THE MECHANISM OF THE EXCRETION OF AMMONIA IN THE DOG

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The excretion of ammonia is dependent upon several factors: the amount and duration of acid intake, presumably affecting variations in intracellular enzyme activity (1–3), the availability of precursors from which ammonia can be formed (4–6), and the pH of the urine (7–9). With respect to the effect of urine pH, the excretion of ammonia resembles that of a number of organic bases, such as quinine (10), nicotine (11), procaine (12), Atabrine® (13), and certain derivatives of 4-aminoquinoline (13), all of which appear in the urine at a rate inversely related to urine pH. The experiments to be reported were designed to reexamine this phenomenon and to compare ammonia excretion with that of selected organic bases in order to provide information concerning 1) the nature of the process by which ammonia enters the urine, 2) the production of ammonia by the cells of the renal tubules, 3) the mechanism of excretion of other weak electrolytes, the output of which is dependent upon urine pH, and 4) to yield an interpretation of the quantitative relations between ammonia excretion and urine pH.

Since the factors which affect the transport of the organic bases are similar to those affecting the movement of ammonia into the urine, it was anticipated that the excretion of these bases might be used as a means of independently evaluating the factors affecting the transport of ammonia as contrasted with those affecting intracellular production. In acute experiments the clearance of quinine was found to serve this purpose since it was influenced by pH only and the excretion of ammonia by both urine pH and by circumstances which might be presumed to influence rate of production. However, it was not possible to extend these principles to chronic experiments since the absolute level of quinine clearance varied at the same pH in different experiments in an unpredictable fashion.

The results of the studies are in accord with the view (9, 14) that ammonia formed in the tubule cell enters the urine almost exclusively in the uncharged state, *i.e.*, as the free base NH_3, and accumulates as NH_4^+. The extent of accumulation is probably limited in the acid range of urine pH by the rate of ammonia production. At higher pH's, production of ammonia is non-limiting and the rate of excretion is dependent upon equilibration of ammonia in the tubule urine with that in tubule cell. Similar considerations also apply to the urinary loss of other weak electrolytes whose excretion is modified by changes in urine pH except insofar as rate of diffusion, rather than rate of production, is presumably the limiting factor in the acid range. The data have further been interpreted to indicate that final changes in urine volume are effected by abstraction of water in the tubule at a site beyond which final ammonia content is achieved.

METHODS

Trained, unanesthetized female dogs weighing approximately 20 kg. were used. The plan of most of the experiments was to compare the excretion of ammonia and of one or more organic bases during progressive elevation of urine pH from acid to alkaline values. In studies designed to examine the effect of urine flow on the excretion of weak bases, an attempt was made to maintain urine pH constant in either the acid or the alkaline range during the infusion of osmotic diuretics or hypotonic solutions. This goal was attained without difficulty only when the urine was strongly acid, though in the extremes of osmotic diuresis, urine pH frequently rose when it was low initially. In urines containing appreciable amounts of bicarbonate the production of diuresis lowers the concentration of bicarbonate without corresponding changes in CO_2 tension and therefore reduces urine pH. Consequently, it was impossible to maintain hydrogen ion concentration constant in the face of varying urine flow when urine pH was above 7.0.

Acidosis and some stimulation of ammonia production were induced by the oral administration of 5 to 6 grams of ammonium chloride on the day prior to most experiments. The organic bases were administered orally in

the following manner: 9-(diethylamino-l-methyl) butylamino-acridine (SN 8439, pKa 8.9),[1] 300 mg. given 12 hours before the experiment; 3-chloro-7-methoxy-9 (diethyl-amino-l-methyl) butylamino-acridine (Atabrine® pKa, 7.3), 100 to 200 mg. per day for 2 to 3 days prior to an experiment; quinine (pKa 8.3), two doses of 500 to 600 mg. each, given 15 hours and 1 to 2 hours prior to the start of an experiment, and 3-chloro-7-methoxy-9 (diethyl-amino) ethylamino-acridine (SN 5228, pKa 7.4), 300 to 400 mg. two hours before the experiment. The disappearance of SN 5228 from plasma was rapid so that it was necessary to administer a constant intravenous infusion of 3 to 4 μg. per min. in order to maintain the plasma level at a constant value.

Effect of urine pH

In studies designed to examine the effects of varying urine pH, a priming injection of creatinine was administered, followed by a sustaining infusion containing creatinine and sodium chloride (350 to 450 μM per min.) during an equilibration period of 30 to 60 minutes. Sodium sulfate, 100 to 200 μM per min., was included in the equilibrating infusion in many of the experiments in order to insure the excretion of acid urine. At the end of the equilibration period, collections of urine were started and the sodium chloride in the infusion was replaced by an equivalent amount of sodium bicarbonate. This generally resulted in a gradual, progressive increase in urine pH to a value between 7.5 and 8.0.

Water diuresis experiments

In experiments at acid pH, diuresis was induced by the administration of approximately 8.0 ml. per min. of a 3 to 5 per cent glucose solution delivering 100 to 200 μM per min. of sodium sulfate. In studies of urine flow at urine pH's above 6.5, the sustaining infusion contained small amounts of $NaHCO_3$, Na_2HPO_4 or K_2HPO_4.

Osmotic diuresis experiments

Osmotic diuresis was produced by the administration of increasing amounts of either 25 per cent mannitol or hypertonic sodium sulfate. One hundred μM per min. of sodium sulfate were infused in the mannitol experiments in order to fix the urine pH in the acid range. All infusions were administered intravenously at a constant rate using a Bowman infusion pump.

Urine was collected from an indwelling catheter. Collection periods were terminated by washing the bladder twice with distilled water. Urine for pH was collected anaerobically at the mid-point of each period in a silicone-coated syringe, the dead space of which was filled with mineral oil. Blood samples were collected at the mid-point of each period from an indwelling arterial needle. Clotting was prevented by the addition of heparin.

Creatinine, for the measurement of the rate of glomerular filtration, was determined in trichloroacetic acid filtrates of plasma and in diluted urine to which trichloroacetic acid was added, by a modification of the method of Folin (15). The pH of urine was measured at room temperature using a Beckman Model G pH meter and syringe-type glass electrode. No correction for temperature was applied. Ammonia in the urine was determined either by the aeration method of Van Slyke and Cullen (16) using an electrometric titration or by the microdiffusion technique of Conway (17). Atabrine® in blood and urine was measured fluorometrically by the double extraction method of Brodie, Udenfriend, Dill, and Downing (18) using heptane as the solvent for the first extraction. SN 8439 and SN 5228 were determined by the same method modified in that the fluorescence was measured in .033 N NaOH to obtain maximum sensitivity for SN 8439 and in .1 N HCl for SN 5228. Quinine was determined by a similar procedure using 1.5 per cent isoamyl alcohol in heptane for the first extraction and measuring the fluorescence in 0.1 N H_2SO_4. The specificity of these procedures for urine was examined and the material isolated from urine by these procedures found to be homogeneous, and identical with that administered, by paper chromatography in a butanol-HCl system for SN 8439 and by a 25-plate countercurrent distribution for quinine.

Plasma concentrations of the acridine derivatives, Atabrine® and SN 8439 showed no consistent trend during the course of experiments. This was to be expected since the amount metabolized or excreted is negligible with respect to the total amount in the body and the half time for disappearance from the plasma is of the order of one week. For this reason determinations of the concentrations of these acridines in the plasma were omitted in many of the experiments and only rates of excretion are presented. Quinine and SN 5228, on the other hand, disappear from the plasma more rapidly and correction has been made for the slowly declining plasma concentrations in the case of quinine by calculation of its clearance and in the case of SN 5228 by infusing it at a constant rate, under which conditions it was found that the plasma concentration remained constant.

THEORETICAL ASPECTS

The behavior of weak electrolytes in living systems is dependent upon the fact that these substances exist in solution as mixtures of unionized and ionized forms in ratios governed by the pH of the medium and the pKa of the conjugate pair. There is ample evidence that the unionized forms penetrate cell membranes relatively freely while the ions do not. This has been demonstrated for NH_3, H_2S, and cresyl blue by Harvey (19), Osterhout (20), and Irwin (21), respectively. Presumably the capacity to penetrate cell membranes is related to lipid solubility and, in general, this property is considerably reduced by ionization. These considerations led Jacobs (22) to the formulation of a general description of the mode of accumulation of weak electrolytes by cells. He concluded that the distribution of weak acids and bases between cells and surrounding fluid at equilibrium is dependent upon the pH of the medium, the pKa of the weak electrolyte and the

[1] Synthesized for us by Dr. Melvin Fish, Laboratory of Chemistry of Natural Products, National Heart Institute.

permeability of the membrane to the uncharged (lipid-soluble) species.

The concept of greater cell permeability to the unionized member of weak electrolyte conjugate pairs has been applied by a number of investigators to an analysis of the excretion of such substances in the urine with the aim of explaining the observed variation of excretion with urine pH (9–14, 23). The uncharged free base has been considered to diffuse into the tubule lumen where it is trapped by combination with hydrogen ion to form the non-diffusible ion.

The ionization of weak bases with only one ionizable group such as NH_3 (pKa 9.3) may be expressed as $B + H^+ \rightleftharpoons BH^+$ and the ionization equilibrium in the form of the Henderson-Hasselbalch equation $pH = pKa + \log \frac{(B)}{(BH^+)}$. The acridine derivatives, Atabrine®, SN 8439 and SN 5228 have two basic amino groups. The ionization is $B.B. + H^+ \rightleftharpoons H^+B.B. + H^+ \rightleftharpoons H^+B.BH^+$. The pKa of the first of these ionizations is approximately 11, so that the reaction of greatest concern in the physiologic range of pH is that involving the singly and doubly charged species. However, the singly charged acridine bases have a high degree of organic solubility and may be presumed to penetrate cells relatively easily. For purposes of brevity these also will be referred to henceforth as "free base." Quinine, although it has two ionizable groups, exists as the uncharged and singly charged species at physiologic pH since the first pKa is 8.3 whereas the second is approximately 3.0.

In a system consisting of two phases, separated by a semi-permeable membrane, and in which the pH of each phase is regulated independently, and assuming movement to occur only by diffusion, the following relationships may be considered to hold as approximations:[2]

$$M_{1,2} = K_B(B)_1 + K_{BH^+}(BH^+)_1 \qquad (1)$$

and

$$M_{2,1} = K_B(B)_2 + K_{BH^+}(BH^+)_2 \qquad (2)$$

where $M_{1,2}$ represents the unidirectional rate of movement or flux of free base, B, plus ion, BH^+, from phase 1 to phase 2 and $M_{2,1}$ the flux in the opposite direction; $(B)_1$ and $(B)_2$ the concentrations of the free base in phases 1 and 2, respectively; and $(BH^+)_1$ and $(BH^+)_2$ the corresponding concentrations of the ion formed by association of the base with hydrogen ion. The constants K_B and K_{BH^+} are permeability factors which include the actual permeability, the thickness of the membrane and the area available for diffusion.

At equilibrium,[3] the rate of movement in both directions is equal so that

$$M_{1,2} = M_{2,1} \qquad (3)$$

and

$$K_B(B)_1 + K_{BH^+}(BH^+)_1 = K_B(B)_2 + K_{BH^+}(BH^+)_2 \qquad (4)$$

[2] These are approximations in that concentrations are used instead of electrochemical potentials and solvent flow is disregarded.

[3] This will be an equilibrium only with respect to the weak electrolyte since a continuous addition of hydrogen ion will be required to maintain a pH gradient in the face of the neutralizing effect of the diffusible base.

The relative concentrations of B and BH^+ in each phase are a function of the pH of that phase and the pKa of the base.

If the membrane is completely impermeable to BH^+, ($K_{BH^+} = 0$), at equilibrium

$$(B)_1 = (B)_2 \qquad (5)$$

If pH_1 and total B in phase 1 remain constant, $\log (BH^+)_2$ will be a linear function of pH_2 with a negative slope of unity. At values of pH_2 below pKa, where $(B)_2$ makes only a small contribution to the total $(B)_2 + (BH^+)_2$, the log of the sum of the total concentrations will also vary approximately as a linear function of pH with a negative slope of unity. Thus there will be an approximately ten-fold increase in the concentration of weak electrolyte ($B + BH^+$) in phase 2 for every unit decrease in pH_2.

If there is some permeability to the ion, the relationship will be non-linear and with a negative slope of less than unity. The terms containing (BH^+) do not drop out of equation 4 and the simplified form, equation 5, does not apply. Instead equation 4 may be rearranged so that

$$\frac{K_B}{K_{BH^+}} = \frac{(BH^+)_2 - (BH^+)_1}{(B)_1 - (B)_2} \qquad (6)$$

This indicates that the disparity in the concentration in the two phases will be a function of the relative permeability of the membrane to the two species, as well as of pH.

Under these conditions, if $(B)_1$ and pH_1 remain constant, total B, $\Sigma(B + BH^+)$ in phase 2 will be a function of pH so long as K_B is not equal to K_{BH^+}. However, the relationship between $\log [(B) + (BH^+)]$ and pH will be complex, the slope depending upon the magnitude of $(pKa - pH_2)$. The larger the latter value, and thus the greater the excess of $(BH^+)_2$ over $(B)_2$, the flatter the relationship between $(B)_2 + (BH^+)_2$ and pH_2, since under these conditions the movement of the ion will make an appreciable contribution to the total flux even though K_B may be several orders of magnitude greater than K_{BH^+}. The negative slope of the relationship between $\log [(B)_2 + (BH^+)_2]$ and pH will approach unity at that pH at which the contribution of the ion to total movement is negligible. This will obtain at pH's close to the pKa of the electrolyte.

The influence of electrical potential across the membrane on movement of the ion has not been included in this formulation. This is justified as a reasonable approximation in the case of ammonia and quinine since movement of the uncharged free base, which is unaffected by the electrical potential gradient, presumably accounts for all but a minute fraction of the total flux. This need not be the case with the 9-amino acridines for which the pertinent equilibrium involves singly and doubly charged ions in the physiologic pH range, since movement of both ions would be modified by an electrical potential.

This general treatment of the distribution of weak electrolytes will serve as a basis for the consideration of the mode of excretion of ammonia, the acridine bases, and other weak electrolytes not transported by specific enzyme systems.[4] If the accumulation of weak bases in the urine

[4] The above derivation also may be applied to weak acids, such as salicylic, which will accumulate in alkaline solutions since the uncharged acid is lipid soluble.

TABLE I

The effect of $NaHCO_3$ on weak base excretion—dog Sp.

Time	Creatinine clearance	Urine flow	Urine pH	Rates of excretion		Clearance of quinine
				Ammonia	SN 8439	
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μg./min.*	*ml./min.*
0	Priming infusion; 2 gm. creatinine in 20 ml. H_2O					
1	Infuse 200 μM/min. Na_2SO_4. 20 mg./min. creatinine in 4.2 ml./min. .86% NaCl					
42	Change infusion to 20 mg./min. creatinine. 380 μM/min. $NaHCO_3$. 250 μM/min. NaCl in 4.2 ml./min. H_2O					
42–60	63	0.4	4.92	34.0	12.5	41.3
60–80	61	0.9	5.00	32.0	12.2	36.8
80–100	64	1.5	5.39	27.0	10.6	32.1
100–114	61	1.6	5.73	15.0	4.8	17.0
114–128	69	1.7	6.32	11.0	5.5	12.5
128–144	66	1.9	6.82	7.3	4.6	10.9
144–158	71	2.2	7.40	4.9	3.9	8.9
158–171	71	2.4	7.60	3.8	2.8	4.6
171–184	73	2.5	7.78	2.6	1.8	4.0

is governed by these equilibrium considerations, it would be anticipated that at the site at which the accumulation occurs, the concentration of weak base in the urine will be a function of pH. To the extent that no further changes in pH, volume, and weak base content of the urine occur, the concentration in the final urine will be a function of the pH. If changes in volume do take place after pH and weak base content have been established, the relationship between pH and weak base concentration will be distorted. However, if the volume flow past the last point of equilibrium remains relatively unchanged, despite alterations in volume in a more distal segment, the rate of excretion of the weak base, rather than the concentration, will be a function of pH. It will be shown that ammonia and the organic bases may be considered to move primarily as the lipid soluble or uncharged form, that equilibrium considerations apply to the excretion of these substances only in alkaline urines, and that rate of production in the case of ammonia or rate of diffusion in the case of the organic bases limits the accumulation and excretion of these substances in the acid range.

RESULTS

Effect of urine pH on base excretion

Data from experiments in which the urine pH was varied over a wide range by the infusion of

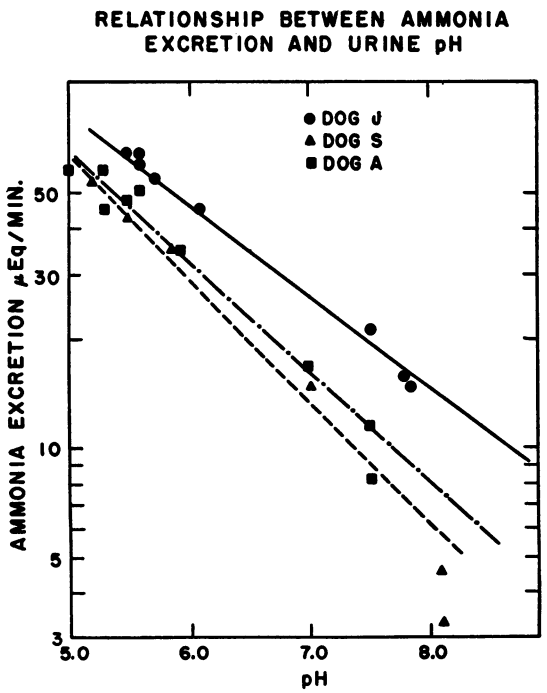

FIG. 1. THE EFFECT OF THE INFUSION OF $NaHCO_3$ ON URINE pH AND THE EXCRETION OF AMMONIA IN ACIDOTIC DOGS

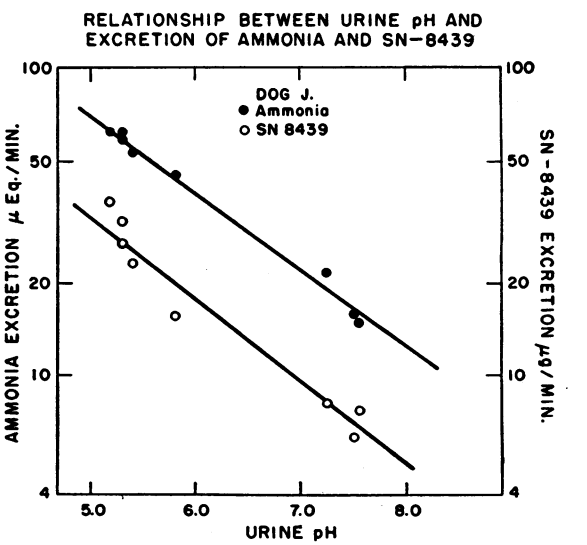

FIG. 2. THE EFFECT OF THE INFUSION OF $NaHCO_3$ ON URINE pH AND THE EXCRETION OF AMMONIA AND SN 8439

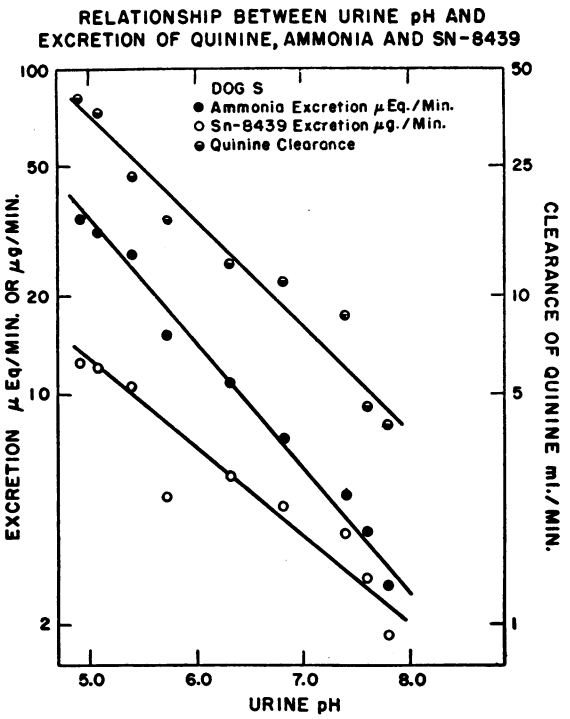

FIG. 3. THE EFFECT OF THE INFUSION OF $NaHCO_3$ ON URINE pH AND THE EXCRETION OF AMMONIA, SN 8439 AND THE CLEARANCE OF QUININE

$NaHCO_3$ are presented in Table I and Figures 1 to 5. These are representative of 27 such experiments in which the excretion of SN 8439, SN 5228, quinine, and Atabrine® either singly or in various combinations[5] was compared with that of ammonia. The uniform depression in ammonia excretion which accompanies alkalinization of the urine is illustrated in Figures 1 (ammonia only) and 2 (8439 and ammonia) and Table I. The change in the excretion of SN 8439 parallels that of ammonia as does the change in the clearance of quinine (Figure 3). It is evident that under the conditions of these experiments the slopes of lines relating the log of the excretion of ammonia and SN 8439 and the log of the clearance of quinine to urine pH are similar though not necessarily identical.[6] The slopes of these lines were independent of the absolute level of the rates of excretion in an individual dog and did not vary appreciably between experiments. As indicated by the data in Figures 4 and 5, the excretion of Atabrine® and of SN 5228 falls off sharply at pH's above 6.5 and 7.0. This was not associated with a fall in plasma concentration of these substances. It should be noted that the pKa of these compounds (± 7.4) are the ones which fall within the pH range of the urine.

Alkalinization of the urine by the infusion of potassium chloride produced results with respect to ammonia and quinine excretion and urine pH indistinguishable from those induced by $NaHCO_3$ (Table II). However, the excretion of SN 8439 diminished to a much lesser extent as urine pH rose, in some experiments actually showing an increase. Similar results with respect to ammonia were obtained when acetazoleamide was injected. The decrease in the excretion of SN 8439 under these conditions seemed uniformly to be smaller than that of ammonia in the same experiments and that of SN 8439 in other experiments in which bicarbonate was administered, but the difference was far less striking than in the experiments in-

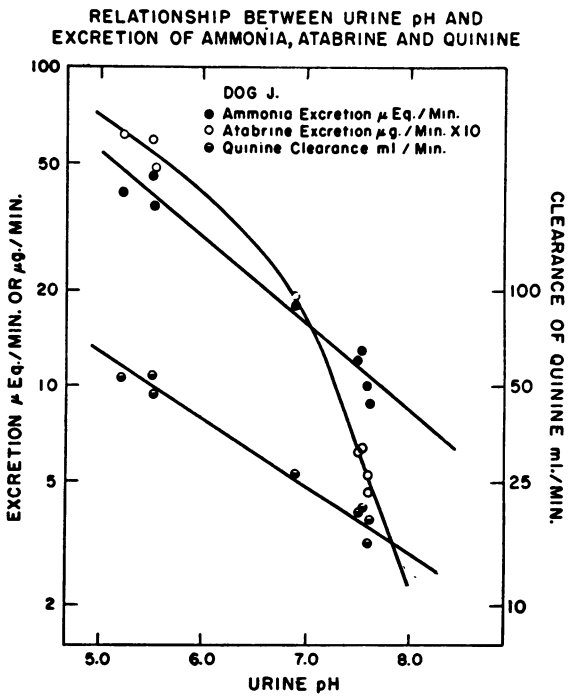

FIG. 4. THE EFFECT OF THE INFUSION OF $NaHCO_3$ ON URINE pH AND THE EXCRETION OF AMMONIA, ATABRINE® AND THE CLEARANCE OF QUININE

[5] Atabrine®, SN 8439, and SN 5228 were not administered in the same experiment since they are not easily distinguishable by the methods used.

[6] Except in the instance where the logarithm of rate of excretion is related to pH by a straight line with a negative slope of unity, there is no theoretical basis for assuming a linear relationship. Lines have been fitted to the data for empirical convenience.

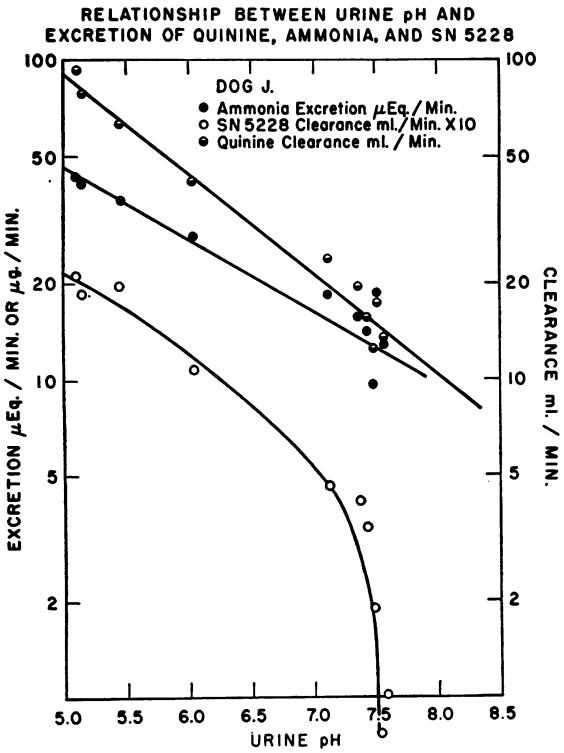

FIG. 5. THE EFFECT OF THE INFUSION OF $NaHCO_3$ ON URINE pH AND THE EXCRETION OF AMMONIA, SN 5228 AND THE CLEARANCE OF QUININE

volving administration of potassium salts and amino acids (see below). The cause of the difference in the effects on the excretion of ammonia and quinine on one hand and SN 8439 on the other in these experiments is not clear. It may be related to an effect on membrane potential since this would affect only the movement of a charged particle (see above). Furthermore, the acridine bases differ from ammonia and quinine in the very marked extent to which they are concentrated in cells. This represents a potential source of a difference in behavior which has not been fully explored.

The effect of amino acids

The infusion of certain amino acids is known to enhance ammonia excretion (5, 6). Twenty-two experiments were performed in which various amino acids (glutamine, dl-alanine, l-glycine, aspartic) were infused into dogs. In most experiments there was a brisk rise in ammonia excretion and a rise in the pH of the urine irrespective of the amino acid used. Table III is illustrative of one such experiment. The clearance of quinine diminished as pH rose in a manner which could not be distinguished from that which occurred when $NaHCO_3$ was infused. As in instances when potassium chloride was administered, the excretion of SN 8439 did not change or diminished less than when equivalent pH changes were produced by the infusion of bicarbonate. In seven experiments the effect of the infusion of dl-alanine on the excretions of ammonia, titratable acid, and bicarbonate was studied. In all, significant alkalinization of the urine occurred. Net effective acidification of the urine, as indicated by the sum of the excretions of ammonia and titratable acid minus that of bicarbonate, did not change consistently. The experiments in Tables IV and V are those in which the largest and most consistent changes occurred.

The effect of urine flow

If ammonia enters the urine by a diffusion process and equilibrium is achieved, changes in

TABLE II

Effect of KCl on excretion of ammonia, SN 8439 and quinine—dog S

Time	Creatinine clearance	Urine flow	Urine pH	Rates of excretion		Clearance of quinine
				Ammonia	SN 8439	
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μg./min.*	*ml./min.*
0	Prime: 2 gm. creatinine					
2–63	Infuse 20 mg./min. creatinine; 500 μM/min. NaCl; 100 μM/min. Na_2SO_4. 2.2 ml./min. H_2O					
63	Substitute 500 μM/min. KCl for NaCl in above infusion					
63–82	103	1.9	5.3	49.5	28.0	33.2
82–105	103	3.0	5.2	43.5	28.9	26.4
105–127	104	4.4	5.9	33.1	29.0	26.9
127–137	113	5.6	6.6	30.2	30.4	27.6
137–146	111	4.4	7.0	25.0	34.5	21.9
146–155	108	4.2	7.2	24.4	38.4	18.4
155–170	102	3.6	7.1	17.6	39.6	16.4
170–184	104	2.5	7.5	10.2	36.8	8.7

TABLE III

Effect of dl-alanine on excretion of ammonia, SN 8439 and quinine—dog J

Time	Creatinine clearance	Urine flow	Urine pH	Rates of excretion: Ammonia	Rates of excretion: SN 8439	Clearance of quinine
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μg./min.*	*ml./min.*
0	Priming injection of 2 gm. creatinine					
1	Infuse 20 mg./min. creatinine.	4.1 ml./min.	.85% NaCl			
66–86	90	3.4	5.32	48	25	—
86–108	94	3.7	5.30	52	26	43
108–130	93	2.9	5.50	48	27	50
132	Add 185 mg./min. dl-alanine to above infusion					
179–197	93	4.1	6.66	96	26	26
197–219	87	4.5	7.06	115	25	21
219–234	88	6.1	6.73	124	26	18

the volume of the receiving fluid (tubule urine) should be reflected in variations in the amount of ammonia which can accumulate in the urine. Alternatively, if equilibrium is not achieved because the extent of the accumulation of ammonia is limited either by the rate at which it can diffuse out of cells or be produced within the cell, changes in urine volume will not influence the rate of excretion. The following experiments were performed to investigate these alternatives.

TABLE IV

Effect of dl-alanine on urine pH and excretion of ammonia, titratable acidity, and bicarbonate—dog J *

Time	Inulin clearance	Urine flow	Urine pH	Rates of excretion: Ammonia	Rates of excretion: Titratable acidity	Rates of excretion: HCO_3^-
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μEq./min.*	*μEq./min.*
0	Prime: 500 mg. inulin					
1	Infuse 10 mg./min. inulin, 100 μM/min. Na_2SO_4.		3% dextrose in H_2O at 7.3 ml./min.			
53–66	100	6.6	4.97	41	14	—
66–77	99	8.6	5.03	38	17	—
77–93	100	10.5	5.53	33	21	3
93–109	102	10.4	4.89	35	27	1
111	Add 300 mg./min. dl-alanine to above infusion					
109–128	105	9.6	5.49	48	27	2
128–142	111	11.1	6.49	77	17	23
142–157	105	11.8	6.56	91	20	34
157–172	101	11.5	6.56	98	22	32
172–186	97	9.2	6.73	95	26	33
186–200	81	7.5	6.81	94	22	33

* Initial plasma total CO_2 23 mM per L.

TABLE V

Effect of dl-alanine on urine pH and excretion of ammonia, titratable acidity, and bicarbonate—dog J *

Time	Creatinine clearance	Urine flow	Urine pH	Rates of excretion: Ammonia	Rates of excretion: Titratable acidity	Rates of excretion: HCO_3
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μEq./min.*	*μEq./min.*
0	Prime: 2 gm. creatinine					
1	Infuse 20 mg./min. creatinine, 100 μM/min. Na_2SO_4.		145 μM/min. NaCl.		11.5 ml./min. H_2O	
60–76	97	3.9	4.91	116	85	—
76–86	96	10.1	4.87	115	83	—
89	Add 200 mg./min. dl-alanine to above infusion					
86–100	98	11.8	5.02	128	62	—
100–111	106	11.3	5.79	171	21	4
111–121	109	11.1	6.30	178	14	12
121–132	105	12.1	6.45	174	16	18
132–143	107	13.6	6.53	174	12	34
143–154	91	14.3	6.60	181	9	43

* Initial plasma total CO_2 10 mM per L.

TABLE VI

Effect of Na_2SO_4 diuresis—dog A

Time	Creatinine clearance	Urine flow	Urine pH	Rates of excretion	
				Ammonia	SN 8439
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μg./min.*
0	Prime: 2 gm. creatinine				
	Infusion I: Right leg—25 mg./min. creatinine; 100 μM/min. Na_2SO_4, H_2O 1 ml./min.				
	Infusion II: Left leg—300 μM/min. Na_2SO_4 in H_2O, 2 ml./min.				
40–55	73	4.3	5.19	38	18
55–65	74	6.0	5.28	38	19
65–77	71	6.4	5.48	39	18
83	Increase speed of Infusion II to 4 ml./min.				
99–112	73	8.0	5.26	40	16
112–124	73	8.7	5.20	41	17
127	Increase speed of Infusion II to 6 ml./min.				
124–131	—	9.5	5.02	42	17
131–145	—	10.4	5.32	—	20
146	Increase speed of Infusion II to 10 ml./min.				
145–157	—	12.1	5.22	39	18

TABLE VII

Effect of mannitol diuresis—dog H

Time	Creatinine clearance	Urine flow	Urine pH	Rates of excretion	
				Ammonia	SN 8439
min.	*ml./min.*	*ml./min.*		*μEq./min.*	*μg./min.*
0	Prime: 2 gm. creatinine				
1	Infuse: 100 μM/min. Na_2SO_4; 20 mg./min. creatinine; 1.0 ml./min. H_2O, left leg				
10	Infuse: 25% Mannitol at 2.5 ml./min., right leg				
29–53	68	8.1	4.72	32	21
53–64	65	8.7	4.68	32	21
66	Increase speed of Mannitol infusion to 5 ml./min.				
64–76	62	9.3	4.68	31	20
76–85	79	13.3	4.68	28	25
85	Increase speed of Mannitol infusion to 8.0 ml./min.				
85–95	71	13.1	4.68	25	23
95–104	69	14.9	4.68	27	22

Irrespective of the means of inducing diuresis, variations in urine flow exerted no influence on the rates of excretion of ammonia or other organic bases if the urine were maintained acid throughout. This is illustrated for water diuresis in Figure 6. Data for the excretion and concentration of SN 8439 were similar. Tables VI and VII are illustrative of experiments in which osmotic diuresis in the acid range did not alter base excretion.

As noted earlier, experiments of a similar nature were difficult to perform successfully when the urine pH was higher. However, urine flow does modify the rates of excretion of the weak bases in the alkaline range. With respect to water diuresis, this conclusion was reached by comparing excretion rates at the same pH but different urine flows. In the experiments summarized in Table VIII the periods were not necessarily consecutive. Figure 7 is constructed from data obtained during

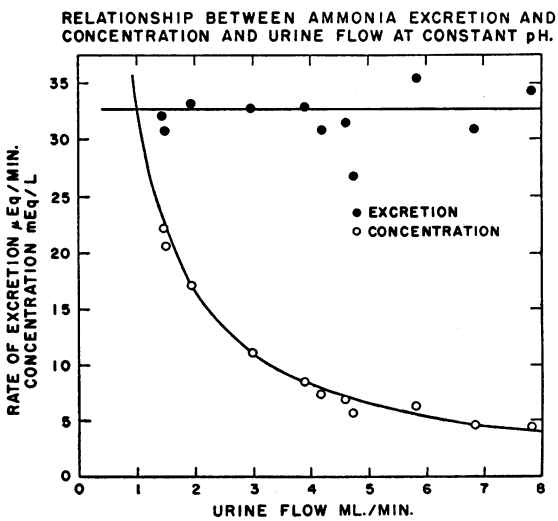

FIG. 6. THE EFFECT OF H_2O DIURESIS ON THE EXCRETION AND CONCENTRATION OF AMMONIA

Urine pH varied from 5.1 to 5.3.

osmotic diuresis produced by the infusion of 25 per cent mannitol. Both ammonia excretion and quinine clearance rose as urine flow increased despite relative constancy of pH between 6.9 and 7.2. It is difficult to specify the exact acidity at which a transition occurs from the range in which changes in urine flow do not modify excretion to that in which urine flow is a factor. However, rate of flow seemed to influence weak base excretion when the urine pH rose above 6.0 to 6.25. It may be noted (Table VIII) that in three of the four experiments with urine pH's below 6.25 the excretion of ammonia changed with urine flow whereas that of SN 8439 did not.

TABLE VIII

Effect of urine flow in water diuresis at high urine pH

Experiment	Urine pH	Urine flow	Excretion of ammonia	Excretion of SN 8439	Clearance of quinine
		ml./min.	*μEq./min.*	*μg./min.*	*ml./min.*
1*	7.93	1.1	2.3	7	
	7.84	2.2	3.9	11	
2	7.92	8.4	2.3	1.6	9.9
	8.00	3.0	1.8	1.0	4.6
3	7.72	4.9	6.4	6.5	5.0
	7.76	2.2	2.5	3.6	2.1
4	7.51	13.3	6.1		6.0
	7.54	1.2	2.3		1.1
5*	7.33	4.1	2.5	4.0	
	7.35	8.0	5.0	5.7	
	7.38	3.7	2.4	3.8	
6	7.06	13.7	11		10
	7.09	3.1	7.5		4.0
7	6.95	4.0	11	21	
	6.82	1.1	5.2	19	
8*	6.88	1.7	7.1	11	
	6.82	3.4	11	19	
9*	6.41	2.3	13	12	
	6.28	6.5	21	18	
10	6.25	1.2	8.8	13	
	6.27	3.7	20	13	
11*	6.10	1.9	15	11	
	6.13	5.3	20	12	
12	6.08	9.1	26	19	
	6.09	4.3	19	13	
13*	6.06	6.2	25	31	
	6.11	10.1	32	33	

* Data from these experiments represent consecutive periods.

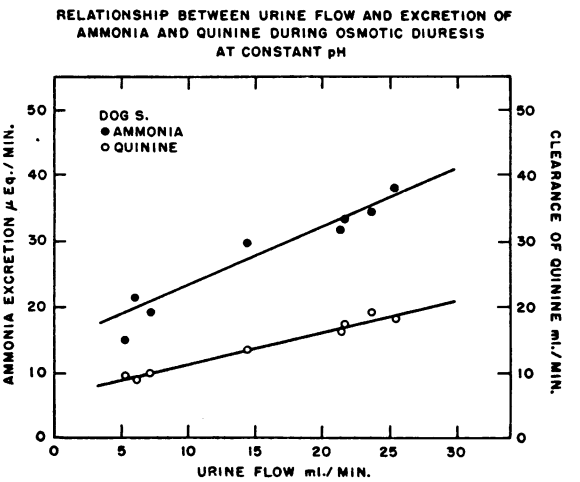

FIG. 7. THE EFFECT OF MANNITOL DIURESIS ON THE EXCRETION OF AMMONIA AND THE CLEARANCE OF QUININE
Urine pH varied from 6.9 to 7.2.

DISCUSSION

The reproducible relationship between the excretion of the weakly basic electrolytes and urine pH and the similarity of behavior of bases with similar ionization constants are consonant with the view that these substances diffuse out of cells primarily in the lipid soluble form and accumulate in the urine as the ion to which the cell membrane is less permeable. This mechanism is operative throughout the entire range of urine pH, though important differences exist in the mode of accumulation in the acid and alkaline ranges of urine hydrogen ion concentration.

The excretion of weak bases in acid urines

In any system in which the time available for diffusion is limited by the movement of one phase past the other, as in the renal tubule, equilibrium may not be achieved when this requires movement of a large amount of substance across a small concentration gradient. This is the situation with regard to ammonia. The concentration gradient over which all the ammonia must diffuse is the difference between the concentration of free ammonia in the cell and free ammonia in the urine. Each of these is only a very small fraction of the total ammonia in the respective phases. Urine pH exerts a minimal influence on the concentration gradient for free ammonia but has a considerable influence on the amount of ammonia which must

diffuse across this gradient in order that equilibrium be achieved. A decrease of three units in pH requires a thousand fold increase in the movement of the lipid soluble member for the achievement of equilibrium. This reasoning may be applied in a general way to weakly basic electrolytes when there is differential permeability of the cell membrane to the individual species and when the value $pH_1 - pH_2$ is large. In a diffusion limited system, such as is proposed in the acid range of urine pH, equilibrium is not achieved; therefore, the flux from phase 2 to 1 is negligible whereas that from 1 to 2 is determined only by the permeability of the membrane, the diffusion coefficient and the phase 1 concentration of the substance. It thus makes little difference what the characteristics of phase 2 may be as to either volume or pH so long as the volume is large enough and the pH low enough. In the case of a substance, such as ammonia, which is produced in phase 1 (the cell) the capacity to produce may not be adequate to replace it as rapidly as it leaves. The concentration in phase 1 will fall and it will enter phase 2 at a rate equal to the rate at which it can be produced. Such a system might be called production-limited and again the rate of entry into phase 2 will not be affected by changes in phase 2.

Experiments designed to determine whether such considerations apply in the dog [7] have shown that in the acid range of urine pH changes in volume of urine do not have an effect on the excretion of weak electrolytes. This is the basis for the conclusion that equilibrium is not achieved in this pH range. The limiting factor in the case of ammonia is presumably the rate of intracellular production, in the case of the organic bases rate of diffusion. For either type of substance (ammonia or exogenous base) the contribution of each individual segment of the tubule urine is maximal so long as the urine pH is low enough. The urinary pH below which production limits the excretion of ammonia would not likely be identical with that at which the entry of the various exogenous bases becomes diffusion-limited. However, although the data do not define the various pH ranges sufficiently to make possible a precise separation, it would appear from Table VIII that a difference between ammonia and SN 8439 excretion probably exists in the range of urine pH between 6.0 and 6.25. It is also unlikely that the curve relating urine pH to base excretion should have the same slope throughout the entire pH range in view of the differences in mechanism of accumulation at varying pH's (see below). The data have sufficient scatter to mask considerable differences in slope at the extremes of pH.

In order to account for the observed effect of alterations of pH on ammonia excretion in acid urines in contrast to the lack of effect of volume changes, it is necessary to consider that diffusion of ammonia out of cells is not limited to an isolated segment of the tubule but extends over an area various portions of which may differ in their contribution depending on the luminal pH. The longer the segment of tubule over which the urine pH is maintained in the production limited range the greater the number of cells which can contribute maximally to ammonia excretion. Urine excreted at a particular acid pH must have had hydrogen ions added more rapidly or have had a lower initial concentration of buffer and have been acid over a longer segment of tubule than any less acid urine. If the urine of lower pH has been sufficiently acid to receive ammonia maximally (*i.e.*, was in the pH range where production limits ammonia entry) over a longer segment of tubule it will accumulate more ammonia.

The excretion of weak bases in neutral and alkaline urines

In more alkaline urines, alterations in urine volume exert an appreciable effect on ammonia excretion. Consequently, equilibrium considerations presumably apply at the site of entry of weak bases in the urine. However, even in this range the data indicate that rate of excretion is more closely related to pH than is concentration. This has been mentioned previously (see above). Such a finding is in accord with the view that final volume adjustments in the kidney tubule occur at a site distal to electrolyte transport (25, 26) and that variations in ultimate urine volume are greater than those at the site of entry of the weak bases studied. At some point in the tubule, urine pH

[7] The applicability of these data to man is clear since experiments relating urine pH to ammonia and Atabrine® excretion have given similar results, as have experiments in which urine flow was modified. Quinine clearance, on the other hand, was not related to urine pH as it is in the dog (24).

may be considered to determine the concentration of ammonia; more distally, in an electrolyte impermeable area, abstraction of water distorts the relationship between electrolyte concentration and pH but not that between rate of excretion and pH.[8]

If diffusion equilibrium of weak bases is achieved, the negative slope of the line relating total excretion to pH should approach unity as an approximation when the ionized form is completely non-permeating. The slope of less than unity observed with ammonia, quinine and SN 8439 may therefore be consequent to slight but significant movement of the ionized form. On the other hand, the urinary excretion of Atabrine® and SN 5228, both of which have pKa's in the neighborhood of 7.4, diminishes much more rapidly at high urine pH's than that of the other compounds studied (Figures 4 and 5). This would be expected even if there were some permeation by the ion since at pH's close to the pKa the relative contribution of the less diffusible form to the total movement is negligible because of the limited concentration at which the ion is present. Under these circumstances the negative slope of the line relating log of excretion to pH should approach unity. This condition is never achieved in the case of SN 8439 or ammonia since their pKa's exceed the maximal urine pH attainable.

The general formulation of the equilibrium distribution of weak electrolytes was based on the assumption that the concentration of ammonia in the cell is constant despite continuous diffusion into the urine. The applicability of this assumption is supported by the parallelism between the excretion of SN 8439, quinine and ammonia, since the interstitial concentrations of the exogenous bases are virtually unaffected by changes in urinary excretion. This implies that the net production of ammonia is more or less equal to the rate of excretion. If the net production of ammonia were not determined by its rate of loss from cells, excretion would be unaffected by urine pH except for transient periods of accumulation in cells and unloading accompanying rises and falls in urine pH, respectively.

The maintenance of a constant intracellular concentration requires either that formation of ammonia occur by a reversible enzymatic process or that ammonia formed in the course of an irreversible reaction be channeled into both the urine and a metabolic pool. Intracellular concentration would not change appreciably if ammonia were produced by a reversible reaction since under these conditions end product concentration is a determinant of the reaction rate. The second possibility that diffusion of NH_3 into the urine (and blood) and cellular utilization are, in a sense, competitive processes is not unreasonable. If such a mechanism were involved, when less NH_3 entered the urine, as at high pH, an increase in the rate of utilization by the second process would tend to stabilize the intracellular concentration. In fact, the alternative, the production of ammonia by a reversible reaction, constantly at or near equilibrium, is not consonant with the role of enzyme adaptation in increasing ammonia excretion at all urine pH's (1–3). At the equilibrium of a reversible process the ammonia concentration in cells would be unaffected by changes in enzyme concentration since enzymes speed the attainment of equilibrium but do not affect the concentrations of the various reactants at equilibrium. Either alternative mechanism of concentration stabilization would account for ammonia excretion when the urine is sufficiently acid since the rapid loss from cells to urine would lead to production at a maximal rate and minimal utilization in alternative pathways.

The effect of the administration of amino acids is presumably an augmentation of the rate of production and an increase in the intracellular concentration of ammonia. The present amino acid experiments do not, in themselves, permit a conclusion as to the mechanism by which the ammonia enters the urine. The observed rise in pH could be attributed to the titration of hydrogen ion by the free ammonia diffusing into the urine. Alternatively, it is recognized that these changes could also be due to a direct exchange of NH_4^+ for Na^+ if the exchanging NH_4^+ were substituted for an equivalent amount of exchanging hydrogen

[8] Although, in a tubule permeable to CO_2, the urine pH will be affected by the process of concentration (27), a ten-fold diminution in volume will change the pH by as much as one unit if the urine is completely unbuffered. It is unlikely that this degree of concentration occurred in these experiments and buffer is never completely absent from the urine.

ion.[9] In any event, the fundamental physiologic role ascribed to ammonia excretion, fixed base conservation, was not evident in these experiments. For the most part, there was little difference between the increase in ammonia excretion and the change in the excretion of bicarbonate minus the change in titratable acid. Under physiologic conditions, the excretion of ammonia presumably serves to promote the reabsorption of sodium in exchange for hydrogen by minimizing the pH gradient between cells and urine (9). The reaction of NH_3 and H^+ to form NH_4^+ in the tubule urine is thought to facilitate further secretion of hydrogen ions in exchange for sodium. In such experiments as those of Lotspeich and Pitts (5) in which no change in urine pH or titratable acid accompanied the increase in ammonia excretion when amino acids were infused, such a mechanism may be considered to have been operating. The reason for the difference between the results of Lotspeich and Pitts (5) and those of the present experiments with respect to urine pH is not clear. In any case, no base conservation can result if ammonium ion merely replaces hydrogen ion in the urine. The mechanisms for hydrogen ion and ammonia secretion must be coextensive and the entry of ammonia permit the further secretion of hydrogen ion.

SUMMARY AND CONCLUSIONS

Experiments have been performed to investigate the mechanism of excretion of ammonia and other weak bases in the dog.

The excretion of these substances (ammonia, quinine, Atabrine®, SN 8439 and SN 5228) diminishes as urine pH rises when the change is effected by the infusion of $NaHCO_3$. Alkalinization of the urine by the injection of potassium chloride or acetazoleamide results in a similar diminution in ammonia and quinine excretion. The effect on the excretion of SN 8439 is less marked.

The accumulation of organic bases in the urine is apparently dependent upon the differential permeability of the tubule cell membrane to the lipid soluble and insoluble members of the ion pairs. These substances diffuse across the membrane as the free base (or singly charged member in the case of Atabrine®, SN 8439 and SN 5228) and accumulate in the urine as the less permeating lipid insoluble species. In the acid range of urine pH accumulation is limited by the maximal rate of intracellular production in the case of ammonia and by the maximal rate of diffusion out of cells in the case of the exogenous bases. Consequently, alterations in urine flow do not affect the rate of excretion. In more alkaline urines, however, urine flow influences excretion rate so that diffusion of the bases between cells and tubule urine presumably approaches equilibrium. In either instance, the relationship between urine pH and rate of excretion rather than concentration of base indicates that final abstraction of water occurs at a site distal to that at which weak electrolyte content is determined.

Amino acids augment ammonia excretion by enhancing the rate of production of ammonia and by elevating the intracellular concentration. It has not been possible to distinguish between the various mechanisms which could account for the rise in urine pH noted in these experiments. In any event, base conservation was not effected, since in general the rise in ammonia excretion did not appreciably exceed the sum of the fall in titratable acid plus the rise in bicarbonate excretion.

It is concluded that the excretion of ammonia and weak bases is not ascribable to active transport of these substances into the urine but to passive diffusion conditioned by the acidification of the urine.

[9] All agents known to inhibit hydrogen ion secretion which have been studied have been found to increase potassium excretion (28). The fact that potassium excretion did not increase when urine pH rose as a result of amino acid administration is in accord with the view that the rise in urine pH may not be attributable to inhibited acidification.

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