Renal Tubular Effects of Chronic Phosphate Depletion

STANLEY GOLDFARB, GEORGE R. WESTBY, MARTIN GOLDBERG, and ZALMAN S. AGUS

From the Renal Electrolyte Section, Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT The effects of chronic phosphate depletion on renal tubular function were evaluated by micropuncture and free water clearance studies in the dog. Proximal tubular punctures demonstrated that chronic hypophosphatemia led to a reduction in ratio of tubular fluid to plasma inulin in late superficial tubules from 1.59±0.08 in control animals to 1.29±0.06 in phosphate-depleted dogs, with proportional inhibition of calcium and sodium reabsorption. The chronic decrease in proximal tubular fluid reabsorption was confirmed by the analysis of sustained water diuresis in conscious, phosphate-depleted dogs, before and after repletion of body PO_4 stores, and in control animals. Urine flow rate/100 ml glomerular filtration rate (V/GFR) was significantly higher in PO_4 depletion than control (15.8±1.1 vs. 10.7±0.82). In addition, acetazolamide infusion did not increase V/GFR in phosphate-depleted dogs (15.8±1.1 vs. 17.16±0.9), supporting the conclusion that inhibition of proximal tubular fluid reabsorption was responsible for the elevated urine flow rate. PO_4 repletion over 5 days reduced V/GFR to 9.2±0.7 despite no change in urine osmolality and no change in GFR, further suggesting a specific reversible alteration in proximal tubular reabsorption in phosphate depletion.

Although hypercalciuria was a constant finding in phosphate depletion (fractional excretion of calcium of 2.04±0.4% vs. 0.47±0.13% in controls), the enhanced distal delivery of calcium was not a crucial factor; acute phosphate infusion reduced urinary calcium excretion to control values without affecting the reduced proximal tubular reabsorption in either intact or thyroparathyroidectomized phosphate-depleted dogs. The change in distal nephron calcium reabsorption was independent of parathyroid hormone (PTH) levels since infusion of PTH failed to alter urinary calcium excretion.

We conclude that chronic phosphate depletion leads to a reversible, sustained inhibition in proximal tubular reabsorptive function as well as a specific decrease in distal nephron calcium reabsorption. This latter reabsorptive defect is sensitive to phosphate infusion but not corrected by PTH.

INTRODUCTION

Chronic phosphate depletion produces alterations in function of many different organ systems. Previous studies have documented a series of disturbances in renal tubular function including a marked hypercalciuria (1–3), as well as lowered tubular maximum (Tm)[1]HCO_3 (4), and Tm glucose (5). Although these observations remain controversial, they are suggestive of disturbances in proximal tubular reabsorption. The intrarenal mechanism and sites of the altered calcium transport in this syndrome have not yet been clarified. The hypercalciuria could result from either a specific inhibitory effect of phosphate depletion upon calcium transport in the distal nephron, or from a generalized, nonspecific decrease in proximal tubular reabsorption which would in turn produce a delivery of calcium in excess of distal nephron reabsorptive capacity. The presumed absence of parathyroid hormone in this syndrome could produce increased calcium excretion either alone or in combination with alteration in tubular calcium transport.

The present series of experiments in chronically phosphate-depleted dogs was designed to investigate these problems by first characterizing the state of proximal tubular reabsorption and then evaluating the

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[1] *Abbreviations used in this paper*: CH_2O, free water clearance; Cosm, osmolar clearance; CPD, chronic phosphate depletion; GFR, glomerular filtration rate; PTH, parathyroid hormone; TF/P, ratio of tubular fluid to plasma; TF/UF, ratio of tubular fluid to ultrafilterable plasma; Tm, tubular maximum; TPTX, thyroparathyroidectomized; V, urine flow rate (ml/min).

roles of distal nephron calcium delivery and parathyroid hormone secretion in the hypercalciuria of phosphate depletion.

METHODS

Female mongrel dogs weighing between 9 and 11 kg were studied either with micropuncture techniques or during sustained water diuresis in the conscious state as follows.

Micropuncture studies

Hydropenic fasted animals were anaesthetized with 20 mg/kg intravenous sodium pentobarbital and received supplemental doses as required. The animals were intubated and ventilated with a Harvard respirator (Harvard Apparatus Co., Inc., Millis, Mass.). Surgical preparation of the animals for clearance and micropuncture studies was performed as previously described from this laboratory (6). Priming doses of [^{3}H]inulin, 100 μCi/kg were given followed by sustaining infusions of inulin in 0.9% saline at a rate of 0.5 ml/min. Three to six late proximal tubules were selected for puncture using Lissamine Green transit time. The last appearance of dye in tubular convolutions was taken as the end point. Punctures were obtained over a 60-min period beginning 30–45 min after Lissamine Green injection.

Study I. Effects of chronic phosphate depletion

To characterize proximal tubular function, three groups of animals were studied as described above. To avoid systematic methodological errors, animals in the following three groups were studied randomly.

Group I. Seven dogs who had been maintained on standard laboratory show for at least 1 wk served as a control for the micropuncture procedure itself.

Group II. 10 animals were studied after 6–8 wk of phosphate depletion produced by being fed 250 g/day of a basal diet and 100 ml/day of aluminum hydroxide gel. The basal diet, (ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio) modified from Coburn and Massry (1), contained less than 50 mg phosphate/kg of diet. Animals which did not spontaneously feed underwent gastric intubation. Serum phosphate was determined to be 1.5 mg/dl or lower in all animals 1 wk before their selection for study.

Group III. Seven animals received the basal low-phosphate diet which was supplemented with 100 mg/day of neutral sodium phosphate (phospho-soda, C. B. Fleet Co., Inc., Lynchburg, Va.) for 4–6 wk. This group served as a control for any effects of chronic administration of the basal diet on renal function.

Study II. Effects of acute phosphate infusion

To determine the mechanisms by which acute phosphate repletion corrects the hypercalciuria of phosphate depletion, three groups of animals were studied. After collections of tubular fluid and initial whole kidney clearance determination as described above, an intravenous infusion of neutral sodium phosphate (Na_2HPO_4:NaH_2PO_4, 4:1, pH 7.4, 300 mosmol/kg) was begun at a rate of 33.6 μmol/0.32 ml per min and continued for 75 min. Recollection of tubular fluid was then performed as previously described (7) and repeat clearance studies were obtained while continuing the phosphate infusion. The following groups were studied:

Group IV. Six chronically phosphate-depleted animals prepared as described above.

Group V. Four chronically phosphate-depleted animals acutely thyroparathyroidectomized 90–120 min before beginning micropuncture studies.

Group VI. Six normal animals, maintained on a standard diet, were acutely thyroparathyroidectomized and then studied to serve as a control for the effects of PO_4 infusion.

Sustained water diuresis studies in conscious animals

To relate observations made during the above series of micropuncture studies to whole kidney function, a series of studies during sustained water diuresis was performed in conscious dogs after phosphate depletion, chronic phosphate repletion, and parathyroid hormone administration. A series of similar studies was performed in normal diet dogs as controls.

Animals were fasted overnight but allowed free access to water. The next morning, a no. 18 French intravenous catheter was placed in the external jugular vein for blood sampling and a no. 20 French intravenous catheter was inserted into a cephalic vein for infusion of solution. The urinary bladder was catheterized with a no. 12 French Foley catheter. The animals were then given 20 ml/kg body wt of tap water by orogastric tube, and infusions of inulin in 0.9% saline were begun at a rate of 0.5 ml/min with concentrations of inulin calculated to achieve plasma levels of approximately 25 mg/dl. A solution of 2.5% dextrose was then begun at a rate of 5 ml/min for 60 min and was adjusted to match urine flow rate. When urine flow was stable (urine flow rate for three consecutive 10-min collection periods within ±10%) and urine osmolality was less than 60 mosmol/kg, three 20-min collections of urine were obtained. After the 60-min control period, each animal received an infusion of 5 mg/kg acetazolamide over 5 min and another three 20-min collections were performed. Blood samples for insulin, sodium, calcium, phosphate, and osmolality were obtained at 30-min intervals before, during, and at the conclusion of the study. Two basic protocols were used.

Study III. Effects of chronic phosphate depletion and chronic repletion

Group VII. Four normal dogs maintained on standard laboratory chow for 2 wk before study were studied during sustained water diuresis and acetazolamide infusion as described. After completion of this study, the animals were maintained on a normal diet for 5–7 days and then restudied using the same protocol. This group provides normal values for this study and also serves as a control for stability over a 1-wk period.

Group VIII. Four dogs, chronically phosphate depleted as described above were studied during water diuresis. After completion of the studies, each animal was administered 1 g of phosphate as neutral sodium phosphate for 5 days while maintained on the basal low PO_4 diet. After repletion of phosphate, these animals were restudied during water diuresis and acetazolamide infusion.

Study IV. Effects of PTH infusion

Group IX. Six chronically phosphate-depleted dogs underwent sustained water diuresis as described above, although a urine osmolality of less than 60 mosmol/kg was not a prerequisite for completion of these studies. After

TABLE I

*Clearance and Micropuncture Data in Phosphate-Depleted and Control Dogs**

	Clearance						Proximal tubule				
	C_{In}	UF_{PO_4}	$UF_{Ca^{++}}$	C_{Na}/C_{In}	C_{Ca}/C_{In}	C_{PO_4}/C_{In}	TF/P_{In}	TF/P_{Na}	$TF/UF_{Ca^{++}}$	FR_{Na}	FR_{Ca}
	ml/min	*mg/dl*	*meq/liter*		%						%
Group I, normal diet controls, $n = 7$											
Mean	16.1	4.7	2.99	0.28	0.47	3.23	1.59	0.98	0.98	34	37
SEM	2.1	0.5	0.88	0.13	0.13	0.55	0.08	0.03	0.04	3	5
Group II, CPD, $n = 16$											
Mean	13.0	1.2	2.92	0.50	2.04	0.38	1.29	0.99	1.05	17	16
SEM	0.9	0.2	0.03	0.11	0.44	0.14	0.06	0.06	0.04	4	6
P value	NS	<0.01	NS	<0.02	<0.02	<0.025	<0.01	NS	NS	<0.01	<0.01
Group III, supplemented low phosphate diet, $n = 7$											
Mean	16.0	7.0	3.07	0.11	0.44	7.14	1.55	0.99	1.06	34	32
SEM	1.5	0.5	0.02	0.02	0.08	3.7	0.06	0.05	0.05	2	3
P value	NS	<0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS

* C_{In}, C_{Na}, C_{Ca}, C_{PO_4}, clearance of inulin, sodium, calcium and phosphate in the micropuncture kidney respectively; C_{Na}/C_{In}, C_{Ca}/C_{In}, and C_{PO_4}/C_{In}, fractional excretion of sodium, calcium and phosphate. UF_{PO_4} and $UF_{Ca^{++}}$, concentration of plasma ultrafilterable calcium and phosphate, respectively. TF/P_{In} and TF/P_{Na} refer to ratio of tubular fluid to plasma inulin and sodium concentrations and $TF/UF_{Ca^{++}}$, the ratio of tubular fluid to plasma ultrafilterable calcium. FR, fractional sodium and calcium reabsorption to the point of puncture in the proximal tubule calculated as $1 - (TF/P_{Na})/(TF/P_{In})$ and $1 - (TF/UF_{Ca^{++}})/(TF/P_{In})$. *P* value refers to the significance of the difference of the mean when compared to group I.

three 20-min control collection periods during a steady state, highly purified parathyroid hormone (Wilson Laboratories, Chicago, Ill., 1,100 U/mg) was infused at a rate of 60 U/h, and 20-min urine collections were continued for 120 min.

Analytical techniques

Urine inulin, sodium, phosphate, and calcium, and serum ultrafiltrable calcium, phosphate, inulin, and sodium were measured as previously described (7). [^{3}H]Inulin activity in tubular fluid, urine, and plasma was determined in a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) (6). Tubular fluid concentration of sodium and calcium was determined by electron microprobe analysis as previously described (6). Osmolality was measured with an osmometer (Osmette Precision Systems, Inc., Sudbury, Mass.).

The clearances of inulin, calcium, sodium, and phosphate were calculated in the usual manner. Osmolar clearance (Cosm) was calculated with the following formula: Cosm = Uosm × V/Posm where Uosm = osmolality of the urine (milliosmoles per kilogram of water), Posm = osmolality of the serum (milliosmoles per kilogram of water), and V = urine flow rate (milliliters per minute). Free water clearance (CH_2O) was calculated from the following formula: $CH_2O = V - Cosm$.

Statistical analyses were performed utilizing Student's *t* test for paired or nonpaired variables where appropriate.

RESULTS

Micropuncture studies

Effects of chronic phosphate depletion. The results of the clearance and micropuncture data from groups I–III are depicted in Table I. Since the control collection period of group II and IV animals represented studies in identically prepared animals, the control period of these two groups were pooled for purposes of comparison to normophosphatemic animals. Chronic phosphate depletion (group II) produced significant hypophosphatemia ($P_{PO_4} = 1.24 \pm 0.21$ mg/dl) and an increase in fractional calcium excretion to 2.04±0.44% whereas both glomerular filtration rate and plasma ultrafiltrable calcium were unchanged compared to normophosphatemic animals in groups I and III. There was also a slight increase in fractional excretion of sodium to 0.50±0.11%. Fractional excretion of phosphate was reduced in phosphate-depleted animals to 0.38±.14% of filtered phosphate compared to either group I or II animals. In the proximal tubule, ratio of tubular fluid to plasma (TF/P)inulin was markedly reduced during phosphate depletion to 1.29±0.06 compared to 1.59±0.08 in group I (Fig. 1) whereas ratio of tubular fluid to ultrafilterable plasma (TF/UF) Ca and TF/P Na were not different from the control group. Therefore, chronic phosphate depletion reduced the percentage of fractional reabsorption of sodium (17±4%) and calcium (16±6%) in proportion to the decrease in fluid transport in the proximal tubule. A comparison of the data from groups I and III revealed that other than an increase in serum phosphate, there were no effects of the low phosphate diet with phosphate supplement. Thus, whereas serum phosphate rose to 7.0±0.52 mg/dl in group III dogs, there were no differences either in the clear-

ances of inulin, sodium, calcium, or phosphate, or in the proximal tubular reabsorption of sodium, water, or calcium between the two normophosphatemic groups.

Effects of acute phosphate infusion. The data from the recollection micropuncture experiments in groups IV–VI are presented in Table II, as the mean ±SEM of data before (control) and after infusion of neutral sodium phosphate (recollection). Acute intravenous infusion of phosphate to chronically phosphate-depleted dogs produced a small decrease in both glomerular filtration rate (GFR) and fractional excretion of sodium whereas ultrafilterable plasma phosphate concentration rose significantly from 1.15±0.39 to 4.75±0.82 mg/dl. Fractional excretion of calcium fell markedly from 2.08±0.61 to 0.32±0.08%. Despite the rise in serum phosphate concentration, urinary phosphate excretion remained less than 0.5% of filtered load. In the proximal tubule, however, there were no changes in the fractional reabsorption of fluid, sodium, or calcium after intravenous phosphate. To determine if parathyroid hormone secretion was an important determinant of the decrease in calcium excretion associated with phosphate infusion, studies were repeated in thyroparathyroidectomized dogs. Infusion of phosphate to chronically phosphate-depleted, acutely thyroparathyroidectomized (TPTX) dogs (group V) did not change either GFR or fractional excretion of sodium, although plasma phosphate rose to 2.45±0.51 from 0.8 ±0.14 mg/dl. Fractional excretion of phosphate was extremely low but fell even further to 0.35±0.14%

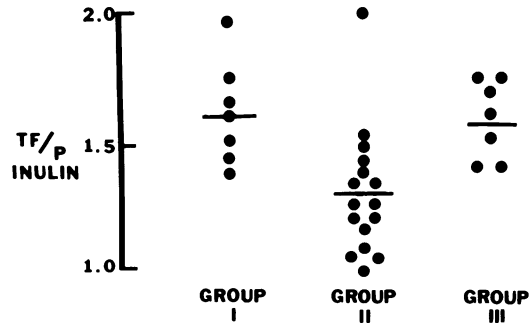

FIGURE 1 TF/P inulin in late, superficial proximal tubules in normal diet controls (group I), chronic phosphate depletion (group II), and low phosphate diet with phosphate supplements (group III). Each point represents the mean value of three to six tubules in each dog; the horizontal bar represents the mean value for each group.

after intravenous phosphate infusion. Fractional excretion of calcium fell significantly to 0.44±0.13 from 2.20±0.97%, similar to the changes observed in the group IV animals. In the proximal tubule there was again no alteration in fractional reabsorption of either sodium or calcium although serum ultrafilterable Ca^{++} was lowered by the phosphate infusion. Infusion of phosphate to normophosphatemic acutely TPTX animals (group VI) produced no significant change in GFR, or fractional excretion of sodium or calcium. Ultrafilterable plasma phosphate increased from 6.06±0.84 to 9.45±0.84 mg/dl and ultrafilterable calcium fell slightly from 3.02±0.07 to 2.68±0.05 meq/liter. As a result of this elevation in plasma ultra-

TABLE II
*Effects of Intravenous Phosphate Infusion**

	Clearance												Proximal tubular reabsorption					
	C_{In}		UF_{PO_4}		$UF_{Ca^{++}}$		C_{Ca}/C_{In}		C_{Na}/C_{In}		C_{PO_4}/C_{In}		H_2O		Na		Ca	
	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R	C	R
	ml/min		*mg/dl*		*meq/liter*						%							
Group IV, CPD, n = 6																		
Mean	14.2	12.6	1.2	4.8	2.95	2.80	2.08	0.32	0.49	0.29	0.32	0.17	21	20	21	21	14	17
SEM	1.7	1.9	0.4	0.8	0.08	0.04	0.61	0.08	0.14	0.10	0.05	0.05	4	2	3	4	4	4
P	<0.05		<0.01		NS		<0.05		<0.05		NS		NS		NS		NS	
Group V, TPTX CPD, n = 4																		
Mean	13.1	16.1	0.8	2.5	2.92	2.55	2.20	0.45	0.27	0.09	1.00	0.35	19	16	22	19	14	9
SEM	1.8	4.3	0.1	0.5	0.08	0.1	0.97	0.13	0.22	0.03	0.07	0.14	2	3	1	2	1	5
P	NS		<0.02		<0.01		<0.05		NS		<0.05		NS		NS		NS	
Group VI, TPTX: controls, n = 6																		
Mean	18.0	16.1	6.1	9.5	3.02	2.68	0.60	0.53	0.16	0.32	0.43	15.10	34	27	36	33	36	30
SEM	1.3	0.7	0.8	0.8	0.07	0.05	0.17	0.14	0.04	0.08	0.27	2.95	2	4	2	4	4	3
P	NS		<0.05		<0.01		NS		NS		<0.01		NS		NS		NS	

* C, mean of clearance and micropuncture data simultaneously obtained during control period. R, mean of recollection clearance and micropuncture data obtained 75 min after initiation of intravenous phosphate infusion. All clearance data represent values from micropunctured kidney. Proximal tubule reabsorption of water to the point of puncture is calculated as 1-(plasma/tubular fluid) inulin.
Remainder of abbreviations as in Table I. P value refers to significance of difference of means between recollection period and control period in each group.

filterable phosphate, the percentage of fractional excretion of phosphate rose from 0.43±0.27 to 15.10±2.95% of filtered load. In the proximal tubule, there were no significant changes in the fractional reabsorption of sodium, calcium, or fluid.

Sustained water diuresis studies

Effects of chronic phosphate depletion and repletion. To determine the significance of micropuncture data from superficial nephrons in relation to whole kidney proximal tubular reabsorptive function, a series of studies was performed in conscious animals during sustained water diuresis. The data obtained in normal control animals (group VII) and chronically phosphate-depleted dogs (before and after dietary repletion) (group VIII A and B) are presented in Table III. Cosm, V, CH_2O, and Uosm, as well as urinary phosphate excretion, were not different during the control period. The serum phosphate concentration in these normal animals was lower than that found in nondiuretic, normal diet animals (group I), a probable consequence of the hypotonic glucose infusions necessary to produce a sustained water diuresis. The effects of this glucose infusion account for the low phosphate excretion in these animals (6). The fraction of the filtered load delivered out of the proximal tubule (V/GFR) and the fraction excreted as free water (CH_2O/GFR) however, were markedly increased in the phosphate-depleted dogs.

The effects of phosphate repletion were evaluated by restudying the depleted animals 5 days after dietary supplementation with 1 g of neutral sodium phosphate daily. As shown in Table III, phosphate repletion increased plasma ultrafilterable phosphate to 2.67±0.03 from 0.36±0.03 mg/dl. There was no change in GFR with repletion, and a significant fall in both V and CH_2O, as a fraction of the filtered load, to normal values. In contrast, there was no change in any parameter with restudy of the normal animals, indicating that correction of the abnormalities in proximal tubular delivery was not merely a function of the time interval between the repeat studies.

In Table III, the effects of acetazolamide infusion in the three groups is shown. After the control period, acetazolamide produces a marked rise in V, V factored for GFR in normal diet animals, and in phosphate-depleted animals that have been repleted with oral phosphate. However, during phosphate depletion, this agent has no effect on V despite comparable urinary osmolality in each of the three experimental groups. As may be seen in Table III, when the period after acetazolamide is compared between normal, phosphate-repleted, and phosphate-depleted animals, the differences between groups with respect to V and CH_2O are abolished. The only parameter which distinguishes phosphate-depleted animals from normophosphatemic animals after acetazolamide is a markedly lower serum ultrafilterable phosphate level.

Effects of parathyroid hormone (PTH) infusion. Table IV depicts the effects of the administration of highly purified PTH to six chronically phosphate-depleted dogs. The data are expressed as the mean ±SEM of the clearance values 1 h before and 1–2 h after PTH infusion. There were no changes in GFR,

TABLE III

*Studies during Sustained Water Diuresis and after Acetazolamide Infusion in Normal Controls and in Phosphate-Depleted Dogs before and after Phosphate Repletion**

		C_{in}		UF_{PO_4}		V		Uosm		V/C_{in}		CH_2O/C_{in}		UV_{PO_4}	
		C	ACZ	C	ACZ	C	ACZ	C	ACZ	C	ACZ	C	ACZ	C	ACZ
		ml/min		*mg/dl*		*ml/min*		*mosmol/kg*		%		%		*μg/min*	
Group VII	Mean	50.8	46.4‡	2.7	3.1	5.3	7.1	46.2	85.1	10.7	16.1	8.9	10.7	0.21	0.32
Normal diet	SEM	7.5	5.1	0.2	0.1	0.5	0.5	4.7	4.2	0.8	0.9	0.8	0.4	0.05	0.01
	P	NS		NS		<0.025		<0.001		<0.01		NS		NS	
Group VIII(A)	Mean	31.7	28.9	§0.4	0.4‖	5.1	4.8	42.3	78.3	§15.8	17.1	§13.4	12.2	0.11	0.14
Phosphate	SEM	3.9	3.2	0.03	0.02	0.9	0.6	3.7	4.1	1.1	0.9	0.9	0.7	0.01	0.02
depletion	*P*	NS		NS		NS		<0.001		NS		NS		NS	
Group VIII(B)	Mean	37.2	36.1	2.7	3.3	3.5	5.4	36.8	70.3	¶9.2	14.1	¶8.4	11.9	0.14	1.6
After	SEM	2.2	1.5	0.1	0.15	0.4	0.6	4.3	4.3	0.7	0.2	0.9	1.8	0.04	1.4
phosphate	*P*	NS		<0.05		<0.01		<0.001		<0.02		NS		NS	
repletion															

* C, control period of water diuresis study. ACZ, period of acetazolamide infusion. V, urine flow rate; Uosm urine osmolality; CH_2O, clearance of solute free water, $UVPO_4$ = urinary excretion of phosphate. *P* refers to significance of difference between mean values before and after acetazolamide infusion in each group.

‡ Refers to *P* value less than 0.05 when compared to period after acetazolamide in group VIII phosphate-depleted animals.

§ Refers to *P* value of less than 0.01 compared to period before acetazolamide infusion in group VII normal diet animals or group VIII phosphate-repleted animals.

‖ Refers to *P* value less than 0.01 when compared to period after acetazolamide infusion of group VII normal diet or group VIII phosphate-repleted animals.

¶ Refers to *P* value less than 0.01 when compared to control period before acetazolamide in group VIII phosphate-depleted animals. Each period before or after acetazolamide represents the mean value of three 20-min clearance periods.

ultrafilterable calcium, or phosphate concentration. There was a small natriuresis during the 2nd h as fractional excretion of sodium rose from 0.49±0.21 to 1.33±0.39% but fractional excretion of phosphate remained at markedly reduced levels despite the PTH infusion. The markedly increased fractional calcium excretion of 4.09±1.36 tended to fall to 3.25±1.21 during the 1st h and 3.91±1.35 in the 2nd h. These changes however, were not statistically significant; thus, acute PTH infusion did not correct the hypercalciuria characteristic of chronic phosphate depletion when renal hemodynamic parameters were stable.

TABLE IV
*Effects of PTH Infusion in CPD**

	Control	1st h	2nd h
C_{In}, *ml/min*	29.33±2.77	30.13±2.58	32.62±4.06
UF_{PO_4}, *mg/dl*	0.5±0.12	0.6±0.15	0.75±0.10
$UF_{Ca^{++}}$, *meq/liter*	2.95±0.14	2.90±0.13	2.86±0.16
C_{Na}/C_{In}, %	0.49±0.2	0.51±0.1	1.33±0.4‡
C_{Ca}/C_{In}, %	4.09±1.4	3.25±1.2	3.91±1.4
C_{PO_4}/C_{In}, %	1.07±0.16	0.96±0.15	0.88±0.19

* Values are expressed as mean±SEM. Control refers to clearance periods during hour before PTH infusion. 1st and 2nd h refer to data obtained during 1st and 2nd h of infusion.
‡ = $P < 0.05$ compared to control.

DISCUSSION

The results of the present study suggest that chronic phosphate depletion produces at least two independent effects upon renal tubular function: sustained inhibition of proximal tubular transport and decreased calcium reabsorption in the distal nephron which is independent of changes in PTH levels.

Earlier studies of the renal effects of chronic phosphate depletion (CPD) suggested that there might be alterations of some components of proximal tubular function in this condition. For example, the Tm for bicarbonate, primarily a proximal tubular function, has been shown to be significantly reduced in phosphate depletion. Although this was attributed to alterations in intracellular pH (4), reduction of proximal tubular sodium transport also would be associated with inhibition of bicarbonate reabsorption (9). Such a possibility has been supported by earlier clearance studies which suggested that CPD may lead to inhibition of sodium reabsorption in the proximal tubule of the dog (10). In addition, evidence for a decrease in Tm glucose has been obtained in phosphate depletion (5), compatible with the concept of a generalized decrease in proximal tubular function. Other investigators, however, have been unable to document this decrease in Tm glucose (11). As phosphate and glucose appear to compete for transport in the proximal tubule (6), it is possible that hypophosphatemia may enhance glucose transport and severe phosphate depletion may depress proximal function accounting for the discrepancy in the two studies (5, 11).

Our micropuncture data clearly suggest a reduction in superficial proximal tubular fluid transport in chronically phosphate-depleted animals when compared to controls. That this alteration is not due to some component of the diet other than phosphate depletion per se is demonstrated by the normal values for proximal tubular reabsorption which we obtained in the animals given the experimental diet supplemented with phosphate. However, there are several possible objections to extrapolating a low TF/P inulin ratio to indicate inhibition of proximal tubular fluid reabsorption. For example, if the tubular site of puncture was consistently earlier in chronically phosphate-depleted animals, TF/P inulin would be reduced, but fluid reabsorptive rate would be unaffected. The introduction of such observer bias or of a systematic error in the collection or analysis of tubular fluid samples was carefully avoided. Specifically, control animals in group I were studied in an alternating fashion with animals in groups II and IV (CPD).

In addition, recent studies have suggested that there are important electrophysiological (12), and therefore possibly reabsorptive, differences between superficial and juxtamedullary cortical nephrons. Thus, it may not be justified to extend observations from superficial nephrons to include the entire proximal tubule population. To circumvent these problems, we evaluated whole kidney segmental fluid transport utilizing CH_2O studies. In these experiments, animals were evaluated after induction of CPD in a manner identical to the group II micropunctured animals. This series of water diuresis experiments was designed to verify the conclusions reached in the micropuncture studies.

Under conditions of sustained water diuresis and maximal vasopressin suppression, the ratio of V to GFR (V/GFR) has been used as an index of delivery of filtrate from the proximal tubule to the distal nephron (13, 14). During states in which distal nephron delivery of filtrate is enhanced, changes in CH_2O/GFR may also reflect such an increased delivery if the solute reabsorptive function of distal nephron diluting sites is unimpaired (14). In our studies, CPD was associated with a significantly greater V/GFR and CH_2O/GFR when compared to control animals with comparably low urine osmolality. Moreover, the reversibility of these abnormalities with dietary repletion of phosphate further supports the primary role of phosphate depletion in the genesis of the proximal

tubular dysfunction. Such analyses during water diuresis have been used to evaluate the tubular sites of action of various drugs and physiological maneuvers (14). However, such studies have potential pitfalls in interpretation since a number of theoretical assumptions underlie their use. For example, an increased V during water diuresis does not necessarily imply decreased fluid reabsorption in the proximal tubule. It could indicate decreased water abstraction from the descending limb of Henle's loop and decreased nonantidiuretic hormone-dependent water reabsorption across the collecting duct epithelium. These two phenomena might occur if medullary solute concentration was "washed-out" by some experimental maneuver. Thus, it is possible that CPD could alter medullary solute accumulation and thereby lead to the increased V/GFR and CH_2O/GFR seen in the animals studied during sustained water diuresis. Also, if collecting duct permeability to water was decreased, diminution of "back flux" of water across this epithelium could be manifested as a rise in V and free water excretion. Thus, clearance studies alone although inferential, could not definitively identify the tubular sites of altered fluid reabsorption.

It is because of the possibility of alternate interpretations of such clearance data that the importance of the parallel series of micropuncture experiments becomes apparent. Two groups of phosphate-depleted animals were prepared in identical fashion and micropuncture was performed in one and the clearance studies in the other. This allowed direct comparison of parameters of proximal tubular function by two different experimental techniques and thereby facilitated specific interpretation of the observed data. Thus, whereas alternate explanations could be employed to analyze either micropuncture or clearance data alone, the combined experimental approach strongly supports the conclusion that inhibition of fluid reabsorption occurs in proximal convoluted tubules of the chronically phosphate-depleted dog.

The observations on the response in V/GFR and CH_2O/GFR to acetazolamide infusion in phosphate-depleted animals further suggests inhibition of proximal tubular fluid reabsorption. Acetazolamide has been shown to specifically inhibit filtrate reabsorption in the proximal tubule through inhibition of carbonic anhydrase (8). This agent has been employed to evaluate segmental tubular function during water diuresis and consistently produces an increase in V/GFR and a variable increase in CH_2O/GFR, confirming micropuncture studies which localize its site of action to the proximal tubule (8). In the present studies, acetazolamide produced no change in V/GFR in phosphate-depleted animals while significantly increasing this parameter in normal control and phosphate-repleted dogs. If the high rate of V/GFR seen in phosphate-depleted animals represents inhibition of proximal tubular fluid reabsorption, then infusion of an agent known also to inhibit proximal tubular reabsorption would be expected to produce little further effect. However, if medullary solute "washout" or decreased water permeability of the collecting duct was the mechanism of the increased V/GFR, an agent which leads to inhibition of proximal tubular fluid reabsorption would lead to a further rise in V/GFR as delivery to distal nephron sites increases. Thus, absence of such an effect of acetazolamide in phosphate-depleted dogs leads to verification of the conclusion that phosphate depletion produces inhibition of proximal tubular fluid reabsorption. Moreover, the analysis of free water excretion data demonstrate that acetazolamide infusion normalizes the large differences between hypophosphatemic and normophosphatemic controls further corroborating the assumption that the increased free water excretion in phosphate depletion is due to enhanced delivery of filtrate to the distal nephron sites of urinary dilution.

In addition, a recent observation from our laboratory supports the validity of the conclusion that increased V/GFR and CH_2O/GFR represent increased filtrate delivery from the proximal tubule and not wash out of medullary solute concentration. Maximum urine osmolality after 12 h of fluid deprivation in chronically phosphate-depleted rats was greater than 1,900 mosmol/kg and identical to normal controls.[2] Thus, although no direct study of medullary solute gradients is available in chronic phosphate depletion, these data suggest that significant medullary washout is not a feature of phosphate depletion.

The present studies do not define the mechanism of the defect in proximal tubule reabsorptive function. A large body of information has accrued on the effects of phosphate depletion on red cells (15, 16), phagocytes (17), hepatocytes (18), skeletal muscle (19), and neural tissue (20). Generally, intracellular phosphate levels are low as are intracellular ATP levels (20). Thus, abnormalities seen in phosphate depletion have been attributed to deficiencies of cellular energy stores (16, 19). The possible role of similar abnormalities in the cellular metabolism of proximal tubular epithelia remains unknown. It is interesting however, that acute phosphate infusion in our studies did not correct the abnormality whereas dietary repletion restored proximal tubular reabsorption to

[2] Emmett, M., S. Goldfarb, Z. S. Agus, and R. Narins. Unpublished observations. Urine osmolality was 1,959±73 mosmol/kg in five phosphate-depleted rats (3 wk of 0.03% phosphate diet) and 2,096±44 in five pair fed control diet animals ($P > 0.3$). Urine osmolality was determined after 1 U pitressin tannate in oil intramuscularly after 12 h of fluid deprivation.

normal values over 5 days. The infusion of phosphate in intact animals would be expected to produce an increase in PTH secretion (21). Thus, the failure of acute phosphate infusions to correct the defect in proximal tubular fluid and calcium reabsorption of phosphate depletion in this group could represent the simultaneous occurrence of two mutually antagonistic effects on the proximal tubule; phosphate repletion could act to normalize the reabsorptive defect found in phosphate depletion whereas an acute rise in PTH levels would lead to inhibition of proximal tubular fluid reabsorption (7). The studies in TPTX, phosphate-depleted dogs (group V) obviate this possibility. In these animals, acute phosphate infusion also failed to correct the altered proximal tubular reabsorption of calcium and fluid. It is also possible that other competitive factors were present to influence proximal reabsorption. For example, the small phosphate infusion could produce enough expansion of extracellular fluid volume to inhibit proximal fluid reabsorption whereas the correction of phosphate deficiency would now act to restore fluid reabsorption to normal. The net effect of these opposing factors could also result in no change from base line. The infusion of phosphate into TPTX normophosphatemic dogs however, did not produce a significant fall in TF/P inulin, and therefore suggests that volume expansion was not produced by these infusions. On the other hand, whereas acute correction of the defect in proximal tubular function was not demonstrable in these micropuncture studies, chronic reversibility was clearly shown in animals repleted with oral phosphate supplements and studied by sustained water diuresis.

The mechanism of hypercalciuria with phosphate depletion has been studied in both man and experimental animals. Factors which could contribute to increased urinary calcium excretion include increase in filtered load, and (or) decreased proximal tubular reabsorption which would increase delivery of filtered calcium to the distal nephron. Calcium reabsorption in the distal nephron could be diminished by a decrease in circulating levels of PTH (22) with phosphate depletion or by a specific transport defect associated with phosphate depletion.

Our data suggest that neither an increase in filtered load nor increased delivery of filtrate out of the proximal tubule play an important role in producing hypercalciuria. These conclusions derive from the observation that the acute infusion of phosphate to chronically phosphate-depleted dogs abolished hypercalciuria but did not alter the massive delivery of calcium to the distal nephron. Since final urinary calcium excretion may be independent of distal nephron delivery, these data are more compatible with inhibition of some component of distal nephron calcium reabsorption by phosphate depletion as the mechanism of hypercalciuria. Enhanced intestinal calcium absorption and (or) increased bone resorption associated with phosphate depletion (3) would be expected to suppress secretion of PTH. Decreased circulating levels of PTH in this syndrome therefore, may represent a potential hormonal mechanism for the inhibition of distal nephron calcium transport. Previous studies evaluating this hypothesis have been inconclusive. For example, the administration of parathyroid extract to phosphate-depleted dogs reduced urinary calcium excretion but not to normal levels (1). This was interpreted as evidence for the presence of PTH-independent inhibition of calcium transport. However, parathyroid extract produces striking renal hemodynamic effects which could modify the hypocalciuric response to PTH. In our studies, the administration of highly purified PTH failed to lower fractional excretion of calcium to normal levels whereas GFR and filtered load of calcium were stable. On the other hand, as has been previously demonstrated (23), acute infusion of phosphate completely abolished the hypercalciuria in both intact and TPTX phosphate-depleted dogs. These data suggest that some component of calcium transport beyond the proximal tubule which is independent of PTH is altered by CPD to produce hypercalciuria. Our studies do not define the cellular mechanisms involved in this event. It is possible that the lack of a hypocalciuric response to PTH is the result of a deficit of some tubular constituent, such as phosphate, rather than a primary cellular resistance to the actions of the hormone but, again, the present data cannot resolve this question.

Previous studies have suggested that the inhibition of calcium transport produced by a decrease in serum phosphate is associated with a systemic rather than a direct renal effect (1). Although chronic expansion of the extracellular fluid volume produces hypercalciuria, it is unlikely that such a mechanism is operative in phosphate depletion since hypercalciuria persists when depleted animals are placed on a salt-free diet (1). Chronic metabolic acidosis also is associated with increased urinary calcium excretion (24). Previous studies have been unable to document consistent changes in acid-base status in phosphate depletion (1, 4), and measurement of arterial pH in our animals did not reveal evidence of metabolic acidosis. Studies in our laboratory in phosphate-depleted rats however, have documented the presence of a renal tubular acidosis which is masked by bicarbonate release from bone (25). It therefore remains possible that intracellular pH may be altered by phosphate depletion in the absence of overt changes in arterial pH and play a role in the development of hypercalciuria, analagous to the syndrome of

incomplete renal tubular acidosis (26). Measurements with 55-dimethyl-2,4 oxazolidinedione however, have suggested an increase in muscle intracellular pH associated with phosphate depletion (4). Therefore, the role of changes in systemic acid-base balance in producing the hypercalciuria of phosphate depletion remains unclear. Other factors which may alter calcium transport and which remain to be defined include alterations of distal nephron tubular fluid pH by phosphate infusion, phosphate-induced changes in intracellular calcium concentration, or the possibility of a humoral factor which inhibits calcium reabsorption during phosphate depletion (27).

Although these studies were not designed to evaluate the renal handling of phosphate during CPD, several points do emerge from an analysis of the urinary excretion of phosphate in the various experimental groups. As has been previously shown, urinary phosphate excretion is extremely low in phosphate-depleted animals and may not rise with elevations in serum phosphate (28). Thus, in group IV micropuncture studies, the elevation of serum ultrafilterable phosphate from 1.2±0.4 to 4.8±0.8 mg/dl produced no rise in the fractional excretion of filtered phosphate (0.32±0.05–0.17±0.05%). After administration of PTH to chronic phosphate-depleted dogs (group IX), there was no increase in phosphate excretion. These results confirm previous observations of the effects of PTH on phosphate reabsorption in chronically phosphate-deprived animals (29). In general, the phosphate-depleted dog would appear to demonstrate resistance to both the phosphaturic and hypocalciuric effects of PTH.

In summary, our studies using both micropuncture and CH_2O techniques have defined an effect of CPD on reabsorptive function in both the proximal tubule and distal nephron. The reabsorption of fluid, calcium, and sodium is significantly reduced in the proximal tubule but only calcium reabsorption is altered in the distal nephron, accounting for the hypercalciuria. The proximal tubular abnormality was not altered by acute phosphate infusion but responded to chronic repletion with dietary phosphate. Acute infusion of phosphate but not PTH corrects the hypercalciuria in both intact and TPTX dogs. Thus, alterations in phosphate balance independent of PTH modulate urinary calcium excretion by affecting calcium reabsorption in the distal nephron. The relevance of these observations to other models of hypercalciuria such as idiopathic hypercalciuria with hypophosphatemia in man (30) remains to be defined with careful studies of proximal tubular transport. In addition, the use of phosphate depletion as a model characterized by sustained inhibition of proximal tubular reabsorption may facilitate the study of the importance of the proximal tubule in various pathophysiological states of altered sodium balance.

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