The Phosphaturic Effect of Sodium Bicarbonate and Acetazolamide in Dogs

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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 (Tm_P). 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. The fraction of filtered P that was excreted increased during urinary alkalinization regardless of whether the pH of the blood increased ($NaHCO_3$ infusion) or decreased (acetazolamide administration).

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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 cannulas at the midpoint of each period;[1] 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 $NaHCO_3$ i.v. (four of them pretreated with ammonium chloride). $NaHCO_3$ 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-

[1] Venous blood was used in two of the alkalinization experiments and in all of those shown in Table IV.

HCO_3 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* $NaHCO_3$ 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_{IN}, and urinary pH. In five experiments the control periods were obtained during the infusion of 0.25–0.4 mEq of $NaHCO_3$ per min, and in one experiment they were obtained during urea–NaCl infusion, after which 50 mmoles of NH_4Cl (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 $NaHCO_3$ again after these intermediate urinary acidification periods.

The effect of infusing more NaCl, or Na_2SO_4, was studied in 10 experiments. In five, the control observations made during the infusion of 128 mM NaCl [2] 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_2SO_4 which yielded somewhat higher rates of Na^+ administration than those used in the usual $NaHCO_3$ experiments. The priming infusions delivered 3 mEq of Na^+ per min for 10 min and the sustaining infusions either 1.13 mEq (as NaCl) or 0.75 mEq (as Na_2SO_4) 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 AutoAnalyzer.[3] 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.[3]

The filtered load of P (F_P) was taken as $C_{In} \times$ plasma P and tubular reabsorption of P as $F_P -$ urinary P excretion (U_PV). 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^+ concentrations, averaging them, and reconverting the averages to pH values. Urinary OH^- concentration was calculated from the formula $OH^- = 10^{-14} \times$ antilog pH. In making these calculations, H^+ *concentration* was assumed to be equal to H^+ *activity.*

RESULTS

The changes of blood pH averaged only +0.10 ± 0.02 (1 SD) during $NaHCO_3$ infusion, −0.01 ± 0.04 after acetazolamide given during NaCl infusion, and −0.04 ± 0.04 after acetazolamide given during $NaHCO_3$ infusion. Plasma P changed relatively little from control values during the experimental periods, averaging −1% during $NaHCO_3$ 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

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

[3] Technicon Instrument Corp., Chauncey, N. Y.

TABLE I

Effect of $NaHCO_3$ Infusion on Urinary Inorganic Phosphate Excretion

Time	V	Urine pH	C_{In}	Plasma P	P		C_P	C_P/C_{In}
					Filtered	Excreted		
min	*ml/min*		*ml/min*	*μg/ml*	*mg/min*		*ml/min*	%
	4 g NH_4Cl per os at −16 hr							
0	Infuse 200 ml of 154 mM NaCl.							
20	Inulin prime, 2.9 g i.v.							
25	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 $NaHCO_3$ 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_{In}, inulin clearance; C_P, phosphorus clearance.

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

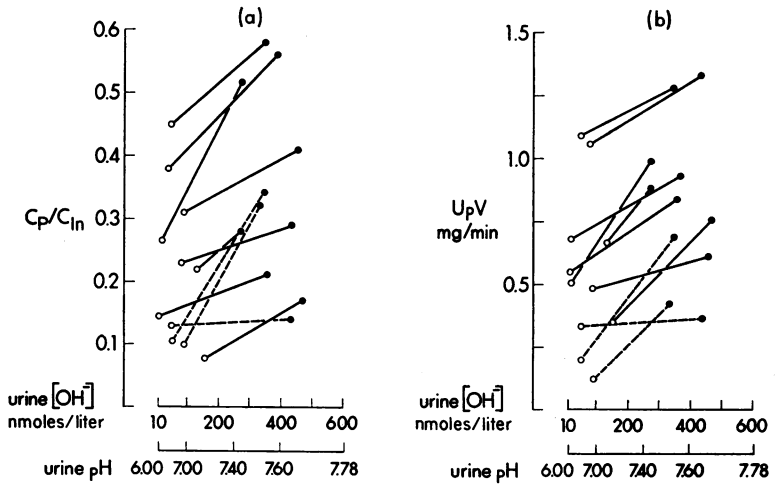

FIGURE 1 Relation between phosphate/inulin clearance (C_P/C_{In} [*a*]), urinary phosphate excretion (U_PV [*b*]), and urinary alkalinity in dogs given sodium bicarbonate. ○, control values; ●, values during urinary alkalinization. Solid lines refer to dogs with constant or decreased filtered loads of 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 ± 0.09) and from +0.44 to +0.92 pH units after acetazolamide that was given during NaCl infusion (mean +0.67 ± 0.09). When acetazolamide was given during $NaHCO_3$ infusion urinary pH increased only by +0.13 to +0.27 units (mean +0.19 ± 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).[4]

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_PV (average 49%, range +15 to +117%, Fig. 1 b and Table I). F_P increased

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

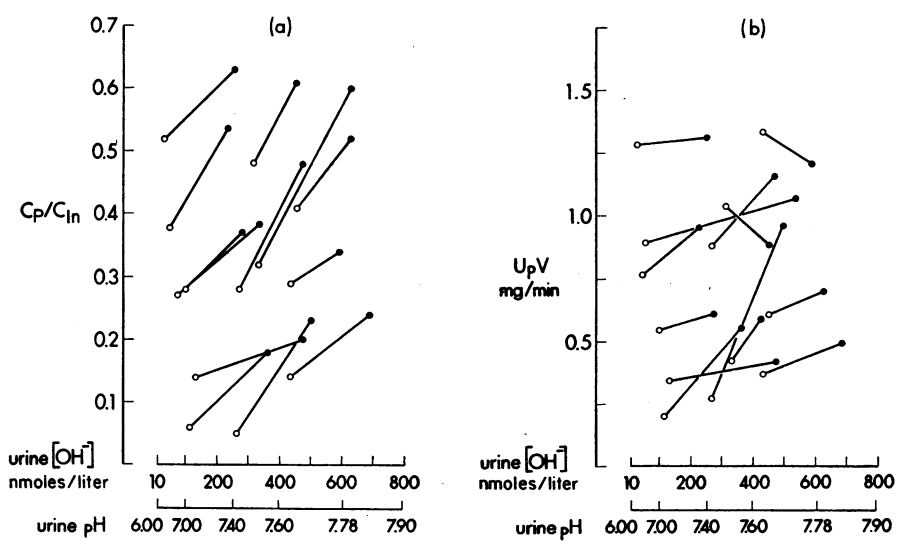

FIGURE 2 Relation between phosphate/inulin clearance (*a*), urinary phosphate excretion (*b*), and urinary alkalinity in dogs given acetazolamide. Symbols are the same as in Fig. 1. F_P decreased in all of these dogs after acetazolamide administration.

TABLE II

Effect of Acetazolamide on Urinary Inorganic Phosphate Excretion

Time	V	Urine pH	C_{In}	Blood pH	Plasma P	P		C_P	C_P/C_{In}
						Filtered	Excreted		
min	*ml/min*		*ml/min*		*μg/ml*	*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_P/C_{In} and U_PV 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_P.

Administration of acetazolamide was followed invariably by a decrease of F_P (average 17% in the 13 studies, range −7 to −26%) that was largely a consequence of a decrease of C_{In} (average 14%, range −9 to −23%). C_P/C_{In} 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_P, 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_PV decreased (by 15 and 9% as compared with F_P decreases of 15 and 24% respectively), acetazolamide had been given during $NaHCO_3$ 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_P/C_{In} (a value equal to U_PV/F_P, the excretion fraction of filtered P) was similar regardless of whether acetazolamide or $NaHCO_3$ had been given.

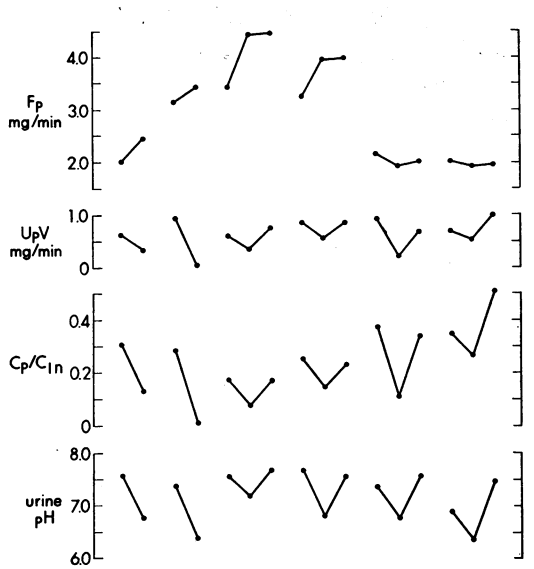

FIGURE 3 Filtered load (F_P) and urinary excretion of phosphate in six dogs in relation to urinary pH changes. The first point in each of the first five studies is the average of the values in each dog during sodium bicarbonate infusion and, in the sixth dog, during urea-NaCl infusion; the second point 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.

Urinary acidification was induced in six experiments by the intragastric instillation of NH_4Cl after the control periods had been obtained (Fig. 3). C_P/C_{In} and U_PV decreased remarkably when the urine became acid even though F_P actually increased in the first four studies shown. In the last four experiments the dogs were then given $NaHCO_3$ again to reestablish urinary alkalinization, 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_2SO_4, was examined in order to learn whether

TABLE III

Comparison of Inorganic Phosphate Excretion in Alkaline and in Acid Urine

Time	V	Urine pH	C_{In}	Blood pH	Plasma P	P		C_P	C_P/C_{In}
						Filtered	Excreted		
min	*ml/min*		*ml/min*		*μg/ml*	*mg/min*		*ml/min*	%
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 $NaHCO_3$ (333 μmoles $NaHCO_3$ 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* $NaHCO_3$.								
125	Give 50 ml of 0.5 M NH_4Cl by gastric tube.								
140	Give 50 ml of 0.5 M NH_4Cl 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 $NaHCO_3$ at 5 ml/min.								
216	Change sustaining infusion to inulin, 5 mg/ml, with 15 mg/ml urea in 128 mM NaCl–100 mM $NaHCO_3$ at 5 ml/min (500 μmoles $NaHCO_3$ 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 $NaHCO_3$ and acetazolamide might be related to either decreased tubular reabsorption of sodium or perhaps expansion of extracellular fluid volume. Neither infusing Na_2SO_4, 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_PV/F_P		$U_{Na}V/F_{Na}$		Reabsorbed Na		V	
	Control*	Diuresis‡	Control	Diuresis	Control	Diuresis	Control	Diuresis
	%		%		*μEq/min*		*ml/min*	
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_PV, urinary phosphate excretion; $U_{Na}V$, urinary sodium excretion; F_P, filtered phosphate; F_{Na}, 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_2SO_4–1.5% urea at 5 ml/min.

TABLE V

Effect of Saline Infusion on Urinary Inorganic Phosphate Excretion

Time	V	Urine pH	C_{In}	Plasma P	P		C_P	C_P/C_{In}	$U_{Na}V/F_{Na}$	Reabsorbed Na
					Filtered	Excreted				
min	*ml/min*		*ml/min*	*μg/ml*	*mg/min*		*ml/min*	%	%	*μEq/min*
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_P/C_{In} 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_P/C_{In} 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 $NaHCO_3$ 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 $NaHCO_3$ infusion (1). However, Malvin and Lotspeich found that $NaHCO_3$ did decrease the Tm_P, as did both acetazolamide administration and hyperventilation (2). In our own studies of the effects of $NaHCO_3$ and acetazolamide in dogs given P infusions, we also found that Tm_P decreased.[5] However, plasma P tended to decrease during $NaHCO_3$ 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 $NaHCO_3$ or acetazolamide, and this increase was often accompanied by an increase of the P filtered load. Therefore, the cause of the decreased Tm_P 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 $NaHCO_3$ 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_P, 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 $NaHCO_3$ was infused later, GFR increased and plasma P often decreased sharply.[5]

[5] 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 $NaHCO_3$ 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 $NaHCO_3$ 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 accompaned by major decreases of F_P secondary to decreases in both plasma P and GFR.[5]

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.[6]

The mechanism of the phosphaturic effect of $NaHCO_3$ 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 $NaHCO_3$ 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., $1 - U_{Na}/F_{Na}$) decreases after acetazolamide or $NaHCO_3$ administration, and it is conceivable that the fractional reabsorption of P might decrease concomitantly. However, when fractional sodium excretion was increased by infusing Na_2SO_4 or more NaCl (either as an isotonic or slightly hyperosmotic solution), C_P/C_{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 $NaHCO_3$ 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 $NaHCO_3$-induced phosphaturia in our experiments must be sought elsewhere.

[6] 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 $H_2PO_4^-$ and $HPO_4^=$ are different, as was suggested by Carrasquer and Brodsky (24). If, for example, the divalent ion $HPO_4^=$ was less readily reabsorbed by the renal tubules than the monovalent form $H_2PO_4^-$, the predominance of the divalent anion in alkaline urine (pK 6.8 for $H_2PO_4^- \rightleftharpoons HPO_4^=$) 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 $H_2PO_4^-$ to $HPO_4^=$. 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 H_2CO_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 $H_2PO_4^-$ to $HPO_4^=$ 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 H^+ from the cells (26). In this view, the effect of carbonic anhydrase is to minimize the alkalinity of H^+-secreting cells. Accordingly, inhibition of the enzyme (as by acetazolamide) during H^+ secretion would cause cell pH to rise (27), and it is likely that cell pH also increases during $NaHCO_3$ infusion. Thus, the common factor might be an intracellular shift towards $HPO_4^=$, 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 $NaHCO_3$ 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 NH_4Cl 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.

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