarce. *b,* In comparison to the aspect shown in *a,* the osteoid seam thickness has decreased, osteoclasts are less numerous, and the marrow fibrosis has disappeared. (Methylmetacrylate, basic fuchsine, ×300.)

relative osteoblastic activity was also reduced. In these children, the main action of 25-(OH)D_3 was related to bone mineralization which became normal in patient 1 and greatly improved in patient 2. The effect of 25-(OH)D_3 on marrow fibrosis and osteoclast surface was also marked. It was particularly notable in the two children (patients 3 and 4) with severe lesions of osteitis fibrosa on their radiographs. Their diffuse marrow fibrosis nearly disappeared. Woven bone was replaced by a tissue formed of parallel oriented bundles of collagen. Osteoblast surface was reduced and osteocytic osteolysis was scarce (Fig. 2, a and b). In these children the main action of 25-(OH)D_3 was related to bone formation, which became normal in patient 3 and below normal in patient 4.

For three patients (1, 2 and 4), after three months of 25-(OH)D_3 therapy, the radiologic examination showed improvement or healing of the metaphyseal lesions and of the lesions of resorption. For the fourth subject (patient 3) who had received smaller doses of 25-(OH)D_3, i.e., 40μg/day for three months, then 25μg/day for the subsequent three months, no changes were detectable on roetgenograms although histological improvement was evident. Normalization of plasma calcium concentrations in the two hypocalcemic children (patients 1 and 2) was obtained at four and ten weeks, respectively. Plasma phosphorus concentrations were variable. At the end of 25-(OH)D_3 therapy, plasma alkaline phosphatase concentrations had returned to normal values in patient 4, had decreased in patients 1 and 2, and had remained unchanged in patient 3.

c. Tolerance to pharmacologic doses of 25-(OH)D_3. Doses of 25-(OH)D_3 equal to 100μg/day or higher were given to three children. These doses were well tolerated by one patient (Table 3, patient 1), not only during the one year of this investigation but also during the following year, up to the time of transplantation when 25-(OH)D_3 therapy was stopped. However, for the two others, 25-(OH)D_3 had overt toxic effects. Both these patients developed severe hypercalcemia. 25-(OH)D_3 intoxication occurred after three months' therapy in patient 2 who was pre-



a b

Fig. 2. *Histologic aspect of bone of patient 4: (a) before and (b) after 25-(OH)D_3. a,* The thin calcified trabeculae are surrounded by osteoid seams covered by a layer of round-shaped, active osteoblasts (*long arrow*); osteoclasts (*short arrow*) are eroding calcified tissue; marrow spaces are filled with fibrous tissue (*star*). *b,* In comparison to the aspect shown in *a,* the calcified trabecula is large, osteoid tissue has disappeared; the active osteoblasts and the marrow fibrosis have greatly decreased. (Methylmetacrylate, basic fuchsine, ×300.)

viously hypocalcemic, and after six weeks in patient 4 who was normocalcemic when 25-(OH)D_3 was started. In the first case (Fig. 3), 25-(OH)D_3 was stopped, the patient was given a low calcium diet (100 mg of calcium a day), and repeated intramuscular injections of porcine extractive calcitonin (Calcitar, Laboratoires Armour–Montagu, Paris, France), i.e., 3 × 60 MRC U/day. Ten days later, his plasma calcium concentration became normal. For this child the only clinical manifestation of hypercalcemia had been tachycardia. For the second patient the intoxication was much more severe. Clinical manifestations included severe hypertension, tachycardia, headache, mental aberration and convulsions. She was hemodialyzed with a fluid devoid of calcium, was given a low calcium diet and 80 MRC U/day of calcitonin. These treatments decreased her plasma calcium concentrations to 12.0 to 13.0 mg/100 ml. As no further improvement could be obtained, subtotal parathyroidectomy was performed. At surgery three hyperplastic parathyroid glands were found and removed.

Group II: Patients with essentially normal radiograms, started on a program of IHD (Tables 1 and 4, Fig. 4 and 5)

Most patients included in this second group had an acquired kidney disease (Table 1). The duration of severe renal failure before IHD was less than 14 months for three children (patients 6, 8 and 9) treated with 25-(OH)D_3 plus calcium (group II-A), and four subjects of five (patients 10, 11, 12 and 14) treated with calcium supplementation (group II-B). All except two children were hypocalcemic. The two exceptions were patient 6 who had been on IHD for three months and patient 14 who had been treated with peritoneal dialysis after the removal of a solitary kidney. Eight of nine were hyperphosphatemic when the first biopsy specimen was obtained. The histologic and morphometric studies demonstrated that not a single child of either subgroup had a normal

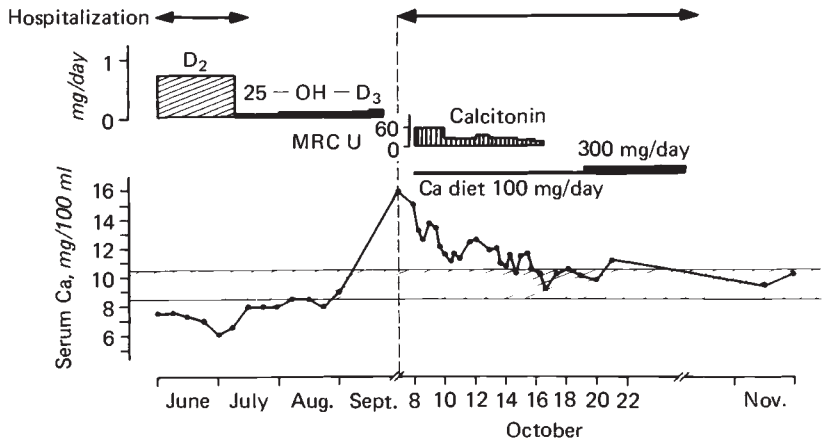


Fig. 3. *Changes in serum calcium concentrations of patient 2 intoxicated with 25-(OH)D_3.*

bone structure. The patient who was anephric (patient 14) and treated by peritoneal dialysis for one month had a marked diminution of bone formation as illustrated by unmeasurable osteoid volume and osteoblast surface. His osteoclast surface was normal and he had no marrow fibrosis. All other subjects had resorptive lesions with increased osteoclast surfaces. Marrow fibrosis was observed on the biopsy specimens of two children from group II-A (patients 6 and 7), and one from group II-B (patient 10). Osteoblast surface was slightly increased in patient 6, at the lower limits of normal, or clearly below normal in the others.

a. Patients treated with 25-(OH)D_3 plus orally administered calcium (Group II-A). Among the four subjects treated with 25-(OH)D_3 and calcium, orally, two (patients 6 and 7) were maintained on therapy for 16 and 8 months, respectively, before the second biopsy was performed. For patients 8 and 9, treatment was of shorter duration, and was interrupted one or more times because plasma calcium concentration had increased to 11.0 to 11.5 mg/100 ml (Table 4). Both children given long-term 25-(OH)D_3 therapy had increased osteoid volume and osteoclast surface, and marrow fibrosis on their first biopsy specimen. Normal mineralization, disappearance of marrow fibrosis and diminution of their osteoclast surface were observed on their second biopsy specimen. For the latter two patients of group II-A, despite interruptions in 25-(OH)D_3 therapy, histology also indicated a decrease in osteoclast surface in one case (patient 8), and a normalization of this parameter in the case of patient 9. The mean osteoid seam thickness decreased during treatment in all the children of group II-A. The relative osteoblastic activity decreased in three cases (patients 6, 8 and 9) and did not change in one case (patient 7). It should be noted that the child (patient 8), whose plasma calcium concentration rose to 11.5 mg/100 ml after 50μg/day of 25-(OH)D_3 for three weeks, had undergone bilateral nephrectomy at the initiation of this treatment (Table 4). For the children of group II-A, plasma calcium concentrations varied from 8.5 to 11.5 mg/100 ml and plasma phosphorus from 2.1 to 11.0 mg/100 ml (Fig. 4, a and b). Plasma alkaline phosphatase concentrations remained within the normal range. X-ray examination showed the disappearance of the discrete lesions of grade + or (+) for patients 6 and 7, and no detectable modifications for patients 8 and 9.

b. Patients treated with calcium, orally, alone (group II-B). Among the children given calcium supplements orally without additional 25-(OH)D_3 therapy (group II-B), the follow-up was of three months' duration for patient 14 who underwent kidney transplantation. During this short period of time, this patient developed increased osteoclast surface. For the other subjects the second biopsy was performed eight months to one year after the beginning of IHD and calcium supplementation orally. Histology did not show the appearance of severe osteodystrophy. However, osteoid volume and mean osteoid seam thickness increased in patients 11 and 12. The aggravation observed for patient 11 might have been related to the fact that this child was dialyzed with a relatively low calcium fluid, i.e., 3.0 mEq/liter, but this was not the case for patient 12 who was dialyzed for 12 months with a fluid containing 3.5 mEq/liter of calcium. Osteoid volume and mean osteoid seam thickness decreased in patients 10 and 13 but, nevertheless, remained abnormally high. The relative osteoblastic activity increased in four out of five children (patients 10, 12, 13 and 14). Osteoblast surface increased slightly in two cases (patients 12 and 13), remained very low in patient 11, and was

Table 4. Quantitative histology of patients with near normal radiograms, started on a program of

Patient No.		Therapy						
		25-(OH)D_3			Calcium, orally			
		Daily dose		Duration	Dose	Duration	Bone	Calcified
		μg	*μg/kg*	*months*	*g/day*	*months*	volume	volume
6	Before 25-(OH)D_3 + Ca						24.6	22.2
	After 25-(OH)D_3 + Ca	50	1.5	16	0.75	16	21.6	21.1
7	Before 25-(OH)D_3 + Ca						25.5	20.6
	After 25-(OH)D_3 + Ca	50	1.1	8	1.50	8	17.4	17
8	Before 25-(OH)D_3 + Ca						18.5	17.7
	After 25-(OH)D_3 + Ca	50	2.5	21 days	0.50	21 days		
		0		1½	0	1½		
		50	2.5	2½	0.25		17.0	16.4
9	Before 25-(OH)D_3 + Ca						20.3	18.8
	After 25-(OH)D_3 + Ca	25	1	4	0.375	4		
		0		1	0	1		
		50	2	1½	0.50	7½	18.2	17.1
		0		3				
		50	2	3				
10	Before Ca						33.6	20.6
	After Ca				0.75	8	20.3	16.4
11	Before Ca						15.5	14.8
	After Ca				0.50	8	20.8	17.4
12	Before Ca						28.0	25.9
	After Ca				0.75	12	31.6	26.3
13	Before Ca						30.0	23.2
	After Ca				0.75	9	21.6	19.3
14	Before Ca						19.5	19.5
	After Ca				0.50	3	18.9	18.6
Normal controls N = 13 (mean ± 1 SD)							22.0 ±1.87	21.1 ±1.73

[a] Osteobl. = osteoblast; S = surface; mo = month.

greatly augmented in patient 10. This latter child had marrow fibrosis at the time this investigation was initiated: her fibrosis increased. No lesions of marrow fibrosis appeared in the other subjects. Osteoclast surface had further increased in patient 10; decreased, although remaining very high in patients 11 and 13; and was not modified in patient 12.

In patients of group II-B, plasma calcium concentrations were variable from a minimal value of 8.3 to a maximal value of 11.1 mg/100 ml and plasma phosphorus concentrations ranged from 2.6 to 8.2 mg/100 ml (Fig. 5, a and b). The plasma alkaline phosphatase concentrations remained elevated throughout the study in patient 10. For the other subjects the alkaline phosphatases increased slightly (patients 12 and 13) or were unchanged (patients 11 and 14). Radiographic examination of group II-B children did not show noticeable modifications during this study except in one individual (patient 10) in whom the bone resorption increased.

Discussion

The present data show that at pharmacologic doses, i.e., 25 to 200μg/day, 25-(OH)D_3 is a potent drug for the treatment of osteodystrophy in children with terminal renal failure. The morphometric results clearly indicate that 25-(OH)D_3 treatment acts on matrix formation, mineralization and resorption. The five children studied initially had severe bone lesions at the time 25-(OH)D_3 therapy was initiated. Plasma

intermittent hemodialysis and given 25-(OH)D_3 plus calcium, orally, or calcium, orally, alone[a]

Bone histology							Remarks
Osteoid volume	Osteoid surface	Mean seam thickness (μm)	Osteoblast surface	Osteobl S / Osteoid S %	Marrow fibrosis	Osteoclast surface	
2.4	23.5	26.7	13.4	57	4.8	18.9	
0.5	17.1	10.5	3.1	18.1	0	8.2	Binephrectomy 2 mo before the second bone biopsy
4.9	54.0	9.2	5.0	9.3	2.6	5.4	
0.4	18.3	3.8	2.0	10.9	0	3.2	
0.8	16.5	6.1	5.8	35.1	0	8.2	Binephrectomy just before 25-(OH)D_3 therapy, plasma calcium (11.5 mg/100 ml)
0.6	21.0	5.8	4.3	20.5	0	5.6	
1.5	32.6	9.1	6.3	19.3	0	5.1	Plasma calcium (11.0 mg/100 ml)
1.1	27.2	4.8	4.5	16.5	0	1.9	
12.6	82.6	36.4	2.2	2.7	2.3	5.9	
3.8	56.9	13.4	19.2	33.9	4.3	7.8	
0.7	9.4	17.4	3.9	41.5	0	11.2	
3.4	15.7	41	0	-	0	6.7	Binephrectomy 5 mo before the second biopsy
2.1	16.7	6.3	1.6	9.6	0	7.8	
5.3	37.6	34.6	6.3	16.8	0	7.4	
6.8	39.3	28.8	2.6	6.6	0	7.9	
2.3	11.6	25.2	6.6	56.9	0	3.1	
0	0.6		0	-	0	2.2	
0.2	5.9	19.3	2.8	47.5	0	4	Anephric
0.9 ±0.42	22.0 ±7.60	9.3 ±3.74	8.3 ±4.44	36.9 ±14.33	0	1.8 ±0.72	

alkaline phosphatase activities were elevated in all. No relationship was established between plasma calcium, plasma phosphorus concentrations and the type or severity of the histologic abnormalities seen on biopsy. The lack of reliability of these latter biochemical parameters for the prediction of histologic or X-ray lesions in patients with chronic renal failure is in agreement with our past experience [1] and with the observations of others [18-24]. Among the five patients, the severity and distribution of bone lesions, i.e., osteoid volume and osteoid surface, augmentation of osteoblast and osteoclast surfaces, presence of marrow fibrosis and of osteolytic lacunae, were similar in the subjects who had never received supplementary vitamin D and in those previously treated with vitamin D. These results, and the fact that for one subject (Table 3, patient 5) five months of vitamin D therapy between the first and the second biopsy did not improve bone abnormalities, suggest that vitamin D, *at the doses utilized in this study* (345 to 685μg/day or 13,000 to 28,400 IU/day), is unable to promote healing of severe skeletal lesions in terminal renal failure.

In the group of children with essentially normal radiograms at the institution of intermittent hemodialysis, the first iliac crest biopsy specimen showed lesions of resorption in eight patients out of nine, a finding in agreement with the observations of other investigators [25-28].

Four subjects were given calcium supplements

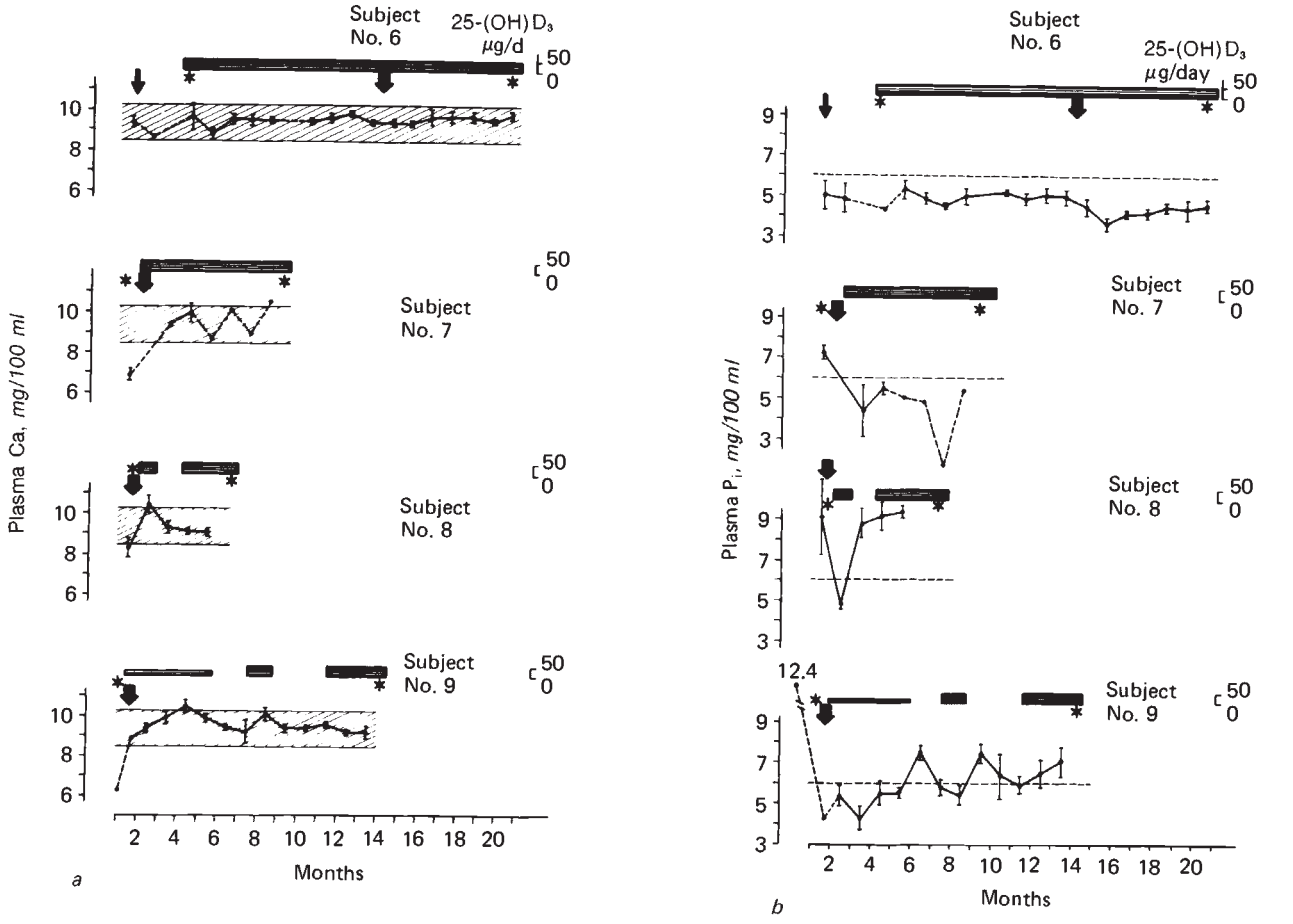


Fig. 4. *Plasma calcium (a) and phosphorus (b) concentrations of patients of group II-A.* Each *point* corresponds to the mean value obtained on eight to ten plasma concentrations measurements per month; *vertical bars* represent ± 1 SEM; *Arrows* indicate the beginning of IHD: *thin arrows* indicate dialysate containing 6 mg/100 ml of calcium, *thick arrows* indicate dialysate containing 7 mg/100 ml of calcium. For each subject the two *stars* indicate the first and the second bone biopsy. *Dashed area* shows the upper and lower normal values for serum calcium; *dotted lines* indicate 6 mg/100 ml of serum phosphorus concentration; this is the limit of acceptable hyperphosphatemia.

orally (0.25 to 1.5 g/day) plus 25 to 50μg/day of 25-(OH)D_3. An improvement of the histologic lesions was observed for these patients, although none of them had a completely normal bone at the end of the study. For five patients given calcium, orally, alone, results were less satisfactory. Mean seam thickness, indicating the balance between matrix formation and mineralization, was found normalized in one subject (Table 4, patient 10); a defect in the process of mineralization appeared (patients 12 and 14), remained unchanged (patient 13) or was aggravated (patient 11) in the others. Marrow fibrosis, when present, had increased (patient 10).

These two groups of patients were comparable at the beginning and during the study in many respects: age groups, proportion of congenital disease with a long duration versus acquired disease with a shorter duration, number of anephric patients (Table 4), variations of individual plasma calcium and phosphorus concentrations (Fig. 4 and 5), roentgenographic findings. But, they were not identical. The oldest subject (patient 7), an adolescent aged 15 yr, in group II-A, treated with 25-(OH)D_3 and calcium supplement, was given a larger dose of calcium than the other subjects. One subject in each group (patients 6 and 11) was started on IHD with a dialysate of low calcium concentration (3mEq/liter). After ten months of IHD, patient 6 was shifted to dialysis with a 3.5 mEg/liter calcium dialysate, whereas patient 10 was dialyzed throughout the investigation against the lower calcium bath. Due to the small number of subjects in each group and to these differences in the manner in which they were treated, it is difficult to ascertain that the better therapeutic results achieved

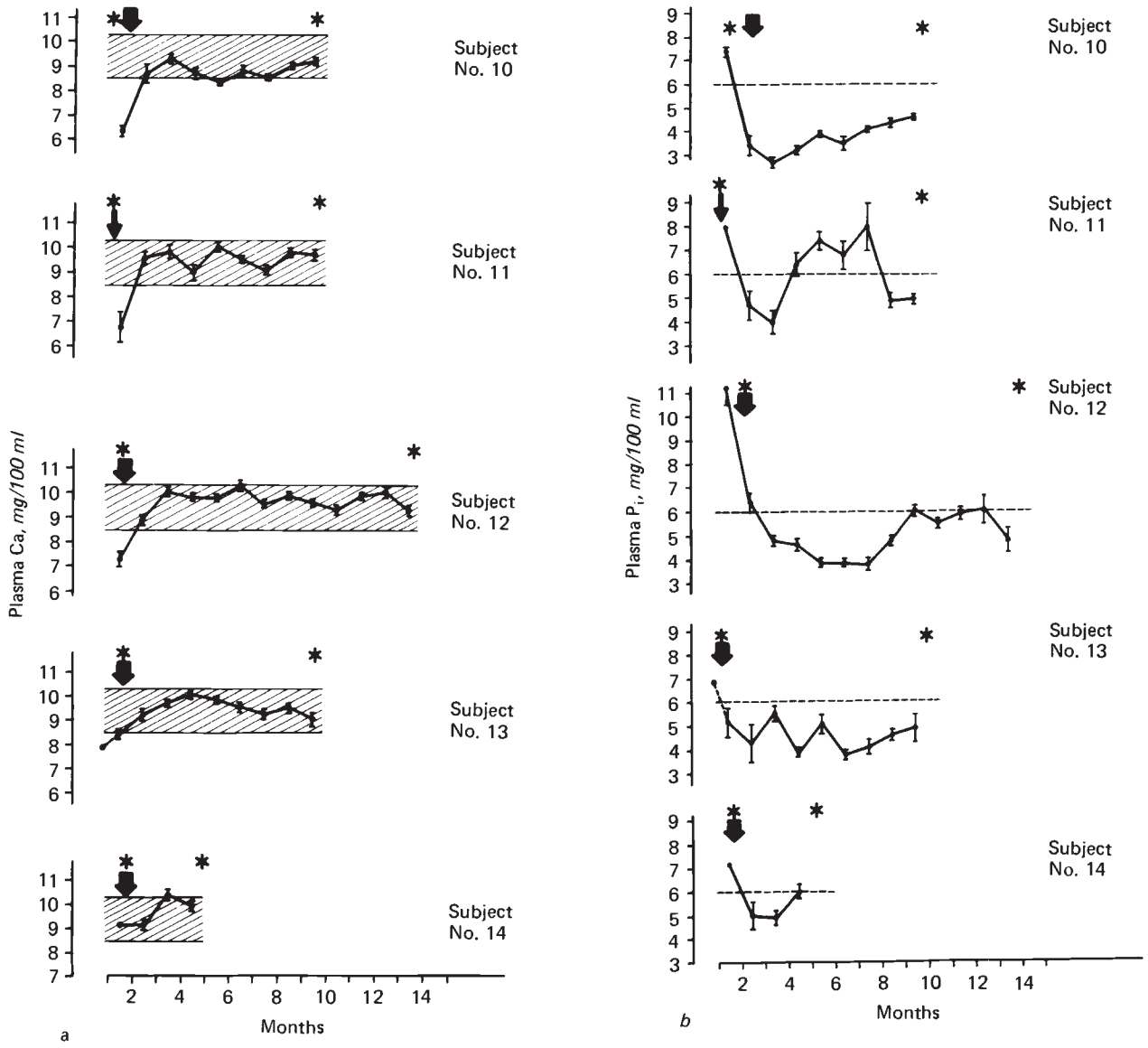


Fig. 5. *Plasma calcium (a) and phosphorus (b) concentrations of patients of group II-B.* Symbols are the same as for Fig. 4.

with 25-(OH)D_3 may be attributable solely to this vitamin D metabolite.

Finally, concerning the tolerance to long-term therapy with 25-(OH)D_3, the present data show two points: *1*) the tolerance to pharmacologic doses of 25-(OH)D_3 is variable among children with terminal renal failure; *2*) such doses may lead to episodes of hypercalcemia even in anephric patients. In our investigation, we were unable to relate the degree of sensitivity to the hypercalcemic action of 25-(OH)D_3 to factors such as daily doses of the drug (expressed in μg or μg/kg of body wt); total dose; the presence or absence of a previous vitamin D therapy; plasma calcium, phosphorus, alkaline phosphatase concentrations; the type and severity of X-ray and histologic bone lesions at the time therapy was started. As for the second point, the episodes of mild or severe hypercalcemia that occurred in some patients demonstrate that when pharmacologic doses are used, 25-(OH)D_3, like vitamin D [29], does not require the presence of the kidney to induce intoxication.

Recent studies [30] have shown that after direct administration of 25-(OH)D_3 its serum concentration is directly correlated to doses and that its half-life is short, whereas after the administration of pharmacologic amounts of vitamin D, serum level of endogenous 25-(OH)D_3 is variable, unpredictable and its decay is prolonged. These data confirm our clinical impression and that of other investigators that in kidney diseases, as in many other pathologic states of

man, therapeutic results are more easily achieved with 25-(OH)D_3 than with vitamin D [11, 31–33]. However, such treatment with 25-(OH)D_3 necessitates careful and repeated medical surveillance. Monitoring of serum 25-(OH)D_3 concentrations during therapy should prevent intoxications.

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