The Effects in the Rat of Varying Intakes of Dietary Calcium, Phosphorus, and Hydrogen Ion on Hyperparathyroidism Due to Chronic Renal Failure

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ABSTRACT Renal failure of 4 wk duration in rats led to parathyroid enlargement, increased bone resorption, and decreased tubular reabsorption of phosphate by the remnant kidney. The degree of hyperparathyroidism was influenced by each of the three dietary factors investigated. In the first study increasing calcium intake reduced the size of the parathyroids by increasing calcium and reducing phosphate absorption. In the second study phosphate intake was linearly related to parathyroid gland size in the uremic animals and associated with rising plasma phosphate levels. In the last study acidosis led directly to increased bone resorption but small parathyroid glands associated with elevated ionized calcium levels. Alkalosis lowered the serum ionized calcium and led to parathyroid enlargement and the expected associated findings. It was shown that parathyroid weight reflected both metabolic activity as judged by amino acid uptake, and the content of immunoassayable parathyroid hormone. In all studies gland weight was inversely related to serum ionized calcium.

INTRODUCTION

Abnormalities in bone and in calcium and phosphorus metabolism in patients with chronic renal failure have been the subject of intensive investigation and numerous reports for at least forty years (1–5). From these investigations two major types of bone pathology have been identified, the first characterized by defective mineralization and presenting as rickets or osteomalacia, depending on age, the second showing increased bone resorption, reactive marrow fibrosis, and associated with hyperplasia of the parathyroids (6–8). Two theories have been advanced to explain these findings. In the presence of the calcification defect an abnormality in vitamin D metabolism with inadequate formation of 1,25-dihydroxycholecalciferol has been suggested (9, 10). In the case of hyperparathyroidism with osteitis fibrosa, phosphate retention leading to parathyroid stimulation has been supported by experimental data (11–13). The situation in humans is complex, as it is usual to find varying degrees of both osteomalacia and osteitis fibrosa present simultaneously (14), whilst hyperparathyroidism is almost invariably of importance (15). Thus multiple factors are probably acting together, making the recognition of the contribution of each very difficult.

It was thought worthwhile, therefore, to explore systematically a number of factors that might influence parathyroid secretion in a rat model with chronic renal failure and this paper describes the effects of varying calcium, phosphorus, and hydrogen ion intakes. Each one of these variables affected parathyroid function.

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METHODS

Animals and diet. The experimental details have been described previously (16, 17). Male Holtzman rats between 90 and 95 g were subjected to unilateral nephrectomy and segmental infarction by arterial ligation of the opposite kidney (18) on day 1 and were termed uremics. Control animals had the kidneys exposed and then replaced. The animals were sacrificed on day 28 in the postabsorptive state by aortic bleeding. Food intake in the uremic group was maintained as similar as possible and the intakes of the controls were restricted to match the uremics. The diet was a semisynthetic one prepared in our laboratory to contain casein 12%, dextrose 15%, corn starch 15%, corn oil 15%, vitamin mix 0.02%, salt mix 5–9%, and water to 100%. Diets all contained 0.3% potassium, 0.5% sodium, and 0.1% magnesium. The calcium and phosphorus content, shown in Table I, was derived from calcium carbonate and ammonium phosphate. All chemicals were reagent grade and

TABLE I

Calcium and Phosphorus Content, Net Acid Excretion on Each Diet, Weight Gain, and Total Mean Daily Food Intake

	Calcium			Phosphorus						Acid-base		
	Low	Med	High	0.2%	0.3%	0.4%	0.5%	0.6%	0.7%	Acid	Neut	Base
Ca, %	0.16	0.87	1.71	0.87	0.86	0.88	0.88	0.89	0.90	0.73	0.76	0.80
P, %	0.63	0.68	0.67	0.19	0.29	0.41	0.50	0.58	0.69	0.73	0.72	0.75
Total acid, *meq/day**	1.41	1.35	1.51	0.85	0.96	0.86	0.90	1.1	0.97	3.3	0.5	−0.96
Food intake, *g/day*												
Control	10.0	9.8	9.7	9.0	9.1	9.0	9.1	9.0	9.2	8.8	9.1	9.0
Uremic	10.3	9.9	10.2	9.3	9.2	9.2	9.3	9.3	9.5	7.9	9.1	9.0
Weight gain, *g/day*												
Control	3.1	3.3	2.9	2.5	2.4	2.6	2.6	2.6	2.7	2.5	2.7	2.5
Uremic	2.3	2.6	2.6	1.7	1.9	2.2	2.2	2.1	2.5	1.1	2.0	1.7

* In test animals. See text for details.

the casein was acid washed.[1] Chromium sesquioxide was added to all diets as a nonabsorbable internal marker to give a final concentration of 0.2% (19). The diet was adequate in composition for all known nutrients (20, 21); however, the total quantities fed were inadequate for maximal growth because the intake was limited to match the most anorectic uremic group. Animals were individually caged and only fed when the previously given diet had been consumed, so that total food intake throughout the complete study was quantitated. Deionized water containing 5% dextrose was provided ad lib. The 24-h urine acid excretion values shown in Table I were obtained by placing normal unoperated rats on the test diet for 7 days before collection. When the diets were devised, the acid excretion was first determined and if not suitable the salt mixture was arbitrarily adjusted so that in the final diet the desired acid excretion was obtained. In order to balance urea formation from added ammonium chloride, urea was added to some of the diets in the proportion of 1 mol urea/2 mol ammonium chloride. Pilot studies confirmed similar 24-h urinary urea excretions under these circumstances. All animals received dimethylchlortetracycline intraperitoneally 10 mg/kg on days 13, 14, 21, and 22, and had a 24-h urine collection before sacrifice. Five or six animals in each group were transferred to balance cages for a 7-day balance study from the 2nd to 3rd wk.

Biochemistry. Analytical methods were similar to those described previously (17, 22). Calcium in serum and urine was measured by atomic absorption spectrophotometry and also in stool, diet, and tissues after preliminary ashing at 450°C for 16 h. Blood taken anerobically from the aorta was used to fill 2-ml Vacutainer blood specimen tubes (Becton, Dickinson & Co., Rutherford, N. J.), and the serum ionized calcium measured with an Orion flow-through electrode (Orion Research, Inc., Cambridge, Mass.). After this was completed, pH, and in some studies P_{CO_2} and bicarbonate (from the Henderson-Hesselbach equation), were measured on the same sample with an Instrumentation Laboratory Model 113 analyzer (Instrumentation Laboratory, Inc., Lexington, Mass.). Creatinine and phosphorus were determined on heparinized plasma and urine with standard Auto-Analyzer II methods (23). The percent tubular reabsorption of the phosphorus (TRP)[2] was calculated from the 24-h urine collected before sacrifice using the formula

$$\left(1 - \frac{U_P/P_P}{U_{Cr}/P_{Cr}}\right) \times 100,$$

where P and Cr represent phosphorus and creatinine in plasma (P) or urine (U).

Bone and parathyroids. The diaphysis of the left femur was used for determination of ash weight and calcium and phosphorus content, which were expressed as a percentage of the fresh weight, i.e., in g/100 g. Mineral appositional rates in microns per day were measured between the two tetracycline labels on hand-ground 20-μm thick transverse sections of the right femur. The last lumbar vertebra was stained after decalcification and the number of trabeculae undergoing resorption were measured with a line technique and expressed as a percentage of the total number of trabeculae counted (24).

Both parathyroid glands were removed immediately after the animal was exsanguinated and weighed together on a Cahn electrobalance (Cahn Div., Ventron Instruments Corp., Paramount, Calif.). In the calcium study the dry weights are recorded after lyophilization. Comparison of freeze-dried and fresh weights on the same glands in 41 samples ranging in fresh weight from 0.12 mg to 1.24 mg gave a correlation coefficient $r = 0.92$. In a separate calcium study 60 animals were each injected with 20 μCi [1-^{14}C]α-amino-isobutyric acid (AIB) intraperitoneally 24 h before sacrifice. Parathyroid glands from these animals were processed as described by Raisz, O'Brien, and Au, and Au, Engerman, and Raisz and we have employed their parathyroid activity index (PAI) to compare results between animals (25, 26). $PAI = G/Bwt \times S$ where G is the total disintegrations per minute in the glands, Bwt is body weight, and S is the disintegrations per minute per microgram of serum water. The ratio T/S was also calculated from $(G/TW)/S$ where TW is the tissue water content in micrograms per gland. Serum was taken to be 92%

[1] Nutritional Biochemicals Corporation, Cleveland, Ohio.

[2] *Abbreviations used in this paper:* AIB, α-aminoisobutyric acid; NPX, reduction in renal mass; PTH, parathyroid hormone; PTX, parathyroidectomized; TRP, tubular reabsorption of phosphorus.

water and glandular water content after drying found to be 77.8%±0.9 in 13 normal glands and 80.7%±1.0 in 12 uremics. A value of 79% was taken for all glands. In the study involving parathyroidectomized animals (PTX) (Table IX) the glands were removed surgically at the same time as reduction in renal mass was effected (NPX).

Measurement of immunoassayable parathyroid hormone (PTH) in the glands was carried out on either both glands from a single animal or a pool from several normal or uremic rats. Hormone was extracted by shaking at 4°C for 24 h in 0.1 ml of a solution containing 8 M urea in 0.2 M hydrochloric acid. The proteins were precipitated with 10% trichloroacetic acid and washed, and the precipitate dissolved in 0.85% saline containing 5% trasylol and 10% outdated blood bank plasma. The assay procedure was similar to that described previously by others (27, 28), with a 1/3,000 dilution of chicken antisera developed in our laboratory as antibody. Unknowns and suitable bovine standards in barbital buffer containing EDTA, outdated plasma, and trasylol were incubated with shaking for 4 days at 4°C, then 3,000 cpm of ^{125}iodine-labeled bovine hormone added. After a further 3-day incubation, a charcoal dextran mixture was added and both supernate and precipitate were counted to determine binding. Good sensitivity in the range of 50–10,000 pg of bovine PTH/tube was obtained with hormone damage averaging 7.5%.

Statistics. Data is presented as the mean ± standard error with the number of samples in brackets. Differences between groups were examined by a principal component method of analysis in which the eigenvectors employed bone ash and parathyroid weight. From these eigenvalues an analysis of variance was carried out (29). In addition each variable was examined with a two-way analysis of variance with significance levels at either the 5% or 1% level tested and these are indicated in the tables. For selected variables, when a significant F value was found, the least significant difference test was applied to examine differences between means (30). All processing utilized the McGill IBM 360-75 computer and programs BMDO-ID, 3D, IM, and 2V (31).

RESULTS

Diet calcium content varied

Blood and urine. The blood and urine values are shown in Table II. Ionized calcium was decreased in the uremics, particularly on the low calcium diet, and total calcium was likewise reduced on the low intake in the uremics and equaled the control value on the high diet. Arterial pH values were the same in all groups. TRP was lower in the uremics on all diets and increased progressively as the dietary calcium rose.

Bone and parathyroids. The data in Table III show that calcium deprivation was associated with a decrease in bone ash more marked in uremics than in controls. Both calcium and phosphorus decreased along with the ash, and there were small but significant changes in the organic fraction. Histological examination in a few animals of the epiphysial plate at the lower end of the femur was normal, and undecalcified sections showed no osteoid excess. At all diet levels the uremics had more bone resportive areas than the controls, and the values doubled between the high and low calcium diets.

On all diets the uremic parathyroid glands were larger than the controls and as the calcium intake increased the glands became smaller. There was a significant inverse correlation between the weight of the uremic glands and serum ionized calcium, $r = 0.5$, $P < 0.01$.

Immunoassayable PTH was quantitated from a pool

TABLE II
Diet Calcium Varied

Group	Calcium intake	Creatinine	Ionized Ca	Total Ca	P	TRP
		mg/100 ml	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%
Control	Low	0.44±0.02 (17)	4.2±0.04 (17)	9.5±0.1 (17)	7.3±0.1 (17)	58.3±5.4 (15)
Uremic	Low	0.94±0.06 (23)	3.6±0.06 (24)	8.6±0.2 (24)	7.6±0.2 (23)	14.8±9.4 (22)
Control	Medium	0.42±0.03 (13)	4.4±0.04 (13)	9.4±0.2 (13)	7.1±0.2 (13)	75.5±4.2 (10)
Uremic	Medium	0.93±0.05 (17)	4.3±0.05 (18)	9.5±0.1 (19)	7.5±0.3 (19)	42.5±5.2 (17)
Control	High	0.49±0.02 (12)	4.4±0.04 (12)	9.6±0.2 (12)	7.5±0.1 (12)	81.4±2.8 (12)
Uremic	High	0.94±0.10 (17)	4.2±0.08 (16)	9.6±0.1 (16)	7.6±0.3 (17)	71.3±1.9 (17)
P	Between diets	NS	1%	1%	NS	1%
	Uremics vs. controls	1%	1%	5%	NS	1%

TABLE III
Bone Values, Diet Calcium Varied

Group	Calcium intake	Weight	Ash	Organic	Ca	P	Parathyroid wt	Trabecular resorption
		g	%	%	%	%	*mg dry wt*	
Control	Low	0.498±0.01 (17)	44.6±0.94 (16)	24.3±0.3 (16)	16.65±0.5 (16)	9.05±0.2 (16)	0.107±0.01 (17)	5.1±0.7 (16)
Uremic	Low	0.476±0.01 (24)	41.2±0.78 (24)	24.2±0.3 (24)	14.48±0.6 (23)	8.47±0.2 (24)	0.169±0.01 (23)	18.1±1.5 (24)
Control	Medium	0.564±0.01 (13)	53.7±0.46 (13)	23.0±0.2 (13)	20.39±0.4 (13)	9.66±0.3 (13)	0.109±0.01 (23)	3.8±0.4 (12)
Uremic	Medium	0.551±0.01 (19)	50.7±0.65 (19)	23.4±0.2 (19)	18.61±0.4 (19)	9.54±0.2 (19)	0.129±0.01 (18)	10.0±1.1 (19)
Control	High	0.553±0.01 (12)	53.7±0.57 (12)	22.8±0.2 (12)	20.32±0.5 (12)	10.02±0.2 (12)	0.078±0.01 (12)	4.1±0.5 (12)
Uremic	High	0.556±0.01 (17)	52.6±0.49 (16)	23.3±0.2 (16)	19.63±0.3 (17)	9.62±0.3 (17)	0.106±0.01 (16)	9.5±1.4 (17)
P	Between diets	1%	1%	1%	1%	1%	1%	1%
	Uremics vs. controls	NS	1%	5%	1%	NS	1%	1%

of uremic and control glands from a separate group of animals. These were extracted as described under Methods and serial dilutions in triplicate of each extract compared with the bovine PTH standards. The results are shown in Fig. 1 and indicate immunochemical similarity between uremic and nonuremic hormone over the range studied. Another uremic and control pool of glands, each weighing 3.2 mg, was extracted, serially diluted, and assayed. Over a fivefold range there was no significant difference between uremics and controls, indicating similar hormone content for the same gland weight. In a further experiment six control and six uremic animals whose individual paired parathyroid fresh weight varied from 0.1 to 0.5 mg had PTH levels from 0.3 to 2.4 ng, correlation coefficient $r = 0.702$, $P < 0.01$ between individual gland weight and hormone content. The uptake of AIB was measured in a separate study and the results shown in Table IV. At each dietary level the parathyroids were metabolically more active and this activity decreased as the calcium intake increased. Correlation of the individual gland weight with AIB uptake gave a correlation coefficient $r = 0.73$, $P < 0.001$. Division of the gland radioactivity by the water content to give the radioactivity per microgram of gland water as compared with serum, the T/S ratio, showed no difference between uremics and controls. This indicates that the glands are more active in the uremics because of an increase in size rather than due to any change in metabolism per unit weight of tissue.

Balance. Calcium balance was similar in uremics and controls at each level of intake (Table V).

Urine phosphorus was high on the low-calcium diet and fell progressively as calcium intake increased. Both calcium and phosphate balances were less positive on the low-calcium diet. Fecal phosphate was consistently higher in the uremics at each diet level as was fecal calcium on the medium and high diet, and in both controls and uremics as calcium intake rose, fecal phosphate increased further.

Statistics. Analysis of variance from the principal components gave differences significant at the 1% level between diets and also between uremics and controls.

Diet phosphate content varied

Blood and urine. Arterial pH, P_{CO_2}, and bicarbonate were similar in all groups. The total serum calcium was elevated in the controls on the 0.2%, 0.3%, and 0.4% phosphate diets (Table VI). Subsequently as the phosphate intake increased, total calcium in the uremics dropped below the control values. Ionized calcium was similar with higher levels in the uremics as compared with the controls on the 0.2% diet, the same at 0.3%, and subsequently lower than the controls. On all diets serum phosphorus levels were inversely related to ionized calcium (Fig. 2). TRP fell as dietary phosphate increased and at every diet level TRP was lower in the uremics.

Bone and parathyroids. Femur weight and length was similar in all groups.

Ash weight was reduced in the 0.2% uremic group, rose to equal the control in the 0.4% group and thereafter fell below the control value (Table VII). The low

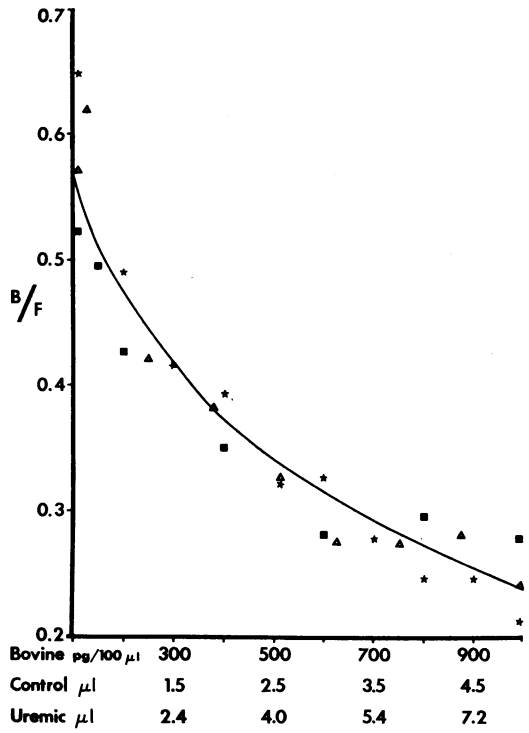

FIGURE 1 Serial dilution of extracts from control ■ and uremic △ parathyroid glands, together with bovine hormone standard ★. On the abscissa is shown either the quantity of partially purified bovine PTH in pg/100 µl or the number of microliters of either control or uremic gland extract. Each point is the mean of triplicate determinations. Uniform binding affinity is seen over the whole range. B/F, bound/free peptide.

ash content combined with the high serum and urine calcium in the 0.2% group is notable. The organic fraction was increased in the uremics, notably in the 0.2% group.

Parathyroid weights were lowest on the 0.2% diet and increased progressively as the phosphate content rose, however, a marked difference was apparent between controls and uremics, for in the former a phosphate intake of 0.5%, 0.6%, or 0.7% caused no further change in parathyroid weight, whilst in the uremics the weight increased progressively with each diet increment and the disparity between control and uremic gland size also increased. The linear relationship for the means of each group between ionized calcium and parathyroid weight is shown in Fig. 3. The point for the uremic 0.2% diet is aberrant, as the glands are larger than would be expected from the ionized calcium level. The reasons for this are unknown. Parathyroid size was paralleled by vertebral bone resorption, which was comparable in uremics and controls until the 0.5% diet, but after this resorption was higher in the uremics. The cortical and medullary cross-sectional areas of the femur were measured in uremic and control animals on the 0.2% and 0.7% diets. On the former diet the ratio of cortical to medullary area was 0.66, whilst on the 0.7% diet it was 0.76, $P < 0.01$. The lower ratio on the 0.2% diet was due to the relative enlargement of the medullary cavity produced by endosteal resorption. As an index of soft tissue calcification the cardiac calcium content was measured in all animals. Mean values ranged from 4.0 to 4.2 mg calcium/100 g fresh weight, there being no difference between groups or between uremics and controls.

Balance. Phosphorus balance was similar in controls and uremics (Table VIII). On the 0.2% and 0.3% diets intake was below the required needs and fecal phosphorus was low. Calcium balance was more positive on all diets in the uremics than the controls. In the 0.2% group the fecal calcium was higher in the uremics so that the higher serum calcium in these animals could not have been due to increased absorption of calcium from the gut. Unlike the calcium study in which increasing calcium intake decreased phosphate absorption, in this study calcium absorption was independent of dietary phosphate over the range studied. Urine calcium was elevated on the 0.2% diet and fell progressively as the phosphorus intake increased.

Statistics. Analysis of variance from the principal components gave significant differences between controls and uremics and between the diets at the 1% level.

TABLE IV
AIB Uptake by the Parathyroid Glands

Group	Calcium intake	PAI*	T/S‡
Control	Low	6.9±0.9 (11)	6.1±0.7 (11)
Uremic	Low	16.7±2.8 (12)	6.7±0.6 (12)
Control	Medium	5.1±0.8 (12)	5.1±0.5 (12)
Uremic	Medium	10.0±1.1 (11)	5.0±0.4 (11)
Control	High	4.3±0.6 (11)	5.8±0.5 (12)
Uremic	High	5.7±0.8 (11)	5.7±1.2 (11)
P between diets		1%	NS
Uremic vs. control		1%	NS

* Parathyroid activity index.
‡ Parathyroid to serum ratio. See Methods for calculation.

TABLE V

Balance Data, Diet Calcium Varied

Element studied	Group	Calcium intake	No. animals	Intake	Stool	Stool as percent of intake	Urine	Balance
				mg/day	*mg/day*	*%*	*mg/day*	*mg/day*
Calcium	Control	Low	8	15.2±0.5	1.3±0.2	8.5±1.4	0.3±0.0	+13.6±0.5
	Uremic	Low	7	15.4±0.4	1.2±0.1	7.5±0.8	0.2±0.0	+14.1±0.4
	Control	Medium	7	81.8±3.1	50.9±2.4	62.2±1.2	1.8±0.4	+28.1±1.6
	Uremic	Medium	8	82.5±2.8	59.4±5.1	71.8±5.3	1.5±0.2	+21.6±4.4
	Control	High	7	161.7±6.2	130.0±4.6	80.6±1.7	3.0±0.7	+28.6±3.8
	Uremic	High	7	166.8±4.9	137.9±5.1	82.6±1.1	3.3±0.5	+25.6±1.3
P Between diets				—	—	—	1%	1%
Uremic vs. control				—	NS	5%	NS	NS
Phosphorus	Control	Low	8	59.8±2.0	4.3±0.6	7.1±0.8	45.2±4.9	+10.3±3.5
	Uremic	Low	7	60.8±1.8	5.2±0.5	8.6±0.8	45.1±2.6	+10.5±3.5
	Control	Medium	7	63.0±2.4	19.1±0.9	30.4±1.0	24.9±1.9	+19.0±1.2
	Uremic	Medium	8	63.6±2.2	25.6±2.3	40.3±3.1	17.8±2.0	+20.2±2.4
	Control	High	7	64.9±2.5	34.0±1.4	52.7±4.9	9.6±1.4	+21.2±2.0
	Uremic	High	7	66.9±2.0	39.1±2.1	58.4±2.3	7.7±1.0	+20.1±1.6
P Between diets				—	—	—	1%	1%
Uremic vs. control				—	1%	1%	NS	NS

Uremics differed from controls on all diets except the 0.4% phosphate.

In a separate study the effect of varying phosphate intake was examined in intact and PTX uremic rats after 28 days. The results are shown in Table IX with the uremic animals showing the expected decreases in ionized calcium and TRP, and an increase in trabecular resorption as compared with the control group. In the PTX uremic animals on the 0.7% phosphate diet, marked hypocalcemia and hyperphosphatemia was found with a decreased bone resorption and higher TRP. As the phosphate intake was lowered, plasma phosphate fell and serum calcium rose with a corresponding rise in TRP, and on the 0.2% P diet an increase in trabecular resorption from 3.3% to 6.6%, $P < 0.01$.

Diet hydrogen ion content varied

In this study, unlike all the others, the source of calcium was calcium monohydrogen phosphate, $CaHPO_4 \cdot 2\ H_2O$.

Blood and urine. The data are shown in Table X, where it can be seen that there was an uncompensated metabolic acidosis in the acid uremic group and normal values for the other groups. However, in the uremics the mean pH rose steadily from 7.26 to 7.43 to 7.45, and similarly with serum bicarbonate, 14.1 to 20.7 to 22.5. The controls were unchanged in all groups. Total calcium was similar but ionized calcium in the acid uremics was higher than in the control group, similar to the control in the neutral and lower than the corresponding control in the basic group. There was a correlation in the uremics between ionized calcium and serum pH or bicarbonate, $r = -0.5$, Fig. 4. Serum phosphorus was higher in the uremics than the controls. Unlike the phosphate study, in this study the elevated plasma phosphorus was associated with higher values for ionized calcium. TRP was lower in the uremics and showed no change with diet.

Bone and parathyroids. As shown in Table XI the femur was lighter and shorter in all uremic groups but the change was most marked in the acid and alkaline

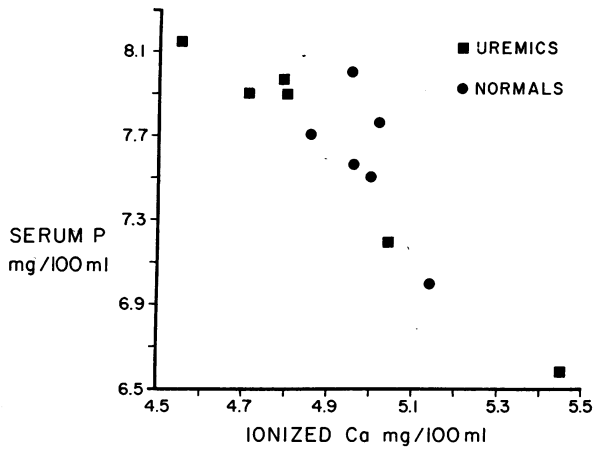

FIGURE 2 Serum ionized calcium and serum phosphate. Mean ionized calcium and phosphate values are inversely related to each other, $r = -0.35$ for all 238 samples, $P < 0.01$.

TABLE VI

Blood and Urine Values at Time of Sacrifice. Diet Phosphate Varied

Group	P Intake	Total Ca	Ionized Ca	P	Creatinine clearance	TRP
	%	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ml/min*	%
Control	0.2	11.1±0.2 (20)	5.14±0.08 (20)	7.0±0.1 (20)	0.75±0.04 (18)	99.7±0.1 (18)
Uremic	0.2	12.0±0.2 (22)	5.45±0.10 (22)	6.6±0.3 (20)	0.31±0.02 (22)	99.2±0.1 (22)
Control	0.3	10.6±0.1 (23)	4.96±0.05 (23)	7.6±0.3 (22)	0.77±0.03 (22)	98.4±0.4 (21)
Uremic	0.3	11.1±0.1 (27)	5.04±0.06 (27)	7.2±0.2 (26)	0.31±0.02 (28)	96.9±0.8 (27)
Control	0.4	10.5±0.1 (19)	5.02±0.04 (19)	7.9±0.2 (19)	0.78±0.03 (18)	91.6±1.2 (18)
Uremic	0.4	10.8±0.2 (18)	4.80±0.08 (18)	7.9±0.2 (19)	0.37±0.02 (17)	87.5±1.6 (17)
Control	0.5	10.6±0.1 (17)	5.00±0.06 (17)	7.5±0.2 (17)	0.77±0.05 (16)	83.9±1.4 (16)
Uremic	0.5	10.4±0.1 (22)	4.79±0.04 (22)	7.9±0.2 (22)	0.31±0.03 (21)	69.9±3.0 (21)
Control	0.6	10.4±0.1 (17)	4.86±0.04 (17)	7.7±0.2 (18)	0.77±0.03 (18)	77.3±1.6 (18)
Uremic	0.6	10.2±0.1 (16)	4.71±0.05 (16)	7.9±0.3 (15)	0.33±0.05 (16)	60.5±4.8 (15)
Control	0.7	10.5±0.1 (19)	4.95±0.05 (19)	8.1±0.2 (19)	0.74±0.05 (19)	62.8±3.8 (19)
Uremic	0.7	10.4±0.2 (19)	4.65±0.10 (19)	8.1±0.5 (19)	0.33±0.04 (19)	36.7±3.4 (18)
P Between diets		1%	1%	1%	NS	1%
Uremic vs. control		1%	5%	NS	1%	1%

animals. Ash content was reduced in all uremics, although more so in the alkaline group, and the organic fraction was increased. The parathyroid weights corresponded to the ionized calcium values, with the smallest glands in the acid uremics and the largest in the alkaline uremics, $r = -0.47$, $P < 0.01$. The controls were unaffected by dietary intake. Vertebral resorption was similar in the controls and elevated in all the uremic groups. It is notable that in the acid group the parathyroids were not enlarged, yet resorption was comparable to the neutral group with larger glands. Femoral appositional rates were similar in all groups varying between 2.8 and 4.5 μm/day.

Balance. The data in Table XII show that intake was less for the acid uremics, nevertheless, a trend was apparent with both uremics and controls showing progressively more positive balances for calcium and phosphorus at the diet goes from acid through neutral to alkaline. Urine calcium was highest on the acid diet and decreased as the diets became alkaline.

Statistics. Principal component analysis showed no difference between diets but a significant difference between controls and uremics at the 1% level. The absence of any diet effect from the pooled data would be expected, as the effect on bone disease is similar in the acid and alkaline groups.

DISCUSSION

The assessment of parathyroid activity in this model has been primarily dependent on the measurement of parathyroid weight. Histology of removed glands has shown them to consist of almost intact parathyroid tissue with on rare occasions a small piece of adherent thyroid. In the uremic animals the parathyroid cells appeared different morphologically, with a larger amount of cytoplasm that looked translucent, resembling the

TABLE VII

Bone and Parathyroids. Diet Phosphate Varied

Group	P intake	Ash	Organic	Ca	P	Parathyroid wt	Vertebra resorption
	%	%	%	%	%	*mg*	%
Control	0.2	53.3±0.4 (20)	23.1±0.1 (20)	17.7±0.2 (20)	10.0±0.1 (20)	0.151±0.01 (20)	6.8±0.7 (20)
Uremic	0.2	50.5±0.6 (22)	23.9±0.5 (22)	16.2±0.3 (22)	9.5±0.1 (22)	0.202±0.01 (22)	7.0±0.1 (22)
Control	0.3	54.8±0.4 (23)	22.4±0.1 (23)	18.3±0.2 (23)	10.2±0.1 (23)	0.207±0.02 (23)	5.5±0.5 (22)
Uremic	0.3	53.5±0.4 (28)	23.0±0.1 (28)	18.0±0.4 (28)	10.0±0.1 (28)	0.211±0.01 (28)	4.9±0.6 (28)
Control	0.4	54.6±0.5 (19)	22.6±0.1 (19)	17.9±0.3 (19)	10.1±0.1 (19)	0.221±0.02 (19)	7.1±0.9 (18)
Uremic	0.4	54.9±0.5 (19)	22.8±0.1 (19)	18.4±0.2 (18)	10.3±0.1 (18)	0.267±0.02 (19)	5.9±0.7 (18)
Control	0.5	54.8±0.6 (17)	22.3±0.1 (17)	19.0±0.3 (17)	10.2±0.1 (17)	0.254±0.02 (17)	5.6±0.8 (17)
Uremic	0.5	53.4±0.9 (22)	22.8±0.1 (22)	17.6±0.3 (22)	10.0±0.2 (22)	0.324±0.02 (22)	9.7±0.9 (21)
Control	0.6	55.2±0.5 (19)	22.4±0.1 (19)	18.2±0.3 (19)	10.2±0.1 (19)	0.256±0.02 (19)	5.1±0.7 (19)
Uremic	0.6	52.3±0.7 (16)	22.6±0.2 (16)	17.4±0.4 (16)	10.0±0.1 (16)	0.356±0.04 (16)	17.0±3.6 (14)
Control	0.7	55.0±0.5 (19)	22.4±0.1 (19)	18.5±0.3 (19)	10.1±0.1 (19)	0.255±0.02 (19)	4.4±0.6 (19)
Uremic	0.7	53.8±0.4 (19)	22.5±0.4 (19)	17.7±0.3 (19)	10.0±0.1 (19)	0.384±0.04 (19)	10.7±1.5 (19)
P Between diets		1%	1%	1%	1%	1%	5%
Uremic vs. controls		1%	1%	1%	NS	1%	1%

hyperplastic cells described in humans with secondary hyperparathyroidism (*32*). The high correlation coefficient of 0.92 between fresh and dry glands indicates that as the glands enlarge in size the cell solids are increasing proportionally, suggesting that weight corresponds to a functional parameter in the glands. Confirmation for this is derived both from the AIB data, which showed a definite relationship between gland weight and metabolic activity as defined by the accumulation of the amino acid, and from measurement of PTH levels in the glands, which showed that per unit weight uremics and controls had similar amounts of hormone. For each animal the uremic glands contained more PTH because they were on the average heavier.

The effects of hyperparathyroidism were manifested in the two primary target organs, bone and kidney. In the latter a lowered TRP was invariably found in the uremics as compared with their controls. The skeleton showed a decrease in ash and mineral content, which could have been due to a change in the bone accretion or resorption. The tetracycline data in the acid-base study indicated that the uremics were forming bone at the same rate as the controls and this was confirmed in a separate study [3] from that reported here, in which the calcium intake was varied in a similar way and the accretion rate calculated from the tetracycline bands. Similar values for uremics and controls were found. The measured parameter of resorption was always elevated in the uremics when the parathyroids were enlarged, indicating that osteopenia was due to resorption in excess of accretion. The importance of the parathyroids in determining both the TRP and percent resorption was shown by the PTX uremic animals on the 0.7% phosphate diet,

[3] Unpublished observations of the author.

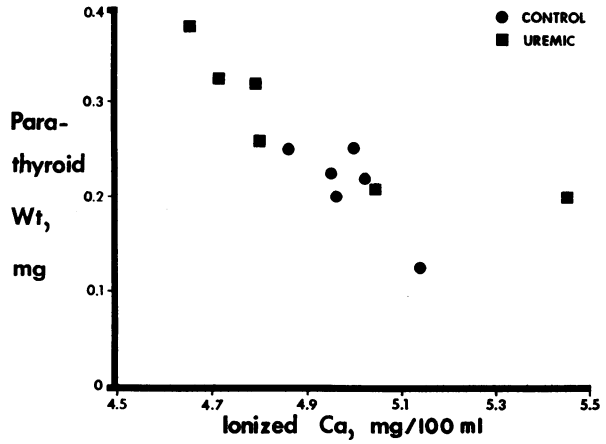

FIGURE 3 Ionized calcium and parathyroid weight. Mean values are shown for each group with linear distribution, whether uremic or control. The square at the extreme right side of the figure is for the uremic 0.2% group where the gland size is inappropriately large for the calcium level. For the uremics (123 samples) $r = -0.42$ and for uremics and controls (238 samples) $r = -0.35$, $P < 0.01$.

who had reduced bone resorption and values for TRP comparable to those of control nonuremic animals.

The effect of calcium in reducing parathyroid activity in the uremics in the first study was in part due to greater calcium absorption as the dietary intake increased, and this was reflected in the rise in serum and urine calcium. An additional factor was that increasing dietary calcium reduced phosphate absorption, as shown by the rising fecal phosphate and falling urine phosphate as the diet calcium increased. A decrease in phosphate absorption would be equivalent to reduction in phosphate intake and this was shown in the phosphate study to reduce parathyroid size.

No evidence was found for calcification in sites other than the skeleton. In an unreported study, measurement of skin and stomach calcium content in normal and uremic animals on three levels of calcium intake failed to show any differences between the groups, and similar negative results for cardiac calcification were found in the phosphate study. This indicates that under the conditions of these studies renal insufficiency per se does not lead to metastatic calcification; however, vascular calcification has not been specifically looked for.

The phosphate study showed a similarity of response in the control and uremic animals. Thus in both groups reduction in phosphate intake below 0.4% led to a decrease in gland size associated with a rise in serum ionized calcium. By whatever process inorganic phosphate changes ionized calcium, it appears to do so equivalently in both uremics and controls. Differences between these groups is apparent, however, as the phosphate intake rises, for in the controls no change in gland size occurred from the 0.5% phosphate diet on, whereas the uremic glands increased progressively. This was associated with elevation of the serum phosphate and further depression of ionized calcium. In the presence of decreased renal mass, excretion of the extra dietary load of phosphate could only be accomplished by elevation of the serum level, thus permitting an increase in the amount filtered by the glomeruli. In the controls the increased intake could be adapted to by reduction in tubular reabsorption without detectable rise in the plasma phosphorus. It should be noted, however, that the serum phosphate levels were the minimal ones, as the animals were killed in the fasting state. Even the con-

TABLE VIII
Balance Data. Phosphate Intake Varied

Group	P intake	No. Animals	Calcium					Phosphorus				
			Intake	Stool	Stool as percent of intake	Urine	Balance	Intake	Stool	Stool as percent of intake	Urine	Balance
	%		*mg/day*	*mg/day*	%	*mg/day*		*mg/day*	*mg/day*	%	*mg/day*	*mg/day*
Control	0.2	6	69.2±0	29.3±2.5	42.4±3.7	22.5±1.6	+17.3±2.0	14.8±0	1.7±0.2	11.8±1.8	0.2±0.07	+12.9±0.2
Uremic	0.2	6	69.1±0.1	37.2±0.7	53.8±1.0	10.5±1.3	+21.4±1.0	14.8±0.02	1.6±0.1	10.9±0.8	0.2±0.02	+13.1±0.1
Control	0.3	6	67.6±0.4	31.6±2.5	46.8±3.7	5.7±1.0	+30.3±2.1	22.8±0.1	4.9±0.5	21.2±1.9	1.2±0.5	+16.6±0.9
Uremic	0.3	6	68.1±0.2	29.9±2.4	43.9±3.3	6.2±0.8	+32.0±1.7	22.9±0.1	5.0±0.7	21.8±3.2	2.1±0.1	+15.8±1.4
Control	0.4	6	70.0±0	35.4±2.1	50.6±3.0	3.3±0.6	+31.3±1.6	32.4±0	8.4±1.1	26.0±3.5	3.2±0.7	+21.9±0.9
Uremic	0.4	6	70.0±0	30.6±1.3	43.8±1.8	4.2±0.5	+35.2±1.1	32.4±0	7.5±0.6	23.2±1.8	2.3±0.7	+22.5±0.6
Control	0.5	6	70.4±0	34.5±0.9	48.9±1.3	3.3±0.7	+32.6±0.9	40.1±0	8.8±0.5	22.1±1.3	9.4±1.4	+21.7±1.3
Uremic	0.5	6	70.4±0	33.4±1.5	47.5±2.2	2.3±0.3	+34.7±1.5	40.1±0	12.0±0.8	30.0±2.1	5.4±0.6	+22.6±1.3
Control	0.6	6	71.6±0	34.6±2.3	48.4±3.2	2.7±0.4	+34.2±1.9	46.2±0	10.9±0.8	23.6±1.7	14.6±1.2	+20.6±1.5
Uremic	0.6	6	71.6±0	31.4±2.9	43.9±4.1	1.4±0.4	+38.8±2.8	46.2±0	13.0±1.6	28.2±3.5	11.3±1.0	+21.8±1.2
Control	0.7	6	72.0±0	37.7±1.8	52.3±2.5	1.4±0.3	+32.9±1.9	55.4±0	14.1±0.9	25.5±1.6	23.7±1.4	+17.5±1.6
Uremic	0.7	6	72.0±0	33.6±1.0	46.7±1.4	1.3±0.2	+37.0±0.9	55.4±0	14.5±0.8	26.2±1.5	17.0±2.4	+23.8±2.4
P Between diets			—	NS	NS	1%	1%	—	1%	5%	1%	1%
Uremic vs. control			—	NS	NS	1%	1%	—	NS	NS	1%	NS

TABLE IX

Effect of Parathyroidectomy and Varying Phosphate Intake

Group	P Intake	Blood				TRP	Trabecular resorption
		Creatinine	Total Ca	Ion Ca	P		
	%	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	%	%
Sham NPX*, sham PTX	0.7	0.40±0.01 (16)	10.1±0.1 (15)	4.7±0.1 (15)	6.9±0.1 (16)	69.0±1.4 (16)	4.5±0.6 (16)
NPX, sham PTX	0.7	0.79±0.04 (18)	10.0±0.1 (17)	4.3±0.1 (17)	6.3±0.1 (18)	39.8±5.1 (18)	8.8±1.4 (18)
NPX, PTX	0.7	0.84±0.02 (19)	6.6±0.2 (19)	2.0±0.1 (19)	15.5±0.6 (19)	71.2±3.8 (17)	3.3±0.4 (19)
NPX, PTX	0.4	0.88±0.04 (10)	8.0±0.4 (10)	3.2±0.2 (10)	10.1±0.6 (10)	93.6±1.5 (10)	2.7±0.3 (10)
NPX, PTX	0.2	0.85±0.03 (18)	10.8±0.3 (18)	4.5±0.2 (17)	6.1±0.3 (18)	99.0±0.2 (16)	6.6±0.7 (18)
P Between diets‡		NS	1%	1%	1%	1%	1%

* NPX, uremic.

‡ *P* values calculated only for NPX, PTX groups.

trols might show slight deviations after feeding and the uremics may have been considerably higher than recorded. Short-lived diurnal changes in plasma phosphate and reciprocal depression in ionized calcium, while sufficient stimulus for increasing PTH secretion, may not be sustained enough to lead to glandular hyperplasia (33, 34). In the uremics on the higher phosphate intake serum phosphorus was permanently elevated and calcium depressed, leading to continuous parathyroid stimulation and hence enlargement. Of central importance is the process whereby hyperphosphatemia leads to a decrease in serum ionized calcium levels. That this occurs in the presence of intact renal function and in the absence of the parathyroid glands is well established (35). While acute elevation in plasma phosphate could lead to formation of calcium phosphate salts with their se-

TABLE X

Blood Values at Time of Sacrifice and Urine Values. Diet H^+ Content Varied

Group	pH	P_{CO_2}	Bicarb	Total Ca	Ion Ca	P	Creatinine clearance	TRP
		mm Hg	*meq/liter*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*ml/min*	%
Acid control	7.47±0.01 (30)	31.8±0.7 (29)	22.7±0.4 (29)	10.2±0.1 (30)	4.70±0.03 (30)	7.2±0.1 (30)	0.70±0.03 (29)	66.7±2.1 (29)
Acid uremic	7.26±0.04 (17)	30.5±0.9 (18)	14.1±1.4 (17)	10.2±0.1 (19)	5.10±0.12 (18)	8.8±0.5 (20)	0.32±0.05 (19)	28.9±5.1 (19)
Neutral control	7.46±0.01 (20)	32.7±1.2 (20)	22.3±0.5 (20)	10.0±0.1 (20)	4.68±0.03 (20)	7.3±0.1 (20)	0.69±0.06 (20)	69.9±1.7 (20)
Neutral uremic	7.43±0.01 (21)	32.3±0.9 (21)	20.7±0.4 (21)	10.1±0.1 (22)	4.60±0.04 (21)	7.7±0.2 (22)	0.27±0.02 (22)	31.9±4.7 (22)
Basic control	7.46±0.01 (28)	32.2±0.7 (28)	22.4±0.4 (28)	10.0±0.1 (28)	4.69±0.04 (28)	7.2±0.2 (28)	0.73±0.04 (28)	70.9±2.2 (28)
Basic uremic	7.45±0.01 (33)	33.2±1.0 (33)	22.5±0.4 (33)	9.7±0.1 (33)	4.28±0.10 (33)	7.6±0.3 (32)	0.25±0.02 (32)	35.8±3.0 (32)
P Between diets	1%	NS	1%	NS	5%	NS	NS	NS
Uremic vs. controls	1%	NS	1%	NS	1%	1%	1%	1%

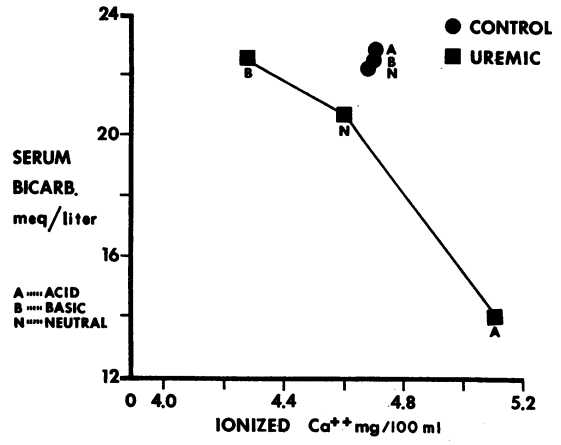

FIGURE 4 Mean values for serum bicarbonate and ionized calcium. The controls are all similar but the uremic means are linearly related to the bicarbonate values, $r = -0.52$, $P < 0.01$.

questration in soft tissue or bone, this would not explain the data in Fig. 2, where serum calcium and phosphorus levels are inversely correlated over a wide range. The uremic PTX rats on different phosphate intakes showed a reciprocal relationship between serum phosphate and calcium levels. Comparison of the NPX animals with the NPX, PTX on the same 0.7% phosphate diet (Table IX) shows that maintenance of near-normal levels of both calcium and phosphate in blood in the uremics was due to action of PTH on renal TRP. That phosphate was the determinant of the serum calcium level was confirmed by the restoration of normocalcemia in NPX, PTX animals on the 0.2% low phosphate diet. It has previously been shown in the rat by sensitive morphometric techniques that serum phosphate levels are inversely related to endosteal resorption, and it would appear that plasma phosphate levels are one of the signals to which bone resorbing cells are sensitive (35). The biochemical process whereby hyperphosphatemia leads to hypocalcemia in these circumstances is unknown, and neither is it clear why production of increased quantities of PTH is unable to cause reversion of the serum levels of calcium and phosphorus to normal.

Hypercalcemia (Table VI) was not related entirely to the degree of bone resorption and was most marked on the lowest phosphate diet, 0.2%, associated with an increase in the bone organic fraction, presumably osteoid. In the presence of hypophosphatemia normal mineralization was not possible and the excess calcium was excreted in the urine. In the uremics on the 0.2% and 0.3% diets the plasma phosphate levels were lower than the controls, there was less bone ash, and the renal clearance of calcium was decreased as compared with the control group. The severer osteomalacia and decreased renal excretion would both lead to higher serum calciums, in addition to any effect on endosteal resorption.

The reason for the lower plasma phosphorus in the uremics on the 0.2% and 0.3% diets is unexplained, as fecal and urine losses were similar to the controls, suggesting that internal redistribution within phosphate pools may have taken place. This was also observed in the NPX, sham-PTX animals (Table IX).

TABLE XI
Bone and Parathyroids. Diet H^+ Content Varied

Group	L. femur weight	Ash	Organic	Ca	P	Parathyroid weight	Vertebra resorption
	g	%	%	%	%	*mg*	%
Acid control	0.512±0.009 (30)	56.3±0.4 (30)	22.9±0.1 (30)	19.7±0.2 (30)	9.7±0.1 (30)	0.274±0.023 (30)	5.7±0.5 (29)
Acid uremic	0.448±0.019 (20)	53.9±0.7 (20)	23.1±0.2 (20)	18.5±0.5 (20)	9.2±0.1 (20)	0.294±0.019 (20)	15.3±1.6 (19)
Neutral control	0.522±0.006 (20)	56.5±0.4 (20)	22.9±0.1 (20)	19.9±0.2 (20)	9.7±0.1 (20)	0.234±0.017 (20)	7.1±1.1 (20)
Neutral uremic	0.509±0.007 (22)	55.1±0.5 (22)	23.3±0.1 (22)	19.0±0.3 (22)	9.5±0.1 (22)	0.344±0.034 (22)	15.8±2.1 (21)
Alkaline control	0.517±0.008 (28)	56.6±0.3 (28)	22.9±0.1 (28)	19.9±0.2 (28)	9.8±0.1 (28)	0.240±0.014 (28)	7.0±0.7 (27)
Alkaline uremic	0.477±0.009 (33)	53.5±0.5 (33)	23.4±0.1 (33)	18.5±0.3 (33)	9.2±0.1 (33)	0.436±0.033 (33)	18.2±1.8 (33)
P Between diets	NS	NS	NS	NS	NS	1%	NS
Uremic vs. control	1%	1%	1%	1%	1%	1%	1%

TABLE XII

Balance Data. Diet H^+ Content Varied

Element studied	Group	No. animals	Intake	Stool	Stool as percent of intake	Urine	Balance
			mg/day	*mg/day*	%		
Calcium	Acid control	5	87.6±0.0	65.1±3.1	74.3±3.5	4.0±0.6	+18.5±3.2
	Acid uremic	6	56.1±7.3	40.7±2.6	76.0±5.4	4.8±0.7	+10.6±4.6
	Neutral control	5	91.2±0.0	61.0±1.0	66.9±1.1	1.7±0.4	+28.5±0.8
	Neutral uremic	6	91.2±0.0	64.9±2.7	71.2±3.0	1.7±0.4	+24.5±2.6
	Basic control	4	96.0±0.0	59.4±1.3	61.9±1.4	1.8±0.7	+34.7±1.9
	Basic uremic	5	95.5±0.0	63.2±2.3	66.1±2.3	1.3±0.4	+31.0±1.9
P Between diets			—	1%	1%	1%	1%
Uremic vs. control			—	1%	NS	NS	NS
Phosphorus	Acid control	5	87.6±0.0	36.5±2.6	41.6±3.0	21.2±1.2	+29.9±1.8
	Acid uremic	6	56.1±7.3	20.1±3.2	36.1±3.4	19.2±2.5	+16.8±4.1
	Neutral control	5	86.4±0.0	37.7±1.3	43.7±1.5	15.0±1.9	+36.6±0.9
	Neutral uremic	6	86.4±0.0	39.4±2.6	45.6±3.0	17.4±1.8	+29.6±1.6
	Basic control	4	90.0±0.0	30.6±4.2	34.0±4.6	18.4±1.2	+41.7±4.5
	Basic uremic	5	89.6±0.4	39.6±1.8	44.2±1.9	14.7±1.1	+35.3±1.1
P Between diets			—	1%	5%	NS	1%
Uremic vs. control			—	NS	NS	NS	1%

Finally, within the dietary range studied, phosphate had no effect on calcium absorption from the gut. This is in agreement with previous observations in rats with normal renal function (36).

In the third study, where hydrogen ion intake was varied, the alteration in serum ionized calcium in the uremics could not be explained solely by an effect on serum protein-binding induced by changes in pH. Taking a figure of 0.2 mg/100 ml alteration in ionized calcium for every 0.1 unit change in pH gives an expected increase of ionized calcium in the acid uremics of 0.34 mg/100 ml (37–39). The observed change was 0.5 mg/100 ml. Likewise in the alkaline uremics, the pH change was 0.02 units whilst the change in ionized calcium was 0.32 mg/100 ml. Thus in both acid and alkaline groups there appears to be other processes operating to alter ionized calcium beyond the limits expected solely from a change in binding. That pH was important is shown by the correlation coefficient $r = 0.56$, $P < 0.001$ for the relationship between pH and ionized calcium for all the uremics, and 0.85 if only the acid uremics are considered. In the acid group the decrease in bone ash and high trabecular resorption values in the presence of normal-sized parathyroid glands indicates a direct effect of acidosis on bone resorption similar to that induced by parathyroid over activity and resembling the findings in normal rats given ammonium chloride to drink (40). The rise in serum ionized calcium was probably due to release of bone mineral into extracellular fluid, which would explain the higher serum inorganic phosphate levels in the acid uremics.

The alkaline uremic group had the lowest levels of ionized calcium. As calcium absorption was maximal and urine calcium the lowest of all groups, this indicates that the animals were not calcium deficient. The decrease in bone ash and calcium content are what would have been expected with the enlarged parathyroids and by exclusion, soft tissue calcification appears to have occurred, although no direct measurements to confirm this are available.

The large urine losses of calcium in the acid group would be expected to lead to a compensatory increase in intestinal calcium absorption. On the contrary, absorption was decreased, suggesting a specific effect of pH on calcium absorption and confirming a previous observation in uremic patients (4). Support for this is found in the progressively lower fecal calcium and more positive calcium balance as the diet goes from acid to alkaline. Whether the effect of pH on absorption is vitamin D-mediated or is a direct effect on the intestine remains to be investigated.

Unlike the phosphate study, in the acid-base study plasma phosphate was changing in the same direction as the ionized calcium. Thus the highest calcium and phosphorus values were in the acid uremics and the lowest in the alkaline uremics. The inference is therefore that pH was primarily controlling ionized calcium, by release from bone or soft tissues, rather than in the previous study where phosphate was the controlling factor. Here phosphate was released or sequestered in association with calcium and the two moved in tandem.

These studies have been confined to determining the

importance of calcium, phosphorus, and hydrogen ion in modulating parathyroid activity in order to understand better the pathogenesis of uremic osteodystrophy. It has been shown that each of the three factors is important and acts on the parathyroid glands by alteration of the circulating ionized calcium level. An additional effect of acidosis directly on bone was also demonstrated. As noted in the introduction, the study of renal osteodystrophy in man has been complicated by the admixture in varying degrees of osteomalacia and osteitis fibrosa, representing the response of the skeleton to impaired calcification and hyperparathyroidism, respectively. In this rat model the calcification defect, although present as shown by the consistent increase in the organic fraction in the uremics, has been of minor degree and constant in all studies except when hypophosphatemia occurred. This has made it possible to examine more precisely the role of the parathyroid glands in producing bone disease and the nature of their contol in renal failure. Accelerated bone resorption, increased fractional phosphate excretion, decreased serum ionized calcium, and elevated phosphate levels are all present in humans with chronic renal failure and have been shown to be characteristic of this rat model. Metabolic acidosis is usual in man and can readily be induced in the uremic rat. We would infer from these similarities that the same pathways are involved in the causation of hyperparathyroidism in man as in the rat and if this is the case then our findings indicate that prophylactic measures involving a generous calcium intake with restricted phosphate, together with maintenance of a low normal serum bicarbonate, would be expected to minimize the development of secondary hyperparathyroidism in patients. The changes in the calcification process and their interrelationship with hyperparathyroidism and vitamin D metabolism will require further investigation for clarification.

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