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EXCRETION OF H^+ AND NH_4^+ BY THE URINARY BLADDER OF THE ACIDOTIC TOAD AND THE EFFECT OF SHORT-CIRCUIT CURRENT ON THE EXCRETION

LOY W. FRAZIER AND JOHN C. VANATTA

University of Texas (Southwestern) Medical School, Dallas, Texas 75235; Southern Methodist University, Institute of Technology, Dallas, Texas 75222 (U.S.A.)

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SUMMARY

1. *In vivo* and *in vitro* studies on the urinary bladder of the acidotic toad *Bufo marinus* were performed to determine if the bladder does excrete H^+ and NH_4^+.

2. In *in vivo* studies using acidotic toads the titratable acidity produced by the bladder *per se* averaged 23.81 ± 4.73 (S.E.) μmoles/48 h. The NH_4^+ excretion averaged 25.48 ± 4.17 μmoles/48 h.

3. Evidence suggests that the urinary bladder is the principal organ for the production of urinary titratable acidity in this species. The bladder contribution to total NH_4^+ excretion amounts to only a very small percent of total excretion.

4. The *in vitro* excretion rate of titratable acidity in bladders from acidotic toads was greater than in bladders from control toads, but the NH_4^+ excretion was unchanged.

5. *In vitro* studies with the bladder mounted between 2-ml leucite chambers show that the excretion rate of H^+ into the mucosal medium is 16.95 ± 3.38 nmoles/100 mg bladder per min. The excretion rate for NH_4^+ was found to average 4.06 ± 0.78 nmoles/100 mg bladder per min.

6. Short-circuit current (s.c.c.) studies performed on acidotic toad bladders show that neither H^+ or NH_4^+ excretion are effected by abolishing the trans-bladder potential difference. This indicates that H^+ excretion in the bladder may be active.

INTRODUCTION

The toad urinary bladder has several functions analogous to the distal nephron of the mammalian kidney. LEAF *et al.*[1] reported that the toad bladder reabsorbs Na^+. CRABBE[2] showed that the Na^+ reabsorption in the toad bladder is stimulated by aldosterone. The toad bladder also serves as a reservoir from which water can be reabsorbed in response to dehydration. The reabsorption is controlled by vasopressin[3,4]. *In vivo* studies reported by KALLUS AND VANATTA[5] indicate that the toad bladder also excretes K^+.

Abbreviations: s.s.c., short-circuit current; PD, potential difference.

SCHILB AND BRODSKY[6] have reported that the urinary bladder of *Pseudemys scripta* acidifies the mucosal medium while transferring Na^+ and Cl^- from the mucosal to serosal fluid. They have shown that this acidification process is accomplished by active transport of HCO_3^- from the mucosal to serosal medium. STEINMETZ[7] and STEINMETZ *et al.*[8] on the other hand have confirmed the acidification of the mucosal medium by the turtle bladder and have demonstrated that this acidification is due to active H^+ excretion and not HCO_3^- reabsorption.

The experiments reported here were performed to determine: (1) if the toad bladder excretes H^+ and NH_4^+; (2) if such secretion was increased by a metabolic acidosis; and (3) if the H^+ excretion is against an electrical chemical gradient by using the short-circuiting technique.

MATERIALS AND METHODS

Bufo marinus of either sex were used in all experiments. The toads were supplied by Charles P. Chase of Miami, Fla. They are originally collected near Barranquilla, Colombia, South America. They were kept in deionized water without food from time of receipt until use. Toads used in the urine-collection experiments were placed in large plastic funnels which drained into beakers containing 5 ml of toluene. Their bladders were emptied by catheterizing at the beginning of the experiment. At the end of the 48-h period the bladder was again emptied and the aliquot added to the urine specimen. If the urine specimen became contaminated with fecal material during the collection period, it was discarded. Specimens on each toad were collected about every 12 h and kept at 4°. At the end of 48 h the specimens were pooled and the analyses performed.

For purposes of this study an acidotic toad is designated as one receiving a standard load of five 8-ml doses of 120 mM NH_4Cl by stomach tube over a 48-h period. Control toads received no such loading. Blood specimens were obtained at the time of sacrifice by cardiac puncture. CO_2 combining power was performed by the method of PETERS AND VAN SLYKE[9]. All pH measurements were made on a Corning Model 10 pH meter with an expanded scale using a Beckman combination electrode.

In vivo experiments

Toads were anesthetized by partial immersion in 10 % ethanol[10]. The bladder orifice entering the cloaca was visualized with the aid of a nasal speculum. The bladder was catheterized with polyethylene tubing, drained and washed 3 times with a 3 mM sodium phosphate buffered Ringer solution, pH 7.10, that was HCO_3^- free. The solution contained in mM, in addition to 3 mM sodium phosphate, NaCl 114.0; KCl 3.0; $CaCl_2$ 0.9. 25 ml of this Ringer solution (mock urine) was then placed in the bladder and mixed thoroughly. A 5-ml aliquot was then removed for analysis and the bladder orifice was sutured closed using a running-lock suture and checked for leaks. This isolates the urinary bladder from the cloaca.

The method of checking for leaks was to visually observe the sutured bladder orifice while pressing on the abdominal area over the bladder. If a leak was observed the toad was discarded.

The anatomy of the toad's excretory system is different than that of the mammal in that the ureters open into the cloaca and not the urinary bladder. The bladder

then is an *in vivo* closed sac preparation. Each ureter was catheterized with polyethylene tubing that drained to the outside. This is an added precaution against contamination by renal urine.

The toads were allowed to recover from the anesthetic and were then loaded with NH_4Cl solution to produce acidosis. At the end of 48 h the toads were sacrificed and the bladder content was removed and the volume recorded. The mock urine was analyzed for pH, titratable acidity and NH_4^+. The NH_4^+ was determined by both the formaldehyde titration method and Nesslerization[11]. The standard deviation of the difference between the two methods was $\pm$ 3.6 %.

In vitro experiments

Acidotic toads were sacrificed by pithing. The hemi-bladders were removed and mounted between leucite chambers, each of which held 2 ml of the indicated bath. Pooled plasma from acidotic toads was placed on the serosal side. In all experiments except 6–10 given in Table II the plasma was frozen and then thawed to eliminate CO_2 in solution. In Expts. 6–10 of Table II the pooled plasma was fresh and was not frozen and thawed before use. This plasma, pH 7.30, obviously contained some CO_2 although no precaution was taken to guard against loss to the atmosphere. A 1.5 mM phosphate Ringer solution, pH 6.85, was placed on the mucosal side. The concentration of electrolytes was the same as in the *in vivo* experiments except for the reduction in the concentration of phosphate buffer. 100 % O_2 was bubbled through the mucosal medium throughout the experiment. At the end of the flux period wet weight was obtained on the bladders and NH_4^+ and pH was determined on the mucosal and serosal medium. Using the Henderson–Hasselbalch equation[12] and the molar concentration of phosphate buffer the amount of H^+ added to the mucosal medium could be calculated. The NH_4^+ was determined by a modification of the method of CHANEY AND MARBACH[13] using a Bausch and Lomb Spectronic 20.

The short-circuit current (s.c.c.) technique was a modification of the method described by USSING AND ZERAHN[14]. Both the mucosal and serosal chambers contained 2 ml of the respective solutions. The cross-sectional area of each chamber was 1.98 cm^2. The mucosal solution was the same as given above except the phosphate buffer was reduced to 0.6 mM. The serosal solution was pooled plasma from acidotic toads. Agar bridges were prepared by adding 2 % agar to the 0.6 mM phosphate buffer. This was done to minimize pH changes associated with current flow. Potential differences (PD) were measured using calomel electrodes immersed in Ringer solution with agar bridges between the electrode bath and incubation chambers. Asymmetry of the electrodes and bridges was checked before and after each experiment and was less than 1.0 mV. Membrane potentials were measured on a Keithley Model 610B electrometer. S.c.c. measurements were made with a direct-current micro-ammeter, having a full scale reading of 100 μA.

The H^+ and NH_4^+ mucosal secretion was measured over two consecutive 60-min periods in each bladder. Seven bladders were maintained in the open-circuited state during the first period followed by short-circuiting in the second period (Series A). Six bladders were short-circuited in the first period followed by the open-circuited period (Series B). The PD was recorded every 5 min during the open-circuited state and the values averaged over the 60-min period. S.c.c. was maintained continuously throughout the short-circuited period and readings recorded every 5 min and aver-

aged for the 60-min period. S.c.c. was calculated as net Na^+ flux and reported as nmoles/100 mg bladder per min.

It was found by using an inert membrane with 0.6 mM phosphate Ringer solution in both chambers and a s.c.c. of 1200 $\mu A \cdot min$ that there was a slight but consistant alkalization of the mucosal medium (0.04 pH unit). This current was the average current that was required to maintain the bladder in a short-circuited state over a 60-min period. In the calculation of the H^+ flux, the observed flux was corrected for the effect of H^+ which the above pH change represents. This was an increase in H^+ flux over the observed flux of about 30 %.

RESULTS

Effect of loading toads with NH_4Cl

The CO_2 combining power was determined on ten normal toads and found to be 20.1 $\pm$ 1.0 (S.E.) mM. In a series of ten toads which had received a standard load of NH_4Cl by stomach tube, the CO_2 combining power was 6.9 $\pm$ 0.97 mM. This method of loading did produce a marked metabolic acidosis.

Excretion of titratable acidity and ammonia by acidotic toads

Total urinary (bladder and renal) excretion of titratable acidity and NH_4^+ were determined on a group of ten acidotic toads. Urine pH ranged from 5.32 to 7.20. Titratable acidity averaged 24.1 $\pm$ 3.80 μmoles/48 h period. NH_4^+ excretion was found to be 371.0 $\pm$ 49.9 μmoles/48 h period.

In vivo excretion of titratable acidity and NH_4^+ by the toad bladder

In Table I are reported the pH of the mock urine and the H^+ and NH_4^+ excretion by the bladder. It is evident by comparing the values from Table I with the total H^+ and NH_4^+ excretion reported above, that during acidosis the bladder contributes

TABLE I

In vivo EXCRETION OF H^+ AND NH_4^+ BY THE URINARY BLADDER OF THE ACIDOTIC TOAD

The preoperative pH measurement was made on an aliquot removed just before the bladder orifice was sutured closed.

Toad	*Preoperative pH*	*Postoperative pH*	*Vol.* of "mock" urine recovered*	*H^+ (titratable acidity) (μmoles/48 h)*	*NH_4^+ (μmoles/48 h)*
A	7.05	5.65	19.0	37.50	18.30
B	7.12	4.00	12.0	28.10	13.10
C	7.00	5.92	16.5	3.39	49.50
D	7.05	6.49	21.5	14.40	28.30
E	7.15	5.31	7.8	14.70	13.60
F	6.92	4.65	13.5	32.40	35.60
G	7.00	5.20	21.4	46.00	23.50
H	7.10	5.90	11.0	19.30	32.90
I	6.99	5.50	19.6	38.80	33.90
J	7.05	4.97	2.4	3.54	6.17
			Mean $\pm$ *S.E.*	23.81 $\pm$ 4.73	25.48 $\pm$ 4.13

* Volume of mock urine remaining in each bladder at the beginning of the experiment was 20 ml except for Toad J which contained only 15 ml.

as much as 95–100 % of the total titratable acidity. The NH_4^+ excretion contributed by the bladder was only 6–7 % of the total excretion.

Also shown in Table I are the volumes of mock urine recovered. In eight of the ten toads there was good recovery of mock urine, allowing for some water reabsorption. In Toads E and J the small volume recovered is probably due to a slow leak from the bladder, with or without water reabsorption. The values of titratable acidity and NH_4^+ of these two toads are then lower than would be expected due to the slow leak of buffer. Values for these two toads were included because in this experiment we were primarily concerned with demonstrating the ability of the bladder *per se* to acidify the mock urine *in vivo* and not with quantitation.

In vitro excretion rate of H^+ and NH_4^+ in the toad bladder

In Table II are reported the rates of excretion of H^+ and NH_4^+ into the mucosal

TABLE II

In vitro EXCRETION RATES OF H^+ AND NH_4^+ IN THE URINARY BLADDER FROM ACIDOTIC TOADS WITH PLASMA ON THE SEROSAL SIDE

Plasma from acidotic toads was the serosal media. The initial pH was 8.03 in Expts. 1–5, and 7.30 in Expts. 6–10. The mucosal medium was 2 ml of phosphate Ringer solution, pH 6.85 in all experiments. The initial NH_4^+ concentration in the serosal medium was 30.9 nmoles/ml in Expts. 1–5 and 24.0 nmoles/ml in Expts. 6–10. The initial NH_4^+ concentration of the mucosal medium was zero. The excretion rate was measured over 120 min.

*Sample**	*Final pH*	*Final NH_4^+ concn. (nmoles/ml)*	*Mucosal H^+ excretion (nmoles/100 mg bladder** per min)*	*Mucosal NH_4^+ excretion (nmoles/100 mg bladder** per min)*
1M	6.75	49.7	19.70	10.30
1S	8.07	38.6	—	—
2M	6.77	64.8	4.85	4.22
2S	8.06	47.2	—	—
3M	6.77	48.4	4.65	2.94
3S	8.03	10.7	—	—
4M	6.69	71.3	5.12	2.48
4S	8.02	248.0***	—	—
5M	6.78	29.7	7.56	3.13
5S	8.03	88.8	—	—
6M	6.38	26.7	32.60	1.88
6S	7.30	56.9	—	—
7M	6.44	52.2	20.40	2.92
7S	7.28	30.4	—	—
8M	6.60	28.0	30.30	3.38
8S	7.48	17.9	—	—
9M	6.53	71.8	24.20	6.19
9S	7.60	17.9	—	—
10M	6.64	32.2	20.10	3.16
10S	7.65	23.1	—	—
		Mean ± S.E.	16.95 ± 3.38	4.06 ± 0.78

* M = mucosal medium; S = serosal medium.

** 100 mg bladder is expressed as wet weight.

*** Since both the mucosal and serosal NH_4^+ values were high on single determinations the bladder was probably a high NH_3 producer, however, analytical error cannot be excluded. The minimal serosal pH change is probably associated with increased glutamic acid formation.

medium by the bladder. Also shown are the pH values and NH_4^+ concentration of both the serosal and mucosal medium. It can be seen that in each case there was an acidification of the mucosal medium. NH_4^+ concentration increased in all the mucosal samples and in six of the ten serosal samples.

A series of control bladders were also performed. The bladders and pooled plasma of the control series were from normal non-loaded toads. In eight of the ten control bladders, there was no H^+ excretion into the mucosal medium. The average H^+ excretion in the 10 bladders was 2.00 ± 1.79 nmoles/100 mg bladder per min. The difference between the acidotic bladders and the control bladders was significant ($P < 0.005$). NH_4^+ excretion in the control bladders was 6.19 ± 1.75 nmoles/100 mg bladder per min. This was not significantly different from the acidotic bladders ($P > 0.10$).

In Table III are reported the values of net excretion of H^+ and NH_4^+ by the

TABLE III

In vitro EXCRETION RATES OF H^+ AND NH_4^+ IN THE URINARY BLADDER FROM ACIDOTIC TOADS WITH RINGER SOLUTION ON BOTH SURFACES

Initially the mucosal and serosal medium were both pH 7.05. The initial NH_4^+ concentration in the mucosal medium was zero and in the serosal medium it was 8.5 nmoles/ml. The mucosal medium in each experiment was 2 ml of phosphate Ringer solution. The serosal medium contained 0.61 mM L-glutamine in the phosphate Ringer solution. The excretion rate was measured over 120 min.

*Sample**	*Final pH*	*Final NH_4^+ concn. (nmoles/ml)*	*Mucosal H^+ excretion (nmoles/100 mg bladder** per min)*	*Mucosal NH_4^+ excretion (nmoles/100 mg bladder** per min)*
1M	6.81	7.7	25.23	1.00
1S	7.11	25.8	—	—
2M	6.85	6.7	16.82	0.72
2S	7.15	32.8	—	—
3M	6.81	15.7	42.04	1.70
3S	7.16	35.5	—	—
4M	6.80	5.9	31.00	0.90
4S	7.17	30.1	—	—
5M	6.90	4.0	38.01	1.31
5S	7.11	23.0	—	—
6M	6.94	4.4	15.66	0.84
6S	7.05	32.1	—	—
7M	6.70	124.0	13.20	2.61
7S	7.68	118.5	—	—
8M	6.78	79.7	9.21	1.58
8S	7.20	87.9	—	—
9M	6.95	25.6	4.71	0.80
9S	7.10	43.2	—	—
10M	6.78	44.4	7.91	0.75
10S	7.20	64.4	—	—
		Mean ± S.E.	20.38 ± 4.12	1.22 ± 0.19

* M = mucosal medium; S = serosal medium.
** 100 mg bladder is expressed as wet weight.

bladder into the mucosal medium. Ringer solution was used on the serosal side in order to reduce the concentration gradient of NH_4^+ across the bladder, which existed when plasma was used. L-Glutamine was added as a precursor for NH_3 formation[15]. In each case there was an acidification of the mucosal medium. NH_4^+ concentration was found to increase in both the mucosal and serosal samples. The average H^+ excretion in these bladders was slightly but not significantly higher than in those which had pooled plasma on the serosal side ($P > 0.50$). The average NH_4^+ excretion in these bladders was significantly lower ($P < 0.01$). Since the serosal medium contained a known volume and concentration of buffer, in this experiment, the amount of alkalization was calculated as base excreted into the serosal medium. The average excretion of base was 7.46 ± 2.05 nmoles/100 mg bladder per min, which was significantly lower than the H^+ excretion into the mucosal medium ($P < 0.05$).

S.c.c. experiments were performed to determine if H^+ is excreted into the mucosal medium in response to the trans-bladder PD. Table IV shows the results of these experiments. It can be seen that H^+ excretion was not reduced by abolishing the trans-bladder PD and was in fact slightly higher in the s.c.c. state ($P < 0.025$).

TABLE IV

THE EFFECT OF S.C.C. ON H^+ AND NH_4^+ EXCRETION RATES OF THE URINARY BLADDER FROM ACIDOTIC TOADS

The mucosal medium was 2.0 ml of 0.6 mM phosphate buffered Ringer solution. The serosal solution was 2.0 ml of pooled plasma from acidotic toads. The initial pH of the mucosal medium was 6.70 and that of the serosal medium was 7.97. In Series A experiments the bladder was maintained in the open-circuited state for the first hour flux period followed by a 1-h flux period in the short-circuited state. In Series B experiments the procedure was reversed s.c.c. was applied first followed by the open-circuited state. S.c.c. measurements were expressed as net Na^- flux and averaged 151 ± 18 nmoles/100 mg bladder per min.

Expt.	*Mucosal H^+ excretion* (*nmoles/100 mg bladder* per min*)		*Mucosal NH_4^+ excretion* (*nmoles/100 mg bladder* per min*)		*PD*** (*mV*)
	Open-circuited	*S.c.c.*	*Open-circuited*	*S.c.c.*	
Series A					
1	10.2	10.9	1.70	1.03	8.4
2	10.0	13.6	4.37	3.06	15.9
3	20.8	17.1	4.99	7.77	9.9
4	14.4	17.3	2.97	2.02	34.5
5	14.0	21.1	1.72	2.08	33.8
6	19.7	19.0	3.02	2.48	43.9
7	15.6	18.6	1.55	1.44	14.1
Series B					
1	21.4	21.4	1.55	2.17	13.2
2	10.9	13.0	2.90	3.47	24.6
3	9.8	9.0	2.24	2.81	54.5
4	5.4	8.1	2.69	3.79	27.9
5	19.0	21.9	2.07	2.38	21.5
6	22.4	27.6	5.85	3.93	5.8
Mean ± S.E.	14.9 ± 1.5	16.8 ± 1.6	2.89 ± 0.38	2.96 ± 0.47	
	$P < 0.025$***		$P > 0.45$***		

* 100 mg bladder is expressed as wet weight.
** PD is the average reading over the 1-h period in which the bladder was open-circuited.
*** Calculated from mean difference.

Although the H^+ excretion is statistically different the reason is not immediately obvious. The increase seen during s.c.c. is of about the same magnitude as the correction factor applied for current flow and for this reason we are not considering it to be of physiological importance. NH_4^+ excretion was found not to be effected by the application of s.c.c. ($P > 0.45$). A correlation coefficient was also determined between the H^- excretion and the trans-bladder PD during the open-circuited state ($r = -0.319$). There was no noticeable effect on H^+ or NH_4^+ excretion caused by passage of time. The sequence of applying s.c.c. was also found not to have an effect on the excretion rates. This is evidenced by similar patterns of response in both A and B series of experiments.

DISCUSSION

The results of both *in vivo* and *in vitro* experiments show that the toad urinary bladder excretes H^+ and NH_4^+ when the toad is in a metabolic acidosis. This is another function of the toad urinary bladder which is analogous to the mammalian distal nephron.

When comparing the results from the *in vivo* studies with those of total excretion rates it becomes obvious that the bladder is an important physiological organ for the excretion of H^+ as titratable acidity. It appears from these studies that the urinary bladder is the major organ for excreting titratable acidity in the acidotic toad.

In this paper we have considered H^+ transport as being measured by titratable acidity alone. If NH_4^+ excretion in the toad is entirely by NH_3 diffusion across cell membranes into the urine, or mucosal medium, then the reaction $NH_3 + H^+ \rightarrow NH_4^+$ represents an error in this assumption, and total H^+ excretion should be the sum of titratable acidity and NH_4^+ excretion.

In the *in vitro* experiments the NH_4^+ production is a small fraction of the H^+ as measured by titratable acidity, and whether it is or is not included in calculating total H^+ production does not alter any of the conclusions presented.

In the *in vivo* experiments the question arises as to the role of the kidney in H^+ production. It cannot be determined from this study just what the role of the kidney is in acidification of the urine. The kidney obviously plays an important part in the conservation of Na^+ by NH_4^+ production. Whether it can alter the pH of the urine has not been determined. It is evident that the bladder can account for the major change in the pH of the urine.

Experimental evidence also shows the bladder is a contributor to total NH_4^+ excretion although to a much lesser extent than H^+ excretion. The fact that the NH_4^+ excretion rate does not increase in metabolic acidosis is surprising. It is possible that our control toads are in a mild starvation acidosis and are stimulated to maximum NH_4^+ production by the bladder, but not to maximum H^+ excretion.

It was noted in Table II that NH_4^+ concentration increased in both the mucosal and serosal medium. This would indicate that the bladder is producing NH_4^+ during metabolic acidosis. Because of the NH_4^+ excretion rates found in the control toads another possibility must exist. The bladder could contain a certain cellular level of NH_4^+ which could diffuse out of the cell as NH_3 during *in vitro* preparations. This possibility has not been evaluated in this study.

In Table II it can be seen that there were varied changes in the serosal pH

values in Expts. 6–10. As mentioned previously in the methods section, the plasma used in these experiments was not frozen and thawed before use. This plasma obviously contained some CO_2 and the serosal pH changes in Expts. 6–10 are meaningless These pH values probably represent mainly CO_2 loss to the atmosphere.

There can be no meaningful interpretation of pH changes in the mucosal medium as compared with the serosal medium because the ionic strength of the plasma buffers on the serosal side was greater than in the mucosal side. Since it was the purpose of this experiment to establish an *in vitro* method for studying acidification of the urine by the toad bladder, the nicety of quantitating the serosal pH change was not a part of the design of the experiments.

It is also noted in Table II that the mucosal H^+ excretion was higher in Expts, 6–10, than it was in Expts. 1–5. This could be due to the fact that in Expts. 1–5 the H^+ excretion was against a much greater transcellular pH gradient than in Expts. 6–10. Since it was assumed that CO_2 was present in the serosal medium of Expts. 6–10 the possibility must be considered that CO_2 is contributing to the mucosal H^+ excretion. However, since the mucosal H^+ excretion in the experiments given in Table III were of the same magnitude and this system contained no exogenous CO_2 or HCO_3^- this possibility seems unlikely.

It is possible that the metabolic CO_2 produced by the toad bladder contributes to H^+ excretion. This could occur by either supplying intracellular H^+ from H_2CO_3 to be transported, or by diffusion of or formation of H_2CO_3 in the mucosal medium and the subsequent reabsorption of bicarbonate. Active reabsorption of HCO_3^- has been reported in the turtle bladder by SCHILB AND BRODSKY[6]. To evaluate the role of metabolic CO_2, the respiratory quotient of the toad bladder was assumed to be 1.0 and the maximum O_2 consumption reported in the literature was used[16]. A calculation then reveals that the maximum amount of H^+ that could be obtained *via* CO_2 production would be approximately 12.5 nmoles/100 mg bladder per min. We found an excretion rate of 16.95 nmoles/100 mg bladder per min. It is apparent then that if CO_2 production is involved in H^+ excretion then another mechanism must also be operative. The purpose of the present study was not to investigate the cellular mechanisms of H^+ excretion and therefore the possibility of endogeneous CO_2 having a part in this must await further investigation.

It should be noted in Table II, Sample 4S, that there was a large increase in NH_4^+ with a change in pH of only –0.01 unit. Part of this difference could be explained if the glutamic acid formed from glutamine accumulated selectively in the serosal medium, but this would not explain it entirely. It is also noted that NH_4^+ accumulated more in the serosal medium than in the mucosal medium in three out of ten cases in Table II and eight out of ten cases in Table III. This seems in contradiction to the mechanism of NH_3 diffusion down a pH gradient and ionic trapping of the NH_4^+ across a selectively permeable membrane as proposed by JACOBS[17]. These current findings would indicate a need to evaluate further the mechanism of NH_3 or NH_4^+ movement in the toad bladder.

In Table III it can be seen that the NH_4^+ excretion rate was lower with Ringer solution than when plasma was used on the serosal side (Table II). Since the Ringer solution contained an amount of glutamine similar to that found in other amphibia[15], this suggests that there is something other than glutamine in the plasma which contributes to NH_4^+ formation.

In the s.c.c. experiments pooled plasma was used on the serosal side because the average excretion rate of NH_4^+ was significantly higher than when Ringer solution was used. The H^+ concentration achieved in the mucosal medium ranged from 13 to 15 times that of the serosal medium at the end of the flux period. This then confirms that the H^+ excretion was against an electrochemical gradient and suggests that it is an active process. Since the mucosal medium was bicarbonate free the change in pH must be by H^+ excretion and not by HCO_3^- reabsorption as in the case of the turtle bladder reported by SCHILB AND BRODSKY[6].

STEINMETZ[7] and STEINMETZ *et al.*[8] have reported, on experiments performed in the absence of exogenous CO_2 and HCO_3^- that H^+ excretion occurs when the spontaneous PD is abolished by s.c.c. Findings in their s.c.c. experiments are similar to what we have found in the acidotic toad bladder. In addition, we have shown that the toad bladder excretes a greater amount of H^+ in response to a metabolic acidosis and is also capable of excreting NH_4^+.

It is interesting to speculate on the relationship between the results reported here and those reported by LEAF *et al.*[18]. They reported that Na^+ transport in the toad bladder is stimulated by acidification of the mucosal medium. Our findings in correlation with theirs suggest that there is a physiological reinforcing mechanism whereby the toad during metabolic acidosis conserves more Na^+.

The urinary bladder of the toad, having these two excretory functions, can serve as a histologically simpler structure in which to study H^+ and NH_4^+ excretion at the cellular level.

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