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Research Article

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Relationship between Phosphaturia and Acute Hypercapnia in the Rat

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ABSTRACT Standard clearance studies were performed in mechanically ventilated intact and acutely thyroparathyroidectomized (TPTX) rats to document and characterize the effect of hypercapnia (HC) on urinary phosphorus excretion (U_PV). HC as compared to normocapnia (NC) was associated with an increase in U_PV in intact (62.5 vs. 7.93 μg/min) and TPTX (30.5 vs. 0.59 μg/min) rats, an increase in filtered load of phosphorus in intact (218 vs. 191 μg/min) and TPTX (243 vs. 146 μg/min) rats, an increase in blood bicarbonate concentration in intact (27.8 vs. 26.0 meq/liter) and TPTX (24.5 vs. 22.3 meq/liter) animals, and a decrease in blood pH in intact (7.15 vs. 7.42) and TPTX (7.07 vs. 7.39) rats. Additional TPTX rats with NC and HC were studied during phosphorus infusion at a comparable filtered load of phosphorus (NC = 307 μg/min and HC = 328 μg/min). U_PV was 18.5 μg/min in NC and 85.2 μg/min in HC animals. Intact NC animals infused with $NaHCO_3$ achieved a blood bicarbonate of 45.9 meq/liter compared to 26.0 meq/liter in intact NC NaCl-infused rats. U_PV was 10.0 μg/min in the $NaHCO_3$ and 7.93 μg/min in NaCl-infused animals. In intact HC animals infused with $NaHCO_3$, blood pH was 7.36 compared to 7.42 in NC intact NaCl-infused animals. U_PV was 83.2 μg/min in the HC bicarbonate-infused and 7.93 μg/min in the NC NaCl-infused rats. These experiments demonstrate that elevated blood carbon dioxide tension per se increases U_PV. Increases in filtered load of phosphorus and blood bicarbonate which are associated with HC contribute to the phosphaturia as does parathyroid hormone. The phosphaturia is not dependent upon reduction of extracellular pH.

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INTRODUCTION

Previous studies have demonstrated that acute hypercapnia in animals with intact parathyroid glands is associated with an increase in the urinary excretion of phosphorus (1–9). The exact mechanism responsible for this phosphaturia remains unknown. A rise in the plasma phosphorus has been noted by some investigators (1, 4, 10–12) and consequently the phosphaturia has been attributed to an increase in the filtered load of phosphorus (1, 4, 9). However, in those studies a possible contributory role by other major determinants of phosphorus excretion either was not evaluated or the studies were not designed in such a way as to permit an adequate evaluation of their potential contribution. Factors known to increase urine phosphorus excretion which might become operative during acute hypercapnia include an increase in the release of parathyroid hormone (PTH)[1] (13–15) an enhancement of PTH effect on the kidney (16–18), and extracellular fluid (ECF) volume expansion (19–21). Furthermore, changes in either the blood or urine pH (22–26) or an increase in the blood bicarbonate concentration or renal reabsorption of bicarbonate (23, 24, 27, 28) might also influence renal phosphate reabsorption.

The present experiments were designed to examine the nature of the phosphaturia that occurs in association with acute hypercapnia. The results indicate that

[1] *Abbreviations used in this paper*: B, bicarbonate infused; B_{HCO_3}, blood bicarbonate concentration; $BpCO_2$, blood carbon dioxide tension; B_{pH}, blood pH; ECF, extracellular fluid; EF_{Na}, excreted fraction of filtered sodium; EF_p, excreted fraction of filtered phosphorus; F_p, filtered load of phosphorus; GFR, glomerular filtration rate; HC, hypercapnia; NC, normocapnia; P, phosphorus infused; P_{Ca_I}, ionized plasma calcium; P_{Ca_T}, total plasma calcium; P_P, plasma phosphorus; PTH, parathyroid hormone; RBF, renal blood flow; T_{HCO_3}, tubular bicarbonate reabsorption; TPTX, thyroparathyroidectomized; $U_{cAMP}V$, urinary cyclic AMP excretion; $U_{Ca}V$, urinary calcium excretion; $U_{Cl}V$, urinary chloride excretion; $U_{Na}V$, urinary sodium excretion; U_PV, urinary phosphorus excretion; V, urine flow.

an increase in the filtered load of phosphorus (F_P), stimulation of endogenous PTH release or enhancement of its effect on the kidney, ECF volume expansion, an increase in blood or renal reabsorption of bicarbonate, and extracellular acidosis are not the major mechanisms responsible for the phosphaturia. Instead, a direct or indirect renal effect of the increase in blood carbon dioxide tension appears to be a likely determinant of the phosphaturia observed during acute hypercapnia.

METHODS

The studies were performed on 40 female Holtzman Sprague-Dawley rats weighing 200–260 g. Animals were fed a diet (Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.) containing 1.20 g of calcium, 0.86 g of phosphorus, and 530 IU of vitamin D per 100 g, and were maintained on a time cycle of 12 h of light and 12 h of darkness. Experiments were always initiated on nonfasted rats between the hours of 8:00 and 11:00 a.m. Anesthesia was induced with intraperitoneal sodium pentobarbital (45 mg/kg body wt) as a solution of 15 mg in 1 ml of a NaCl solution (osmolality: 300 mosmol/kg H_2O) with administration of small additional doses as needed. Animals were placed on a thermostatically controlled warming table to maintain their body temperature between 37° and 38°C. Intubation was accomplished via a tracheostomy with a polyethylene (PE 240) endotracheal tube, and animals breathed room air supplemented with 100% O_2 during the subsequent surgery. Thyroparathyroidectomy, if required, was then performed utilizing blunt dissection. The right femoral artery and vein were then cannulated with polyethylene tubing (PE 50) through an inguinal incision. Mean arterial blood pressure was monitored continuously with a Statham pressure transducer (model no. P23Db, Statham Instruments, Div. Gould Inc., Oxnard, Calif.). All blood samples for chemical determinations were obtained from the arterial line. The bladder was cannulated with PE 100 tubing. A hypertonic NaCl solution (osmolality: 400 mosmol/kg H_2O) was given i.v. in small intermittent doses (total dose: 1 ml/100 g body wt) to replace estimated fluid losses during surgical preparation. Upon the completion of surgery, 0.5 ml of the hypertonic NaCl solution containing 1 μCi [carboxyl-^{14}C]inulin (New England Nuclear, Boston, Mass.) and 2.5 μCi ^{3}H-*p*-aminohippuric acid (New England Nuclear) was injected i.v. This was followed by a continuous sustaining infusion of the same hypertonic NaCl solution administered at a rate calculated to deliver a volume equivalent to 4.5% of the body weight/h. The latter solution contained 0.032 μCi [carboxyl-^{14}C]inulin/ml and 2.8 μCi ^{3}H-*p*-aminohippuric acid/ml. In some experiments a sustaining infusion of different composition was utilized (see below). At this point muscle relaxation was induced with i.v. tubocurarine chloride (E. R. Squibb & Sons, Princeton, N. J.) at an initial dose of 0.12 mg, and the animal was placed on a mechanical respirator (small animal respirator, model no. 680, Harvard Apparatus Co., Inc., Millis, Mass.). Additional doses of tubocurarine chloride were given as needed.

After completion of all surgery and placement of the animal on assisted ventilation, the following experimental protocol was initiated. Each experiment included a 40-min period of equilibration followed by four 20-min clearance periods. In those animals subjected to thyroparathyroidectomy at least 2 h elapsed from the completion of the surgical procedure to initiation of the first clearance period. At the beginning of the equilibration period, the sustaining infusion administered during surgery was altered as described below, depending on the experimental condition under study. Blood samples were drawn at the completion of surgery, at the midpoint of the equilibration and clearance periods, and at the end of the fourth clearance period. Urine was always collected under oil during the equilibration period and each of the four clearance periods. Only animals with a blood oxygen saturation of at least 90 mm Hg were studied as described below. Eight groups of animals were studied.

Healthy control animals (group NC [normocapnia]). Five animals were studied during the constant infusion of hypertonic NaCl alone (osmolality: 400 mosmol/kg H_2O). The inspired gas mixture was 30% O_2 and 70% N_2, and the respirator was adjusted to maintain the blood carbon dioxide tension ($BpCO_2$) at approximately 40 mm Hg.

Acute hypercapnia (group HC). Five animals were studied in the same manner as NC animals except the inspired gas mixture was adjusted to contain 10% CO_2, 30% O_2, and 60% N_2 with the respirator adjusted to maintain the $BpCO_2$ at approximately 80 mm Hg.

Thyroparathyroidectomized animals (group TPTX). Five animals were studied in a manner identical to the NC animals except they underwent surgical thyroparathyroidectomy at least 2 h before the start of the clearance periods.

Thyroparathyroidectomy with acute hypercapnia (group HC-TPTX). Five animals were studied as in group HC except an acute thyroparathyroidectomy was performed as in the TPTX animals.

Bicarbonate-loaded animals (group B). Five rats were

TABLE I
Effects of HC

	B_{pCO_2}	B_{pH}	B_{HCO_3}	T_{HCO_3}	GFR	P_P	F_P	U_PV
	mm Hg		*meq/liter*	*μeq/min*	*ml/min*	*mg/100 ml*	*μg/min*	*μg/min*
NC	41.2	7.42	26.0	80.7	3.08	6.19	191	7.93
±SD	3.0		1.3	21.7	0.74	1.01	51	6.01
(n = 5)								
HC	81.5	7.15	27.8	89.2	3.19	6.89	218	62.5
±SD	3.5		2.6	22.3	0.58	0.97	41	9.7
(n = 5)								
P*	<0.001	<0.001	<0.02	>0.2	>0.6	<0.05	>0.05	<0.001

* NC vs. HC animals.

studied in a manner identical to NC animals except the rats received a constant infusion of $NaHCO_3$ (osmolality: 400 mosmol/kg H_2O) in place of the hypertonic NaCl solution.

Compensated metabolic alkalosis (group HC-B). Five animals were studied in a manner similar to those in group B (sodium bicarbonate infusion) except the inspired gas mixture was 10% CO_2, 30% O_2, and 60% N_2.

Phosphate-loaded thyroparathyroidectomized animals (group P-TPTX). Five animals were studied in a manner identical to the TPTX animals except the infusate contained sodium phosphate (40 mg/100 ml) and the osmolality was adjusted to 400 mosmol/kg H_2O by the addition of NaCl. The pH of the solution was 7.4 with the molar ratio of Na_2HPO_4/NaH_2PO_4 being 4:1.

Phosphate-loaded thyroparathyroidectomized animals with acute hypercapnia (group HC-P-TPTX). Five animals were studied in a manner identical to the P-TPTX animals (sodium phosphate infusion) except the inspired gas mixture was 10% CO_2, 30% O_2, and 60% N_2.

Glomerular filtration rate (GFR) was determined by inulin clearance which was calculated by dividing urinary excretion rate of ^{14}C-radioactivity by plasma ^{14}C-radioactivity. Renal plasma flow (RPF) was determined by *p*-aminohippuric acid clearance which was calculated by dividing the urinary excretion rate of ^{3}H-radioactivity by plasma ^{3}H-radioactivity. Renal blood flow (RBF) was calculated from the relationship: RBF = RPF/1-hematocrit. The radioactivity in the blood and urine specimens was counted by liquid scintillation spectrometry (Isocap/300, Searle Analytic Inc., Des Plaines, Ill.).

Concentration of sodium and potassium in plasma and urine were determined by flame photometry (model 143, Instrumentation Laboratory, Inc., Lexington, Mass.). Plasma and urine osmolalities were determined with a vapor pressure osmometer (model 5130, Wescor Inc., Logan, Utah). Total calcium concentration in urine and plasma was determined with a fluorometric titrator (Calcette, Precision Systems, Inc., Sudbury, Mass.). Ionized calcium in plasma was measured on blood specimens drawn and centrifuged anaerobically with a flow-through calcium electrode (model 99-20, Orion Research Inc., Cambridge, Mass.). Plasma and urine phosphorus concentrations were measured with a Pierce Phosphorus Auto/Stat TM Kit (Pierce Chemical Co., Rockford, Ill.).

Urine pH and pCO_2 and blood pH, pCO_2, and pO_2 determinations were performed with a pH blood gas analyzer (model 213, Instrumentation Laboratory). Plasma and urine bicarbonate concentrations were calculated from the pH and pCO_2 data by the Henderson-Hasselbalch equation. For calculation of blood bicarbonate, a pKa of 6.1 and a CO_2 solubility coefficient of 0.0301 were used. A CO_2 solubility coefficient of 0.0309 was used for the calculation of urine bicarbonate. The pKa used for urinary bicarbonate was derived from the relationship: $pKa = 6.33 - 0.5\sqrt{Na^+ + K^+}$ with Na^+ and K^+ representing the urinary concentration of these ions in Eq/l. Urinary cyclic AMP concentrations were measured by the Gilman competitive binding assay (29).

Statistical methods. Statistical comparisons were made utilizing Student's unpaired *t* test. All data listed in the tables represent the mean±SD of 20 observations derived from five animals in each group studied.

RESULTS

The results of the studies in intact NC and HC animals are summarized in Table I. The presence of respiratory acidosis is obvious. Although the blood bicarbonate concentration (B_{HCO_3}) was slightly greater in HC animals, the mean values for the tubular reabsorption of bicarbonate (T_{HCO_3}) did not differ significantly between the two groups. Urine pH averaged 5.63 in NC and 5.78 in HC animals ($P > 0.05$). Plasma phosphorus concentration (P_P) was significantly greater in HC animals, but the filtered load of phosphorus, although greater in HC animals, was not statistically different from that measured in NC animals. A marked increase in absolute urinary phosphorus excretion (U_PV) and the excreted fraction of filtered phosphorus (EF_P) was observed in HC animals. GFR, total plasma calcium (P_{Ca_T}), ionized plasma calcium (P_{Ca_I}), urinary calcium excretion ($U_{Ca}V$), and urinary cyclic AMP excretion ($U_{cAMP}V$) were not significantly different in the two groups of animals. Mean values for RBF, urine flow (V), urinary sodium excretion ($U_{Na}V$), the excreted fraction of filtered sodium (EF_{Na}), and urinary chloride excretion ($U_{Cl}V$) were not significantly different in the two groups, thus indicating that the degree of ECF volume expansion was similar in HC and NC animals. However, urinary potassium excretion (U_KV) was greater in HC animals ($P < 0.02$) and there was a small but significant increase in the blood bicarbonate concentration. Thus, acute HC in intact animals produced

in Intact Rats

EF_P	P_{Ca_T}	P_{Ca_I}	$U_{Ca}V$	$U_{cAMP}V$	RBF	V	$U_{Na}V$	EF_{Na}	$U_{Cl}V$	U_KV
%	*mg/ 100 ml*	*mg/ 100 ml*	*μg/min*	*pmol/ min*	*ml/ min*	*μl/ min*	*μeq/ min*	%	*μeq/ min*	*μeq/ min*
4.1	8.12	4.29	7.40	259	12.3	96.2	20.7	4.62	25.4	3.15
2.7	0.44	0.33	3.92	133	2.1	42.1	8.4	1.70	9.6	0.43
29.7	8.52	4.30	7.64	239	12.7	82.0	20.2	4.37	24.2	3.54
7.3	0.78	0.50	2.34	110	2.3	27.1	5.8	0.87	7.3	0.56
<0.001	>0.05	>0.9	>0.8	>0.6	>0.5	>0.2	>0.8	>0.5	>0.6	<0.02

TABLE II
Effects of HC

	B_{pCO_2}	B_{pH}	B_{HCO_3}	T_{HCO_3}	GFR	P_P	F_P	U_PV
	mm Hg		*meq/ liter*	*μeq/ min*	*ml/ min*	*mg/ 100 ml*	*μg/ min*	*μg/ min*
NC-TPTX	37.9	7.39	22.3	60.0	2.68	5.45	146	0.59
±SD	2.7		1.4	10.2	0.38	0.67	29	0.45
($n = 5$)								
HC-TPTX	86.2	7.07	24.5	68.8	2.80	8.73	243	30.51
±SD	5.4		1.9	11.2	0.34	0.98	29	6.43
($n = 5$)								
P*	<0.001	<0.001	<0.001	<0.02	>0.2	<0.001	<0.001	<0.001

* NC-TPTX vs. HC-TPTX animals.

hyperphosphatemia and a marked increase in U_PV, largely in the absence of other significant alterations in renal function.

To assess the contribution of endogenous PTH to the phosphaturia associated with HC, NC-TPTX and HC-TPTX animals were studied (Table II). As was true of the intact animals, B_{HCO_3} was slightly but significantly greater in HC-TPTX animals. T_{HCO_3} was also slightly greater in HC-TPTX animals, undoubtedly reflecting the increase in filtered bicarbonate secondary to an increase in B_{HCO_3} and GFR. Mean urine pH was 5.52 in NC-TPTX and 5.65 in HC-TPTX animals, the difference just achieving statistical significance ($P < 0.05$). P_P, F_P, U_PV, and EF_P were all significantly greater in HC-TPTX animals, but there were no significant differences in mean P_{Ca_T}, P_{Ca_I}, and $U_{Ca}V$. $U_{cAMP}V$ was significantly greater in HC-TPTX rats. Mean values for RBF, V, $U_{Na}V$, EF_{Na}, and $U_{Cl}V$ were not significantly different in the two groups of animals. Mean U_KV was similar in the two groups ($P < 0.02$). These experiments documented the occurrence of hyperphosphatemia and phosphaturia during acute HC, even when the release of PTH had been prevented.

To assess the possible contribution of the increase in F_P to the phosphaturia of HC, studies were performed in normocapnic phosphate-infused TPTX (NC-P-TPTX) and similarly prepared hypercapnic (HC-P-TPTX) animals (Table III). B_{HCO_3} and T_{HCO_3} were slightly, although not significantly greater in the HC-P-TPTX animals. Average urine pH was 5.73 in NC-P-TPTX and 5.85 in HC-P-TPTX animals ($P > 0.01$). P_P and F_P were not significantly different in the two groups, yet U_PV and EF_P were again significantly greater in the HC animals. P_{Ca_T} and $U_{Ca}V$ were not different in the two groups; however, P_{Ca_I} was slightly greater in the HC-P-TPTX animals, the values just achieving statistical significance. $U_{cAMP}V$ was greater in HC-P-TPTX animals. Mean values for RBF, V, $U_{Na}V$, EF_{Na}, $U_{Cl}V$, and U_KV were not significantly different between the two groups of animals. These studies demonstrated that the phosphaturia of acute HC does not occur merely as a consequence of an increase in F_P.

Next, studies were performed in intact bicarbonate-infused normocapnic (NC-B) and bicarbonate-infused hypercapnic (HC-B) animals to assess the possible contribution of the increased concentration of B_{HCO_3} to the phosphaturia (Table IV). The results are compared with those of experiments performed in intact NC animals. As expected, a marked metabolic alkalosis

TABLE III
Effects of HC

	B_{pCO_2}	B_{pH}	B_{HCO_3}	T_{HCO_3}	GFR	P_P	F_P	U_PV
	mm Hg		*meq/ liter*	*μeq/ min*	*ml/ min*	*mg/ 100 ml*	*μg/ min*	*μg/ min*
NC-P-TPTX	38.7	7.41	23.6	71.3	2.99	10.1	307	18.5
±SD	3.0		3.1	16.3	0.35	1.6	70	6.4
($n = 5$)								
HC-P-TPTX	81.6	7.09	24.3	75.5	3.07	10.6	328	85.2
±SD	7.1		3.5	18.8	0.39	1.2	68	8.8
($n = 5$)								
P*	<0.001	<0.001	>0.5	>0.4	>0.5	>0.2	>0.3	<0.001

* NC-P-TPTX vs. HC-P-TPTX animals.

in TPTX Rats

EF_P	P_{Ca_T}	P_{Ca_I}	$U_{Ca}V$	$U_{cAMP}V$	RBF	V	$U_{Na}V$	EF_{Na}	$U_{Cl}V$	U_KV
%	*mg/ 100 ml*	*mg/ 100 ml*	*μg/ min*	*pmol/ min*	*ml/ min*	*μl/ min*	*μeq/ min*	%	*μeq/ min*	*μeq/ min*
0.4	8.91	4.24	8.05	106	10.2	72.4	16.7	4.29	22.4	3.53
0.3	0.87	0.46	2.54	38	1.4	30.4	6.5	1.71	7.9	0.77
12.6	8.66	4.34	8.80	157	10.6	80.9	18.1	4.38	23.4	2.95
2.4	0.93	0.79	1.40	60	1.5	26.7	6.1	1.48	5.6	0.71
<0.001	>0.3	>0.8	>0.2	<0.01	>0.3	>0.3	>0.4	>0.8	>0.6	<0.02

was induced in the NC-B animals with bicarbonate infusion. Urine pH was 7.98 in NC-B and 5.63 in NC animals. The B_{pCO_2}, however, was not significantly different from that observed in NC animals. As a consequence of the increased B_{HCO_3}, the T_{HCO_3} was significantly greater in NC-B rats. P_P was significantly less in the alkalotic animals and as a result of this change and the slight decrease in GFR, F_P was also significantly less in NC-B as compared to NC animals. U_PV was not different between the two groups (NC vs. NC-B), but EF_P was slightly greater in NC-B rats. P_{Ca_T} was not different, but P_{Ca_I} and $U_{Ca}V$ were decreased in the NC-B animals. $U_{cAMP}V$ was markedly decreased in the animals with metabolic alkalosis. Mean values for RBF, V, and $U_{Cl}V$ were significantly less in NC-B animals, whereas $U_{Na}V$, EF_{Na}, and U_KV were significantly greater ($P < 0.01$). Because EF_P was only slightly greater in the animals with marked increases in B_{HCO_3}, it seems unlikely that the increase in B_{HCO_3} contributed in a major way to the phosphaturia of HC.

To evaluate the possible relationship of extracellular pH or H^+ concentration to the phosphaturia, the extracellular acidosis of HC was minimized by infusion of sodium bicarbonate (Table IV). The blood pH (B_{pH}) was almost restored to levels comparable to those observed in NC animals, although the difference between the two groups was still statistically significant. B_{pCO_2}, B_{HCO_3}, and T_{HCO_3} were all significantly greater in HC-B animals. Urine pH was 7.84 in HC-B and 5.63 in NC animals. P_P, U_PV, and EF_P were increased in the HC-B animals; however, F_P was not significantly greater in the HC-B rats. P_{Ca_I} and $U_{Ca}V$ were decreased but P_{Ca_T} was not different in the two groups. $U_{cAMP}V$ was not significantly different between the two groups (HC-B vs. NC). The mean values for RBF and $U_{Cl}V$ were significantly less in HC-B animals when compared to NC animals, but $U_{Na}V$, EF_{Na}, and U_KV were significantly greater in HC-B animals. V was not different in the two groups. These experiments demonstrate that the phosphaturia of HC occurs even when the associated extracellular acidosis has been minimized.

DISCUSSION

The present studies demonstrate that acute HC is associated with an increased U_PV in rats with intact parathyroid glands. Several known factors could contribute to such a phosphaturia. The possible role of

in P-TPTX rats

EF_P	P_{Ca_T}	P_{Ca_I}	$U_{Ca}V$	$U_{cAMP}V$	RBF	V	$U_{Na}V$	EF_{Na}	$U_{Cl}V$	U_KV
%	*mg/ 100 ml*	*mg/ 100 ml*	*μg/ min*	*pmol/ min*	*ml/ min*	*μl/ min*	*μeq/ min*	%	*μeq/ min*	*μeq/ min*
6.3	7.87	3.09	4.59	183	10.9	77.8	18.0	3.97	21.7	2.74
2.6	0.58	0.18	1.15	49	1.4	44.4	3.4	1.21	4.1	0.60
26.7	7.99	3.52	4.27	225	10.4	58.9	18.6	4.03	19.4	2.77
4.5	0.48	0.38	1.29	66	1.0	17.5	4.9	0.92	5.4	0.44
<0.001	>0.5	<0.05	>0.4	<0.05	>0.1	>0.05	>0.6	>0.8	>0.1	>0.8

TABLE IV
Effects of Bicarbonate Infusion

	B_{pCO_2}	B_{pH}	B_{HCO_3}	T_{HCO_3}	GFR	P_P	F_P	U_PV
	mm Hg		*meq/ liter*	*μeq/ min*	*ml/ min*	*mg/ 100 ml*	*μg/ min*	*μg/ min*
NC	41.2	7.42	26.0	80.7	3.08	6.19	191	7.93
±SD	3.0		1.3	21.7	0.74	1.01	51	6.01
($n = 5$)								
NC-B	41.3	7.66	45.9	105	2.70	5.05	136	10.0
±SD	3.4		7.8	17	0.30	0.58	20	4.6
($n = 5$)								
*P**	>0.9	<0.001	<0.001	<0.001	<0.05	<0.001	<0.001	>0.2
HC-B	84.7	7.36	47.0	118	2.99	7.14	213	83.2
±SD	4.2		4.8	14	0.35	1.03	35	11.4
($n = 5$)								
*P**	<0.001	<0.001	<0.001	<0.001	>0.6	<0.01	>0.1	<0.001

* Compared to NC animals.

those factors during HC was explored in some detail. It seemed most likely that the phosphaturia might be related to release of endogenous PTH with subsequent reduction of renal phosphorus reabsorption (13–15). However, several findings offered no support for such a mechanism. First, a major stimulus for PTH release, lowering of the plasma calcium concentration (30), was not observed in intact HC animals. Second, an appropriate end-organ response to elevated levels of circulating PTH did not occur, inasmuch as $U_{cAMP}V$ remained unchanged during HC. The most compelling evidence against a major role for PTH was the demonstration that HC still provided a potent phosphaturic stimulus when PTH release was prevented by acute thyroparathyroidectomy. The effectiveness of acute thyroparathyroidectomy in reducing plasma PTH activity is supported by the finding that EF_P and $U_{cAMP}V$ were lower in NC and HC animals subjected to thyroparathyroidectomy than in their counterparts. These observations suggest that PTH does not play a major role in mediating the phosphaturia of acute HC.

Extracellular volume expansion is also known to increase U_PV (19–21). This mechanism appears to be an unlikely possibility inasmuch as NC and HC animals were subjected to the same degree of volume expansion using identical rates of infusion with infusates of the same composition. That success was achieved in the maintenance of comparable extracellular volumes in all groups of animals is supported by the similarity of values for V, $U_{Na}V$, EF_{Na}, GFR, and RBF in all experimental groups.

F_P was found to be increased in HC animals, a factor that could possibly contribute to the phosphaturia. Others have reported a similar finding and have suggested that the increased F_P was responsible for the phosphaturia (1, 4, 9). Indeed, an elevation of the P_P concentration was observed by us in intact HC animals when compared with their NC counterparts, although the rise in F_P did not achieve statistical significance. However, a statistically significant increase in both the P_P concentration and F_P was found in HC animals subjected to acute thyroparathyroidectomy when compared to their NC counterparts. Thus, it was still felt necessary to examine the potential role of an increased P_P concentration and F_P on the phosphaturia of HC. NC- and HC-TPTX animals were studied at comparable levels of plasma and filtered phosphorus which were achieved by sodium phosphate infusion. The results indicated that HC produces phosphaturia independent of the increase of the P_P concentration or F_P, although it must be admitted that elevations of either may contribute to phosphaturia in a minimal way.

The possibility that the phosphaturia could be the result of an increase in the ultrafilterability of phosphate occurring as a direct consequence of HC was also entertained, but considered highly unlikely. Previous studies in rats have demonstrated that the ultrafilterable fraction is at least 0.93 (31). Parathyroidectomy (32, 33) and phosphate loading (31–33) appear to have no discernible effect on the ultrafilterable fraction of phosphate as determined in in vitro systems. Moreover, LeGrimellec et al. (34) have shown that the filterability of phosphate across the glomerular filtration barrier is essentially identical to the value measured across cuprophane. Thus, it appears that the maximum increment in filtered load which could be attributed to a change in phosphate ultrafilterability must be less than 10%. A change of this magnitude could not produce the phosphaturia observed in HC animals.

The relationship between a decrease in ECF pH and U_PV is unclear. This and earlier studies have demonstrated that respiratory and metabolic acidosis are

in Intact NC and HC Rats

EF_P	P_{CaT}	P_{Ca_i}	$U_{Ca}V$	$U_{cAMP}V$	RBF	V	$U_{Na}V$	EF_{Na}	$U_{Cl}V$	U_kV
%	*mg/ 100 ml*	*mg/ 100 ml*	*μg/ min*	*pmol/ min*	*ml/ min*	*μl/ min*	*μeq/ min*	%	*μeq/ min*	*μeq/ min*
4.07	8.12	4.29	7.40	259	12.3	96.2	20.7	4.62	25.4	3.15
2.70	0.44	0.33	3.92	133	2.1	42.1	8.4	1.70	9.6	0.43
7.41	8.49	1.99	4.49	80	9.49	115.7	26.8	6.73	7.38	3.76
3.32	0.82	0.32	1.42	62	1.7	24.7	4.3	0.11	5.99	0.82
<0.001	>0.05	<0.001	<0.01	<0.001	<0.001	<0.05	<0.01	<0.001	<0.001	<0.01
39.6	8.12	3.40	4.68	195	11.0	111.6	30.4	6.83	4.47	3.55
5.8	1.20	0.53	2.80	62	1.2	35.4	7.2	1.41	2.24	0.63
<0.001	>0.9	<0.01	<0.02	>0.05	<0.05	>0.2	<0.001	<0.001	<0.001	<0.05

accompanied by phosphaturia (1–9, 35–38). However, the earlier studies were not designed to assess the influence of major known determinants of U_PV. In particular, studies of metabolic acidosis (35–38) did not attempt to define the role of compensatory changes in $BpCO_2$. Some investigators (23–25) have suggested that in the presence of a more acid renal tubular fluid at the site or sites of phosphate reabsorption, $H_2PO_4^-$ relative to $HPO_4^=$, would be increased and that, assuming $H_2PO_4^-$ to be the more readily reabsorbed ionic species, tubular reabsorption of phosphate would increase. Therefore, if this mechanism is operative and important, a decrease in U_PV would be expected to occur during respiratory or metabolic acidosis if one assumes that the luminal fluid at the site or sites of phosphate reabsorption is more acid than in the nonacidotic state. The early proximal tubule represents one site for phosphate reabsorption (39–43) and, at this site, the luminal fluid pH should approach systemic pH. The distal nephron has also been shown by some investigators (39, 40, 44, 45) to reabsorb phosphate, and, for its more distal portion, urine pH should reflect tubular fluid pH. Thus, experimental conditions in which the pH of the blood and urine are lower should be associated with a decreased U_PV. Direct attempts to evaluate the importance of this mechanism in the proximal tubule have yielded conflicting results. Bank et al. (25), utilizing the microperfusion technique in the rat, demonstrated enhanced reabsorption of the $H_2PO_4^-$ ion species, whereas Baumann et al. (26) demonstrated greater reabsorption of the $HPO_4^=$ ion species.

To assess the possible effects of acid-base alterations on U_PV, HC-B and NC-B animals were studied (Tables I and IV). Phosphaturia was not prevented by normalization of the ECF pH with bicarbonate infusion (HC-B animals). These results suggest that changes in the ionic species ($H_2PO_4^-$ vs. $HPO_4^=$) in the early proximal tubule or ECF acidosis per se provide an unlikely explanation for the phosphaturia of HC. In addition, inasmuch as urine pH was not significantly different ($P > 0.05$) in intact NC and HC animals, it appears unlikely that changes in tubular fluid pH in the terminal nephron can account for the phosphaturia. Interestingly, U_PV was actually greater in HC-B than in HC animals ($P < 0.001$), suggesting that some function of bicarbonate infusion served to enhance the phosphaturia associated with HC. This could have been the result of a change in ECF pH, B_{HCO_3}, or urine pH because all were significantly greater ($P < 0.001$) in the HC-B animals. However, NC-B animals also had significantly different B_{pH}, B_{HCO_3}, and urine pH than HC animals, but did not demonstrate phosphaturia. Therefore, the only acid-base disturbance that was consistently associated with phosphaturia was an elevation of the B_{pCO_2}. It would thus appear that alterations of B_{HCO_3}, B_{pH}, urine pH, and presumably of tubular fluid pH cannot account for the phosphaturia of HC. On the other hand, although ECF pH was normalized in HC-B animals, intracellular fluid pH may still have been less than normal because in certain in vitro studies where intracellular pH has been measured, poor cellular penetration of bicarbonate has been demonstrated in some cell types (46). It is possible that the phosphaturic effect of the increase in B_{pCO_2} is mediated via a reduction of intracellular pH in those tubular cells responsible for phosphate reabsorption.

The present studies demonstrated that an elevation in P_P concentration was associated with an increase in $U_{cAMP}V$ in the presumed absence of endogenous PTH. NC-P-TPTX animals had a significantly greater $U_{cAMP}V$ than NC-TPTX animals not receiving a phosphate infusion (183 vs. 106 pmol/min; $P < 0.01$). In addition,

HC-P-TPTX animals had significantly greater $U_{cAMP}V$ than HC-TPTX animals not receiving phosphate (225 vs. 157 pmol/min; $P < 0.01$). This study was not designed to determine whether increases in U_{cAMP} were of renal origin; however, it is possible that phosphate infusion could raise intracellular phosphate levels thereby stimulating increased production of cAMP with a resultant incrase in $U_{cAMP}V$. One might speculate that by activating the cellular cAMP system, phosphate infusion could ultimately lead to enhanced excretion of phosphate providing a convenient mechanism for preventing phosphate retention during phosphate loading.

An interesting association was also observed between $U_{cAMP}V$ and bicarbonate infusion. Intact NC-B animals had a markedly reduced $U_{cAMP}V$ when compared to intact NC animals not receiving bicarbonate ($P < 0.001$). Yet, U_PV did not decrease in the NC-B animals. Similar findings have been reported by Rodriquez et al. (47). When compared to NC animals, P_{Ca_I} fell significantly in the NC-B animals ($P < 0.001$). The expected finding would have been an increase both in the $U_{cAMP}V$ and U_PV as PTH was released in response to the decrease in ionized calcium. Although the mechanism is obscure, bicarbonate infusion in animals with intact parathyroid glands appears to dissociate $U_{cAMP}V$ from U_PV.

The present results failed to confirm earlier in vitro observations which have demonstrated an inverse relationship between P_{Ca_I} concentration and BpH (48). There was no significant difference noted in the P_{Ca_I} concentrations between intact HC ($B_{pH} - 7.15$) and intact NC animals ($B_{pH} - 7.42$). Our findings are consistent with those of Höffken et al. (49) who failed to observe a significant rise in P_{Ca_I} levels in intact rats in acute respiratory acidosis. The failure to observe such an inverse relationship may have been due to the effect of plasma phosphate on plasma concentrations of P_{Ca_I}. Elevation of the P_P concentration was associated with depression of P_{Ca_I} concentration in both NC- and HC-TPTX animals (NC-P-TPTX vs. NC-TPTX, $P < 0.01$; HC-P-TPTX vs. HC-TPTX, $P < 0.01$). Inasmuch as HC animals had a significant rise in their P_P concentrations in comparison with NC animals, the anticipated rise in P_{Ca_I} in response to respiratory acidosis may have been prevented. Therefore, acidosis, at least of acute respiratory etiology, did not produce an incresae in P_{Ca_I} levels under the conditions of this study.

The present experiments were not designed to identify the source of the phosphate excreted in the urine of HC animals. In animals not receiving a phosphate infusion, the source of the increased phosphate must have derived from an intracellular pool, but it is not known which cell types contribute to such a pool that responds so readily to HC. However, during metabolic acidosis it has been demonstrated that the intracellular phosphate content of erythrocytes is decreased (50) and such a phenomenon could conceivably occur during respiratory acidosis.

In summary, acute HC was found to produce hyperphosphatemia and phosphaturia. The phosphaturia was not dependent upon endogenous PTH, an increase in F_P, an associated extracellular acidosis, or an increase in B_{HCO_3} concentration. Instead the findings suggest that the increase in B_{pCO_2} has a direct effect on the kidney which is mainly responsible for the phosphaturia observed during acute HC. Because HC is an extremely potent stimulus of phosphaturia, future studies directed toward examination of renal phosphorus reabsorption should consider this variable in the design of the experimental protocol.

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