On the Pathogenesis of Hyperparathyroidism in Chronic Experimental Renal Insufficiency in the Dog

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ABSTRACT Healthy adult dogs were subjected to stepwise reduction of nephron population so as to create the transition from normal renal function to advanced renal insufficiency. Studies were performed at each level of renal function. Glomerular filtration rate (GFR), renal phosphate clearance, and serum radioimmunoassayable parathyroid hormone (PTH) levels were measured. Two groups of animals were studied. In one, phosphorous intake was maintained at 1200 mg/day. As GFR declined, fractional phosphate excretion rose reciprocally, and PTH levels increased over 20-fold. In the second group, phosphorous intake was maintained at less than 100 mg/day. As GFR fell, fractional phosphate excretion changed little, and no increment in PTH levels occurred. The data suggest that the control system regulating phosphate excretion contributes importantly to the pathogenesis of secondary hyperparathyroidism in advancing renal insufficiency.

INTRODUCTION

Hyperparathyrodism has been recognized for many years as a potentially serious complication of chronic renal disease (1–5). However, the full clinical significance of this abnormality has only become apparent since the advent of techniques for prolonging the life of patients with end-stage renal disease. The exposition of the details of the evolution of secondary hyperparathyroidism, therefore, is important not only from the point of view of scientific inquiry; it probably represents an essential prerequisite to the development of effective techniques of preventing osteitis fibrosa in chronic uremia.

There is growing evidence that an increase in parathyroid hormone (PTH)[1] activity in patients with advancing renal disease contributes to the maintenance of external phosphate balance by increasing phosphate excretion per nephron (6, 7). The possibility also exists that the stimulus to the increase in PTH production arises from disruptions in phosphate balance attendant upon nephron destruction. We recently have proposed a theoretical schema for the pathogenesis of hyperparathyroidism in advancing renal disease, which envisions progressivly increasing levels of PTH as a manifestation of the adaptation in the control system governing phosphate excretion (8). The hypothesis states that whenever nephron losses occur and GFR diminishes, phosphate excretion must decrease transiently. The unexcreted phosphate will result in a finite elevation of the serum phosphate concentration, ionized serum calcium concentration then will decrease reciprocally, and the latter serves as a stimulus to increased secretion of PTH. The increased serum levels of PTH will decrease fractional reabsorption of phosphate in the residual nephrons, thereby increasing phosphate excretion per nephron. This relative phosphaturia will effect a reduction of plasma phosphate concentration towards normal, and ionized calcium concentration will increase towards normal, but PTH activity must remain permanently elevated, for if it were to return to its initial level, phosphate retention again would ensue and ionized calcium concentration again would fall. Each time GFR falls the same cycle should recur, allowing for the continuing preservation of external phosphate balance throughout much of

This work was presented in part at the 62nd Annual Meeting of the American Society for Clinical Investigation, and an abstract has been published: 1970. *J. Clin. Invest.* **49**: 89a.

Received for publication 3 August 1970 and in revised form 28 September 1970.

[1] *Abbreviations used in this paper:* PTH, parathyroid hormone level; GFR, glomerular filtration rate; TRP, fractional phosphate reabsorption.

the natural history of chronic renal disease. According to this formulation, secondary hyperparathyroidism begins with the onset of renal disease and progresses inexorably thereafter. When enteric absorption of calcium decreases due to the advent of vitamin D resistance, a second potent force would contribute to the progression of hyperparathyroidism.

The present studies were designed to examine the hypothesis. The nephron population of adult dogs was reduced experimentally in several stages so as to create, in a series of controlled steps, the transition from normal renal function to uremia. Studies were performed at each level of renal function. Two groups of animals were studied. The first received a diet containing 1200 mg of phosphorus per day. The second was maintained on a synthetic diet which contained less than 100 mg of phosphorous per day. The patterns of renal excretion of phosphate and the circulating levels of parathyroid hormone were measured in both groups of animals at each stage of study.

METHODS

Healthy, adult, female mongrel dogs were employed in these studies. In a preliminary operation, the urethra was ligated, and a permanent cystostomy tube was sewn into the bladder and brought out through the lower anterior abdominal wall in order to facilitate accurate urine collection and to obviate the need for repeated bladder catheterizations. The initial studies were performed with both kidneys intact. Thereafter approximately 70% of the nephron population of one kidney was destroyed by ligation of most of the second and third order branches of the ipsilateral renal artery (9). After stabilization, studies were repeated. Subsequently about 70% of the nephron population of the contralateral kidney was eliminated through a second partial infarction. Studies were repeated at this level of renal function. Thereafter, one of the two "remnant" kidneys was removed, leaving only the small residual population of nephrons in the remaining remnant kidney to sustain life. The final series of studies then was performed. By following this sequence, GFR was reduced in stepwise fashion from approximately 60 ml/min, to 40, 20, and then 5–10 ml/min. After each surgical procedure, at least 1 wk was allowed for recovery and stabilization.

Studies were performed on two groups of animals. The first group, consisting of seven dogs, received a diet of commercial dog food which provided 1200 mg of phosphorus, 1600 mg of calcium, and 63 g of protein per day. All feedings were supervised to make sure that the entire day's ration was ingested. The second group (six dogs) was fed a synthetic diet (Nutritional Biochemicals Corporation, Cleveland, Ohio) which was composed of 20% protein, 70% carbohydrate, and 10% fat. 150 g of this diet was administered by gastric tube twice daily, providing a total caloric intake of 1200 cal. The total daily intake of phosphorus was less than 100 mg while that of calcium was 1200 mg. Both groups of dogs were maintained on their respective diets throughout the entire period of study, which ranged from 5 to 8 wk.

In five uremic dogs which had been maintained on the low phosphate intake, additional studies were performed after completion of the foregoing sequence.. In three of these animals, a single dose of 600 mg of elemental phosphorous (as potassium phosphate[2]) was given by gastric tube in order to observe the short-term effects of phosphate administration. Three control clearance periods were obtained, phosphate was administered, and five additional clearance periods of approximately 1 hr each were obtained. In the other two dogs, the diet was switched from the low phosphorous to the 1200 mg phosphorous diet, and the latter was maintained for 1 wk. Studies then were repeated to observe the change in the patterns of phosphate excretion and in the levels of parathyroid hormone.

GFR was measured as the exogenous creatinine clearance, and phosphate clearance was measured concurrently. Three or more 15- to 45-min clearance periods were obtained during each study.

Blood samples were obtained through an indwelling jugular vein catheter. Creatinine was determined according to the method of Bonsnes and Taussky (10); phosphate was determined according to the method of Gomori (11); calcium was measured with an atomic absorption spectrophotometer (IL Model 153); and ionized calcium was measured anaerobically using a flow-through electrode (12) (Orion, Inc., Cambridge, Mass.).

Serum PTH was assayed by a modification of the radioimmunoassay technique of Berson, Yalow, Aurbach, and Potts (13) as modified by Reis and Canterbury (14). A single antiserum to bovine PTH, prepared in a rooster, was used throughout these studies. This antiserum (laboratory identification No. 71) was different from that employed in our previous reports on the assay of human PTH, but possessed binding characteristics to bovine hormone similar to those obtained with the original chicken antiserum (14–16). It was used in a final dilution of 1:7500 in a total incubation volume of 0.3 ml. Highly purified PTH (Wilson Laboratories), was labeled with ^{131}I at a specific activity of 300–500 mCi/mg. It was added to the incubation mixture in a volume of 0.1 ml, 75,000 cpm/0.1 ml. The lowest dilution of test serum in the final incubation volume was 1:9; hyperparathyroid serum could be assayed in final dilutions of 1:60 or higher. The immunologic cross-reactivity between canine and bovine PTH was sufficient to detect circulating PTH in normal canine sera. Sera derived from parathyroidectomized dogs contained no assayable PTH. All test sera gave assay curves parallel to the standard curve. The coefficient of variation of replicate samples assayed at two widely different concentrations was 10%. Replicates that did not agree within 20% were discarded, and repeat assays were performed. Coded samples were analyzed at four different concentrations. The code was broken at the termination of the experiments. The PTH levels reported are in arbitrary units (microliter equivalents per milliliters) relating the potency of the test sera to that of a standard hyperparathyroid serum, which was assigned a potency of 1000 μlEq/ml (15). This standard serum was obtained from a dog with experimentally induced severe chronic renal failure. In the assay, 100 μlEq/ml corresponds to 2.0 ng of highly purified bovine PTH (Fig. 1).

[2] Administered as a potassium-phosphate solution, pH 7.4. Kindly supplied as Hyper-Phos-K by Dr. A. Wolbarsht, Davies Rose Hoyt Pharmaceutical Division, Needham, Mass.

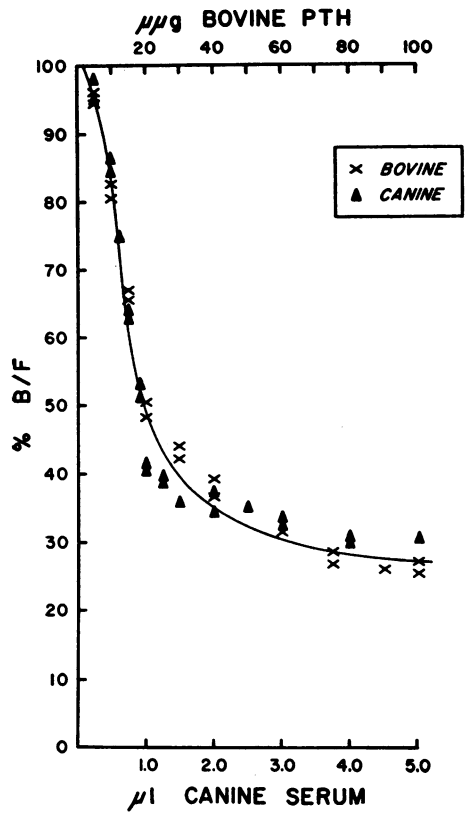

FIGURE 1 Standard curve comparing canine hyperparathyroid serum with highly purified bovine PTH. The ordinate represents the bound to free hormone ratio (B/F). A value of 100% is assigned to the B/F ratio obtained in the absence of unlabeled hormone.

RESULTS

Sequential observations on a representative dog, maintained on a 1200 mg phosphorous intake, are shown in Table I (dog A). In the control studies with both kidneys present, glomerular filtration rate was 64 ml/min, fractional phosphate reabsorption (TRP) was 91%, and serum parathyroid hormone concentration was 40 μlEq/ml. After partial infarction of one kidney, GFR was reduced to 37 ml/min, TRP was 90%, and the PTH level increased to 70 μlEq/ml. With partial infarction of the second kidney, GFR was further decreased to 16 ml/min, and TRP fell to 60%. PTH at this stage of renal function was 150 μlEq/ml. The final set of observations was obtained after removal of one of the two remnant kidneys. GFR was reduced to 5 ml/min, TRP to 8%, and now the PTH level was increased to 730 μlEq/ml.

In Table I (dog B), the results of a representative study on a dog maintained on the low phosphate diet are presented. The pattern of reduction of GFR is closely comparable to that in dog A. However, in striking contrast to the data obtained for the animal who had received a 1200 mg phosphorous diet, TRP in dog B remained above 90% throughout the period of study, PTH did not increase even in the presence of marked nephron reduction and chemical stigmata of renal insufficiency.

Fig. 2 A and B, are group plots relating TRP to GFR in six dogs on the 1200 mg phosphorous diet and seven dogs on the low phosphorous intake. In the former group (Fig. 2 A), TRP decreased in a hyperbolic fashion as GFR fell in a manner quite similar to that which has previously been observed in a similar plot using group data from patients with chronic renal disease (7). The relationship observed in the animals on the low phosphorous (Fig. 2 B), is strikingly different. No tendency exists for TRP levels to fall progressively as GFR diminishes. With the exception of one study on one dog, none of the values for TRP was below 83%, and all but three remained above 90%.

The relationship between PTH and GFR for five dogs maintained on the 1200 mg phosphorous diet and seven dogs maintained on the low phosphorous diet is shown in Fig. 3. In the former group, PTH increased markedly as GFR fell and at the lowest levels of GFR, PTH values were extremly high. In the low phosphorous group, on the other hand, serum PTH concentrations remained within the normal range even at the lowest levels of GFR.

TABLE I

Serial Measurements of GFR, Fractional Phosphate Reabsorption, and Serum PTH in Two Representative Dogs Subjected to Progressive Reduction of Nephron Populations

Dog A was maintained on a 1200 mg/day phosphorous intake; dog B was maintained on less than 100 mg/day of phosphorous.

Condition	GFR	C_{PO_4}	TRP	PTH
	ml/min	*ml/min*	%	*μlEq/ml**
Dog A				
Control	64.0	5.66	91	40
Nephron reduction				
1	37.0	3.60	90	70
2	16.0	6.24	60	150
3	5.0	4.16	8	730
Dog B				
Control	61.0	0.40	99	28
Nephron reduction				
1	47.0	1.63	97	41
2	18.0	0.13	99	42
3	12.0	0.21	98	36

C_{PO_4} represents phosphate clearance; TRP, fractional phosphate reabsorption; and PTH, serum parathyroid hormone level.

* PTH is expressed in arbitrary units (see Methods).

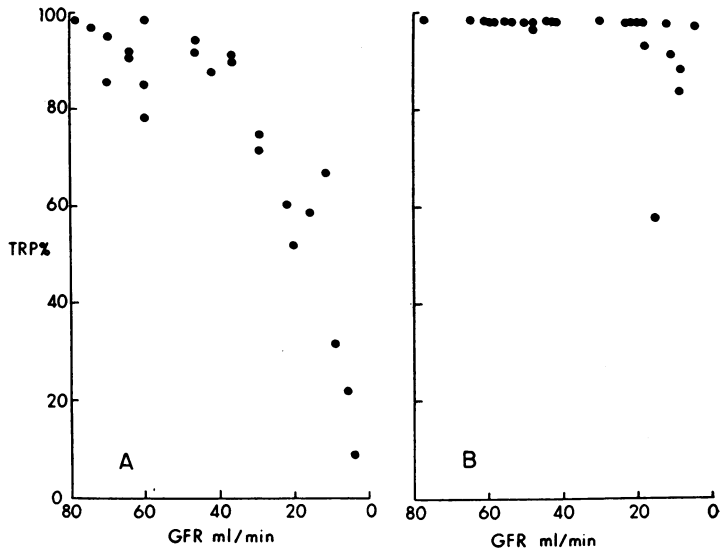

FIGURE 2 The relationship between fractional phosphate reabsorption (TRP) and GFR in two groups of dogs. Fig. 2 A represents data obtained in a group of six dogs maintained on a 1200 mg/day phosphorous diet. Fig. 2 B represents data from a group of seven dogs maintained on a diet containing less than 100 mg of phosphorous per day.

In three dogs maintained on the low phosphorous intake through the sequential studies described above, the effects of a single oral dose of 600 mg of phosphorous on TRP and PTH were measured. The data are shown in Table II. GFR ranged from 8 to 17 ml/min. During the control clearance periods, prior to phosphorous administration, TRP averaged 89.6% (range 82.5%–98.2%), and PTH averaged 53 μlEq/ml (range 47–57). 600 mg of phosphorous were then given by gastric tube. Serum phosphate values increased markedly in all three animals. Serum calcium values decreased after phosphate administration, and ionized calcium fell concurrently. The value for TRP fell strikingly, approximating zero in each of the three dogs, and PTH levels increased to a final mean value almost 4 times as great as the control value.

The effects of continued administration of 1200 mg of phosphorous per day for 1 wk were studied in two dogs in which secondary hyperparathyroidism had been prevented by administering a low phosphorous diet during the evolution of renal insufficiency. In the first dog, GFR averaged 11 ml/min. Before the phosphorous administration was commenced, TRP was 99% and PTH 45 μlEq/ml. After 1 wk on the 1200 mg phosphorous diet, TRP had fallen to 42%, and PTH had risen to over 600 μlEq/ml. The results in the second dog were similar. TRP fell from 88 to 30%, and PTH rose from 50 to 500 μlEq/ml.

Table III presents the mean values for plasma phosphate and total calcium concentrations in the two groups of animals at each stage of study. Phosphate values were similar in both groups with the exception of the values observed at the lowest level of GFR. However, none of the differences, including the latter, was significant. Calcium concentrations were slightly but significantly greater in the animals on the low phosphate diet after the first and second phases of nephron reduction; however, at the lowest level of GFR calcium values were not significantly different between the two groups.

DISCUSSION

The progression of chronic renal disease is attended by a series of remarkable adaptations in the patterns of solute excretion by the residual nephrons. In general, these adaptations lead to increasing rates of solute excretion per nephron as the number of excretory units diminishes. Such an adaptation appears to account for the continued regulation of phosphate excretion in advancing renal disease (6, 7). As GFR falls, phosphate excretion per nephron increases due to progressive inhibition of fractional reabsorption of phosphate in the residual nephrons, and indirect evidence suggests that parathyroid hormone plays a central role in this adaptation (6, 7).

We have proposed a hypothesis that explains the mechanisms of adaptation (8). Each time GFR diminishes due to nephron destruction, phosphate excretion must decrease transiently, and transient hyperphosphatemia ensues. The latter would result in reciprocal hypocalcemia. It is the decrease in ionized calcium rather

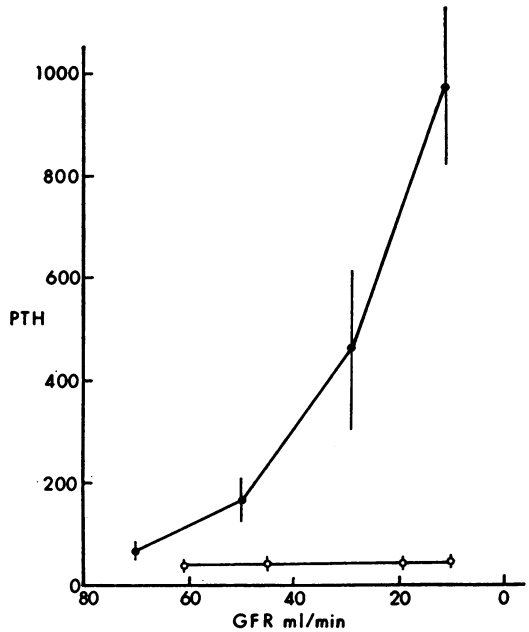

FIGURE 3 The relationship between parathyroid hormone levels and GFR in two groups of dogs: those maintained on a 1200 mg per day phosphorous diet (closed circles) and those maintained on a diet containing less than 100 mg of phosphorous per day (open circles). The vertical lines represent ±1 SEM. PTH is expressed in μlEq/ml (see Methods).

than the elevation of phosphate per se that stimulates the parathyroid glands to increase the rate of hormone secretion. According to this formulation, therefore, hyperparathyroidism occurs, at least in part, as a manifestation of an adaptive change in the control system which serves to promote the maintenance of external phosphate balance.

The present studies support this hypothesis. In dogs maintained on a fixed, normal intake of phosphate, progressive reduction of the population of nephrons was associated with increasing levels of circulating PTH. This experimental model confirms the pattern of increasing hyperparathyroidism in man with chronic renal disease. Clinical studies have demonstrated increasing levels of PTH in rough proportion to the severity of renal insufficiency (15, 17). The contrast between dogs on normal and reduced phosphate intakes is striking: the low phosphate diet completely prevented the development of hyperparathyroidism. Thus, dietary phosphate plays a key role in the stimulation of parathyroid overactivity. In all of the studies, where PTH rose, TRP fell, indicating that, under appropriate experimental conditions, TRP is a reliable indirect measure of PTH activity.

According to all known information, hyperphosphatemia per se does not stimulate PTH secretion. Phosphate affects PTH only when it induces concomitant hypocalcemia. This is evident from controlled studies in cows (18) and, more recently in man (19). The oral administration of phosphate in man initiates a brisk elevation of serum PTH, apparently stimulated by a small decrease of ionized calcium. The control system is rapid and sensitive. It is apparent from the present studies and those previously reported, that the phosphate-initiated, calcium-mediated PTH control system operated remarkably effectively in the maintenance of phosphate homeostasis as the nephron population is reduced.

If the mechanism examined in this study applies to man, it may not be the only one responsible for the evolution of secondary hyperparathyroidism in chronic renal disease. Vitamin D resistance decreases enteric absorption of calcium, and thus contributes to a decrease in the concentration of ionized calcium in the extracellular fluid, independent of any change in plasma phosphate concentrations (20, 21). It is not yet known when vitamin D resistance begins in the course of chronic renal disease (22), but in far advanced renal disease, vitamin D resistance could represent the dominant factor in sustaining and increasing the level of parathyroid hormone secretion.

The possibility that vitamin D resistance contributed to the pathogenesis of secondary hyperparathyroidism in the dogs maintained on the 1200 mg phosphorous diet in the present studies seems remote. If vitamin D resistance were a factor, it should have led to the development of hyperparathyroidism in the animals on the low phosphorous intake as well; indeed the calcium intake of the low phosphorous group was lower than that of the high phosphorous dogs. Thus, it seems unlikely that any appreciable degree of vitamin D resistance occurred within the period of approximately 2 months during which the present animals were studied.

The possibility exists that in the animals maintained on the low phosphorous diet, demineralization of the skeleton occurred due to phosphate depletion, and the calcium entering the extracellular fluid from bone suppressed the parathyroid glands, thereby preventing the evolution of hyperparathyroidism. To evaluate this possibility, preliminary experiments have been performed in which phosphorous intake was reduced during the genesis of renal insufficiency in direct proportion to the decrement in GFR rather than to very low levels. Thus if GFR was reduced by 30%, phosphorous intake was reduced by 30% (i.e. from 1200 to 800 mg/day), and so on through the evolution of renal insufficiency. This regimen should obviate any risk of developing phosphate depletion. In the results obtained to date, TRP has remained high throughout, which suggest that secondary hyperparathyroidism is prevented by proportional reduc-

tion in phosphorous intake as well as by more marked restriction of phosphorous intake.

The present studies have demonstrated that early secondary hyperaparthyroidism can be prevented even in the presence of marked renal insufficiency by maintaining a low phosphorous intake. By implication, therefore, the progressive rise in the levels of PTH, which appears to begin with the earliest stages of nephron destruction, is at least in part an expression of a salutary adaptation in the control system which maintains external phosphate balance. However, because the parathyroid hormone has effects beyond those on the tubular transport of phosphate, the adaptations may ultimately lead to deleterious skeletal effects. The possibility exists that if

TABLE II

The Effects of a Single Oral Dose of 600 mg of Phosphorous on Fractional Phosphate Reabsorption and Parathyroid Hormone Levels in Three Uremic Dogs in Whom Secondary Hyperparathyroidism Had Been Prevented by the Sustained Administration of a Low Phosphorous Diet

Dog	Time	GFR	P_{PO_4}	P_{Ca}	I_{Ca}	TRP	PTH
	min	*ml/min*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*%*	*μlEq/ml*
Creatinine prime and sustain (2.5% DW at 2 ml/min) started at −46 min							
1	0–14	8.0	5.2	10.8	5.7	81.8	55
	14–29	7.8	5.2	10.5		82.9	
	29–34	7.5	5.4	10.4		82.8	
	Mean	7.8	5.3	10.6		82.5	
Phosphorus 600 mg given orally at 34 min							
	34–104	7.8	8.5	9.9		57.8	
	104–164	7.9	10.0	9.9		18.7	
	164–225	7.2	12.2	9.2	4.7	−13.1	100
	225–284	7.7	12.4	9.1		4.0	
	284–344	7.4	10.5	9.5	4.8	1.8	210
Creatinine prime and sustain (2.5% DW at 2 ml/min) started at −45 min							
2	0–63	14.9	5.4	10.1	5.6	86.6	47
	63–117	12.8	5.2	9.3		88.5	
	117–166	10.9	4.5	9.7		89.4	
	Mean	12.9	5.0	9.7		88.2	
Phosphorus 600 mg given orally at 166 min							
	166–227	13.2	6.1	9.3		68.0	
	227–287	13.0	8.8	9.0		19.9	
	287–347	12.0	10.3	8.9	4.8	− 1.1	95
	347–407	11.5	10.7	9.2		8.6	
	407–461	11.5	10.7	9.0		13.7	129
Creatinine prime and sustain (2.5% DW at 2 ml/min) started at −79 min							
3	0–20	21.7	3.4	10.1	5.6	97.1	57
	20–40	14.2	3.2	10.2		98.7	
	40–60	14.2	3.4	10.1		98.9	
	Mean	16.7	3.3	10.1		98.2	
Phosphorus 600 mg given orally at 60 min							
	60–120	15.2	4.4	9.9		85.8	
	120–180	14.7	7.2	9.0		40.0	
	180–240	15.4	7.0	9.4	4.8	27.7	90
	240–300	15.9	6.8	9.2		27.6	
	300–360	15.5	4.6	9.3		−1.0	270

I_{Ca} represents ionized calcium concentration. Creatinine was infused in the sustain solution at the rate of 3.75 mg/min. P_{PO_4} = plasma phosphate level, P_{Ca} = plasma calcium level.

TABLE III

Serum Phosphate and Calcium Values at Each Stage of Study in Dogs on a 1200 mg Phosphorous Diet vs. Dogs on a Diet Containing Less Than 100 mg of Phosphorous/day

	Phosphate		Calcium	
	1200 mg P diet	Low P diet	1200 mg P diet	Low P diet
Condition	n6	n7	n6	n7
	mg/100 ml		*mg/100 ml*	
Control	4.7 ±0.3	4.5 ±0.33	10.4 ±0.34	10.2 ±0.21
	NS		NS	
Nephron reduction				
1	4.0 ±0.23	3.8 ±0.53	9.5 ±0.34	10.6 ±0.16
	NS		$P < 0.02$	
2	4.5 ±0.29	3.9 ±0.33	9.9 ±0.22	11.5 ±0.37
	NS		$P < 0.01$	
3	6.4 ±1.18	4.2 ±0.57	10.1 ±0.41	10.7 ±0.45
	NS		NS	

Values represent the mean ± one standard error of the mean. NS, not significant ($P > 0.05$).

phosphorous intake were reduced in direct proportion to the decrease in GFR beginning early in chronic renal disease, secondary hyperparathyroidism could be prevented at least until the onset of vitamin D resistance without risk of phosphate depletion and osteomalacia. However, more work is required before the results of the present study can be translated into effective and safe therapeutic recommendations.

ACKNOWLEDGMENTS

Dr. Bricker is a recipient of a U. S. Public Health Service Research Career Award.

This investigation was supported by National Institutes of Health Grants AM-09976, AM-05248, and AM-08572.

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