J. exp. Biol. **119**, 199–209 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

PARTITIONING OF REGULATORY SITES IN *BUFO MARINUS* DURING HYPERCAPNIA

BY BRUCE L. TUFTS AND DANIEL P. TOEWS

Department of Biology, Acadia University, Wolfville, Nova Scotia B0P 1X0, Canada

Accepted 28 May 1985

SUMMARY

Ureters were cannulated in specimens of *Bufo marinus* (L.) in order to partition the regulatory contributions of the kidney and skin. The *in vivo* roles of the kidney, skin and internal calcareous deposits in the response of these animals to chronic hypercapnia were then evaluated.

There was no compensatory adjustment by the skin and only a minimal regulatory response by the kidney. Major adjustments which have been attributed to combined skin and urinary tract in previous studies must therefore come from the urinary bladder. Removal of the bladder as a regulatory site in these animals completely eliminated the compensatory elevation of HCO_3^- in the extracellular fluid. Mobilization of internal calcareous deposits as a source of HCO_3^- was found to contribute 50 % of the compensatory response of these animals during hypercapnia.

INTRODUCTION

Although both the extent and the time course of pH regulation during acid-base disturbances are well documented in various amphibians, the underlying mechanisms and sites involved in the regulatory adjustments of these animals are less well defined. Although amphibians have several major regulatory sites (skin, bladder, kidneys, lungs, gills and buccopharyngeal mucosa), the majority of studies have concentrated on the regulatory capability of an individual site (i.e. the perfused kidney or the isolated hemibladder) and few have attempted to integrate the relative contribution of each organ in a study of the whole animal. This is probably because it is difficult to partition the numerous regulatory sites.

The changes in acid-base status of the anuran *Bufo marinus* resulting from chronic hypercapnia (5 % CO_2 exposure) have been well documented (Boutilier, Randall, Shelton & Toews, 1979*a*; Toews & Heisler, 1982), as has the animal's ventilatory response to elevated CO_2 (Macintyre & Toews, 1976; Boutilier *et al.* 1979*a*). The response includes a partial pH compensation as a result of elevations in both extra- and intracellular bicarbonate (Toews & Macintyre, 1978; Boutilier *et al.* 1979*a*; Toews & Heisler, 1982). The source of this compensatory bicarbonate is a question which remains unresolved. Toews & Heisler (1982) found that much of the increase (50 %)

Key words: Kidney, hypercapnia, bladder, skin.

may result from increased ammonia excretion. However, these authors did not differentiate between urinary tract and cutaneous routes. Although not an osmoregulatory site *per se*, the calcareous deposits of the endolymphatic sacs of anuran amphibians have also been suggested as a source of HCO_3^- during hypercapnia (Simkiss, 1968; Robertson, 1972). The objective of this study was to define not merely the capacity of these sites to combat acid-base imbalance, but the actual regulatory contributions to the rise in internal HCO_3^- of the skin, kidney and calcareous deposits in the response of *Bufo marinus* to chronic hypercapnia.

MATERIALS AND METHODS

Adult *Bufo marinus* of both sexes, obtained from Charles D. Sullivan Co. Inc. (Nashville, TN, U.S.A.), were used for all experiments. The animals were kept in tilted, fibreglass aquaria (0·8 × 0·7 × 0·4 m) and provided with tap water at one end.

Toads were randomly chosen and chronically cannulated in the femoral artery (using the method of Boutilier *et al.* 1979*a*) for blood sampling and in both of the ureters for urine collection. Clay-Adams polyethylene tubing (P.E. 90) was used to cannulate the ureters. This technique involved a 1-cm dorsal incision, approximately 5 mm lateral to the spine and 2–3 cm proximal to the cloaca. Separation of the muscle layers ventral to this incision exposed the ureters. Prior to insertion, the cannula was lubricated with an inert vegetable oil to reduce friction in the fragile ureters and flared at the proximal end to prevent slippage. Upon insertion the cannula was fed *via* the ureter through the cloaca, and beyond the animal. The proximal end was then slipped through the puncture site into the ureter itself and secured in a position between the kidney and the initial puncture site. In this way, the steady drip of urine from the kidney was routed *via* the cannula to the exterior. To prevent slippage, the cannula was anchored to the wall of the cloaca using Ethicon-Mersilene surgical suture and a cyanoacrylate glue (Bostik no. 7332).

Following a 24-h recovery period, the animal was moved to the experimental chamber (described in Boutilier *et al.* 1979*a*) and a volume of water equal to three times the weight of the animal in grams was evenly distributed in the chamber.

After a 3- to 5-h adjustment period, during which time room air flowed through the chamber from gas mixing pumps (Wösthoff Digamix, type M/300a, Bochum, F.R.G.), control samples were taken. Approximately 0·6–0·7 ml of blood was first removed from the femoral catheter and a small portion immediately analysed for pH and blood gases. The remaining sample was centrifuged and plasma stored frozen for later electrolyte analysis. Although the effect of freezing of samples of plasma, urine and water was not assessed, the samples did not show any abnormalities such as volume loss or appearance of precipitate after thawing. Immediately following the blood sample, ureteral urine for pH and P_{CO_2} measurements was collected. Several methods were tried to minimize diffusive loss of CO_2 during urine collection. However, since this could not be avoided, a pH-logP_{CO_2} plot was constructed for toad ureteral urine. Urine for this series of determinations was collected from toads with ureteral catheters which were exposed to 5 % CO_2-95 % air for several hours prior to and during urine collection. Ureteral urine (10 ml) was equilibrated (in shaken tonometric vessels at 25 °C) with 1 %, 2 %, 4 %, 5 % and 7 % CO_2, the balance of

which was air. These water-saturated, CO_2-air mixtures were from Wösthoff gas mixing pumps. Urine was equilibrated for 1 h with each CO_2-air mixture and then immediately analysed for P_{CO_2} and pH. This semi-log relationship plot was found to be of the linear form $y = a + bx$ where $a = 24{\cdot}4$, $b = -3{\cdot}4$ and $r^2 = 0{\cdot}99$. The ureteral urine pH could then be read directly off the line from blood P_{CO_2} values if it is assumed that CO_2 equilibration occurs between arterial blood and urine in the toad, *Bufo marinus* (Long, 1982*a*,*b*). Ureteral urine was then allowed to flow into a tared glass test tube for 1 h as an indicator of urine flow rate. This sample was subsequently frozen for later analysis of titratable acidity and electrolytes. In some cases, collection of this sample persisted for more than 1 h when insufficient amounts of urine were obtained to measure all parameters after the first hour. Approximately 2 ml of water from the experimental chamber was also removed during the control period and frozen for later electrolyte analysis. Following the control period, the gas mixing pumps were adjusted to provide a 5 % CO_2-95 % air mixture in the experimental chamber.

The first hypercapnic blood, ureteral urine and water samples were taken after 1 h of CO_2 exposure and samples were treated identically to control samples. The same parameters were also measured at times of 2, 4, 12 and 24 h of exposure and after 24 h of recovery (24 h in flowing air). To provide enough urine for the measurement of all parameters, however, urine flow and titratable acidity samples were collected at the offset times of 3 h (5 h for titratable acidity only) and 23 h of hypercapnia and 23 h of recovery.

Blood and urine pH and P_{CO_2} measurements were made using Radiometer electrodes and a Radiometer PHM 72 acid-base analyser (Radiometer, Copenhagen, Denmark). The pH electrode was calibrated using Radiometer precision buffer solutions S1500 and S1510. The P_{CO_2} electrode was calibrated with 1 % and 5 % CO_2 with gases delivered by gas mixing pumps. The pH and P_{CO_2} electrodes were calibrated at temperatures thermostatically controlled to be the same as the animal (25 °C).

Plasma and ureteral urine HCO_3^- concentrations were calculated using the Henderson-Hasselbalch equation. The values used for αCO_2 and pK′ for plasma were those of Boutilier *et al.* (1979*a*); 0·033 and 6·05 respectively. The αCO_2 constant for ureteral urine was determined experimentally using the technique of Van Slyke, Sendroy, Hastings & Neill (1928). Approximately 10 ml of ureteral urine were acidified with lactic acid to eliminate HCO_3^- in solution. The urine was then equilibrated with 100 % CO_2 and P_{CO_2} and total CO_2 measurements were taken. Since $[HCO_3^-]$ is non-existent, αCO_2 is determined using the equation:

$$\alpha CO_2 = \frac{T_{CO_2}}{P_{CO_2}} \text{ (mmol l}^{-1}\text{ mmHg}^{-1}\text{)}.$$

The value for αCO_2 was 0·028. The value for urine pK′ was 6·31 (R. G. Boutilier, unpublished data).

Plasma, ureteral urine and water Na^+ and K^+ analysis were done using a model IL 443 Instrumentation Laboratory Flame Photometer (Instrumentation Laboratory, Lexington, MA). Plasma Cl^- was determined with a Radiometer CMT 10 chloride titrator (Radiometer, Copenhagen, Denmark) and ureteral urine and water Cl^- measurements were made with a Buchler-Cotlove chloridometer (Buchler

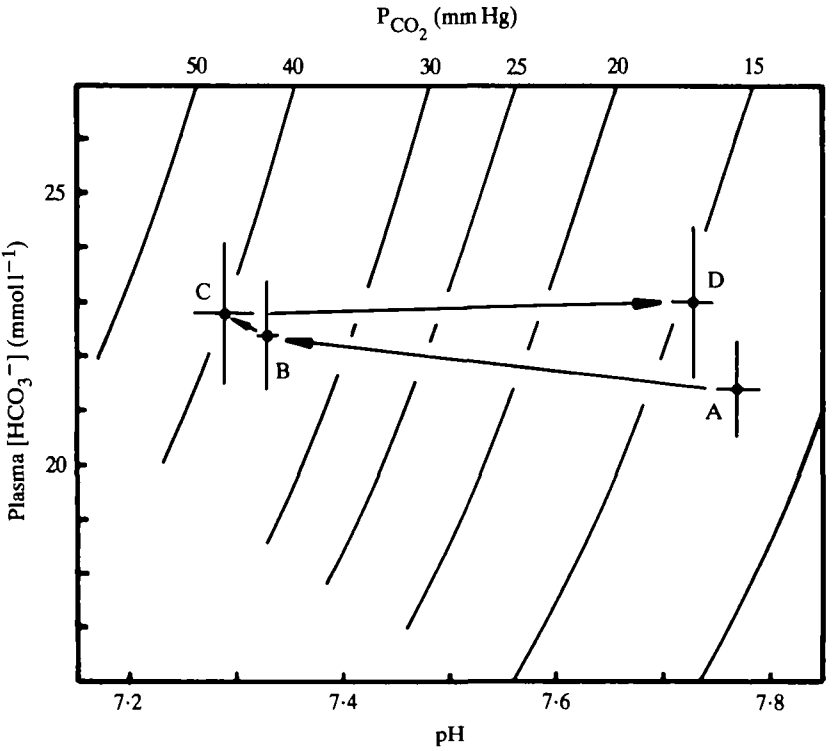

Fig. 1. Relationship between blood pH, P_{CO_2} and plasma HCO_3^- concentration and time of exposure to 5% CO_2 ($N = 11$). Mean normal values are represented by point A, mean 1 h values by point B, mean 24 h values by point C, and mean 24 h recovery values by point D. All values are means ± S.E.M.

Table 1. *Calculated pH and HCO_3^- concentration of ureteral urine*

	Time (h)						
	N	1	2	4	12	24	24 Rec
pH	6·33 ± 0·03	6·04 ± 0·05*	6·00 ± 0·04*	6·03 ± 0·05*	6·00 ± 0·05*	6·00 ± 0·03*	6·29 ± 0·05*
HCO_3^- (mequiv/l^{-1})	0·36 ± 0·01	0·52 ± 0·03*	0·53 ± 0·03*	0·52 ± 0·03*	0·53 ± 0·03*	0·53 ± 0·01*	0·38 ± 0·02*

N = normal; 1, 2, 4, 12 and 24 h = hours of exposure to 5% CO_2; 24 Rec = 24 h of recovery in air. Values are means ± one S.E.M. Asterisks denote significant ($P < 0·05$) difference from the normal.

Instruments, Inc., Fort Lee, NJ, U.S.A.). All Ca^{2+} measurements were made using a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer (Perkins-Elmer Corp., Norwalk, CT, U.S.A.). Ammonia in ureteral urine and water was determined with a micro-modification (McDonald, 1983) of the salicylate-hypochlorite method of Verdouw, van Echteld & Dekkers (1978). The method of McDonald & Wood (1981) was used for titratable acidity [TA−HCO_3^-] determinations using a Canlab (Canlab, McGaw Supply Ltd) combination pH electrode (Type H5503-21) coupled to a Radiometer PHM-84 research pH meter.

Significance of the results was assessed using a two-way analysis of variance. Differences were accepted as being significantly different at the 0·05 level.

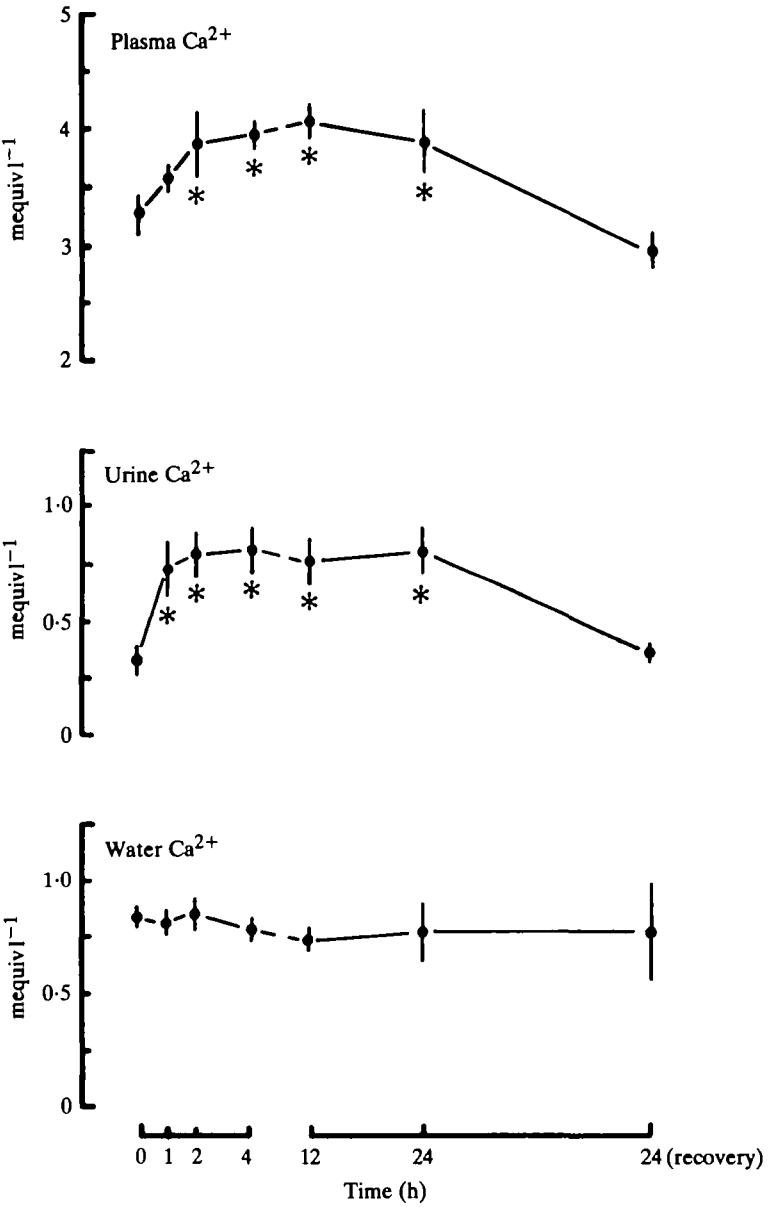

Fig. 2. Relationship between plasma ($N = 9$), ureteral urine ($N = 9$) and water ($N = 7$) Ca^{2+} concentrations and time of exposure to 5% CO_2. Ca^{2+} values are means ± S.E.M. Asterisks denote significant ($P < 0{\cdot}05$) differences from normal.

RESULTS

Mean arterial blood pH (pH_a) fell significantly from a control level of 7·77 to 7·33 in the first hour of hypercapnia. The pH_a remained significantly depressed from this control level for the remainder of the hypercapnic exposure time. However, after 24 h

Table 2. *Electrolyte composition (mequiv l^{-1}) of plasma, ureteral urine, and ambient water*

	Time (h)							
	N	1	2	4	12	24	24 Rec	
Na^+–plasma	106·5 ±3·6	109·8 ±5·0	110·8 ±3·6	101·2 ±7·1	106·7 ±6·7	104·1 ±6·6	105·9 ±6·9	$N = 11$
Na^+–urine	2·9 ±0·7	7·0 ±2·6	16·6 ±8·4*	7·6 ±4·2	8·5 ±3·1	4·3 ±2·1	1·5 ±0·7	$N = 9$
Na^+–water	0·3 ±0·1	0·3 ±0·1	0·4 ±0·1	0·5 ±0·3	0·8 ±0·4	0·6 ±0·3	0·4 ±0·3	$N = 7$
K^+–plasma	2·7 ±0·3	2·8 ±0·2	2·8 ±0·2	2·5 ±0·2	3·8 ±0·8	3·5 ±0·6	2·5 ±0·2	$N = 11$
K^+–urine	0·32 ±0·09	0·53 ±0·20	1·13 ±0·45*	0·51 ±0·16	0·73 ±0·22	0·50 ±0·16	0·17 ±0·11	$N = 9$
K^+–water	0·10 ±0·04	0·07 ±0·04	0·10 ±0·04	0·21 ±0·08	0·31 ±0·10*	0·33 ±0·10*	0·22 ±0·05	$N = 7$
Cl^-–plasma	90·8 ±5·1	89·1 ±4·7	90·1 ±5·8	87·4 ±5·4	78·9 ±5·3	87·1 ±5·5	83·2 ±6·5	$N = 11$
Cl^-–urine	1·7 ±0·4	4·6 ±1·8	4·6 ±2·6	3·9 ±1·7	4·2 ±1·4	3·5 ±1·2	1·3 ±0·6	$N = 8$
Cl^-–water	1·2 ±0·1	1·2 ±0·1	1·2 ±0·1	1·2 ±0·2	1·2 ±0·2	1·2 ±0·2	1·2 ±0·2	$N = 7$

N = normal; 1, 2, 4, 12 and 24 h = hours of exposure to 5% CO_2; 24 rec = 24 h of recovery in air.
Values are means ± one S.E.M. Asterisks denote significant ($P < 0·05$) difference from the normal.
N = number of measurements.

of recovery, it returned to 7·73 which was not significantly different from normal levels. The control P_{CO_2} of the blood was 12·4 mmHg and rose significantly to 35·8, 38·6, 35·6, 38·2 and 39·0 mmHg after 1, 2, 4, 12 and 24 h of hypercapnia, respectively. Similarly in recovery, it returned to a value (14·5 mmHg) not significantly different from normal. Plasma HCO_3^- did not significantly deviate from the normal (21·4 mmol l^{-1}) during the entire experiment. The relationship between these three parameters during the experiment may be seen graphically in Fig. 1.

The calculated pH of ureteral urine (Table 1), like blood pH, fell significantly from its control value of 6·33 to 6·04 during hypercapnia. It remained significantly lowered for the duration of the experiment, but returned to 6·29 during the recovery period. From the normal value of 0·36 mmol l^{-1}, ureteral urine [HCO_3^-] rose significantly during hypercapnia, but it reached a maximum value of only 0·53 mmol l^{-1}. During the recovery period, [HCO_3^-] was still significantly elevated but had fallen to 0·38 mmol l^{-1}.

The plasma and ureteral urine [Ca^{2+}] were both significantly elevated during hypercapnia, while ambient water [Ca^{2+}] remained unchanged (Fig. 2). Plasma [Ca^{2+}] began at a mean control value of 3·3 mequiv l^{-1} and after 2, 4, 12 and 24 h of hypercapnia rose to 3·9, 4·0, 4·1 and 3·9 mequiv l^{-1}, respectively. It returned to 3·0 mequiv l^{-1} during the recovery period. The urine [Ca^{2+}] was initially 0·33 mequiv l^{-1} and hypercapnic levels of 0·72, 0·79, 0·81, 0·77 and 0·82 mequiv l^{-1} after 1, 2, 4, 12 and 24 h, respectively, were all significantly elevated from the normal. Following the usual trend, in recovery, the value of 0·37 had not been altered significantly from the normal. The initial water [Ca^{2+}] of 0·84 mequiv l^{-1} was unchanged during the experiment.

[Na^+], [K^+] and [Cl^-] in the plasma (Table 2) were not significantly different in hypercapnia from their control levels which were 106·5, 2·7 and 90·8 mequiv l^{-1} respectively. Ureteral urine [Cl^-] was unchanged from its mean normal level of 1·7 mequiv l^{-1}, but ureteral urine [Na^+] and [K^+] did show a transient, but significant, increase after 2 h of elevated CO_2 exposure. Normal urine [Na^+] and [K^+]

Table 3. *Urine flow, urine titratable acidity minus bicarbonate, total urinary acid efflux and ammonia concentration in ureteral urine and ambient water*

Time (h)	[NH_4^+] Urine (mequiv l^{-1}) ($N=8$)	[NH_4^+] Water (mequiv l^{-1}) ($N=7$)	Urine [TA−HCO_3^-] (mequiv l^{-1}) ($N=5$)	Total urinary acid efflux ($N=5$)	Urine flow (ml 100 g^{-1} h^{-1}) ($N=7$)
N	1·1 ± 0·2	18·0 ± 3·9	3·3 ± 1·4	9·4 ± 3·4	2·5 ± 0·4
1	1·0 ± 0·2	24·1 ± 7·2			
2	1·1 ± 0·3	34·6 ± 5·6			
3			0·8 ± 0·9		
4	1·1 ± 0·2	54·1 ± 9·6		1·4 ± 2·5	2·0 ± 0·2
5			1·2 ± 2·0		
12	1·0 ± 0·1	75·4 ± 6·8*			
24	1·4 ± 0·4	127·2 ± 20·2*	3·0 ± 1·3	8·2 ± 1·9	1·9 ± 0·1
24 Rec	1·1 ± 0·2	84·6 ± 24·5*	1·3 ± 0·4	2·6 ± 0·6	1·4 ± 0·1

N = normal; 1, 2, 4, 12 and 24 h = hours of exposure to 5% CO_2; 24 Rec = 24 h of recovery in air.
Values are means ± one S.E.M.
Asterisks denote significant ($P < 0·05$) difference from the normal.
N = number of measurements

were 2·9 and 0·32 mequiv l^{-1}, while their 2-h hypercapnia values rose to 16·6 and 1·13 mequiv l^{-1}, respectively. Following this, water [Na^+] and [Cl^-] also remained unchanged from their normal means of 0·3 and 1·2 mequiv l^{-1} respectively, while [K^+] in the ambient water accumulated significantly. After 12 and 24 h of hypercapnia, the [K^+] in the water was 0·31 and 0·33 mequiv l^{-1} respectively, which is significantly above the control concentration of 0·10 mequiv l^{-1}.

Mean ureteral urine [NH_4^+] (Table 3) did not significantly deviate from 1·1 mequiv l^{-1} in hypercapnia, while ammonia in the water did significantly accumulate from the control value of 18·0 μequiv l^{-1} to 75·4 and 127·2 μequiv l^{-1} after 12 and 24 h respectively. After 24 h of recovery in fresh water, there was a significant increase from the control to 84·6 μequiv l^{-1}.

Neither the [$TA - HCO_3^-$] of the ureteral urine nor the urine flow rate were significantly changed during hypercapnia from their normal values of 3·3 mequiv l^{-1} and 2·5 ml $100\,g^{-1}h^{-1}$ respectively. Calculated from these values and corresponding NH_4^+ levels (within 1 h), the total urinary acid efflux [$TA - HCO_3^- + NH_4^+$] × (urine flow rate) (McDonald & Wood, 1981) was also not significantly altered during hypercapnia from its control value of 9·4 μequiv $100\,g^{-1}h^{-1}$.

DISCUSSION

Yoshimura, Yata, Yuasa & Wolbach (1961) conducted a set of experiments on the difference between ureteral and bladder urine in the bullfrog *Rana catesbeiana*. The authors found that differences in the urine composition and urine flow between the two sites only became apparent when the animals were out of water. This was explained by the fact that dehydration stimulates reabsorption of water and electrolytes by the urinary bladder in this species.

The arterial acid-base status of bladder by-passed toads in the present experiments is similar to that of catheterized animals with full urinary potential (Boutilier *et al.* 1979*a*; McDonald, Boutilier & Toews, 1980; Toews & Heisler, 1982). However, exposure of these animals to a 5 % hypercapnic insult does not duplicate the previously documented arterial response of the intact animal (Boutilier *et al.* 1979*a*; Toews & Heisler, 1982). Although arterial pH and P_{CO_2} changes follow a pattern similar to that described by previous authors with a slight metabolic component to the acidosis during the first hour, by-passing the bladder prevents the compensatory response. Elimination of the bladder as an exchange surface incurs a loss of the ability to elevate plasma HCO_3^- to the levels characteristic of hypercapnia in the fully intact animal. Reabsorption of HCO_3^-, or the functional equivalent of H^+ or NH_4^+ excretion, must apparently take place across the bladder epithelium. The increased kidney urine HCO_3^- levels that we found could, therefore, be re-routed back into the animal at this site. The urinary bladder of *Bufo marinus* is a well vascularized membranous structure capable of retaining fluid volumes in excess of 30 % gross body weight (Boutilier *et al.* 1979*b*). This regulatory site has been found to delay the adverse internal effects of dehydration (Boutilier *et al.* 1979*b*) and numerous studies have documented its capacity for ion exchange. Acidification of the urine by the toad bladder has been documented both *in vitro* (Ziegler, Ludens & Fanestil, 1974; Frazier & Vanatta, 1972, 1973) and *in vivo* (Frazier & Vanatta, 1971). The *in vitro* preparations

elicited an increase in proton and NH_4^+ excretion rates during respiratory and metabolic acidosis (Frazier & Vanatta, 1971, 1973).

The potential of the kidney of higher vertebrates in compensation for acid-base imbalance is well known and, recently, a substantial renal contribution towards the neutralization of various acid or base loads has been shown in fish (Cameron, 1980; McDonald & Wood, 1981; Cameron & Kormanik, 1982). However, the present experiments show that the response of the *Bufo marinus* kidney to a hypercapnic acid load is limited.

Neither urine flow, nor total urinary acid excretion were significantly altered for the duration of hypercapnic exposure. Somewhat surprisingly, in response to an acidosis, $[HCO_3^-]$ increased significantly in the ureteral urine after the first hour and remained elevated until the recovery period. If retained in the animal, this quantity of HCO_3^-excreted in the urine plus the stoichiometric HCO_3^- equivalent resulting from the calculated ureteral urine pH change would have amounted to only 10% of the compensatory increase in plasma HCO_3^- found by Boutilier *et al.* (1979*a*), assuming a 24·7% extracellular fluid volume in *Bufo marinus* (Thorson, 1964).

This lack of a substantial regulatory renal response has also been previously documented in other anurans. Experiments by Yoshimura *et al.* (1961) on *Rana limnocharis*, in which urine was collected *via* a cloacal catheter, showed that urine H^+ and NH_4^+ excretion rates remained constant during respiratory acidosis (6·4% CO_2) while the urine $[HCO_3^-]$ increased. Although the authors explain that their cannulation technique allowed the exposure of the urine to the bladder epithelium, these results are very similar to those in our experiments.

In accordance with the results of Yoshimura *et al.* (1961), ureteral urine $[Na^+]$ in our experiments was found to increase during hypercapnia. There is evidence for possible Na^+/H^+ ion exchange in the urinary epithelia of *Bufo marinus* (Yoshimura *et al.* 1961; Frazier & Vanatta, 1971, 1973). Our data suggest that H^+ uptake is countered by Na^+ excretion in the kidney and this process is accelerated during respiratory acidosis. This could account to some extent for the increased $[HCO_3^-]$ in the ureteral urine since there is no evidence in our data for Cl^-/HCO_3^- exchange by the kidney. Any Na^+/H^+ exchange in the opposite direction which could acidify the urine and thus be accelerated to assist in compensation for an acidosis must be located at the site of the urinary bladder. Long (1982*a*,*b*) found that HCO_3^- reabsorption may be associated with net K^+ secretion by the kidney of *Bufo marinus*. A slight increase in this process could account for the small increase in urine $[K^+]$ we found during hypercapnia.

The contribution of the skin to the buffering of the acid load also appears to be negligible. Vanatta & Frazier (1981) obtained normocapnic NH_4^+ excretion values of 0·42 μequiv $50\,g^{-1}h^{-1}$ for the skin of *Rana pipiens* (assuming the average weight of a *Rana pipiens* to be 50 g). In comparison, excretion of NH_4^+ from the skin of *Bufo marinus* during hypercapnia in our experiments was little different at 0·52 μequiv $50\,g^{-1}h^{-1}$. The majority of the NH_4^+ excretion during hypercapnia in *Bufo marinus* can probably be attributed to normal NH_4^+ excretion which would not elevate internal $[HCO_3^-]$ over and above normocapnic conditions.

Increases in ureteral urine and plasma $[Ca^{2+}]$ implicate internal calcium carbonate deposits as a possible source of HCO_3^-. Our work supports previous studies by Sulze

(1942), Simkiss (1968) and Robertson (1972). Simkiss (1968) theorized that the dissolution of the crystalline deposits of the endolymphatic sacs could produce HCO_3^- which would be available for buffering in keeping with the equation:

$$CaCO_3 \rightleftarrows Ca^{2+} + CO_3^{2-}$$
$$HCO_3^- \rightleftarrows H^+ + CO_3^{2-}.$$

The potential amount of HCO_3^- from this internal source can be estimated from our values for ureteral urine flow rate, maximum hypercapnic levels of Ca^{2+} in ureteral urine and plasma, and the values of Thorson (1964) for extracellular and intracellular fluid volume. Assuming that the increase in calcium concentrations in urine and plasma represent the dissolution of the calcium carbonate deposits, these increases in calcium concentration would produce the stoichiometric equivalent of approximately 1·0 mequiv kg^{-1} of HCO_3^- in 24 h in *Bufo marinus*.

From the data of Toews & Heisler (1982) (assuming that skeletal muscle represents the majority of the animal's tissue, and could therefore be regarded as typical of intracellular space) and that of Boutilier *et al.* (1979*a*) for intracellular and extracellular HCO_3^- increases during hypercapnia, the actual total HCO_3^- gain can be estimated. We estimated that the total HCO_3^- gain in *Bufo marinus* (over and above that provided by chemical buffering) was approximately 2·0 mequiv kg^{-1}. Therefore, the approximate percentage of the HCO_3^- increase normally found in these animals which could result from the production of HCO_3^- from the dissolution of calcareous deposits [assuming the hypothesis of Simkiss (1968) to be correct] is 50 %. Buffering of the intracellular compartment has priority over the extracellular compartment in amphibians (Toews & Heisler, 1982) and therefore this HCO_3^- may be accumulating intracellularly.

There is an interesting charge difference in the ureteral urine electrolyte data. The $([Na^+] + [K^+] + [Ca^{2+}]) - ([Cl^-] + [HCO_3^-])$ value for the control period is 4·03 mequiv l^{-1}, whereas the same value at 2 h hypercapnia is 13·06 mequiv l^{-1}. It is unlikely that this 9 mequiv l^{-1} difference is phosphate since we have measured it in toad urine during hypercapnia (unpublished data) and the levels are too low. Pitts (1974) explains that citrate is not only a common organic anion in mammalian urine, but it also plays a specific role in solubilizing calcium. Since $CaCO_3$ dissolution is suggested by our data, it is possible that the missing anion could be citrate or another organic calcium-binding agent.

In summary, the present experiments represent the first documented *in vivo* response of the isolated kidney of an anuran to respiratory acidosis. The results of these experiments indicate that the regulatory adjustment by the kidney during hypercapnia is minimal and would not account for the documented internal increase in HCO_3^-. Contributions of the urinary tract during this type of stress must therefore come from regulatory adjustments of the bladder epithelium. The contribution of the skin towards the generation of internal HCO_3^- also appears negligible. However, the results of these experiments indicate that the dissolution of internal calcareous deposits may amount to as much as 50 % of the documented internal HCO_3^- increase. It therefore appears that the major regulatory adjustments used by *Bufo marinus* to counteract the acid load imposed by hypercapnia are two-fold. Firstly, these animals mobilize a large buffer store of $CaCO_3$ to produce HCO_3^-, and secondly, they readjust

ion exchanges across the bladder epithelium which also serves to elevate internal HCO_3^- concentrations.

Financial support for this study was provided by an NSERC operating grant to DPT and an NSERC graduate fellowship to BLT. Technical assistance by Edgar Spalding, Pam Brown and Dennis Mense was greatly appreciated. We would also like to thank D. J. Randall for assistance in the preparation of the manuscript.

REFERENCES

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1979*a*). Acid-base relationships in the blood of the toad, *Bufo marinus*. I. The effects of environmental CO_2. *J. exp. Biol.* **82**, 331–344.

BOUTILIER, R. G., RANDALL, D. J., SHELTON, G. & TOEWS, D. P. (1979*b*). Acid-base relationships in the blood of the toad, *Bufo marinus*. II. The effects of dehydration. *J. exp. Biol.* **82**, 345–355.

CAMERON, J. N. (1980). Body fluid pools, kidney function, and acid-base regulation in the freshwater catfish, *Ictalurus punctatus*. *J. exp. Biol.* **86**, 171–185.

CAMERON, J. N. & KORMANIK, G. A. (1982). The acid-base responses of gills and kidneys to infused acid and base loads in the channel catfish, *Ictalurus punctatus*. *J. exp. Biol.* **99**, 143–160.

FRAZIER, L. W. & VANATTA, J. C. (1971). 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* **241**, 20–29.

FRAZIER, L. W. & VANATTA, J. C. (1972). Evidence that a plasma factor(s) stimulates H^+ and NH_4^+ excretion in the toad urinary bladder. *Proc. Soc. exp. Biol. Med.* **139**, 336–339.

FRAZIER, L. W. & VANATTA, J. C. (1973). Characteristics of H^+ and NH_4^+ excretion by the urinary bladder of the toad. *Biochim. biophys. Acta* **311**, 98–108.

LONG, W. S. (1982*a*). Renal electrolyte handling, acid-base status, and urinary acidification in *Bufo marinus*. *Am. J. Physiol.* **243**, F60–F66.

LONG, W. S. (1982*b*). Bicarbonate reabsorption and potassium handling in isolated perfused kidneys of *Bufo marinus*. *Am. J. Physiol.* **243**, F67–F73.

MCDONALD, D. G. (1983). The interaction of environmental calcium and low pH on the physiology of the rainbow trout, *Salmo gairdneri*. I. Branchial and renal net ion and H^+ fluxes. *J. exp. Biol.* **102**, 123–140.

MCDONALD, D. G., BOUTILIER, R. G. & TOEWS, D. P. (1980). The effects of enforced activity on ventilation, circulation and blood acid-base balance in the semi-terrestrial anuran, *Bufo marinus*. *J. exp. Biol.* **84**, 273–287.

MCDONALD, D. G. & WOOD, C. M. (1981). Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri* at low environmental pH. *J. exp. Biol.* **93**, 101–118.

MACINTYRE, D. H. & TOEWS, D. P. (1976). The mechanics of lung ventilation and the effects of hypercapnia on respiration in *Bufo marinus*. *Can. J. Zool.* **54**, 1364–1374.

PITTS, R. F. (1974). *Physiology of the Kidney and Body Fluids*, 3rd Edn. Chicago: Year Book Medical.

ROBERTSON, D. R. (1972). Influence of the parathyroids and ultimobranchial glands in the frog (*Rana pipiens*) during respiratory acidosis. *Gen comp. Endocr. (Suppl.)* **3**, 421–429.

SIMKISS, K. (1968). Calcium and carbonate metabolism in the frog (*Rana temporaria*) during respiratory acidosis. *Am J. Physiol.* **214**, 627–634.

SULZE, W. (1942). Uber die physiologische Bedeuting des Kalkasackchenapparates der Amphibien. *Arch. ges. Physiol.* **246**, 250–257.

THORSON, T. B. (1964). The partitioning of body water in amphibia. *Physiol. Zool.* **37**, 395–399.

TOEWS, D. P. & HEISLER, N. (1982). The effects of hypercapnia on intracellular and extracellular acid-base status in the toad *Bufo marinus*. *J. exp. Biol.* **97**, 79–86.

TOEWS, D. P. & MACINTYRE, D. H. (1978). Respiration and circulation in an apodan amphibian. *Can. J. Zool.* **56**, 998–1004.

VANATTA, J. C. & FRAZIER, L. W. (1981). The epithelium of *Rana pipiens* excretes H^+ and NH_4^+ in acidosis and HCO_3^- in alkalosis. *Comp. Biochem. Physiol.* **68**A, 511–513.

VAN SLYKE, D. D., SENDROY, J., JR., HASTINGS, A. B. & NEILL, J. M. (1928). The solubility of carbon dioxide at 38 °C in water, salt solution, serum and blood cells. *J. biol. Chem.* **78**, 765–799.

VERDOUW, H., VAN ECHTELD, C. J. A. & DEKKERS, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Res.* **12**, 399–402.

YOSHIMURA, H., YATA, M., YUASA, M. & WOLBACH, R. A. (1961). Renal regulation of acid-base balance in the bullfrog. *Am. J. Physiol.* **201**, 980–986.

ZIEGLER, T. W., LUDENS, J. H. & FANESTIL, D. D. (1974). Role of carbonic anhydrase in urinary acidification by the toad bladder. *Am. J. Physiol.* **227**, 1132–1138.