# Prostaglandins as Mediators of Acidification in the Urinary Bladder of *Bufo marinus*[1] (43046)

LOY W. FRAZIER AND THOMAS YORIO

*Department of Physiology, Baylor College of Dentistry, Dallas, Texas 75246 and Department of Pharmacology, Texas College of Osteopathic Medicine, Forth Worth, Texas 76107*

*Abstract.* **Experiments were performed to determine whether prostaglandins (PG) play a role in $H^+$ and $NH_4^+$ excretion in the urinary bladder of *Bufo marinus*. Ten paired hemibladders from normal toads were mounted in chambers. One was control and the other hemibladder received $PGE_2$ in the serosal medium ($10^{-5}$ *M*). $H^+$ excretion was measured by change in pH in the mucosal fluid and reported in units of nmol (100 mg tissue)$^{-1}$ (min)$^{-1}$. $NH_4^+$ excretion was measured colorimetrically and reported in the same units. The control group $H^+$ excretion was 8.4 ± 1.67, while the experimental group was 16.3 ± 2.64 ($P < 0.01$). The $NH_4^+$ excretion in the experimental and control group was not significantly different. Bladders from toads in a 48-hr $NH_4^+Cl$ acidosis (metabolic) did not demonstrate this response to $PGE_2$ ($P > 0.30$). Toads were put in metabolic acidosis by gavaging with 10 ml of 120 m*M* $NH_4^+Cl$ 3 × day for 2 days. In another experiment, we measured levels of PG in bladders from control (N) and animals placed in metabolic acidosis (MA). Bladders were removed from the respective toad, homogenized, extracted, and PG separated using high-pressure liquid chromatography and quantified against PG standards. The results are reported in ng (mg tissue)$^{-1}$. $PGE_2$ fraction in N was 1.09 ± 0.14 and in MA was 3.21 ± 0.63 ($P < 0.01$). $PGF_{1\alpha}$, $F_{2\alpha}$ and $I_2$ were not significantly different in N and MA toads. Bladders were also removed from N and MA toads, and incubated in Ringer's solution containing [$^3$H]arachidonic acid (0.2 μCi/ml) at 25°C for 2 hr. Bladders were then extracted for PG and the extracts separated by thin layer chromatography. PG were identified using standards and autoradiography, scraped from plates, and counted in a scintillation detector. The results are reported in cpm/mg tissue × hr ±SEM. In MA toads, PG6-keto-$F_{1\alpha}$ = 1964 ± 342, $PGF_{2\alpha}$ = 1016 ± 228, and $PGE_2$ = 904 ± 188; in N animals PG6-keto-$F_{1\alpha}$ = 625 ± 280, $PGF_{2\alpha}$ = 364 ± 85, and $PGE_2$ = 404 ± 104; ($P < 0.01$, <0.025, <0.05, respectively). We conclude that $PGE_2$ may be an important mediator of $H^+$ excretion in toad urinary bladder and that endogenous $PGE_2$ levels are increased in response to MA.** [P.S.E.B.M. 1990, Vol 194]

It has been known for some time that the urinary bladder of *Bufo marinus* excretes both $H^+$ and $NH_4^+$ (1–3) and hence contributes to the overall acid base balance of the animal. Additionally, it was shown in these same studies that during stimulation by metabolic acidosis, the bladder increases its capacity to excrete both of these ions. The skin of *Rana pipiens*, another tissue analogous to the mammalian distal nephron, also has been shown to have the ability to excrete $H^+$ and $NH_4^+$ and this excretion increases during metabolic acidosis (4, 5).

Prostaglandins (PG) are known to be mediators involved in many physiologic mechanisms. PG have been reported to act in regulation of water reabsorption during antidiuretic hormone stimulation in the toad urinary bladder (6). It also has been suggested by Kokko (7) that PG may serve as possible natriuretic factors in the mammalian nephron. In addition, a preliminary report has suggested a possible role for PG in $H^+$ excretion in the skin of *R. pipiens* (8).

The purpose of this study was to determine whether exogenous PG might regulate $H^+$ or $NH_4^+$ excretion in toad urinary bladder. With the use of high-pressure liquid chromatography and thin layer chromatography, we measured endogenous levels of PG as well as turnover rate of PG in both normal and metabolic acidotic toads. We also determined the dose response for PG in both the normal and acidotic toad urinary bladder.

[1] Presented in part at the FASEB Meeting, March 1989, New Orleans, LA.

Received October 5, 1989. [P.S.E.B.M. 1990, Vol 194]
Accepted January 6, 1990.

0037-9727/90/1941-0010$2.00/0

## Materials and Methods

Hemibladders from the toad *B. marinus* of either Columbian or Mexican origin were used in these studies. The animals were supplied by Carolina Biological Supply, Burlington, NC, and were maintained in the laboratory in an amphibian tank and fed once a week. Toads were either in a normal state or in an $NH_4Cl$-induced acidosis. In the experiments with exogenous PG, paired hemibladders were used, one serving as the control and the other as the experimental bladder. Metabolic acidosis was induced by gavaging 3 × day for 2 days with 10 ml of 120 m*M* $NH_4Cl$. Hemibladders were removed after double pithing and mounted between Lucite chambers, each of which held 2 ml of the indicated solution. The mucosal solution was a 1.5 m*M* $PO_4$ buffered Ringer's solution containing in m*M*: NaCl, 114.5; KCl, 3.0; $CaCl_2$, 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. The serosal solution was the same as the mucosal solution for the control hemibladders, but the experimental serosal solution also contained either $PGE_2$ or $PGF_{2\alpha}$ at various indicated concentrations. All PG as well as the quinacrine and mezerein used in these studies were obtained from Sigma Chemical Co., St. Louis, MO. In one experiment $10^{-8}$ *M* mezerein was placed in the serosal bath instead of the PG. The mucosal fluid was bubbled with room air throughout the experiment.

$H^+$ fluxes were determined in paired hemibladders. Following a 15-min equilibration period, the mucosal fluid was changed and a 2-hr flux period was begun. At the end of this time, the bladder was removed and the wet weight obtained. In all experiments, the $H^+$ excretion was calculated from change in pH of the mucosal medium using the Henderson-Hasselbach equation. The concentration of $NH_4^+$ was determined colorimetrically according to the method of Chaney and Marbach (9). The excretion rates were calculated and reported as nmol (100 mg tissue wt weight)$^{-1}$ (min)$^{-1}$. Differences between the paired hemibladders were analyzed using a paired Student's *t* test. The dose-response analysis was represented as the mean difference between the experimental and control hemibladders.

Prostaglandin analyses were performed on bladders from normal and metabolic acidotic toads. PG were quantified after rapid extraction using an Octadecylsilyl silica column (Sep-Pak $C_{18}$ cartridge from Waters Associates, Millford, MA). The bladders were removed, weighed, placed in cold Ringer's solution (2–4°C), and homogenized. The medium was then acidified to pH 3.5 with formic acid. The acidified sample was applied to the column and the column washed with 20 ml of water to remove the polar lipids; 20 ml of 15% ethanol was passed through the column followed by 20 ml of water to remove the ethanol. The water was removed with petroleum ether and the fatty acids were then eluted with 20 ml of petroleum ether:chloroform (65:25 v/v). PG were removed using 10 ml of 100% methyl formate. The PG eluate was dried under $N_2$ and reconstituted in high-pressure liquid chromatography mobile phase.

The PG were analyzed according to the method of Terragno *et al.* (10), using a dual piston pump Beckman model 244 system with a variable wave length UV detector as a monitoring device, a single reverse phase Octadecylsilyl silica (25 cm × 14.5 mm) ultrasphere $C_{18}$ column and a UV detector at 192.5 nm. All separations were accomplished using a mobile phase of acetonitrile:phosphoric acid (32.8:67.2 v/v) at a flow rate of 1.7 ml/min (Fig. 1). The amount of PG fraction was determined by comparing against known standards and reported as ng (mg tissue)$^{-1}$.

PG were also determined in another group of 10 toads that were put in metabolic acidosis as described above. This group of toads in addition to receiving the $NH_4Cl$ loading was loaded with quinacrine hydrochloride at a dose of 10 μg/g of body weight. The toads received this dose two times daily, beginning the day before $NH_4Cl$ loading and continuing for 72 hr. All injections were made in the dorsal lymph sac of the animal. At the end of this time, the bladders were removed and analyzed for $PGE_2$ and given above.

PG synthesis was measured in normal and acidotic toad urinary bladders. Quarter bladders were placed in 2 ml of Ringer's containing [$^3$H]arachidonic acid (0.2 μCi/ml) (New England Nuclear, Boston, MA) and labeled for 2 hr at 25°C. The tissue was homogenized and extracted with acidified acetone (5% HCl in acetone) at 4°C and dried under $N_2$. The samples were then redissolved in 50 μl of chloroform and spotted on silica gel plates for thin layer chromatography. The solvent used in the chromatography chamber contained in ml: ethyl acetate, 110; iso-octane, 50, glacial acetic acid, 20; and water, 100. The PG were identified using PG standards and autoradiography. The PG were scraped from the plate and counted in a Beckman scintillation detector. All samples were counted to an accuracy of <2% and were corrected for background. The results are reported in cpm (μ*M* tissue $PO_4$)$^{-1}$ (hr)$^{-1}$. Tissue $PO_4$ was determined by the modified method of Chalvardjian and Rudnicki (11). Differences in PG concentrations were determined using the non-paired Student's *t* test.

## Results

Shown in Table I are the results of exogenous $PGE_2$ on $H^+$ and $NH_4^+$ excretion in urinary bladder. In bladders from normal toads, it is clear that $PGE_2$ stimulates $H^+$ excretion by approximately 100% ($P < 0.01$). $NH_4^+$ excretion was not stimulated by $PGE_2$ in the

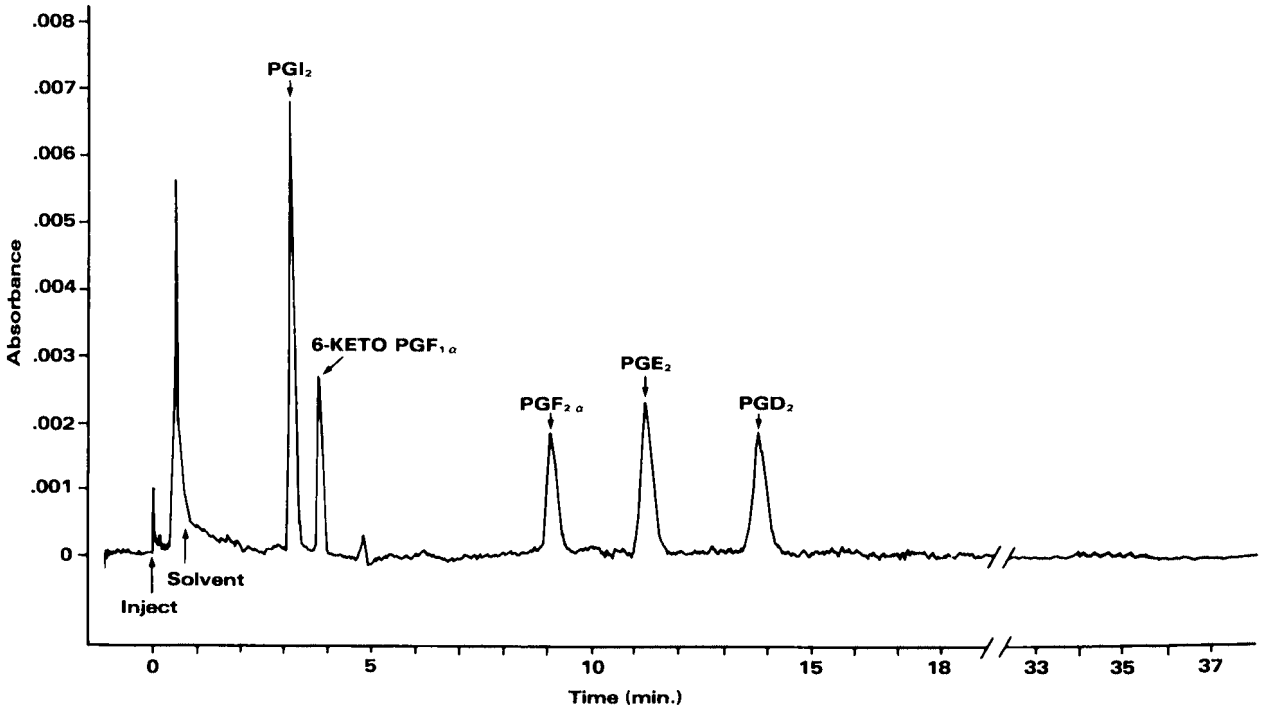


**Figure 1.** High-pressure liquid chromatogram showing separation of prostaglandins. A mixture of the compounds was applied to an Ultrasphere $C_{18}$ column (250 × 4.6 mm) and eluted isocratically with premixed solvents consisting of 0.017 *M* $H_3PO_4$:$CH_3CN$ (67.2:32.8 v/v), having a flow rate of 1.7 ml/min; UV detection at 192.5 nm.

**Table I.** Effect of Exogenous $PGE_2$ on $H^+$ and $NH_4^+$ Excretion in Toad Urinary Bladder

| State of toad | Excretion in nmol (100 mg tissue)$^{-1}$ (min)$^{-1}$ | | | |
|---|---|---|---|---|
| | $H^+$ Excretion[a] | *P* | $NH_4^+$ Excretion[a] | *P* |
| Normal ($PGE_2$, $10^{-5}$ *M*) | 16.3 ± 2.64 | <0.01 | 5.5 ± 1.32 | NS[c] |
| Normal (control) | 8.4 ± 1.67 | | 3.2 ± 0.48 | |
| Metabolic acidosis[b] ($PGE_2$, $10^{-5}$ *M*) | 25.5 ± 4.41 | NS | 4.2 ± 0.92 | <0.025 |
| Metabolic acidosis[b] (control) | 32.7 ± 6.31 | | 7.3 ± 1.40 | |

[a] Each value represents the average of 10 bladders ± SEM.
[b] Toads were in a 48-hr $NH_4Cl$-induced acidosis before bladders were removed.
[c] NS, not significant.

normal bladders. Bladders from toads in metabolic acidosis (high $H^+$ excretion rate) did not show further stimulation by $PGE_2$ (not significant). However, the $NH_4^+$ excretion was decreased in this group of toads compared with the control group.

To determine whether this response to $PGE_2$ was seen with other prostanoids, the effect of exogenous $PGF_{2\alpha}$ on $H^+$ and $NH_4^+$ excretion in toad bladder was determined. In the normal bladder, there was no effect on either $H^+$ or $NH_4^+$ excretion (not significant) by $PGF_{2\alpha}$ ($10^{-5}$ *M*). Likewise, bladders from toads in metabolic acidosis were not altered by this same dose of $PGF_{2\alpha}$ (data not shown).

Tables II and III show the dose response of $PGE_2$ in normal and acidotic toad urinary bladders. In the normal bladder, $PGE_2$ stimulated $H^+$ excretion at $10^{-5}$ and $10^{-6}$ *M*. At $10^{-7}$ *M*, there was an inhibition of $H^+$ excretion. There was no effect on $H^+$ excretion at lower concentrations. $PGE_2$ did not stimulate $NH_4^+$ excretion at any of the doses tested. Table III shows that $PGE_2$, at any concentration tested, had no effect on $H^+$ excretion in the bladder from acidotic toads.

In the next series of experiments, PG concentrations in urinary bladders from normal toads and toads in metabolic acidosis were determined to see whether endogenous PG were changed during an altered metabolic state (Table IV). Of the four PG detected, only $PGE_2$ was significantly increased above normal during

**Table II.** Dose-Response Analysis of the Effect of $PGE_2$ on $H^+$ and $NH_4^+$ Excretion in Normal Toad Urinary Bladder

| $PGE_2$ in serosal solution ($M$) | Excretion in nmol (100 mg bladder)$^{-1}$ (min)$^{-1}$ | | | |
|---|---|---|---|---|
| | $H^+$ Excretion[a] (experimental-control) | $P^b$ | $NH_4^+$ Excretion (experimental-control) | $P$ |
| $10^{-5}$ (10)[c] | 7.8 ± 2.16 | <0.01 | 2.2 ± 0.90 | NS |
| $10^{-6}$ (10) | 5.0 ± 1.23 | <0.025 | 0.0 | NS |
| $5 \times 10^{-7}$ (10) | 1.1 ± 2.31 | NS[d] | −0.5 ± 0.53 | NS |
| $10^{-7}$ (10) | −3.7 ± 1.98 | <0.03 | 0.5 ± 0.57 | NS |
| $10^{-8}$ (9) | 1.6 ± 2.49 | NS | 0.5 ± 1.00 | NS |

[a] Mean difference ± SE.
[b] Calculated from the mean difference.
[c] Number of paired hemibladders.
[d] NS, not significant ($P > 0.05$).

**Table III.** Dose-Response Analysis of the Effect of $PGE_2$ on $H^+$ and $NH_4^+$ Excretion in Toad Urinary Bladder from Acidotic Toads

| $PGE_2$ in serosal solution ($M$) | Excretion in nmol (100 mg bladder)$^{-1}$ (min)$^{-}$ | | | |
|---|---|---|---|---|
| | $^1H^+$ Excretion[a] (experimental-control) | $P^b$ | $NH_4^+$ Excretion (experimental-control) | $P$ |
| $10^{-5}$ (12)[c] | −7.3 ± 5.36 | NS[d] | −3.2 ± 1.2 | NS |
| $10^{-6}$ (8) | 8.0 ± 5.86 | NS | 0.1 ± 1.3 | NS |
| $10^{-7}$ (10) | 1.3 ± 3.03 | NS | −1.5 ± 1.0 | NS |

[a] Mean difference ± SE.
[b] Calculated from the mean difference.
[c] Number of paired hemibladders.
[d] NS, not significant ($P > 0.05$).

**Table IV.** Prostaglandin Concentrations in Toad Urinary Bladder from Normal and Metabolic Acidotic Toads

| PG Fraction | Concentration in ng (mg tissue)$^{-1}$ | | |
|---|---|---|---|
| | Normal toad | Metabolic acidotic toad | $P$ |
| $PGI_2$ | 4.45 ± 1.20[a] | 9.70 ± 5.01[a] | NS |
| 6-Keto-$PGF_{1\alpha}$ | 6.58 ± 2.10 | 13.65 ± 7.14 | NS |
| $PGF_{2\alpha}$ | 1.73 ± 1.05 | 0.20 ± 0.08 | NS |
| $PGE_2$ | 1.09 ± 0.14 | 3.12 ± 0.63 | <0.025 |

[a] Each value represents the average of 10 bladders ± SEM.

**Table V.** Incorporation of $^3H$ into PG from Arachidonic Acid in Normal and Acidotic Toad Urinary Bladder

| PG Fraction | Normal toads[a] ($n = 7$) | Acidotic toads[a] ($n = 8$) | $P$ |
|---|---|---|---|
| 6-Keto-$F_{1\alpha}$ | 625 ± 280 | 1964 ± 342 | <0.01 |
| $F_{2\alpha}$ | 364 ± 85 | 1016 ± 228 | <0.025 |
| $E_2$ | 404 ± 104 | 904 ± 188 | <0.05 |

[a] Expressed as cpm ($\mu$mol tissue $PO_4$)$^{-1}$ (hr)$^{-1}$ ± SEM.

acidosis ($P < 0.025$). $PGE_2$ was increased by approximately 150% as seen in the representative values in the table. Shown in Table V is the incorporation of [$^3H$] arachidonic acid into PG in toad urinary bladder. All fractions, 6-keto-$F_{1\alpha}$, $F_{2\alpha}$ and $E_2$ demonstrated increased incorporation of $^3H$ from arachidonic acid when the toad was in metabolic acidosis ($P < 0.01$, <0.025, and 0.05, respectively).

The concentration of $PGE_2$ was determined in the bladders from toads that were in a $NH_4Cl$ metabolic acidosis and also treated with quinacrine hydrochloride, a known inhibitor of phospholipase $A_2$ (12). Quinacrine abolished the increase in $PGE_2$ which was seen during metabolic acidosis. $PGE_2$ formation went from 3.12 ± 0.63 ng (mg tissue)$^{-1}$ in MA animals to 0.63 ± 0.04 ng (mg tissue)$^{-1}$ in MA animals treated with quinacrine ($P < 0.005$).

Table VI demonstrates the effect of mezerein, a known activator of protein kinase C activity. Mezerein placed on the serosal surface of the bladder resulted in approximately a 50% increase in $H^+$ excretion ($P < 0.05$). There was no stimulation of $NH_4^+$ excretion observed at this concentration of mezerein.

## Discussion

Exogenous $PGE_2$, when placed on the serosal side of the normal toad bladder at $10^{-5}$ $M$ concentration, was found to increase $H^+$ excretion by approximately 100%. There was no stimulation of $NH_4^+$ excretion observed at this concentration. When the animal was

**Table VI.** Effect of Mezerein on $H^+$ and $NH_4^+$ Excretion in Normal Toad Urinary Bladder

| | Excretion in nmol (100 mg bladder)$^{-1}$ (min)$^{-1}$ | | | |
|---|---|---|---|---|
| | $H^+$ Excretion | $P$ | $NH_4^+$ Excretion | $P$ |
| Experimental ($10^{-8}$ $M$ mezerin) | 7.6 ± 1.21[a] | | 1.9 ± 0.34[a] | |
| | | <0.05 | | NS[b] |
| Control | 5.0 ± 1.15[a] | | 2.5 ± 0.63[a] | |

[a] Average of 10 paired hemibladders ± SEM.
[b] NS, not significant.

placed in a metabolic acidosis, this stimulation by exogenous $PGE_2$ was absent. During metabolic acidosis, the urinary bladder was excreting $H^+$ at maximal rates (2) and we assume that no further stimulation by $PGE_2$ was possible. It has been reported that $PGE_2$ was an important regulator of antidiuretic hormone water permeability response in the toad urinary bladder (6), and that changes in extracellular pH can enhance $PGE_2$ production in toad urinary bladder epithelium (13). Our studies suggest that $PGE_2$ may also play a role in regulating $H^+$ excretion in the toad bladder during acid base changes in the animal. We did observe in the animals in metabolic acidosis that $PGE_2$ actually inhibited $NH_4^+$ excretion. The reason for this inhibition is not apparent from this study.

In the frog skin, a tissue similar to toad urinary bladder, it was found that exogenous $PGF_{2\alpha}$ inhibits $H^+$ excretion at low concentrations ($ED_{50} = 5 \times 10^{-8}$ $M$) (8). Therefore, we wanted to test this compound in the toad bladder. Our results indicated that $PGF_{2\alpha}$, at concentrations similar to $PGE_2$ that enhanced proton excretion, had no effect on $H^+$ excretion in the toad bladder. This was most likely due to a dose-response and species differences or in predominate receptor types.

The dose-response analysis revealed that $PGE_2$ was effective in stimulating $H^+$ excretion at $10^{-5}$ and $10^{-6}$ $M$ concentrations in bladders from normal toads. These concentrations are in the same general range for $PGE_2$ effects on $Na^+$ transport in other epithelial tissue. It has been reported that $PGE_2$, at a concentration of $10^{-6}$ $M$, stimulated the short circuit current in the toad urinary bladder (14). Larsson and Anggard (15) also reported that $PGE_2$ decreased the potential difference across the cortical tubule. At these concentrations, $PGE_2$ stimulates adenylcyclase activity (16) and increasing intracellular cAMP concentrations have been shown to enhance $H^+$ excretion (17). Such effects on adenylcyclase could account for the stimulation of $H^+$ excretion by $PGE_2$ in control animals. At low $PGE_2$ concentrations adenylcyclase is inhibited in toad urinary bladder (16, 18). It was interesting to note that at $10^{-7}$ $M$, $PGE_2$ inhibited $H^+$ excretion in the toad bladder. A dose response for this inhibitory effect could not be ascertained, since $5 \times 10^{-7}$ $M$ and $10^{-8}$ $M$ had no additional effect on $H^+$ excretion. The reason for this lack of dose response will have to be investigated in future experiments, but it could reflect the opposite actions of $PGE_2$ on $H^+$ excretion at low versus high doses. In turtle urinary bladder, $PGE_2$ was found to be a potent inhibitor of $H^+$ excretion (19). There was no effect on $H^+$ excretion found between $10^{-5}$ and $10^{-7}$ $M$ in the toad that was in metabolic acidosis. Alternatively, the enhanced $H^+$ excretion by $PGE_2$ could have occurred as a result of an enhanced $Na^+$ transport, thereby increasing $CO_2$ production. This increase in $CO_2$ could, through carbonic anhydrase, provide an increased proton supply.

We were able to isolate and separate four different PG or metabolites from the bladder. $PGI_2$, $PGF_{2\alpha}$, and $PGE_2$, along with the chief metabolite of $PGI_2$, 6-keto-$PGF_{1\alpha}$, were found in bladders from both normal toads and toads in metabolic acidosis. However, only $PGE_2$ was found to increase significantly in response to a metabolic acidosis. This is consistent with the finding of Forrest and Goodman (13) that serosal acidification inhibits the water flow response to vasopressin in part by stimulating the biosynthesis of PGE. This suggests that the decrease in pH of body fluid stimulates the synthesis of $PGE_2$, which in turn may stimulate $H^+$ excretion.

In an earlier study by Frazier (20), it was shown that the vasopressin-stimulated water flow response in the toad urinary bladder was reduced by placing the animal in a $NH_4Cl$ acidosis. It was suggested in this study that the acid pH brings about an increased production of PG, which in turn inhibits the bladder's response to vasopressin. This also supports the hypothesis that PG synthesis may be increased by an acidic pH and hence may lead to PG stimulation of $H^+$ excretion.

In Figure 1, it is clear that another fraction of PG was noted coming off of the column at approximately 14 min. This correlated with our $PGD_2$ standard. However, this fraction was seen in only 3 of the 10 normal toads and 1 of the 10 toads in metabolic acidosis. We, therefore, did not consider this an important and/or a consistent finding.

In determining PG synthesis from its precursor arachidonic acid, we measured the incorporation of $^3H$ from arachidonic acid into the PG fractions. All three fractions isolated, 6-keto-$F_{1\alpha}$, $F_{2\alpha}$, and $E_2$ had an increased incorporation when the toad was placed in metabolic acidosis. These results suggest that the formation of PG from arachidonic acid is stimulated by metabolic acidosis and this may be related to an increased activity of phospholipase $A_2$. Studies on the enzymatic activity of the arachidonic acid cascade during acidosis will have to be completed before this can be confirmed.

Quinacrine, an inhibitor of phospholipase $A_2$ (21), prevented the increase in $PGE_2$ concentrations in animals placed in metabolic acidosis; in fact, the concentration fell below that seen in normal toads. These results suggest that an increase in phospholipase $A_2$ activity is involved in the response of the PG to acidosis. This also supports the hypothesis that acidosis stimulates phospholipase $A_2$ activity in the bladder and this in turn leads to an increase in the concentration of $PGE_2$ which enhances $H^+$ excretion.

Changes in phospholipid turnover are associated with alterations in the metabolic state of the toad (22). Such effects could alter the activity of the phospholipid-dependent enzyme, protein kinase C. Protein kinase C may thus also be involved in the regulation of $H^+$ excretion in the toad bladder. In our experiments with mezerein, a non-phorbol activator of protein kinase C, we found a 50% increase in $H^+$ excretion in bladders exposed to mezerein. This was not as great as the stimulation observed with $PGE_2$ (100%) but was presumptive evidence that the activation of kinase C may be involved in the stimulation of $H^+$ excretion.

Phorbol esters, activators of protein kinase C, also have been shown to alter $Na^+/H^+$ exchange in many tissues (23) and to inhibit proton excretion in intestinal epithelial cells (24). However, additional studies are yet needed to uncover the role of "second messengers" in the acidification process in renal epithelia as it relates to changes in the metabolic state.

The results of our study support the following conclusions: (i) The urinary bladder of *B. marinus* contains significant amounts of $PGI_2$, 6-keto-$PGF_{1\alpha}$, $PGF_2$, and $PGE_2$ and during $NH_4Cl$-induced acidosis $PGE_2$ is increased. (ii) Exogenous $PGE_2$ stimulates $H^+$ excretion in the urinary bladder at a concentration of $10^{-5}$ and $10^{-6}$ *M* when placed on the serosal surface. (iii) Metabolic acidosis in the toad results in enhanced phospholipase $A_2$ activity resulting in increased synthesis of $PGE_2$. (iv) $PGE_2$ may be an important mediator of $H^+$ excretion and plays a regulatory role in the maintenance of acid base balance in the urinary bladder of *B. marinus*.

This work was supported in part by a Research Enhancement Grant from Texas College of Osteopathic Medicine and Baylor College of Dentistry Research Funds.

We wish to thank Mrs. Teresa Traylor for her expert technical assistance during this study and Ms. Robin Shepherd for typing and proofing the manuscript.

1. Frazier LW, Vanatta JC. Excretion of $H^+$ and $NH_4^+$ by the urinary bladder of the acidotic toad and the effect of short-circuit current on the excretion. Biochim Biophys Acta **214**:20–29, 1971.
2. Frazier LW. Characteristics of proton excretion in normal and acidotic toad urinary bladder. Biochim Biophys Acta **817**:75–84, 1985.
3. Ludens JH, Fanestil DS. Acidification of urine by the isolated urinary bladder of the toad. Am J Physiol **223**:1338–1344, 1972.
4. Vanatta JC, Frazier LW. The epithelium of *Rana pipiens* excretes $H^+$ and $NH_4^+$ in acidosis and $HCO_3^-$ in alkalosis. Comp Biochem Physiol **68A**:511–513, 1981.
5. Frazier LW. Acidification of the bathing fluid by the skin of *Rana pipiens* in metabolic acidosis. Comp Biochem Physiol **85A**:57–61, 1986.
6. Handler JS. Vasopressin-prostaglandin interactions in the regulation of epithelial cell permeability to water. Kidney Int **19**:831–838, 1981.
7. Kokko JP. Effect of prostaglandins on renal epithelial electrolyte transport. Kidney Int **19**:791–796, 1981.
8. Page RD, Buttar S, Yorio T. $PGF_{2\alpha}$ regulation of $H^+$ excretion in the integument of *Rana pipiens*. FASEB J **3**:A563, 1989.
9. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem **8**:130–132, 1962.
10. Terragno A, Rydzik R, Terragno NA. High performance liquid chromatography and UV detection for the separation and quantitation of prostaglandins. Prostaglandins **21**:101–112, 1981.
11. Chalvardjian A, Rudnicki E. Determination of lipid phosphorus in the nanomolar range. Anal Biochem **36**:225–226, 1970.
12. Yorio T, Bentley PJ. Mechanism of action of aldosterone: The role of phospholipase A. Nature **271**:78–81, 1978.
13. Forrest JN, Goodman DBP. Prostaglandin $E_2$ mediates the effect of pH on ADH-stimulated water flow but vasopressin does not stimulate prostaglandin $E_2$ production in the toad urinary bladder. Clin Res **28**:445A, 1980.
14. Halushka PV, Levanko A, Auber M. Prostacyclin stimulates short circuit current in the toad (*Bufo marinus*) urinary bladder. In: Samuelsson B, Ramwell PW, Paoletti R, Eds. Advances in Prostaglandin Thromboxane Research. Raven Press: New York, Vol **8**: pp1269–1272, 1980.
15. Larsson C, Anggard E. Mass spectrometric determinations of prostaglandin $E_2$, $F_{2\alpha}$, and $A_2$ in the cortex and medulla of the rabbit kidney. J Pharm Pharmacol **28**:326–328, 1976.
16. Yorio T, Henry SL, Hodges DH, Caffrey JL. Role of calcium and prostaglandins in the antidiuretic hormone response. Effect of ionophore A23187. Biochem Pharmacol **32**:1113–1118, 1983.
17. Frazier LW. The effect of catecholamines on $H^+$ and $NH_4^+$ excretion in toad urinary bladder. Comp Biochem Physiol **75C**:321–326, 1983.
18. Omachi RS, Robbie DE, Handler JS, Orloff J. Effects of ADH and other agents on cyclic-AMP accumulation in toad urinary bladder epithelium. Am J Physiol **226**:1152–1157, 1974.
19. Ascer K, Lowenstein I, Mirtz B, Schwartzman M, Lief P. Prostaglandin $E_2$ inhibits $H^+$ excretion in the turtle bladder. Kidney Int **25**:228A, 1984.
20. Frazier LW. The effect of acid-base changes on vasopressin-stimulated water flow in toad urinary bladder. Life Sci **26**:1843–1849, 1980.
21. Rainsford KD. Inhibitors of eicosanoids metabolism. In: Curtis-Prior PB, Ed. Prostaglandins: Biology and Chemistry of Prostaglandins and Related Eicosanoids. New York: Churchill Livingstone, pp52–68, 1988.
22. Frazier LW, Yorio T. Phospholipid changes during adaptation to acidosis in urinary bladder of *Bufo marinus*. Proc Soc Exp Biol Med **190**:339–343, 1989.
23. Grinstein S, Elder B, Furuya W. Phorbol ester induced changes in cytoplasmic pH in neutrophils: Role of exocytosis in $Na^+$-$H^+$ exchange. Am J Physiol **248**:C379–C386, 1985.
24. Shimada T, Hoshi T. $Na^+$ dependent elevation of the acidic cell surface pH (microclimate pH) or rat jejunal villus cells induced by cyclic nucleotides and phorbol esters: Possible mediators of the regulation of the $Na^+/H^+$ antiporter. Biochim Biophys Acta **937**:328–332, 1988.