Changes in Serum and Urinary Calcium during Phosphate Depletion: Studies on Mechanisms

JACK W. COBURN and SHAUL G. MASSRY

From the Medical and Research Services, Wadsworth Hospital, Los Angeles Veterans Administration Center, and the Departments of Medicine, Cedars-Sinai Medical Center and UCLA School of Medicine, Los Angeles, California 90073

ABSTRACT The changes in serum calcium and the renal handling of this ion were evaluated during phosphate depletion. 96 renal clearance studies were carried out in 10 dogs before and after prolonged phosphate depletion (30–160 days) and after repletion. Depletion was produced by reducing phosphate intake and administering aluminum hydroxide gel while intakes of sodium, calcium, and magnesium were constant. With phosphate depletion, serum phosphorus fell to less than 1.0 mg/100 ml and diffusible serum calcium either remained unchanged or rose transiently. Glomerular filtration rate (GFR) fell by 15 to 53%. Despite the reduced filtered load of calcium, its fractional excretion increased in most experiments. This hypercalciuria was not dependent upon changes in sodium or magnesium excretion, or the urinary concentration of complexing anions, and persisted after sodium restriction. Phosphate repletion reversed the effects on GFR and calcium excretion. The intravenous infusion of small quantities of phosphate (0.04 mmole/min) into either intact or thyroparathyroidectomized (T-PTX), phosphate-depleted animals caused a significant reduction in fractional excretion of calcium, but the intrarenal infusion of 0.02 mmole/min of phosphate into one kidney failed to produce an ipsilateral effect. The administration of parathyroid extract reduced fractional calcium excretion, but the latter remained significantly elevated. After T-PTX, fractional calcium excretion did not increase in the phosphate-depleted animals. Furthermore, serum calcium was normal after T-PTX until serum phosphorus increased slightly, and only then did hypocalcemia develop. These observations indicate that (*a*) phosphate depletion produces hypercalciuria through a reduction in tubular reabsorption of calcium which is not due to changes in the tubular reabsorption of other ions; this effect is not reversed by the direct intrarenal infusion of phosphate; (*b*) a state of functional hypoparathyroidsm occurs during phosphate depletion which may, in part, cause reduced tubular reabsorption of calcium; (*c*) other extra renal mechanism(s), possibly related to events occurring in bone as a result of phosphate depletion, may have an effect on urinary calcium excretion; and (*d*) in the phosphate-depleted state, parathyroid hormone is not required for the maintenance of a normal level of serum calcium.

This work was presented in part before the Western Society for Clinical Research, 28 January 1967, Carmel, Calif., and before The Endocrine Society, 17 June 1967, Bal Harbour, Fla.

Dr. Massry is an Established Investigator of the American Heart Association.

Received for publication 12 November 1969 and in revised form 6 February 1970.

INTRODUCTION

A clinical entity of phosphate depletion, characterized by weakness, anorexia, malaise, and bone pain, has been described after the long-term use of antacids which render phosphate nonabsorbable in the gastrointestinal tract (1). Among the abnormalities observed during phosphate depletion are demineralization of bone and a marked negative calcium balance primarily caused by increased urinary losses. Such a marked increase in urinary calcium excretion has been reported during phosphate depletion in humans (1, 2), sheep (3), and rats (4). Conversely, the acute administration of phosphate causes a significant reduction in urinary calcium (5–9). The mechanism through which phosphate affects the renal handling of calcium have not been well delineated.

Urinary calcium represents the difference between the quantity filtered in the glomeruli and that reabsorbed by the renal tubules (10); an increase in the filtered load of calcium and/or a decrease in the renal tubular reabsorption can produce hypercalciuria. Several hor-

TABLE I

Composition of Diets

Diet	Normal	Low phosphate
I. Each 100 g of diet contains:		
1) Basic diet, *952 g*		
Sucrose, *g*	440	440
Lard, *g*	210	210
Beef fibrin, *g*	230	230
Corn oil, g	48	48
Choline, *g*	4	4
Vitamin fortification mixture,* *g*	20	20
2) Mineral mixture, *g*	48	48
II. Each 100 g mineral mixture contains:		
NaCl	10.53	10.53
$CaCO_3$	36.20	29.26
Ca-gluconate	—	19.89
$Ca\ HPO_4$	7.47	—
$MgSO_4$	14.55	14.46
$MnSO_4$	0.372	0.372
KCl	—	4.99
K-citrate	—	7.22
K-acetate	—	8.74
K_2HPO_4	25.00	—
Fe-citrate	4.465	4.465
$CuSO_4$	0.0293	0.0293
KI	0.0781	0.0781
$CoCl_2$	0.0293	0.0293
$ZnCl_2$	0.0244	0.0244
III. Daily mineral intake:		
Sodium, *mEq/day*	28	28
Potassium, *mEq/day*	33	33
Magnesium, *mg/day*	201	196
Calcium, *mg/day*	1130	1095
Phosphorus, *mg/day*	710	43

* Vitamin fortification mixture, supplied by Nutritional Biochemicals Corp., Cleveland, Ohio.

monal or nonhormonal factors affect the rate of urinary calcium excretion: an augmentation in sodium excretion, produced by volume expansion (11–13), osmotic agents (14), and various diuretics (14–16), is associated with calciuresis. Acute (17, 18) and chronic (19, 20) increases in urinary magnesium and an increment in the urinary excretion of complexing anions, such as citrate (21), sulphate (22), and gluconate (23), also augment the rate of calcium excretion. The administration of parathyroid extract enhances the renal tubular reabsorption of calcium (24–27), and parathyroidectomy is followed by a rise in the renal clearance of calcium (24). Calcium excretion is also heightened under the long-term effect of excess mineralocorticoids (28, 29).

Phosphate depletion also may affect the level of serum calcium. An elevation of the concentration of serum calcium was reported in puppies (30) and rats (31); furthermore, Lotz, Zisman, and Bartter reported an increase of blood calcium levels to normal in hypoparathyroid humans after phosphate depletion (1). The present study was undertaken to evaluate the possible mechanisms that might contribute to the changes in serum and urinary calcium during phosphate depletion.

METHODS

96 clearance studies were carried out in 10 trained, adult female mongrel dogs, weighing 12–25 kg, which had been previously subjected to episiotomy. Phosphate depletion was produced by feeding the animals an artificial phosphate restricted diet, modified from that described by Manitius, Levitin, Beck, and Epstein (32) and by the administration of aluminum hydroxide gel, (supplied as unflavored Amphogel, courtesy of Dr. J. E. Pickering, Wyeth Laboratories, Marietta, Pa.) 90 ml/day, for periods ranging between 30 and 160 days. Before phosphate depletion and during repletion periods, the diet was similar except a normal quantity of phosphate was added. Each animal received 325–350 g of either the normal or low phosphate diet at the same time each morning except on days when experiments were carried out, and the dogs were conditioned to consume the entire daily ration within a few minutes. The constituents of the normal and low phosphate diets are shown in Table I; both diets supplied 1800 calories and 6000–6500 U of vitamin D_2 per day and provided similar intakes of minerals except for phosphate.

Clearance studies were carried out during (*a*) a normal phosphate intake after the dogs had received the normal diet for at least 8 days; (*b*) phosphate depletion, manifested by hypophosphatemia and the absence of phosphate in the urine; and (*c*) in three animals, during phosphate repletion produced by resumption of the normal diet for periods of 10–60 days. In five animals, which had received the low phosphate diet for at least 30 days, sodium intake was reduced to less than 2 mEq/day by withholding NaCl from the diet; after 5 days, clearance studies were performed, and thereafter, sodium intake was restored to its previous level. The sequence of dietary intake in the different animals is shown in Table II.

The great majority of clearance studies (87 experiments) were carried with the dogs unanesthetized and standing in loosely fitting, canvas slings. To ensure water diuresis and to minimize the influence of calcium-complexing anions in the urine, a water load of 2% of body weight was given by gavage; and, throughout the experiments, 2.5% dextrose in water was given intravenously at a rate equal to urine flow. Glomerular filtration rate was measured by exogenous creatinine clearance. Urine was collected from an indwelling Foley catheter, and the bladder was washed with air at the end of each clearance period. Blood samples were obtained from the external jugular vein by venipuncture at the midpoint of each period. In each of these studies, clearance periods of 15–30 min duration were collected between 9:00 to 10:30 a.m. These observations served either as base line observations at different degrees of phosphate depletion or, in certain instances, as controls for other test procedures carried out during phosphate depletion. In each dog, at least 6 days elapsed between the individual clearance studies. The following experimental maneuvers were carried out after collection of the base line urine samples: (*a*) in seven animals, the clearance studies were continued until 1:00 p.m. to evaluate the effect of diurnal rhythm on electrolyte excretion in the state of phosphate depletion. (*b*) In 10 dogs, a neutral sodium phosphate solution, constituted to deliver 0.04 mmole of phosphorus per min, was infused into a peripheral vein for 60–120 min. Clearance collections were obtained during the infusion and for 60–90 min thereafter.

TABLE II

Effect of Phosphate Depletion on Glomerular Filtration Rate and Renal Excretion of Calcium and Sodium

Dog No.	Diet	Duration diet	Body weight	C_{Cr}	Sp	S_{Ca} Total	S_{Ca} Diff.	F_{Ca}	$U_{Ca}V$	S_{Na}	$U_{Na}V$
		days	*kg*	*ml/min*	*mg/100 ml*	*mg/100 ml*		*mg/min*	*μg/min*	*mEq/liter*	*μEq/min*
1	N	8	18.2	59.5	2.67	9.9	6.8	4.05	3	139	4
	L	19	18.2	41.8	0.40	9.6	5.7	2.34	57	137	2
2	N	11	18.2	86.7	4.07	9.4	6.2	4.38	9	140	19
	L	41	17.3	57.4	0.20	8.7	5.6	3.28	140	140	62
3	N	10	22.7	88.3	—	—	—	—	—	—	—
	L	39	24.5	64.3	0.43	10.4	6.9	4.43	57	142	4
	L	58	22.3	68.8	0.32	10.7	6.2	4.26	163	138	2
4	N	11	17.7	42.2	5.76	10.4	6.5	2.75	42	142	3
	L	11	16.8	36.5	0.90	10.9	7.2	2.65	142	136	3
5	N	15	20.5	62.5	3.45	10.8	7.0	4.36	6	139	7
	L	35	20.5	50.5	1.87	11.9	7.6	3.81	105	142	4
	L	55	20.5	44.7	3.12	10.7	6.8	3.05	20	141	4
	N	10	18.6	31.3	3.84	10.8	7.2	2.23	46	140	5
	N	22	18.2	41.2	4.10	10.5	6.9	2.86	36	140	3
	L	17	19.1	41.3	1.13	10.4	7.3	3.01	45	140	7
	L	28	18.6	38.9	0.50	11.3	7.2	2.81	90	141	9
	L	34	18.6	32.8	1.29	11.0	7.1	2.33	26	139	6
	L	88	19.1	38.9	0.60	10.4	6.6	2.57	47	139	2
	N	40	17.7	43.7	3.50	9.7	6.3	2.76	12	136	3
6	L	35	13.2	32.7	2.09	9.9	6.4	2.07	90	141	3
	L	42	13.2	34.7	1.95	10.2	6.8	2.37	15	142	1
	N	14	13.2	31.3	3.55	10.8	7.2	2.24	1	145	2
	N	49	13.6	58.6	5.51	9.7	6.2	3.66	34	145	2
	N	59	13.6	50.9	4.48	9.4	5.4	2.76	32	146	3
	L	24	14.1	43.3	1.00	11.3	6.9	2.97	317	139	2
	L	87	14.1	34.5	0.15	9.7	6.5	2.28	127	138	2
	L	151	13.2	32.1	0.56	9.9	6.9	2.22	105	137	4
7	N	18	12.3	34.9	3.10	10.4	6.1	2.14	27	144	5
	L	50	10.9	26.4	0.85	10.5	6.6	1.75	116	142	1
	L	56	10.9	19.4	1.32	11.4	7.4	1.44	179	139	4
	L	64	11.0	30.9	0.50	9.7	6.2	1.90	73	140	2
	N	15	10.9	35.9	4.12	9.5	6.2	2.21	9	142	6
8	N	11	15.9	61.6	4.21	9.8	6.4	4.02	10	142	6
	L	6	16.5	51.2	1.60	8.9	5.8	2.97	21	140	10
	L	12	16.5	39.3	2.64	9.5	6.9	2.73	22	140	18
	L	19	15.9	49.4	0.30	8.7	5.7	2.84	262	139	144
9	N	9	18.2	78.9	4.22	8.9	6.4	5.06	2	142	4
	L	41	20.5	53.1	1.93	11.0	7.3	3.88	247	141	2
	L	60	20.5	46.5	1.74	10.6	6.5	3.04	175	144	4
10	N	11	19.5	67.7	4.22	9.6	6.4	4.35	6	144	3
	L	22	20.5	48.2	1.77	10.7	6.5	3.13	56	142	60
	L	45	19.5	48.0	2.07	10.3	7.0	3.35	138	141	2

Each data point represents the mean of 3–5 clearance periods or plasma values obtained between 9 and 11 a.m. Abbreviations: N = normal phosphate diet, L = low phosphate diet, C_{Cr} = exogenous creatinine clearance, Sp = serum phosphorus, S_{Ca} = serum calcium, Diff. = diffusible, $U_{Ca}V$ = urinary calcium, S_{Na} = serum sodium, and $U_{Na}V$ = urinary sodium. The results of other studies during phosphate depletion are shown as control data in Tables IV and V.

(*c*) And, in seven experiments, parathyroid extract (supplied as Lilly parathyroid extract, courtesy of Dr. A. S. Ridolfo, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Ind.) was injected intramuscularly in a dose of 100 U each hour for 3 hr; clearances were measured during the time of parathyroid extract (PAE) administration and for 90 min after the last injection. To evaluate the potency of the PTE and ascertain its effect on serum calcium, larger quantities of PTE were injected into four animals; they received 200 U at 8 a.m., 10 a.m., 12 noon, and 2 p.m. and 4 p.m., and blood samples were obtained just before each injection and 2 hr after the last injection.

In five animals, thyroparathyroidectomy (T-PTX) was carried out after the dogs had been maintained with the phosphate depletion regimen for 66–86 days. The animals received a similar oral water load and 1 hr later were anesthetized with intravenous pentobarbital. Arterial blood pH and P_{CO_2} were measured with a Radiometer pH meter (London Co., Cleveland, Ohio) utilizing the Astrup method. The animals were ventilated with a Harvard respirator pump (Harvard Apparatus Co., Millis, Mass.) with its tidal volume adjusted to maintain the arterial blood pH between 7:40 and 7.44. Control clearances were obtained immediately before and during the surgical procedure, the thyroid and the parathyroid glands were then removed, and clearance observations were extended for 270–300 min after the removal of the glands. Two animals died within 24 hr after surgery, and the other three were maintained on the low phosphate diet for 35–90 days after T-PTX. 11 clearance studies were carried out in the phosphate-depleted T-PTX dogs in the unanesthetized state in a manner similar to that described above. The effect of an intravenous infusion of phosphate (0.04 mmole/min) was evaluated in five experiments in the same manner as carried out before T-PTX. In addition, blood samples were obtained in the fasting state on 3–5 days each week after T-PTX for the measurement of serum calcium and phosphorus. On three occasions in one dog (No. 10) and on one occasion in the other two (Nos. 5 and 9), aluminum hydroxide gel was withheld and an oral neutral potassium phosphate solution (Hyper-Phos-K, supplied courtesy of Dr. A. Wolbarsht of the Davies Rose & Co., Ltd., Boston, Mass.) was given for 2–4 days to provide an additional 1000 mg of phosphorus per day.

In four intact, phosphate-depleted animals, the effect of an intrarenal infusion of a small amount of phosphate was evaluated. Anesthesia and the state of hydration and respiration were regulated in a manner similar to that carried out during thyroparathyroidectomy. Both ureters were exposed via a suprapubic incision and catheterized with polyethylene catheters. The left kidney and left renal artery were approached through a flank incision, and a ½ inch, 26 gauge, hubless needle was inserted into the left renal artery 2–3 cm distal to its origin from the aorta and directed toward the latter. Saline (0.85%) was infused into the renal artery at a rate of 0.066 ml/min both during a stabilization period of 60–90 min after the manipulation of the renal artery and while three to four control clearance periods of 10–15 min duration were collected simultaneously from the separate kidneys. The saline solution was then replaced with a neutral phosphate solution in saline which was infused at the same rate and delivered 0.02 mmole of phosphorus per min. Clearance periods of 3–10 min duration were then obtained simultaneously from each kidney for an additional 80–90 min. Blood samples were obtained at 10- to 15-min intervals from the right femoral artery, and the concentrations of calcium, sodium, and creatinine in arterial blood were used to calculate the clearances from both kidneys.

Blood and urine samples were analyzed for creatinine, sodium, and phosphate by standard Technicon autoanalyzer techniques (Technicon Corporation, Tarrytown, N. Y.). Calcium and magnesium in urine, serum, and ultrafiltrates of serum were measured with the Perkin-Elmer atomic absorption spectrophotometer (Perkin-Elmer Corp., South Pasadena, Calif.), model 303, utilizing the method of Zettner and Seligson (33). Citrate concentration was measured by the method of Beutler and Yeh (34) in urine samples obtained from three dogs during certain experiments during both phosphate depletion and repletion. In 20 experiments during both the normal phosphate intake and phosphate depletion, pH was measured in fresh urine samples using the Radiometer pH meter. These measurements were discontinued when it was found that urine pH did not vary during phosphate depletion or repletion. Urine samples were tested during each experiment for glucose and acetone utilizing qualitative "dip-stick" methods (Combistix and Ketostix, The Ames Co., Elkhart, Ind). The water content of serum was determined by refractometry (Goldberg Refractometer, American Optical Company, Buffalo, N. Y.). Diffusible calcium was determined in ultrafiltrates of serum prepared anaerobically at room temperature in Lavietes chambers (35). In each experiment, at least two samples were taken for the preparation of ultrafiltrates during the control period and another two during the subsequent experimental procedure (e.g. PTE administration, intravenous PO_4 infusion, etc.). The per cent diffusibility of calcium was calculated from the following formula:

$$\text{Per cent diffusibility} = \frac{\text{diffusible calcium mg/100 ml serum}}{\text{total calcium mg/100 ml serum}} \times 100$$

In any given experiment, the per cent diffusibility evidenced no appreciable change. Therefore, the mean per cent diffusibility of calcium in each experiment was used to calculate the diffusible calcium for those clearance periods where direct measurements were not made. The diffusible magnesium was measured and calculated in a similar way. All clearances of calcium and magnesium are expressed in terms of their diffusible fractions.

RESULTS

The animals remained relatively healthy in appearance and maintained their body weight over the prolonged periods they received the low phosphate diet. Two animals (Nos . 5 and 6) which ingested the low phosphate diet for the longest period of time became sluggish and were less active during periods of exercise.

After administration of the low phosphate diet and aluminum hydroxide gel for 2–3 wk, the serum phosphorus concentrations fell to very low levels, 0.2–1.0 mg/100 ml, and urinary phosphorus was invariably undetectable in the urine (< 0.002 mg/ml). Occasionally, the level of serum phosphorus rose spontaneously to levels of 1.0–2.0 mg/100 ml without any change in dietary regimen. The effect of phosphate depletion (26 studies) and repletion on serum calcium, glomerular filtration rate (GFR), and the renal excretion of cal-

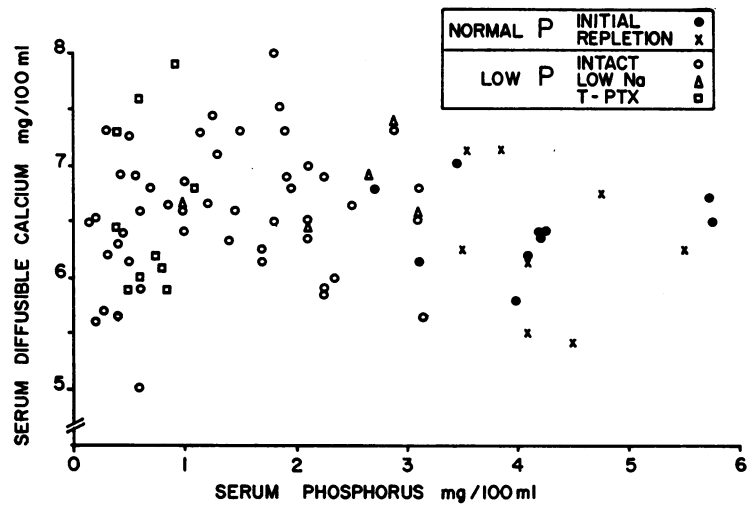

FIGURE 1 The relation between serum levels of diffusible calcium and the level of serum phosphorus during phosphate depletion. Each data point represents the mean of two determinations during base line measurements in a single experiment. *Low Na* indicates data after 5 days of sodium restriction and *T-PTX*, those after thyroparathyroidectomy.

cium and sodium are shown in Table II, and additional base line clearance studies during phosphate depletion (17 experiments) are shown as "control" data in Tables IV and V. In each animal, there was a fall in GFR, ranging from 14 to 53%, during phosphate depletion; with subsequent phosphate repletion, GFR rose significantly in two of the three animals which were so studied (dog Nos. 6 and 7).

In 5 of the 10 animals (Nos. 5–7, 9, 10), serum calcium rose by 1 to 2.5 mg/10(ml during phosphate depletion, although the calcium concentration returned to normal in most instances despite continued phosphate

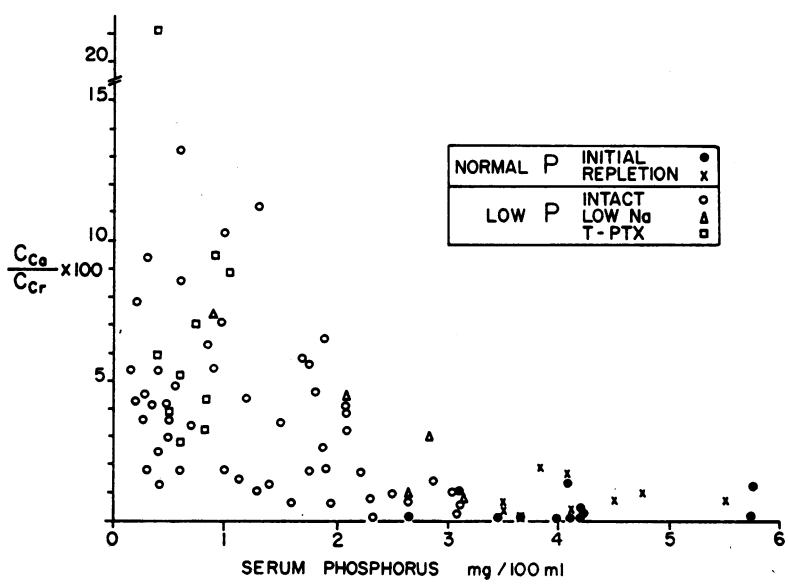

FIGURE 2 The relationship between the percentage of filtered calcium excreted ($C_{Ca}/C_{Cr} \times 100$) and the levels of serum phosphorus in unanesthetized dogs during phosphate depletion. Each data point represents the mean of three to four base line clearance periods in a single experiment. *Low Na* indicates observations after 5 days of sodium restriction and *T-PTX*, those after thyroparathyroidectomy.

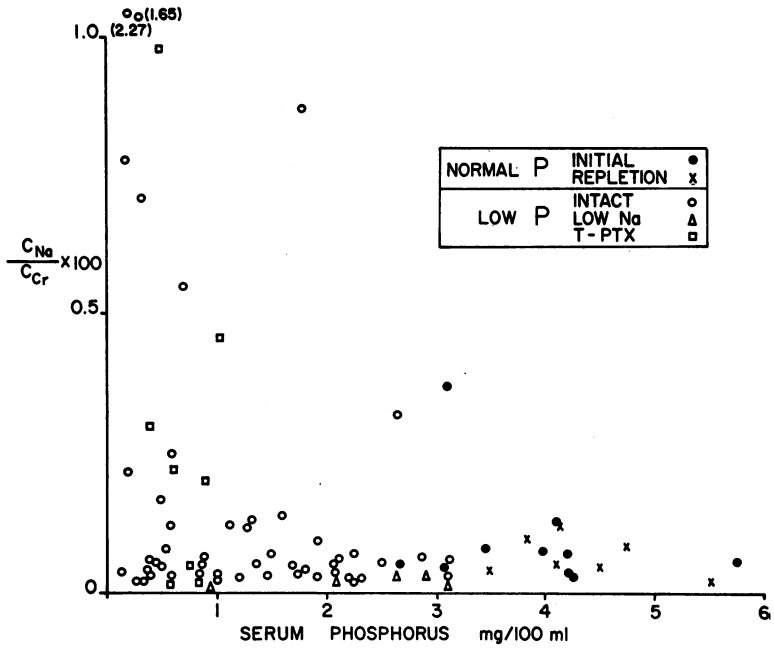

FIGURE 3 The relationship between the percentage of filtered sodium excreted ($C_{Na}/C_{Cr} \times 100$) and the levels of serum phosphorus in unanesthetized dogs during phosphate depletion. Each data point represents the mean of three to four base line clearance periods in a single experiment. *Low Na* indicates observations after 5 days of sodium restriction and *T-PTX*, those after thyroparathyroidectomy.

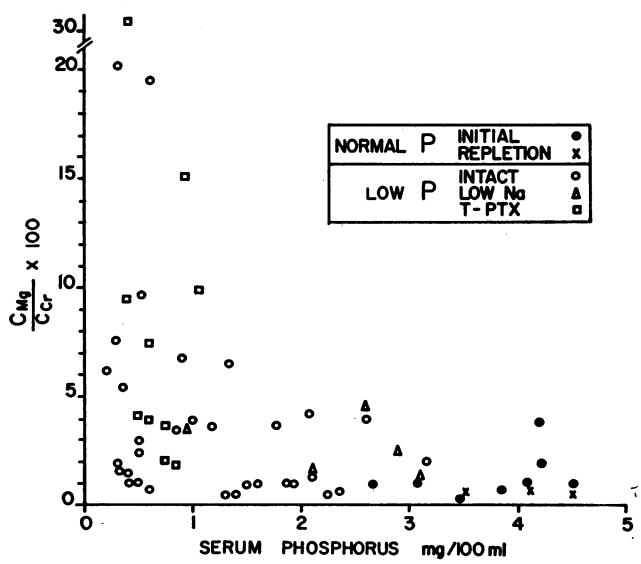

FIGURE 4 The relationship between the percentage of filtered magnesium excreted ($C_{Mg}/C_{Cr} \times 100$) and the levels of serum phosphorus in unanesthetized dogs during phosphate depletion. Each data point represents the mean of three to four base line clearance periods in a single experiment. *Low Na* indicates observations after 5 days of sodium restriction and *T-PTX*, those after thyroparathyroidectomy. (In 28 experiments shown in Figs. 2 and 3, urinary magnesium levels were not measured.)

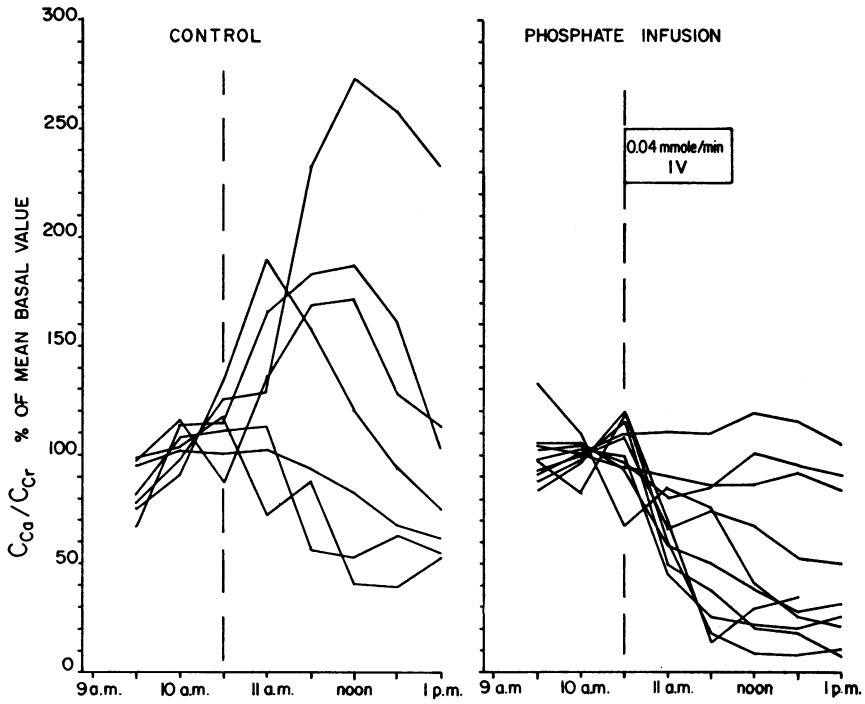

FIGURE 5 A comparison of the pattern of fractional calcium excretion in 10 phosphate-depleted dogs receiving a small intravenous infusion of phosphate (on the right) with that of 7 of these dogs studied over the same hours of the day without phosphate (on the left). Values obtained between 9 a.m. and 10:30 a.m. are considered as base line, and the data are expressed as per cent of these basal values. Each line represents an individual animal.

depletion and hypophosphatemia. The relationship between diffusible calcium level and phosphorus concentration in sera for the entire population, including eight studies carried out in the early phases of phosphate depletion, which were not included in Tables II, IV, and V, is shown in Fig. 1. The diffusible calcium concentrations rose slightly as serum phosphorus fell to levels between 1.0 and 2.0 mg/100 ml, but the former tended to decrease as serum phosphorus dropped below 1.0 mg/100 ml. The percentage of diffusibility of serum calcium in the phosphate-depleted dogs was 65 ±0.49%, a mean value similar to that of normal dogs studied in our laboratory.

With the significant fall in glomerular filtration rate and a slight but variable increase in diffusible calcium concentration, the quantity of calcium filtered at the glomeruli usually decreased with phosphate depletion. Despite this fall in filtered calcium, urinary calcium increased and, in some experiments, reached rates as high as 250–430 μg/min. This hypercalciuria was associated with no change in urinary flow but resulted from an increase in the concentration of calcium in the urine. The relation between the percentage of the filtered calcium excreted and the serum phosphorus level is shown in Fig. 2 for all experiments. The percentage of filtered calcium excreted increased from control values of 0.35 ±0.14% (mean ±SE) to 3.61 ±0.64% ($P < 0.001$) with serum phosphorus levels between 1.0 and 2.0 mg/100 ml, and to 5.32 ±0.65% ($P < 0.001$) with serum phosphorus levels less than 1.0 mg/100 ml. In five phosphate-depleted dogs evaluated after dietary restriction of sodium, the fractional excretion of calcium was similar (Fig. 2). With phosphate repletion, the per cent of filtered calcium excreted decreased to 0.77 ±0.19%, values not different from those observed before phosphate depletion ($P = 0.1$).

During phosphate depletion, there was neither a significant nor consistent change in serum sodium concentration, urinary sodium excretion (Table II), or in the per cent of filtered sodium excreted (Fig. 3). Phosphate depletion was not associated with any consistent change in serum magnesium levels, although the fractional excretion of magnesium increased in some of the experiments (Fig. 4). Furthermore, the increases in urinary calcium concentration which occurred during phosphate depletion were independent of changes in the concentration of citrate in the urine of the three dogs in which the latter was measured.

TABLE III

A Representative Experiment Demonstrating the Effect of an Intravenous Infusion of Phosphate on Renal Calcium Handling in a Phosphate Depleted Dog

Time	V	C_{Cr}	Sp	S_{Ca} Total	S_{Ca} Diff.	F_{Ca}	$U_{Ca}V$	$\frac{C_{Ca}}{C_{Cr}} \times 100$	$U_{Na}V$	$\frac{C_{Na}}{C_{Cr}} \times 100$
min	*ml/min*	*ml/min*	*mg/100 ml*	*mg/100 ml*		*mg/min*	*μg/min*		*μEq/min*	
−95	Waterload 600 ml by stomach tube									
−40	Priming dose creatinine 1000 mg									
	Infusion I started 8 mg/min creatinine delivered in 2.5% dextrose/water at 1 ml/min Infusion II started and adjusted to equal urine flow 2.5% dextrose in water									
0–20	3.3	35.4	0.25	10.2	7.4	2.62	171	6.58	3	0.07
20–40	4.5	32.0	0.25	10.1	7.3	2.34	172	7.30	5	0.11
40–60	5.9	32.3	0.20	10.1	7.3	2.36	184	7.82	5	0.11
60–90	7.0	35.7	0.20	10.0	7.2	2.57	268	10.44	9	0.20
90–120	5.2	37.2	0.20	10.2	7.3	2.72	294	10.12	22	0.43
120	Infusion III 0.04 mM phosphate/min delivered as neutral phosphate in 2.5% dextrose in water at 1.0 ml/min									
120–150	3.8	42.0	0.35	10.0	7.2	3.02	131	4.33	9	0.15
150–180	4.8	45.6	0.35	9.8	7.0	3.19	104	3.24	7	0.12
180–210	3.9	42.7	0.40	10.3	7.4	3.16	55	1.72	15	0.25
210–240	4.4	47.0	0.45	10.1	7.2	3.38	51	1.49	32	0.50

Abbreviations: V = urine volume, C_{Cr} = exogenous creatinine clearance, Sp = serum phosphorus, S_{Ca} = serum calcium, Diff. = diffusible, F_{Ca} = filtered calcium, $U_{Ca}V$ = urinary calcium excretion, C_{Ca} = clearance of diffusible calcium, $U_{Na}V$ = urinary sodium excretion, C_{Na} = clearance of sodium.

A representative experiment demonstrating the effect of the intravenous infusion of a small quantity of phosphate is shown in Table III, and the summary of all such experiments, in Table IV. The intravenous infusion of phosphate, 0.04 mmole/min, was followed by an increase of serum phosphate concentration of 0.65 ±0.20 mg/100 ml (mean ±SE) and a fall in urinary calcium excretion of 54 ±15 μEq/min ($P < 0.01$) and in the percentage of its filtered load excreted of 2.47 ±0.68% ($P < 0.01$). This decrease in fractional excretion of calcium was not associated with a fall in its filtered load and occurred between 10:30 a.m. and noon, a time of the day when urinary calcium usually increased as part of the diurnal rhythm. Fig. 5 depicts the changes in fractional calcium excretion that occurred in seven phosphate-depleted dogs as a result of the diurnal rhythm during the morning hours and in 10 experiments when phosphate was infused intravenously over the same morning hours.

The results of the seven experiments in which injections of parathyroid extract were given to phosphate-depleted dogs are shown in Table V. Glomerular filtration rate increased in each experiment with an increase in filtered calcium of 29 ±7.8%. Urinary calcium fell in three dogs (Nos. 2, 3, 6) and was unchanged in the others, and the fraction of the filtered calcium excreted decreased in each instance; however, the latter remained above 1% in all but one experiment. Serum calcium increased only slightly in three of such studies; however, when four dogs (Nos. 2, 3, 9, 10) were given larger quantities of PTE for a longer period of time, serum calcium increased by 2.5, 3.1, 1.5, and 1.3 mg/100 ml, respectively.

The acute effect of thyroparathyroidectomy (T-PTX) on the renal handling of calcium in the phosphate-depleted dogs is summarized in Table VI. Unlike the definite increase in fractional excretion of calcium reported in normal dogs after T-PTX (24), there was either a decrease or no change in the fractional excretion of calcium in phosphate-depleted dogs. In three T-PTX animals which were maintained on the phosphate depletion regimen, augmented urinary calcium and high fractional excretion of filtered calcium persisted at levels comparable to those noted before removal of the parathyroid glands (Fig. 2).

After T-PTX, serum calcium level was entirely dependent upon the concentration of serum phosphorus. Serum calcium remained normal as long as marked hypophosphatemia persisted, but, whenever serum phosphorus rose either spontaneously or after the oral administration of phosphate, marked hypocalcemia and tetany appeared (Fig. 6). The inverse relationship be-

TABLE IV

A Summary of the Effect of Intravenous Infusion of Phosphate on Renal Calcium Handling in Phosphate Depleted Dogs

Dog No.	Duration diet	Period	Sp	C_{Cr}	S_{Ca}		F_{Ca}	$U_{Ca}V$	$\frac{C_{Ca}}{C_{Cr}} \times 100$	$U_{Na}V$	$\frac{C_{Na}}{C_{Cr}} \times 100$
					Total	Diff.					
	days		*mg/100 ml*	*ml/min*	*mg/100 ml*		*mg/min*	*μg/min*		*μEq/min*	
1	25	C	2.35	31.4	11.0	6.8	2.14	33	1.77	3	0.06
		P	4.57	35.0	10.8	6.6	2.36	13	0.58	6	0.11
2	35	C	0.60	38.9	10.4	6.5	2.56	48	1.75	2	0.03
		P	1.45	41.4	10.6	6.7	2.76	56	2.00	2	0.03
3	70	C	0.59	34.1	8.1	5.0	1.74	144	8.41	12	0.25
		P	0.86	34.6	8.0	5.0	1.73	56	3.29	8	0.18
4	18	C	0.70	26.5	9.6	6.8	1.81	60	3.33	19	0.55
		P	1.40	30.9	9.7	6.7	2.25	44	2.11	31	0.80
5	76	C	1.92	45.2	10.4	6.8	3.16	72	2.35	7	0.11
		P	2.91	37.0	9.9	6.4	2.38	12	0.51	2	0.03
6	100	C	0.21	35.4	10.1	6.6	2.32	222	8.50	12	0.25
		P	0.39	44.2	10.0	6.7	2.82	52	1.76	24	0.40
7	96	C	0.47	34.3	9.8	6.1	2.72	96	4.58	3	0.06
		P	1.13	39.2	9.2	5.8	2.71	21	0.93	1	0.02
8	47	C	0.35	40.0	8.2	5.5	2.12	101	4.37	33	0.71
		P	0.43	38.2	8.1	5.2	2.03	8	0.42	5	0.09
9	53	C	1.74	49.4	10.6	6.5	3.04	175	5.47	2	0.03
		P	2.09	54.4	10.3	5.8	3.14	167	5.26	4	0.05
10	53	C	0.60	46.5	12.1	7.1	2.75	434	13.18	5	0.07
		P	0.84	47.5	11.7	6.8	2.62	375	11.62	7	0.10

Duration of diet indicates the number of days on the low phosphate diet. Abbreviations: C = mean of 3–5 control periods collected before infusion of phosphate, P = mean of 3–5 periods collected during and immediately after infusion of a neutral phosphate solution delivering 0.04 mM/min for 60–120 min, Sp = serum phosphorus, C_{Cr} = exogenous creatinine clearance, S_{Ca} = serum calcium, Diff. = diffusible, F_{Ca} = filtered calcium, $U_{Ca}V$ = urinary calcium, C_{Ca} = clearance of diffusible calcium, $U_{Na}V$ = urinary sodium, and C_{Na} = clearance of sodium.

tween the levels of serum calcium and phosphorus in the three T-PTX animals is demonstrated in Fig. 7. The intravenous infusion of 0.04 mmole/min of phosphate into T-PTX, phosphate-depleted dogs caused a significant fall in urinary calcium of 133 ±18 μEq/min ($P < 0.01$) and in the percentage of filtered calcium excreted of 4.37 ±0.75% ($P < 0.01$).

When phosphate was infused directly into a single renal artery, there was no ipsilateral effect on calcium excretion. At a later phase of the experiment, when systemic blood concentration of phosphate rose slightly, the fractional calcium excretion from both kidneys fell proportionately (Table VII).

DISCUSSION

The results of the present study indicate that phosphate depletion in the dog, as in other species (1–4), is associated with increased urinary calcium. The augmented urinary calcium, which was present in most experiments, occurred despite a definite and significant fall in its filtered load, indicating that the tubular reabsorption of calcium is reduced. The observations of the present study further demonstrate that the increase in calcium excretion is not related to changes in the renal excretion of other ions. First, a reduction in the tubular reabsorption of calcium has been shown to occur under circumstances which depress the renal reabsorption of sodium (11–14). In the present study, hypercalciuria occurred in the absence of consistent changes in the renal excretion of sodium. In a few instances, the percentage of filtered sodium excreted was increased during phosphate depletion; however, this change was of a small magnitude and the simultaneous augmentation in calcium excretion was disproportionately greater. Second, urinary calcium also increases with the augmented magnesium excretion caused by magnesium loading (17–20). In the studies carried out in humans by Lotz et al. (1), phosphate depletion was induced using magnesium-aluminum-hy-

TABLE V

Effect of Injections of Parathyroid Extract of Renal Handling of Calcium in Phosphate Depleted Dogs

Dog No.	Duration diet	Period	C_{Cr}	Sp	S_{Ca} Total	S_{Ca} Diff.	F_{Ca}	$U_{Ca}V$	$\frac{C_{Ca}}{C_{Cr}} \times 100$	$U_{Na}V$	$\frac{C_{Na}}{C_{Cr}} \times 100$
	days		*ml/min*	*mg/100 ml*	*mg/100 ml*		*mg/min*	*μg/min*		*μEq/min*	
2	28	Control	54.1	0.20	8.6	5.7	3.30	140	2.45	62	0.78
		PTE	59.7	0.10	8.5	5.7	3.45	99	1.83	137	1.58
3	46	Control	62.3	0.30	10.5	7.2	4.49	82	1.76	2	0.02
		PTE	82.3	0.39	10.8	7.5	6.04	33	0.56	6	0.05
5	63	Control	37.6	1.50	11.3	7.2	2.72	100	3.53	4	0.06
		PTE	58.0	1.98	12.4	8.0	4.62	107	2.19	16	0.19
5	64*	Control	33.8	2.23	10.4	6.9	2.34	42	1.74	4	0.08
		PTE	39.2	2.25	11.0	7.3	2.85	40	1.29	5	0.08
6	115	Control	32.9	0.40	9.5	6.3	2.07	117	5.40	3	0.06
		PTE	41.9	0.36	10.0	6.6	2.76	73	2.58	28	0.46
9	46	Control	47.0	1.70	9.3	6.1	2.90	172	5.88	4	0.11
		PTE	56.9	1.54	9.3	6.2	3.51	181	5.19	9	0.29
10	60	Control	38.1	1.21	9.6	6.7	2.54	110	4.37	1	0.02
		PTE	47.4	0.94	9.6	6.4	3.02	116	3.79	3	0.04

Control indicates the mean of 3–4 periods collected before the injection of parathyroid extract and PTE indicates the mean of 3–4 periods collected after the intramuscular injection of Lilly parathyroid extract, 100 U each hour × 3. Duration of diet indicates the number of days on the low phosphate diet. Abbreviations: C_{Cr} = exogenous creatinine clearance, Sp = serum phosphorus, S_{Ca} = serum calcium, Diff. = diffusible, F_{Ca} = filtered calcium, $U_{Ca}V$ = urinary calcium, C_{Ca} = clearance of diffusible calcium, $U_{Na}V$ = urinary sodium, and C_{Na} = clearance of sodium.

* This study was carried out during a second period of phosphate depletion which followed phosphate repletion.

TABLE VI

Summary of Experiments on the Acute Effects of Thyroparathyroidectomy in Phosphate Depleted Dogs

Dog No.	Period	C_{Cr}	Sp	S_{Ca} Total	S_{Ca} Diff.	F_{Ca}	$U_{Ca}V$	$\frac{C_{Ca}}{C_{Cr}} \times 100$	$U_{Na}V$	$\frac{C_{Na}}{C_{Cr}} \times 100$
		ml/min	*mg/100 ml*	*mg/100 ml*		*mg/min*	*μg/min*		*μEq/min*	
5	Control	42.3	0.75	11.1	7.5	3.18	428	12.45	51	0.90
	PTX	58.6	1.01	11.0	7.2	4.20	263	6.13	125	1.67
7	Control	65.4	1.13	7.8	5.0	3.29	49	2.09	3	0.04
	PTX	26.2	3.00	7.3	5.0	1.32	2	0.17	1	0.05
8	Control	54.6	1.08	8.9	6.1	3.33	175	5.21	15	0.22
	PTX	71.8	1.00	8.8	6.0	4.28	121	2.84	40	0.47
9	Control	66.4	2.44	8.3	5.7	3.80	163	4.50	19	0.20
	PTX	86.1	2.57	7.2	4.9	4.19	68	1.56	86	0.75
10	Control	44.9	0.93	11.0	7.3	3.30	585	19.80	133	2.23
	PTX	48.4	0.87	11.3	7.6	3.68	760	20.40	97	1.53

Control represents the mean of 3–5 periods carried out under anesthesia before thyroparathyroidectomy; PTX represents the mean of 5–8 clearance periods collected between 2 and 4½ hr after complete removal of the parathyroid gland. Abbreviations: C_{Cr} = exogenous creatinine clearance, Sp = serum phosphorus, S_{Ca} = serum calcium, Diff. = diffusible, F_{Ca} = filtered calcium, $U_{Ca}V$ = urinary calcium, C_{Ca} = clearance of diffusible calcium, $U_{Na}V$ = urinary sodium, C_{Na} = clearance of sodium.

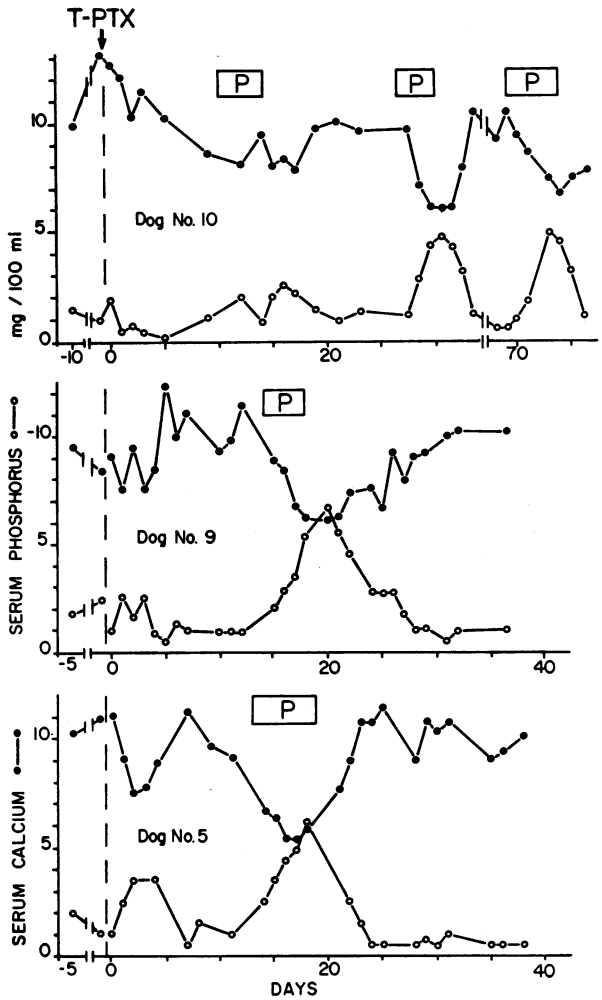

FIGURE 6 Chronological alterations in serum calcium and phosphorus in three thyroparathyroidectomized (T-PTX) phosphate-depleted dogs. P indicates the period when oral phosphate, 1 g/day, was added to the regimen.

droxide, raising the possibility that the increased magnesium intake might underlie the hypercalciuria. In the present study, the phosphate-binding antacid contained no magnesium, and dietary magnesium intake was the same during control periods and phosphate depletion. Magnesium excretion was increased in some of the present experiments during phosphate depletion; this change is most likely due to the same mechanisms which produce hypercalciuria rather than a cause of increased urinary calcium. Third, an increase in complexing anions is apparently not responsible for the hypercalciuria since the change in urinary calcium concentration was unrelated to citrate concentration and since acetoacetate and acetone were always absent from the urine. Although urinary sulphate concentration was not measured, it is extremely unlikely that this ion could appear spontaneously in the urine in a concentration large enough to cause this degree of hypercalciuria, e.g. with 5–12% of filtered calcium excreted. In addition, these studies were carried out during water diuresis with large volumes of dilute urine, a situation which minimizes the effect of complexing anions (11).

The state of phosphate depletion might produce decreased tubular reabsorption of calcium either by a direct effect on the kidney or by triggering extrarenal mechanisms that may affect calcium reabsorption. The results of the present study indicate that the first possibility is remote, since a small amount of phosphate reduced calcium excretion when given intravenously while the direct infusion of phosphate into a single renal artery failed to produce an ipsilateral effect.

Among the known extrarenal or humoral factors that might cause an increase in urinary calcium are starvation (36), a carbohydrate load (37), acidosis (38), an excess of mineralocorticoids (28, 29), and a decrease in the activity of the parathyroid glands. The caloric intake during phosphate depletion was adequate and the animals maintained stable body weights; observations which exclude starvation as a cause of the hypercalciuria. The small quantity of dextrose (3–6 g/hr) administered to both normal and phosphate-depleted dogs, failed to cause hypercalciuria in the normal animals. Although a careful evaluation of acid-base status was not carried out, chronic acidosis does not seem likely since urine pH remained high in both the normal and phosphate-depleted state; and when arterial blood pH and P_{CO_2} were measured during studies carried out under anesthesia, no evidence for metabolic acidosis was found. Hypercalciuria occurs after the long-term effect of an excess of mineralocorticoids as a result of expansion of extracellular fluid volume secondary to cumulative sodium retention (39). The persistence of hypercalciuria when phosphate-depleted animals received a salt-free diet would vitiate the possibility that expansion of extracellular fluid volume due to an excess of mineralocorticoids could cause the hypercalciuria.

A reduction in the activity of the parathyroid glands leads to a decreased renal tubular reabsorption of calcium (24). There are several features of phosphate depletion which suggest that the activity of the parathyroid glands is inhibited. It has been shown that hypoplasia of these glands occurs in rats after the intake of a diet deficient in its phosphate content (40). During phosphate restriction, inhibition of the parathyroid glands is probably caused by the elevation of serum calcium, a feature which has been clearly observed in certain animal models of phosphate depletion (30, 31) and was transiently noted in certain dogs in the present study. The presence of a normal or slightly elevated serum calcium level does not militate against hypo-

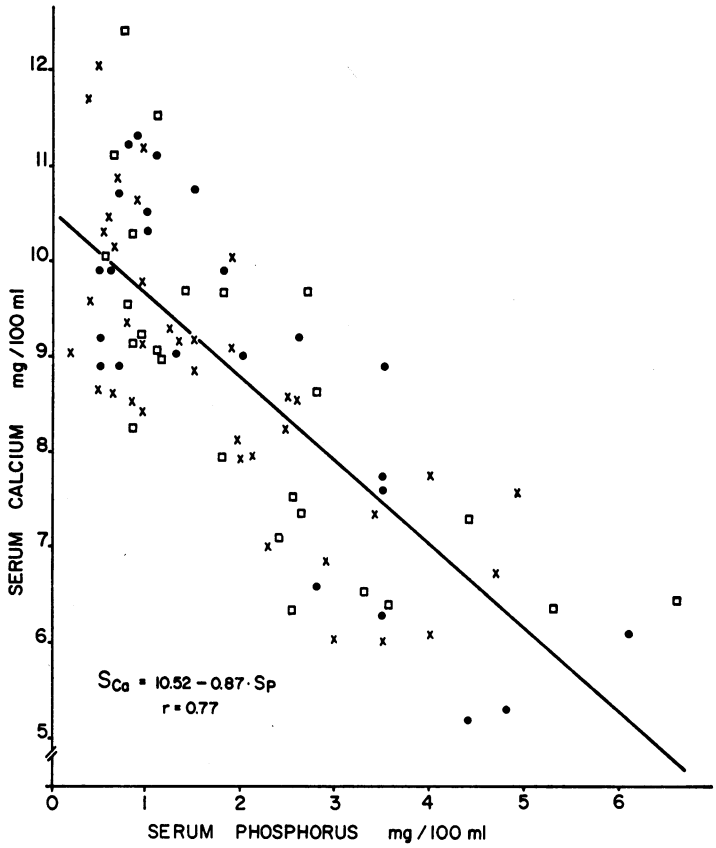

FIGURE 7 The relation between all values of serum calcium and phosphorus concentrations in three thyroparathyroidectomized, phosphate-depleted dogs which were observed as serum phosphorus changed, either spontaneously or after oral supplementation of phosphate. Each symbol represents a different dog. S_{Ca} indicates serum calcium and S_p, serum phosphorus.

function of the parathyroid glands. Indeed, our observations that the levels of serum calcium were elevated or normal after thyroparathyroidectomy as long as marked hypophosphatemia persisted indicate that parathyroid hormone is neither responsible for the hypercalcemia nor is it required for the maintenance of a normal serum calcium concentration in the phosphate-depleted state. This concept is supported by the work of Shikita, Tsurufuji, and Ito (31) who found that serum calcium was slightly elevated in phosphate-depleted rats both before and after parathyroidectomy, and of Lotz et al. (1) who observed an increase of blood calcium to normal levels in hypoparathyroid humans during phosphate depletion. Furthermore, the observations in T-PTX, phosphate-depleted dogs that serum calcium levels changed abruptly with small reciprocal alterations of the already low concentrations of phosphorus suggest that calcium may move to and from extracellular fluid in response to blood phosphate levels with a calcium-phosphorus molar product which is well below the solubility product presumed to exist under physiological conditions (41).

The lack of an acute rise in fractional calcium excretion after thyroparathyroidectomy in phosphate-depleted dogs and the maintenance of a similar degree of hypercalciuria before and after removal of the parathyroid glands are consistent with the concept that inhibition of the parathyroid glands is an important mechanism responsible for the hypercalciuria. However, other factors may also be operative: although the administration of parathyroid extract reduced fractional excretion of calcium, PTE failed to reduce the latter to the levels observed in the same dogs receiving the normal diet. Glomerular filtration rate and, hence, the filtered load of calcium increased after the administration of PTE, but it seems improbable that this acute augmentation of GFR and filtered calcium could account for the persistent hypercalciuria after PTE, since it has been shown that a similar increase in filtered calcium after

TABLE VII

An Illustrative Experiment Demonstrating the Effect of the Direct Infusion of Phosphate into the Left Renal Artery in a Phosphate Depleted Dog

Time	V		C_{Cr}			S_{Ca}		F_{Ca}		$U_{Ca}V$		$\frac{C_{Ca}}{C_{Cr}} \times 100$		$U_{Na}V$		$\frac{C_{Na}}{C_{Cr}} \times 100$	
	E	C	E	C	Sp	Total	Diff.	E	C	E	C	E	C	E	C	E	C
min	*ml/min*		*ml/min*		*mg/100 ml*	*mg/100 ml*		*mg/min*		*μg/min*				*μEq/min*			
Dog No. 6																	
−290	Water load, 500 ml by gastric tube																
−230	Anesthetized with pentobarbital																
−220	Solution No. 1, 2.5% dextrose in water given intravenously at a rate equal to urine flow																
−220−−90	Both ureters catheterized by a suprapubic incision and left renal artery and kidney exposed via a flank incision																
−80	Needle placed in left renal artery and solution II, 0.85% NaCl, infused at 0.066 ml/min																
−70	Creatinine prime, 800 mg, and solution III, delivering creatinine at 6 mg/min in 2.5% dextrose in water given at 1.0 ml/min																
0–12	1.13	1.07	16.0	17.9	0.64	8.1	5.0	0.81	0.89	77	72	9.60	8.00	23	19	1.24	0.95
12–30	1.29	1.17	18.4	16.5	0.59	8.1	5.0	0.93	0.90	82	67	9.31	8.10	25	20	1.18	1.05
30–45	2.20	2.16	17.1	16.5	0.55	8.2	5.0	0.86	0.83	64	67	7.34	8.06	18	20	0.93	1.07
45	Solution II replaced by solution IV, containing neutral sodium phosphate (0.30 mM/ml) in 0.85% NaCl, and given at 0.066 ml/min into the left renal artery to deliver 0.02 mM phosphate/min																
45–55	1.59	1.47	17.5	17.1	0.61	8.1	4.9	0.86	0.84	59	57	6.88	6.80	19	17	0.94	0.87
55–65	0.98	0.89	17.3	17.2	0.72	8.2	5.0	0.83	0.83	41	43	4.93	5.23	15	16	0.79	0.83
65–75	0.47	0.39	18.9	17.8	0.76	8.3	5.1	0.96	0.91	40	39	4.15	4.30	17	16	0.78	0.78
75–85	0.85	0.79	20.9	17.9	0.77	8.2	5.0	1.00	0.90	37	37	3.66	4.13	16	13	0.69	0.72
85–105	1.30	1.12	17.6	17.9	0.87	8.1	5.0	0.88	0.91	37	41	4.09	4.48	18	18	0.92	0.89
105–125	0.93	0.81	16.0	15.6	1.10	8.0	4.9	0.80	0.78	26	27	3.18	3.50	16	14	0.88	0.79
125–145	0.42	0.34	18.0	18.8	1.20	8.0	4.9	0.88	0.92	23	21	2.63	2.22	14	12	0.72	0.60

Abbreviations: V = urine volume, E = experimental kidney (left), C = control kidney (right).

an acute rise in GFR has little effect on urinary calcium (42). In addition, Pronove, Bell, and Bartter (43) reported that an increase in calcium excretion occurs in patients with primary hyperparathyroidism after phosphate deprivation. These observations and the acute reduction in urinary calcium after the infusion of small quantities of phosphate to intact or T-PTX phosphate-depleted dogs indicate that a decrease in parathyroid activity cannot be implicated as the sole determinant of alterations of urinary calcium.

It is possible that the renal handling of calcium may be affected by events occurring in bone as a result of phosphate depletion. Among the principal features of the latter condition are negative calcium balance and reduction in bone mineral content (44); and although experimental studies have generally demonstrated osteomalacia in growing animals during phosphate depletion (30), Lotz, Ney, and Bartter (2) showed an increase in bone resorption as measured by isotopic techniques. Furthermore, Rasmussen, Anast, and Arnaud (45) have offered experimetnal support for the original view of Albright and Reifenstein (46) that a fall in blood calcium or phosphorus may enhance reabsorption of bone in the absence of parathyroid hormone. Rasmussen and coworkers (45) also demonstrated that increased resorption of bone produced by an acute decrease in the ionized calcium concentration of blood was associated with phosphaturia in the absence of the parathyroid glands and despite a fall in the level of blood phosphorus. It is reasonable to postulate that events associated with an increase in bone reabsorption produced by hypophosphatemia might contribute to increased urinary calcium excretion during phosphate depletion. The nature of such possible events are not elucidated by the present study.

An increase in absorption of calcium in the gastrointestinal tract may be associated with augmented renal calcium excretion. Controversy exists concerning the effect of phosphate depletion on calcium absorption; thus, increased absorption has been reported to occur in some humans (1) while a reduction in calcium absorption has been noted in rats (4) and sheep (47). Freeman and McLean (30) suggested that calcium absorption may be increased in puppies during phosphate depletion, but they did not provide evidence to support this. Even if enhanced gastrointestinal calcium absorption occurs during phosphate depletion, this may only partly account for the hypercalciuria since both in humans (1) and animals (4, 44, 48) there is markedly negative calcium balance.

The demonstration that glomerular filtration rate fell in each animal with phosphate depletion and returned to control levels after phosphate repletion deserves special comment. Lotz et al. (1) reported no change in inulin clearance after phosphate depletion in two patients; however, their serum phosphorus levels were normal, which may indicate that phosphate depletion was only mild. In a patient studied in our laboratory

(unpublished data) endogenous creatinine clearance fell significantly during phosphate depletion and increased promptly with phosphate repletion. The mechanism(s) responsible for the reduction in glomerular filtration rate are not clear. It is unlikely that structural damage to the kidney due to transient hypercalcemia or persistent hypercalciuria, per se, is responsible. Chronic impairment of renal function due to hypercalcemia (49) or hypercalciuria (50) is associated with deposition of calcium in the kidney and often with areas of scarring. Histological evaluation of the kidneys from the phosphate-depleted dogs of the present study was entirely normal, and the calcium contents of both renal cortex and medulla were normal in the two animals where these measurements were made. The return of GFR to normal with phosphate repletion further supports a functional nature of this abnormality. Alterations in renal hemodynamics and/or cardiac output possibly produced by phosphate depletion, per se, may provide an alternative explanation.

ACKNOWLEDGMENTS

Technical support was provided by Miss Sybil Gordon, John Steppe, and Howard Davidson and secretarial assistance by Miss Catherine Weckesser and Mrs. Shirley Lant.

This work was supported in part by U. S. Public Health Service Training Grant TI AM 5383.

REFERENCES

1. Lotz, M., E. Zisman, and F. C. Bartter. 1968. Evidence for a phosphorus-depletion syndrome in man. *N. Engl. J. Med.* **278**: 409.
2. Lotz, M., R. Ney, and F. C. Bartter. 1964. Osteomalacia and debility resulting from phosphorus depletion. *Trans. Ass. Amer. Physicians Philadelphia* **77**: 281.
3. Young, V. R., G. P. Lofgreen, and J. R. Luick. 1966. The effects of phosphorus depletion and of calcium and phosphorus intake, on the endogenous excretion of these elements by sheep. *Brit. J. Nutr.* **20**: 795.
4. Day, H. G., and E. V. McCollum. 1939. Mineral metabolism, growth, and symptomatology of rats on a diet extremely deficient in phosphorus. *J. Biol. Chem.* **130**: 269.
5. Albright, F., W. Bauer, D. Claflin, and J. R. Cockrill. 1932. Studies in parathyroid physiology. III. The effect of phosphate ingestion in clinical hyperparathyroidism. *J. Clin. Invest.* **11**: 411.
6. Bernstein, D. S., and R. Newton. 1966. The effect of oral sodium phosphate on the formation of renal calculi and on idiopathic hypercalcuria. *Lancet.* **2**: 1105.
7. Hebert, L. A., J. Lemann, Jr., J. R. Petersen, and E. J. Lennon. 1966. Studies of the mechanism by which phosphate infusion lowers serum calcium concentration. *J. Clin. Invest.* **45**: 1886.
8. Massry, S. G., E. Mueller, A. G. Silverman, and C. R. Kleeman. 1968. Inorganic phosphate treatment of hypercalcemia. *Arch. Intern. Med.* **121**: 307.
9. Coburn, J. W., and S. G. Massry. 1969. The role of chronic changes in phosphate intake and acute phosphate infusion on the relationship between renal handling of calcium and sodium. *Clin. Res.* **17**: 129.
10. Lassiter, W. E., C. W. Gottschalk, and M. Mylle. 1963. Micropuncture study of renal tubular reabsorption of calcium in normal rodents. *Amer. J. Physiol.* **204**: 771.
11. Walser, M. 1961. Calcium clearance as a function of sodium clearance in the dog. *Amer. J. Physiol.* **200**: 1099.
12. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. 1967. Effect of NaCl infusion on urinary Ca^{++} and Mg^{++} during reduction in their filtered loads. *Amer. J. Physiol.* **213**: 1218.
13. Blythe, W. B., H. J. Gitelman, and L. G. Welt. 1968. Effect of expansion of the extracellular space on the rate of urinary excretion of calcium. *Amer. J. Physiol.* **214**: 52.
14. Wesson, L. G., Jr. 1962. Magnesium, calcium and phosphate excretion during osmotic diuresis in the dog. *J. Lab. Clin. Med.* **60**: 422.
15. Duarte, C. G. 1967. Effects of Furosemide and Ethacrynic acid on the renal clearance of phosphate, ultrafilterable calcium and magnesium. *Clin. Res.* **15**: 357.
16. Parfitt, A. M. 1969. The acute effects of mersalyl, chlorothiazide and mannitol on the renal excretion of calcium and other ions in man. *Clin. Sci. (London).* **36**: 267.
17. Barker, E. S., J. R. Elkinton, and J. K. Clark. 1959. Studies of the renal excretion of magnesium in man. *J. Clin. Invest.* **38**: 1733.
18. Massry, S. G., J. W. Coburn, and C. R. Kleeman. 1969. Renal handling of magnesium in the dog. *Amer. J. Physiol.* **216**: 1460.
19. Briscoe, A. M., and C. Ragan. 1966. Effect of magnesium on calcium metabolism in man. *Amer. J. Clin. Nutr.* **19**: 296.
20. Heaton, F. W., A. Hodgkinson, and G. A. Rose. 1964. Observations on the relation between calcium and magnesium metabolism in man. *Clin. Sci. (London).* **27**: 31.
21. Chen, P. S., Jr., and W. F. Neuman. 1955. Renal excretion of calcium by the dog. *Amer. J. Physiol.* **180**: 623.
22. Walser, M., and A. A. Browder. 1959. Ion association. III. The effect of sulphate infusion on calcium excretion. *J. Clin. Invest.* **38**: 1404.
23. Bernstein, D., C. R. Kleeman, R. E. Cutler, J. T. Dowling, and M. H. Maxwell. 1962. Comparison of renal clearance of calcium during infusions of calcium gluconate. *Proc. Soc. Exp. Biol. Med.* **110**: 671.
24. Kleeman, C. R., D. Bernstein, R. Rockney, J. T. Dowling, and M. H. Maxwell. 1961. Studies on the renal clearance of diffusible calcium and the role of the parathyroid glands on its regulation. *Yale J. Biol. Med.* **34**: 1.
25. Widrow, S. H., and N. G. Levinsky. 1962. The effect of parathyroid extract on renal tubular calcium reabsorption in the dog. *J. Clin. Invest.* **41**: 2151.
26. Bethune, J. E., R. A. Turpin, and H. Inoue. 1968. Effect of parathyroid extract on divalent ion excretion in man. *J. Clin. Endocrinol. Metab.* **28**: 673.
27. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. 1968. Role of serum Ca, parathyroid hormone, and NaCl infusion on renal Ca and Na Clearances. *Amer. J. Physiol.* **214**: 1403.
28. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. 1968. The effect of long-term desoxycorticosterone acetate administration on the renal excretion of calcium and magnesium. *J. Lab. Clin. Med.* **71**: 212.
29. Suki, W. N., R. S. Schwettmann, F. C. Rector, Jr., and D. W. Seldin. 1968. Effect of chronic mineralocorticoid

administration on calcium excretion in the rat. *Amer. J. Physiol.* **215**: 71.
30. Freeman, S., and F. C. McLean. 1941. Experimental rickets. Blood and tissue changes in puppies receiving a diet very low in phosphorus, with and without vitamin D. *Arch. Pathol.* **32**: 387.
31. Shikita, M., S. Tsurufuji, and Y. Ito. 1962. Adaptation in renal phosphorus excretion under the influence of parathyroids; a study of ureterally catheterized rats. *Endocrinol. Jap.* **9**: 171.
32. Manitius, A., H. Levitin, D. Beck, and F. H. Epstein. 1960. On the mechanism of impairment of renal concentrating ability in potassium deficiency. *J. Clin. Invest.* **39**: 684.
33. Zettner, A., and D. Seligson. 1964. Application of atomic absorption spectrophotometry in the determination of calcium in serum. *Clin. Chem.* **10**: 869.
34. Beutler, E., and M. K. Y. Yeh. 1959. A simplified method for the determination of citric acid. *J. Lab. Clin. Med.* **54**: 125.
35. Lavietes, P. H. 1937. Anaerobic ultrafiltration. *J. Biol. Chem.* **120**: 267.
36. Bell, N. H. 1969. Observations concerning the effects of fasting on collagen metabolism in man. *J. Clin. Endocrinol. Metab.* **29**: 338.
37. Lemann, J., Jr., W. F. Piering, and E. J. Lennon. 1969. Possible role of carbohydrate induced calciuria in calcium oxalate kidney-stone formation. *N. Engl. J. Med.* **280**: 232.
38. Lemann, J., Jr., J. R. Litzow, and E. J. Lennon. 1967. Studies of the mechanism by which chronic metabolic acidosis augments urinary calcuim excretion in man. *J. Clin. Invest.* **46**: 1318.
39. August, J. T., D. H. Nelson, and G. W. Thorn. 1958. Response of normal subjects to large amounts of aldosterone. *J. Clin. Invest.* **37**: 1549.
40. Stoerk, H. C., and W. H. Carnes. 1945. The relation of the dietary Ca: P ratio to serum Ca and to parathyroid volume. *J. Nutr.* **29**: 43.
41. Neuman, W. F., and M. W. Neuman. 1958. The Chemical Dynamics of Bone Mineral. University of Chicago Press, Ill. 23.
42. Massry, S. G., and C. R. Kleeman. 1969. Effect of increase in glomerular filtration rate with and without extracellular volume expansion on urinary calcium and magnesium. *Clin. Res.* **17**: 439.
43. Pronove, P., N. H. Bell, and F. C. Bartter. 1961. Production of hypercalciuria by phosphorus deprivation on a low calcium intake: a new clinical test for hyperparathyroidism. *Metab. (Clin. Exp.).* **10**: 364.
44. Jones, J. H. 1938. The metabolism of calcium and phosphorus as influenced by the addition to the diet of salts of metals which form insoluble phosphates. *Amer. J. Physiol.* **124**: 230.
45. Rasmussen, H., C. Anast, and C. Arnaud. 1967. Thyrocalcitonin, EGTA and urinary electrolyte excretion. *J. Clin. Invest.* **46**: 746.
46. Albright, F., and E. C. Reifenstein, Jr. 1948. The parathyroid glands and metabolic bone disease. Williams & Wilkins Co., Baltimore, Md. 13.
47. Young, V. R., W. P. C. Richards, G. P. Lofgreen, and J. R. Luick. 1966. Phosphorus depletion in sheep and the ratio of calcium to phosphorus in the diet with reference to calcium and phosphorus absorption. *Brit. J. Nutr.* **20**: 783.
48. Young, V. R., J. R. Luick, and G. P. Lofgreen. 1966. The influence of dietary phosphorus intake on the rate of bone metabolism in sheep. *Brit. J. Nutr.* **20**: 727.
49. Carone, F. A., F. H. Epstein, D. Beck, and H. Levitin. 1960. The effects upon the kidney of transient hypercalcemia induced by parathyroid extract. *Amer. J. Pathol.* **36**: 77.
50. Sanderson, P. H. 1959. Functional aspects of renal calcification in rats. *Clin. Sci. (London).* **18**: 67.