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Impaired Distal Nephron Acidification in Chronically Phosphate Depleted Rats

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Abstract. Renal tubular bicarbonate reabsorption and acidification were evaluated in phosphate depleted rats (PD) and controls. After 33 days of phosphate depletion, urine pH of PD rats ($N = 5$, 6.36 ± 0.15) was significantly higher than control ($N = 5$, 5.64 ± 0.09, $P < 0.005$) following an NH_4Cl load. Urinary titratable acid of PD rats (9.6 ± 1.8) was significantly reduced compared to control (117.2 ± 19.7 μEq/3 h, $P < 0.001$), whereas NH_4^+ excretion was not different. The plasma HCO_3^- thresholds at which bicarbonaturia occurred (approximately 25 mEq/l) were identical in controls and phosphate depleted rats during isotonic bicarbonate infusion. The higher urine pH of phosphate depleted rats following NH_4Cl administration was not due to low urinary phosphate as 3-day phosphate depleted rats could normally acidify urine after NH_4Cl (pH $= 5.86 \pm 0.09$, $N = 6$ vs. control 5.87 ± 0.08, $N = 6$, $P =$ N.S.) despite urinary phosphate excretion as low as in 33-day PD rats. These data indicate the presence of impaired distal tubular acidification in chronically phosphate depleted rats.

Key words: Phosphates – Acidosis, renal tubular – Ammonium chloride – Bicarbonate.

Introduction

Chronic phosphate depletion may affect the renal regulation of acid-base balance. Gold et al. [8] have shown that chronic phosphate depletion in dogs reduced both the plasma bicarbonate threshold at which bicarbonate appeared in the urine and the maximum tubular reabsorption of bicarbonate. Renal bicarbonate wasting during chronic phosphate depletion has also been reported by Emmett et al. [6] in rats although critical evaluation of renal tubular bicarbonate reabsorption by bicarbonate loading was not performed. In addition, systematic acidosis, bicarbonaturia, and impaired urinary acidification have been reported in patients with osteomalacia and secondary hyperparathyroidism [7, 16, 19, 20]. It is possible that these alterations in acid-base homeostasis during hyperparathyroidism are related to phosphate depletion from excessive phosphaturia. This study was designed to evaluate renal tubular bicarbonate reabsorption and renal acidification in chronically phosphate depleted rats.

Methods

Female Sprague-Dawley rats weighing 180–200 g were placed in individual metabolic cages. Phosphate depletion was produced in 5 rats by feeding a phosphate deficient diet (ICN Pharmaceuticals, Inc., Cleveland, Ohio) supplemented with 145 mmol/kg diet of NaCl and KCl [Phosphate-depleted rats (PD)]. Five control rats were pair-fed the phosphate deficient diet supplemented with 80 mmol/kg diet of neutral sodium phosphate and neutral potassium phosphate yielding a 0.5% phosphate diet [6]. Water was given ad libitum. The animals were kept in separate metabolic cages for 33 days. Twenty-four hour urines were collected on day 30 for measurement of sodium, potassium, calcium, and phosphate excretions.

NH_4Cl Loading Test. On days 31 and 32, both experimental and control rats recieved 1 mM/100 g body weight of a 1.0 M NH_4Cl solution via a stomach tube at 8:00 a.m. and 5:00 p.m. On day 33, the rats were weighed, anesthetized with ether, and cannulated through the femoral artery, femoral vein, and bladder with polyethylene tube No. 50. Following surgery, the rats were placed in restraining cages and allowed to recover for approximately 1 h prior to measurement of baseline arterial pH and pCO_2. The measurement of pH and pCO_2 (Instrumentation Lab., Inc., Model IL 113) required less than 0.5 ml of blood which was recovered from the instrument with minimal

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sample loss, and returned to each animal immediately after use. Two milliliter per 100 g body weight of 0.5 M NH_4Cl (1 mM/100 g) was then given to each animal via a stomach tube. Immediately after acid loading, urine was collected for 3 h and 1.5 ml of arterial blood was obtained at the midpoint for measurement of creatinine and phosphate. At the end of 3 h, 2.0 ml of arterial blood was taken for measurement of calcium and phosphate. This blood specimen and the midpoint sample were collected, immediately centrifuged and the plasma was separated from the red blood cells. The red blood cells were then mixed with appropriate amounts of fresh rat plasma and returned to the original donor animals so that no blood loss occurred throughout the experiment. An additional 0.5 ml of blood was taken at the end of 3 h and immediately returned following measurement of pH and pCO_2. Serum bicarbonate (HCO_3^-) was calculated using the Henderson-Hasselbalch equation with a pK′ value of 6.1 and a solubility coefficient of 0.0301. Urine pH was measured with a Beckman pH meter (Model 76). Urinary titratable acid (TA) was measured by titrating the urine with 0.01 N NaOH solution to a pH of 7.40 and urinary ammonia (NH_4^+) was determined using the indophenol color reaction as modified by Chaney and Marbach [2]. Plasma and urine calcium levels were measured with a Perkin-Elmer atomic absorption spectrophotometer Model 303 (Perkin-Elmer Corp., South Pasadena, Calif.), phosphate and creatinine with a Technicon autoanalyzer, and Na and K with a flame photometer (Beckman Instruments Co.).

A separate group of 6 rats, phosphate depleted by feeding a phosphate deficient diet for 3 days, was subjected to the same NH_4Cl loading test except that NH_4Cl was given only on the day of the experiment (1 mM/100 g body weight). A corresponding control group ($N=6$) was tested in a similar fashion.

Bicarbonate Infusion. Immediately following the acid-loading test, each animal was infused through the femoral vein with a sodium bicarbonate solution, 150 mEq/l, at a rate of 0.1 ml/min/100 g body weight for apptoximately 120–160 min. All urine samples were collected under oil in preweighed tubes at 8–12 min intervals with the volume measured as weight divided by a specific gravity of 1.0. Urine pH and pCO_2 were measured with the IL 113. 0.5 ml of arterial blood was obtained at the midpoint of each urine collection for determination of pH and pCO_2 with the samples being returned to each animal immediately thereafter. Bicarbonate titration was continued until the urine pH was 7.5 or above and plasma bicarbonate greater than 28 mEq/l in all animals. The urine pH was measured with a pH M64 Research pH meter (Radiometer Copenhagen) whenever it was below 6.8. Urine HCO_3^- was calculated using a pK′ of $6.33-0.5\sqrt{Na^+ + K^+}$ and a solubility coefficient of 0.0309. Phosphate was also measured in each urine collection. Creatinine clearance (Ccr) was measured again at the last urine collection.

Statistical analyses were performed utilizing the appropriate *t*-test for either paired or unpaired variables.

Table 1. 24-Hour excretion of sodium, potassium, calcium and phosphate in phosphate depleted and control rats[a]

Diet	Sodium mEq	Potassium mEq	Calcium mg	Phosphate µg
I Normal phosphate ($N=5$)	3.3 ± 0.52	3.2 ± 0.42	0.79 ± 0.37	9475 ± 2766
II Phosphate depletion ($N=5$)	3.3 ± 0.30	3.3 ± 0.39	15.4 ± 6.33	28.3 ± 5.3
P value[b] I–II	NS	NS	< 0.001	< 0.001

[a] Values are mean ± S.E.M. obtained on 30th day of diet administration.
[b] *P* values refer to the significance by student *t*-test. NS = not significant ($P > 0.05$)

Table 2. Effect of acid loading on phosphate depleted rats and controls[a]

	Blood gases					
	pre-acid loading			post-acid loading		
	pH	pCO_2 mmHg	HCO_3 mEq/l	pH	pCO_2 mmHg	HCO_3 mEq/l
I Normal phosphate $N=5$	7.44 ±0.03	36.0 ±1.2	24.1 ±1.3	7.25 ±0.03	27.4 ±2.9	12.0 ±1.7
II Phosphate depletion $N=5$	7.44 ±0.02	37.2 ±1.9	24.6 ±0.4	7.31 ±0.01	27.8 ±1.2	13.8 ±0.7
P value Gr I – Gr II	NS	NS	NS	NS	NS	NS

[a] p_{Ca}, plasma calcium; p_p, plasma phosphorus; C_{cr}, creatinine clearance; V, urine flow rate; TA, titratable acid; U_{ca}, urinary calcium; U_p, urinary phosphorus. These values are mean ± S.E.M.

Results

The amounts of diet ingested by controls and PD rats were 14.0 ± 0.03 and 13.9 ± 0.2 g/day, respectively. Despite similar diet intake, PD rats gained 24 ± 3.4 g body weight, whereas controls gained 33 ± 2.3 g ($0.05 < P < 0.06$) over 33 days.

Table 1 shows 24-h urine excretions of sodium, potassium, calcium and phosphate for both groups. Twenty-four hour sodium and potassium excretions were unaffected by phosphate depletion whereas calcium excretion was significantly increased. Marked calciuria during chronic phosphate depletion has been previously reported [3–5, 14].

NH_4Cl Loading. Table 2 presents data obtained prior to and during the acid challenge with NH_4Cl. Blood pH, pCO_2 and HCO_3^- prior to the last NH_4Cl loading on day 33 were not significantly different between PD rats and controls. One and one-half hours after NH_4Cl administration, serum phosphate levels were significantly lower in PD rats compared to controls. At the end of 3 h, however, serum phosphate levels in PD rats had risen significantly to levels not different from the controls. Mean serum calcium of phosphate depleted animals was greater than controls at 3 h post NH_4Cl. Blood pH, pCO_2, and HCO_3^- decreased to the same levels in both groups at the end of 3 h. Ccr, urine flow rate and ammonia excretion measured over the 3-h period were not significantly different between the phosphate depleted rats and the control group. Three-hour calcium excretion was significantly greater in phosphate depleted rats compared to controls In addition, all phosphate depleted animals failed to acidify their urine to the same extent as controls as urine pH was significantly higher in PD rats compared to control rats. The urine pH of these specimens represent average values for the 3-h period rather than instantaneous values during sustained acidosis. Therefore, comparison of urine pH was also made at the very beginning of bicarbonate infusion while the animals were still quite acidemic. Serum bicarbonate levels at this time were 13.1 ± 1.10 and 15.7 ± 0.94 mEq/l ($P < 0.10$) for normal controls and PD rats respectively. Despite comparable degrees of acidosis, instantaneous urine pH of PD animals (6.57 ± 0.18) was significantly higher than that of normal rats (5.87 ± 0.01, $P < 0.01$). Thus, the increased pH value for the 3-h post NH_4Cl loading period in PD rats accurately reflects impaired distal acidification and is not due to proximal bicarbonaturia that may have occurred at the beginning of the 3-h collection. In addition, we have obtained similar results in identical experiments using phosphate depleted animals receiving aluminum hydroxode gel in their diets. The arterial pH for six phosphate depleted animals receiving aluminum hydroxide gel was 7.26 ± 0.05 and minimal urinary pH was 6.54 ± 0.27 whereas corresponding control values were 7.25 ± 0.03 and 5.64 ± 0.09 respectively.

Mean urine pH following NH_4Cl of animals phosphate depleted for 3 days (5.86 ± 0.09, $N = 6$) was not significantly different from the mean urine pH of corresponding controls (5.87 ± 0.08, $N = 6$). The urinary phosphate excretion of 3-day phosphate depleted rats was significantly lower than control (17.5 ± 8.3 vs. 3510 ± 520 µg/3 h, $P < 0.001$, respectively), but did not differ from that of 33-day phosphate depleted rats.

Bicarbonate Infusion. Figure 1 is a plot of HCO_3^- excretion vs. plasma HCO_3^- level using data obtained

Plasma (mg/100 ml)			Urine post-acid loading						
mid acid loading p_p	post acid loading		C_{cr} ml/min/100 g	V µl/min	pH	TA µEq/3 h	NH_4^+ µEq/3 h	U_{ca} mg/3 h	U_p µg/3 h
	p_{Ca}	p_p							
5.39 ±0.12 (P = NS)[b]	8.20 ±0.48	5.72 ±0.21	0.60 ±0.02	34.4 ±5.0	5.64 ±0.09	117.2 ±19.7	897.8 ±68.8	0.49 ±0.12	3820 ±552
4.22 ±0.23 ($P < 0.001$)[b]	9.52 ±0.33	6.18 ±0.37	0.65 ±0.03	37.4 ±1.1	6.36 ±0.15	9.63 ±1.84	726.1 ±73.9	3.27 ±0.47	22.7 ±4.5
< 0.005	< 0.05	NS	NS	NS	< 0.005	< 0.001	NS	< 0.001	< 0.001

[b] P values refer to the significance of the difference of the mean plasma phosphorus levels between mid-acid loading and post-acid loading by paired t-test

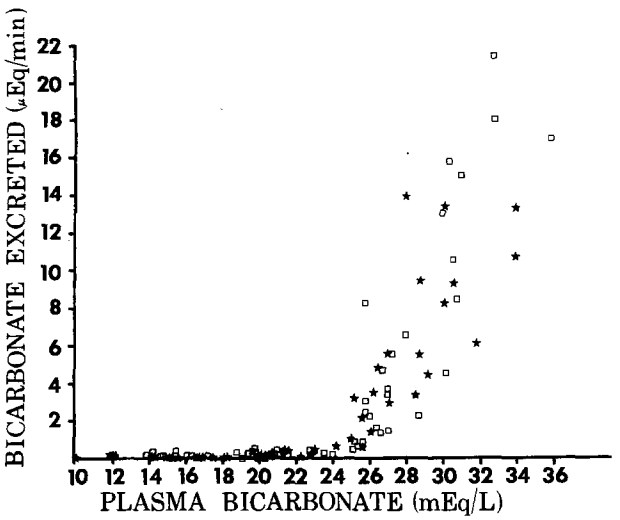

Fig. 1. The relationship between urinary bicarbonate excretion and plasma bicarbonate concentration during isotonic sodium bicarbonate infusion in control rats and phosphate depleted rats. (*) Normal phosphate, (□) phosphate depletion

during bicarbonate infusion. The plasma HCO_3 threshold at which bicarbonaturia occurred was identical in controls and phosphate depleted rats. The calculation of the maximal rate of bicarbonate reabsorption (T_mHCO_3) was not undertaken in this study since use of creatinine clearance rather than inulin clearance in the formula will result in an underestimation of the T_m. This is a consequence of endogenous creatinine clearance being approximately one half of inulin clearance in the rat [13]. Furthermore, since we replaced all of the blood withdrawn for measurement of HCO_3^-, whereas previous investigators have not, a component of relative volume expansion is present in our studies which will minimize $T_mHCO_3^-$. Hence, estimation of the T_mHCO_3 was deliberately omitted from the protocol.

Discussion

The results of this investigation confirm certain aspects of previous studies. Specifically, the findings of hypercalciuria, significantly elevated serum calcium, and a marked increase in serum phosphate following an NH_4Cl load of phosphate depleted rats are consistent with earlier reports of increased bone resorption and impaired distal nephron calcium reabsorption during phosphate depletion [6, 10]. That phosphate depleted rats maintained systemic acid-base parameters identical to controls in the face of an equivalent acid load despite the fact that acid excretion of phosphate depleted animals was significantly lower than normal, also suggests increased buffering of acid by bone. Goldfarb et al. have indicated that impaired tubular reabsorption of calcium is more important than the increased filtered load in the production of hypercalciuria during phosphate depletion [10].

Gold et al. [8] demonstrated reduction of both the plasma threshold at which bicarbonate appears in the urine and the maximal tubular reabsorptive capacity for bicarbonate ($T_mHCO_3^-$) in severely phosphate depleted dogs. The present study, however, failed to reveal an abnormal tubular response to isotonic sodium bicarbonate infusion in rats phosphate depleted for 33 days. The plasma bicarbonate thresholds of phosphate depleted rats (Fig. 1) were identical to those of normal controls. These thresholds (approximately 25 mEq/l) are within the reported range for unanesthetized female Spraque-Dawley rats at comparable blood pCO_2 levels [17]. It should be emphasized, however, that the bicarbonate infusion was performed after 3 days of NH_4Cl administration. It is conceivable that this stimulation of hydrogen ion secretory capacity could have masked a defect in proximal tubule bicarbonate reabsorption. This may partially explain why tubular bicarbonate reabsorption appeared normal at commonly observed plasma bicarbonate levels in this study whereas Gold et al. [8] and Emmett et al. [6] demonstrated otherwise. Another reason for the difference between this study and that of Gold et al. may be related to the severe phosphate depletion and hypophosphatemia induced by the protocol of Gold and co-workers whereas less intense phosphate depletion occurred in our study. Species differences could also play a role in the different results. Gold et al. [9] invoked the concept of more severe phosphate depletion to account for their finding of impaired glucose reabsorption which differed from Harter et al.'s report of increased maximal glucose transport during phosphate depletion [11].

The finding that ammonium excretion in PD animals was similar to that of normal controls despite the fact that urine pH of PD rats was significantly higher deserves comment. One might conclude that ammonia excretion or production is actually higher in PD animals to permit an equivalent excretion of ammonium at a higher urine pH. Emmett et al. [6], however, have shown that renal cortical ammoniagenesis is not different between PD rats and normal controls. In addition, Leonard and Orloff have demonstrated that ammonia excretion by rats is not linearly related to urine pH [12]. Their studies showed no change in ammonia excretion as urine pH increased from 5.5 to 6.5. The urine pH values of PD rats and control rats in our study fall within this range. Thus, it is reasonable to conclude that in rats with the current degree of phosphate depletion, ammonia excretion is normal.

The present study has documented impaired distal tubular acidification during chronic phosphate depletion. The evidence for this is the finding that urine pH of control rats was significantly lower than that of phosphate depleted animals following an NH_4Cl load. In contrast, Emmett et al. [6] concluded that the hydrogen ion secretory defect in phosphate depletion was limited to the proximal tubule on the basis of a normal increase in urinary pCO_2 in two phosphate depleted rats following bicarbonate loading. However, complete assessment of the status of distal tubular acidification cannot be made on the basis of urinary pCO_2 alone. For example, urinary pCO_2 may be elevated despite impaired distal acidification if increased back diffusion of hydrogen ion is responsible for the defect. This has been shown to be the case in amphotericin B induced renal acidosis [18]. Furthermore, Arruda et al. have demonstrated that in highly alkaline urine, urinary pCO_2 is mainly determined by the concentration of urinary bicarbonate and as a consequence, urinary pCO_2 cannot be used solely to indicate distal hydrogen ion secretion [1]. The urinary bicarbonate levels at the time of pCO_2 measurement in the study of Emmett et al. were 120 and 144 mEq/l. Such concentrations are high enough to substantially elevate urinary pCO_2 without the addition of hydrogen ion [1].

The mechanism of the distal acidification defect in phosphate depleted rats cannot be determined from this study. It might be suggested that low urinary phosphate is responsible for the failure of the phosphate depleted rats to normally acidify their urine after NH_4Cl. By acting as an unreabsorbable anion, phosphate may be providing a stimulus for hydrogen ion secretion in controls that is not present in the urine of phosphate depleted rats. However, the fact that 3-day phosphate depleted animals responded normally to NH_4Cl loading despite levels of urinary phosphate as low as those in 33-day phosphate depleted rats argues against this explanation. Furthermore, the small amounts of phosphate present to buffer the urine should allow phosphate depleted rats to more readily lower urine pH than controls. Although the distal acidification defect revealed by abnormally high urine pH following NH_4Cl loading is not due to lack of unreabsorbable anion, alternative mechanisms could involve back diffusion of hydrogen ion or a partial defect in hydrigen ion secretion. The present study, however, does not allow determination of the type of mechanisms. The observation of elevated intracellular pH during phosphate depletion [8] is compatible with either a secretory or back diffusion defect in distal acidification. Low intracellular hydrogen ion concentration would favor back diffusion of hydrogen ion from the tubular lumen as well as limit hydrogen ion secretion. Theoretically, the degree of phosphate depletion could influence the extent of elevation of intracellular pH and therefore determine the severity of a distal nephron acidification defect.

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