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)D₃ 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
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) 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 stages, 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)D3 (13–14) and then by the kidney into 1,25(OH)2D3 and other highly polar metabolites (15–18). Patients with chronic renal disease have low levels of 1,25(OH)2D3 secondary to the failure of the kidney to hydroxylate 25(OH)D3 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)D3 and 1,25(OH)2D3, the latter being more potent. 1,25(OH)2D3 alone or synergistically with parathyroid hormone (PTH) stimulates osteoclastic activity and bone resorption. 25(OH)D3, although less potent than 1,25(OH)2D3, also has this capacity (23), furthermore 25(OH)D3 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)D3 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 fibroretro 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 supplementation 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)D3 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)D3 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 47Ca was given by orogastric tube with 120 mg Ca (as calcium in the diet) and other addition, calcium, trivalent cations, and to human subjects. The technique employed has been described previously (25). 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)D3 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 47Ca was given by orogastric tube with 120 mg Ca (as calcium in the diet) and other

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

To obtain further information about the effects of CRD on calcium absorption, 47Ca 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.

Fig. 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.

Refer to the text for details of the studies.

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>Measurement of Calcium Absorption in Normal Dogs and Dogs with Experimental CRD</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>GFR (ml/min)</td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
</tr>
<tr>
<td>Normal</td>
<td>66.9 ± 3.6</td>
</tr>
<tr>
<td>CRD</td>
<td>8.9 ± 2.8</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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)D₃. 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)D₃, (c) CRD receiving 40 μg of 25(OH)D/ wk, and (d) CRD receiving 40 μg of 25(OH)D/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)D₃ 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/ day for 8 days; the other four received 40 μg of 25(OH)D/ 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)D₃. 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)D₃ 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)D₃ levels in the 14 dogs are shown in Table IV. The mean values 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)D₃, 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,
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)D₃, the serum immunoreactive PTH concentrations were low or undetectable throughout the study (the lower limit of PTH detectability is 20 pg/ml). The serum 25(OH)D₃ 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

<table>
<thead>
<tr>
<th>Group (N)</th>
<th>GFR</th>
<th>Total</th>
<th>Ionized</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/min</td>
<td>mg/100 ml</td>
<td>mg/100 ml</td>
<td></td>
</tr>
<tr>
<td>I (4)</td>
<td>12.7±2.28</td>
<td>10.2±0.22</td>
<td>4.30±0.29</td>
<td>4.40±0.38</td>
</tr>
<tr>
<td>II (4)</td>
<td>12.9±3.76</td>
<td>10.4±0.25</td>
<td>4.64±0.13</td>
<td>3.81±0.20</td>
</tr>
<tr>
<td>III (6)</td>
<td>11.2±2.09</td>
<td>11.7±0.56</td>
<td>5.74±0.16</td>
<td>2.64±0.28</td>
</tr>
</tbody>
</table>

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

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 osteoid 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

<table>
<thead>
<tr>
<th>Dog</th>
<th>Length of CRD</th>
<th>Absolute volume of cancellous bone</th>
<th>Osteoclast count</th>
<th>Osteoclast resorption surface</th>
<th>Osteoblastic Total osteoid surface</th>
<th>Calcification front</th>
<th>Osteoid volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mo</td>
<td>% total bone volume</td>
<td>mm³ of section</td>
<td>% of calcified surface</td>
<td>% of total surface</td>
<td>% of osteoid surface</td>
<td>% of absolute volume of cancellous bone</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>17.0±0.1</td>
<td>1.9±0.5</td>
<td>1.4±0.2</td>
<td>6.1±0.4</td>
<td>29.0±0.8</td>
<td>16.0±3.0</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>22.0±1.1</td>
<td>0.8±0.01</td>
<td>2.6±0.4</td>
<td>5.4±0.04</td>
<td>58.0±3.2</td>
<td>43.0±4.0</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>22.0±3.3</td>
<td>0.95±0.03</td>
<td>3.3±0.1</td>
<td>5.0±1.3</td>
<td>62.0±0.7</td>
<td>12.0±0.1</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>15.0±1.1</td>
<td>1.6±0.2</td>
<td>2.2±1.0</td>
<td>5.1±1.1</td>
<td>41.0±2.1</td>
<td>18.0±3.0</td>
</tr>
</tbody>
</table>

Group II: Dogs fed a diet in which P was proportionally reduced in relation to the fall in GFR

<table>
<thead>
<tr>
<th>Dog</th>
<th>Length of CRD</th>
<th>Absolute volume of cancellous bone</th>
<th>Osteoclast count</th>
<th>Osteoclast resorption surface</th>
<th>Osteoblastic Total osteoid surface</th>
<th>Calcification front</th>
<th>Osteoid volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>23.0±2.7</td>
<td>0.65±0.1</td>
<td>1.6±0.03</td>
<td>8.5±1.3</td>
<td>15.0±2.0</td>
<td>55.0±4.0</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>21.0±1.2</td>
<td>1.4±0.2</td>
<td>1.9±0.7</td>
<td>4.4±0.4</td>
<td>17.0±3.5</td>
<td>55.0±5.0</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>28.0±0.5</td>
<td>0.3±0.03</td>
<td>1.4±0.2</td>
<td>6.9±1.1</td>
<td>29.0±2.0</td>
<td>31.0±2.0</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>29.0±0.7</td>
<td>0.3±0.07</td>
<td>0.9±0.1</td>
<td>2.9±0.6</td>
<td>17.0±0.7</td>
<td>41.0±2.0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>24.0±0.2</td>
<td>1.3±0.06</td>
<td>2.4±0.3</td>
<td>3.4±0.8</td>
<td>11.0±0.5</td>
<td>38.0±5.0</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>22.0±1.7</td>
<td>0.36±0.07</td>
<td>1.6±0.5</td>
<td>2.1±0.9</td>
<td>9.0±0.6</td>
<td>68.0±4.0</td>
</tr>
<tr>
<td>a</td>
<td>12</td>
<td>30.0±0.3</td>
<td>1.3±0.05</td>
<td>1.1±0.1</td>
<td>9.1±0.4</td>
<td>21.0±1.5</td>
<td>33.0±0.5</td>
</tr>
<tr>
<td>b</td>
<td>18</td>
<td>22.0±0.9</td>
<td>0.5±0.04</td>
<td>0.7±0.3</td>
<td>1.8±0.7</td>
<td>12.0±1.0</td>
<td>42.0±5.0</td>
</tr>
</tbody>
</table>

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)D₃.

DISCUSSION

The data demonstrated that dogs with experimentally induced renal insufficiency, maintained on a constant,
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 prevented 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 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 (O—O) 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)D₃ 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.

**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.
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)D₃ 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 osteoclast 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)D₃ 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)D₃ was excessive. The hypercalcemia may have been secondary to excessive calcium absorption. The dose of 25(OH)D₃ 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)₂D₃ 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)₂D₃. 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)D₃ 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)₂D₃ 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)D₃ (33) and low concentrations of PTH on tubular P reabsorption (34). If 25(OH)D₃ 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)D₃.

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)D₃. 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.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. J. Jowsey for the suggestions regarding the bone biopsies, Dr. Jack Hinman and Mr. Ron McCandlis from the Upjohn Company for supplying the 25(OH)D₃ made available for this study; to Mrs. Sue King, Mrs. Claire Pedersen, and Ms. Jane Lewis for their excellent technical assistance; and to Mrs. Patricia Verplancke and Ms. Patti Lyles for their assistance in the preparation of this manuscript.

This investigation was supported by National Institutes of Health grants AM-09976 and AM-05248, from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

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