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Research Article

Acid-base disturbances may develop secondary to the changes in renal tubular function and bone dynamics which attend phosphate depletion (PD). This work characterizes the acid-base status of rats fed a low phosphate diet. After 18 days, PD rats had marked calciuria (pair-fed controls: 0.3 +/- 0.2; PD 32.2 +/- 2.5 mueq/h; P less than 0.001), severe bicarbonaturia (controls: 0; PD 17.6 +/- 0.2 meq/h; P less than 0.001), and negative net acid excretion (controls: 44.5 +/- 2.9; PD: --6.6 +/- 2.5 meq/h; P less than 0.001), but plasma pH, HCO3, and PCO2 were equal in both groups. After 45 days, plasma HCO3 fell to 21.1 +/- 0.9 meq/liter in PD (controls: 23.6 +/- 0.5 meq/liter; P less than 0.05), while bicarbonaturia (controls: 0.4 +/- 0.2; PD: 3.8 +/- 1 mueq/h; P less than 0.02) and calciuria were present but diminished. These data suggested the coexistence of bone HCO3 mobilization and renal HCO3 wasting in PD. To test this thesis, bicarbonaturia was eliminated by nephrectomy. 24 h later plasma HCO3 was higher in PD rats (controls: 19.3 +/- 0.02; PD: 22.6 +/- 0.8 meq/liter; P less than 0.05), consistend with the presence of extrarenal HCO3 production. After inhibition of bone resorption with colchicine (1 mg/kg), plasma HCO3 decreased to 16.8 +/- 0.6 meq/liter in PD rats (controls): 26.4 +/- 1 meq/liter; […]

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The Pathophysiology of Acid-Base Changes in Chronically Phosphate-Depleted Rats

BONE-KIDNEY INTERACTIONS

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A B S T R A C T Acid-base disturbances may develop secondary to the changes in renal tubular function and bone dynamics which attend phosphate depletion (PD). This work characterizes the acid-base status of rats fed a low phosphate diet. After 18 days, PD rats had marked calciuria (pair-fed controls: 0.3±0.2; PD 32.2±2.5 μeq/h; $P < 0.001$), severe bicarbonaturia (controls: 0; PD 17.6±0.2 μeq/h; $P < 0.001$), and negative net acid excretion (controls: 44.5±2.9; PD: −6.6±2.5 μeq/h; $P < 0.001$), but plasma pH, HCO_3, and PCO_2 were equal in both groups. After 45 days, plasma HCO_3 fell to 21.1±0.9 meq/liter in PD (controls: 23.6±0.5 meq/liter; $P < 0.05$), while bicarbonaturia (controls: 0.4±0.2; PD: 3.8±1 μeq/h; $P < 0.02$) and calciuria were present but diminished. These data suggested the coexistence of bone HCO_3 mobilization and renal HCO_3 wasting in PD. To test this thesis, bicarbonaturia was eliminated by nephrectomy. 24 h later plasma HCO_3 was higher in PD rats (controls: 19.3±0.02; PD: 22.6±0.8 meq/liter; $P < 0.05$), consistent with the presence of extrarenal HCO_3 production. After inhibition of bone resorption with colchicine (1 mg/kg), plasma HCO_3 decreased to 16.8±0.6 meq/liter in PD rats (controls): 26.4±1 meq/liter; $P < 0.001$) while bicarbonaturia persisted. These data indicate that the plasma HCO_3 in PD is the net result of renal HCO_3 wasting and bone HCO_3 mobilization. These combined effects maintain normal

blood HCO_3 initially (18 days) but with time (45 days), bone resorption diminishes and the acidifying renal tubular defect predominates.

INTRODUCTION

Chronic phosphate depletion may alter acid-base balance by its effects on bone and kidney. Thus, bone contains large stores of alkali which, along with calcium, may be mobilized in certain pathologic conditions (1). Although unproven, the enhanced bone resorption, known to occur in phosphate depletion (2), could theoretically mobilize enough alkali to produce a metabolic alkalosis. The renal effects of phosphate depletion, however, could result in a different acid-base disturbance.

The hypophosphaturia which accompanies phosphate depletion should impair acid excretion, since phosphate serves as a major urinary buffer. In addition, the ability of the kidney to increase ammonia buffer synthesis and excretion to compensate for the lack of phosphate may also be restricted by phosphate depletion (3, 4). Finally Gold et al. (5) have recently shown that phosphate depletion in the dog impairs renal bicarbonate reabsorption. These combined effects of bicarbonate wasting and restricted buffer excretion should theoretically lead to a systemic acidosis.

Previously, Coburn and Massry were unable to clearly identify abnormalities in acid-base homeostasis in phosphate depletion, perhaps because of coexisting and offsetting disturbances (6). The present study was designed to define the effects of phosphate depletion on acid-base homeostasis in rats. Attention is directed specifically toward clarifying the roles played by bone and kidney.

METHODS

Male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) weighing 200 g were housed in individual metabolic cages in a temperature-, humidity-, and light-controlled environment. Experimental animals were fed a phosphate-deficient diet supplemented with 145 mmol/kg diet of both NaCl and KCl. Analysis of phosphate in the acid-ashed diet (ICN Pharmaceuticals Inc., Cleveland, Ohio) revealed less than 30 μg/100 g diet. The amount of diet ingested at 13, 18, and 40 days was 13.8±1.6, 15.5±2.2, and 16.7±2.3 g/day. Thus, no more than 5 μg of phosphate was ingested daily and the cumulative ingestion of phosphate during the first 40 days of feeding was less than 0.2 mg. Control animals were pair-fed the same diet supplemented with 80 mmol of both neutral sodium phosphate and neutral potassium phosphate per kg of diet, yielding a 0.5% phosphate diet. The calcium and vitamin D content of both diets were identical and equal to 0.4% and 2.2 U/g, respectively. Water was provided ad libitum. Urine was collected under mineral oil with phenylmercuric nitrate and thymol preservative. During the 24-h urine collections, food, but not water, was withheld. Blood was collected by aortic exsanguination under light anesthesia provided by 125 mg/kg of amobarbital. Arterial blood and urinary collections were obtained at 13, 18, and 45 days after the start of feeding.

Effects of phosphate depletion. To characterize changes in acid-base homeostasis with time, 54 phosphate-depleted and 54 control rats were prepared as described above. Equal numbers of rats from each group were sacrificed at 13, 18, and 45 days, after the collection of 24-h urine specimens and arterial blood. To determine rates of ammonia production, slices of renal cortex from three control and three phosphate-depleted rats were incubated in Krebs-Ringer bicarbonate buffer at pH 7.4 and the rate of ammonia production from 10 mM glutamine was assayed as previously described (7). In addition, two control and two phosphate-depleted rats were infused with bicarbonate and urine collected under oil for the measurement of pH, P_{CO_2}, and bicarbonate concentration.

Effects of bilateral nephrectomy. To evaluate the effects of the elimination of urinary bicarbonate excretion on systemic acid-base parameters, arterial blood was collected 24 h after bilateral nephrectomy in five control and five phosphate-depleted rats who had been maintained on their respective diets for 14 days. Light anesthesia was produced with amobarbital (125 mg/kg) and the kidneys were removed through a midline incision with care being taken to preserve adrenal vessels. Food and water were withheld during the anephric period.

Effects of colchicine. To determine the effects of inhibition of bone resorption upon acid-base status, four additional animals in each group were given 1 mg/kg of colchicine intraperitoneally, after 20 days of the appropriate diet. 2 h later, a 24-h urine collection was begun, after which the animals were sacrificed and urine and aortic blood analyzed.

Methods. Arterial and urine pH was measured with a Radiometer pH meter (model 27, Radiometer Co., Copenhagen, Denmark). Total CO_2 was determined with a Natelson microgasometer (model 650). Bicarbonate and P_{CO_2} were calculated using a pK' of 6.1 and a solubility coefficient of 0.0301 mmol/liter per torr for plasma and a urinary pK' of $6.33 - 0.5\sqrt{Na^+ + K^+ + NH_4^+}$ and a solubility coefficient of 0.0309 mmol/liter per torr for urine.

Titratable acid (TA)[1] was calculated from the urine pH and

the measured excretion rate of phosphate. The pK_2 of phosphoric acid was assumed to be 6.8 in plasma and the pK_2 in urine was calculated by the method of Schwartz et al. (8). Over the pH range encountered, creatinine contributed less than 1% to the TA and was therefore neglected. Urinary ammonia was measured by the method of Chaney and Marbach (9). Net acid excretion was calculated as $(TA + NH_4)$ $- HCO_3$. Plasma and urinary sodium and potassium, calcium, phosphorus, and creatinine were measured as previously described (10). Muscle potassium was measured in boiled extracts from the hind limb of control and phosphate-depleted rats, and was expressed as mmol/kg wet weight. Renal cortical ATP was assayed in quick-frozen tissue by previously published methods (11).

RESULTS

General response to phosphate depletion (Table I). Although phosphate-depleted rats gained less weight than pair-fed controls, the differences were small and all animals appeared healthy throughout the study. Diarrhea did not occur in either group. The plasma phosphate decreased after 13 days of depletion, but soon normalized and remained so for the duration of the study. The marked hypophosphaturia, however, was sustained for the entire 45-day period. Mild hypercalcemia (5.0±0.9 meq/liter) was found at 13 days and also normalized by 18 days. Renal function, as reflected by the serum creatinine and creatinine clearance, remained normal in both groups throughout the study. Phosphate-depleted (PD) rats demonstrated a persistently lower serum potassium at 18 and 45 days than controls, despite both groups having the same muscle potassium concentration at 18 days (control: 100.6±1.5; PD: 102.9±2.1 meq/kg wet weight).

Early (18 days) acid-base effects of phosphate depletion. After 18 days of phosphate depletion, striking increases in calcium and bicarbonate excretion were found (Fig. 1). Calcium excretion in phosphate-depleted rats was 3.1 meq/kg per day while that of bicarbonate was 1.65 meq/kg per day. The marked urinary alkalinity of phosphate-depleted rats was associated with a decreased excretion of TA and ammonia, resulting in negative net acid excretion ($-6.6±2.5$ μeq h). In contrast, pair-fed controls were excreting large amounts of net acid (44.5±2.9 μeq/h). Despite the marked bicarbonaturia present in phosphate-depleted rats, arterial parameters did not significantly differ from controls (Fig. 2).

Renal cortical ammoniagenesis from glutamine was no different in the two groups (control: 233±2.4, PD: 245±4.2 mmol/kg wet weight per h). Sodium bicarbonate infusions caused an equal increase in the urinary P_{CO_2} in phosphate-depleted and control rats. Thus, the urinary P_{CO_2} was 92 and 132 torr in the control rats when urine bicarbonate concentration was 114 and 130 meq/liter, respectively, while urine P_{CO_2} in the phosphate-depleted rats was 100 and 110 torr when bicarbonate concentration was 120

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[1] *Abbreviations used in this paper*: PD rats, phosphate-depleted rats; TA, titratable acid.

TABLE I
Effects of 13, 18, and 45 Days of Phosphate Depletion on Body Weight, Creatinine Clearance, and Electrolyte Metabolism*

	Body weight		Creatinine clearance		Plasma						Urine			
					Phosphate		Calcium		Potassium		Phosphate		Potassium	
	C	PD	C	PD	C	PD	C	PD	C	PD	C	PD	C	PD
	g		ml/min/kg		mg/dl		meq/liter		meq/liter		µg/h		µeq/h	
13 Days														
Mean	253	245	3.0	2.8	6.3	3.7	4.6	5.0	4.0	4.1	529	1.7	26.3	27.6
SEM	1.6	3.0	0.7	0.8	0.1	0.3	0.1	0.1	0.1	0.1	42	0.4	3.2	3.2
n	21	21	21	21	21	21	21	21	21	21	21	21	21	21
P	<0.05		NS		<0.001		<0.05		NS		<0.001		NS	
18 Days														
Mean	267	256	3.4	4.3	7.2	7.1	4.6	4.8	4.6	3.7	658	2.3	21.0	49.1
SEM	2.6	2.8	0.4	0.4	0.2	0.3	0.1	0.1	0.1	0.1	62	0.2	2.2	5.0
n	24	24	24	24	24	24	24	24	24	24	24	24	24	24
P	<0.01		NS		NS		NS		<0.001		<0.001		<0.001	
45 Days														
Mean	292	297	5.5	4.1	5.2	5.8	4.3	4.0	4.9	4.5	534	7.2	15.5	11.0
SEM	4.4	4.3	1.0	0.7	0.2	1.0	0.1	0.2	0.1	0.1	32	1.0	3.5	1.2
n	9	9	9	9	9	9	9	9	9	9	9	9	9	9
P	NS		NS		NS		NS		<0.05		<0.001		NS	

* C and PD refer to control and phosphate-depleted rats, respectively. n refers to number of animals in each group. P values refer to the significance of the mean difference between C and PD.

and 144 meq/liter. Simultaneous plasma P_{CO_2} ranged between 30 and 35 torr in all four rats during the study.

Analysis of renal cortical ATP concentration in five animals from each group after 18 days of feeding revealed no significant differences (control: 1.5±0.06; PD: 1.7±0.08 mmol/kg wet weight renal cortex).

Late (45 days) acid-base effects of phosphate depletion. Fig. 3 contrasts the values for the excretion of calcium and bicarbonate and the plasma bicarbonate concentration found at 18 days with those values found at 45 days in controls and phosphate-depleted rats. While no changes were found in controls, phosphate-depleted rats demonstrated striking and parallel decreases in calcium and bicarbonate excretion. The values for both parameters at 45 days were significantly lower than those found at 18 days ($P < 0.001$), although they still remained greater than controls (calcium: $P < 0.05$; bicarbonate: $P <0.02$). Whereas control rats showed a slight but statistically insignificant increase of their plasma bicarbonate concentration at 45 days, phosphate-depleted rats demonstrated a decrease (control: 23.6 ± 0.5, PD: 21.1±0.9 meq/liter; $P < 0.05$). Although the serum bicarbonate concentration of phosphate-depleted animals fell at 45 days, substantial amounts of bicarbonate remained in their urine (3.8±1 µeq/h). The arterial pH and P_{CO_2} tended to fall in phosphate-depleted rats at 45 days but the decrease did not

reach statistical significance (pH control: 7.48±0.01; PD: 7.46±0.02; P_{CO_2} control: 34.1±0.6; PD: 30.2±1.2 torr). Due to the reduced bicarbonaturia, net acid excretion rose in phosphate-depleted rats but remained well below that of controls (control: 40.0±1.9; PD: 12.8±1.8 µeq/h; $P < 0.001$).
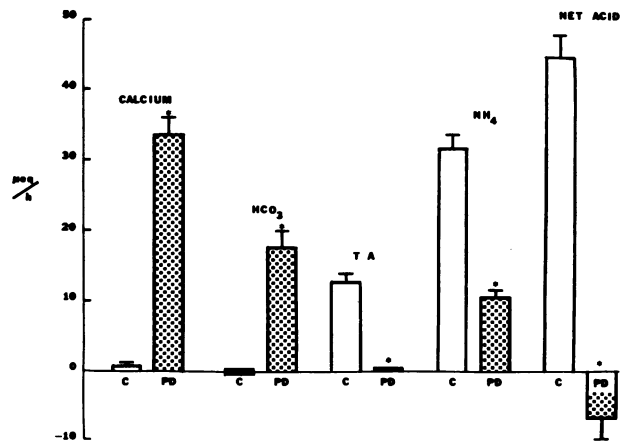

FIGURE 1 Effect of 18 days of phosphate depletion on urinary excretion of calcium, bicarbonate, titratable acid, ammonium, and net acid. C refers to control rats (open bars) fed low phosphate diet with phosphate supplement and PD refers to phosphate-depleted rats (shaded bars). Each bar represents mean±SEM of each group. * = $P < 0.01$ compared to controls.

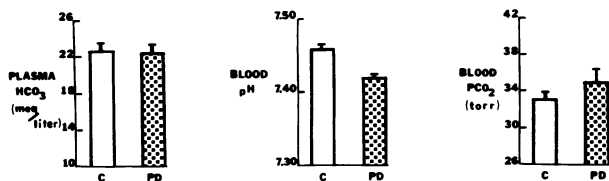FIGURE 2 Arterial bicarbonate, pH, and P_{CO_2} in control and phosphate-depleted rats. Abbreviations as in Fig. 1.

The maintenance of a normal serum bicarbonate concentration at 18 days despite marked bicarbonaturia suggests the presence of an extra-renal source of bicarbonate, while the continued bicarbonaturia at 45 days in the face of a decreased filtered load suggests urinary bicarbonate wasting. The next two series of experiments were performed to further delineate these possibilities. In the first series, the response of the plasma bicarbonate concentration to the elimination of urinary bicarbonate wasting by nephrectomy was studied, while the second series defined the plasma response to inhibition of bone resorption with colchicine.

Effects of nephrectomy. 24 h after bilateral nephrectomy, after 14 days of diet, the serum creatinine concentration rose equally in both groups (control: 4.1±0.05; PD: 4.2±0.1 mg/dl). At this time (Fig. 4), control rats had a serum bicarbonate concentration of 19.3±0.2 meq/liter, which was significantly lower than the 22.6±0.8 meq/liter found in phosphate-depleted rats ($P < 0.005$). Although the arterial pH and P_{CO_2} fell in controls, they were not significantly different from phosphate-depleted rats (Fig. 3). After nephrectomy, the plasma calcium in phosphate-depleted rats was not statistically different from that of controls (control: 4.5±0.11; PD: 4.8±0.13 meq/liter; NS), but

FIGURE 3 Effect of 45 days of phosphate depletion on urinary calcium and bicarbonate excretion and plasma bicarbonate. Data represent mean±SEM for phosphate-depleted rats (dotted line) and control rats (solid line) fed low phosphate diet with phosphate supplement.

a marked hyperphosphatemia occurred in controls (control: 12.7±0.6; PD: 6.0±0.4 mg/dl; $P < 0.001$).

Effects of colchicine (Fig. 5). After 20 days of diet, the administration of colchicine decreased the plasma calcium in phosphate-depleted rats, whereas no change was found in controls. Bicarbonate excretion in control rats fell from 2.3±0.7 μeq/h to zero in response to colchicine, and net acid excretion rose from 18.3±1.1 to 53.2±11.9 μeq/h. The striking increase in acid excretion was associated with a significant increase in plasma bicarbonate concentration, from 22.2±0.6 to 26.4±1.0 meq/liter ($P < 0.05$). In sharp contrast to controls, colchicine treatment of phosphate-depleted rats was associated with continued bicarbonaturia (control: 0; PD: 5.8±2.4 μeq/h; $P < 0.001$) despite the abrupt fall in plasma bicarbonate concentration from 23.5±0.8 to 16.8±0.6 meq/liter; $P < 0.001$. Although arterial pH and P_{CO_2} both fell in the phosphate-depleted rats after colchicine (pH control: 7.56±0.03; PD: 7.45 ±0.04; P_{CO_2} control: 30.5±1.2; PD: 25.3±3.1 torr), the changes were not significant.

DISCUSSION

This study demonstrates that 18 days of phosphate depletion is characterized by marked hypercalciuria and massive bicarbonaturia, despite the absence of significant changes in the filtered load of calcium or bicarbonate. As a consequence of the hypophosphaturia and high urine pH, the excretion of titratable acid and ammonia was markedly reduced, causing net acid excretion to actually become negative. Despite this enhanced loss of alkali, plasma acid-base parameters remained unchanged. Increased bicarbonate excretion in the absence of a systemic alkalosis can be due to either a failure of renal tubular reabsorption, or increased availability from the diet, intermediary metabolism, or mobilization of body stores.

Although previous studies have suggested the presence of renal tubular bicarbonate wasting in this syndrome (5), the absence of a systemic acidosis clearly excludes pure renal bicarbonate wasting as the sole cause of the bicarbonaturia. A dietary source of alkali is excluded by the absence of bicarbonate diuresis in pair-fed controls. Phosphate depletion could have caused metabolic changes which in turn resulted in the overproduction of alkali. It has recently been shown, for example, that such metabolic derangements as glucose intolerance and hyperinsulinemia are caused by the depletion of phosphate (12). However, in the absence of alkali ingestion, only gastrointestinal and renal losses of acid are known to enhance net synthesis of bicarbonate. Since the kidneys were excreting alkali and not acid and since gastric losses were not observed to occur,
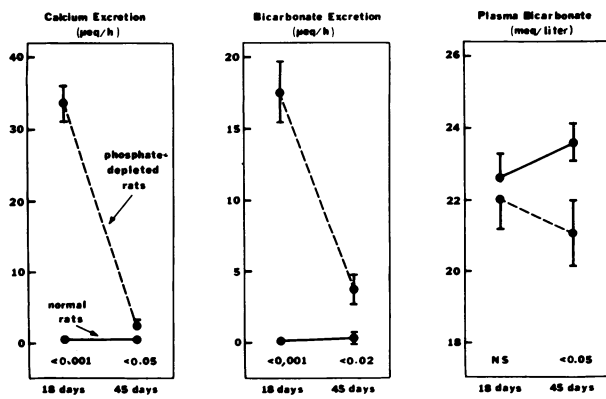
these possibilities may be safely excluded. We are unaware of any abnormality of cellular metabolism which causes excessive alkali production. It is conceivable that redistribution of hydrogen ions from the extracellular to the intracellular space resulted in a small increase in plasma bicarbonate which in turn led to bicarbonaturia. Intracellular pH, however, has been shown to rise in phosphate depletion, thereby excluding this possibility (5). For the present, enhanced metabolic production of bicarbonate, does not appear to be a tenable explanation for the bicarbonaturia. It seems likely, therefore, that the excreted bicarbonate must have derived from some endogenous store, mobilized by the stimulus of phosphate depletion.

To document the presence of this extra-renal source of bicarbonate in phosphate depletion we abolished urinary bicarbonate wasting by nephrectomizing rats from both groups. Normally, this leads to the retention of fixed acids, causing the production of a progressive metabolic acidosis. The presence of another bicarbonate source in the phosphate-depleted rats, however, might be expected to decrease the severity of this acidosis. The higher plasma bicarbonate concentration found in phosphate-depleted rats 24 h after nephrectomy provides strong support for this thesis. The body stores of bicarbonate in cellular and extracellular water approximate 20 meq/kg (13). If the observed loss of 1.65 meq/kg per day of bicarbonate from phosphate-depleted rats was solely derived from these compartments, a severe acidosis should have developed. The absence of even mild acidosis clearly indicates that the bicarbonate must have come from some bicarbonate-rich, nonextracellular fluid source.

Recent studies suggest that bone serves as a vast reservoir for bicarbonate and therefore is a potential source of the excessive urinary bicarbonate in our studies. Thus, Kildeberg et al. (14) have shown that the deposition of alkaline calcium salts in the bones of growing infants accounts for a large portion of their daily acid load. In addition, the demonstration that acid ingestion is associated with a positive hydrogen balance despite the absence of a falling plasma bicarbonate concentration, but with a coexistent negative calcium balance, suggests that alkaline bone salts serve as a buffer for the ingested acid (1, 15). As enhanced bone resorption is a prominent feature of phosphate depletion (2), it seems likely that bone dissolution, with release of its alkali, is the source of the bicarbonaturia observed in our study. This conclusion is strengthened by the finding of an associated hypercalciuria and by the response of phosphate-depleted rats to colchicine.

Although both groups of rats ingested approximately 60 mg of calcium daily, controls excreted 0.1 − 0.5 mg/day while phosphate-depleted rats excreted 15–20
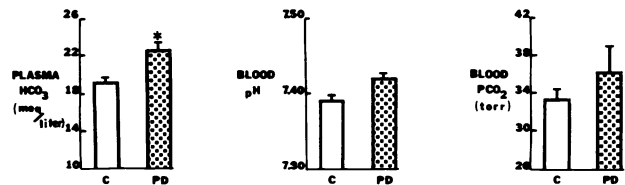

FIGURE 4 Effects of bilateral nephrectomy on arterial bicarbonate, pH, and PCO_2 after 14 days of phosphate depletion. Bars represent mean±SEM of values obtained 24 h after nephrectomy in control rats (open bars) and phosphate-depleted rats (shaded bars). * = $P < 0.05$.

mg/day. This marked calciuria in depleted rats could have resulted from enhanced absorption of dietary calcium and (or) enhanced mobilization and excretion of bone reserves.

Sammon et al. (16) have shown that 200-g rats absorb approximately one-half of their ingested calcium and that more than 97% is deposited in bone. Although phosphate depletion enhances calcium absorption (17), the quantitative increase is not well defined (18). Thus, although enhanced absorption could account for the hypercalciuria, three lines of evidence suggest that bone absorption played the major role. Firstly, Baylink et al. (2) have shown that phosphate depletion results in marked increases in bone resorption. This could account for the calciuria by itself, or indirectly by the failure of bone, to take up the enhanced load of dietary calcium. Secondly, since our rats were fasting during their 24-h urine collection, the hypercalciuria is much less likely to reflect the dietary load. Lastly, the marked hypocalcemia resulting from the inhibition of bone resorption by colchicine (see below) adds strong support to the dominant role of bone resorption.
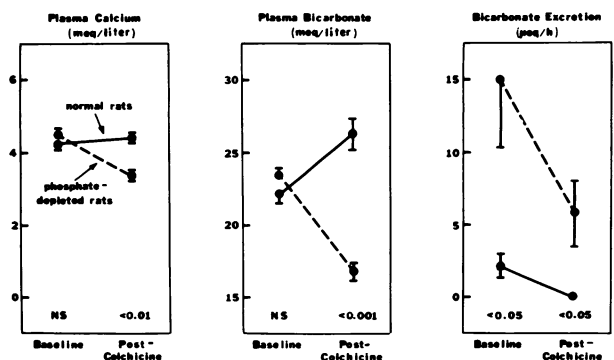
Colchicine has been shown to decrease net bone

FIGURE 5 Effects of colchicine on plasma calcium, plasma bicarbonate, and bicarbonate excretion after 20 days of phosphate depletion. Points represent mean±SEM. Base line refers to 20-day control and phosphate-depleted rats sacrificed before colchicine. Post-colchicine refers to 20-day control and phosphate-depleted rats sacrificed 24 h after receiving 1 mg/kg of colchicine intraperitoneally.

resorption in vitro and in vivo by impairing the response of osteoclasts to parathyroid hormone and by directly inhibiting osteoclast function (19–21). It therefore, appears that colchicine is well suited for demonstrating the bone-kidney interactions on acid-base balance in phosphate depletion. If a normal serum bicarbonate was the resultant of the offsetting effects of bone-bicarbonate mobilization and renal bicarbonate wasting, the acute inhibition of bone resorption by colchicine, in the face of continued bicarbonate wasting, should result in marked acidosis.

As predicted, the plasma bicarbonate concentration fell by almost 7 meq/liter in phosphate-depleted rats after colchicine therapy. This fall is probably best explained by two effects. First, the suppression of bone resorption markedly reduced entry of bicarbonate into the extracellular spaces and allowed for the net deposition of alkaline calcium salts into previously depleted bone. This is analogous to the "hungry bone syndrome" seen after the acute removal of parathyroid hormone in patients with hyperparathyroidism that is associated with hypocalcemia and a reduction in serum bicarbonate (22). While this mechanism probably played the major role, impairment of renal bicarbonate reabsorption also contributed to the fall in serum bicarbonate in our rats. The continued bicarbonaturia in the colchicine-treated, phosphate-depleted rats, despite their marked reduction in the filtered load of bicarbonate, implies defective reabsorption. If this urinary bicarbonate were derived from the cellular and extracellular spaces, it would account for a decrease in plasma bicarbonate concentration of approximately 1.5 meq/liter.

Control rats responded to colchicine by reabsorbing all filtered bicarbonate and increasing titratable acid and ammonia excretion. This marked increase in net acid excretion resulted in the synthesis of enough new bicarbonate to increase its plasma level from 22.2 ± 0.5 to 26.4 ± 1.0 meq/liter. These changes, which are opposite in direction from those of phosphate-depleted rats, lend even more credance to the above conclusions. The mechanism by which colchicine enhances renal bicarbonate synthesis is not explained by these data. While it has been shown that colchicine therapy impairs the renal response of rats to antidiuretic hormone (23) and parathyroid hormone (24), no direct effects or renal acid-base balance have been shown. It is possible that the variable sodium, potassium and phosphate wasting caused by colchicine (24) reflects a slight impairment of proximal tubular sodium and phosphate transport. Reclaimation of some of this sodium at the distal exchange sites could result in the enhanced excretion of acid (25). Further studies will be required to define this interesting question.

The presence of a bicarbonate reabsorptive defect is also suggested by our studies, at 18 days, when phosphate-depleted rats exhibit hypokalemia and tendency toward hypercalcemia. Both of these electrolyte changes as well as the presumed functional hypoparathyroidism of phosphate depletion[2] normally act to increase renal bicarbonate reabsorption (26–28). The failure of these alterations to effect an increase in the plasma bicarbonate concentration, especially in the face of the increased load of bone-bicarbonate being presented to the kidneys, strongly suggests that renal bicarbonate reabsorption is defective in the rats made phosphate deficient. At 18 days this defect is masked by the enhanced release of bicarbonate from bone. Thus, the data suggest the presence of a primary defect in renal tubular bicarbonate reabsorption in phosphate depletion which is masked by enhanced release of alkali from bone. The mild, but persistent, hypokalemia that developed at 18 days (Table I) was almost certainly secondary to the bicarbonate wasting. It is well recognized that bicarbonaturia impairs the net reabsorption of potassium from the glomerular filtrate (29). The apparent improvement in the hypokalemia at 45 days is consistent with the diminished bicarbonaturia, kaliruia, and mild acidosis found at this time.

The mechanism of the defect in renal tubular bicarbonate transport is not apparent from our data. Previous studies in phosphate depletion have demonstrated impairment of both glucose and bicarbonate reabsorption (5, 30), and recent studies in the dog have shown a generalized defect in proximal tubular sodium reabsorption (31). The ability of our phosphate-depleted rats to normally increase urinary P_{CO_2} in response to sodium bicarbonate loading is indirect evidence of normal distal tubular hydrogen ion secretion (32), indicating that the hydrogen secretory defect in phosphate depletion is limited to the proximal tubule. The mechanism of these changes in tubular function remains undefined. Our data demonstrating a normal renal cortical ATP level, however, would suggest that deficiency of high energy phosphate production is not involved, as has been suggested in other tissues (6).

It is possible, although unlikely, that the constant exposure of the kidney to the large bicarbonate load coming from bone was responsible for its altered acid-base response. If bicarbonate mobilization were the primary factor and a slight but imperceptible metabolic alkalosis existed to explain the data, then there should have been a demonstrable inhibition of renal ammoniogenesis. Studies of renal cortical slices, however, revealed a normal response in our phosphate-depleted rats. Phosphate is known to play a vital role

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[2] Slatopolsky, E. Personal communication.

in renal ammonia synthesis. The hydrolysis of glutamine to glutamate with release of the amide nitrogen as free ammonia is catalyzed by glutaminase I. The activity of this enzyme has been shown to be highly dependent on the presence of phosphate (3). The metabolic disposal of glutamine's carbon skeleton is linked to its active transport out of the mitochondria in exchange for phosphate (4). When phosphate is not available these mitochondrial end products accumulate, causing feedback inhibition of glutaminase I activity and, thereby, of ammoniagenesis (4). It is of interest, therefore, that despite signs of marked phosphate depletion, in vitro renal cortical ammoniagenesis from glutamine was unchanged. The questions of whether more severe depletion is required to show the impairment, or whether other anions may substitute for phosphate, remain to be explored.

The mechanism of alterations in urinary calcium excretion in phosphate depletion remains undefined (6); however, it seems clear that resorbed bone must be the ultimate source, similar to bicarbonate. In this context, it is interesting that at 45 days the 24-h urinary excretion of bicarbonate and calcium were both greatly diminished, although still greater than control. In the face of sustained normal renal function, these data suggest a decrease in bone resorption, possibly related to accumulating osteoid which would decrease the surface area available for resorption. The continued loss of urinary bicarbonate with diminished release from bone is most likely responsible for the decrease in plasma bicarbonate concentration observed in these rats compared to controls. The mechanism for the changes in calcium excretion in the absence of changes in filtered load is not apparent from our data. It is possible that tubular reabsorption is altered by some factor such as parathyroid hormone which responds to changes in bone resorption. If phosphate balance were less negative at 45 days than at 13 or 18 days, then a diminished stimulus to bone resorption might obtain and thereby explain the falling bicarbonaturia and calciuria. Since cumulative phosphate balance became progressively more negative, this explanation appears untenable. It is true, however, that despite ongoing phosphate loss plasma phosphate normalized at 18 days. This observation was first made by Schneider and Steenbock (33) who demonstrated that plasma phosphate normalized after the addition of vitamin D to the diet of phosphate-deprived rats. The protocol of the present study similarly included vitamin D in the diet and the avoidance of phosphate binding agents resulting in more modest degrees of depletion. Under these experimental conditions it would appear that bone phosphate is able to normalize the plasma level. It is also conceivable that there is an adaptation by peripheral tissues other than bone to chronic phosphate depletion resulting in alterations of cellular stores, enabling plasma phosphate to rise toward normal. Obviously, further experiments are required to satisfactorily answer this question.

In summary, this study demonstrates that the prevailing plasma bicarbonate concentration found in chronic phosphate depletion is the net result of renal bicarbonate wasting and the release of bone bicarbonate stores. Initially, (18 days), these effects are offsetting and there are no demonstrable changes in systemic acid-base parameters. With continued phosphate depletion, however, bone resorption diminishes and the acidifying renal tubular defect predominates, producing a fall in plasma bicarbonate concentration.

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