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Characteristics of proton excretion in normal and acidotic toad urinary bladder

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This study, performed on the urinary bladder of *Bufo marinus*, was to investigate the characteristics of H^+ excretion in the normal and metabolic acidotic toad. Experiments were run in modified Ussing chambers in the presence and absence of exogenous CO_2. Amiloride in the mucosal medium ($1.5 \cdot 10^{-4}$ M) inhibited H^+ excretion in the acidotic bladder but this inhibition was reversed in the presence of 5% CO_2. Na^+-free muscosal medium revealed a component of H^+ excretion in the normal toad that is Na^+-dependent and not reversed by 5% CO_2. Acetazolamide (10^{-3} M) had no effect on H^+ excretion in normal toad but inhibits excretion in the acidotic toad both in the presence and absence of exogenous CO_2. In both the presence and absence of exogenous CO_2 and in the presence of varying pH gradients, the bladders from acidotic toads excreted H^+ at a greater rate and were able to generate greater pH gradients than in normal bladders. Voltage clamp experiments revealed that H^+ excretion in the acidotic toad was inhibited by a mucosal potential difference of -20 to -100 mV but this inhibition was completely reversed by 5% CO_2. In the normal toad bladder H^+ excretion was stimulated by a mucosal potential difference of -20 to -100 mV in both the presence and absence of exogenous CO_2. Our evidence suggests the possibility of two H^+ excretory mechanisms in toad urinary bladder each with its' own set of characteristics dependent on the acid-base state of the animal. Proposed models are presented for these mechanisms.

The urinary bladder of the toad *Bufo marinus* has been used as a model for studying sodium and water transport in the mammalian renal distal tubule. In addition, the urinary bladder of *B. marinus* excretes hydrogen ion in both in vivo and in vitro preparations [1]. This H^+ excretion has been shown to be an active, electrogenic process located on the apical membrane [2–4]. In studies from this laboratory we have shown that the acidification rate is decreased by amiloride and Na^+ substitution in the mucosal medium, when the animal was in metabolic acidosis [5]. Additionally, in bladders from acidotic animals, H^+ excretion was inhibited by acetazolamide when only endogenous CO_2 was present but acetazolamide had no effect when the serosal media contained 5% CO_2/HCO_3^- buffer [2]. On the other hand, Ludens and Fanestil [3] measured the acidification rate by reverse short-circuit current in urinary bladders from normal, non-acidotic *B. marinus*. Their 'acidification current' was found to be dependent on amiloride, not altered by removal of Na^+ from the mucosal medium and inhibited by acetazolamide even in the presence of 5% CO_2/HCO_3^- buffer [3,6].

In a report, Ziegler et al. [7] demonstrated that acidification in the toad urinary bladder was a linear function of the potential difference. In the same study they also demonstrated that the rate of acidification could be increased by a favorable potential difference no matter if the potential difference was the spontaneous transepithelial potential difference due to Na^+ transport or a potential difference imposed on the bladder from an exter-

nal source. On the other hand, previous results from this laboratory [1] have shown that by removing the favorable spontaneous potential difference across the bladder with short-circuit current actually increased the acidification rate slightly.

The present study was done to characterize further the mechanism of proton excretion in the toad urinary bladder in both normal toads and toads in metabolic acidosis. Additionally, we examined the effect of transepithelial potential difference on urinary acidification in both the normal and acidotic toad. The results indicate that H^+ excretion in the urinary bladder from normal and acidotic toads are different and each has its' own unique characteristics. We propose two models for proton excretion in the toad urinary bladder based upon these characteristics.

Materials and Methods

Hemibladders from toads (*B. marinus*) of Mexican origin were used in all experiments. Two different groups of toads were used: (1) those in normal acid-base balance, and (2) those in metabolic acidosis. Metabolic acidosis was produced by gavaging with 10 ml of a 120 mM NH_4Cl solution three times daily over a 48-h period. The toads were supplied by Carolina Biological Supply of Burlington, NC. In all experiments except the pH stat experiments, the H^+ excretion was calculated from the change in pH and the concentration of buffer in the mucosal solution. H^+ excretion was then normalized for wet weight of the bladder and time and reported in units of $nmol \cdot (100\ mg\ bladder\ wet\ wt.)^{-1} \cdot min^{-1}$. A Radiometer Model PHM74 digital pH meter was used for all pH determinations. The standard phosphate-buffered Ringer's solution used in most experiments contained in mM: NaCl, 114.5; KCl, 3.0; CaCl, 0.9; and sodium phosphate 1.5; the final pH was adjusted to 6.80–7.00 by titrating the phosphate buffer with 0.12 M HCl or 0.12 M NaOH.

Hemibladders were removed from double-pithed toads, rinsed in the Ringer's solution and mounted between Lucite chambers, each of which held 2 ml of the indicated solution. In all experiments, the bladders were in the open-circuit state except in the experiments in which we voltage-clamped the bladders to study the effect of potential difference on H^+ excretion. The flux periods were always begun 15–30 min after the bladder had been mounted on the chambers to allow for equilibration. The flux period was either for 60 or 120 min. Routine care of the toads as well as details of all the above methods have been described previously from this laboratory [1,2,5].

Experiments with pH stat technique. Toads were killed by pithing and the bladder removed. One hemibladder was mounted as a sheet between two modified Ussing chambers, each of which held 2 ml of bath. The other hemibladder was mounted as a sac around a cannulae that contained a pH electrode and a pipette for adding 0.001 M NaOH. The bathing solution on both the mucosal and serosal surface was a PO_4-buffered Ringer's solution, pH 7.00. The H^+ excretion was measured in the Ussing chambers from change in pH of the mucosal solution following a 2-h flux period. The mucosal fluid in the sac preparations was maintained at 7.00 by addition of 0.001 M NaOH during the same 2-h period. This was done using a Radiometer pH stat assembly. The amount of H^+ excreted was calculated from the amount of NaOH added. The values were normalized for bladder weight and reported in units of $nmol \cdot (100\ mg\ bladder)^{-1} \cdot min^{-1}$.

Experiments with amiloride, acetazolamide, and Na^+-free media. Paired hemibladders were used in all experiments and mounted in the modified Ussing chambers. The Ringer's solution was as given above except in the case where 5% CO_2/HCO_3^- Ringer was used in the serosal medium. This CO_2/HCO_3^- Ringer solution was the same except $NaHCO_3^-$, 24 mM, was substituted for an equal quantity of NaCl and the solution equilibrated and gassed continuously with 5% CO_2/95% O_2. One hemibladder served as the control and the other hemibladder received the indicated treatment. One group received amiloride ($1.5 \cdot 10^{-4}$ M) in the mucosal bath, another group received acetazolamide (10^{-3} M) in the serosal bath, and the third group contained Na^+-free (choline chloride) bath on the mucosal side. This concentration of amiloride has been shown by Bently [8], Ziegler et al. [7], and Ludens and Fanestil [3] to be adequate to block Na^+ transport across this epithelia. In the experiments free of exogenous CO_2, the serosal medium was bubbled continuously

throughout the experiment with 100% O_2. The flux period in all experiments was 2 h in duration. The H^+ excretion was calculated from change in pH of the mucosal media. Statistics were performed as the paired *t*-test on each group of paired hemibladders.

Experiments at varying initial mucosal pH. Bladders from normal and acidotic toads were used and mounted on the 2-ml chambers. All flux periods were 2h in duration. At the end of that time, the mucosal and serosal samples were collected and pH was determined. H^+ excretion was calculated from the change in pH of the mucosal medium. The first experiments were carried out in the absence of exogenous CO_2. Phosphate-buffered Ringer solution was in both the serosal and mucosal chambers. The initial serosal pH was 7.0 in all experiments. The initial mucosal pH was adjusted and varied from 6.0 to 8.0.

In the second group of experiments, the same protocol as above was used except, (1) the serosal medium was gassed throughout the experiment with 5% CO_2 and contained 24 mM $NaHCO_3^-$, (2) the initial serosal pH was 7.40, and (3) the initial mucosal pH was varied from pH 5.5 to pH 8.0.

Experiments with potential difference clamped at various voltages. Hemibladders were removed and mounted between modified Ussing chambers each of which held 2 ml of the indicated solution. The mucosal solution in all experiments was a phosphate-buffered Ringer solution indicated above; the final pH was 7.0. In two sets of experiments (normal and acidotic toads), the serosal solution was the same phosphate-buffered Ringer solution and was bubbled throughout the experiment with 100% O_2. These experiments were designated as 'No exogenous CO_2'. Two other groups of experiments (normal and acidotic toads) were run in which the serosal solution contained 24 mM $NaHCO_3^-$ substituted in equimolar amounts for NaCl. The serosal solution in these experiments was gassed throughout the experiment with 5% CO_2/95% O_2, final pH 7.40. These experiments are designated at '5% CO_2 present'.

Each hemibladder was allowed to equilibrate for a 15–30-min period and then voltage-clamped at the respective potential difference for a 1-h period. H^+ excretion was measured simultaneously during this same 1-h period. The potential difference was measured using calomel electrodes immersed in Ringer solution with agar bridges between the electrode bath and incubation chambers. Assymmetry between the electrodes was less than 1 mV. Membrane potentials were measured on a Keithley Model 610B electrometer. Short-circuit current was applied with a direct-current microammeter, having a full-scale reading of 200 μA, and the voltage-clamped at the indicated potential difference.

Chloride substitution experiments. These experiments were run on paired hemibladders. One hemibladder served as control and received the same solutions as indicated above. The other hemibladder received a solution in both the serosal and mucosal chamber in which the chloride had been replaced by an equimolar quantity of sodium gluconate and are referred to as 'chloride-free'. These hemibladders were allowed to equilibrate for a 15–30 min period and then the test period began. The flux period was for 120 min. The chambers were oxygenated as given in the above experiments. Chloride was determined by the method of Schales and Schales [9] on both the mucosal and serosal solutions before and after each experiment.

Results

In Table I are the results of the experiments in which we compared the two methods of measuring acidification in the bladder; (1) the pH stat method, and (2) from the change in pH of a known buffered solution. These experiments were done in bladders from both normal toads and toads in metabolic acidosis. It can be seen in Table I that there is no significant difference in the two methods of measuring H^+ excretion in either the normal or acidotic toad ($P > 0.50$).

In Fig. 1 are the results showing the effect of amiloride on H^+ excretion. These experiments were done on normal and acidotic toad bladders and in the presence and absence of exogenous CO_2. Amiloride had no effect in the bladders from normal toads or in the bladders from acidotic toads that were enriched with 5% CO_2 in the serosal bath. However, in bladders from acidotic toads and in a phosphate buffer free of exogenous

TABLE I

COMPARISON OF H^+ EXCRETION AS DETERMINED BY pH STAT AND CHANGE IN pH METHODS IN BLADDERS FROM NORMAL AND ACIDOTIC TOADS

All experiments were performed in the absence of exogenous CO_2. pH stat was done with a Radiometer pH stat assembly. Paired *t*-test was used for obtaining *P* values. H^+ excretion is reported in nmol·(100 mg bladder)$^{-1}$·min^{-1} ± S.E.

State of animal	*N*	H^+ excretion	
		from change in pH	pH stat
Normal	8	26.7 ± 0.96	26.2 ± 1.43
		$P > 0.50$	
Metabolic acidosis	10	29.2 ± 2.72	30.5 ± 1.95
		$P > 0.50$	

CO_2, significant reduction in H^+ excretion ($P < 0.02$) was observed.

On the other hand, when all sodium in the mucosal medium was replaced with choline there was a significant reduction in H^+ excretion (see Fig. 2) in the groups free of exogenous CO_2. In bladders from normal toads and enriched with 5% CO_2 there was an inhibition of H^+ excretion at the border-line level of significance ($P < 0.050$. In bladders from acidotic toads and enriched with 5% CO_2 there was no effect on H^+ excretion ($P > 0.30$). Na^+ analyses were done on all mucosal samples at the end of the flux period and all choline-substituted baths contained less than 0.5 mM/l of Na^+.

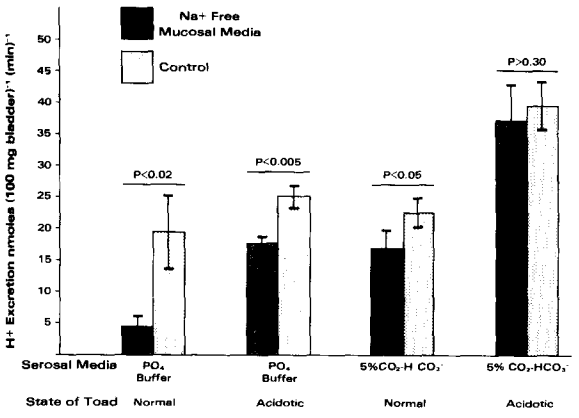

Fig. 2. The effect of Na^+-free mucosal medium on H^+ excretion in normal and acidotic bladders in the presence and adsence of exogenous CO_2. Each group represents the average ± S.E. of 12 paired hemibladders. Choline chloride replaced NaCl on an equimolar basis in the mucosal bath. In addition, a 1.5 mM K_2HPO_4/KH_2PO_4 was used as a buffer.

Fig. 3 demonstrates the effect of acetazolamide on H^+ excretion. It appears that there is a clear distinction here between acidification in normal bladders and those from acidotic animals. Normal

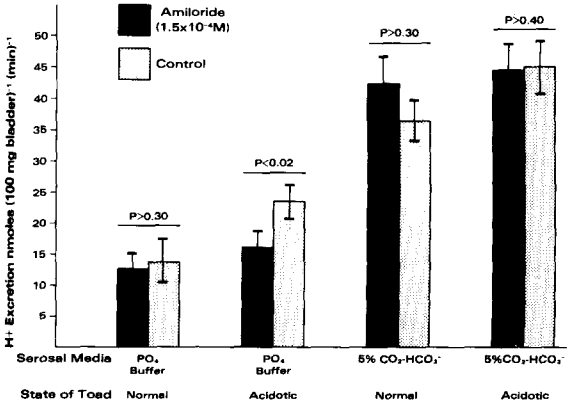

Fig. 1. The effect of amiloride on H^+ excretion in urinary bladders from normal and metabolic acidotic toads in the presence and absence of exogenous CO_2. Each group represents the average ± S.E. of 12 paired hemibladders. Amiloride ($1.5 \cdot 10^{-4}$ M) was placed in the mucosal bath. Both the mucosal and serosal bath were 2 ml each in volume.

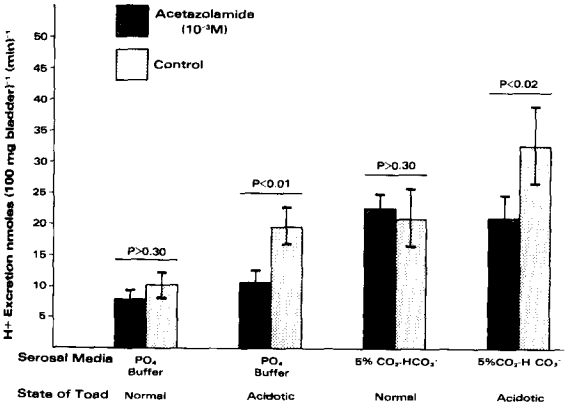

Fig. 3. The effect of acetazolamide on H^+ excretion in normal and acidotic bladders in the presence and absence of exogenous CO_2. Each group represents the average ± S.E. of ten paired hemibladders. Acetazolamide was added to the serosal bath in a final concentration of 10^{-3} M.

bladders were not affected by acetazolamide in either the phosphate buffer or the 5% CO_2/HCO_3^- buffer; $P > 0.30$ in both cases. In contrast to this, in bladders from acidotic animals, H^+ excretion was inhibited in both phosphate buffer and 5% CO_2/HCO_3^- buffer ($P < 0.01$ and < 0.02, respectively).

Shown in Fig. 4 is the effect of mucosal pH or H^+ gradient on H^+ excretion in normal and acidotic toads. There was no exogenous CO_2 present in this system. At every mucosal pH, except 7.50 and 5.50, the acidification rate in the acidotic bladders was greater than in normal bladders.

Fig. 5 shows the H^+ excretion into the mucosal medium with changing H^+ gradient in normal and acidotic toads. In this experiment, the serosal medium contained 24 mM $NaHCO_3^-$ and was gassed continuously with 5% CO_2/95% O_2. Again

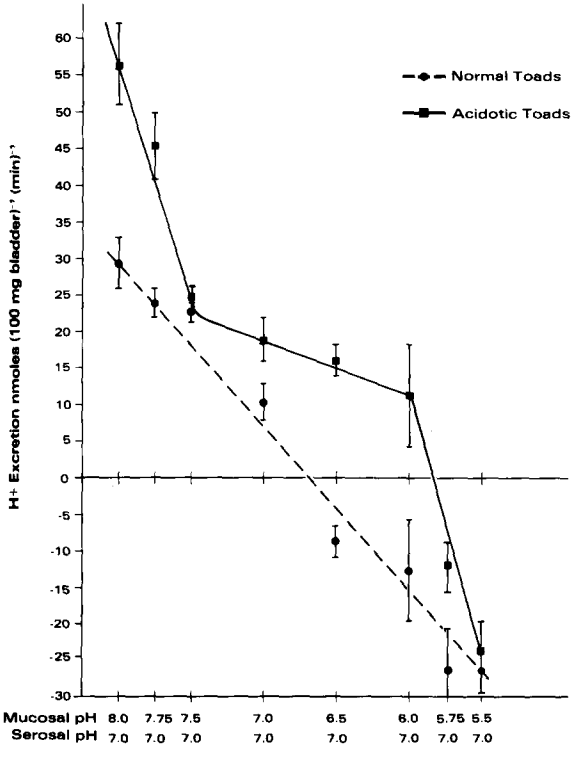

Fig. 4. H^+ excretion in bladders from normal and acidotic toads at varying initial mucosal pH. The serosal pH was the same initally in all experiments (pH 7.0). The mucosal pH was the indicated pH at the beginning of the flux period. There was no exogenous CO_2 in the system. 100% O_2 was bubbled continuously into the mucosal bath. Each point on the graph represents the average of ten bladders ±S.E.

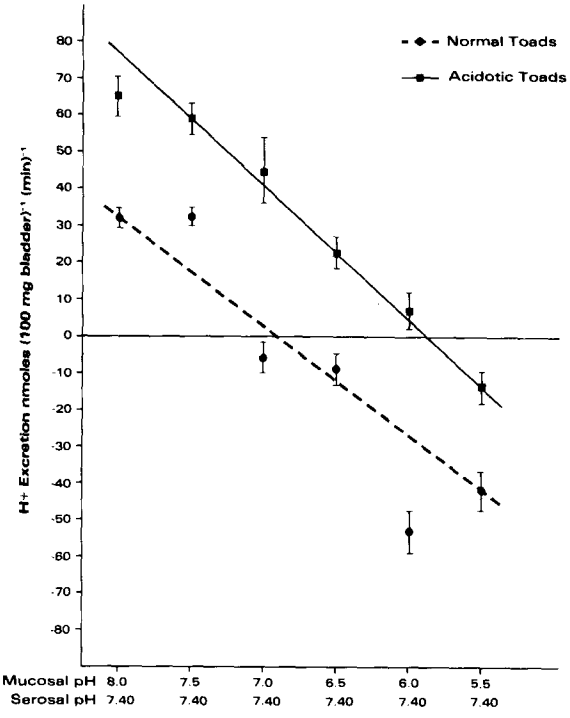

Fig. 5. H^+ excretion in bladders from normal and acidotic toads at varying initial mucosal pH values. The serosal bath contained 24 mM HCO_3^- and was gassed continuously with 5% CO_2/95% O_2 to maintain the pH at 7.40. The initial mucosal pH was varied from 5.5 to 8.0. Each point on the acidotic bladder line represents the average of ten bladders ±S.E. Each point on the normal bladder line represents the average of eight bladders ±S.E.

at every mucosal pH the acidification rate in the acidotic bladder was greater than in normal bladders.

Shown in Fig. 6 are the results of voltage clamping at various potential difference values on H^+ excretion in acidotic toad urinary bladder. In the absence of CO_2 it can be seen that at a favorable potential difference for H^+ excretion, there was an inhibition of acidification (-20 to -100 mV mucosal with respect to serosal). However, when 5% CO_2 was present this inhibition was reversed and H^+ excretion was voltage-independent over the entire range from $+100$ to -100 mV (mucosal with respect to serosal).

Fig. 7 shows the results of the same experiments but performed in bladders from toads in normal acid-base balance. In the absence of exogenous CO_2 there was no effect from -10 to $+100$ mV.

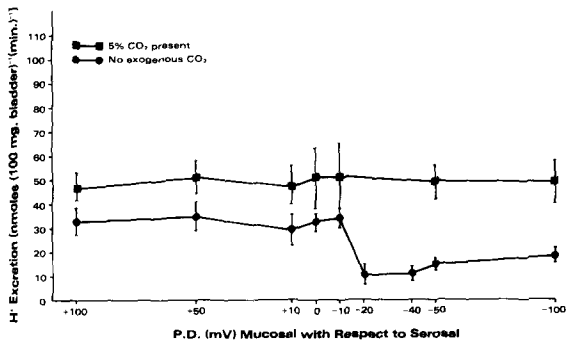

Fig. 6. Graph showing the effect of transepithelial voltage clamping on H^+ excretion in the toad urinary bladder. All animals were in metabolic acidosis. Each point represents the average of ten hemibladders ± S.E. In the experiments labeled no exogenous CO_2, there was a matched phosphate-buffered Ringer solution on each side of the bladder. In the experiment labeled 5% CO_2 present, the serosal bath contained 24 mM $NaHCO_3^-$ gassed with 5% CO_2/95% O_2. PD, potential difference.

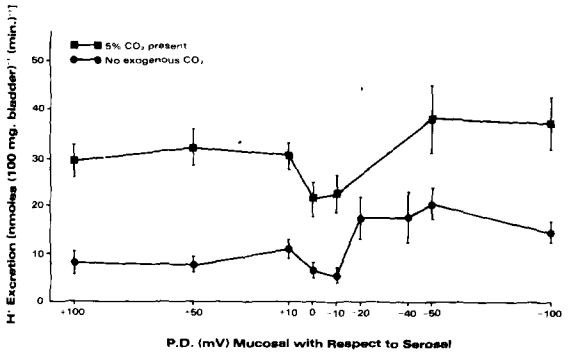

Fig. 7. Graph showing the effect of potential difference (PD) on H^+ excretion in the toad urinary bladder. All animals were in normal acid-base balance. Each point represents the average of ten hemibladders ± S.E. In the experiments labeled no exogenous CO_2, there was a matched phosphate-buffered Ringer solution on each side of the bladder. In the experiment labeled 5% CO_2 present, the serosal bath contained 24 mM $NaHCO_3^-$ gassed with 5 CO_2/95% O_2.

However, at −20 to −100 mV (mucosal with respect to serosal) there was a significant increase in H^+ excretion compared to that at zero potential difference. When exogenous 5% CO_2 was added to this system the same stimulation was observed. There was also an increase in H^+ excretion in the presence of 5% CO_2 when the potential difference was +10 to +100 mV.

Shown in Table II are the results of the studies in which gluconate was substituted for chloride in both the serosal and mucosal baths. It is clear that in the normal toad there is a component of the H^+ excretory system that is dependent on chloride ($P < 0.01$). There was also an inhibition of H^+ excretion in the acidotic bladders in the presence of 5% CO_2. In the acidotic bladders with no exogenous CO_2 present there was also a slight depression of H^+ excretion but not at a level of significance ($P > 0.50$). Cl^- analyses were done on the baths before and after each experiment and found to contain less than 0.5 mM/l of Cl^-.

TABLE II

THE EFFECT OF GLUCONATE SUBSTITUTION FOR CHLORIDE ON H^+ EXCRETION IN TOAD BLADDER

Control and experimental fluxes are given in units of nmol·(100 mg bladder)$^{-1}$·min^{-1}. Each value is the average of N for that group. Mean difference is the average value obtained by subtracting each experimental value from each control value (control − experimental) ± S.E. The P values were obtained using the paired t-test.

State of toad	CO_2 in media	N	Control (chloride)	Experiment (chloride-free)	Mean difference ± S.E. (P value)
Normal	no exogenous CO_2	8	10.53	8.05	2.48 ± 0.84 (< 0.01)
Acidotic	no exogenous CO_2	10	14.46	8.07	6.39 ± 3.58 (> 0.05)
Normal	5% CO_2	10	23.09	15.35	7.74 ± 3.51 (< 0.05)
Acidotic	5% CO_2	8	22.72	12.78	9.94 ± 2.70 (< 0.01)

Discussion

Two methods of measuring the acidification rates in urinary epithelia are used in studying the acidification mechanism. The pH stat method [10] and the change in pH of a known buffer solution over a given unit of time [1] have been assumed to be equivalent methods of measuring acidification rates. The pH stat technique offers the advantage of keeping the H^+ gradient across the epithelia constant during the course of the experiment. However, the pH stat method cannot be used with reliability in the presence of exogenous CO_2, because the NaOH in the titrant is buffered by carbonic acid produced from the CO_2 bubbling into the solution. On the other hand, in the pH change of a known buffer there is an increasing, albeit small, gradient of H^+ developed during the course of the experiment. It is important then to demonstrate that these two methods of measuring proton excretion were indeed equivalent. Our experiments in Table I clearly indicate that over the time-course of our experiments (normally 1–2 h) there was no difference in the two methods of measuring the acidification rate in the toad urinary bladder.

Amiloride was found to inhibit H^+ excretion only in the acidotic toad in the absence of exogenous CO_2. The reason for inhibition in this instance was probably due to the fact that by blocking Na^+ entry into the cell there was a reduction in CO_2 production from Na^+ metabolism. It has been reported that up to 50% of the bladders endogenous CO_2 arises from the Na^+ transport mechanism [11]. When exogenous CO_2 was present there was no inhibition of H^+ excretion. This is consistent with the report of Ludens and Fanestil [3] who measured H^+ excretion in the toad urinary bladder using reverse short-circuit current. H^+ excretion was not inhibited in the normal toad bladder by amiloride. This could be due to the fact that H^+ excretion in this group is not at the increased level of the acidotic animal and hence not limited by the amount of endogenous CO_2 from Na^+ metabolism.

The experiments substituting choline for Na^+ in the mucosal media suggest that in the normal toad there is a H^+ excretory mechanism that is dependent on mucosal Na^+ and that when the animal is placed in acidosis a H^+ excretory mechanism is stimulated that appears independent of mucosal Na^+. In the acidotic toad in the presence of 5% CO_2 there is maximal H^+ excretion and no difference was found when the mucosal Na^+ was removed. In this state, the H^+ excretory mechanism dependent on mucosal Na^+ may still be present but relatively small compared to the total H^+ excretion and hence not demonstrable at these high levels of H^+ excretion.

The studies with acetazolamide provides strong evidence to support the concept that there are two different H^+ excretory systems in the toad urinary bladder. One system in the normal toad that is not dependent on carbonic anhydrase and a second system stimulated by a metabolic acidosis that is dependent on carbonic anhydrase activity and sensitive to acetazolamide. A report by Ziegler et al. [6] demonstrated this same inhibition of H^+ excretion by acetazolamide in acidotic animals. An earlier report [3] reported an inhibition of H^+ excretion by acetazolamide in the normal toad urinary bladder. This finding in the normal toad is different from our findings and the reason for this discrepancy is not apparent at this time.

Several reports have indicated the presence of carbonic anhydrase in both normal and acidotic urinary bladder [6,12,13]. These studies found no change in the carbonic anhydrase activity or amount, when the animal was in acidosis. Our hypothesis would predict that carbonic anhydrase would only be involved and active with the H^+ excretory system that is stimulated during acidosis.

In order to characterize further the H^+ excretory system in the toad urinary bladder we performed experiments at varying pH gradients in the presence and absence of exogenous CO_2. In the presence of 5% CO_2 (Fig. 5) the acidotic animals at every pH gradient was found to secrete H^+ at a higher rate than the normal. Additionally, the normal toad bladder can excrete H^+ only in the face of about 0.4–0.5 pH units transbladder gradient. Whereas, the bladder from acidotic toads can excrete H^+ against a pH gradient of 1.4–1.5 pH units, a 3-fold increase in the strength of the H^+ excretory system.

The point at which the line of H^+ excretion crosses the zero line (Figs. 4 and 5) is taken to be the point at which there is no net excretion of H^+.

Above this point, there is net addition of H^+ to the mucosal fluid, while below this line the back-flux of H^+ into the cell and serosal fluid becomes greater than the cell mucosal flux of H^+.

In the absence of exogenous CO_2 (Fig. 4), the bladders from acidotic animals always secreted H^+ at a greater rate than the normal bladders, except at mucosal pH of 7.5 and 5.5. At a mucosal pH of 5.5, the back-flux (mucosal cell) of H^+ may be nearing a maximal rate and this could account for the fact that the normal and acidotic bladders are the same at this point. The apparent back-flux could be the result of HCO_3^- or OH^- secretion as suggested by Weiner [14] for the toad bladder. This would then lead to a maximum alkalization rate. It is not clear why the H^+ excretion should be the same in both groups of bladders at mucosal pH of 7.5. Again, as in the experiments in Fig. 5, it is apparent that the H^+ excretion in bladders from acidotic toads is distinctly different from the normal toads. The bladders from acidotic toads are excreting H^+ at a much greater rate and are capable of maintaining a greater pH gradient.

In bladders from acidotic toads (Fig. 6) it was found that in the absence of exogenous CO_2 there was an inhibition of H^+ excretion when the mucosal potential difference was clamped between -20 and -100 mV. This gradient should have favored H^+ excretion into the mucosal fluid. However, when the bladders were enriched with 5% CO_2 the H^+ excretion in acidotic bladders was independent of potential difference across the entire physiological range of $+100$ to -100 mV, mucosal with respect to serosal. It is obvious that increasing the potential difference from -20 to -100 mV inhibits H^+ excretion by limiting in some way the availability of CO_2. It has been shown that Na^+ transport in the toad urinary bladder accounts for as much as 50% of the total CO_2 production [10,15]. In addition, it has also been shown that net mucosal to serosal flux of Na^+ in toad bladder is reduced linearly as a function of imposing an unfavorable transepithelial potential difference [16,17]. In light of these studies, we believe that the inhibition of H^+ excretion observed was due to a limitation of available CO_2 derived from Na^+ metabolism that was reduced by unfavorable voltage clamping.

A report by Al-Awqati et al. [18] demonstrated that in the turtle urinary bladder both an unfavorable pH gradient or an unfavorable electrical gradient would inhibit H^+ excretion in this tissue. Our findings are similar in the toad urinary bladder with respect to the pH or H^+ gradient. However, our findings differ with respect to the electrical gradient and point to a difference in these two tissues with regard to electrical gradients. It should be pointed out that the turtle bladder excretes H^+ at a much higher rate than does the toad bladder, but whether this could explain any of this difference is not apparent from this study.

Experiments in normal bladders indicated a voltage-sensitive component of the H^+ excretion in these bladders. This was seen in both the presence and absence of exogenous CO_2. There was also a slight stimulation of H^+ excretion in the presence of 5% CO_2 when the potential was clamped between $+10$ and $+100$ mV mucosal positive with respect to serosal. The cause of this stimulation is not clear from this study and will have to await further investigation.

Chloride is involved in the H^+ excretion in the bladders from normal toads. Our results in bladders from acidotic toads are not as clear-cut with respect to chloride. In the acidotic bladder and in the absence of exogenous CO_2, gluconate substitution had a slight but not significant inhibitory effect on H^+ excretion. Whereas in this same group of bladders with 5% CO_2 there was a significant inhibition of H^+ excretion in the absence of chloride. This could be due to a HCO_3^--Cl^- exchange as reported for the turtle bladder [19,20] or a component of the H^+-excretory system that involves a H^+-Cl^- cotransport system. Our present study with gluconate did not delineate between these two possible roles of chloride in H^+ excretion. It is interesting to note that a report by Sobaslai et al. [21] suggest a possible association between the processes of Cl^- and H^+ and/or HCO_3^- transport.

In Fig. 8 is shown a proposed model for H^+ excretion in the toad urinary bladder when the toad is in normal acid-base balance. There is an active H^+ pump on the apical border of the cell that is sensitive to transepithelial potential difference, independent of carbonic anhydrase activity, requires the presence of mucosal Na^+, and not related directly to cellular Na^+ metabolism. The

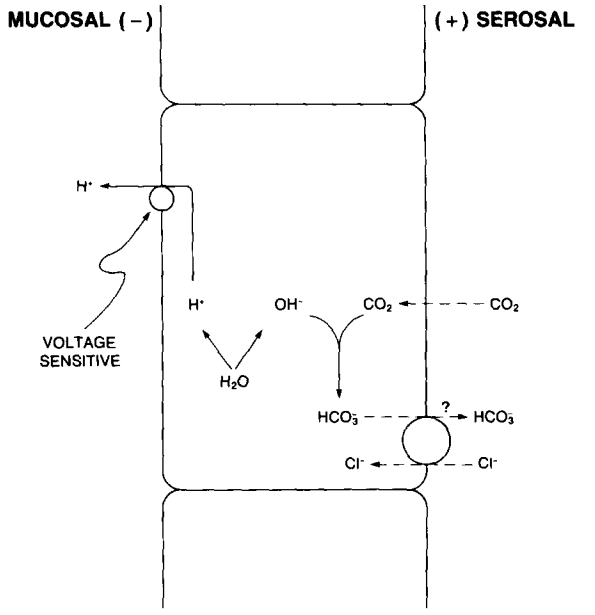

Fig. 8. A proposed model for H^+ excretion in the toad urinary bladder during normal acid-base balance. (−) Indicates the mucosal side negative with respect to the serosal side (+) positive in the spontaneous state.

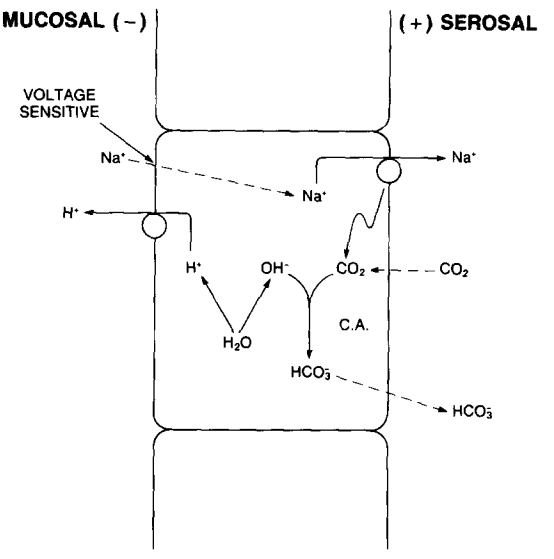

Fig. 9. A proposed model for H^+ excretion in the toad urinary bladder during metabolic acidosis. C.A. indicates carbonic anhydrase. (−) Indicates the mucosal side negative with respect to the serosal side (+) positive in the spontaneous state.

CO_2 necessary for the intracellular neutralization of OH^- would come from cellular metabolism other than that concerned with Na^+ transport. The electromotive force for this H^+-excretory pump is much weaker when compared to the toad in acidosis, only being able to develop and maintain a transepithelial pH gradient of 0.4–0.5 pH units. There is also a component of this H^+ system that may be dependent on HCO_3^--Cl^- exchange on the serosal border of the cell.

Fig. 9 is a proposed model for H^+ excretion in the toad urinary bladder when the animal is in metabolic acidosis. This is an active metabolic pump located on the apical border of the cell and this system is independent of transepithelial potential difference over the range −100 to +100 mV. This H^+ pump is dependent in part on CO_2 derived from Na^+ metabolism or supplied from the serosal compartment. Additionally, it is dependent on carbonic anhydrase activity for maximal excretion rates, and does not require mucosal Na^+. This pump in the acidotic animal has a larger electromotive force than the normal animal, being able to pump against a transepithelial pH gradient of 1.4–1.5 pH units and at a higher rate of excretion.

The possible role of Cl^- in the HCO_3^--exit step across the basolateral membrane was omitted from this figure. This was done since our experiments concerning the role of Cl^- in the acidotic animal were not strong as in the normal animal. Additional studies will have to be done in this area.

The model in Figs. 8 and 9 would predict that there would be a concurrent serosal alkalization with mucosal acidification. We have shown in a previous study [2] that this indeed occurs. However, there was not an equivalent stoichiometry between mocusal H^+ excretion and alkalization of the serosal fluid. This difference in stoichiometry was explained by a portion of the HCO_3^- being buffered by intracellular buffers. It should be noted that the models presented do not attempt to delineate one particular cell type in the toad urinary bladder, but rather they represent a composite cell type of the urinary bladder.

The question always arises when studying acidification mechanisms; is the acidification a result of addition of H^+ to the mucosal fluid or the removal of a HCO_3^- molecule with subsequent reabsorption? Earlier studies [2–4] have clearly shown that in the toad urinary bladder the acidification of the mucosal fluid occurs by addition of H^+ and not reabsorption of HCO_3^-.

In summary, our study has provided evidence that supports the concept of two H^+ excretory systems in the toad urinary bladder, each with its' own characteristics. It is not clear if these are two separate and distinct H^+ pumps in parallel or the same pump that may be stimulated by acidosis and takes on a new set of characteristics.

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