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### Research Article

The role of parathyroid hormone (PTH) and of  $\text{Ca}^{++}$  in the regulation of bicarbonate absorption ( $\text{RHCO}_3$ ) and its response to extracellular volume expansion (VE) was studied in  $\text{HCO}_3^-$ -loaded dogs.

VE lowered  $\text{RHCO}_3$  in both intact (from 24.8 to 22.0 mmol/liter GFR,  $P < 0.01$ ) and thyroparathyroid-ectomized (TPTX) (from 24.5 to 18.0 mmol/liter GFR,  $P < 0.001$ ) dogs; glomerular filtration rate (GFR) and filtered  $\text{HCO}_3^-$  did not change. Both groups showed a significant increase in the fractional excretion of sodium ( $C_{\text{Na}} \times 100/\text{GFR}$ ), calcium ( $C_{\text{Ca}} \times 100/\text{GFR}$ ), and chloride ( $C_{\text{Cl}} \times 100/\text{GFR}$ ) and a decrease in phosphorus reabsorption. Fractional clearance of phosphate ( $C_{\text{P}} \times 100/\text{GFR}$ ) rose in both groups but did not achieve significance.

Infusion of purified parathyroid extract (PTE) decreased  $\text{RHCO}_3$  in intact dogs (from 24.6 to 22.5 mmol/liter GFR,  $P < 0.025$ ) and in TPTX dogs (from 26.9 to 22.6 mmol/liter GFR,  $P < 0.05$ ). No change was noted in GFR, renal blood flow (RBF), filtered  $\text{HCO}_3^-$ , or fractional excretion of sodium, calcium, or chloride in either group. There was a significant increase in fractional phosphorus clearance and a decrease in phosphorus reabsorption in each group.

Infusion of  $\text{Ca}^{++}$  raised ultrafilterable  $\text{Ca}^{++}$  from 5.7 to 7.9 mg/100 ml in intact and from 4.9 to 7.2 mg/100 ml in TPTX dogs;  $\text{RHCO}_3$  increased in intact (from 22.9 to 26.9 mmol/liter [...])

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# Effects of Volume Expansion, Purified Parathyroid Extract, and Calcium on Renal Bicarbonate Absorption in the Dog

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**ABSTRACT** The role of parathyroid hormone (PTH) and of  $\text{Ca}^{++}$  in the regulation of bicarbonate absorption ( $\text{RHC}\text{O}_3$ ) and its response to extracellular volume expansion (VE) was studied in  $\text{HCO}_3^-$ -loaded dogs.

VE lowered  $\text{RHC}\text{O}_3$  in both intact (from 24.8 to 22.0 mmol/liter GFR,  $P < 0.01$ ) and thyroparathyroidectomized (TPTX) (from 24.5 to 18.0 mmol/liter GFR,  $P < 0.001$ ) dogs; glomerular filtration rate (GFR) and filtered  $\text{HCO}_3^-$  did not change. Both groups showed a significant increase in the fractional excretion of sodium ( $\text{C}_{\text{Na}} \times 100/\text{GFR}$ ), calcium ( $\text{C}_{\text{Ca}} \times 100/\text{GFR}$ ), and chloride ( $\text{C}_{\text{Cl}} \times 100/\text{GFR}$ ) and a decrease in phosphorus reabsorption. Fractional clearance of phosphate ( $\text{C}_P \times 100/\text{GFR}$ ) rose in both groups but did not achieve significance.

Infusion of purified parathyroid extract (PTE) decreased  $\text{RHC}\text{O}_3$  in intact dogs (from 24.6 to 22.5 mmol/liter GFR,  $P < 0.025$ ) and in TPTX dogs (from 26.9 to 22.6 mmol/liter GFR,  $P < 0.05$ ). No change was noted in GFR, renal blood flow (RBF), filtered  $\text{HCO}_3^-$ , or fractional excretion of sodium, calcium, or chloride in either group. There was a significant increase in fractional phosphorus clearance and a decrease in phosphorus reabsorption in each group.

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Infusion of  $\text{Ca}^{++}$  raised ultrafilterable  $\text{Ca}^{++}$  from 5.7 to 7.9 mg/100 ml in intact and from 4.9 to 7.2 mg/100 ml in TPTX dogs;  $\text{RHC}\text{O}_3$  increased in intact (from 22.9 to 26.9 mmol/liter GFR,  $P < 0.025$ ) and in TPTX dogs (from 26.6 to 28.6 mmol/liter GFR,  $P < 0.05$ ). The GFR, RBF, and the fractional excretion of sodium, chloride, and calcium did not change in either group. The reabsorbed phosphate increased in both groups, and fractional phosphorus clearance fell in the intact group but did not change significantly in the TPTX group.

Superimposition of PTE on hypercalcemia in TPTX dogs resulted in a decrease in  $\text{RHC}\text{O}_3$  (from 27.3 to 23.9 mmol/liter GFR,  $P < 0.001$ ), which was accompanied by an increase in the fractional excretion of phosphate and a decrease in the reabsorbed phosphate. In this group of TPTX dogs hypercalcemia caused a drop in RBF from 135.6 to 105.8 ml/min with no change in GFR. The RBF returned to control value with PTE infusion.

It is concluded that: (a) the lowering of  $\text{RHC}\text{O}_3$  by VE is not dependent solely on stimulation of PTH by the lowered  $\text{Ca}^{++}$ , (b) PTE acts directly on the renal tubules to lower  $\text{RHC}\text{O}_3$ , (c)  $\text{Ca}^{++}$  enhances  $\text{RHC}\text{O}_3$  and this effect is exerted in the absence of PTH and calcitonin, (d) neither the effects of  $\text{Ca}^{++}$  nor of PTH appear to be mediated by altered hemodynamics, although this cannot be excluded in  $\text{Ca}^{++}$ -infused TPTX dogs, (e)  $\text{Ca}^{++}$  enhanced phosphate reabsorption in the absence of PTH; this may be a specific effect of hypercalcemia on phosphate reabsorption or the nonspecific consequence of the rise in serum phosphorus.

## INTRODUCTION

Most of the hydrogen ion secreted by the kidney goes towards reabsorption of the filtered bicarbonate. This

process of bicarbonate reclamation depends on carbonic anhydrase (1) and varies directly with the level of blood  $P_{CO_2}$  (2) and inversely with intracellular potassium (3) and the serum chloride (4) or the volume of the extracellular fluid (5). Bicarbonate reabsorption ( $R_{HCO_3}$ )<sup>1</sup> has also been shown to vary inversely with renal blood flow (RBF) (6).

In past studies it had been shown that the infusion of parathyroid extract (PTE) results in increased bicarbonate excretion (7-10). Conversely, the infusion of calcium was shown to increase hydrogen ion secretion and the excretion of ammonia (11). These observations, coupled with the clinical association of metabolic acidosis with primary (12, 13) and secondary (14-16) hyperparathyroidism and metabolic alkalosis with hypoparathyroidism (17) have led to the suggestion that parathyroid hormone (PTH) may exert a regulatory role in acid-base balance (16).

It is not clear from published reports whether the effect of PTE is specifically that of PTH or is a non-specific consequence of the renal hemodynamic effects of a nonhormonal protein present in PTE. Also, it is not clear whether calcium specifically enhances hydrogen ion secretion or if it simply suppresses PTH secretion thereby eliminating the wasting of bicarbonate caused by it. Furthermore, whether PTH acts on the threshold or on the capacity for bicarbonate reabsorption has not been elucidated. Finally, if indeed PTH depresses bicarbonate reabsorptive capacity it is possible that it may play a role in bringing about the depression of bicarbonate reabsorption observed during expansion with saline or with bicarbonate solution (5). Alkalosis (18) and volume expansion (19) both lower the ionized calcium and may stimulate PTH secretion (20). The reported depression of bicarbonate reabsorption by volume expansion, therefore, may have been the nonspecific consequence of stimulation of PTH secretion.

To investigate these possibilities the effects of bicarbonate and of balanced salt solution and also of calcium and PTH on bicarbonate absorption were studied in bicarbonate-loaded intact and thyroparathyroidectomized (TPTX) dogs.

## METHODS

Studies were performed on 49 mongrel dogs of either sex weighing 9-28 kg. Anesthesia was induced with sodium pentobarbital, 32 mg/kg body wt i.v., and additional doses given as needed. All animals were intubated with an endo-

<sup>1</sup> Abbreviations used in this paper: GFR, glomerular filtration rate; PTE, parathyroid extract; PTH, parathyroid hormone; RBF, renal blood flow;  $R_{HCO_3}$ , bicarbonate reabsorption;  $R_{HCO_3}/GFR$ ,  $R_{HCO_3}$ , corrected to GFR; RPF, renal plasma flow; TPTX, thyroparathyroidectomized;  $U_{Ca}$ , ultrafilterable calcium.

tracheal tube and their respiration supported by a Harvard large-animal respirator (Harvard Apparatus Co., Inc., Millis, Mass.). The  $P_{CO_2}$  was maintained between 35 and 45 mm Hg by varying the frequency of respiration.

The femoral arteries and veins were cannulated through bilateral femoral incisions. Blood pressure was monitored with a Statham pressure transducer (Statham Instruments, Inc., Oxnard, Calif.) from one arterial line. The other artery was used to obtain blood samples anaerobically for the measurement of the pH and  $P_{CO_2}$ . The ureters were cannulated with polyethylene catheters through a small midline suprapubic incision for the collection of urine under oil. The left renal vein was catheterized through the spermatic or ovarian vein using a left subcostal incision. A minimum of 1 h was allowed for recovery before starting each experiment.

In all experiments, a loading dose of inulin, 50 mg/kg body wt, was given followed by a continuous infusion of 50 mg/kg/h for the duration of the experiment. An infusion of 0.7 M  $NaHCO_3$  was used to raise the serum bicarbonate above threshold and to maintain the serum bicarbonate between 30 and 36 mmol/liter.

The renal plasma flow (RPF) was calculated from the extraction of inulin by using Wolf's formula (21), and the RBF was calculated from the relation:  $RBF = RPF/1 - \text{hematocrit}$ .

Glomerular filtration rate (GFR) was determined by inulin clearance. Determinations of  $Na^+$ ,  $K^+$ , inulin,  $Ca^{++}$ , and phosphorus in the plasma and urine were made as described by Hebert, Rouse, Eknayan, Martinez-Maldonado, and Suki (22). Chloride was determined in a Cotlove Chloridometer (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N. J.).

Plasma and urine bicarbonate were calculated from the pH and  $P_{CO_2}$  by using the Henderson-Hasselbach equation as previously published (6, 23). Numerous determinations of  $HCO_3^-$  in this manner did not differ significantly from  $HCO_3^-$  values determined simultaneously by the manometric method using the Natelson microgasometer (Scientific Industries, Inc., Springfield, Mass.). Statistical analysis was performed by the paired *t* test where only one experimental condition was induced, and by analysis of variance with one degree of freedom where two experimental conditions were induced.

Eight groups of animals were studied:

*Group I.* Five dogs were studied to determine the effects of continued  $HCO_3^-$  infusion. The serum bicarbonate level was raised above 30 mmol/liter and a steady state was maintained with 0.7 M sodium bicarbonate solution. The infusion of sodium bicarbonate will be referred to as "bicarbonate loading." After a steady state was reached, blood and urine samples were collected every 10-20 min depending on the urine flow rate, and continued for a 3-h period. The first 3-4 collections were used as the control period and the remainder of the collections were considered as the experimental period.

*Group II.* Seven intact animals were bicarbonate loaded and the first four collections were used as control. The animals were then volume expanded with isotonic saline containing 13 meq KCl/liter given at 2 ml/kg/min for 30 min then at 1 ml/kg/min for the remainder of the experiment. Blood and urine samples were collected every 10-20 min for approximately 2 h.

*Group III.* Thyroparathyroidectomy was performed on five dogs after the endotracheal tube was in place by using a technique previously described (24). 1½ h was allowed before starting the experiment to permit circulating PTH

levels to decline. The remainder of the experiment was the same as described for group II.

**Group IV.** Six intact animals were bicarbonate loaded and the first four periods were used as control. The animals were then given 100 U PTE (purified parathyroid extract lot no. 153727, Wilson Laboratories, Chicago, Ill.) intravenously over 3 min and 5 U/min was infused for the remainder of the collection periods in the manner described by Hellman, Au, and Bartter (10).

**Group V.** Six animals underwent thyroparathyroidectomy, and after the previously designated time lapse, the experiment was performed in a manner identical to group IV.

**Group VI.** A routine control period was collected in six intact dogs. The animals were then infused with calcium chloride, 0.75 mg/kg/min (25), until the serum calcium rose above 10.6 mg/100 ml (required  $75 \pm 25$  min). The serum calcium was determined every 20 min thereafter, and the rate of calcium infusion was adjusted to maintain serum calcium between 10.6 and 13.6 mg/100 ml. Urine and blood samples were collected for approximately 2 h.

**Group VII.** Eight animals underwent thyroparathyroidectomy and were then bicarbonate loaded. The dogs were then infused with calcium in a manner identical to group VI.

**Group VIII.** Six TPTX animals were loaded with bicarbonate. After the control period, the dogs were made acutely hypercalcemic in the manner described for group VI and three collections made. PTE was then infused as for group IV while maintaining the serum calcium in a steady state.

## RESULTS

The results of studies performed on intact animals receiving a sustained infusion of 0.7 M  $\text{NaHCO}_3$  are summarized in Table I. The hematocrit before the infusion of  $\text{NaHCO}_3$  was  $36.0 \pm 3.4$  and fell to  $32.8 \pm 2.7$  in the control period; it remained stable thereafter and was  $32.6 \pm 2.3$  in the experimental period. From Table I it is apparent that there were no significant changes between the control period and the experimental period, with the exception of a slight rise in

the filtered bicarbonate. Specifically there was no significant fall in  $\text{RHC}_3$  corrected to GFR ( $\text{RHC}_3/\text{GFR}$ ) or in the hematocrit, nor rise in the fractional clearances of sodium, chloride, calcium, or phosphate. Thus the continued administration of bicarbonate for a duration comparable to that in the other experiments performed does not in itself alter renal bicarbonate absorption or further expand extracellular volume.

The effects of volume expansion with isotonic saline in bicarbonate-loaded intact dogs (group II) and TPTX dogs (group III) are shown in Table II. Volume expansion resulted in a drop in the  $\text{RHC}_3/\text{GFR}$  from 24.8 to 22.0 mmol/liter,  $P < 0.01$ , in intact animals and in a similar but more marked decrease in the  $\text{RHC}_3/\text{GFR}$  from 24.5 to 18 mmol/liter,  $P < 0.001$ , in the TPTX animals. The serum potassium concentration did not change significantly; ultrafilterable calcium (UfCa) dropped from 5.2 to 4.2 mg/100 ml,  $P < 0.001$ , in intact dogs, and from 5.1 to 4.2 mg/100 ml,  $P < 0.025$ , in TPTX dogs, and the fractional clearances of sodium, chloride, and calcium rose significantly in both groups. The hematocrit fell significantly in both groups. Fractional clearance of phosphate rose in both groups but did not attain significance; reabsorbed phosphate corrected to the GFR, however, fell significantly in both groups. The more profound depression of  $\text{RHC}_3$  in the TPTX group is probably related to the greater increase in the clearances of sodium and of chloride effected in this group.

The effects of infusion of PTE in intact (group IV) and TPTX (group V) dogs are shown in Table III. Infusion of PTE caused a significant fall in the  $\text{RHC}_3/\text{GFR}$  from 24.6 to 22.5 mmol/liter,  $P < 0.025$ , in intact dogs and a similar but more pronounced fall in TPTX dogs (from 26.9 to 22.6 mmol/liter,  $P < 0.05$ ). The clearance of sodium rose significantly in TPTX dogs;

TABLE I  
Effects of Sustained Bicarbonate Infusion (Group I)

	GFR	RBF	$\text{BHCO}_3$	$\text{FHCO}_3$	$\text{RHC}_3$		$\text{P}_P$	$\text{P}_K$	Hct	$\text{C}_{\text{Na}}$	$\text{C}_{\text{Cl}}$	$\text{C}_{\text{Ca}}$	$\text{C}_P$	$\text{R}_P$
					$\times 100$	$\times 100$				$\times 100$	$\times 100$	GFR		
	ml/min		mmol/liter	$\mu\text{mol}/\text{min}$	mmol/liter	mg/100 ml	meq/liter	%	%	%	%	%	mg/liter	
Control	31.0	162.7	31.9	1002	24.5	5.5	4.60	3.20	32.8	6.20	0.92	3.60	22.6	33.0
SEM	7.1	30.2	0.5	246	1.0	0.2	0.58	0.16	2.7	1.00	0.36	0.94	6.6	5.0
Experimental	32.8	132.2	32.6	1062	24.1	5.5	4.50	3.20	32.6	6.30	0.68	3.25	30.2	30.0
SEM	7.6	31.2	0.4	263	1.3	0.2	0.40	0.15	2.3	0.93	0.22	0.96	2.3	4.0
P	>0.05	>0.2	>0.2	<0.05	>0.3		>0.5	>0.6	>0.7	>0.8	>0.5	>0.6	>0.1	>0.2

$\text{BHCO}_3$ , blood bicarbonate;  $\text{FHCO}_3$ , filtered bicarbonate;  $\text{P}_P$ , plasma phosphorus;  $\text{P}_K$ , plasma potassium; Hct, hematocrit;  $\text{C}_{\text{Na}} \times 100/\text{GFR}$ , fractional clearance of sodium;  $\text{C}_{\text{Cl}} \times 100/\text{GFR}$ , fractional clearance of chloride;  $\text{C}_{\text{Ca}} \times 100/\text{GFR}$ , fractional clearance of calcium;  $\text{C}_P \times 100/\text{GFR}$ , fractional clearance of phosphorus,  $\text{R}_P/\text{GFR}$ , reabsorbed phosphorus corrected to 1,000 ml GFR. The values listed are the means of all collections in a respective period.

TABLE II  
Effects of Volume Expansion (VE) in Intact (Group II) and TPTX (Group III) Dogs

	GFR	BHCO <sub>3</sub>	FHCO <sub>3</sub>	RHCO <sub>3</sub>		UfCa	PP	PK	Hct	C <sub>Na</sub>	C <sub>Cl</sub>	C <sub>Ca</sub>	C <sub>P</sub>	R <sub>P</sub>
				× 100	× 100					× 100	× 100	R <sub>P</sub>		
				GFR	GFR					GFR	GFR	GFR		
	ml/min	mmol/liter	μmol/min	mmol/liter	mg/100 ml	mg/100 ml	meq/liter	%	%	%	%	mg/liter		
Group II														
Control	47.2	34.4	1605	24.8	5.2	5.2	2.6	30	7.9	2.3	6.0	34.3	35.3	
SEM	10.1	1.1	336	1.6	0.3	0.55	0.11	1.49	2.0	1.5	2.5	3.9	5.7	
VE	51.1	33.8	1711	22.0	4.2	4.1	2.7	25	15.7	10.9	16.6	45.5	22.3	
SEM	10.7	1.0	349	1.5	0.3	0.39	0.17	2.37	3.8	3.9	5.4	5.1	3.4	
P	>0.05	>0.7	>0.2	<0.01	<0.001	<0.025	>0.3	<0.01	<0.02	<0.02	<0.025	>0.05	<0.05	
Group III														
Control	38.7	32.1	1237	24.5	5.10	4.20	3.00	33.0	6.8	2.5	5.7	15.0	36.0	
SEM	9.5	0.5	304	1.1	0.16	0.38	0.36	1.6	1.3	1.0	1.4	7.3	5.0	
VE	36.0	31.5	1126	18.0	4.20	3.60	3.20	27.0	20.2	15.2	16.9	27.0	28.0	
SEM	6.7	0.3	210	1.4	0.16	0.38	0.70	1.3	4.2	4.2	4.1	11.5	6.0	
P	>0.3	>0.3	>0.3	<0.001	<0.025	>0.1	>0.2	<0.01	<0.02	<0.05	<0.025	>0.05	<0.05	

For explanation see Table I.

the clearance of chloride and the hematocrit did not change significantly in either group. The clearance of calcium was also unchanged in the two groups but the clearance of phosphate rose and phosphate reabsorption fell significantly in both groups.

Hypercalcemia induced acutely in intact dogs (group VI) was followed by a rise in RHCO<sub>3</sub>/GFR from 22.9 to 26.9 mmol/liter,  $P < 0.025$ , (Table IV). This does

not appear to be due to suppression of PTH since RHCO<sub>3</sub>/GFR in TPTX dogs (group VII, Table IV) also rose from 26.6 to 28.6 mmol/liter,  $P < 0.05$ . The clearances of sodium, chloride, and calcium did not change significantly. The hematocrit was unchanged in group IV, but fell significantly in group VII. The clearance of phosphate fell significantly in the intact group but not in the TPTX group. Phosphorus reab-

TABLE III  
Effects of PTE in Intact (Group IV) and TPTX (Group V) Dogs

	GFR	RBF	BHCO <sub>3</sub>	FHCO <sub>3</sub>	RHCO <sub>3</sub>		UfCa	PP	PK	Hct	C <sub>Na</sub>	C <sub>Cl</sub>	C <sub>Ca</sub>	C <sub>P</sub>	R <sub>P</sub>
					× 100	× 100					× 100	× 100	R <sub>P</sub>		
					GFR	GFR					GFR	GFR	GFR		
	ml/min	ml/min	mmol/liter	μmol/min	mmol/liter	mg/100 ml	mg/100 ml	meq/liter	%	%	%	%	mg/liter		
Group IV															
Control	26.2	140.2	31.9	840	24.6	6.00	5.10	2.90	35.0	6.46	2.7	4.4	30.0	37.0	
SEM	4.9	26.0	0.2	153	1.2	0.22	0.61	0.08	1.5	1.50	1.3	1.3	5.4	7.0	
PTE	26.6	136.0	31.9	855	22.5	5.80	4.90	2.80	33.0	7.11	1.6	4.1	39.8	32.0	
SEM	4.2	10.4	0.4	139	1.1	0.26	0.69	0.07	2.2	0.82	0.7	0.7	2.4	6.0	
P	>0.80	>0.80		>0.10	<0.025	<0.05	>0.4	>0.3	>0.1	>0.40	>0.10	>0.60	<0.05	<0.05	
Group V															
Control	28.8	167.2	32.5	928	26.9	5.00	4.60	3.10	29.0	3.67	0.40	1.37	9.7	42.0	
SEM	5.2	49.3	0.5	156	0.9	0.22	0.27	0.13	4.0	0.53	0.07	0.38	4.8	1.0	
PTE	27.6	166.6	32.5	889	22.6	4.90	4.20	2.80	29.0	6.73	0.60	2.03	26.7	34.0	
SEM	4.9	43.8	0.5	147	0.9	0.22	0.33	0.12	3.3	0.81	0.25	0.35	4.9	2.0	
P	>0.5	>0.975		>0.6	<0.05	>0.7	>0.3	<0.02		<0.02	>0.30	>0.10	<0.001	<0.005	

For explanation see Table I.

TABLE IV  
Effects of Acute Hypercalcemia in Intact (Group VI) and TPTX (Group VII) Dogs

	GFR	RBF	BHCO <sub>3</sub>		FHCO <sub>3</sub>		RHCO <sub>3</sub>			C <sub>Na</sub>	C <sub>Cl</sub>	C <sub>Ca</sub>	C <sub>P</sub>	R <sub>P</sub>		
			GFR	RBF	BHCO <sub>3</sub>	FHCO <sub>3</sub>	GFR	UfCa	P <sub>P</sub>	P <sub>K</sub>	Hct	× 100	× 100	× 100	× 100	R <sub>P</sub>
												GFR	GFR	GFR	GFR	
	ml/min	ml/min	mmol/liter	μmol/min	mmol/liter	mg/100 ml	meq/liter	%	%	%	%	mg/liter				
Group VI																
Control	23.9	121.9	33.6	810	22.9	5.70	4.50	2.90	32.0	8.30	1.72	4.6	37.8	28.0		
SEM	4.3	26.5	1.0	159	0.7	0.15	0.48	0.16	2.3	2.39	0.72	1.5	4.0	4.0		
Hypercalcemia	22.4	100.9	33.6	779	26.9	7.90	5.20	2.90	32.0	5.40	1.74	4.4	21.9	41.0		
SEM	3.8	16.6	1.0	150	0.8	0.29	0.35	0.12	2.2	0.57	0.43	0.7	5.2	3.0		
P	>0.3	>0.2		>0.4	<0.025	<0.001	<0.05	>0.8		>0.05	>0.95	>0.8	<0.05	<0.005		
Group VII																
Control	28.1	118.6	33.3	922	26.6	4.9	4.80	3.10	29.3	4.4	0.70	2.90	8.6	43.0		
SEM	5.7	25.0	0.6	182	0.7	0.20	0.28	0.10	1.9	0.46	0.09	0.46	3.1	2.0		
Hypercalcemia	25.5	103.0	34.1	859	28.6	7.2	6.10	3.10	26.8	4.2	1.10	4.30	14.4	53.0		
SEM	5.6	23.4	1.1	176	0.8	0.31	0.18	0.12	1.8	0.92	0.33	1.30	3.5	2.0		
P	>0.1	>0.2	>0.2	>0.2	<0.05	<0.001	<0.005	>0.8	<0.05	>0.8	>0.2	>0.3	>0.1	<0.05		

For explanation see Table I.

sorption, however, increased with calcium infusion in both intact and TPTX dogs. This may represent either a direct effect of calcium to enhance phosphorus absorption not mediated by suppression of PTH, or a passive consequence of the rise in plasma phosphorus.

To investigate the interaction between calcium and PTE and to further ascertain that the drop in RHCO<sub>3</sub>/GFR induced by PTE was not simply due to sustained bicarbonate infusion, TPTX dogs were first made hypercalcemic and then PTE was given (Table V). Hypercalcemia caused a rise in RHCO<sub>3</sub>/GFR from 25.3 to 27.3 mmol/liter, *P* < 0.005, a change that is similar to that seen in group VII dogs (Table IV). The super-

imposition of PTE infusion in the face of sustained hypercalcemia caused a drop in RHCO<sub>3</sub>/GFR from 27.3 to 23.9 mmol/liter, which is significantly lower than the value during hypercalcemia (*P* < 0.001) and even lower than the control period (*P* < 0.05). The induction of hypercalcemia in this group was associated with a significant drop in RBF from 135.6 to 105.8 ml/min even though the rise in UfCa (5.6 to 7.4 mg/100 ml) was comparable to that seen in groups VI and VII. The RBF returned to near the control level (141.3 ml/min) with the infusion of PTE without a change in the ultrafilterable calcium. The clearance of phosphate did not change with hypercalcemia and rose

TABLE V  
Effects of Acute Hypercalcemia and of Superimposed PTE Infusion in TPTX Dogs (Group VIII)

	GFR	RBF	BHCO <sub>3</sub>		FHCO <sub>3</sub>		RHCO <sub>3</sub>			C <sub>Na</sub>	C <sub>Cl</sub>	C <sub>Ca</sub>	C <sub>P</sub>	R <sub>P</sub>		
			GFR	RBF	BHCO <sub>3</sub>	FHCO <sub>3</sub>	GFR	UfCa	P <sub>P</sub>	P <sub>K</sub>	Hct	× 100	× 100	× 100	× 100	R <sub>P</sub>
												GFR	GFR	GFR	GFR	
	ml/min	ml/min	mmol/liter	μmol/min	mmol/liter	mg/100 ml	meq/liter	%	%	%	%	mg/liter				
Control	28.5	135.6	32.1	913	25.3	5.60	5.30	3.10	31.0	5.30	0.82	2.20	10.3	47.0		
SEM	4.0	19.0	0.6	131	0.5	0.15	0.36	0.15	0.9	0.57	0.28	0.49	2.7	2.0		
Hypercalcemia	26.4	105.8	32.0	840	27.3	7.40	6.60	3.20	30.0	4.80	1.50	2.90	10.9	60.0		
SEM	3.7	18.9	0.4	107	0.7	0.14	0.29	0.08	1.3	0.68	0.59	0.58	1.4	3.0		
PTE	28.2	141.3	31.7	894	23.9	7.20	5.90	3.10	31.0	7.10	1.40	4.20	34.1	41.0		
SEM	3.6	16.7	0.3	114	0.3	0.10	0.32	0.13	0.8	0.43	0.31	0.55	2.4	3.0		
P (control vs. hypercalcemia)	>0.05	<0.05	>0.05	>0.05	<0.005	<0.005	<0.005	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	<0.005		
P (control vs. PTE)	>0.05	>0.05	>0.05	>0.05	<0.05	<0.005	<0.05	>0.05	>0.05	<0.05	>0.05	<0.005	<0.005	<0.05		
P (hypercalcemia vs. PTE)	>0.05	<0.05	>0.05	>0.05	<0.001	>0.05	<0.025	>0.05	>0.05	<0.05	>0.05	>0.05	<0.001	<0.001		

For explanation see Table I.

after PTE. However, there was a rise in the reabsorbed phosphorus during hypercalcemia and a fall to below control after the infusion of PTE. The clearances of both sodium and calcium increased with the infusion of PTE but did not change with hypercalcemia alone. The hematocrit remained relatively constant throughout the experiment.

The values of  $R_{HCO_3}/GFR$  in the control periods of experiments on intact dogs (groups I, II, IV, and VI) were remarkably close and averaged  $24.2 \pm 0.6$  mmol/liter. Parathyroidectomy raised  $R_{HCO_3}$  in groups V and VII, and to a lesser extent in group VIII; it had seemingly little effect on animals in group III. Taken together, however,  $R_{HCO_3}$  in this group averaged  $25.9 \pm 0.4$  mmol/liter, a value that is significantly higher than that of intact dogs ( $P < 0.025$ ).

## DISCUSSION

The present studies were designed to examine the specific effects of volume expansion, purified PTE, and calcium on  $R_{HCO_3}$  in the intact and the TPTX dog.

Previous studies have demonstrated that volume expansion causes a decrease in reabsorption of bicarbonate (5, 26), calcium (19), and phosphate (22, 24). Our studies in bicarbonate-loaded intact dogs confirm these findings. The concentration of ultrafilterable calcium fell, however, and this coupled with the alkalosis (20) could have stimulated PTH secretion. Since PTH has been shown to increase urinary bicarbonate excretion (7–10), it is possible that the observed decrease in bicarbonate reabsorption was the consequence of the elaboration of PTH and not of volume expansion per se. To resolve this question TPTX animals were studied. Despite hypoparathyroidism  $R_{HCO_3}$  fell significantly with volume expansion thus excluding the possibility that the effect of volume expansion is mediated entirely through PTH. In confirmation of our previous studies, volume expansion of these acutely TPTX dogs did result in a fall in phosphate reabsorption (22) even though the clearance of phosphate did not change significantly.

As early as 1935 Ellsworth and Nicholson (7) noted that the infusion of PTE was followed by a rise in urinary pH, bicarbonate, sodium, and phosphate. They postulated that PTE produced an increase in sodium excretion and secondarily increased phosphate and bicarbonate excretion. Kleeman and Cooke (8) and Nordin (9) reported similar findings but were unable to determine from their data whether the changes were the result of an increase in GFR or of a direct effect of PTE on the renal tubule. The effects of PTE have been shown to precede any rise in the GFR by Hellman et al. (10). They postulated that the PTE inhibits sodium-for-hydrogen ion exchange in the renal tubule and that the inhibition is mediated by direct interfer-

ence with the ability of the kidney to maintain a hydrogen ion gradient between the renal tubular cells and tubular fluid. These findings have clinical significance since primary hyperparathyroidism (12, 13) and hyperparathyroidism secondary to intestinal malabsorption (14, 15) and to renal failure are associated with systemic acidosis. It has also been shown that the acidosis can be blunted or corrected by parathyroidectomy (16). These observations have led to the suggestion that PTH causes the systemic acidosis by depressing tubular absorption of bicarbonate. This view, however, has been challenged by a number of authors. Kurtzman, Karlin-sky, and Sager (27) could not demonstrate a decrease in  $R_{HCO_3}$  when they infused 60 U PTE/h in dogs. Other investigators (28–30) have attributed the changes in bicarbonate homeostasis in hyperparathyroidism to phosphate depletion induced by PTH rather than to the direct action of the hormone on  $R_{HCO_3}$ .

To help resolve this conflict we studied the effects of a purified PTE, that is devoid of hemodynamic effects (31, 32), in intact and TPTX dogs loaded with bicarbonate. The infusion of PTE in a dose similar to that previously employed by Hellman et al. (10) led to a fall in bicarbonate absorption in both groups, but more marked in the TPTX group. The infusion of PTE produced no change in GFR or RBF. Thus, PTE appears to exert a direct effect on the renal tubules to inhibit hydrogen ion secretion and  $R_{HCO_3}$ , and this effect is not dependent on the induction of a state of phosphate depletion. Since PTE has been shown to inhibit proximal tubular sodium and water absorption (31, 32) it is possible that  $R_{HCO_3}$  in this segment of the nephron is also inhibited. It may be argued, however, that the fall in bicarbonate absorption in our experiments may have been due to the expansion of extracellular volume consequent to the continued infusion of bicarbonate solution. In experiments on dogs receiving bicarbonate solution for a total duration comparable to that of the experiments on PTE, however,  $R_{HCO_3}$  remained stable. Furthermore, the hematocrit in the experimental periods was unchanged from that in control in five of the six groups not receiving saline solution. Finally, in four of the six groups not given saline, the fraction of the filtered chloride excreted was less than 1% and does not rise significantly in any of the groups during the experimental periods. These observations militate against significant expansion by bicarbonate solution in the present experiments both in the control and experimental periods. Thus, it appears that the fall in  $R_{HCO_3}$  was not spontaneous, but rather the consequence of PTE infusion. The difference between our data and those of Kurtzman et al. (27) may be attributed to the larger dose employed in the present studies. However, since they did observe a fall in  $R_{HCO_3}$  after PTE, their conclusion differs from

ours primarily because  $R_{HCO_3}$  also fell in their control animals thereby obscuring the effects of PTE. There is no disagreement, however, with respect to the depression of  $R_{HCO_3}$  by PTE in TPTX animals.

Metabolic alkalosis has been associated with extra-parathyroid hypercalcemia (33, 34). The mechanism for the alkalosis has not been elucidated, but it is possibly the consequence of suppression of PTH secretion since hypoparathyroidism has been associated with alkalosis (17), and thyroparathyroidectomy in our experiments significantly raises  $R_{HCO_3}$ . Calcium, however, has been shown to stimulate tubular secretion of hydrogen ion (11) and could conceivably enhance tubular  $R_{HCO_3}$ . Indeed, when we induced acute hypercalcemia in intact dogs  $R_{HCO_3}$  rose and a similar rise was observed in TPTX dogs. Thus, calcium appears to enhance bicarbonate absorption by a direct effect on the renal tubules not dependent on the suppression of PTH. Calcitonin also is secreted after elevation of the serum calcium, and although its effect on bicarbonate absorption is variable in different species (35, 36) it does not appear to play a role in mediating the effect of calcium on bicarbonate absorption since calcium exerted an effect in animals that were also thyroidectomized and free of the potential for secreting this hormone.

Since hypercalcemia and hyperparathyroidism frequently coexist we investigated the interrelationship of calcium and parathyroid hormone action on the renal tubule. PTE was infused into TPTX dogs made hypercalcemic. Hypercalcemia caused a rise in  $R_{HCO_3}$  and an increase in the reabsorption of phosphate as shown in a similar group of dogs discussed above. Although RBF fell somewhat in this group, GFR was not altered. The infusion of PTE returned the  $R_{HCO_3}$  to below the control value. The RBF and the reabsorbed phosphate returned to control values. Therefore, it is apparent that calcium exerts a separate action on the renal tubule and this effect can be suppressed with PTE. These studies also lend further support to the conclusion that PTE does depress tubular absorption of bicarbonate. This is the probable explanation for the observation that patients with primary hyperparathyroidism may be acidotic despite hypercalcemia.

Of interest in this group of studies is the demonstration that calcium infusion enhanced phosphate reabsorption even when the parathyroid glands had been ablated, thus supporting the view that a phosphate transport system may exist which is independent of PTH and responsive to calcium (37). Lavender and Pullman have also observed increased phosphate absorption when they infused calcium into the renal arteries of dogs (38). Wen, on the other hand, could not detect a decrease in phosphate clearance after the infusion of  $CaCl_2$  into dogs (39). Also at variance with

our studies are the observations of Eisenberg (40) who reported increased clearance of phosphate after infusion of calcium for 72 h in hypoparathyroid patients. His patients, however, were hypocalcemic at the outset and became normocalcemic after the calcium infusion. They were also preloaded with an acidic mixture of sodium phosphate, and they received a prolonged infusion of calcium and also received 2 liters of half-isotonic saline every 24 h. The role of each of these factors in bringing about the increased clearance and decreased absorption of phosphate remains to be elucidated. Thus, the data on the effect of  $Ca^{++}$  on phosphate reabsorption are quite conflicting. Because of the inconsistency between phosphate clearance and reabsorption in our TPTX dogs made hypercalcemic, it is possible that the apparent enhancement of phosphate reabsorption is simply a passive consequence of the rise in serum phosphorus.

In summary, the lowering of  $R_{HCO_3}$  by volume expansion does not depend solely on the stimulation of PTH secretion. PTH, however, does appear to act directly on the renal tubule to lower  $R_{HCO_3}$ . Calcium enhances  $R_{HCO_3}$  in the presence and in the absence of PTH and thyrocalcitonin. The enhanced  $R_{HCO_3}$  by calcium can be suppressed by elevated levels of PTH. The effects of volume expansion, calcium, and PTH do not appear to be mediated by changes in renal hemodynamics although, in the case of calcium infusion in TPTX dogs, this cannot be completely excluded. Calcium also appears to enhance phosphate reabsorption independent of PTH; this apparent effect, however, may have been the passive consequence of increased plasma phosphorus.

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#### REFERENCES

1. Leaf, A., W. B. Schwartz, and A. S. Relman. 1954. Oral administration of a potent carbonic anhydrase inhibitor ("Diamox"). I. Changes in electrolyte and acid-base balance. *N. Engl. J. Med.* 250: 759-764.
2. Rector, F. C., Jr., D. W. Seldin, A. D. Roberts, Jr., and J. S. Smith. 1960. The role of plasma  $CO_2$  tension and carbonic anhydrase activity in the renal reabsorption of bicarbonate. *J. Clin. Invest.* 39: 1706-1721.
3. Rector, F. C., Jr., H. A. Bloomer, and D. W. Seldin. 1964. Effect of potassium deficiency on the reabsorption of bicarbonate in the proximal tubule of the rat kidney. *J. Clin. Invest.* 43: 1976-1982.
4. Schwartz, W. B., C. van Ypersele de Strihou, and J. P. Kassierer. 1968. Role of anions in metabolic alkalosis



- and potassium deficiencies. *N. Engl. J. Med.* **279**: 630-639.
5. Kurtzman, N. A. 1970. Regulation of renal bicarbonate reabsorption by extracellular volume. *J. Clin. Invest.* **49**: 586-595.
  6. Hebert, C. S., M. Martinez-Maldonado, G. Eknoyan, and W. N. Suki. 1972. Relation of bicarbonate to sodium reabsorption in dog kidney. *Am. J. Physiol.* **222**: 1014-1020.
  7. Ellsworth, R., and W. M. Nicholson. 1935. Further observations upon the changes in the electrolytes of the urine following the injection of parathyroid extract. *J. Clin. Invest.* **14**: 823-827.
  8. Kleeman, C. R., and R. E. Cooke. 1951. The acute effects of parathyroid hormone on the metabolism of endogenous phosphate. *J. Lab. Clin. Med.* **38**: 112-127.
  9. Nordin, B. E. C. 1960. The effect of intravenous parathyroid extract on urinary pH, bicarbonate and electrolyte excretion. *Clin. Sci. (Oxf.)*. **19**: 311-319.
  10. Hellman, D. E., W. Y. W. Au, and F. C. Bartter. 1964. Evidence for a direct effect of parathyroid hormone on urinary acidification. *Am. J. Physiol.* **209**: 643-650.
  11. Richet, G., R. Ardaillou, C. Amiel, and M. Lecestre. 1963. Acidification de l'urine par injection intraveineuse de sels de calcium. *J. Urol. Nephrol.* **69**: 373-398.
  12. Siddiqui, A. A., and D. R. Wilson. 1972. Primary hyperparathyroidism and proximal renal tubular acidosis: report of two cases. *Can. Med. Assoc. J.* **106**: 654-659.
  13. Barzel, U. S. 1972. The differential diagnosis of hypercalcemia. *Ann. Intern. Med.* **76**: 825-826.
  14. Muldowney, F. P., R. Freaney, and D. McGeeney. 1968. Renal tubular acidosis and amino-aciduria in osteomalacia of dietary origin. *Quart. J. Med.* **37**: 517-539.
  15. Muldowney, F. P., J. F. Donohoe, R. Freaney, C. Kampff, and M. Swan. 1970. Parathormone-induced renal bicarbonate wastage in intestinal malabsorption in chronic renal failure. *Ir. J. Med. Sci.* **3**: 221-231.
  16. Muldowney, F. P., D. V. Carrole, J. F. Donohoe, and R. Freaney. 1971. Correction of renal bicarbonate wastage by parathyroidectomy. Implications in acid-base homeostasis. *Quart. J. Med.* **40**: 487-498.
  17. Barzel, U. S. 1969. Systemic alkalosis in hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **29**: 917-918.
  18. Moore, E. W. 1970. Ionized calcium in normal serum, ultrafiltrates, and whole blood determined by ion-exchange electrodes. *J. Clin. Invest.* **49**: 318-334.
  19. Massry, S. G., J. W. Coburn, L. W. Chapman, and C. R. Kleeman. 1967. Effect of NaCl infusion on urinary  $Ca^{++}$  and  $Mg^{++}$  during reduction in their filtered loads. *Am. J. Physiol.* **213**: 1218-1224.
  20. Kaplan, E. L., B. J. Hill, S. Locke, and G. W. Peskin. 1971. Acid-base balance and parathyroid function: metabolic alkalosis and hyperparathyroidism. *Surgery (St. Louis)*. **70**: 198-204.
  21. Wolf, A. V. 1950. *The Urinary Function of the Kidney*. Grune & Stratton, Inc., New York. p. 65.
  22. Hebert, C. S., D. Rouse, G. Eknoyan, M. Martinez-Maldonado, and W. N. Suki. 1972. Decreased phosphate reabsorption by volume expansion in the dog. *Kidney Int.* **2**: 247-252.
  23. Stinebaugh, B. J., S. A. Barton, W. N. Suki, G. Eknoyan, and M. Martinez-Maldonado. 1971. Renal handling of bicarbonate: effect of mannitol diuresis. *Am. J. Physiol.* **220**: 1271-1274.
  24. Suki, W. N., M. Martinez-Maldonado, D. Rouse, and A. Terry. 1969. Effect of expansion of extracellular fluid volume on renal phosphate handling. *J. Clin. Invest.* **48**: 1888-1894.
  25. Suki, W. N., G. Eknoyan, F. C. Rector, Jr., and D. W. Seldin. 1969. The renal diluting and concentrating mechanism in hypercalcemia. *Nephron.* **6**: 50-61.
  26. Slatopolsky, E., P. Hoffsten, M. Purkerson, and N. S. Bricker. 1970. On the influence of extracellular fluid volume expansion and of uremia on bicarbonate reabsorption in man. *J. Clin. Invest.* **49**: 988-998.
  27. Kurtzman, N. A., M. L. Karlinsky, and D. S. Sager. 1973. Effects of infusion of cyclic AMP and parathyroid hormone on renal bicarbonate reabsorption. *Clin. Res.* **21**: 283. (Abstr.)
  28. Coburn, J. W., and S. G. Massry. 1970. Changes in serum and urinary calcium during phosphate depletion: studies on mechanism. *J. Clin. Invest.* **49**: 1073-1087.
  29. Kleeman, C. R., and O. S. Better. 1973. Disordered divalent ion metabolism in kidney disease: comments on pathogenesis and treatment. *Kidney Int.* **4**: 73-79.
  30. Gold, L. W., S. G. Massry, A. I. Arief, and J. W. Coburn. 1973. Renal bicarbonate wasting during phosphate depletion. A possible cause of altered acid-base homeostasis in hyperparathyroidism. *J. Clin. Invest.* **52**: 2556-2562.
  31. Agus, Z. S., J. B. Puschett, D. Senesky, and M. Goldberg. 1971. Mode of action of parathyroid hormone and cyclic adenosine 3',5'-monophosphate on renal tubular phosphate reabsorption in the dog. *J. Clin. Invest.* **50**: 617-626.
  32. Agus, Z. S., L. B. Gardner, L. H. Beck, and M. Goldberg. 1973. Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium and phosphate. *Am. J. Physiol.* **224**: 1143-1148.
  33. Heinemann, H. O. 1965. Metabolic alkalosis in patients with hypercalcemia. *Metab. (Clin. Exp.)*. **14**: 1137-1152.
  34. Willis, M. R. 1971. Value of plasma chloride concentration and acid-base status in the differential diagnosis of hyperparathyroidism from other causes of hypercalcemia. *J. Clin. Pathol. (Lond.)*. **24**: 219-227.
  35. Aldred, J. P., R. R. Kleszynski, and J. W. Bastian. 1970. Effects of acute administration of porcine and salmon calcitonin on urine electrolyte excretion in rats. *Proc. Soc. Exp. Biol. Med.* **134**: 1175-1180.
  36. Ardaillou, R., P. Vuagnat, G. Milhaud, and G. Richet. 1967. Effets de la thyrocalcitonine sur l'excretion rénale des phosphate, du calcium et des ion  $H^+$  chez l'homme. *Nephron.* **4**: 298-314.
  37. Glorieux, F., and C. R. Scriver. 1972. Loss of a parathyroid hormone-sensitive component of phosphate transport in x-linked hypophosphatemia. *Science (Wash. D. C.)*. **175**: 997-1000.
  38. Lavender, A. R., and T. N. Pullman. 1963. Changes in inorganic phosphate excretion induced by renal arterial infusion of calcium. *Am. J. Physiol.* **205**: 1025-1032.
  39. Wen, S.-F. 1974. Micropuncture studies of phosphate transport in the proximal tubule of the dog. The relationship to sodium reabsorption. *J. Clin. Invest.* **53**: 143-153.
  40. Eisenberg, E. 1965. Effects of serum calcium level and parathyroid extracts on phosphate and calcium excretion in hypoparathyroid patients. *J. Clin. Invest.* **44**: 942-946.