Biochimica et Biophysica Acta, 311 (1973) 98–108

BBA 76345

CHARACTERISTICS OF H^+ AND NH_4^+ EXCRETION BY THE URINARY BLADDER OF THE TOAD

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(Received December 27th, 1972)

SUMMARY

1. This paper deals with some of the characteristics of H^+ and NH_4^+ excretion in the toad urinary bladder. The evidence that H^+ and NH_4^+ are excreted by the urinary bladder has been previously published.

2. Studies were performed to investigate the characteristics of H^+ and NH_4^+ excretion in the toad urinary bladder.

3. Studies using anaerobic conditions as well as studies using CN^- demonstrate that H^+ excretion is partially dependent on aerobic metabolism but can proceed at reduced rates under anaerobic conditions.

4. Both H^+ and NH_4^+ excretion appear to have some relationship to mucosal Na^+. However, the exact nature of this relationship is not clear from this study.

5. Neither H^+ or NH_4^+ excretion are affected by the absence of Cl^- in the bathing media.

INTRODUCTION

Previous studies from our laboratory have shown that the urinary bladder of *Bufo marinus* can acidify the urine and produce and excrete NH_4^+ (ref. 1). The acidification process has also been shown to occur *via* H^+ excretion as opposed to HCO_3^- reabsorption[3]. In addition, H^+ excretion has been shown to occur against an electrochemical gradient[1]. This meets one criteria for active transport. In this paper we deal with the effects of metabolic inhibitors on H^+ transport. Inhibition by a metabolic inhibitor is another criteria for active transport.

The purpose of the present study was to characterize further the H^+ and NH_4^+ excretory mechanisms. Also, to determine if the active excretion of H^+ is linked to metabolic energy production or the active transport of another ion. The present findings indicated that one component of the H^+ excretory system may be directly linked to Na^+ reabsorption and that another component is independent of Na^+ reabsorption and dependent on metabolic energy. The findings on NH_4^+ excretion are not as clear cut as are those dealing with H^+ excretion.

MATERIALS AND METHODS

General

The source and the routine care of toads, instrumentation, the procedure for inducing acidosis and the methods of measuring H^+ and NH_4^+ excretion were as previously described[1–3]. Heparinized plasma was obtained by cardiac puncture from pithed toads. Bladders from toads in metabolic acidosis were used in all studies reported here.

Toads were sacrificed by pithing. The hemibladders were removed and mounted between lucite chambers, each of which held 2 ml of the indicated bath. The exposed surface area of each bladder was 3 cm^2. A rubber "O" ring was placed between the chambers to minimize edge damage. The bladders were allowed to equilibrate for 15 min. The mucosal chamber was then drained and washed twice with the indicated Ringer solution. The flux period was begun at the end of the second draining and the appropriate solution placed in the mucosal chamber. The flux period was for 120 min unless otherwise noted. Humidified 100% O_2 was bubbled into the mucosal medium throughout each experiment except where noted otherwise. At the end of the flux period both wet and dry weight were obtained on the bladders. The H_2O content of each bladder was calculated. This content was within normal limits in all bladders, therefore, wet weight was used as the basis for the normalized flux. The pH and NH_4^+ were determined on both the mucosal and serosal samples. In all experiments where pooled plasma from acidotic toads was used as the serosal medium the plasma was frozen and then thawed before used to reduce CO_2 in solution to near zero. The HCO_3^- concentration was not changed by this procedure.

The Na^+ analyses were performed on a Beckman model B spectrophotometer with a flame attachment. Chloride was determined by iodometric titration[5], modified so that it was capable of detecting 0.5 mM Cl^-. NH_4^+ was determined colorimetrically[6] and read on a Bausch and Lomb spectrophotometer. Na^+ fluxes were measured using ^{22}Na supplied by ICN Corp. of Irvine, Calif. Gamma counting was done on a Tracerlab scaler-spectrometer attached to a well-type scintillation detector. Standard counting techniques, and calculations were used in calculating the Na^+ flux. Counts were to statistical accuracy of 1%.

Calculations

The H^+ was calculated using a pK_a for the phosphate buffer pair of 6.50. This value was obtained both empirically in Ringer solution and by calculation to correct for the ionic strength[4]. A typical result is given below.

The initial pH of the mucosal media was 6.80 as stated. In the two series of experiments with Mg and K substitution in the mucosal media, the pH fell during the flux period to a final value between 6.27 and 6.60 in the series. A typical analysis is taken from one bladder in which Mg was substituted (one of the bladders making up the series reported in Table IV). This bladder weighed 19.02 mg, and the pH change of the mucosal solution was from 6.80 to 6.38 over the 120-min period. With 2 ml of 1.5 mM phosphate buffer as the mucosal media, there was a total of 3.0 μmoles of phosphate buffer present. At pH 6.80, using the Henderson–Hasselbalch equation we find that there is 1.00 μmoles of $H_2PO_4^-$, and 2.0 μmoles of HPO_4^{2-}. At the pH of 6.38 at the end of the flux period, the quantities are again calculated and we find

that there is now 1.71 μmoles of $H_2PO_4^-$, and 1.29 μmoles of HPO_4^{2-}. This indicates that 0.71 μmole of H^+ has been added to the system. This bladder in the chamber weighed 19.02 mg. Calculating then: (0.71 μmole H^+ per bladder per 120 min)·(100 mg/19.02 mg) (120 min) = 0.0311 μmole per 100 mg bladder per min, or 31.1 nmoles/100 mg bladder per min.

Solutions

All Ringer solutions involved are described as modifications of a Ringer solution used as the mucosal media designated, 1.5 mM phosphate Ringer solution. This solution contained in mM: NaCl, 114.0; KCl, 3.0; $CaCl_2$, 0.9; with sodium phosphate buffer, pH 6.80, 1.5 mM. The 0.6 mM phosphate Ringer solutions contained the reduced concentration of the phosphate buffer, pH 6.80, and had NaCl increased to 114.9 mM.

The 1.5 mM phosphate Ringer solution with glutamine, used as a serosal medium, was modified in that the pH was 7.00 and 0.6 mM glutamine was added. Choline Ringer, K^+-Ringer and Mg^{2+}-Ringer solutions contained equivalent amounts of the appropriate salts as substitutes for the NaCl. They each contained 1.5 mM potassium phosphate buffer, pH 6.80. Sucrose was added to the Mg^{2+}-Ringer solution to make it isotonic.

The 1.0, 4.0, 10.0, and 18.0 mM Na^+-Ringer solutions contained the stated concentration of Na^+ as NaCl, with sufficient choline chloride to make the sum of choline and NaCl concentrations equal to 114.5 mM. There was 0.6 mM potassium phosphate buffer, pH 6.80, in each solution, and KCl and $CaCl_2$ in the quantities indicated above. The 25 mM bicarbonate Ringer solution contained in mM: NaCl, 89.5; KCl, 3.0; $CaCl_2$, 0.9; $NaHCO_3$, 25.0 and glutamine, 0.6. The solution was equilibrated with 5% CO_2 and the resulting pH was 7.20.

The 0.6 mM phosphate–sulfate Ringer solution, and the 1.5 mM phosphate–sulfate Ringer solution with glutamine were pH 6.80 and 7.00, respectively. Each contained equivalent amounts of the appropriate sulfate salts as substitutes for the NaCl, KCl, and $CaCl_2$ salts. Sucrose was added to each to make it isotonic. The 1 mM Mg^{2+}-Ringer solution contained in mM: NaCl, 112; KCl, 3.0; $CaCl_2$, 0.9; $MgCl_2$, 1.0; sucrose, 1 mM and 1.5 mM potassium phosphate buffer, pH 6.80.

In the cyanide experiments, 10^{-3} M NaCN was added to the serosal media. This resulted in a change in the pH of the serosal medium to 8.10. In the amiloride experiments, 10^{-5} M amiloride was added to the mucosal media. Amiloride was supplied by Merck Sharp and Dohme Research Laboratories, West Point, Pa.

Experimental and control fluxes

Paired hemibladders were used for control and experimental observations in the cyanide, amiloride and the cationic substitution experiments. The flux observed on each hemibladder was normalized for the wet weight of the bladder and expressed in nmoles/100 mg bladder per min. The difference in the normalized fluxes of the pair of hemibladders (experimental–control) was determined, and the mean difference calculated for each series. There were 10 pairs in each series unless otherwise stated. In the experiments with varying Na^+ concentration, hemibladders were paired as indicated.

In the anaerobic experiments and the sulfate experiments, each hemibladder

served as its own control, with three fluxes being determined on each bladder. These, in order, were control, experimental, and control fluxes. The difference between control and experimental fluxes and the mean difference for each series was determined as described above. There were 10 hemibladders in each series.

The details as to which solution was used as the serosal and mucosal medium is given in the heading of the tables reporting the results of the respective experiments. The chambers contained 2 ml of media on each side of the bladder.

In the experiment using sulfate media, Cl^--free media was obtained by washing each chamber three times with the respective sulfate media over a 30-min period between the first control and the experimental flux. At the end of this washing period the chamber was drained, and fresh Cl^--free sulfate media was placed in each chamber. At the end of the 60-min flux period the chambers were drained. A Cl^- determination was made on aliquots of both the mucosal and the serosal media obtained at the end of the flux period.

In the experiments with reduced O_2 tension, the plasma and mucosal bath were washed free of dissolved O_2 by overlaying with nitrogen, and shaking gently, and repeating this at least three times. The mucosal media was then bubbled with nitrogen (minimum purity, 99.96%). However, this gas passed through rubber tubing so we have designated the atmosphere as low partial pressure of O_2 because the tubing is not completely impermeable to gases.

RESULTS

Cyanide

It can be seen from the results in Table I that CN^- inhibits H^+ excretion, both in the presence of 5% CO_2 and in the presence of only endogenous CO_2. The

TABLE I

EFFECT OF CN^- ON THE EXCRETION OF H^+ AND NH_4^+ BY THE TOAD URINARY BLADDER

Excretion is in nmoles/100 mg bladder wet wt per min. The mucosal medium in all experiments was 1.5 mM phosphate Ringer solution. In the endogenous CO_2 experiments, the serosal medium was 1.5 mM phosphate Ringer solution with glutamine. In the 5% CO_2 experiments the serosal medium was 25.0 mM $NaHCO_3$ Ringer solution with glutamine bubbled with 5% CO_2. $n = 10$ in each series.

Procedure	*H^+ extretion*	*H^+ mean difference**	*NH_4^+ excretion*	*NH_4^+ mean difference**
A. Control	30.26		4.18	
		-20.33 ± 4.83 ($P < 0.005$)		-1.47 ± 0.54 ($P < 0.01$)
CN^- 10^{-3} M	9.93		2.71	
B. Control 5% CO_2	46.15		2.14	
		-42.86 ± 5.46 ($P < 0.005$)		$+0.68 \pm 0.28$ ($P < 0.05$)
CN^- 10^{-3} M 5% CO_2	3.27		2.82	

* $\pm$ S.E. of the mean.

3.27 nmoles/100 mg wet wt per min H^+ excretion with CN^- and with 5% CO_2 aerating the serosal medium could be due to CO_2 diffusing across the bladder to react with the phosphate in the mucosal medium. This then would be the maximum rate for this process, and the stimulation of H^+ transport as evidenced by the difference in the control of the A series and the control of the B series is not likely due to CO_2 diffusion alone. Since these two control series do not represent paired hemibladders, a fine interpretation of the difference is not justified.

NH_4^+ excretion was inhibited by CN^- in phosphate media, but not inhibited in bicarbonate media. The explanation of this difference in response is not apparent.

Amiloride

In Table II the results of the experiments with amiloride are reported. Amiloride produced a 20% inhibition of hydrogen excretion, but had no effect on NH_4^+ excretion.

Low O_2 tension

In Table III are reported the results of studies at low partial pressures of oxygen. Low oxygen produced about a 45% reduction in H^+ excretion, but had no effect on NH_4^+ excretion. The persistence of some H^+ excretion under conditions of low oxygen tension are explained either by an oxidative pathway functioning at very low partial O_2 pressures or by energy derived from glycolytic pathways. On the other hand the persistence of some H^+ excretion in the presence of 10^{-3} M CN^- suggest that H^+ excretion can be carried out by energy derived from glycolytic pathways.

Sulfate experiments

The experiments using sulfate media to obtain a Cl^--free media are reported in Table III. These results clearly indicate that Cl^- in either the serosal or mucosal

TABLE II

EFFECT OF AMILORIDE ON THE EXCRETION OF H^+ AND NH_4^+ BY THE TOAD URINARY BLADDER

Excretion is in nmoles/100 mg bladder wet wt per min. Serosal medium was 1.5 mM phosphate Ringer solution with glutamine. Control mucosal medium was 0.6 mM phosphate Ringer solution. Amiloride was added to the mucosal medium of the experimental group. $n=10$ in each series.

Procedure	*H^+ excretion*	*H^+ mean difference**	*NH_4^+ excretion*	*NH_4^+ mean difference**
Control	18.10		1.25	
		-4.13 ± 1.37 ($P<0.01$)		0.01 ± 0.10 ($P>0.90$)
Amiloride 10^{-5} M	14.30		1.24	

* $\pm$ S.E. of the mean.

TABLE III

STUDIES OF H^+ AND NH_4^+ EXCRETION USING HEMIBLADDERS AS THEIR OWN CONTROLS

Series A, effect of low O_2 tensions. Series B, effect of Cl^--free mucosal and serosal media. Excretion is in nmoles/100 mg bladder wet w per min. In Series A, the mucosal medium was 0.6 mM phosphate Ringer solution, and the serosal medium was pooled plasma from acidotic toads. In the period of low O_2 tension the media were aerated with nitrogen as described in the text before placing on the bladder. The mucosal media were bubbled with virtually O_2-free N_2 during the flux period. In Series B the control mucosal medium was 0.6 mM phosphate Ringer solution and the serosal medium was 1.5 mM phosphate Ringer solution with glutamine. For the Cl^--free media, the mucosal medium was 0.6 mM phosphate–sulfate Ringer solution and the serosal medium was 1.5 mM phosphate–sulfate Ringer solution with glutamine. $n = 10$ for each series.

Procedure	*H^+ excretion*	*H^+ mean difference**	*NH_4^+ excretion*	*NH_4^+ mean difference**
A. Control	27.35		4.85	
		-11.20 ± 2.29 ($P < 0.005$)		-0.27 ± 0.33 ($P > 0.20$)
Low O_2	16.15		4.58	
		-12.44 ± 2.64 ($P < 0.005$)		$+0.08 \pm 0.58$ ($P > 0.02$)
Control	28.59		4.50	
B. Control Cl^-	14.96		1.51	
		-2.46 ± 1.70 ($P > 0.05$)		-0.37 ± 0.34 ($P > 0.15$)
SO_4^{2-}	12.50		1.14	
		-1.53 ± 1.50 ($P > 0.15$)		-0.42 ± 0.24 ($P > 0.05$)
Control Cl^-	14.03		1.56	

* $\pm$S.E. of the mean.

media is not essential for either H^+ or NH_4^+ excretion. The Cl^- determination on each mucosal and serosal sample was zero using a method capable of detecting 0.5 mM Cl^-.

Mucosal cationic substitution

The results of the experiments in which choline, K^+, and Mg^{2+} were substituted for Na^+ in the mucosal media are reported in Table IV. There was about 75% reduction in H^+ transport produced by the choline Ringer solution, and a 40% reduction by the K^+-Ringer solution. The Mg^{2+}-Ringer solution actually stimulated H^+ excretion. The effect on NH_4^+ excretion is not in the same direction as the effect on H^+ excretion in each experiment.

Sodium was determined on an aliquot of each mucosal media at the end of the flux period. The sodium concentration was usually less than 1 mM, and was less than 2 mM in all experiments. This sodium represents serosal to mucosal leak during

TABLE IV

EFFECT OF CATIONIC SUBSTITUTIONS IN MUCOSAL MEDIA ON H^+ AND NH_4^+ EXCRETION BY THE TOAD URINARY BLADDER

Excretion is given in nmoles/100 mg bladder wet wt per min. The serosal medium was pooled plasma from metabolic acidotic toads in Series A–C. The serosal medium was 25 mM $NaHCO_3$ Ringer solution bubbled with 5% CO_2 in Series D. The control mucosal media was 1.5 mM phosphate Ringer solution. The choline, K^+-, or Mg^{2+}-Ringer solutions were the mucosal media on the indicated experiments. $n = 10$ for each series.

Series	*H^+ excretion*	*H^+ mean difference**	*NH_4^+ excretion*	*NH_4^+ mean difference**
A. Na^+	9.00		5.43	
		-6.60 ± 1.66 ($P < 0.005$)		-2.29 ± 0.94 ($P < 0.05$)
Choline	2.40		3.14	
B. Na^+	19.52		4.11	
		-7.75 ± 2.58 ($P < 0.01$)		-0.39 ± 0.55 ($P > 0.50$)
K^+	11.77		3.72	
C. Na^+	16.95		4.06	
		10.99 ± 3.53 ($P < 0.01$)		-1.44 ± 0.60 ($P < 0.05$)
Mg^{2+}	27.94		2.62	
D. Na^+ 5% CO_2	60.66		2.46	
		-12.60 ± 2.47 ($P < 0.005$)		0.11 ± 0.41 ($P > 0.80$)
Choline 5% CO_2	48.06		2.57	

* $\pm$S.E. of the mean.

the flux period, with possibly some contribution from sodium coming from the bladder wall.

Because Mg^{2+}-Ringer solution stimulated H^+ transport, and we did not have Mg^{2+} in any of our Ringer solutions, we decided to try the effect of a more physiological concentration of Mg^{2+} by using 1 mM Mg^{2+}-Ringer solution. It was recognized that those experiments in which plasma had been used, there was some Mg^{2+} present in the serosal medium, but still it seemed desirable to test the effect of small amounts of this ion on each side of the bladder. The fluxes in nmoles/100 mg bladder per min were for H^+ 15.17 in Mg^{2+}-free medium, 13.14 in 1 mM Mg^{2+} medium; and for NH_4^+, 3.16 and 3.47, respectively. The mean differences of 2.03 ± 4.54 for H^+ excretion, and 0.31 ± 0.70 for NH_4^+ excretion indicates that there is no effect of the 1 mM Mg^{2+} on the flux of either of these ion species.

Variation in Na^+ concentration, mucosal media

The results of these experiments are shown in Fig. 1. The hemibladders used

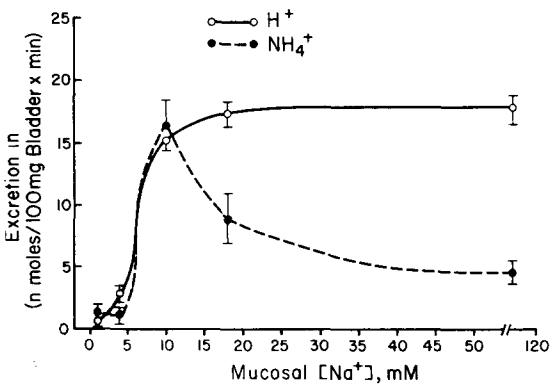

Fig. 1. Graph showing the effect of varying Na^+ concentration by choline substitution on the H^+ and NH_4^+ excretion. The serosal medium in each case was 2.0 ml of pooled plasma from metabolic acidotic toads. The mucosal medium was 2.0 ml of ^{22}Na-labeled Na^+-Ringer solution with the indicated amount of Na^+. 1.0 mM and 4.0 mM Na^+ were run as paired hemibladders. Likewise 10.0 mM and 18.0 mM Na^+ were run as paired hemibladders. The experiments at 117.0 mM Na^+ were not run as paired hemibladders. Ten experiments were performed at each Na^+ concentration. The vertical bar represents 1 S.E. The average mucosal to serosal Na^+ flux $\pm$ 1 S.E. in nmoles/100 mg bladder wet wt per min at the various concentrations were as follows: 1 mM, 47.0 ± 7.83; 4 mM, 114.0 ± 10.7; 10 mM, 109.0 ± 14.1; 18 mM, 127.0 ± 19.9; 117 mM, 134.0 ± 25.3.

for 1, 4, 10, and 18 mM Na^+ concentrations were paired as indicated and were from the same shipment of toads. The bladders used for the 117 mM Na^+ concentration experiment were from a different shipment of toads and were done three months later. It is noted that the 1 mM Na^+ concentration in this series gave a greater inhibition of H^+ transport than the choline Ringer solution gave in the series reported in Table IV. The cause for this difference in results is not apparent.

DISCUSSION

Our experiments with either CN^- or low O_2 tensions demonstrate that the H^+ excretion is linked to aerobic metabolism of the cell. This lends further support to our hypothesis that H^+ excretion in the toad bladder involves an active transport system. As mentioned previously in the CN^- experiments the presence of 5% CO_2 atmosphere gave an extremely low H^+ excretion. This acidification could be regarded as the amount due to diffusion of CO_2 from a high serosal medium into the mucosal medium and subsequent hydration. As can be seen this could account for only about 7% of the total H^+ excretion observed in the paired hemibladders that had received no CN^-.

It might be argued that lactic acid production might account for some part of the acidification observed in the mucosal medium. In 1959 Leaf *et al.*[7] made a study of sodium transport and respiration in the urinary bladder of the toad. They found that during anaerobiosis lactic acid production was increased about 7-fold over the amount produced during aerobic metabolism in bladders from normal toads. They were further able to show that the lactate ion distributed asymmetrically across the bladder and accounted for the acidification observed in both the

mucosal and serosal media during anaerobiosis. We considered that the bladder from acidiotic toads might be different than from normal toads, and might use lactic acid to acidify the urine. However, we concluded that this was not the case since there is increased lactic acid production during anaerobiosis, but there was not an increased acidification.

Finn *et al.*[8] have demonstrated active transport of Cl^- from the mucosal to serosal side in the toad urinary bladder. Likewise, Gonzalez *et al.*[9] have demonstrated the same transport system in the turtle bladder. It is possible that Cl^- transport might be coupled in some way to that of H^+ transport. The demonstration that H^+ and NH_4^+ excretion both continue despite a virtual absence of Cl^- in the system is strong evidence against such coupled transport.

The relationship between mucosal Na^+ concentration and H^+ excretion is not clear. If one focuses on the data in which choline is substituted for Na^+, and the Na^+ concentration is varied, one concludes that there is an interrelationship between the two. The most likely explanation would be the effect of mucosal Na^+ concentration on Na^+ reabsorption, and a linkage of Na^+ reabsorption with H^+ excretion. When one looks at the results using a Na^+-free K^+-Ringer solution as the mucosal medium, we see that H^+ excretion is inhibited, but on the other hand the rate of excretion is still about 60% of the control value.

Similarly, amiloride inhibits H^+ excretion. Since amiloride blocks the entry of Na^+ into the epithelial cell[10] and thus inhibits the Na^+ pump, this suggests a linkage between Na^+ reabsorption and H^+ excretion. Looking at the H^+ excretion that remains in the presence of amiloride, though, shows that 80% of the H^+ excretion is not blocked. This 80% excretion is then either not associated with Na^+ reabsorption, or else is associated with reabsorption which is not blocked by amiloride.

If one looks at the Na^+-free Mg^{2+}-Ringer solution, one finds a stimulation of H^+ excretion. This would indicate that H^+ excretion is not dependent on Na^+ reabsorption.

Certainly more work is indicated in this area. The following statements seem consistent with the data presently available. (1) If H^+ excretion is linked with Na^+ reabsorption in the toad bladder, this mechanism must account for only 20–40% of the H^+ excretion in the acid-loaded toad, and some other mechanism must account for the remainder. (2) The combination of choline substitution and low Na^+ concentration in the mucosal medium gives greater inhibition of H^+ excretion than does low Na^+ concentration of the mucosal medium achieved by other means.

The reason that NH_4^+ excretion peaked at 10 mM Na^+ concentration is interesting. This finding might be of significance in that the sodium concentration of urine is usually low, thereby allowing the NH_4^+ excretory system to operate at peak capacity. At this Na^+ concentration NH_4^+ would appear to be following the pattern of H^+ excretion. However, as the mucosal Na^+ was increased the NH_4^+ excretion fell. The reason for this is not at all clear. It has been shown that high concentrations of NH_4^+ salts in the mucosal fluid will inhibit Na^+ transport in the toad bladder[11]. It seems possible then that there might be a reciprocal relationship between NH_4^+ and Na^+; high mucosal Na^+ inhibiting Na^+ excretion. Our findings leave open a very interesting area, the relationship of mucosal Na^+ to the excretion of H^+ and NH_4^+ for future investigations.

There was a wide variability observed between control groups with regards to H^+ excretion. It has been observed throughout this study that there was a variation in control groups in both H^+ and NH_4^+ excretion. Various factors could be contributing to this variation. It is well known that animals collected in the field show more variation than inbred laboratory strains. In addition, amphibia are known to show marked seasonal variation. Also, the geographical source of the toad must be considered.

Davis *et al.*[12] have observed that bladders of *Bufo marinus* obtained from different geographical areas show different effects of aldosterone application on the Na^+ transport system; hence, geographical area could be a contributing factor to the variability observed between control groups.

We considered the possibility that the acidification might be due to microorganisms on the bladder. This seems unlikely for the following reasons: (1) The mucosal side of the bladder was washed with fresh Ringer solution twice before each flux determination; (2) in a previous study[1] we reported that bladders from non-acidotic toads excreted H^+ at a rate which was only 10% of that of bladders from acidotic toads; (3) in another study[3] we reported that acetazolamide will produce a 50% inhibition of H^+ excretion in the urinary bladder; (4) and 2 h is a very short time for a culture of organisms to show much metabolic activity at room temperature in a Ringer solution initially containing no glucose or other common substrates.

In considering similarly whether microorganisms from the mucosal surface of the bladder might contribute to NH_4^+ production, we find that arguments 1 and 4 given in the paragraph above apply. In addition, it is pointed out that the effect of changes in Na^+ concentrations on NH_4^+ production reported in Fig. 1 of this paper are not consistent with NH_4^+ production by microorganisms.

In summary, our data support the concept that H^+ excretion is an active transport system. The transport system is partly dependent on aerobic metabolism but can proceed at slower rates under conditions of low O_2 tension. There is no evidence of coupling between Cl^- transport and H^+ or NH_4^+ excretion. Mucosal Na^+ is important for 20–40% of H^+ excretion. However, the exact role of mucosal Na^+ is not clear at this time. The results obtained with regard to the mechanism of NH_4^+ excretion are not as clear cut as with the H^+ excretion.

ACKNOWLEDGEMENTS

The authors wish to thank Mr Robert C. Lipsey for his technical assistance during this study. This work was supported by a National Science Foundation Grant GB-17610.

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