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THE INFLUENCE OF ANION PENETRATING ABILITY ON
URINARY ACIDIFICATION AND THE EXCRETION
OF TITRATABLE ACID*

BY NORMAN BANK† AND WILLIAM B. SCHWARTZ‡

(From the Department of Medicine, Tufts University School of Medicine, and the Pratt
Clinic-New England Center Hospital, Boston, Mass.)

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Previous studies in the dog have demonstrated that during administration of neutral sodium phosphate the rate of titratable acid excretion is directly proportional to the rate of phosphate excretion and is inversely related to plasma bicarbonate concentration (1). Markedly acid urines containing near theoretical maximum amounts of titratable acid¹ were observed only in the presence of severe metabolic acidosis.

More recently it has been shown that intense acidification of the urine can be achieved without the induction of acidosis (2). Normal subjects stimulated to retain sodium showed a rapid fall in urine pH to approximately 5.0 or below when infused with sodium sulfate, and it was concluded that increased reabsorption of sodium in the presence of a poorly reabsorbable anion was responsible for this phenomenon. Acidification of the urine has also been observed during administration of poorly reabsorbable anions to normal human subjects during quiet standing (3) and to dogs in which glomerular filtration rate was reduced by clamping of the renal artery (4)—experimental procedures known to induce a more complete reabsorption of filtered sodium. These observations raise the possibility that, even without acidosis, large numbers of hydrogen ions might be transported into the glomerular filtrate over a

comparably wide pH gradient if a poorly reabsorbable *buffer* anion were infused under appropriate experimental conditions. In the present study it has been demonstrated that, during the infusion of sodium phosphate (pH 7.4) into dogs whose sodium and chloride excretion had been reduced by dietary salt restriction, excretion of titratable acid rises to near theoretical maximum values in the presence of normal plasma bicarbonate concentrations.

These data suggest the hypothesis that a disproportion between the reabsorption of sodium and anion was a critical factor in regulating not only the acidity of the urine but also the rate of excretion of titratable acid. This hypothesis has been further supported by the demonstration that administration of relatively reabsorbable anions, such as chloride or thiocyanate, to phosphate-loaded dogs is associated with a progressive elevation of urine pH and reduction in titratable acid excretion. On the other hand, administration of poorly reabsorbable anions, such as sulfate or ferrocyanide, is accompanied by sustained or increased acidity of the urine and excretion of near theoretical maximum amounts of titratable acid.

As a possible mechanism to account for these findings, it is proposed that the various anions affect urine pH and acid excretion through their influence on the electrical gradient established by active sodium transport across the renal tubular cells. According to this hypothesis, the magnitude of the gradient is determined by the penetrating ability of the available anions, those which penetrate the tubular epithelium more readily having a greater tendency to follow sodium and thus to reduce the potential difference. *Changes* in urinary acidity have tentatively been attributed to the passive movement of hydrogen ions in response to variations in the transtubular electrical gradient.

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† This work was done during the tenure of a Fellowship from the Life Insurance Medical Research Fund. Present address: Department of Medicine, New York University Medical Center, New York, N. Y.

‡ Established Investigator of the American Heart Association.

¹ In urines where phosphate is the major buffer, values closely approaching the theoretical maximum for titratable acid are attained when urine pH falls to approximately 5.5, since at this point more than 95 per cent of the phosphate (pK_2' approximately 6.8) is in the $H_2PO_4^-$ form.

Although it seems probable that active transport of hydrogen is also involved in the over-all acidifying process, the present data suggest that passive diffusion of hydrogen plays an important role in the mechanism governing urine acidification and the renal excretion of titratable acid.

METHODS

Twenty-one experiments were performed on 21 female mongrel dogs, varying in weight from 10 to 20 kg. The animals were fed 100 g of ground horse meat for 10 to 14 days prior to the day of the experiment. This diet contained approximately 4 mEq Na⁺, 8 mEq Cl⁻, and 6 mEq K⁺ daily. Sixty to 90 minutes before each experiment, 500 ml of water was administered through a gastric tube. Anesthesia was induced by intravenous administration of chloralose, 100 mg per kg, as a 1.5 per cent solution in 2 per cent dextrose. Additional amounts were administered during the course of the experiment as required.

Each experiment was begun with the intravenous infusion of 4 per cent dextrose in water at a rate of 2.4 to 6.0 ml per minute. After an adequate urine flow had been established (approximately 3 to 6 ml per minute), two control urine collections of 10 to 15 minutes each were obtained. The dextrose solution was then replaced by one containing 60 mmoles per L of sodium phosphate (48 mmoles HPO₄²⁻, 12 mmoles H₂PO₄⁻) delivered at 4.2 ml per minute. After collecting urine for four to seven periods of 10 to 15 minutes each, this solution was replaced by one of the following, in millimoles per liter: NaCl, 108 (9 experiments); Na₂SO₄, 54 (4 experiments); NaSCN, 108 (4 experiments); and Na₄Fe(CN)₆, 27 (4 experiments); each was delivered at 4.2 ml per minute. The concentration of sodium and its rate of administration was thus the same during sodium phosphate loading and during subsequent loading with other salts. In order to maintain the osmolarity of all solutions at about 220, appropriate amounts of dextrose were added to the Na₂SO₄ and Na₄Fe(CN)₆ solutions.

Urines were collected through an indwelling bladder catheter and the bladder was emptied by manual compression at the end of each period. pH was determined on aliquots collected anaerobically at the midpoint of each period. Heparinized arterial blood samples were collected for determination of pH, total CO₂, chloride and potassium.

Urine and blood pH were determined anaerobically at 37° C with a Cambridge model R pH meter. CO₂ contents were determined manometrically by the method of Peters and Van Slyke (5). Urine sodium and potassium were measured by a Baird flame photometer using an internal lithium standard. Urine phosphorus was determined by the method of Fiske and Subbarow (6). Chloride was measured in urine and plasma by titration with silver nitrate, using a potentiometric method (7). Urine ferrocyanide was measured by the method of Ber-

liner, Kennedy and Hilton (8), ammonium by the method of Folin and Bell (9), and inorganic sulfate was determined gravimetrically as barium sulfate (5). Thiocyanate was measured in urine and plasma by the method of Barker (10). Chloride was calculated for specimens containing thiocyanate by subtracting the measured thiocyanate concentration from the sum of chloride and thiocyanate as determined by titration with AgNO₃. In specimens containing ferrocyanide, chloride and phosphate were determined after removal of the ferrocyanide by precipitation with ZnSO₄.

The value for titratable acid was calculated from the urine phosphate, urine pH and blood pH.² The pK₂' of phosphate in blood was taken to be 6.8. The same value was used arbitrarily for urine when urine pH was below 6.0, since the error introduced into the calculation of titratable acid is less than 5 per cent even when pK₂' is as much as ± 0.2 unit different from 6.8 (11). The pK₂' was calculated for individual specimens with pH values above 6.0, since in such urines appreciable errors in estimation of titratable acid may result from use of an incorrect pK₂'. On these specimens additional analyses were made for CO₂ and organic acids (11) in order to make a closer approximation of ionic strength. The theoretical considerations and method of calculation of pK₂' from ionic strength have been described in a previous publication from this laboratory (11).

For each urine specimen, titratable acid has been expressed as a percentage of the theoretical maximum titratable acid. This latter value was calculated by subtracting from total phosphate excretion that amount already present as H₂PO₄⁻ at the pH of the plasma (and filtrate). For example, when plasma pH is 7.4 and 20 per cent of the phosphate is thus present as H₂PO₄⁻, the value for theoretical maximum titratable acid is equivalent to 80 per cent of total urine phosphate.

RESULTS

A. Effect of sodium phosphate infusion on urine pH and titratable acid excretion. Tables I to IV show the results of four representative experiments. During the control periods urine pH ranged between 6.32 and 6.83, chloride excretion ranged between 8 and 27 μmoles per minute, and phosphate excretion was 6 μmoles per minute or less. With the infusion of sodium phosphate, phosphate excretion rose to between 149 and 186 μmoles per minute and titratable acid excretion to between 102 and 143 μEq per minute. In all but one of the experimental periods, titratable acid

² The contribution of organic acids to titratable acid excretion was found to be negligible by potentiometric titration of urine from which phosphate had been removed by calcium hydroxide precipitation. The titration was carried out between the original pH of the urine and the pH of the plasma.

ranged between 87 and 99 per cent of the theoretical maximum. Urine pH in each instance fell within 15 minutes to values approximately 0.5 to 1.0 unit below those of the control periods and, as phosphate excretion increased, fell further to a level which ranged between 4.43 and 5.80. Chloride excretion fell in each case from control levels to less than 10 μ moles per minute and sodium excretion rose progressively.

Blood pH ranged between 7.25 and 7.39 during control periods and from 7.26 to 7.35 during phosphate loading. Bicarbonate concentrations were between 18.5 and 24.2 mmoles per L during control periods and between 18.5 and 22.2 during phosphate loading; P_{CO_2} was between 37 and 51 mm Hg during control periods and between 37 and 47 during phosphate loading.

The results of 17 experiments in which sodium phosphate was infused initially and NaCl, Na_2SO_4 or $Na_4Fe(CN)_6$ was given subsequently, are shown in Figures 1 and 2. The periods of sodium phosphate infusion are shown in each figure as solid circles. The other symbols, referring to a latter portion of each study, will be discussed separately. Phosphate excretion is plotted on the abscissa and the percentage of theoretical maximum titratable acid on the ordinate. Two points shown for convenience at 210 μ moles per minute were actually 221 and 255. It is clear that, during infusion of sodium phosphate, titratable acid excretion was

close to the theoretical maximum over the entire range of phosphate excretion. The absolute values for titratable acid excretion rose to a maximum of 195 μ Eq per minute during phosphate infusion. Urine pH during control periods was usually between 6.0 and 7.0 and with the infusion of sodium phosphate fell to levels between 4.4 and 6.0. The average chloride excretion during control periods was 10 μ moles per minute, with a range from 1 to 28. During phosphate loading chloride excretion fell to an average of 4 μ moles per minute, with a range of 1 to 12. There were usually small but significant increases in excretion of both potassium and ammonium during phosphate loading.

Plasma bicarbonate concentration was between 18 and 22 mmoles per L in almost all experiments, and blood pH ranged between 7.25 and 7.44. In the individual experiments, blood pH and total CO_2 changed only slightly during control and phosphate loading periods.

B. Effect of NaCl infusion on urine pH and titratable acid excretion. In Table I are shown the results of a typical experiment in which NaCl was given following a sodium phosphate infusion. With the administration of NaCl, titratable acid excretion fell from 99 per cent of theoretical maximum to 42 per cent. Urine pH rose abruptly from 4.71 to 6.41 and continued to rise to 6.80 as chloride was infused. Chloride excretion rose progressively. Phosphate excretion fell gradually

TABLE I
The effect of a sodium phosphate infusion and a subsequent infusion of sodium chloride on urine pH and titratable acid (T.A.) excretion

Time min	Urine						pK_2'	T.A.	Theor. max. T.A.	Max. T.A. %	Plasma			
	Na	K	NH_4	Cl	P	pH					pH	HCO_3	P_{CO_2}	Cl
	μ moles/min						μ Eq/min			mmoles/L mmHg mEq/L				
0	Infusion: dextrose, 222 mmoles/L at 4.2 ml/min									7.39	21.7	37	106	
50-65	11	28	29	12	5	6.32								
65-80	43	36	28	27	5	6.79				7.34	21.5	41	95	
85	Infusion: Na_2HPO_4 , 48; NaH_2PO_4 , 12; dextrose, 110 mmoles/L; at 4.2 ml/min													
95-110	1	19	32	2	20	5.67	6.80	14	15.5	90				
110-125	8	43	41	2	58	4.59	6.80	44	45	98	7.35	20.4	38	93
125-140	43	59	45	3	109	4.43	6.80	84	85	99				
140-155	95	55	34	4	144	4.96	6.80	110	112	98				
155-170	118	49	31	5	153	5.40	6.80	113	119	95	7.33	22.2	43	93
170-185	117	48	32	5	157	5.42	6.80	116	122	95				
185-200	141	63	36	5	186	4.71	6.80	143	144	99	7.33	22.1	43	91
202	Infusion: NaCl, 108 mmoles/L, at 4.2 ml/min													
215-230	181	37	24	15	149	6.41	6.84	76	116	66				
230-245	212	42	22	32	124	6.60	6.86	52	96	54	7.34	23.7	45	95
245-260	184	38	21	26	101	6.65	6.87	40	78	51				
260-275	173	33	21	28	80	6.80	6.88	26	62	42	7.35	23.5	44	98

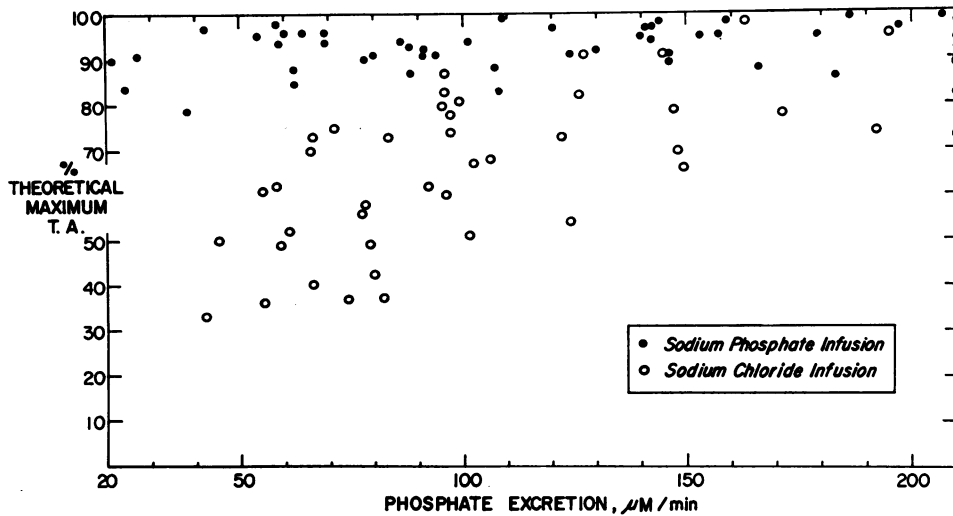


FIG. 1. THE EFFECT OF SODIUM PHOSPHATE INFUSION AND A SUBSEQUENT INFUSION OF SODIUM CHLORIDE ON THE PERCENTAGE OF THEORETICAL MAXIMUM TITRATABLE ACID EXCRETION. For definition of "theoretical maximum titratable acid," see text.

during the next hour but the excretion rates were comparable with those of the preceding phosphate loading periods. Sodium excretion was higher than during the previous periods of phosphate loading.

The highest blood pH was 7.35, a value essentially the same as that during earlier periods when urine pH was between 4.43 and 5.67. Plasma bicarbonate rose about 1.5 mmoles per L and P_{CO_2}

showed no significant change. Plasma chloride concentration rose 7 mEq per L.

The results of all nine experiments are shown in Figure 1 in which the NaCl periods are represented by open circles. The percentage of maximum titratable acid excretion fell progressively to levels well below those seen at comparable rates of phosphate excretion during sodium phosphate administration. Urine pH usually rose to above 6.0

TABLE II

The effect of a sodium phosphate infusion and a subsequent infusion of sodium sulfate on urine pH and titratable acid excretion

Time min	Urine							T.A. $\mu\text{Eq}/\text{min}$	Theor. max. T.A. %	Max. T.A. %	Plasma			
	Na	K	NH_4	Cl	SO_4	P	pH				pH	HCO_3	P_{CO_2}	Cl
0	Infusion: dextrose, 222 mmoles/L, at 4.2 ml/min										7.30	24.2	51	100
80-95	112	45	26	23		6	6.83							
95-110	44	30	28	17		3	6.69				7.32	20.3	41	88
115	Infusion: Na_2HPO_4 , 48; NaH_2PO_4 , 12; dextrose, 55 mmoles/L; at 4.2 ml/min													
125-140	6	43	37	3		42	5.67	30	33	91				
140-150	18	69	37	2		79	5.38	58	62	94				
150-160	36	77	35	2		105	5.23	79	82	96	7.35	20.0	37	88
160-170	63	79	36	2		132	5.22	99	103	96				
170-180	85	75	38	3		149	5.18	113	116	97				
180-192	92	82	38	2		161	5.28	123	128	96				
195	Infusion: Na_2SO_4 , 54; dextrose, 55 mmoles/L; at 4.2 ml/min													
205-215	139	80	39	4	44	144	5.62	105	115	91	7.39	20.9	36	88
215-225	158	79	45	3	68	118	5.12	91	94	97				
225-235	168	72	46	3	72	101	5.04	79	80	99				
235-245	202	76	41	3	83	99	5.11	77	79	98	7.40	22.9	38	88
245-255	218	76	41	3	116	97	5.10	76	77	99				

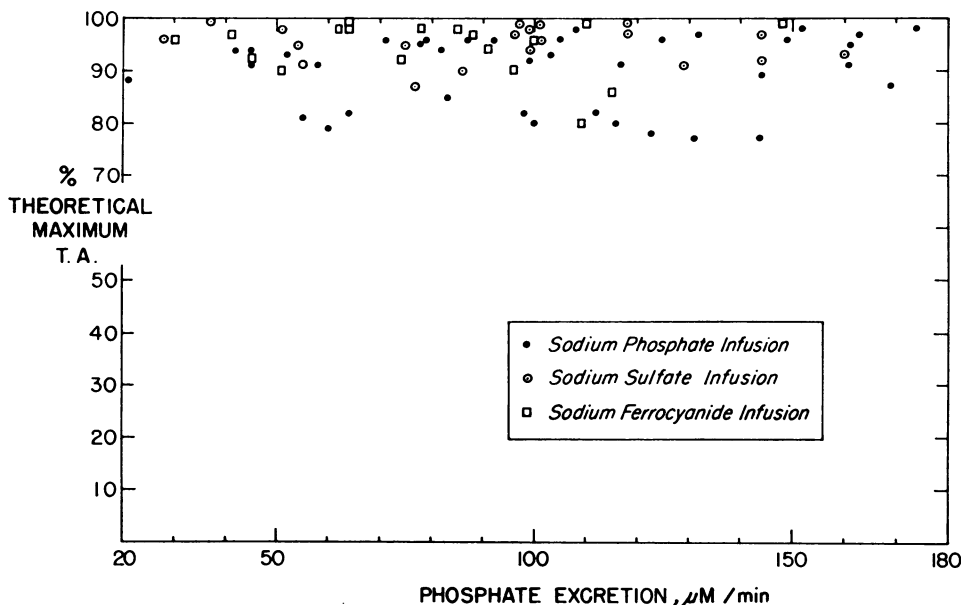


FIG. 2. THE EFFECT OF SODIUM PHOSPHATE INFUSION AND A SUBSEQUENT INFUSION OF SODIUM SULFATE OR SODIUM FERROCYANIDE ON THE PERCENTAGE OF THEORETICAL MAXIMUM TITRATABLE ACID EXCRETION.

shortly after NaCl was begun and continued to rise during the next 40 to 60 minutes to levels between 6.5 and 7.0 as chloride excretion increased from 15 to 130 μ moles per minute. In a few instances, a significant rise in urine pH and fall in titratable acid did not occur until the second period after beginning NaCl administration. Although it is not apparent from the figure, a close correlation existed between the first appearance of increased quantities of chloride in the urine and both the rise in pH and the fall in percentage of theoretical maximum titratable acid.³

Blood pH during NaCl infusion ranged between 7.30 and 7.44, and plasma bicarbonate between 17 and 25 mmoles per L for the group. Changes in these values between the sodium phosphate and sodium chloride periods were small and variable.

C. Effect of Na₂SO₄ infusion on urine pH and titratable acid excretion. In Table II are shown the results of a typical experiment in which

³ In two experiments (not shown) initial acidification of the urine occurred with administration of phosphate but was followed by a sudden spontaneous rise in chloride excretion and pH, possibly due to an osmotic effect of the phosphate load. In a few additional experiments, where control chloride excretion was high (100 to 200 μ Eq per minute), little or no reduction in urine pH occurred during phosphate infusion.

Na₂SO₄ was given following a sodium phosphate infusion. It is apparent that, during Na₂SO₄ administration, titratable acid excretion remained at over 90 per cent of the theoretical maximum, and there was a slight further fall in urine pH. Chloride excretion was 2 to 3 μ moles per minute during phosphate administration and showed no significant change during sulfate infusion. Sodium excretion rose progressively throughout the entire experiment.

Blood pH, bicarbonate and chloride varied only slightly between the phosphate and sulfate infusion periods.

The results of all four experiments are shown in Figure 2, in which the open circles represent periods during Na₂SO₄ infusion. The infusion of this salt was accompanied by excretion of near theoretical maximum amounts of titratable acid at rates of phosphate excretion comparable with those during sodium phosphate infusion. In three of the four experiments urine pH fell to lower levels than those achieved during phosphate infusion. In the fourth, there was no significant change from the acid values achieved during the phosphate periods. Chloride excretion was below 10 μ moles per minute during sulfate loading. Blood pH ranged between 7.30 and 7.40, plasma bicarbonate between

TABLE III

The effect of a sodium phosphate infusion and subsequent infusions of sodium ferrocyanide and sodium chloride on urine pH and titratable acid excretion

Time <i>min</i>	Urine							T.A.	Theor. max. T.A.	Max. T.A. %	Plasma			
	Na	K	NH ₄	Cl	Fe(CN) ₆	P	pH				pH	HCO ₂	Pco ₂	Cl
	<i>μmoles/min</i>							<i>μEq/min</i>			<i>mmoles/L</i>	<i>mmHg</i>	<i>mEq/L</i>	
0	Infusion: dextrose, 222 mmoles/L at 4.2 ml/min										7.27	20.3	46	105
45-60	33	24	41	25	0	4	6.50							
60-70	40	24	42	17		4	6.44				7.25	18.5	44	98
71	Infusion: Na ₂ HPO ₄ , 48; NaH ₂ PO ₄ , 12; dextrose, 55 mmoles/L; at 4.2 ml/min													
80-90	76	34	45	9		82	5.49	58	62	94				
90-100	115	35	42	7		125	5.34	90	94	96				
100-110	149	36	47	5		163	5.32	118	122	97	7.27	18.5	42	99
110-120	174	29	31	6		169	5.80	111	127	87				
122	Infusion: Na ₄ Fe(CN) ₆ , 27; dextrose, 83 mmoles/L; at 4.2 ml/min													
130-140	275	50	36	8	29	148	4.62	110	111	99				
140-150	373	53	34	8	54	110	4.44	82	83	99	7.28	18.2	40	95
150-160	399	50	32	6	63	85	4.45	63	64	98				
160-170	476	57	34	8	78	78	4.65	59	60	98				
172	Infusion: NaCl, 108 mmoles/L at 4.2 ml/min													
180-190	445	64	26	26	62	43	6.37	21	33	64	7.31	19.4	40	95
190-200	413	52	26	43	50	26	6.75	8	20	40				
200-210	308	41	26	43	35	15	6.84	4	11	36				
210-220	289	41	27	62	33	15	6.81	4	11	36	7.30	18.2	38	110

16 and 23 mmoles per L and Pco₂ between 30 and 40 mm Hg. These values were not significantly different from those of the preceding phosphate loading periods.

D. Effect of Na₄Fe(CN)₆ infusion on urine pH and titratable acid excretion. In Table III are shown the results of a typical experiment in which sodium ferrocyanide was given following a sodium phosphate infusion. The infusion of Na₄Fe(CN)₆ was accompanied by a slight rise in the percentage of theoretical maximum titratable acid to values of 98 to 99 per cent. Urine pH fell, from a range of 5.32 to 5.80, to 4.44 to 4.65, and chloride excretion remained below 10 μ moles per minute. During the last four periods, sodium ferrocyanide was replaced by sodium chloride. There was a prompt fall in the percentage of theoretical maximum titratable acid.⁴ Urine pH rose from 4.65 to 6.37 in the first period and continued to rise to 6.84. Ferrocyanide excretion continued in significant amounts and chloride excretion rose progressively. Sodium excretion increased during ferrocyanide infusion and decreased during the final periods of sodium chloride infusion.

Blood pH, plasma bicarbonate and Pco₂ re-

⁴ Since the specific effects of ferrocyanide on the pK₂' of phosphate are not known, a value of 6.8 has been used arbitrarily.

mained essentially constant during the periods of both phosphate and ferrocyanide administration. Plasma chloride concentration was unchanged during ferrocyanide infusion and rose to 110 mEq per L when NaCl was administered.

The results of all four experiments are shown in Figure 2 in which the open squares represent the periods of Na₄Fe(CN)₆ infusion. It is evident that titratable acid excretion was more than 90 per cent of theoretical maximum at rates of phosphate excretion comparable with those during sodium phosphate infusion. Urine pH values in each instance were lower than those achieved during the previous phosphate infusion periods. Chloride excretion remained below 10 μ moles per minute during ferrocyanide infusion.

Blood pH ranged between 7.28 and 7.44, plasma bicarbonate between 17 and 22 mmoles per L and Pco₂ between 28 and 47 mm Hg during sodium ferrocyanide infusion. There were no significant changes from the values of the preceding phosphate loading periods.

E. Effect of NaSCN infusion on urine pH and titratable acid excretion. In Table IV are shown the results of the experiment in which occurred the most prompt response after starting sodium thiocyanate infusion. Urine pH rose from 5.74 to 6.62 and titratable acid excretion fell from 90

TABLE IV

The effect of a sodium phosphate infusion and a subsequent infusion of sodium thiocyanate on urine pH and titratable acid excretion

Time <i>min</i>	Urine							pK ₂ '	T.A. <i>μEq/min</i>	Theor. max. T.A.	Max. T.A.	Plasma			
	Na	K	NH ₄	Cl	SCN	P	pH			%	pH	HCO ₃	Pco ₂	Cl	
	<i>μmoles/min</i>											<i>mmoles/L</i>	<i>mmHg</i>	<i>mEq/L</i>	
0	Infusion: dextrose, 222 mmoles/L at 4.2 ml/min											7.28	22.2	49	99
65-80	10	21	27	12		1	6.75								
80-95	7	17	28	8		1	6.60				7.26	20.7	48	93	
120	Infusion: Na ₂ HPO ₄ , 48; NaH ₂ PO ₄ , 12; dextrose, 55 mmoles/L; at 4.2 ml/min														
130-140	33	36	38	8		53	6.16	30	39	77					
140-155	26	24	24	2		50	5.58	6.80	34	37	92	7.26	20.3	47	91
170-185	104	44	28	4		130	5.72	6.80	87	97	90				
185-200	122	48	36	4		146	5.68	6.80	98	108	91				
200-215	144	49	33	5		149	5.74	6.80	102	114	90				
220	Infusion: NaSCN 108 mmoles/L at 4.2 ml/min														
235-250	231	47	21	8	1	165	6.62	6.80	60	126	48	7.32	21.3	43	86
250-265	164	33	20	8	1	105	6.67	6.87	40	80	50				
265-275	210	42	23	22	2	107	6.79	6.91	35	82	43				
275-285	286	59	28	70	5	107	6.85	6.90	31	82	38	7.30	21.4	45	83

to 48 per cent of theoretical maximum within 20 minutes. Thiocyanate excretion rose to 5 and chloride excretion from 5 to 70 μ moles per minute. A similar increase in chloride excretion following administration of thiocyanate has previously been noted by Rapoport and West (12).

Blood pH, plasma bicarbonate and Pco₂ remained within a normal range throughout the experiment.

In two of the other three experiments, there was a fall in titratable acid excretion from over 90 to less than 60 per cent of theoretical maximum and a progressive rise in urine pH from 5.56 and 5.28 to 6.58 and 6.57, respectively. However, these changes took place more slowly than in the experiments in which chloride was administered, and the maximal changes occurred only after 40 to 60 minutes of thiocyanate infusion. Furthermore, they were accompanied by only a slight rise in chloride and thiocyanate excretion. It is probable that this delay was due to almost complete reabsorption of thiocyanate and chloride proximal to the acidifying site. In the fourth experiment the changes in urine pH and titratable acid were small. At the end of 60 minutes pH had risen only from 5.44 to 5.98, and titratable acid excretion had fallen from 93 to 81 per cent of theoretical maximum. However, it should be noted that no thiocyanate appeared in the urine during this time, and there was no rise in chloride excretion.

In the latter three experiments, blood pH ranged

between 7.25 and 7.35, plasma bicarbonate between 17 and 21 mmoles per L and Pco₂ between 34 and 49 mm Hg during NaSCN infusion. There were no changes from the values of the preceding sodium phosphate periods.

DISCUSSION

The present experiments demonstrate that administration of neutral sodium phosphate to dogs whose sodium chloride excretion has been reduced by dietary salt restriction is associated with a prompt and marked fall in urine pH and excretion of near theoretical maximum amounts of titratable acid. Titratable acid excretion remains close to these maximal values despite a progressive increase in phosphate excretion to as much as 250 μ moles per minute. The results indicate that, over the range of phosphate excretion rates which was studied, acidosis is not necessary for excretion of maximal amounts of acid. These observations are in accord with the view that a disproportion between sodium and anion reabsorption was the critical factor in determining not only the hydrogen ion gradient between blood and urine but also the total excretion of acid. This view is supported by the finding that the percentage of theoretical maximum titratable acid is decreased by the infusion of the readily reabsorbable anions, chloride and thiocyanate, and is sustained or increased by infusion of the poorly reabsorbable anions, sulfate or ferrocyanide.

No complete explanation for the influence of various anions on the process of acid excretion will be possible until more detailed information is available concerning the forces governing ion transfers across the renal tubule. However, it is of considerable interest that the results of the present experiments may readily be accounted for if it is assumed that the mechanism by which the anions affected acid excretion operated through their influence upon the electrical gradient across the renal tubular cells (13, 14). Active transport of sodium [which has recently been demonstrated for the renal tubule (15)] has been shown to account fully for the electrical potential difference (P.D.) across a variety of membranes (16, 17). The magnitude of the P.D. in such membranes depends upon both the rate at which sodium is transported and the resistance of the membrane to the ions that distribute themselves passively and which, by their diffusion, tend to reduce the P.D. (18, 19). In these terms the data in the present experiments might be explained in the following fashion: when sodium phosphate is infused into animals excreting little chloride, sodium reabsorption is increased at a time when only a relatively nonpenetrating anion, phosphate, is present in significant quantities. As a consequence, the P.D. across the tubular cell increases, the lumen becoming more negative, and H^+ moves passively along the electrical gradient into the lumen, titrating the phosphate and reducing the urine pH.⁵ According to this hypothesis, not only phosphate but other anions in the filtrate should also influence the P.D. and, therefore, the acidifying process, in a manner dictated by their ability to penetrate the tubular epithelium. This "penetrating ability" has been shown by Rapoport and West (12) to follow closely the lyotropic series ($SCN^- > Cl^- > HPO_4^- > SO_4^- > Fe(CN)_6^{3-}$) in a fashion similar to that observed for red cells, unicellular organisms, plant cells, and intestine (20, 21). Thus, when anions of low penetrating ability such as sulfate or ferrocyanide are infused, they sustain or increase the P.D. and the transfer of H^+ to the filtrate. On the other hand, it is postulated that

⁵ The simultaneous increase in potassium excretion observed in these experiments could also be accounted for by the movement of intracellular potassium into the filtrate in response to the increased negativity of the lumen.

anions such as chloride or thiocyanate,⁶ which can move readily along the electrical gradient set up by active sodium transport, decrease the P.D. and reduce passive movement of hydrogen into the lumen even when large amounts of phosphate are present. This hypothesis is supported by the demonstration that the potential difference established across isolated frog skin by active sodium transport is much greater when a nonpenetrating anion such as sulfate is substituted in the bathing solution for a relatively penetrating anion such as chloride (19).

In the preceding discussion *changes* in pH and acid excretion have been described in terms of passive movement of hydrogen ions in response to a potential difference established across the renal tubule by active sodium transport. This view in no way excludes the possibility that active hydrogen transport plays an important role in the overall acidifying process but does suggest that anion penetrating ability can exert a critical influence on the mechanism governing urinary pH and the renal excretion of acid.

It is not meant to imply that the presence of large amounts of chloride in the urine is necessarily incompatible with aciduria. It is well known, for example, that during the chloruresis associated with ammonium chloride acidosis urine pH is low (23), and it seems probable that other factors such as a low intracellular pH, a reduced cellular potassium content, or a change in the permeability of the tubular epithelium to chloride might account for these findings. In this regard, it is of interest that permeability of the isolated frog skin to chloride may vary widely, e.g., when trace amounts of copper (10^{-5} M) are added to the bathing solution, chloride becomes virtually nonpenetrating (19). Under these circumstances potential differences of 120 to 130 mv develop across the membrane. Whether a comparable change in permeability as a consequence of metabolic acidosis might account for urinary acidification in the presence of chloruresis, or whether

⁶ It should be noted that thiocyanate inhibits acid secretion by the gastric mucosa without producing a significant change in electrical potential (22). However, in view of the striking differences between the mechanisms of gastric and renal acid formation, it seems improbable that the mode of action of thiocyanate on stomach and kidney is the same.

some other factors are responsible, remains to be determined.

SUMMARY

The influence on acid excretion of anions differing in penetrating ability has been studied in dogs whose sodium and chloride excretion had been reduced by dietary restriction. It was demonstrated that administration of the sodium salt of phosphate, a poorly reabsorbable buffer anion, leads to a marked reduction in urine pH and the excretion of near theoretical maximum amounts of titratable acid. These results indicate that, over the range of phosphate excretion rates which has been studied, maximal excretion of acid can be achieved without the stimulus of acidosis. It has been suggested that a disproportion between sodium and anion reabsorption was the critical factor in determining not only the pH gradient between blood and urine but also the total excretion of acid. This hypothesis is supported by the finding that the percentage of theoretical maximum titratable acid excreted in the urine is decreased by the subsequent infusion of the readily reabsorbable anions, chloride or thiocyanate, and is sustained or increased by the administration of the poorly reabsorbable anions, sulfate or ferrocyanide.

As a possible mechanism to account for these findings, it is proposed that the various anions affected urine pH and acid excretion through their influence on the electrical gradient established by active sodium transport across the renal tubular cells. According to this hypothesis, the magnitude of the gradient is determined by the relative penetrating ability of the available anions, those which penetrate the tubular epithelium more readily having a greater tendency to follow sodium and thus to reduce the potential difference. Changes in urinary pH and titratable acid excretion might thus be attributed to the passive movement of hydrogen ions in response to variations in the transtubular electrical gradient. This view in no way excludes the possibility that active hydrogen transport plays an important role in the over-all acidifying process but does suggest that anion penetrating ability can exert a critical influence on the mechanism governing urinary pH and the renal excretion of acid.

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