

Renal Tubular Sites of Altered Calcium Transport in Phosphate-depleted Rats

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ABSTRACT Increased calcium (Ca) excretion is characteristic of chronic phosphate (PO_4) depletion (PD). To study the changes in tubular transport and the site of the hypocalciuric effect of PO_4 administration, clearance and micropuncture experiments were performed in intact rats pair fed either a control diet (0.5% PO_4) or a PO_4 -depleted (PD) diet (0.01% PO_4) plus $\text{Al}(\text{OH})_3$ and in parathyroidectomized (PTX) PD rats, infused either with saline or with neutral sodium PO_4 .

Intact PD rats, compared with intact rats on a control diet, exhibited a lower plasma ultrafiltrable (UF) PO_4 (5.8 ± 0.5 vs. 7.8 ± 0.3 mg/dl), higher fractional excretion (FE) of Ca (4.1 ± 1.2 vs. $0.6 \pm 0.1\%$), and reduced FE PO_4 (0.1 ± 0.01 vs. $10.2 \pm 1.8\%$). Tubular fluid/plasma inulin was lower in the late proximal tubule of PD rats, associated with increases in fractional delivery (FD) from the proximal tubule of Na and Ca.

The %FD of Ca to the early distal tubule of PD rats was increased (20 ± 3 vs. $11 \pm 2\%$), but this difference was abolished by the late distal tubule (5.1 ± 1.2 vs. $3.3 \pm 0.9\%$). In PTX-PD rats, PO_4 infusion increased plasma UF PO_4 (13.8 ± 0.7 vs. 7.8 ± 0.7 mg/dl). FE of Ca was reduced (1.08 ± 0.35 vs. $4.59 \pm 1.57\%$) without correcting the increased Ca delivery to the late distal tubule.

These data indicate that PD impairs Ca reabsorption in tubular segments before but not within the distal convoluted tubule, so that hypercalciuria is ul-

timately a result of decreased Ca transport either in the terminal nephron or in deeper nephrons where PO_4 infusion stimulates Ca transport independent of parathyroid hormone or changes in the filtered load of Ca.

INTRODUCTION

The renal effects of chronic phosphate depletion (PD)¹ (1) consist of reduced phosphate excretion, glycosuria, bicarbonaturia, and reduced proximal tubular transport of sodium and fluid. Whereas several studies have suggested that tubular sites beyond the late superficial proximal tubule are responsible for the alterations manifested in the final urine, little information is available regarding the specific tubular sites for reduced calcium transport in PD. Several possible nephron sites, however, could potentially play a role in modulating the effects of PD. For example, recent studies with the isolated perfused rabbit tubule have suggested that the pars recta of the proximal tubule is a site of calcium reabsorption (1) and could therefore represent a potential site of the observed effects in PD. Studies in the same experimental preparation have also implicated the ascending limb of Henle's loop as an important distal nephron site of calcium reabsorption (2). The distal convoluted tubule has been shown by micropuncture and stationary micropfusion studies to be a high-capacity site for calcium transport (3). Finally, the terminal nephron has been implicated as a critical site for tubular calcium reabsorption (4).

To delineate the role of the distal nephron in modulating calcium transport in PD, we studied the effects of severe PD and PO_4 repletion in intact and parathyroidectomized rats with micropuncture and microdissection techniques. The studies reported here docu-

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¹ Abbreviations used in this paper: FD, fractional delivery; FE, fractional excretion; PD, phosphate depletion(ed); PTH, parathyroid hormone; PTX, parathyroidectomy(ized); TF, tubular fluid; UF, ultrafiltrable(ility).

ment an important role of segments of the distal nephron in the hypercalciuria of PD.

METHODS

Protocol A

Effects of PD on segmental changes in calcium reabsorption in intact rats. Male Wistar rats (between 175 and 225 g) were paired by weight, individually housed in metabolic cages, and pair fed either a PD diet (Pi content 0.01% by weight) (ICN Nutritional Biochemicals, Cleveland, Ohio) or the same diet supplemented with PO_4 to give a PO_4 content of 0.5% (control diet). The PD diet was prepared by adding NaCl, KCl, and CaCO_3 to give a final composition by weight as follows: Na, 0.73%; K, 1.36%; Ca, 0.61%. The control diet had identical Na, K, and calcium content and differed only in having 0.5% Pi. To assure true depletion of total body PO_4 stores, animals were administered 2.0 ml/d $\text{Al}(\text{OH})_3$ gel. After a period of 32 ± 3 d (mean \pm SE) of diet treatment, PD rats ($n = 12$), alternating with diet control ($n = 11$), were fasted overnight and allowed ad lib. water for 12–14 h preparatory for clearance and micropuncture experiments.

Anesthesia and surgery were performed as previously described (5, 6). [*Methoxy*- ^3H]inulin was infused via the external jugular vein in 0.9% sodium chloride that contained 3.05 meq/liter CaCl_2 at a rate of 2.5 ml/100 g weight per h. Tubular localization was aided by intratubular injection of lissamine green (0.1%) via sharpened pipettes ($\text{OD} \leq 4 \mu\text{m}$). Late proximal tubules were identified as the last convolutions visualized after dye injection. Early distal tubules were identified as the earliest surface convolution outlined by dye after its passage through Henle's loop. Late distal tubules were identified by observing the last surface convolution outlined by the dye after its second submergence. Subsequent latex injections and microdissection by methods previously described (4) confirmed the locations of early and late distal tubule to be within 35 ± 3 and $76 \pm 4\%$, respectively, of the distance from the macula densa to the first branching with another tubule. Body temperature was kept between 36.5 and 37.5°C by an electrically heated plate. Carotid artery blood pressure was monitored, and only those animals with a mean blood pressure over 100 mm Hg were included in the data reported here. After 150 min of equilibration, 3–5 proximal and 2–10 distal tubules were punctured over the next 90–100 min. An oil block equal to 3–5 tubular diameters was injected distal to the flow of tubular fluid. Tubular fluid was collected by free-flow technique. Minimal suction was applied to keep the oil block in place, care being taken to avoid changes in tubular diameter. Three to five urine collections (20- to 35-min each) were simultaneously performed. Arterial blood, $\sim 100 \mu\text{l}$, was collected at the beginning and every 30–40 min throughout the experiment for determination of plasma [*methoxy*- ^3H]inulin, PO_4 , calcium, and Na. Plasma was also obtained by preparing five diet control rats and five PD rats exactly as above and then bleeding via carotid artery after 200 min of saline infusion. An ultrafiltrate was prepared as previously described (4), and the percent ultrafiltrability of calcium and PO_4 was determined.

Protocol B

Effects of PO_4 infusion on segmental changes in calcium reabsorption in parathyroidectomized (PTX)-PD rats. Male Wistar rats (between 175 and 225 g) were PTX by electric cautery under light ether anesthesia. PTX was considered complete if plasma calcium fell to or below 3.8 meq/liter

after an overnight fast. They were then PD as in Protocol A. The surgical and experimental preparations were identical to that in Protocol A with the following changes. In one-half of these animals ($n = 7$), [*methoxy*- ^3H]inulin was delivered in 0.9% NaCl that contained 12 meq/liter of CaCl_2 at 2.5 ml/100 g weight per h. (In a pilot study, this dose of calcium had been shown to maintain normocalcemia [plasma total calcium = 4.5 meq/liter] in these rats during equivalent volume expansion with saline.) These animals served as time controls. In the other half ($n = 7$) (PO_4 infusion group), a neutral ($\text{pH} = 7.4$) solution of sodium phosphate ($\text{Na}_2\text{HPO}_4 \cdot \text{NaH}_2\text{PO}_4 = 4:1$, concentration of $\text{PO}_4 = 400 \text{ mg/dl}$) was infused at a rate of 1.6 mg PO_4 in 0.4 ml/h per 100 g weight via one jugular vein for the duration of the experiment. [*Methoxy*- ^3H]inulin was delivered via another jugular vein in saline solution that contained 25 meq/liter of CaCl_2 at a rate of 2.1 ml/100 g weight per h. This dose of calcium had been previously determined to maintain normocalcemia (plasma total calcium = 4.5 meq/liter) despite PO_4 infusion.

Micropuncture and clearance experiments were otherwise performed exactly as described in Protocol A. Because the total and ultrafiltrable calcium were slightly but significantly lower in the PO_4 -infused group (see Table III), an additional group of time-control rats ($n = 5$) were identically prepared as in Protocol B, but infused with saline that contained only 6 meq/liter of CaCl_2 . This maneuver produced a degree of hypocalcemia comparable to that observed in the PO_4 -infused rats. Only clearance studies were performed in this group to determine if the hypocalciuric effect of PO_4 infusion was a function of induced hypocalcemia. Plasma ultrafiltrate was obtained as in Protocol A on similarly prepared groups of PO_4 -infused rats and their time control.

Analytical methods

Tubular fluid calcium and Na were determined by electron probe microanalysis as previously described (4, 5). Urine and plasma calcium, PO_4 , and Na were determined by methods previously described (6). [^3H]Inulin activity was measured by liquid scintillation spectrometry (5).

Calculations

Glomerular filtration rate was estimated as the clearance of [*methoxy*- ^3H]inulin. Fractional excretion (FE) of Na, calcium, or PO_4 was determined by dividing the clearance of Na, calcium, or PO_4 by the clearance of inulin. Fractional delivery (FD) of Na and calcium were calculated as the ratio of tubular fluid (TF)/plasma or ultrafiltrable (UF) for each ion divided by TF/plasma inulin $\times 100\%$. Fractional reabsorption of calcium = $(1 - \text{FD})$ of calcium $\times 100\%$. Statistical analysis was performed by using Student's *t* test for dependent or independent variables, as appropriate. A *P* value of <0.05 was considered significant. All data are given as mean \pm SEM.

RESULTS

Protocol A

Effects of PD on plasma values and whole kidney function (Table I). Chronic phosphate depletion was associated with a reduced body weight (256 ± 5 vs. 305 ± 10 g) and a lower plasma UF PO_4 (5.8 ± 0.5 vs. $7.8 \pm 0.3 \text{ mg/dl}$) compared with diet controls. The %UF of PO_4 and calcium and the plasma UF calcium

TABLE I
Effect of PD on Weight, Plasma, and Clearance Data in Intact Rats*

	Body weight	Plasma UF PO ₄ †	Plasma UF Ca†	GFR‡	FE Na	FE Ca	FE PO ₄
	g	mg/dl	meq/liter	ml/min	%	%	%
Diet control (n = 11)	305±10	7.8±0.3	2.92±0.03	3.7±0.2	1.3±0.2	0.6±0.1	10.2±1.8
PO ₄ depletion (n = 12)	256±5	5.8±0.5	2.99±0.07	2.7±0.1	2.3±0.8	4.1±1.2	0.10±0.01
P	<0.001	<0.005	NS	<0.001	<0.03	<0.01	<0.001

* Values are mean±SEM.

† Data given refers to values at midpoint of the experiment.

‡ Glomerular filtration rate.

|| Statistical significance between diet-control and PD rats.

concentration were not significantly different in the two groups. The glomerular filtration rate was significantly reduced in PD (2.7 ± 0.1 vs. 3.7 ± 0.3 ml/min per kg body wt). FE sodium was increased in PD (2.3 ± 0.8 vs. $1.3 \pm 0.2\%$), in association with a markedly increased FE calcium (4.1 ± 1.2 vs. $0.6 \pm 0.1\%$). Phosphate was virtually eliminated from the final urine (FE PO₄ = 0.10 ± 0.01 vs. $10.2 \pm 1.8\%$) during PD.

Effects of PD on segmental fluid and Na transport. TF to plasma inulin was significantly reduced by PD (Table II) in the late proximal tubule, which indicates inhibition of fluid reabsorption. The reduced TF/plasma inulin ratio persisted in both the early and late distal convoluted tubules. FD of Na (Table II) was equalized by the early distal punctures and remained comparable in late distal TF.

It is of note that whereas reduced proximal TF reabsorption has not been demonstrated in animals placed on low-PO₄ diets for brief periods (7), it is regularly seen in more severe PD in both clearance and micro-puncture studies (5, 8, 9).

Effect of PD on segmental calcium transport (Fig.

1). In PD rats TF/UF calcium tended to be higher in the late proximal tubule (1.09 ± 0.04 vs. 0.99 ± 0.05), and was significantly higher in the early distal tubule (0.59 ± 0.06 vs. 0.43 ± 0.06 , $P < 0.05$). When factored for the altered fluid transport in PD, there was a significant increase in the FD of calcium (TF/UF [Ca/In]) from the late proximal tubule (PD = $63 \pm 4\%$; control = $51 \pm 3\%$), and to the early distal tubule (PD = $20 \pm 3\%$; control = $11 \pm 2\%$) (Fig. 1). Reabsorption between these two points, when factored for the delivered load out of the late proximal tubule ($[\text{late proximal FD}_{\text{Ca}} - \text{early distal FD}_{\text{Ca}}] / \text{late proximal FD}_{\text{Ca}} \times 100\%$) was significantly reduced in PD (PD = $67 \pm 5\%$, control = $77 \pm 3\%$, $P < 0.05$).

These data suggest that absolute proximal tubular calcium reabsorption was reduced in PD. Because calcium reabsorption in the loop of Henle did not increase with the higher delivered load, it is likely that absolute calcium transport is reduced in this segment as well. Reabsorption between early and late distal tubule, also factored for delivery, as estimated by corresponding terms ($[\text{early distal FD}_{\text{Ca}} - \text{late distal}$

TABLE II
Effect of PD on Segmental Fluid and Na Transport*

	Late proximal			Early distal			Late distal		
	TF/P _I In	TF/P Na	TF/P Na/In	TF/P In	TF/P Na	TF/P Na/In	TF/P In	TF/P Na	TF/P Na/In
			%			%			%
Diet control (n = 11)	2.02±0.04	1.00±0.01	49.5±1.0	4.07±0.18	0.49±0.06	12.3±1.6	12.9±0.9	0.34±0.07	3.03±0.66
PD (n = 12)	1.83±0.07	1.00±0.01	55.8±2.1	3.33±0.16	0.36±0.02	12.2±1.2	7.5±0.6	0.34±0.05	4.85±0.83
P‡	<0.02	NS	<0.03	<0.005	<0.05	NS	<0.001	NS	NS

* Values are mean±SEM.

† P, plasma.

‡ P value refers to statistical comparison between control and PD rats.

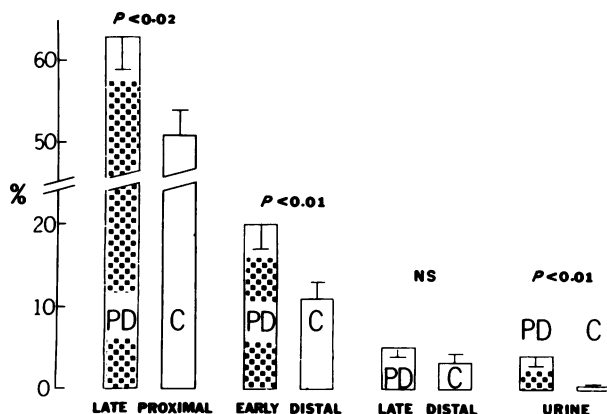


FIGURE 1 Effect of PD on FD of calcium (TF/UF [Ca/In]), plotted in percent (%) on the ordinate. The sites of micropuncture are indicated on the abscissa. Statistical data given refer to comparison between PD and diet control (C).

FD_{ca}/early distal FD_{ca} × 100%) was, however, similar (PD = 73.5 ± 4%, control = 74 ± 4%). Thus, despite increased FD of calcium to the early distal tubule in PD, the distal tubule reduced this load to 5.1 ± 1.2% of the filtered calcium by the late distal site, a value not significantly different from the corresponding value in diet control (3.3 ± 0.9%) (Fig. 1). These data therefore suggest a marked increase in absolute calcium reabsorption in the distal convoluted tubule during PD. No significant reabsorption took place beyond the late distal tubule in PD because the late distal tubular %FD of calcium (5.1 ± 1.2) was not different from that excreted in the final urine (4.1 ± 1.2%). In diet-control animals, on the other hand, significant reabsorption continued beyond the late distal puncture sites, reducing the FD of calcium from 3.3 ± 0.9% in late distal tubule to 0.6 ± 0.1% excreted in the final urine ($P < 0.001$).

Protocol B

Effect of PO₄ infusion on plasma values and whole kidney function (Table III). PO₄ infusion raised the plasma UF phosphate to 13.8 ± 0.7 mg/dl, which was significantly higher than that in the two time-control groups. Plasma UF calcium was decreased by PO₄ infusion to 2.66 ± 0.05 meq/liter, a value significantly lower than that found in the normocalcemic group (2.92 ± 0.07 meq/liter). The %UF of calcium was not different with or without PO₄ infusion (62.3 ± 3% vs. 65.4 ± 2%). When PTX normal diet animals were allowed to become hypocalcemic (2.74 ± 0.04 meq/liter) by infusion of saline that contained only 6 meq/liter of CaCl₂, plasma UF calcium was not different from the PO₄-infused rats (but significantly lower than that of normocalcemic time-control animals). There was no difference in the glomerular filtration rate or the FE of

sodium. FE of calcium was significantly reduced (to 1.08 ± 0.35%) by PO₄ infusion as compared with both the normocalcemic, time-control (4.59 ± 1.57%) and hypocalcemic, time control (5.12 ± 1.62%). The final urine FE of PO₄ was reduced to 0.81 ± 0.63 and 0.09 ± 0.01%, respectively, in these two groups (Table III).

Effects of PO₄ infusion on segmental fluid transport. Chronic PTX had no effect on the inhibition of fluid reabsorption associated with PD since the TF/plasma inulin remained significantly reduced in the PTX-PD rats in the late proximal (1.72 ± 0.08), early distal (3.08 ± 0.14), and the late distal (7.1 ± 0.8) tubules as compared with the corresponding values in the intact rats fed a control diet (Table II). PO₄ infusion had no effect on the altered fluid reabsorption in the PTX-PD rats in either the late proximal (1.83 ± 0.07), early (3.73 ± 0.3), or late distal (8.9 ± 0.5) tubules.

Effect of PO₄ infusion on segmental calcium reabsorption (Fig. 2). In the late proximal tubule, there was no difference in TF/UF calcium (0.99 ± 0.08 vs. 1.04 ± 0.06) or in the %FD of calcium (TF/UF [Ca/In]) between the time-control and the PO₄-infused rats (60 ± 3 vs. 60 ± 5%) (Fig. 2). Similarly, in the early distal tubule, the TF/UF calcium was comparable (0.50 ± 0.10 vs. 0.64 ± 0.09), as was the TF/UF (Ca/In) (18 ± 3 vs. 18 ± 3%, Fig. 2). Thus PO₄ infusion did not correct the increased FD of calcium either to the late proximal or to the early distal tubule. Furthermore in the late distal tubule, regardless of PO₄ infusion, similar values were obtained for the TF/UF calcium (0.50 ± 0.14 vs. 0.61 ± 0.17) and for the TF/UF (Ca/In) (7.3 vs. 7.5%, Fig. 2). However, beyond the late distal tubules, significant reabsorption of calcium continued in the PO₄-infused rats, reducing the FD of calcium from 7.5 ± 2.0 to 1.1 ± 0.4% in the final urine (Fig. 2) ($P < 0.01$). In contrast, no significant reabsorption took place in the terminal segments in the time-control PTX-PD rats.

DISCUSSION

The results of these studies suggest that the striking hypercalciuria of PD results from a complex pattern of tubular alteration. There is reduced transport in the proximal convoluted tubule of PD rats compared with normal-diet controls, but these differences are eliminated by the end of the distal convoluted tubule. In the nephron segments beyond the late superficial distal tubule, including the contribution of deeper nephrons, reduced calcium reabsorption occurs and leads to the hypercalciuria of PD. In addition, this "terminal nephron" site is the locus of enhanced calcium reabsorption after PO₄ infusion in PTX-PD rats.

In this investigation animals were studied after prolonged PD combined with Al(OH)₃ administration to assure that many of the pathophysiologic effects of PD, rather than simple reduction in dietary PO₄, would be

TABLE III
Effect of PO₄ Infusion on Plasma and Clearance Data in PTX-PD Rats*

	Plasma UF PO ₄	Plasma UF Ca	GFR†	FE Na	FE Ca	FE PO ₄
	mg/dl	meq/liter	ml/min	%	%	%
PO ₄ infusion (n = 7)	13.8±0.7	2.66±0.05	2.27±0.26	2.03±0.30	1.08±0.35	0.81±0.63
Time control (normocalcemic) (n = 5)	7.8±0.7§	2.92±0.07	2.39±0.14	2.56±0.48	4.59±1.57¶	0.09±0.01
Time control (hypocalcemic) (n = 5)	7.6±0.2§	2.74±0.04**	2.10±0.13	2.71±0.71	5.12±1.62¶	0.08±0.01

* Values are mean±SEM.

† Glomerular filtration rate.

§ Indicates significant difference at 0.001 level between noninfused time controls and PO₄-infused rats.

^{||} Indicates significant difference at 0.01 level between noninfused time control and PO₄-infused rats.

¶ Indicates significant difference at 0.05 level between noninfused time controls and PO₄-infused rats.

** Indicates significant difference at 0.05 level between the two time-control groups.

obtained (10). More severe PD may account for the reduction of proximal TF and sodium transport, seen as a characteristic trait of severe PD (5, 9), but not evident with milder dietary PO₄ restriction (7).

The mechanism and tubular sites of altered calcium transport in PD have been studied by clearance and micropuncture techniques. Increased filtered load of calcium, although a possible result of PD through the hypercalcemic effect of PD-induced bone resorption and/or intestinal hyperabsorption (10) was not a feature of these studies in the fasted animals (Table I). Similarly this factor has been absent in other studies of PD (5, 11, 12). The hypercalciuria, therefore, is at least in part the result of altered tubular calcium transport. Suppression of parathyroid hormone (PTH) secretion during PD does contribute to a component of the hypercalciuria (11, 12), but the administration of pharmacologic amounts of PTH in acute or subacute infusions failed to entirely correct the hypercalciuria of PD (5, 8, 12). Finally, as demonstrated in these experiments and in previous studies, phosphate infusion rapidly and

completely corrects the hypercalciuria of PD even in PTX animals (5, 13), further suggesting a specific PO₄-sensitive tubular transport defect for calcium in PD.

A micropuncture study of the altered calcium transport in PD has suggested that although reduced calcium transport is found in the proximal tubule, sites beyond the late proximal convoluted tubule were responsible for the hypercalciuria of PD (5). In this study, we have confirmed a reduction in proximal tubular calcium reabsorption during PD, but this effect does not appear to be crucial to the observed hypercalciuria, as will be discussed below. Although the increased calcium delivery out of the late proximal tubule in PD can be attributed to a generalized inhibition of proximal tubule transport (decreased Na and fluid reabsorption), the increased calcium delivery to the early distal tubule (20±3 vs. 11±2, Fig. 1) suggests an additional defect. Despite an increased late proximal Na delivery in PD, tubular segments, presumably the pars recta and the thick ascending limb of the loop of Henle, were able to compensate by augmenting Na reabsorption. Thus, by early distal tubule, the fractional Na delivery was almost identical in PD vs. normal-diet controls (Table II). Reabsorption of calcium by the "loop" has been shown to be load dependent (14, 15). Failure by the loop to compensate for the increased calcium delivery from the proximal convoluted tubule in PD implies possible defects in appropriate adaptation. This impression is reinforced by the observation of reduced reabsorption in the loop if calculated as a percentage of the delivered load of 67±5 in PD vs. 77±3% in diet control.

The conclusion that enhanced calcium delivery is not critical to final urinary calcium excretion in PD derives from the finding that the increase in delivered load of calcium to the early distal tubule is abolished by the late distal tubule (Fig. 1). This observation is consistent with the findings of Costanzo and Windhager (3), with in vivo micropuncture techniques,

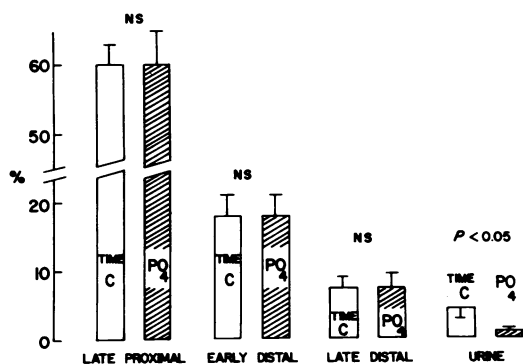


FIGURE 2 Effect of PO₄ infusion on FD of calcium (TF/UF [Ca/In]), plotted in percent (%) on the ordinate. The sites of micropuncture are indicated on the abscissa. Statistical data given refer to comparison between PO₄-infused (PO₄) and non-infused time control (Time C).

which demonstrate that the distal convoluted tubule has a large capacity for calcium transport that increases as delivered load increases. Presumably, PD does not significantly interfere with this reabsorptive system, and, in fact, absolute calcium reabsorption may be higher in PD than in control animals.

Hypercalciuria occurred in the PD animals because virtually no calcium was reabsorbed in the terminal nephron segment in PD animals compared with a reduction of final urinary calcium to <1% of filtered load in normal-diet controls. The "terminal nephron" has been found to be an important site of calcium transport in the rat as well as in the isolated perfused rabbit tubule (4, 16). Moreover, this site has been shown to be sensitive to the effects of PTH to enhance calcium reabsorption. The precise locus of PTH-sensitive calcium transport has not been determined, but recent studies utilizing the isolated perfused rabbit tubule technique have demonstrated that the so-called granular portion of the distal convoluted tubule and the cortical collecting tubule is the apparent site of PTH-sensitive calcium transport (16) as well as PTH-stimulated adenylate cyclase activity (17). Whereas the presence of PTH-stimulated adenyl cyclase has not been localized to various nephron segments in the rat, it is possible that such granular-appearing segments in the collecting tubule system represent the sites of calcium transport in the non-PD and in the PO_4 -infused animals in these experiments. Calcium transport does not occur in the light segment of the cortical collecting duct (16) but data obtained in papillectomized rats suggests the possibility of transport in papillary structures (18), and this site, as well, could play a role in the calciuria of PD. Alternatively, massive calcium delivery from deeper nephrons could account for differences between late distal tubule and final urine. The latter hypothesis seems unlikely in view of the previously documented capacity of granular segments to transport large quantities of calcium and the greater preponderance of granular segments in the terminal portion of juxtamedullary nephrons.

The mechanisms whereby PD leads to reduced calcium reabsorption in the proximal convoluted tubule and terminal nephron remain unknown. Because proximal tubular reabsorption of sodium (5), bicarbonate (19, 20), and glucose (21), are reduced in PD, it is likely that the alteration in calcium reabsorption is related to a nonspecific, generalized reduction in proximal tubular transport. Such an effect may be related to alterations in permeability or to changes in the transport of sodium or bicarbonate as the driving forces for the reabsorption of other organic and nonorganic solutes in this nephron segment. Whereas it is also possible that reduced calcium reabsorption does reflect reduced sodium reabsorption at some critical site in the terminal nephron, reduction of

sodium intake and consequent enhanced sodium reabsorption does not lead to a reduction in the hypercalciuria of PD (13). More likely, PD leads to some specific alteration in calcium reabsorption through changes in cellular metabolism, alterations in intracellular calcium or PO_4 activities, or some alteration in tubular fluid composition.

The effect of PO_4 infusions to reduce urinary calcium excretion is most strikingly seen in PD (5, 13) but is also seen in thyroparathyroidectomized non-PD animals (22). In these studies PO_4 infusions produce a complete correction of the hypercalciuria of PD. This effect is clearly dissociated from a reduction in the filtered load of calcium (Table III) (22). Segmental analysis also indicates that neither the decreased calcium reabsorption nor the decreased Na and/or fluid reabsorption in the proximal tubule was corrected by PO_4 administration. These findings confirmed our previous micropuncture studies in PD dogs, where acute PO_4 infusion was found to have no influence on the inhibited transport in the proximal tubule (5). Our data further show that calcium reabsorption was unaffected by PO_4 infusion in tubular segments between the late proximal and early distal tubules because the FD of calcium to early distal tubule was remarkably similar between the PO_4 -infused and noninfused rats. Similarly, PO_4 administration exerted no effect on calcium reabsorption within the distal convoluted tubule. The fractional calcium excretion was, however, markedly different between the PO_4 -infused and the time-control dogs (Fig. 2). While significant calcium reabsorption took place beyond the late distal tubule of PO_4 -infused rats, no significant reabsorption was observed in the time control (Fig. 2). Thus, PO_4 administration and/or hyperphosphatemia had no appreciable effect on calcium reabsorption in the proximal tubule, the loop of Henle, or the distal convoluted tubule. The absence of an effect of PO_4 infusion on calcium reabsorption in either the proximal tubule or the loop of Henle has also been suggested by previous micropuncture studies of intact rats fed a normal diet (22). Although the studies by LeGrimmelec et al. (22) implied a distal site of hypocalciuric action of PO_4 infusion, the role of endogenous PTH could not be excluded and the precise locus of action could not be determined. Calcium transport within the terminal nephron was, however, significantly increased by PO_4 infusion, as demonstrated in our studies. Taken together, these data indicate that the terminal site of action of PO_4 infusion is not restricted to PD animals.

In conclusion, these micropuncture studies demonstrate the complex renal effects of PD. Whereas reduced proximal tubular reabsorption of sodium and calcium leads to increased delivered loads of each of these ions to the distal nephron, sites beyond the accessible late distal tubule nephron are the critical regula-

tory sites for calcium reabsorption; an impairment at those sites results in hypercalciuria in PD. Our studies further demonstrate virtually normal distal convoluted tubule calcium transport in PD. PO_4 infusion exerts its hypocalciuric effect independently of changes in plasma UF calcium, PTH, and probably the status of PO_4 balance.

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