

## The phosphaturic effect of sodium bicarbonate and acetazolamide in dogs

Milford Fulop, Paul Brazeau

*J Clin Invest.* 1968;47(5):983-991. <https://doi.org/10.1172/JCI105813>.

**Research Article**

Urinary inorganic phosphate excretion was studied before and during the administration of sodium bicarbonate and acetazolamide in dogs that were not given infusions of phosphate. The excretion fraction of filtered phosphate increased after sodium bicarbonate or acetazolamide was given. This phosphaturia was attributed to decreased tubular reabsorption of phosphate consequent to alkalization of either tubular urine or cells.

**Find the latest version:**

<https://jci.me/105813/pdf>



# The Phosphaturic Effect of Sodium Bicarbonate and Acetazolamide in Dogs

MILFORD FULOP and PAUL BRAZEAU

*From the Departments of Medicine and Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461*

**ABSTRACT** Urinary inorganic phosphate excretion was studied before and during the administration of sodium bicarbonate and acetazolamide in dogs that were not given infusions of phosphate. The excretion fraction of filtered phosphate increased after sodium bicarbonate or acetazolamide was given. This phosphaturia was attributed to decreased tubular reabsorption of phosphate consequent to alkalinization of either tubular urine or cells.

## INTRODUCTION

The role of the kidney in phosphate homeostasis is still imperfectly understood. There is particular disagreement about whether acute changes of systemic or urinary pH affect the urinary excretion of orthophosphate (P, [1, 2]). Previous studies that were directed toward answering this question usually entailed the infusion of P in order to estimate its reabsorptive transport maximum ( $T_{mP}$ ). However, phosphate infusion indirectly causes increased parathormone secretion (3) that can, in turn, decrease the tubular reabsorption of P. Accordingly, it would be preferable to evaluate the influence of other variables in the absence of acute loading with exogenous P.

The effect of urinary pH changes on P excretion was therefore examined in clearance experiments with fasting anesthetized dogs not given P.

An abstract of this study appeared in *J. Clin. Invest.* 1967. 46: 1059.

Address requests for reprints to Dr. Milford Fulop, Department of Medicine, Jacobi Hospital, Pelham Parkway South and Eastchester Road, New York 10461.

*Received for publication 3 May 1967 and in revised form 5 July 1967.*

The fraction of filtered P that was excreted increased during urinary alkalinization regardless of whether the pH of the blood increased ( $\text{NaHCO}_3$  infusion) or decreased (acetazolamide administration).

## METHODS

*Subjects and experimental procedures.* The experiments were performed in 29 mongrel female dogs, weighing between 12 and 19 kg, that had been fasted and thirsted for 18 hr before being anesthetized with i.v. pentobarbital, 30 mg/kg. 200–300 ml of 154 mM NaCl was infused i.v. just before the inulin priming dose. Inulin sustaining infusions were usually given in 128 mM NaCl–1.5% urea at a rate of 5 ml/min in order to insure moderate diuresis. Preliminary studies showed that mild urea diuresis did not affect P excretion, in confirmation of other reports (4, 5). Sustaining infusions were given for at least 80 min before control urine collections were started. Urine was collected under paraffin oil through an indwelling catheter, and the 10-min clearance periods were terminated by bladder compression, usually without water or air washout. The completeness of urine collections was confirmed by the very close values of urine flow (V) and inulin clearance ( $C_{in}$ ) in consecutive paired periods, and the data presented in the figures are the average values for each such pair. Heparinized blood specimens were obtained from indwelling arterial canulas at the midpoint of each period;<sup>1</sup> blood pH was measured in specimens collected in oiled syringes usually between consecutive periods.

7 of the dogs received 3–5 g of ammonium chloride orally 18 hr before the experiment. After two to three control urine collections were obtained, six dogs were given acetazolamide i.v. (three of them pretreated with ammonium chloride), and seven dogs were given  $\text{NaHCO}_3$  i.v. (four of them pretreated with ammonium chloride).  $\text{NaHCO}_3$  was usually given at a rate of 3 mEq/min for about 10 min, followed by 0.5–0.6 mEq/min; in several studies, no loading dose was given, and the Na-

<sup>1</sup> Venous blood was used in two of the alkalinization experiments and in all of those shown in Table IV.

HCO<sub>3</sub> sustaining infusion was larger (Table I). Acetazolamide was administered in a single dose of 3–5 mg/kg, sometimes followed by a sustaining infusion of 100–167 µg/kg per min; it was also given in seven other studies during NaHCO<sub>3</sub> infusion. Clearance periods during bicarbonate infusions were begun an average of 24 min after the preceding control period; after acetazolamide administration, they were started an average of 7 min after the preceding urine collection. Despite these relatively short intervals, the consecutive paired experimental periods matched very closely with respect to V, C<sub>IN</sub>, and urinary pH. In five experiments the control periods were obtained during the infusion of 0.25–0.4 mEq of NaHCO<sub>3</sub> per min, and in one experiment they were obtained during urea–NaCl infusion, after which 50 mmoles of NH<sub>4</sub>Cl (0.5 mole/liter) was instilled into the dogs' stomachs. After the urine had become acid (85–150 min later), clearance periods were again obtained. Four of these dogs were then given NaHCO<sub>3</sub> again after these intermediate urinary acidification periods.

The effect of infusing more NaCl, or Na<sub>2</sub>SO<sub>4</sub>, was studied in 10 experiments. In five, the control observations made during the infusion of 128 mM NaCl<sup>2</sup> at 5 ml/min were followed by the infusion of 144 mM NaCl at 10 ml/min. In five others the control observations were followed by priming and sustaining infusions of either NaCl or Na<sub>2</sub>SO<sub>4</sub> which yielded somewhat higher rates of Na<sup>+</sup> administration than those used in the usual NaHCO<sub>3</sub> experiments. The priming infusions delivered 3 mEq of Na<sup>+</sup> per min for 10 min and the sustaining infusions either 1.13 mEq (as NaCl) or 0.75 mEq (as Na<sub>2</sub>SO<sub>4</sub>) per min.

*Analytical.* The concentration of inorganic P in plasma and urine was estimated by the method of Taussky and Shorr (6). Inulin was estimated by the method of Higashi and Peters (7) adapted for the Auto-

<sup>2</sup> Urea was included in all of these infusions at a concentration calculated to deliver 75 mg/min.

Analyzer.<sup>3</sup> Plasma bicarbonate was estimated with a Natelson microgasometer. The pH of blood and urine was measured with a Cambridge model R pH meter at 37°C. Sodium concentrations in plasma and urine were measured with an AutoAnalyzer.<sup>3</sup>

The filtered load of P (F<sub>P</sub>) was taken as C<sub>IN</sub> × plasma P and tubular reabsorption of P as F<sub>P</sub> – urinary P excretion (U<sub>P</sub>V). Corrections were not made for the slight binding of P to plasma proteins (8), for the Donnan effect, or for plasma water concentration, the resultant error presumably being small and consistent in these experiments which involved small changes of blood pH. The average urine pH for each pair of consecutive clearance periods was calculated by converting the individual pH values to H<sup>+</sup> concentrations, averaging them, and reconverting the averages to pH values. Urinary OH<sup>-</sup> concentration was calculated from the formula OH<sup>-</sup> = 10<sup>-14</sup> × antilog pH. In making these calculations, H<sup>+</sup> concentration was assumed to be equal to H<sup>+</sup> activity.

## RESULTS

The changes of blood pH averaged only +0.10 ± 0.02 (1 SD) during NaHCO<sub>3</sub> infusion, –0.01 ± 0.04 after acetazolamide given during NaCl infusion, and –0.04 ± 0.04 after acetazolamide given during NaHCO<sub>3</sub> infusion. Plasma P changed relatively little from control values during the experimental periods, averaging –1% during NaHCO<sub>3</sub> infusion and –4% after acetazolamide administration.

The control urinary pH values were between 6.30 and 7.12 during urea–NaCl diuresis. The changes induced ranged from +0.32 to +1.29 pH

<sup>3</sup> Technicon Instrument Corp., Chauncey, N. Y.

TABLE I  
Effect of NaHCO<sub>3</sub> Infusion on Urinary Inorganic Phosphate Excretion

Time <i>min</i>	V <i>ml/min</i>	Urine pH	C <sub>IN</sub> <i>ml/min</i>	Plasma P <i>µg/ml</i>	P		C <sub>P</sub> <i>ml/min</i>	C <sub>P</sub> /C <sub>IN</sub> <i>%</i>
					Filtered <i>mg/min</i>	Excreted <i>mg/min</i>		
0	4 g NH <sub>4</sub> Cl per os at –16 hr							
20	Infuse 200 ml of 154 mM NaCl.							
25	Inulin prime, 2.9 g i.v.							
	Start sustaining infusion of inulin 4.17 mg/ml in 154 mM NaCl at 6 ml/min							
173–183	6.76	6.68	35.3	70.0	2.47	1.11	15.8	44.8
183–193	6.75	6.71	33.9	69.0	2.34	1.07	15.5	45.6
194	Change sustaining infusion to inulin 4.17 mg/ml in 400 mM NaHCO <sub>3</sub> at 6 ml/min							
217–227	7.47	7.43	34.0	70.0	2.38	1.21	17.3	50.9
227–237	7.10	7.53	32.7	70.0	2.28	1.27	18.2	55.6
237–247	6.83	7.56	32.6	67.0	2.18	1.29	19.3	59.4
247–257	5.90	7.66	30.6	67.0	2.04	1.19	17.7	58.0

V, urine flow; C<sub>IN</sub>, inulin clearance; C<sub>P</sub>, phosphorus clearance.

\* Dog 65-26, 16 kg (urine collections from right ureter).

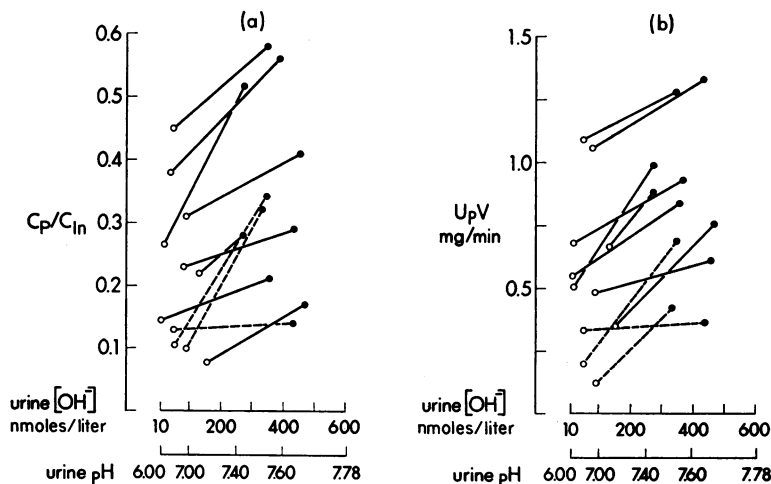


FIGURE 1 Relation between phosphate/inulin clearance ( $C_P/C_{In}$  [a]), urinary phosphate excretion ( $U_{P/V}$  [b]), and urinary alkalinity in dogs given sodium bicarbonate.  $\circ$ , control values;  $\bullet$ , values during urinary alkalinization. Solid lines refer to dogs with constant or decreased filtered loads of P ( $F_P$ ). Dashed lines refer to three dogs in which  $F_P$  increased (5–13%) during bicarbonate infusion.

units during bicarbonate infusion (mean  $+0.69 \pm 0.09$ ) and from  $+0.44$  to  $+0.92$  pH units after acetazolamide that was given during NaCl infusion (mean  $+0.67 \pm 0.09$ ). When acetazolamide was given during  $\text{NaHCO}_3$  infusion urinary pH increased only by  $+0.13$  to  $+0.27$  units (mean  $+0.19 \pm 0.03$ ).

The control  $C_P/C_{In}$  values ranged between 0.05 and 0.60. This variation was at least partly a function of the dogs' ages, the younger animals usually having higher concentrations of plasma P and lower rates of urinary P excretion than the

older ones, as was found by Harrison and Harrison (9).<sup>4</sup>

$F_P$  either decreased or did not change significantly during bicarbonate infusion in 8 of 11 experiments (range  $+1$  to  $-7\%$  as compared to control periods).  $C_P/C_{In}$  nevertheless increased in each case (average 53%, range  $+26$  to  $+118\%$ , Fig. 1 a) as did  $U_{P/V}$  (average 49%, range  $+15$  to  $+117\%$ , Fig. 1 b and Table I).  $F_P$  increased

<sup>4</sup> Differences in antecedent diet may also have played a role in the variation of control  $C_P/C_{In}$  among these dogs.

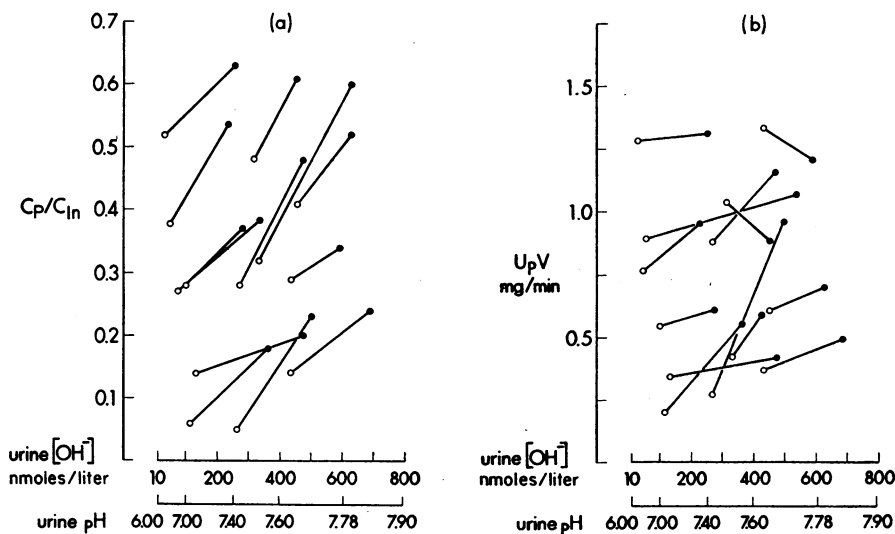


FIGURE 2 Relation between phosphate/inulin clearance (a), urinary phosphate excretion (b), and urinary alkalinity in dogs given acetazolamide. Symbols are the same as in Fig. 1.  $F_P$  decreased in all of these dogs after acetazolamide administration.

TABLE II  
Effect of Acetazolamide on Urinary Inorganic Phosphate Excretion

Time	V	Urine pH	C <sub>In</sub>	Blood pH	Plasma P	P		C <sub>P</sub>	C <sub>P</sub> /C <sub>In</sub>
						Filtered	Excreted		
min	ml/min		ml/min		μg/ml	mg/min	mg/min	ml/min	%
0	Infuse 250 ml of 154 mM NaCl with 4 g urea.								
16	Inulin prime, 3.5 g i.v.								
21	Start sustaining infusion of inulin 5.5 mg/ml with 15 mg/ml urea in 128 mM NaCl at 5 ml/min.								
115-125	2.48	6.86	76.8	7.37	41.8	3.21	0.87	20.9	27.2
125-135	2.62	6.82	80.0	7.37	42.1	3.37	0.91	21.6	27.1
137	80 mg acetazolamide i.v., and add acetazolamide, 8 mg/kg per hr, to sustaining infusion								
146-154	6.09	7.73	70.5	7.36	40.6	2.86	1.07	26.3	37.3
154-162	5.96	7.73	69.8	7.36	38.9	2.72	1.07	27.6	39.5

See Table I for explanation of abbreviations.

\* Dog 67-5, 19 kg.

by 5, 6, and 13% respectively in three other experiments (dashed lines in Figs. 1 a and b), and both C<sub>P</sub>/C<sub>In</sub> and U<sub>P</sub>V increased strikingly in two of them; the increase in the third was trifling. It is especially significant in the former two experiments that the calculated P reabsorption decreased despite the increased F<sub>P</sub>.

Administration of acetazolamide was followed

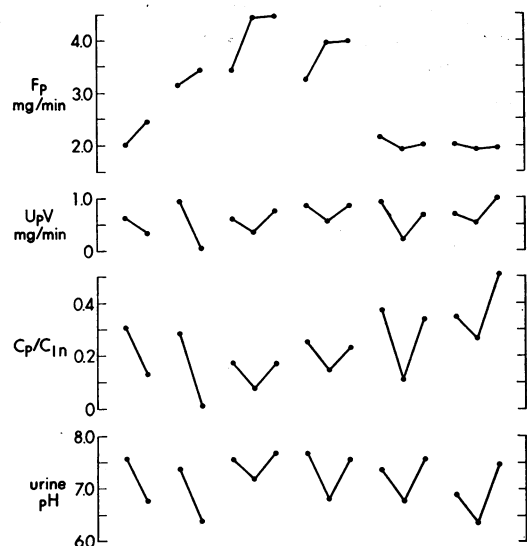


FIGURE 3 Filtered load (F<sub>P</sub>) and urinary excretion of phosphate in six dogs in relation to urinary pH changes. The first point in each of the first five studies is the average of the values in each dog during sodium bicarbonate infusion and, in the sixth dog, during urea-NaCl infusion; the second point is from the periods after ammonium chloride administration; the third point in the last four dogs is from the periods after readministration of sodium bicarbonate.

invariably by a decrease of F<sub>P</sub> (average 17% in the 13 studies, range -7 to -26%) that was largely a consequence of a decrease of C<sub>In</sub> (average 14%, range -9 to -23%). C<sub>P</sub>/C<sub>In</sub> nevertheless increased over the control values in each case (average 84%, range +15 to +360%, Fig. 2 a and Table II). Despite the decreased F<sub>P</sub>, urinary P excretion increased an average of 47% over control values (range +2 to +256%, Fig. 2 b) in 11 of the 13 experiments. In the two animals in which U<sub>P</sub>V decreased (by 15 and 9% as compared with F<sub>P</sub> decreases of 15 and 24% respectively), acetazolamide had been given during NaHCO<sub>3</sub> infusion, and the increase in urinary pH was very small (0.16 and 0.13 units respectively). In experiments in which the increase of urinary pH between initial and final values was similar, the percentage increase of C<sub>P</sub>/C<sub>In</sub> (a value equal to U<sub>P</sub>V/F<sub>P</sub>, the excretion fraction of filtered P) was similar regardless of whether acetazolamide or NaHCO<sub>3</sub> had been given.

Urinary acidification was induced in six experiments by the intragastric instillation of NH<sub>4</sub>Cl after the control periods had been obtained (Fig. 3). C<sub>P</sub>/C<sub>In</sub> and U<sub>P</sub>V decreased remarkably when the urine became acid even though F<sub>P</sub> actually increased in the first four studies shown. In the last four experiments the dogs were then given NaHCO<sub>3</sub> again to reestablish urinary alkalization, and P excretion thereupon increased to about the original control levels (see representative protocol in Table III).

The effect of infusing either more saline, or Na<sub>2</sub>SO<sub>4</sub>, was examined in order to learn whether

TABLE III  
Comparison of Inorganic Phosphate Excretion in Alkaline and in Acid Urine

Time <i>min</i>	V <i>ml/min</i>	Urine pH	C <sub>in</sub> <i>ml/min</i>	Blood pH	Plasma P <i>μg/ml</i>	P		C <sub>P</sub> <i>ml/min</i>	C <sub>P</sub> /C <sub>in</sub> <i>%</i>
						Filtered <i>mg/min</i>	Excreted <i>mg/min</i>		
0	Infuse 250 ml of 154 mM NaCl.								
15	Inulin prime, 3.0 g i.v.								
21	Start sustaining infusion of inulin, 5 mg/ml, with 15 mg/ml urea in 128 mM NaCl at 5 ml/min.								
81	Change sustaining infusion to include 67 mM NaHCO <sub>3</sub> (333 μmoles NaHCO <sub>3</sub> per min).								
100-110	4.65	7.59	98.6	7.37	33.3	3.28	0.86	25.9	26.3
110-120	4.60	7.68	97.5	7.37	32.8	3.20	0.79	24.2	24.8
122	Change sustaining infusion to omit NaHCO <sub>3</sub> .								
125	Give 50 ml of 0.5 M NH <sub>4</sub> Cl by gastric tube.								
140	Give 50 ml of 0.5 M NH <sub>4</sub> Cl by gastric tube.								
185-195	7.60	6.81	103.5	7.27	37.4	3.87	0.55	14.6	14.1
195-205	7.10	6.80	104.5	7.27	38.8	4.05	0.57	14.7	14.0
206	Change sustaining infusion to inulin, 5 mg/ml, with 15 mg/ml urea in 600 mM NaHCO <sub>3</sub> at 5 ml/min.								
216	Change sustaining infusion to inulin, 5 mg/ml, with 15 mg/ml urea in 128 mM NaCl-100 mM NaHCO <sub>3</sub> at 5 ml/min (500 μmoles NaHCO <sub>3</sub> per min).								
225-235	7.80	7.56	111.1	7.37	36.6	4.07	0.85	23.3	21.0
235-245	6.65	7.54	110.6	7.37	35.5	3.93	0.83	23.4	21.1

See Table I for explanation of abbreviations.

\* Dog 66-22, 18 kg.

the phosphaturic effects of NaHCO<sub>3</sub> and acetazolamide might be related to either decreased tubular reabsorption of sodium or perhaps expansion of extracellular fluid volume. Neither infusing Na<sub>2</sub>SO<sub>4</sub>, slightly hypertonic NaCl, nor doubling the rate of isotonic NaCl administration had any effect on urinary P excretion in 9 of the 10 dogs (Tables IV and V) regardless of whether natri-

TABLE IV  
Effect of Sodium Chloride and Sodium Sulfate Infusions on Urinary Inorganic Phosphate Excretion

Dog	U <sub>P</sub> V/F <sub>P</sub>		U <sub>Na</sub> V/F <sub>Na</sub>		Reabsorbed Na		V	
	Control*	Diuresis†	Control	Diuresis	Control	Diuresis	Control	Diuresis
	<i>%</i>		<i>%</i>		<i>μEq/min</i>		<i>ml/min</i>	
A. Isotonic saline infused at 10 ml/min								
1	60.0	58.7					2.0	5.8
2	4.3	8.1	4.1	4.4	15,771	15,280	5.0	6.5
3	24.3	22.8	4.7	9.0	9778	9645	2.8	7.2
4	2.0	1.0	4.2	4.4	11,049	9400	2.7	2.7
5	31.6	31.3	2.3	5.2	11,100	11,210	1.8	4.1
B. Hypertonic saline infused at 5 ml/min								
6	22.9	23.7	2.1	4.9	14,997	15,359	1.7	4.2
7	17.4	20.0	5.7	6.7	11,058	11,413	4.9	5.9
C. Sodium sulfate infused at 5 ml/min								
8	27.0	26.1					1.9	2.8
9	30.1	31.3	7.4	11.5	7642	7245	3.4	4.7
10	29.2	29.6	3.2	9.6	9525	9373	1.5	4.2

U<sub>P</sub>V, urinary phosphate excretion; U<sub>Na</sub>V, urinary sodium excretion; F<sub>P</sub>, filtered phosphate; F<sub>Na</sub>, filtered sodium.

\* Control values are the averages from 2 to 3 consecutive clearance periods during the infusion of 128 mM NaCl-1.5% urea at 5 ml/min.

† Diuresis values are the averages from 2 to 4 consecutive clearance periods during the indicated infusions: A, 144 mM NaCl-0.75% urea at 10 ml/min; B, 226 mM NaCl-1.5% urea at 5 ml/min; C, 75 mM Na<sub>2</sub>SO<sub>4</sub>-1.5% urea at 5 ml/min.

TABLE V  
Effect of Saline Infusion on Urinary Inorganic Phosphate Excretion

Time	V	Urine pH	C <sub>In</sub>	Plasma P	P		C <sub>P</sub>	C <sub>P</sub> /C <sub>In</sub>	U <sub>Na</sub> V/F <sub>Na</sub>	Reabsorbed Na
					Filtered	Excreted				
<i>min</i>	<i>ml/min</i>		<i>ml/min</i>	<i>μg/ml</i>	<i>mg/min</i>		<i>ml/min</i>	%	%	<i>μEq/min</i>
0	Infuse 250 ml of 154 mM NaCl with 3 g urea.									
17	Inulin prime, 2.8 g i.v.									
25	Start sustaining infusion of inulin 4 mg/ml with 15 mg/ml urea in 128 mM NaCl at 5 ml/min.									
115-125	2.0	6.86	80.0	50.1	4.01	1.26	25.2	31.5	2.5	11,307
125-135	1.7	6.69	72.1	50.5	3.64	1.15	22.8	31.6	2.3	10,357
135-145	1.8	6.68	80.3	49.8	3.99	1.26	25.4	31.7	2.1	11,637
147	Change sustaining infusion to inulin 2 mg/ml with 7.5 mg/ml urea in 144 mM NaCl at 10 ml/min.									
175-185	3.4	6.79	77.6	48.3	3.74	1.20	25.0	32.2	4.4	11,097
185-195	4.2	6.82	81.5	47.4	3.86	1.19	25.1	30.8	5.3	11,412
195-205	4.2	6.74	79.6	47.0	3.73	1.16	24.7	31.0	5.4	11,066
205-215	4.5	6.75	81.2	44.8	3.63	1.09	24.3	29.9	5.6	11,264

See Tables I and IV for explanation of abbreviations.

\* Dog 5, 15 kg.

uresis and diuresis or volume expansion predominated. Extracellular fluid volume usually increased during these secondary infusions (see volume infused minus volume excreted per minute) and the *fractional* reabsorption of filtered sodium usually decreased. In the only instance in which C<sub>P</sub>/C<sub>In</sub> did increase significantly (Table IV, dog 2), there was actually only a slight decrease of fractional sodium reabsorption. Although the *absolute* rate of tubular sodium reabsorption decreased by 3% in that dog, the percentage decreases of absolute sodium reabsorption were actually greater in dogs 4 and 9 (15 and 5% respectively) in which C<sub>P</sub>/C<sub>In</sub> did not change. With respect to extracellular fluid volume expansion, which presumably occurred in all the dogs, dog 2 that weighed 18 kg retained much less of the additional infused saline than did dog 4 that weighed 15 kg.

## DISCUSSION

Previous studies of the effect of NaHCO<sub>3</sub> administration on urinary inorganic phosphate excretion have yielded contradictory results. Pitts and Alexander reported that the maximum reabsorption of phosphate in dogs was unaffected by NaHCO<sub>3</sub> infusion (1). However, Malvin and Lotspeich found that NaHCO<sub>3</sub> did decrease the T<sub>mP</sub>, as did both acetazolamide administration and hyperventilation (2). In our own studies of the effects of NaHCO<sub>3</sub> and acetazolamide in dogs given P

infusions, we also found that T<sub>mP</sub> decreased.<sup>5</sup> However, plasma P tended to decrease during NaHCO<sub>3</sub> administration, and glomerular filtration rate (GFR) usually decreased after the administration of acetazolamide. Accordingly, in order to maintain the filtered load of P at a high level, it was necessary for us to increase the rate of P infusion before administering NaHCO<sub>3</sub> or acetazolamide, and this increase was often accompanied by an increase of the P filtered load. Therefore, the cause of the decreased T<sub>mP</sub> observed in those experiments is uncertain because P infusion causes hypocalcemia (10) which evokes increased parathormone secretion (3). Inasmuch as this itself can cause a decrease in the tubular reabsorption of P (11), it is difficult to analyze the effects of other concurrent experimental maneuvers on urinary P excretion when exogenous phosphate is infused.

Attempts to evaluate the influence of NaHCO<sub>3</sub> and acetazolamide on P excretion in the absence of P loading did pose other problems, however. These were that (a) major changes of blood pH may cause large changes of plasma P and GFR, and hence of F<sub>P</sub>, and (b) there are diurnal variations of urinary P excretion. We found in preliminary experiments that moderate systemic acidosis induced by the infusion of HCl at the modest rate of 9-10 mEq/hr often caused GFR to decrease; when NaHCO<sub>3</sub> was infused later, GFR increased and plasma P often decreased sharply.<sup>5</sup>

<sup>5</sup> Fulop, M., and P. Brazeau. Unpublished observations.

Therefore, in order to minimize alterations of blood pH and to maintain both GFR and plasma P stable, we deliberately did not aim to achieve intense aciduria during control periods. We have excluded from this report experiments in which the plasma P concentration was not very stable throughout any given experiment, although the results in those experiments were entirely consistent with the findings reported here. We dealt with the problem of diurnal variation of P excretion in two ways. In the experiments in which control urines were acid, the subsequent collections of alkaline urine were obtained after so little delay that diurnal variation was obviously not a significant factor in the increased phosphaturia. Secondly, when the experimental procedure was reversed so that the control urines were alkaline, P excretion was nevertheless also higher than in the later collections of acid urine (Fig. 3).

Some other investigators who found that  $\text{NaHCO}_3$  infusion caused increased urinary P excretion ascribed this occurrence to the systemic metabolic alkalosis (12, 13). It is possible that systemic alkalosis, by decreasing the plasma concentration of ionized calcium, might evoke increased parathormone secretion and in turn decreased tubular reabsorption of phosphate. However, we found that acetazolamide, which does not cause systemic alkalosis, also enhanced phosphaturia (Table II, Fig. 2, and references 2 and 14). While it is possible that the explanations for the phosphaturic effects of acetazolamide and of  $\text{NaHCO}_3$  are different, it does seem reasonable to seek a unifying explanation. In this regard the effect of respiratory alkalosis on P excretion was naturally also of interest, but it was not possible to study this effect because both brisk hyperventilation and the administration of tris-(hydroxymethyl)aminomethane (THAM) were accompanied by major decreases of  $F_P$  secondary to decreases in both plasma P and GFR.<sup>6</sup>

Renal tubular secretion of P has been demonstrated in certain fish (15), in amphibia (16), and in the chicken (17), but its existence in mammals is unproven (18, 19), although it has been suggested by some experiments (20). If renal tubular secretion of P does occur in dogs, it is possible that the phosphaturia observed in our studies was the consequence of enhanced tubular secretion of P. If phosphate is not secreted by the renal tubules

in dogs, our findings are explained by decreased tubular reabsorption of P.<sup>6</sup>

The mechanism of the phosphaturic effect of  $\text{NaHCO}_3$  and acetazolamide has not been elucidated by the present studies. Assuming that they depressed tubular reabsorption of P through a common mechanism, one can consider four possible explanations. The phosphaturia may have been related to decreased tubular reabsorption of bicarbonate or sodium or to the urinary or intracellular pH changes induced by  $\text{NaHCO}_3$  and acetazolamide. Malvin and Lotspeich suggested that P may compete for tubular reabsorption with the portion of filtered bicarbonate that is reabsorbed independently of carbonic anhydrase activity (2). The present results neither support nor refute that hypothesis.

The fractional reabsorption of filtered sodium (i.e.,  $1 - U_{\text{Na}}/F_{\text{Na}}$ ) decreases after acetazolamide or  $\text{NaHCO}_3$  administration, and it is conceivable that the fractional reabsorption of P might decrease concomitantly. However, when fractional sodium excretion was increased by infusing  $\text{Na}_2\text{SO}_4$  or more NaCl (either as an isotonic or slightly hyperosmotic solution),  $C_P/C_{\text{In}}$  usually did not change (Tables IV and V). Moreover, other studies have shown previously that the brisk natriuresis and diuresis produced by infusing very hyperosmotic NaCl (1), urea (4, 5), or mannitol (5, 22, 23) did not increase P excretion. Incidentally, those earlier studies and our own suggest that the phosphaturic effect of  $\text{NaHCO}_3$  was probably not just a consequence of expanding plasma or extracellular fluid volume which, of course, would not occur after acetazolamide administration. These findings do not exclude the possibility that decreased fractional sodium reabsorption and (or) volume expansion may have played some role in causing the phosphaturia, or that these factors might evoke decreased tubular reabsorption of P in dogs given less preliminary saline loading than those in the present series. However, they do suggest that the major explanation for  $\text{NaHCO}_3$ -induced phosphaturia in our experiments must be sought elsewhere.

<sup>6</sup> Kupfer and Kosovsky have recently pointed out that in some cases of increased phosphaturia ostensibly attributable to decreased tubular reabsorption, a very small portion of the additional urinary P may actually derive from organic P of intracellular origin (21).



In this light, another possible explanation for our findings is that the mechanisms for the renal tubular reabsorption of  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$  are different, as was suggested by Carrasquer and Brodsky (24). If, for example, the divalent ion  $\text{HPO}_4^{2-}$  was less readily reabsorbed by the renal tubules than the monovalent form  $\text{H}_2\text{PO}_4^-$ , the predominance of the divalent anion in alkaline urine ( $\text{pK } 6.8$  for  $\text{H}_2\text{PO}_4^- \rightleftharpoons \text{HPO}_4^{2-}$ ) would account for decreased reabsorption of total inorganic P from alkaline urine. Alkalinization of proximal tubule fluid during bicarbonate infusion (25) clearly favors a shift of equilibrium from  $\text{H}_2\text{PO}_4^-$  to  $\text{HPO}_4^{2-}$ . However, Rector, Carter, and Seldin found that carbonic anhydrase inhibition in rats, despite the decreased reabsorption of bicarbonate, caused proximal tubule fluid to become more acid, ostensibly because the catalytic dehydration of  $\text{H}_2\text{CO}_3$  was delayed under those circumstances (25). If this also occurs in dogs, the phosphaturic effect of acetazolamide could not be explained by a shift from  $\text{H}_2\text{PO}_4^-$  to  $\text{HPO}_4^{2-}$  in proximal tubule fluid. The pH of distal tubule fluid, which presumably does increase during carbonic anhydrase inhibition, may conceivably influence P reabsorption at that site. However, the data from nephron micropuncture studies in rats suggest that most of the reabsorption of P takes place in the proximal tubule (19). If this is also true in dogs, the small fraction of filtered P that reaches the distal tubule would not be sufficient to account for the increments in  $C_P/C_{In}$  observed in some of our experiments.

It is possible that changes of *intracellular* rather than of *luminal* pH in the proximal tubule might account for our findings. One interpretation of the role of carbonic anhydrase in acid-secreting cells is that this enzyme facilitates the cellular generation of carbonic acid, which can then buffer the excess of hydroxyl ions that remains after extrusion of  $\text{H}^+$  from the cells (26). In this view, the effect of carbonic anhydrase is to minimize the alkalinity of  $\text{H}^+$ -secreting cells. Accordingly, inhibition of the enzyme (as by acetazolamide) during  $\text{H}^+$  secretion would cause cell pH to rise (27), and it is likely that cell pH also increases during  $\text{NaHCO}_3$  infusion. Thus, the common factor might be an intracellular shift towards  $\text{HPO}_4^{2-}$ , which then impedes the further reabsorptive transport of P across the tubular epithelium.

While the present experiments indicate that acute urinary alkalinization is accompanied by increased P excretion in dogs, as has also recently been reported by Puschett and Goldberg in humans (28), further studies are needed to assess the effect of longer sustained changes of urinary pH. Nadell administered acetazolamide for several days to humans and found some increase of urinary P excretion, particularly during the 1st day when the urine was most alkaline (29). On the other hand, Martin and Jones reported that mean urinary P excretion decreased slightly in six subjects who ingested 95 mEq of  $\text{NaHCO}_3$  daily for 5 days (30), but their subjects' dietary P intakes were not rigidly controlled throughout. Sartorius, Roemmelt, and Pitts reported that urinary P excretion increased when  $\text{NH}_4\text{Cl}$  was administered for several days to humans (31). However, the increased phosphaturia lagged behind urinary acidification, which implies that this was a compensatory phenomenon rather than a primary effect of urinary acidification.

If it should be found that increased phosphaturia is sustained during chronic urinary alkalinization, this may be a factor in the pathogenesis of certain disorders associated with increased urinary P excretion. Although some authors have reported that P excretion was usually normal in patients with urinary tract calcium phosphate calculi (32), in other series P excretion has been found to be increased in as many as 20% of patients (33). It is therefore possible that increased phosphaturia may be important in the genesis of some calculi. The relationship between basal urinary pH, renal acidifying capacity, and urinary P excretion in such patients should be studied. Increased urinary phosphate excretion also occurs in many patients with renal tubular acidosis. The customary explanation is that this increase is secondary to bone dissolution, which is a consequence of hypercalciuria, acidosis, and (or) secondary hyperparathyroidism (34). Although these may be the major factors, the urinary alkalinity in patients with renal tubular acidosis may also play a role in the pathogenesis of their hyperphosphaturia and renal calcium deposition.

#### ACKNOWLEDGMENTS

Luis Leon, Alma Annexy, and Carol Cheng provided capable technical assistance.

This work was supported by U. S. Public Health Service Grants 55O1FR 5397-05 and FR 50.

## REFERENCES

1. Pitts, R. F., and R. S. Alexander. 1944. The renal reabsorptive mechanism for inorganic phosphate in normal and acidotic dogs. *Am. J. Physiol.* **142**: 648.
2. Malvin, R. L., and W. L. Lotspeich. 1956. Relation between tubular transport of inorganic phosphate and bicarbonate in the dog. *Am. J. Physiol.* **187**: 51.
3. Aurbach, G. D., and J. T. Potts, Jr. 1967. Parathyroid hormone. *Am. J. Med.* **42**: 1.
4. Mudge, G. H., J. Foulks, and A. Gilman. 1949. Effect of urea diuresis on renal excretion of electrolytes. *Am. J. Physiol.* **158**: 218.
5. Wesson, L. G., Jr. 1962. Magnesium, calcium and phosphate excretion during osmotic diuresis in the dog. *J. Lab. Clin. Med.* **60**: 422.
6. Taussky, H. H., and E. Shorr. 1953. A microcolorimetric method for the determination of inorganic phosphorus. *J. Biol. Chem.* **202**: 675.
7. Higashi, A., and L. Peters. 1950. A rapid colorimetric method for the determination of inulin in plasma and urine. *J. Lab. Clin. Med.* **35**: 475.
8. Walser, M. 1960. Protein-binding of inorganic phosphate in plasma of normal subjects and patients with renal disease. *J. Clin. Invest.* **39**: 501.
9. Harrison, H. E., and H. C. Harrison. 1941. The renal excretion of inorganic phosphate in relation to the action of vitamin D and parathyroid hormone. *J. Clin. Invest.* **20**: 47.
10. Hebert, L. A., J. Lemann, Jr., J. R. Petersen, and E. J. Lennon. 1966. Studies of the mechanism by which phosphate infusion lowers serum calcium concentration. *J. Clin. Invest.* **45**: 1886.
11. Pullman, T. N., A. R. Lavender, I. Aho, and H. Rasmussen. 1960. Direct renal action of a purified parathyroid extract. *Endocrinology.* **67**: 570.
12. Siggaard Andersen, O. 1962. Acute experimental acid-base disturbances in dogs. *Scand. J. Clin. Lab. Invest. Suppl.* **14**: 66.
13. Mostellar, M. E., and E. P. Tuttle, Jr. 1964. Effects of alkalosis on plasma concentration and urinary excretion of inorganic phosphate in man. *J. Clin. Invest.* **43**: 138.
14. Berliner, R. W., T. J. Kennedy, and J. Orloff. 1951. Relationship between acidification of the urine and potassium metabolism. *Am. J. Med.* **11**: 274.
15. Smith, W. W. 1939. The excretion of phosphate in the dogfish, *Squalus acanthias*. *J. Cellular Comp. Physiol.* **14**: 95.
16. Walker, A. M., and C. L. Hudson. 1937. The role of the tubule in the excretion of inorganic phosphates by the amphibian kidney. *Am. J. Physiol.* **118**: 167.
17. Levinsky, N. G., and D. G. Davidson. 1957. Renal action of parathyroid extract in the chicken. *Am. J. Physiol.* **191**: 530.
18. Handler, J. S. 1962. A study of renal phosphate excretion in the dog. *Am. J. Physiol.* **202**: 787.
19. Strickler, J. C., D. D. Thompson, R. M. Klose, and G. Giebisch. 1964. Micropuncture study of inorganic phosphate excretion in the rat. *J. Clin. Invest.* **43**: 1596.
20. Nicholson, T. F., and G. W. Shepherd. 1959. The effect of damage to various parts of the renal tubule on the excretion of phosphate by the dog's kidney. *Can. J. Biochem. Physiol.* **37**: 103.
21. Kupfer, S., and J. Kosovsky. 1965. Effect of digoxin on renal intracellular phosphate and phosphate excretion in the dog. *Federation Proc.* **24**: 521. (Abstr.)
22. Wesson, L. G., Jr., and W. P. Anslow, Jr. 1948. Excretion of sodium and water during osmotic diuresis in the dog. *Am. J. Physiol.* **153**: 465.
23. Seldin, D. W., and R. Tarail. 1949. Effect of hypertonic solutions on metabolism and excretion of electrolytes. *Am. J. Physiol.* **159**: 160.
24. Carrasquer, G., and W. A. Brodsky. 1961. Elimination of transient secretion of phosphate by alkalinization of plasma in dogs. *Am. J. Physiol.* **201**: 499.
25. Rector, F. C., Jr., N. W. Carter, and D. W. Seldin. 1965. The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. *J. Clin. Invest.* **44**: 278.
26. Davies, R. E., and F. J. W. Roughton. 1948. Hydrochloric acid production by isolated gastric mucosa. *Biochem. J.* **42**: 618.
27. Berliner, R. W., and J. Orloff. 1956. Carbonic anhydrase inhibitors. *Pharmacol. Rev.* **8**: 137.
28. Puschett, J. B., and M. Goldberg. 1967. Control of phosphate excretion by urinary pH in man. Proceedings of the 1st meeting of the American Society of Nephrology. **54**. (Abstr.)
29. Nadell, J. 1953. The effects of the carbonic anhydrase inhibitor "6063" on electrolytes and acid-base balance in two normal subjects and two patients with respiratory acidosis. *J. Clin. Invest.* **32**: 622.
30. Martin, H. E., and R. Jones. 1961. The effect of ammonium chloride and sodium bicarbonate on the urinary excretion of magnesium, calcium and phosphate. *Am. Heart J.* **62**: 206.
31. Sartorius, O. W., J. C. Roemmelt, and R. F. Pitts. 1949. The renal regulation of acid-base balance in man. IV. The nature of the renal compensations in ammonium chloride acidosis. *J. Clin. Invest.* **28**: 423.
32. Hodgkinson, A., and L. N. Pyrah. 1958. The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin. *Brit. J. Surg.* **46**: 10.
33. Edwards, N. A., and A. Hodgkinson. 1965. Phosphate metabolism in patients with renal calculus. *Clin. Sci.* **29**: 93.
34. Seldin, D. W., and J. D. Wilson. 1966. Renal tubular acidosis. In *The Metabolic Basis of Inherited Disease*. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Company, New York. 2nd edition. 1230.