Phosphate Control and 25-Hydroxycholecalciferol Administration in Preventing Experimental Renal Osteodystrophy in the Dog

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ABSTRACT Previous studies from this laboratory demonstrated that secondary hyperparathyroidism in dogs with chronic renal disease may occur, at least in part, as a consequence of the need for progressive adaptation in renal phosphorus (P) excretion that occurs as glomerular filtration rate falls. However, the studies were of relatively short duration. Moreover, no information emerged regarding a potential role of calcium malabsorption in the pathogenesis of secondary hyperparathyroidism. The short duration of the protocol did not lend itself to the study of the effect of P control or the administration of vitamin D in the pathogenesis of renal osteodystrophy. In the present studies, 14 dogs with experimental chronic renal disease were studied serially for a period of 2 yr. Each animal was studied first with two normal kidneys on an intake of P of 1,200 mg/day. Then, renal insufficiency was produced by 5/6 nephrectomy. The dogs then were divided into three groups. In group I, 1,200 mg/day P intake was administered for the full 2 yr. In group II, P intake was reduced from the initial 1,200 mg/day, in proportion to the measured fall in glomerular filtration rate, in an effort to obviate the renal adaptation in P excretion. In group III, "proportional reduction" of P intake also was employed; but in addition, 20 μg of 25(OH)D_3 were administered orally three times a week.

In group I, parathyroid hormone (PTH) levels rose throughout the 2-yr period reaching a final concentration of 557±70 U (normal 10–60). In group II, values for PTH remained normal throughout the 1st yr, increased modestly between the 12th and the 18th mo, but then did not rise after the 18th mo. In group III, no elevation of PTH levels was observed at any time; however, these animals were hypercalcemic.

Histomorphologic analyses of the ribs of these dogs were performed serially throughout the 2-yr period. A linear relationship was obtained between the osteoclastic resorption surface and the concentration of circulating immunoreactive PTH. The osteoid volume was greater in group I animals when compared to those in group II. None of the morphologic abnormalities associated with renal osteodystrophy were observed in the animals in the third group.

INTRODUCTION

The homeostatic mechanisms governing calcium and phosphorus metabolism are severely challenged by the loss of renal function. The adaptations required for the maintenance of external balance and the internal milieu occur predominantly, though not exclusively, through alterations in two interrelated hormonal systems: parathyroid hormone and vitamin D. These hormonal systems have profound effects on bone, the reservoir of calcium and phosphorus. Excesses or deficiencies of these minerals activate the system in a manner that appears to be directed toward the maintenance of balance often at the expense of bone (1–3).

An increase in the fractional phosphate excretion per nephron is required for the maintenance of external phosphorus balance, as the number of excretory units decrease because a compensatory decrease in

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phosphorus absorption does not occur. The adaptations required for the maintenance of phosphorus balance appear early in the course of the disease, whereas calcium malabsorption may be a relatively late phenomenon (4). Evidence obtained during the past several years has served to implicate the requirement for an adaptive increase in fractional phosphate excretion, which accompanies the fall in glomerular filtration rate (GFR)[1] in chronic renal disease, as an important factor in the pathogenesis of secondary hyperparathyroidism (5, 6). It was reasoned that if, during the evolution of chronic renal disease, the load of phosphorus to be excreted by the kidneys could be decreased in proportion to the fall in GFR, the requirement for an adaptive increase in the fractional phosphorus excretion could be negated and hyperparathyroidism could be minimized or prevented. When "proportional reduction" of phosphorus intake was employed in dogs in which renal mass and GFR were reduced in sequential steps, secondary hyperparathyroidism was in fact prevented (6, 7). These observations were made over a relatively short period of time and the study designed did not allow for either the evaluation of the possible importance of calcium absorption or the effect of proportional reduction of phosphorus intake on renal osteodystrophy.

It is known that calcium malabsorption occurs in chronic renal failure, secondary to alterations in the metabolism of vitamin D (4, 8–12). Vitamin D is first metabolized by the liver into $25(OH)D_3$ (13–14) and then by the kidney into $1,25(OH)_2D_3$ and other highly polar metabolites (15–18). Patients with chronic renal disease have low levels of $1,25(OH)_2D_3$ secondary to the failure of the kidney to hydroxylate $25(OH)D_3$ in the carbon-1 position (19). A number of studies (10, 20–22) have demonstrated that calcium absorption may be normalized by the administration of $25(OH)_2D_3$ and $1,25(OH)_2D_3$, the latter being more potent. $1,25(OH)_2D_3$ alone or synergistically with parathyroid hormone (PTH) stimulates osteoclastic activity and bone resorption. $25(OH)D_3$, although less potent than $1,25(OH)_2D_3$, also has this capacity (23), furthermore $25(OH)D_3$ or a metabolic derivative is important in the "maturation" of collagen and mineral (24).

In this study, we have attempted to normalize the external balance of phosphorus and calcium by restricting phosphorus intake (in direct proportion to the decrease in GFR) and by normalizing calcium absorption by the administration of $25(OH)D_3$ in animals with moderately severe renal disease (20% of normal) over a long period of time (2 yr) in order to evaluate their effects on the development of secondary hyperparathyroidism and renal osteodystrophy.

METHODS

14 female mongrel dogs weighing 15–20 kg were tube fed 400 g of a fibrin-base low phosphorus (P) diet (Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio) daily for 2 yr. During the initial period of the studies, when all of the animals had normal renal function, 1,200 mg/day of supplementary P were added to the diet. After completion of the base-line studies, GFR was reduced in all 14 dogs by ligating most of the arterial branches to one kidney and performing a contralateral nephrectomy. Thereafter the animals were divided into three groups. In group I (4 dogs), the 1,200 mg/day P supplement was continued for the entire 2-yr period of study. In group II (4 dogs), the amount of supplementary P was reduced from the initial 1,200 mg/day in proportion to the measured fall in GFR (e.g., with a 50% reduction in GFR, P intake was reduced to 600 mg/day; with a 75% reduction in GFR, intake was reduced to 300 mg/day, etc.). In group III (6 dogs), the same regimen for proportional reduction of P intake was employed as in group II; however, 20 μg of $25(OH)D_3$ was administered orally three times per week.

Exogenous creatinine clearance was measured every 2–4 mo, with the dogs fasting, unanesthetized, and standing quietly in slings. The technique employed has been described previously (5). During the clearance studies, determinations were made of the concentrations of total serum calcium, ionized serum calcium, and serum P (6). In addition, serum P concentrations were measured at weekly intervals throughout the study. Serum immunoreactive PTH levels were measured at 6-mo intervals by the method previously described (25). $25(OH)D_3$ serum levels were measured by a protein binding assay as previously described (26).

Enteric absorption of calcium was estimated as follows: 20–30 μCi of ^{47}Ca was given by orogastric tube with 120 mg Ca (as calcium in the diet) to fasting animals, and 100 ml of blood were drawn into heparinized syringes at 2, 4, and 24 h and counted directly in an Armac volume counter (Packard Instruments Co., Inc., Downers Grove, Ill.). The blood was reinfused into each dog immediately after counting. 2 days later, 7–10 μCi ^{47}Ca was administered intravenously in 180 ml of 2.5% dextrose in water by constant infusion at 2.0 ml/min. The same diet containing 120 mg Ca was given at the start of the infusion. The radioactivity in the blood was measured 4 h, 2 h, and immediately before the start of the intravenous infusion in order to account for the radioactivity remaining from the previously administered oral ^{47}Ca. The variation between these counts was found to be exceedingly small as were the absolute counts. This procedure was discontinued in the later studies and only a single count was performed just before the start of the intravenous infusion. Blood radioactivity was counted at 2, 4, and 24 h after the start of the intravenous infusion. The schedule and calculations are given in Table I.

Rib biopsies were obtained from dogs before the induction of decreased renal mass and then repeated sequentially during the course of the study. The bones were fixed in a 1:3 mixture (vol/vol) of 8% aqueous glutaraldehyde and 100 mM sodium cacodylate. They were then embedded in methylmethacrylate. A series of sections, 5- to 6-μm thick, were obtained with a R-Jung microtome (American Optical Corp., Buffalo, N. Y.). The 1st, 10th, 20th, 30th, 40th, and 50th sections were selected and stained with toluidine blue (pH 2.8). Each following section, 2nd, 11th, 21st, 31st, 41st, and 51st,

[1] *Abbreviations used in this paper*: CRD, chronic renal disease; GFR, glomerular filtration rate; P, phosphorus; PTH, parathyroid hormone.

TABLE I
Schedule and Calculations Used for the Measurement of Calcium Absorption

$$\frac{\frac{X}{PO\ ^{47}Ca}}{\frac{Y-(Z\times B)}{IV\ ^{47}Ca}}\times 100 = \%\ \text{Ca absorption}$$

X = Blood counts* @ 2, 4, or 24 h after the oral administration of ^{47}Ca
Y = Blood counts @ 2, 4, or 24 h after the intravenous administration of ^{47}Ca
Z = Blood counts immediately before the intravenous administration of ^{47}Ca
B = Biologic decay (fractional disappearance of radioactivity from blood) over 2, 4, or 24 h
PO ^{47}Ca = Total corrected counts given orally
IV ^{47}Ca = Total corrected counts given intravenously

* Corrected for background and decay.

was analyzed under UV light to evaluate tetracycline labeling. This was done in order to compare the measurements of the mineralization front by tetracycline labeling and toluidine blue staining because most but not all dogs were given tetracycline. The two methods gave essentially the same results ($r = 0.93$).

The following parameters were measured with a Zeiss integrating eye piece (no. 2; Carl Zeiss, Inc., N. Y.). The osteoclastic resorption surface (resorption areas lined by one or more osteoclasts) was expressed as a percentage of the mineralized surface of cancellous bone (total cancellous bone surface minus osteoid surface). The total osteoid surface (osteoid surface covered by osteoblasts) was expressed as a percentage of the total cancellous bone surface. The extent of the mineralization front (tetracycline labeled and/or stained with toluidine blue) was expressed as a percentage of the osteoid surface. Other parameters were evaluated with the Zeiss integrating eye piece (no. 1). The absolute volume of cancellous bone (total volume minus marrow volume) was expressed as a percentage of the total bone volume. Osteoclasts (adherent to the bone surface) were counted and expressed per mm^2 of bone.

Each measurement was made on five to six sections and a mean value obtained with the intrabiopsy variation expressed as the standard error of the mean. This permitted an assessment of the significance of the changes obtained during the course of the disease.

RESULTS

Measurement of calcium absorption. Preliminary studies were performed on both normal dogs and dogs with experimental chronic renal disease (CRD) to determine the reliability and reproducibility in our hands of the technique described for the measurement of enteric calcium absorption. A range of values for calcium absorption was achieved by varying the amount of calcium given as carrier (from 20 to 200) and by studying dogs with GFRs ranging from 3.5 to 78 ml/min. Calcium absorption was measured twice in each animal under identical conditions; the interval between measurements varied from 2–12 wk. The results comparing the initial with the follow-up measurements are shown in Fig. 1. The values were closely comparable in most dogs and for the group data. The fractional disappearance of radioactivity from blood was similar in normal dogs and dogs with CRD.

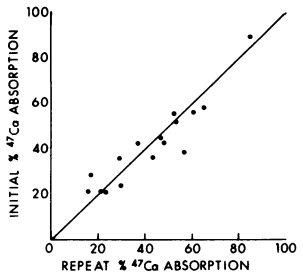

FIGURE 1 Comparison of calcium absorption (% of oral load) determined on two separate occasions. Each point represents the two values for one animal. The spread in values for the group data was achieved by changing the amount of calcium carrier (20–200 mg) and by using animals with a wide range of GFR (3.5–78 ml/min). The initial and repeat studies in each animal were conducted under identical conditions. The correlation coefficient is 0.93.

To obtain further information about the effects of CRD on calcium absorption, ^{47}Ca absorption studies were performed on five normal dogs and five dogs that had a decreased renal mass for 3–4 mo before the study. The results are shown in Table II. Calcium absorption in the normal dogs (mean GFR of 66.9±3.6 ml/min) averaged 38.9% at 2 h, 49.2% at 4 h, and 50.7% at 24 h. By contrast, in the dogs with experimental CRD (mean GFR of 8.9±2.8 ml/min), calcium absorption averaged 20.2% at 2 h, 29.1% at 4 h, and 37.6% at 24 h. The differences in the values at 2 and 4 h are statistically significant ($P < 0.01$ and < 0.02, respectively). Although the mean value for calcium absorption at 24 h was considerably less in the dogs with reduced renal mass than in the normal animals, the

TABLE II
Measurement of Calcium Absorption in Normal Dogs and Dogs with Experimental CRD

	N	GFR	^{47}Ca absorption		
			2 h	4 h	24 h
		ml/min		%	
Normal	(5)	66.9 ±3.6	38.9 ±3.09	49.2 ±4.23	50.7 ±7.35
CRD	(5)	8.9 ±2.8	20.2 ±2.3	29.1 ±3.02	37.6 ±7.27
	P	<0.001	<0.01	<0.02	NS

Refer to the text for details of the studies.

difference did not achieve statistical significance. This pattern is similar to results obtained in humans as previously described by Brickman and collaborators (11).

A final set of absorption studies was performed to determine whether the calcium malabsorption in the dogs with experimental CRD could be reversed by the administration of 25(OH)D_3. This drug was chosen because it has been shown to be more potent than vitamin D in stimulating Ca absorption in uremic animals (20) and because it was readily available in quantities large enough for the execution of the studies. Fig. 2 illustrates the results obtained in one animal studied under four different conditions: (*a*) normal, (*b*) CRD with no supplementary 25(OH)D_3, (*c*) CRD receiving 40 μg of 25(OH)D_3/wk, and (*d*) CRD receiving 40 μg of 25(OH)D_3/day. The induction of renal failure was associated with a substantial decrease in calcium absorption. The abnormality was corrected by the administration of 25(OH)D_3 at both dosage levels. Table III illustrates the composite data obtained from studies on 5 normal dogs and 12 dogs with CRD. Eight of the dogs with CRD were given 40 μg of 25(OH)D_3/day for 8 days; the other four received 40 μg of 25(OH)D_3/wk for 3–6 wk. No evidence of calcium malabsorption was apparent in any of the animals treated with either dosage level of 25(OH)D_3. With the dosage of 40 μg/wk, absorption tended to be slightly less than normal. With the 40 μg/day dose, absorption was greater than normal at 4 and 24 h. On the basis of these results, it was elected to administer 20 μg of 25(OH)D_3 three times per week in the long-term studies on the group III animals.

Long-term studies. Base-line values for GFR, total and ionized serum calcium concentrations, serum P concentrations, PTH levels, and 25(OH)D_3 levels in the 14 dogs are shown in Table IV. The mean values

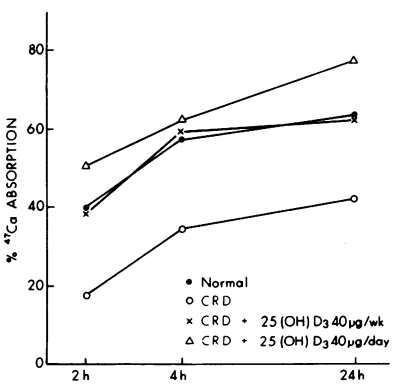

FIGURE 2 Comparison of calcium absorption (expressed as a percentage of the oral load on the ordinate) vs. time in a single dog under four conditions: (● —— ●) normal, (○ —— ○) CRD, (× —— ×) CRD plus 40 μg of 24(OH)D_3/wk, (△ —— △) CRD plus 40 μg of 25(OH)D_3/day.

TABLE III
Calcium Absorption in Normal Dogs and Animals with Experimental CRD Treated with 25(OH)D_3

	⁴⁷Ca absorption		
	2 h	4 h	24 h
	%		
Normal (5)	38.9±3.09	49.2±4.23	50.7±7.35
CRD (8) 40 μg 25(OH)-D_3/day	37.8±6.60*	54.5±7.10*	67.9±6.52*
CRD (4) 40 μg 25(OH)-D_3/wk‡	30.0±3.49*	41.3±5.87*	48.9±5.63*

Data are given as mean±SEM.
* Values are not statistically different from control.
‡ This dose was given for 3–6 wk.

were as follows: GFR, 66.2±3.7 ml/min; total calcium, 9.73±0.16 mg/100 ml; ionized calcium, 4.6±0.2 mg/100 ml; serum P, 4.1±0.3 mg/ml; PTH, 34.7±3.9 μl eq/ml; and 25(OH)D_3, 35.9±2.6 ng/ml. After completion of the base-line studies, reduction in renal mass was produced in all the dogs and they were divided into three groups as described in Methods. The reduction of renal mass was similar in all animals, and the level of renal insufficiency was closely comparable in all three groups. Mean values for GFR, measured at 2- to 4-mo intervals over a 2-yr period,

TABLE IV
Base-Line Studies of GFR, Total and Ionized Serum Calcium, Serum P, PTH, and 25-Hydroxycholecalciferol in 14 Normal Dogs

Dog	GFR	Calcium		P	PTH	25(OH)D_3
		Total	Ionized			
	ml/min	*mg/100 ml*		*mg/100 ml*	*μl eq/ml**	*ng/ml*
1	74	9.65	4.9	3.0	37	24
2	71	8.93	5.4	3.8	42	31
3	86	10.40	5.5	3.6	31	30
4	57	9.05	5.0	4.0	57	27
5	71	8.98	5.6	5.2	37	48
6	55	9.91	5.1	2.6	33	46
7	79	10.20	4.3	5.4	52	38
8	56	10.50	4.6	4.8	19	41
9	49	9.73	4.3	3.7	23	44
10	50	9.78	3.4	6.0	43	33
11	67	9.54	4.4	5.4	13	25
12	58	9.19	3.4	3.0	27	40
13	59	9.55	4.1	2.9	58	53
14	96	10.80	4.7	3.5	14	22
Mean	66.2	9.73	4.6	4.1	34.7	35.9
SEM	±3.7	±0.16	±0.2	±0.3	±3.9	±2.6

* μl eq/ml = microliter equivalent per milliliter of serum.

are shown in Table V. GFR averaged 12.7±2.28 for group I, 12.9±3.76 for group II, and 11.2±2.09 for group III. None of these values differed significantly from the others. In the animal model used in these studies, some degree of compensatory renal hypertrophy occurs regularly; however, at the end of the 2-yr period of study, GFR remained equal in the three groups, averaging 18 ml/min in each. The serum calcium and P concentrations measured during the clearance studies are also shown in Table V. The values for the serum P concentrations measured weekly in each animal in the fasting state throughout the 2-yr period were generally higher than those shown in Table V, averaging 5.35±0.3 mg/100 ml for group I, 4.72±0.1 mg/100 ml for group II, and 4.57±0.1 mg/100 ml in group III. The serum P concentrations obtained during the clearance studies are considered more reliable because they were measured in triplicate and were collected under standardized early morning fasting conditions.

Fig. 3 demonstrates the results of PTH assays performed at 6-mo intervals throughout the 2-yr period in each group of dogs. The serum PTH concentrations rose progressively in the animals in group I which were maintained on the 1,200 mg P diet, and at the end of the 2-yr the values were approximately 10 times normal. In the group II animals, which were maintained on a proportionally reduced P intake, the serum immunoreactive PTH concentrations remained normal throughout the 1st yr; then increased, but only to a modest degree, between 12 and 18 mo; but from 18 mo through the end of the study no significant increment occurred. In the group III animals, which were maintained both on proportional reduction of P intake and 25(OH)D_3, the immunoreactive PTH concentrations were low or undetectable throughout the study (the lower limit of iPTH detectability is 20 pg/ml). The serum 25(OH)D_3 concentration in this group ranged from 100 to 170 ng/ml (normal, 22–53 ng/ml).

Bone histology. Quantitative histologic data from 12 normal dogs and each animal at specific times after the induction of CRD are presented in Table VI. Each parameter is plotted versus time in Figs. 4 and 5.

TABLE V

*Mean Values for GFR, Total and Ionized Serum Calcium, and P in Three Groups of Dogs with Experimental CRD**

Group (N)	GFR	Calcium		P
		Total	Ionized	
	ml/min	*mg/100 ml*		*mg/100 ml*
I (4)	12.7±2.28	10.2±0.22	4.30±0.29	4.40±0.38
II (4)	12.9±3.76‡	10.4±0.25‡	4.64±0.13‡	3.81±0.20‡
III (6)	11.2±2.09‡	11.7±0.56‡	5.74±0.16§	2.64±0.28§

* Data are given as means±SEM.
‡ Values are not statistically different from group I.
§ $P < 0.05$.

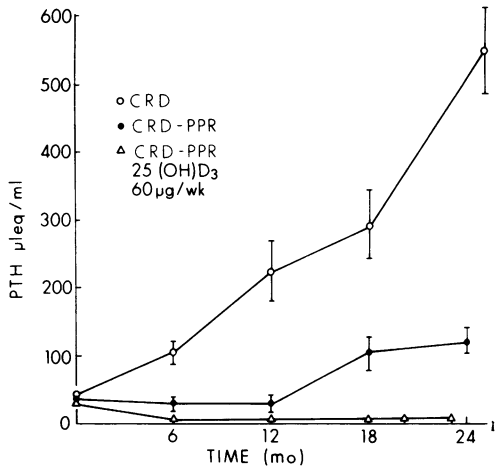

FIGURE 3 Representation of mean±SE for serum PTH levels as a function of time in the three groups of dogs. Group I (○ —— ○) consisted of the four dogs with CRD ingesting 1,200 mg P/day; Group II, as defined in the text (● —— ●), includes the four dogs with CRD maintained on proportional reduction of P (PRP); Group III (△ —— △) includes the six dogs with CRD on the proportional reduction regimen plus supplemental 20 µg of 25(OH)D_3 three times per week.

Ribs from group I dogs exhibited most of the morphological changes observed in chronic uremia in man: increased osteoclast count and osteoclastic resorption surface, increased osteoid volume and surface, and a decrease in the mineralization front. Each change became progressively worse with time. The increase in osteoclastic resorption surface paralleled the increase in the concentration of circulating PTH (Figs. 3 and 4).

When comparisons among the three groups are analyzed, the following changes are observed: (*a*) the absolute volume of cancellous bone decreased progressively in groups I and II but remained unchanged in group III (Fig. 4); (*b*) there was no change in osteoclast count in any group during the first 6 mo. After 18 mo the count was unchanged in group III, slightly increased in group II, and most increased in group I; (*c*) the osteoclastic resorption surface was quite different in the three groups: markedly increased in group I, moderately increased in group II, and normal or even decreased in group III. Each of these changes paralleled the changes in circulating PTH concentrations (Fig. 6); (*d*) the osteoid volume was markedly increased in group I, moderately increased in group II, and unchanged in group III (Fig. 5); (*e*) the osteoid surface was markedly increased in groups I and II and unchanged to decreased in group III (Fig. 5); (*f*) the mineralization front was markedly decreased in group I, modestly decreased in group II during the 1st yr and markedly decreased thereafter; it was effectively increased in group III (Fig. 5).

2 yr after the induction of uremia, one dog from group I and one from group II were treated according to

TABLE VI

Quantitative Histology in Ribs of Normal Dogs and Dogs with Experimental CRD

Dog	Length of CRD	Absolute volume of cancellous bone	Osteoclast count	Osteoclast resorption surface	Osteoblastic surface	Total osteoid surface	Calcification front	Osteoid volume
	mo	*% total bone volume*	*mm² of section*	*% of calcified surface*	*% of total surface*	*% of total surface*	*% of osteoid surface*	*% of absolute volume of cancellous bone*
Group I: Dogs fed a diet containing P, 1,200 mg/24 h								
1a	6	23.2±0.9	0.4±0.04	2.8±0.6	10.0±2.0	47.6±4.5	35.0±3.0	16.1±1.0
b	9	21.4±1.2	0.92±0.12	2.8±0.5	8.0±0.7	30.5±2.3	33.0±6.0	14.0±2.8
c	12	22.1±2.6	1.9±0.2	4.6±0.4	5.0±0.5	27.6±1.6	23.0±4.0	17.0±2.1
d	18	18.4±0.01	2.7±0.8	7.0±0.3	3.3±0.1	49.0±3.4	22.0±3.0	18.0±2.0
2	12	29.0±1.4	1.9±0.4	4.2±0.5	8.6±1.2	36.5±1.1	16.0±2.0	10.0±0.5
3	14	35.7±0.2	2.8±0.2	4.6±0.2	9.2±1.0	37.6±1.6	22.0±2.0	13.0±0.6
4a	6	25.9±2.5	0.69±0.04	2.2±0.1	2.8±0.2	37.0±1.8	34.0±2.0	10.6±0.9
b	9	24.9±0.8	0.88±0.03	3.2±0.1	6.1±1.4	32.7±0.6	24.0±1.0	12.0±0.9
c	18	23.0±1.0	1.50±0.2	5.3±0.2	7.9±3.3	54.0±1.8	21.0±1.0	14.0±1.1
Group II: Dogs fed a diet in which P was proportionally reduced in relation to the fall in GFR								
1	18	17.0±0.1	1.9±0.5	1.4±0.2	6.1±0.4	29.0±0.8	16.0±3.0	9.1±1.3
2a	12	22.0±1.1	0.8±0.01	2.6±0.4	5.4±0.04	58.0±3.2	43.0±4.0	13.1±0.6
b	18	22.0±3.3	0.95±0.03	3.3±0.1	5.0±1.3	62.0±0.7	12.0±0.1	15.7±1.3
3a	14	21.0±1.2	0.90±0.07	1.6±0.6	9.3±2.6	34.0±3.6	27.0±5.0	7.0±1.0
b	18	19.0±0.8	0.7±0.1	2.1±0.1	9.8±0.04	31.0±1.6	20.0±3.0	9.5±2.4
4	18	15.0±1.1	1.6±0.2	2.2±1.0	5.1±1.1	41.0±2.1	18.0±3.0	7.9±1.7
Group III: Dogs fed a diet as group II + 25(OH)D_3, 60 μg/wk								
1	9	23.0±2.7	0.65±0.1	1.6±0.03	8.5±1.3	15.0±2.0	55.0±4.0	2.5±0.2
2	9	21.0±1.2	1.4±0.2	1.9±0.7	4.4±0.4	17.0±3.5	55.0±5.0	2.1±0.3
3a	10	28.0±0.5	0.3±0.03	1.4±0.2	6.9±1.1	29.0±2.0	31.0±2.0	4.3±1.1
b	21	29.0±0.7	0.3±0.07	0.9±0.1	2.9±0.6	17.0±0.7	41.0±2.0	5.1±0.6
4a	15	24.0±0.2	1.3±0.06	2.4±0.3	3.4±0.8	11.0±0.5	38.0±5.0	3.8±0.8
b	27	22.0±1.7	0.36±0.07	1.6±0.5	2.1±0.9	9.0±0.6	68.0±4.0	3.3±0.4
5a	12	30.0±0.3	1.3±0.05	1.1±0.1	9.1±0.4	21.0±1.5	33.0±0.5	3.6±0.05
b	18	22.0±0.9	0.5±0.04	0.7±0.3	1.8±0.7	12.0±1.0	42.0±5.0	5.1±0.6
Normal $n = 12$ Mean±SD		27.1±1.8	0.7±.05	1.75±.19	6.74±.88	25.9±1.95	35.2±2.2	7.67±1.19

Refer to text for details of the studies.

the group III protocol. Individual data points comparing the osteoclastic resorption surface and mineralization front for each dog from group I and II are plotted in Fig. 7. Within 3–6 mo the osteoclastic resorption surface returned to normal and during that same time there was a marked increase in the mineralization front, thus demonstrating a rapid normalization of these important parameters after the administration of 25(OH)D_3.

DISCUSSION

The data demonstrated that dogs with experimentally induced renal insufficiency, maintained on a constant,

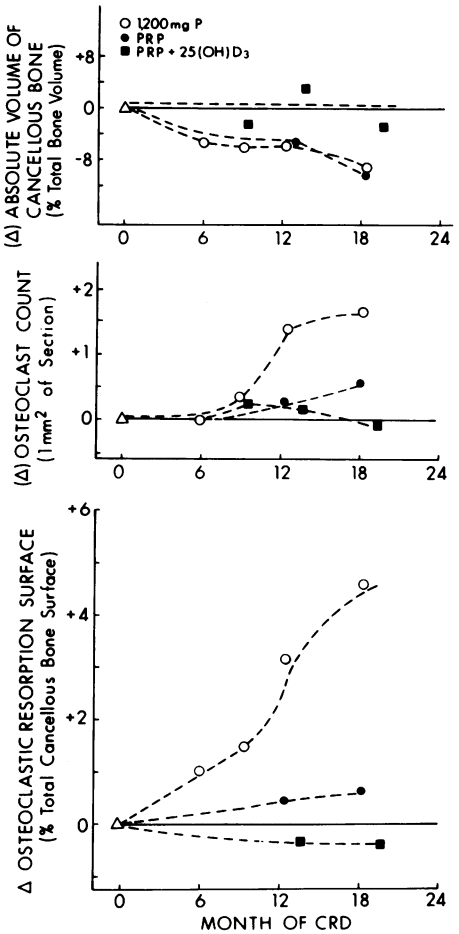

FIGURE 4 Representation of temporal changes in bone histology from ribs obtained in three groups of dogs with CRD. The upper part of the graph illustrates delta changes for absolute volume of cancellous bone; the middle part, the osteoclast count; and the lower part, the osteoclastic resorption surface. Group I (○ —— ○) represents dogs ingesting 1,200 mg P/day; group II (● —— ●) represents dogs maintained on proportional reduction of P (PRP); group III (■ —— ■) represents dogs on proportional reduction of P plus supplemental 20 μg of 25(OH)D_3 three times a week.

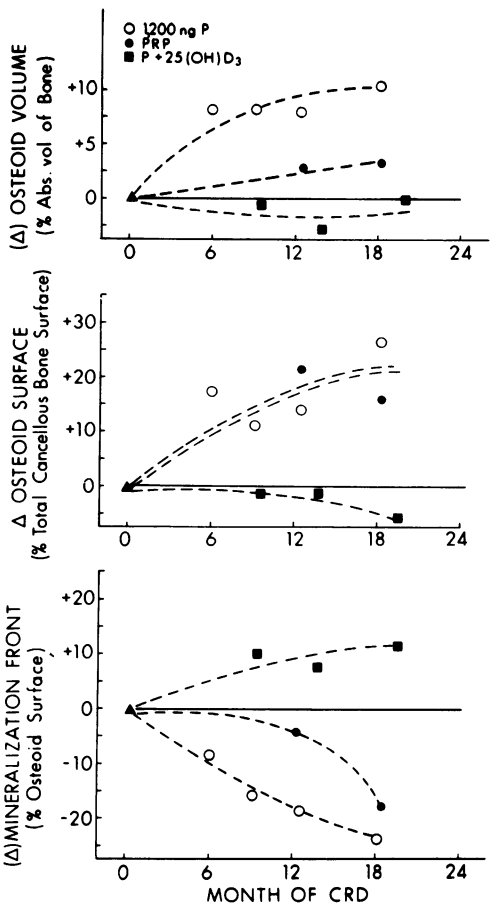

FIGURE 5 Representation of temporal changes in bone histology from ribs obtained in three groups of dogs with CRD. The upper part of the graph illustrates delta changes for osteoid volume; the middle part, osteoid surface; and the lower part, the mineralization front. Other abbreviations as in Fig. 4.

normal intake of P (group I) developed severe progressive hyperparathyroidism. When animals with the same degree of renal insufficiency were maintained on a regimen of proportional reduction of P intake (group II), secondary hyperparathyroidism was completely pevented for a full year. The circulating levels of PTH increased modestly between the 12th and through the 18th mo with no further increment thereafter. These observations serve to support the hypothesis that secondary hyperparathyroidism is, at least in part, a consequence of the physiological adaptation which serves to maintain P homeostasis in the face of a progressive reduction in the number of excretory units (7).

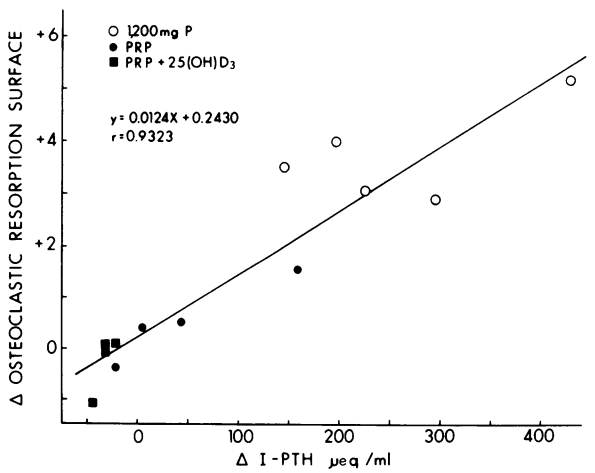

FIGURE 6 Comparison of serum immunoreactive PTH levels and osteoclastic resorption surface for three groups of dogs with CRD. Other abbreviations as in Fig. 4.

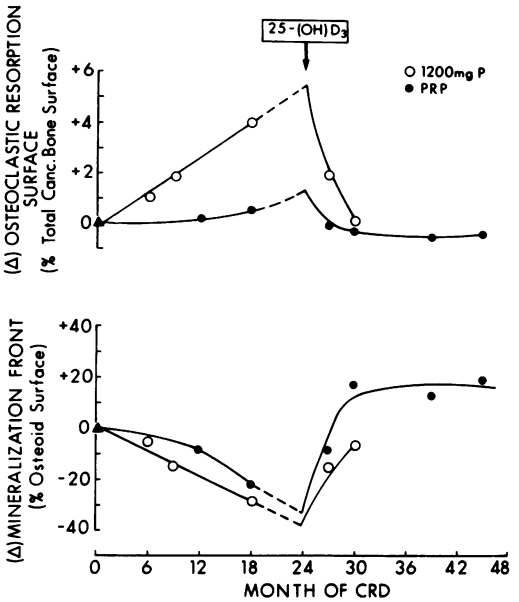

FIGURE 7 Illustration of the results of bone histology in two representative dogs with CRD during the first 24 mo of CRD and after treatment with 20 μg of 25(OH)D_3 three times a week. Other abbreviations as in Fig. 4.

The effect of hyperparathyroidism on bone morphology appears to be an increase in the osteoclastic resorption surface because the change in osteoclastic resorption surface paralleled changes in the serum concentrations of PTH in all groups (Fig. 6). Furthermore, the changes were temporally related (cf. Figs. 3 and 4). The slight increase in osteoclastic resorption surface found in group II at the end of 2 yr also correlates with the failure of proportional reduction of P intake to completely prevent secondary hyperparathyroidism. These data, as well as those from other sources (27), strongly implies a cause and effect relationship between PTH and osteoclastic resorption surface.

There was general agreement between the osteoclast count and the extent of the osteoclastic resorption surface; however, the increase in resorption surface was observed earlier, suggesting that activity increased before the increase in osteoclast number.

The total bone volume decreased in both groups (I and II) that did not receive 25(OH)D_3. The decrease in bone volume in group I can be explained, in part, by excessive resorption. This is further illustrated by the marked decrease in mineralized bone; however, excessive resorption cannot explain the bone loss in group II because the osteoclastic resorption surface was nearly normal. The alternative explanation in this group could be a decrease in bone matrix formation. This explanation remains speculative because apposition rate was not measured.

The volume of osteoid present at any time reflects the relative rates of matrix formation and mineralization. The excessive osteoid volume in group I could have resulted from an increase in matrix formation or a decrease in mineralization; but, inasmuch as neither the appositional rate nor the rate of mineralization was measured, this issue is not resolved.

The histologic findings in animals in group III are in striking contrast to those of groups I and II. The administration of 25(OH)D_3 in combination with proportional reduction of P intake prevented the histologic manifestations of renal osteodystrophy. The absolute volume of bone remained normal as did the osteoclast count, the osteoclastic resorption surface, the osteoid surface, and the osteoid volume. The mineralization front actually increased above normal. Hypercalcemia alone has been shown to be incapable of producing an increase in the mineralization front in uremic man (28). The increase in the mineralization front must, therefore, be ascribed to a more specific mineralization effect of 25(OH)D_3 or some more polar metabolite.

These data suggest that the hypercalcemia occurring under the conditions of the study was not primarily a result of continuing net removal of mineral from bone because the absolute volume of bone and the osteoid volume were normal. These data, plus the fact that these animals were hypercalcemic and the circulating levels of immunoreactive PTH were reduced, suggest that the dose of 25(OH)D_3 was excessive. The hypercalcemia may have been secondary to excessive calcium absorption. The dose of 25(OH)D_3 chosen was based on absorption studies in uremic animals receiving 1,200 mg P/day. Animals with 20% of normal renal function in whom P retention was prevented and in whom the serum P was low might be able to make more 1,25$(OH)_2D_3$ than animals on a normal P diet because P has been implicated as an important factor in the regulation of the activity of the 1-hydroxylase system (29). Therefore, under these conditions, calcium absorption may have been, in fact, greater than normal.

Positive balance and hypercalcemia may have been achieved even if intestinal calcium absorption were normal if renal excretion of calcium were not comparably increased. Maximum rates for calcium excretion/GFR have not been clearly defined. Brickman et al. (30) noted hypercalcemia in uremic patients after the administration of 1,25$(OH)_2D_3$. The same dose given to normal subjects did not produce hypercalcemia even though calcium absorption increased in both groups. The differences were in large measure attributed to a limited capacity for calcium excretion in the uremic patients. This could also be the case in our animals.

The hypercalcemia in the animals in group III was

undoubtedly important in suppressing the circulating levels of immunoreactive PTH. It is, however, not possible to exclude a further effect of $25(OH)D_3$ or a derivative more polar metabolite on the release of PTH in view of recent observations (31–32). Particularly because the concentrations of $1,25(OH)_2D_3$, under these experimental conditions, may have been increased.

The low serum P concentration (2.64 mg/100 ml) in group III animals may represent selective uptake and sequestering of P in tissue. The tubular reabsorption of P in this group was virtually complete in the studies performed in fasting conditions. This finding could be explained by the known effects of $25(OH)D_3$ (33) and low concentrations of PTH on tubular P reabsorption (34). If $25(OH)D_3$ or a more polar metabolite had any effect on P absorption by the gastrointestinal tract, one would expect it to be enhanced (35–36). Thus, the low serum P concentration most likely represents an increase in the uptake of P by bone. Whether the P concentration in other tissue (e.g., muscle) was increased or not is unanswered. This is an important facet which merits further investigation.

These data suggest that in the dog with CRD the obligatory adaptation for renal P excretion plays an important role in the development of secondary hyperparathyroidism and thereby appears to be responsible for the observed increase in the osteoclastic resorption surface. However, the mineralization defect was prevented by the administration of $25(OH)D_3$.

These studies suggest that in the dog with experimental CRD the development of renal osteodystrophy can be prevented by reduction of P in the diet and the simultaneous administration of $25(OH)D_3$. It is premature at this time to prognosticate that similar results will be obtained in humans with CRD if the same therapeutic regimen is used. However, based on the pathogenesis of renal osteodystrophy, the results of this study in the dog, and preliminary results in our patients with CRD, a rational approach for the prevention of bone disease in patients with uremia can now be developed.

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