THE EFFECT OF BUFFER LOADING UPON AMMONIUM EXCRETION IN THE DOG *

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According to present concepts, urinary ammonium and titratable acid represent, quantitatively, hydrogen ions eliminated from the body by the kidney (1). Although it is known that titratable acid excretion depends upon the amount of buffer in the urine and urine pH (2), and that ammonium excretion in man and dog is determined primarily by urine pH (3–6), it is not clear whether there is any fundamental interdependence of these two moieties of acid excretion. In previous studies where both were measured (7–10), variations in urine acidity could have affected either ammonium or titratable acid excretion predominantly and could thus have obscured a significant underlying relationship between the two.

In the present experiments in dogs, titratable acid excretion was increased progressively by intravenous sodium phosphate loading, and the effect on ammonium excretion was studied under conditions of relatively constant urine pH. Because of evidence that ammonium excretion is limited by rate of intracellular NH_3 production in acid but not in alkaline urine (4), experiments were carried out in both urine pH ranges. In one group of animals, ammonium excretion was stimulated by prior induction of metabolic acidosis. It was found that in each of these experimental situations, ammonium excretion was entirely independent of the buffer content of the urine and the rate of titratable acid excretion. Although these observations do not allow any conclusions concerning the intimate nature of ammonium transport into the urine, they suggest that the mechanism regulating ammonium excretion in the dog may be dissociated from total hydrogen ion excretion and those factors that determine the magnitude of Na^+–H^+ exchange. They also support the view that the availability of hydrogen ions does not ordinarily constitute a limiting factor in the accumulation of ammonium in the urine.

METHODS

Fifteen experiments were carried out on 15 female mongrel dogs weighing between 10 and 22 kg. In 10 experiments, systemic acid-base balance was normal and the urine pH was adjusted to either an acid or a more alkaline range by infusion of sodium ferrocyanide or sodium chloride. In the other 5 experiments, ammonium excretion was augmented by inducing metabolic acidosis for 5 to 7 days prior to the acute experiment. Titratable acid excretion was increased progressively by infusion of sodium phosphate in concentrations calculated to deliver sodium at the same rate as during the preceding control periods. Anesthesia was induced by intravenous chloralose, 100 mg per kg, as a 1.5 per cent solution in 4 per cent dextrose. Additional small amounts were given as required.

Group I: Normal acid-base balance. Five animals were maintained on a salt-free diet for 7 to 10 days. After induction of anesthesia, a solution containing 30 mmoles per L of $Na_4Fe(CN)_6$ was administered at 4.5 ml per minute by means of a Bowman infusion pump. When a constant urine flow had been established, several timed urine collections were made. The solution was then replaced by one containing the following in millimoles per liter: Na_2HPO_4, 48; NaH_2PO_4, 12; $Na_4Fe(CN)_6$, 3. Fifteen to 45 minutes after starting this infusion, urine was collected for 5 additional periods.

The other 5 animals in this group were maintained on a normal diet. Sodium chloride, 135 mmoles per L, was infused and after a constant urine flow had been established, several urine specimens were collected. A sodium phosphate infusion was then begun and additional urine specimens obtained.

Group II: Chronic metabolic acidosis. Five dogs were fed 8 g of ammonium chloride daily for 5 to 7 days prior to the acute experiment. In order to assure a markedly acid urine, 0.1 N HCl in 4 per cent dextrose was administered for 30 to 60 minutes at the start of each experiment. Control urine specimens were collected during sodium ferrocyanide infusion. A solution containing 50 mmoles per L of Na_2HPO_4 and 25 mmoles per L of NaH_2PO_4 (pH 7.1) was then substituted for the ferrocyanide and additional urine collections were ob-

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tained. Dextrose was added to each solution to adjust the final osmolarity to between 240 and 270.

Urine was collected through an indwelling catheter and the bladder emptied by manual compression at the end of each period. pH was determined on mid-point aliquots collected without exposure to air. Heparinized blood samples were collected from the femoral artery for determination of pH and CO_2 content.

Urine and blood pH were determined at 37° C without exposure to air, with a Cambridge model R pH meter. Plasma CO_2 content was determined manometrically by the method of Van Slyke and Neill (11). Urine ammonium was measured by the method of Folin and Bell (12) and inorganic phosphorus by that of Fiske and Subbarow (13). Sodium and potassium were determined by flame photometry with lithium as an internal standard. Chloride was measured by titration with silver nitrate, by a potentiometric method (14). Titratable acid was calculated from urine phosphate, urine pH and blood pH. (During phosphate loading, organic buffers contribute only a relatively small amount to total titratable acid excretion.) In this calculation, the pK'_2 of phosphoric acid was taken to be 6.8 in blood and in urine when urine pH was below 6.0. It has been shown that significant deviations in pK'_2 due to variations in urinary ionic strength may introduce appreciable errors in estimation of titratable acid when urine pH is above 6.0. In such urines, pK'_2 was calculated from an estimated ionic strength. An arbitrary addition of 0.025 was made to the ionic strength estimate to account for organic acids, bicarbonate, calcium, and other unmeasured ions. The theoretical considerations and methods of calculation of pK'_2 from ionic strength have been previously described (15).

RESULTS

Group I. The results of one of the experiments in this group are shown in detail in Table I. During the infusion of sodium ferrocyanide, urine

pH varied between 5.04 and 5.30 and ammonium excretion between 27 and 30 μmoles per minute. With the subsequent administration of sodium phosphate, titratable acid excretion increased from 1 to 100 μEq per minute, and urine pH rose only about 0.3 unit. In spite of the marked increase in buffer content of the urine and titratable acid excretion, ammonium excretion was essentially unaltered. Blood pH and bicarbonate were in the low-normal range and did not change appreciably during the course of the experiment.

The results of the five experiments in which aciduria was induced by ferrocyanide infusion are shown in Figure 1 as solid circles. The circle marked by an asterisk represents the average ammonium and titratable acid values for all periods during sodium ferrocyanide administration. Urine pH values were between 4.8 and 5.8 in almost all instances and in individual experiments varied by no more than 0.6 unit. It is clear that increases in titratable acid excretion up to 133 μEq per minute had no discernible effect upon ammonium excretion. Sodium excretion remained essentially constant in three experiments, while in the other two there was a moderate fall when the ferrocyanide solution was replaced by phosphate. Blood pH and bicarbonate values were within normal limits during control periods and showed small increases during phosphate loading.

In Table II are shown the results of an experiment in which urine pH was more alkaline, owing to the administration of sodium chloride.

TABLE I

The effect of a sodium phosphate infusion upon ammonium excretion

Time	Flow	Na	K	Cl	NH₄⁺	P	pH	TA	TA+NH₄⁺	pH	HCO₃	pCO₂
					Urine						Plasma	
min	ml/min	μmoles/min	μmoles/min	μmoles/min	μmoles/min	μmoles/min		μEq/min	μEq/min		mmoles/L	mm Hg
0	Infusion: Na₄FE(CN)₆, 30 mmoles/L; dextrose, 110 mmoles/L at 4.5 ml/min											
65–85	1.5	261	78	4	29	2	5.16	1	30	7.31	20.7	42.5
85–100	1.7	346	87	4	28	2	5.24	1	29			
100–115	1.7	294	67	3	30	2	5.30	1	31	7.30	19.8	41.4
115–130	2.2	273	62	2	27	1	5.04	1	28			
130–145	3.1	357	69	5	30	1	5.29	1	31	7.31	21.7	44.4
147	Infusion: Na₂HPO₄, 48 mmoles/L; NaH₂PO₄, 12 mmoles/L; Na₄Fe(CN)₆, 3 mmoles/L; dextrose, 55 mmoles/L at 4.5 ml/min											
185–200	2.7	284	66	3	23	67	5.53	48	71			
200–215	2.6	263	63	1	24	83	5.54	60	84	7.34	22.7	43.4
215–230	2.2	214	56	2	22	91	5.52	66	88			
230–245	2.1	212	59	2	23	106	5.61	77	100			
245–260	2.3	223	59	2	26	137	5.57	100	126	7.36	22.8	41.5

● NORMAL ACID-BASE BALANCE   △ NORMAL ACID-BASE BALANCE   □ CHRONIC METABOLIC ACIDOSIS
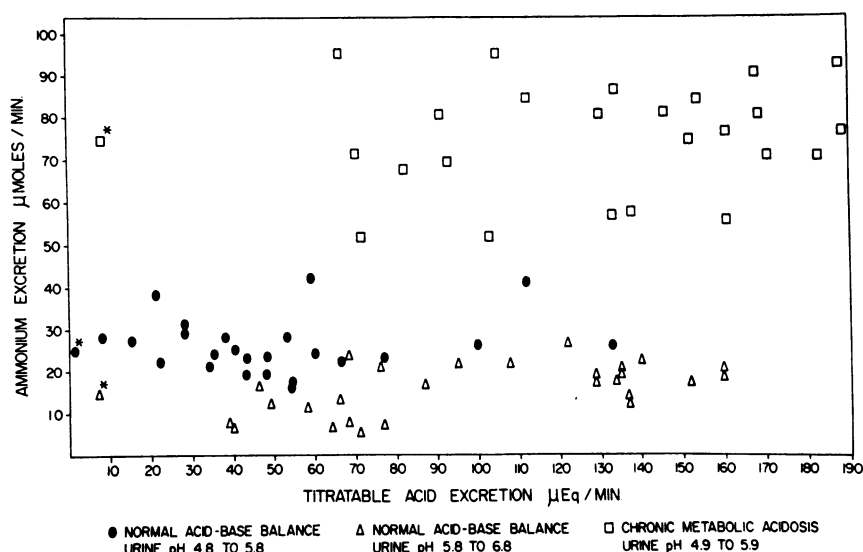URINE pH 4.8 TO 5.8   URINE pH 5.8 TO 6.8   URINE pH 4.9 TO 5.9

FIG. 1. THE EFFECT OF PROGRESSIVE INCREASES IN TITRATABLE ACID EXCRETION UPON THE CONCURRENT RATE OF AMMONIUM EXCRETION.

During the control periods, urine pH varied between 6.46 and 6.60 and ammonium excretion between 18 and 26 μmoles per minute. Titratable acid contributed only small amounts to total acid excretion. With the subsequent infusion of sodium phosphate, titratable acid excretion increased to 135 μEq per minute, while urine pH remained essentially constant. As can be seen from the table, the marked rise in titratable acid excretion was without effect on ammonium under these circumstances. Blood pH and bicarbonate rose moderately during phosphate loading.

The results of all five experiments in which this protocol was followed are shown as triangles in Figure 1. The average of ammonium and titratable acid values during sodium chloride administration is represented by the symbol marked with an asterisk. Urine pH values were between 5.8 and 6.8 in almost all periods and varied by no more than 0.5 unit in individual experiments. It is apparent from the figure that increases in titratable acid excretion up to 160 μEq per minute had virtually no effect upon the simultaneous excretion of ammonium. Sodium excretion rose in one experiment, fell in one, and remained essentially unchanged in the other three.

TABLE II

The effect of a sodium phosphate infusion upon ammonium excretion

| Time | Flow | Urine | | | | | | | | | | Plasma | | |
		Na	K	Cl	NH₄⁺	P	pH	pK₂'	TA	TA+NH₄⁺	pH	HCO₃	pCO₂
min	ml/min	μmoles/ min	μmoles/ min	μmoles/ min	μmoles/ min	μmoles/ min			μEq/ min	μEq/ min		mmoles/L	mm Hg
0		Infusion: NaCl, 135 mmoles/L at 6.8 ml/min												
60–70	5.2	442	73	511	25	14	6.46	6.80	6	31	7.28	19.2	42.2
70–80	7.4	375	74	448	24	13	6.47	6.88	6	30			
80–90	7.0	434	77	496	26	14	6.46	6.84	7	33			
90–100	5.8	505	84	632	19	18	6.55	6.80	7	26	7.32	20.8	41.7
100–110	5.2	515	80	567	18	18	6.60	6.79	7	25			
112		Infusion: Na₂HPO₄, 60 mmoles/L; NaH₂PO₄, 15 mmoles/L; dextrose, 55 mmoles/L at 6.8 ml/min												
135–145	6.5	559	107	371	19	227	6.26	6.79	129	148	7.39	21.1	36.0
145–155	7.4	518	115	239	19	272	6.42	6.81	135	154			
155–165	8.0	624	116	254	18	315	6.55	6.80	134	152	7.36	22.8	41.5
165–175	7.2	691	122	266	18	353	6.64	6.78	129	147			
175–185	7.3	667	123	209	21	365	6.63	6.78	135	156	7.36	23.2	42.4

TABLE III

The effect of a sodium phosphate infusion upon ammonium excretion

	Urine									Plasma		
Time	Flow	Na	K	Cl	NH$_4^+$	P	pH	TA	TA+NH$_4^+$	pH	HCO$_3$	pCO$_2$
min	*ml/min*	*µmoles/ min*	*µmoles/ min*	*µmoles/ min*	*µmoles/ min*	*µmoles/ min*		*µEq/ min*	*µEq/ min*		*mmoles/L*	*mm Hg*
0	Infusion: HCl, 0.1 N; dextrose, 220 mmoles/L at 6.8 ml/min									7.15	9.2	27.2
42	Infusion: Na$_4$Fe(CN)$_6$, 31 mmoles/L; dextrose, 72 mmoles/L at 6.8 ml/min											
60–70	10.4	342	88	124	76	20	5.52	12	88	7.02	6.2	25.0
70–80	10.3	349	82	118	71	17	5.87	9	80			
80–90	9.1	319	68	62	72	17	5.63	9	81			
90–100	6.7	415	74	60	72	16	5.36	9	81	7.01	6.9	28.0
100–110	4.5	481	89	51	76	19	5.20	11	87			
111	Infusion: Na$_2$HPO$_4$, 50 mmoles/L; NaH$_2$PO$_4$, 25 mmoles/L; dextrose, 28 mmoles/L at 6.8 ml/min											
135–145	7.1	374	92	46	81	142	5.48	91	172	7.14	6.9	20.8
145–155	7.4	370	85	48	81	196	5.39	130	211			
155–165	7.9	393	75	46	75	225	5.23	152	227	7.17	6.5	18.5
165–175	7.4	370	70	50	71	253	5.21	171	242			
175–185	6.8	374	68	48	71	274	5.27	183	254	7.16	6.9	19.9

As can be seen from the figure, ammonium excretion was generally higher in the animals with aciduria than in those with more alkaline urine.

Group II. In Table III is shown one of the experiments in which ammonium excretion was enhanced by prior induction of metabolic acidosis. Plasma bicarbonate was lowered further by infusion of 0.1 N HCl. During the administration of sodium ferrocyanide, urine pH varied between 5.20 and 5.87 and ammonium excretion between 71 and 76 µmoles per minute. The contribution of titratable acid to total acid excretion was small. As the result of sodium phosphate (pH 7.1) loading, titratable acid excretion rose to 183 µEq per minute, but it is apparent that this was without effect upon the concurrent excretion of ammonium. Urine pH remained within the narrow range of 5.21 to 5.48 and sodium excretion was essentially constant. Blood bicarbonate did not vary throughout the experiment, but pH rose and pCO$_2$ fell.

The results of the five experiments in this group are shown as squares in Figure 1. The symbol representing the average ammonium and titratable acid values during sodium ferrocyanide infusion is marked by an asterisk. Ammonium excretion was greater in this group than in Group I because of the stimulus of chronic metabolic acidosis. The wide spread in rates of ammonium excretion in the acidotic animals is not the result of differences in urine pH, since this varied by no more than 0.7 unit in individual experiments. It is, rather,

related to differences in the duration of ammonium chloride ingestion. For example, the five points of lowest ammonium excretion are from the experiment in which ammonium chloride was fed for the shortest period of time. It is apparent, however, that increases in titratable acid up to 190 µEq per minute had no significant effect upon the rate of ammonium excretion. Sodium excretion remained essentially constant in all five experiments.

DISCUSSION

The present experimental observations demonstrate that, under conditions of relatively constant urine pH, progressive increases in titratable acid excretion can take place without having any significant effect upon the concurrent rate of ammonium excretion. This was found to be true in the presence of normal acid-base balance and also in chronic metabolic acidosis with markedly augmented rates of ammonium excretion. This dissociation of ammonium from titratable acid excretion is consistent with the view that NH$_3$ diffuses passively into an acid tubular fluid where it is "trapped" by conversion to NH$_4^+$ (4). The rate at which this physicochemical process proceeds depends primarily upon the pH gradient between tubule cell and lumen and should be independent of all changes in composition of the tubule fluid other than that of pH (4). It therefore follows that the addition of large amounts of buffer

to the tubular fluid and the associated acceleration of Na^+-H^+ exchange should not influence ammonium excretion. However, the results of these experiments are also compatible with the hypothesis that NH_4^+ gains access to the urine by an independent Na^+-NH_4^+ exchange mechanism (9, 16), and therefore do not provide critical information concerning the intimate nature of ammonium transport into the urine.

It has been demonstrated repeatedly that ammonium excretion in man and dog is inversely related to urine pH (1, 4, 5, 17), a fact which is most readily explained by the passive diffusion theory. According to the physical laws governing such a process, ammonium excretion should increase tenfold with each unit fall in urine pH if the tubular cell membrane is freely permeable to NH_3 and completely impermeable to NH_4^+ (4, 6). Stated in another way, there should be a linear relationship between urine pH and ammonium excretion when the latter is plotted logarithmically, and the regression coefficient defining the rate of change of log ammonium excretion per unit fall in urine pH should be -1.0 (6). The experimentally observed relationship in man and dog is indeed linear but the calculated regression coefficient is considerably less than unity (3, 4). In order to account for this discrepancy within the context of the diffusion hypothesis, it has been postulated that the rate of intracellular ammonia production limits ammonium excretion in acid urine and is insufficient for an equilibrium state to be achieved (4). This postulate is based on the observation that ammonium excretion varies with urine flow in the alkaline but not in the acid range of urine pH (4). As an alternate explanation, it seems reasonable that the availability or rate of diffusion of hydrogen ions into the tubular fluid might play a significant regulatory role in ammonium excretion, particularly in acid urine, since H^+ is necessary for the conversion of NH_3 to NH_4^+ prior to excretion. However, the present studies show that large increases in hydrogen ion excretion can take place in both alkaline and acid urine at a time when ammonium excretion is already maximal for the urine pH. This suggests that, in experimental situations where only endogenous amounts of buffer are present, hydrogen ions are available in excess and therefore do not constitute a limiting factor in ammonium excre-

tion. Indeed, the present experimental observations indicate that the process regulating ammonium excretion in the dog may be largely independent of total hydrogen ion excretion and those factors that determine the magnitude of the Na^+-H^+ exchange mechanism.

SUMMARY

The relationship between ammonium and titratable acid excretion has been studied in dogs under conditions of relatively constant urine pH. It was found that progressive increases in titratable acid excretion due to sodium phosphate loading had no significant effect upon ammonium excretion in either normal or chronically acidotic animals. This dissociation of ammonium from titratable acid excretion is consistent with the view that ammonia accumulates in the urine by passive diffusion, since this process depends primarily upon the pH gradient between tubular cell and lumen and should be independent of all changes in composition of the tubular fluid other than that of pH. However, an independent Na^+-NH_4^+ exchange mechanism can also account for the present observations and, therefore, conclusions cannot be drawn from these experiments concerning the intimate nature of the tubular mechanism for ammonium excretion.

The finding that large increases in titratable acid excretion can take place without affecting urinary ammonium suggests that, in experimental situations where only endogenous amounts of buffer are present, hydrogen ions are available in excess and do not constitute a limiting factor in ammonium excretion. It appears, rather, that ammonium excretion in the dog may be largely independent of total hydrogen ion excretion and those factors that determine the magnitude of the Na^+-H^+ exchange mechanism.

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