ACID-BASE REGULATION IN THE BULLFROG *(RANA CATESBIANA)* FOLLOWING ACID INFUSIONS AND ENFORCED SUBMERGENCE

ACID-BASE REGULATION IN THE BULLFROG *(RANA CATESBIANA)* FOLLOWING ACID INFUSIONS AND ENFORCED SUBMERGENCE

By

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ABSTRACT

This study examined the acid-base and ionoregulatory responses by the skin and kidney of adult bullfrogs *(Rana catesbiana)* during recovery from non-respiratory acidoses. Acidosis was induced by intravascular infusions of HCl (3,000 μequiv/kg), or NH_4Cl (4,000 μequiv/kg), or by 45 min enforced submergence.

Infused acids were immediately buffered by the extracellular fluids (ECF) and moved rapidly into the intracellular compartment. Clearance of the acid load was slow (> 24 h post-infusion) and only in NH_4Cl loaded frogs was the full amount cleared within 5 days. Excess acid was excreted primarily by increased renal NH_4^+ effluxes. The skin contributed very little to the net acid excretion; instead large "base" losses ("acid" uptake) occurred. Acid infusions also resulted in large ion losses and elevated water uptake across the skin, with electrolytes and water moving down chemical gradients. Frogs infused with HCl died within 96 h; NH_4Cl loaded frogs all survived.

Forced submergence resulted in a severe lactacidosis which was corrected in 12 h by a combination of renal/cutaneous acid excretion (9:1 ratio) and metabolic utilization of lactate and H^+ ($\sim$ 95% of excess acid load). Acid excretion occurred primarily as NH_4^+ efflux by the kidney and skin. A 1:1 exchange of Na^+ influx/NH_4^+ efflux across the skin was found ($r = 0.94$; $P < 0.01$) when $J_{in}Na^+$ was elevated over basal levels (30-40 μequiv/kg·h). Lactate and net acid effluxes by the skin and kidney diminished by 24 h post-disturbance and accounted for 4

to 6% of the total estimated load to the animal. All frogs survived the diving protocol.

Restoration of acid-base state in acidotic bullfrogs by ECF and non-ECF mechanisms, as well as the renal and cutaneous responses to the acidosis, is discussed and compared to that of other vertebrates. It is concluded that anurans, and the bullfrog in particular, are poor regulators of acid-base state but instead are very tolerant to marked disturbances of acid-base balance.

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INTRODUCTION

This thesis examined the ability of the semi-aquatic bullfrog, *Rana catesbiana* (Amphibia, Anura) to restore its acid-base state following induction of a variety of blood acidoses.

Acid-base regulation implies the maintenance of a constant balance between hydrogen ion concentration $[H^+]$ and hydroxyl ion concentration $[OH^-]$ in the intra- and extracellular body fluids (Rahn and Howell, 1978). Normal body function is exceedingly dependent on hydrogen ion concentration. Despite the fact that $[H^+]$ is 6 orders of magnitude less than that of Na^+, the principal cation of the extracellular fluids (ECF), protein function is extremely sensitive to small changes in $[H^+]$. Thus even small acid-base disturbances can substantially affect enzyme mediated cellular processes (Woodbury, 1974).

Acid-base disturbances are of two types: respiratory, where acid-base state is disturbed by either an increase or a decrease in pCO_2; and non-respiratory, where the concentration of fixed or non-volatile acids is altered. The latter is also called a 'metabolic' acid-base disturbance to indicate that these disturbances may result from metabolism. Typically, respiratory disturbances are of short-term nature and corrected by respiratory gas exchange. Non-respiratory disturbances, on the other hand, are usually longer term in nature. These disturbances can usually be at least partially compensated by ventilatory adjustments, but their eventual correction requires a combination of excretion and/or metabolism of the excess acids or bases.

It is the latter disturbance which this thesis will focus on.

Non-respiratory acidosis occurs naturally in lower vertebrates - primarily fish and amphibians - in response to a variety of activities or environmental conditions, i.e. diet, exposure to high ambient H^+, heavy metals, diving apnoea, muscular exercise (for reviews see McDonald, 1983b; Toews and Boutilier, 1983), and has been produced by such experimental treatments as exhaustive exercise (Wood *et al.*, 1977; Boutilier *et al.*, 1980; McDonald *et al.*, 1980; Holeton *et al.*, 1983; Turner *et al.*, 1983), hypoxic exposure (Kobayashi and Wood, 1980), enforced submergence (Jones, 1972a; Emilio and Shelton, 1980; Boutilier, 1981; Shelton and Boutilier, 1982), and by infusions of various acidic substances (i.e. HCl, lactic acid, NH_4Cl - Yoshimura *et al.*, 1961; Wood and Caldwell, 1978; Cameron, 1980; Kobayashi and Wood, 1980; Vanatta and Frazier, 1981; Cameron and Kormanik, 1982; McDonald *et al.*, 1982). Generally speaking, the purpose of these experimentally induced internal acidoses has been to produce a maximal response so as to enable the detection and quantification of the compensatory/corrective processes.

Usually these experimental disturbances produce a marked blood acidosis initially accompanied by an elevation in blood pCO_2. The latter is typically corrected by increased respiratory gas exchange by lungs (Gottlieb and Jackson, 1976; Boutilier *et al.*, 1979a, b; McDonald, *et al.*, 1980; Shelton and Boutilier, 1982), skin (MacKenzie and Jackson, 1978; Boutilier *et al.*, 1980; Emilio and Shelton, 1980) or gills (Cameron, 1978; Cameron and Kormanik, 1982; McDonald *et al.*, 1982; Heisler, 1982; Holeton *et al.*, 1983).

The first line of defence against a non-respiratory acidosis is rapid passive buffering by bicarbonate and non-bicarbonate (mainly protein) buffers in the ECF and intracellular fluids (ICF) (Woodbury, 1974). Secondly, organic acids (i.e. lactic acid) whether infused or endogenously produced may be removed by metabolism, resulting in the gradual restoration of acid-base status (Hochachka *et al.*, 1974; Kobayashi and Wood, 1980; Hultman and Sahlin, 1980). Additionally, excess non-volatile acids can be excreted across various epithelial surfaces. For fish and amphibians the potential surfaces are, respectively, gills and skin in addition to the kidneys (Riegel, 1971; Maetz, 1974). Acid excretion across epithelia may occur by passive electroneutral diffusion or may be facilitated by transepithelial exchange against actively transported ions such as Na^+ and Cl^- (Pitts, 1974; Heisler, 1982; Toews and Boutilier, 1983).

There is now considerable evidence that Na^+ and Cl^- absorption are independent of one another both at the frog skin (Krogh, 1939; Garcia-Romeu *et al.*, 1969; Garcia-Romeu and Ehrenfeld, 1975b) and at the fish gill (Krogh, 1939: Maetz and Garcia-Romeu, 1964; Evans *et al.*, 1982). This was initially demonstrated in the isolated frog skin (Huf, 1935, 1936) and shortly thereafter in intact living frogs (Krogh, 1937, 1938). Krogh (1939) pointed out that active Na^+ and Cl^- uptake must occur against the loss of endogenous cations and anions in order to maintain electroneutrality, and suggested that the likely ions were H^+ and HCO_3^- respectively. More recently, other authors (Maetz and Garcia-Romeu, 1964; Garcia-Romeu and Salibán, 1968; Maetz, 1974; McDonald, 1983b) have suggested that NH_4^+ and OH^- could also serve as counterions.

There is currently good evidence for the existence of these exchanges in both fish and amphibians. For example, in the goldfish, *Carassius auratus* (Garcia-Romeu and Maetz, 1964) and in the Chilean and European frogs, *Calyptocepalella gayi* (Garcia-Romeu *et al.*, 1969), *Rana esculenta* (Garcia-Romeu and Ehrenfeld, 1975a) exposure to Na^+-free or Cl^--free media (i.e. choline chloride; sodium sulphate) led to Cl^-/base exchange and Na^+/acid exchange, respectively. These, and other studies (Maetz and Garcia-Romeu, 1964; Garcia-Romeu and Saliban, 1968), indicated that the likely exchanges were Na^+/H^+ (NH_4^+) and Cl^-/HCO_3^- (OH^-) and further suggested their potential role in the regulation of internal acid-base state.

More recent studies on fish have examined these exchanges with the view to determining whether their relative intensities can be manipulated to correct an acid-base disturbance (Cameron, 1978; Cameron and Kormanik, 1982; Heisler, 1982; Holeton *et al.*, 1983; Wood, Wheatly and Hōbe, 1983). The evidence for this occurring is, at present, inconclusive. However, a recent study by Wood, Wheatly and Hōbe (1983) on the rainbow trout has shown that compensation of a metabolic alkalosis was accompanied by an excretion of base together with a stimulation of Cl^- influx and reduction of Na^+ influx.

Similar investigations using amphibians have not been conducted to the extent performed on fish. Several studies have, in fact, shown that the *in vitro* frog skin (Fleming, 1957; Frazier and Vanatta, 1980) and the skin of whole living frogs (Friedman *et al.*, 1967; Garcia-Romeu *et al.*, 1975a) is capable of acidifying the external medium by manipulation of external Na^+ and Cl^-. However, only one study on the intact living frog, *Rana pipiens*, (Vanatta and Frazier, 1981) has demonstrated

elevated rates of acidification of the external medium (i.e. 'H^+' excretion) in response to 'metabolic acidosis'. Since these authors did not examine Na^+ and Cl^- movements there are currently no data concerning the involvement of ionic exchanges in the cutaneous compensation of internal acid-base disturbances in amphibians.

In higher vertebrates it appears that the kidney is the sole organ responsible for long-term acid-base regulation. Naturally or experimentally induced loads of non-volatile acids or bases to the body fluids, while initially buffered intra- and extracellularly and provoking partial respiratory compensation, are corrected ultimately by increased excretion in the urine (Riegel, 1971; Pitts, 1974; Woodbury, 1974). Similarly, the kidneys of fish (Wood and Caldwell, 1978; Kobayashi and Wood, 1980; McDonald and Wood, 1981; Cameron and Kormanik, 1982; Wheatly *et al.*, 1983) and of anuran amphibians (Yoshimura *et al.*, 1961; Long, 1982a) will excrete acid at elevated rates in response to experimentally induced acidosis. Thus for fish at least, there is now good evidence that two organs, the gills and the kidneys, are capable of ultimately correcting internal acid-base disturbances. However it is not yet fully clear how the excretion of acid or base loads is partitioned between these two sites. Some current studies indicate a predominant role for the gills (Heisler, 1982; Holeton *et al.*, 1983) while others give a greater importance to the kidneys (Wood and Caldwell, 1978; McDonald and Wood, 1981). However, only one study (Cameron and Kormanik, 1982), on freshwater catfish infused with acid and base loads, has examined the responses of both organs simultaneously. In this study branchial acid and base clearance occurred at a greater rate than

renal clearance, but since the response was followed for only 22 h it may be that the typically longer-term renal response (Wood and Caldwell, 1978; Kobayashi and Wood, 1980; McDonald and Wood, 1981) may not have been observed.

In amphibians, in contrast to fish, the partitioning of experimentally induced acid loads between renal and cutaneous sites of exchange/excretion has not been examined. Thus, the objectives of the present study were threefold: 1) to investigate, in bullfrogs, *Rana catesbiana*, blood, ECF and non-ECF regulation of acid-base state in response to experimentally induced blood acidosis; 2) to evaluate the role of the skin in acid-base regulation; and 3) to examine the relative contributions of skin and kidney in excretion of excess acid.

The bullfrog was chosen for these studies because its large size (up to 1 kg) permitted ease of cannulation and repetitive blood sampling. Furthermore the bullfrog is a good *in vivo* preparation with which to examine cutaneous and renal mechanisms of acid-base regulation for when immersed in water the animal will utilize both the urinary system and skin for ion exchanges with the water (Alvarado and Moody, 1970; Shoemaker and Nagy, 1977; Alvarado, 1979; Koefoed-Johnsen, 1979).

Adult bullfrogs were made acidotic by infusions of acid or by prolonged enforced submergence. Acid infusion procedures as a means of acid-loading the ECF have been used with success in mammals (Levine and Nash, 1973; Guntupalli *et al.*, 1982), fish (Cameron and Kormanik, 1982; Wood and Caldwell, 1978; Kobayashi and Wood, 1980; McDonald *et al.*, 1982) and amphibians (Yoshimura *et al.*, 1961; Vanatta and Frazier, 1981; Long, 1982a). Hydrochloric acid (HCl) was employed because the load

constitutes a known amount of fixed acid which is added to the ECF. As such, the resulting acidosis can only be corrected by excretion of excess H^+ (Kobayashi and Wood, 1980).

Such a rapid loading of H^+ is, however, unphysiological so that ammonium chloride (NH_4Cl) infusions were also performed. Since the pK' for the ammonia buffer system is about 9.3 (Pitts, 1974), NH_4^+ only slowly dissociates to NH_3 and H^+. The NH_3 so formed is very permeable to membranes and rapidly diffuses out of the ECF and into the ICF and/or, preferably, to the external medium. This results in a gradual and progressive non-respiratory acidosis as an equimolar H^+ load is left behind. Additionally NH_4^+ provides highly efficient precursors for urea synthesis (Unsworth *et al.*, 1969) via the activation of enzymes in the arginine urea pathway (Carlisky, 1970; McBean and Goldstein, 1970; Jungreis, 1976). One molecule of urea synthesized in this fashion utilizes two NH_2^- and thus releases $2H^+$ for each molecule of urea formed. This situation more closely mimics the onset of an endogenously produced non-respiratory acidosis than do HCl infusions. The disadvantage, of course, is that it is difficult to quantify the resulting H^+ load.

Other experiments were conducted in order to simulate a more natural metabolic acidosis, i.e. lactacidosis. A prolonged apnoea induced by enforced submergence was chosen over other methods such as Faradic stimulation (Hutchison and Turney, 1975; Hutchison and Miller, 1979; Boutilier *et al.*, 1980; Hutchison *et al.*, 1981) or manual/mechanical handling (Putnam, 1979; McDonald *et al.*, 1980). The latter two methods either do not raise circulating lactate to levels high

enough to produce the sustained metabolic acidosis required for the study, or are tedious and present technical problems when using cannulated animals. The former was chosen because of its simplicity to perform using cannulated frogs and because some of the cardiac, circulatory, ventilatory and acid-base adjustments in diving amphibians have been characterized (Shelton and Jones, 1965; Jones 1972b; Jones and Shelton, 1964; Emilio and Shelton, 1980; Meyers *et al.*, 1980; Boutilier, 1981; Shelton and Boutilier, 1982). During the apnoeic period of the dive considerable accumulations of lactate and metabolic CO_2 occur in the tissues and blood as a result of the progressive exhaustion of O_2 stores and the onset of anaerobic glycolysis (Hochachka *et al.*, 1974; Dejours, 1981; Boutilier, 1981). Soon after termination of the dive blood PCO_2 and lactate concentration are highest, followed by gradual decrease to resting values (Boutilier, 1981; Shelton and Boutilier, 1982; Toews and Boutilier, 1983).

MATERIAL AND METHODS

1. Experimental Animals

Bullfrogs, *Rana catesbiana*; 295-490 g bladder-empty body weight) of both sexes were obtained from Lemberger and Associates, Germantown, WI, U.S.A. The bullfrogs had been collected in the late spring to summer (1981 through 1983) in Wisconsin; as a result most females used were gravid.

Upon receipt all bullfrogs were kept in tanks filled to a depth of 3 to 8 cm with continuously flowing water (Table 1). Bullfrogs received in spring-summer, 1982 and spring, 1983 were administered chloramphenicol (50 µg/100 g; Rogar-Mycine Succinate, BTI Products, Inc., London, Ont.) intraperitoneally as a prophylaxis against outbreaks of redleg infections, *Aeromonas hydrophilia* (Gibbons and Kaplan, 1959; Reichenbach-Klinke and Elkan, 1965; Gibbs, 1973). The frog holding tanks contained partially submerged foam and brick ledges upon which the frogs could emerge from the water. The frogs were acclimated to laboratory conditions for 2 to 6 weeks and starved to minimize fecal contamination of urine during experimentation. After this period individual bullfrogs were acclimated for 2 to 5 days to the water used in the ensuing experiment. At the onset of each experiment the frogs used appeared to be healthy, although a few later showed signs of redleg. Data from these animals were not used in the subsequent analyses.

Table 1. Composition of holding tank water (HTW) and of experimental water used for each series. [ion] in μequiv/l ± 1 S.E.M.; TA in equiv H^+ required to titrate 1 L of water to pH 4.0; T=21°C.

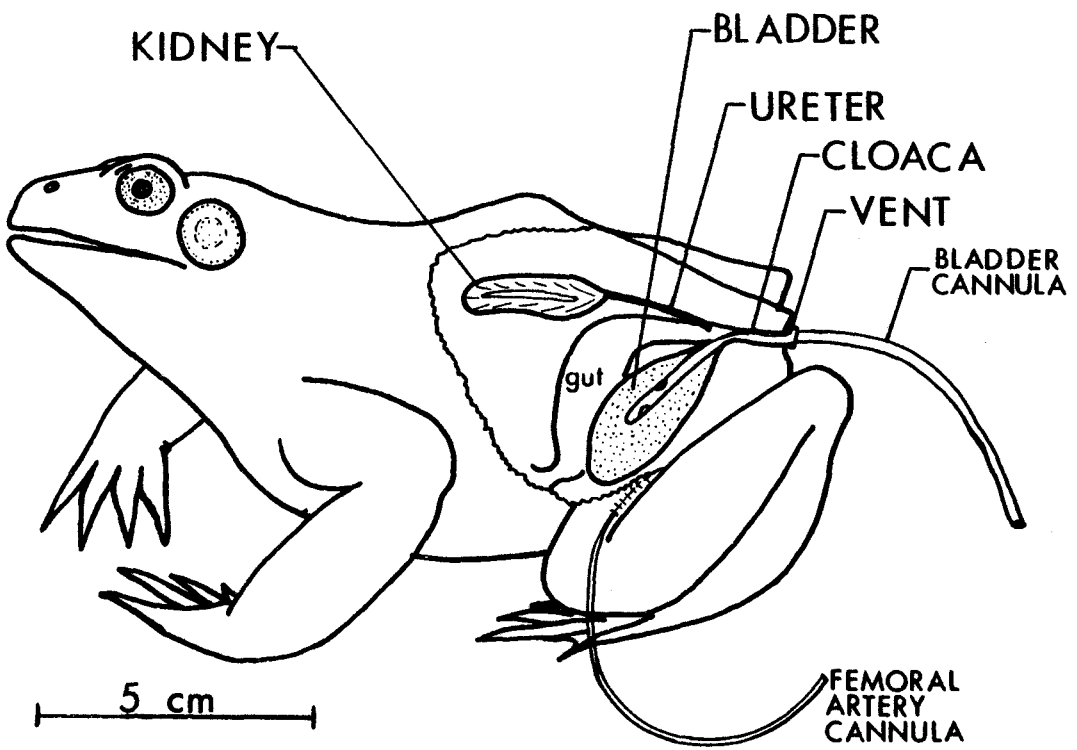
Series	pH	TA	Na^+	Cl^-	K^+	Ca^{++}	P_i	$^{22}Na^+$	$^{36}Cl^-$
HTW	7.52	388	9,100	4,100	130	4,640	5.6	--	--
1 & 2 controls	7.77 (7.64-7.97)	730	217 ± 3	302 ± 3	18.0 ±0.3	53.4 ±0.2	0.0 $\mu mol \cdot l^{-1}$	1.0 μCi/l	0.5 μCi/l
0.1 N-HCl 3	7.63 (7.56-7.71)	440	209 ± 3	213 ± 2	15.4 ±1.2	237 ± 7	1.0 $\mu mol \cdot l^{-1}$	--	--
0.1 N-HCl 4	7.60 (7.47-7.74)	482	209 ± 1	191 ± 5	13.3 ±0.4	317 ± 2	4.2 ±0.9 $\mu mol \cdot l^{-1}$	--	--
1.0 M NH_4Cl cannulated 5	7.52 (7.48-7.57)	342	204 ± 2	308 ±21	14.0 ±1.1	291 ± 4	9.8 ±2.3 $\mu mol \cdot l^{-1}$	--	--
1.0 M NH_4Cl cannulated 6	7.60 (7.51-7.71)	433	212 ± 1	209 ± 3	35.0 ±1.8	392 ± 3	0.3 $\mu mol \cdot l^{-1}$	1.0 μCi/l	0.5 μCi/l
7 0.1 N-HCl	8.28 (8.19-8.38)	1,750	815 ± 3	890 ±10	44.3 ±1.3	2,000 ± 32	0.0 $\mu mol \cdot l^{-1}$	5.6 μCi/l	4.0 μCi/l
8 forced submergence	8.30 (8.21-8.41)	1,680	592 ± 6	839 ± 5	42.5 ±0.4	1,906 ± 16	0.38 $\mu mol \cdot l^{-1}$	5.6 μCi/l	4.0 μCi/l

2. Animal Preparation

Bullfrogs to be fitted with indwelling femoral artery catheters and/or bladder catheters were anaesthetized in an aerated solution of tricaine methane sulfonate (1.5 g/l) pH adjusted to ∿ 7.2. The femoral artery of one leg was occlusively cannulated by the procedure of Boutilier *et al.* (1979a). Bladder catheters, (polypropylene feeding tubes, C.R. Bard, Inc., Murray Hill, N.J.) were fitted immediately following installation of the femoral catheters. The bladder catheters (Fig. 1) were modifed at the insertion end to form internal and external seals around the cloacal opening and tightly secured *via* a purse-string stitched into the vent integument. Following recovery from surgery, the patency of this seal was verified by dye injection. Bladder, rather than ureteral catheterization, was used for urine collection because the former is simpler, faster and less invasive. Also, since the bullfrogs were maintained in a hydrated state no difference in urine composition between bladder and utereral urine was expected (Yoshimura *et al.*, 1961). Following surgery the frogs were allowed to recover for 36 to 72 h at 20-22^{o}C in 1 ℓ experimental chambers containing 250 to 500 ml of water of the composition to be used in the ensuing experiment (Table 1). During experimentation the bullfrogs were kept in water to avoid dehydration, to maintain urine production, and to facilitate measurement of transcutaneous ion and water fluxes. Urine was collected into covered vials *via* a siphon of 1 to 7 cm of H_2O.

Fig. 1. Schematic drawing of a bullfrog fitted with indwelling bladder and femoral artery cannulae. The paired kidneys are connected to the cloaca via the ureters. The urinary bladder lies ventral to the gut and cloaca, and possesses its own opening to the cloaca. The bladder cannula has a thickened area at the site of attachment to the vents. This 'plug' limited cannula movements and provided a tight seal to prevent the voiding of urine.

BULLFROG (Rana catesbeiana)



3. Experimental Protocol

Eight experimental series were performed (Table 2).

Series 1. Uncannulated controls

The purpose of this series was to establish baseline measurements of the total (skin plus urinary tract) water, acid and ion fluxes of completely intact animals. Bullfrogs (N=3, 318-490 g) were placed in flux chambers containing 250.0 ml partially deionized water (Table 1) for 5 consecutive 12 h flux periods. Prior to the first flux period and at the end of each flux period: 1) water was drained, volume determined, and samples collected for measurements of ions, $^{36}Cl^-$, $^{22}Na^+$, urea, ammonia and titratable alkalinity (TA); 2) chamber plus bullfrog was weighed and change in bullfrog weight recorded; and 3) 250.0 ml water replaced. One-way fluxes of Na^+ and Cl^- across the skin were determined by monitoring the rate of disappearance of ^{22}Na and ^{36}Cl from the bathing medium (Kirschner, 1970).

Series 2. Cannulated controls

The intention here was to establish baseline measurements of (i) blood parameters, (ii) to determine cutaneous acid, ion, and water fluxes, (iii) urinary acid and ion excretion, (iv) the effects of volume expanding the ECF, and (v) the effects of increasing the volume of the bathing medium.

Bullfrogs (N=3, 350-370 g) fitted with arterial and bladder catheters were placed in flux chambers containing 100.0 ml soft water

Table 2. Experimental protocol for studying acid-base regulation in bullfrogs.

Series	N	Wt (g)	Water	Treatment
1	3	397.3 ±49.4	soft	uncannulated controls
2	3	358.2 ± 6.4	soft	cannulated controls-volume loaded, i.v.
3	4	394.2 ±14.4	soft	1.0 N-HCl infusion; 3,000 μeq/kg, i.v.
4	4	354.3 ± 8.2	soft	0.1 N-HCl infusion; 3,000 μeq/kg, i.v.
5	4	420.0 ±23.3	soft	1.0 M-NH_4Cl infusion; 4,000 μeq/kg, i.v.
6	2	335.4 ±32.6	soft	1.0 M-NH_4Cl infusion; 4,000 μeq/kg, d.l.s.
7	2	117.5 ± 4.5	hard	0.1 N-HCl infusion; 2,000 μeq/kg, i.v.
8	5	366.2 ±28.7	hard	45 min enforced submergence

Notes: 1) weights are means ± 1 S.E.M.

2) water composition given in Table 1.

3) i.v. = intravascular infusion; d.l.s. = dorsal lymph sac infusion

(Table 1) for 6 successive 12 h flux periods. This was immediately followed by 4 consecutive 12 h flux periods with 250.0 ml soft water. Mid-way during the 6th flux period the bullfrogs were volume loaded (2.5 ml glass distilled H_2O over 10 min) *via* the femoral artery cannula. Urine was collected continuously for analyses and vials replaced at the end of each flux period. Water replacement in chambers, and determination of Na^+ and Cl^- unidirectional fluxes, were as described above.

Series 3. HCl Infusion (1.0 N; 3,000 μeq/kg)

Bullfrogs (N=4, 350-420 g) fitted with femoral artery and bladder catheters were acid-infused following a 48 h control period (4 x 12 h flux periods). During the control period blood was sampled daily, and urine and water collected at the end of each flux period for analysis. The acid infusion was performed over a 3 h period and consisted of three 30 min infusions of 1,000 μeq/kg spaced by 30 min intervals in which blood pH was monitored. The intention was to achieve a depression in blood pH of 0.5 pH units.

Following acid infusion, blood parameters, urinary ion and acid excretion, and transcutaneous ion, acid and water fluxes were monitored for 72 h. Total blood volume removed was about 8.0 ml, about 30-40% of the reported blood volume (50-65 ml/kg; Thorson, 1964), or 13-20% of the extracellularl fluid volume (200 to 270 ml/kg; Thorson, 1964). Blood samples were replaced with an equal volume of heparinized (40 iμ/ml) Mackenzie saline (de la Lande *et al.*, 1962).

Unidirectional fluxes of Na^+ and Cl^- across the skin were determined by intramuscular injection of 500 μl of labelled Mackenzie

saline (12 μCi/ml $^{22}Na^{+}$; 6 μCi/ml $^{36}Cl^{-}$) immediately prior to acid infusions. The appearance of $^{22}Na^{+}$ and $^{36}Cl^{-}$ in the water was measured at the end of each flux period. This procedure also enabled estimations of the Na^{+} and Cl^{-} spaces of the animals.

Series 4. HCl Infusion (0.1 N; 3,000 μeq/kg)

In this series, bullfrogs (N=4, 340-410 g) fitted with femoral artery and bladder catheters were infused with 500 μl labelled saline followed by the same acid load as in Series 3, but at a 10 fold dilution. Infusion was continuous at 1,000 μeq/kg·h and the disturbance produced was followed for 96 h.

Series 5. NH_4Cl Infusion (1.0 N; 4,000 μeq/kg; pH=7.46)

Bullfrogs (N=4, 365-460 g) fitted with femoral artery and bladder catheters were infused with labelled saline followed by 4.0 mmol/kg NH_4Cl at a rate of 8.0 mmol/kg·h. The resulting disturbance was followed for 168 h (7 days).

Series 6. NH_4Cl Infusion (as in Series 5)

Two unoperated, uncatheterized bullfrogs (302 g, 368 g) were loaded with 4.0 mmol/kg of 1.0 M NH_4Cl by a single injection into the dorsal lymph sac following emptying of the flux chambers. After 30 min had elapsed for equilibration of infused NH_4Cl to occur, labelled water (Table 1) was added to the chambers so that unidirectional Na^{+} and Cl^{-} fluxes could be determined. Ion, acid and water exchanges between animal and bathing medium were followed for 120 h post-infusion.

Series 7. HCl Infusion (0.1 N; 2,000 μeq/kg) - Tap water

The purpose of this series was to determine if there were differences in unidirectional and net fluxes of water and ions between HCl infused frogs in partially de-ionized water *vs.* those in normal tap water.

Three bullfrogs (113-122 g) fitted with femoral artery and bladder catheters were infused with acid (1,000 μequiv/kg·h) following a 48 h control period. Unidirectional fluxes of Na^+ and Cl^- were determined 24 h prior to, and following, acid infusion by measuring the disappearance of $^{22}Na^+$ (5.6 μCi/l) and $^{36}Cl^-$ (4.2 μCi/l) from the water. Ion, acid and water exchanges across the skin, renal excretion, and blood acid-base and ion status was followed for 24 h following acid infusion.

Series 8. Forced Submergence - Tap water

Bullfrogs (N=5; 295-440 g) fitted with femoral artery and bladder catheters were forcibly submerged for 35-45 min following a 48-96 h control period. The apparatus consisted of a 10 L plastic container with a removable plastic screen. When this container was filled with water the screen prevented the submerged bullfrog from gaining access to air. To initiate the dive the bullfrog was placed in the partially filled container (water of the same composition used during recovery - see Table 1), allowed to submerge and the container immediately filled to well above the level of the screen. Prior to and during the dive the water in the diving chamber was bubbled with N_2 to remove O_2 from the

water. During the dive period the animal's activity was carefully monitored and the dive terminated when the bullfrog became flacid following a frenzied bout of escape activity. Just prior to dive termination a blood sample was drawn to determine end-of-dive blood parameters. The bullfrog was then transferred back to its flux chamber and the recovery from submergence followed for 72 h.

4. Analytical Procedures

a) Blood acid-base parameters

Blood samples (180-600 µl) were drawn anaerobically using gas-tight Hamilton syringes. Arterial blood pH (pHa), total CO_2 (TCO_2), and hematocrit were determined immediately on 180 µl of the collected sample. Blood pH was determined by injecting 50 µl aliquots into a thermostatted (21°C) Radiometer BMS MK2 blood micro system connected to a Radiometer PHM 71-MK2 acid-base analyser. The calibration of the pH electrode was checked before measurements with precision buffers (Analytical Products, Inc., Belmont, CA.). Blood (80 µl) was injected into a heparinized hematocrit tube for determination of hematocrit. The tube was sealed and centrifuged for 5 min (International Clinical Centrifuge). Blood and plasma TCO_2 measurements were made on 40 µl aliquots by the electrode and cuvette method of Cameron (1971) or on a Corning 965 CO_2 analyser. Each sample was bracketed with $NaHCO_3$ standards to increase the precision of these techniques.

b) Blood metabolites

Lactate analyses were performed on 50 µl or 100 µl of whole

blood immediately deproteinized in two volumes of ice-cold perchloric acid (8% w/v) and then centrifuged at 5,000 g for 6 min. The supernatant was analyzed for lactate with Sigma reagents (Sigma #726-UV, 1981).

Ammonia and glucose determinations were performed on 100 µl plasma samples deproteinized in 400 µl cold trichloroacetic acid (12.5% w/v) and then centrifuged at 5,000 g for 6 min. The supernatant (200 µl) was analyzed for NH_4^+ by colorimetric assay using a micro-modification of the salicylate-hypochlorite method of Verdouw *et al.* (1978). Glucose was determined by the o-toluidine method of Hyvarinen and Nikkila (1962) using Sigma reagents (Sigma #635, 1980).

Plasma concentrations of inorganic phosphate (P_i) and urea were determined on 50 µl plasma samples using micro-modifications of colorimetric assays: phosphomolybdate reduction for P_i (Sigma #670, 1971), and the BUN procedure for urea (Sigma #535, 1981).

Plasma protein was determined by the Biuret method using Sigma reagents (Sigma #540, 1980) and correlated to plasma refractive index using a refractometer (American Optical Goldberg). Subsequent protein determinations were performed solely by refractometry. Plasma osmolarity was determined using a Wescor vapour pressure osmometer.

c) Ions

Concentrations of Na^+, K^+ and Ca^{2+} in plasma, urine and water were determined after appropriate dilution by flame photometry (EEL Mark II for Na^+, K^+; Coleman 20 for Ca^{2+}) or by atomic absorption spectrophotometry (Varian AA-1275). Chloride levels were determined

by coulometric titration (Radiometer CMT-10 or Buchler-Cotlove chloridometers). Urine and water concentrations of lactate, NH_4^+, urea, P_i were determined by micro-modifications of techniques described above. $^{22}Na^+$ was measured by gamma counting in a well counter (Nuclear Chicago Model 1085), and $^{36}Cl^-$ determined by liquid scintillation counting (Beckman LS230 or LS233) with correction for $^{22}Na^+$ beta emission.

d) Urine Acidity

Urine pH and titratable acidity ($TA\text{-}HCO_3^-$) were determined immediately after collection as described in Wood and Caldwell (1978) and McDonald and Wood (1981). $TA\text{-}HCO_3^-$ was determined as a single value in the double titration procedure recommended by Hills (1973) using a Radiometer micro pH electrode (Type E5021) coupled to a Radiometer PHM-84 pH meter. Titrants used were 0.02 N-HCl and 0.02 N-NaOH or 0.10 N-HCl and 0.10 N-NaOH. The final end point of titration for all samples was the mean blood pH on the day before acid infusion or forced submergence was conducted. This value represents the titratable acid content of the urine relative to a fixed value. It is thus more appropriate for assessing changes in absolute acid efflux over time and for comparisons of cutaneous and renal fluxes (McDonald and Wood, 1981). Total renal acid output was calculated as the sum of ($[TA\text{-}HCO_3^-]$ x urine flow rate) and the ammonium efflux ($[NH_4^+]$ x urine flow rate).

e) Cutaneous ion and acid fluxes

Net fluxes (J_{net}) of Na^+, K^+, Ca^{2+}, Cl^-, NH_4^+, P_i, urea, lactate (in μequiv/kg·h or μmol/kg·h) were calculated from changes in their concentrations in the water and changes in water volume. Sodium and

chloride influx values (J_{in}) were determined by two techniques:

(i) When isotope was added to the water J_{in} was determined by the disappearance of radioactivity from the water according to the following equation (modified from equation 5 of Kirschner, 1970; McDonald *et al.*, 1983):

$$J_{in} = \frac{(\ln Q^*out_o - \ln Q^*out_t)}{t \cdot W} \cdot Q_{out} \qquad (1)$$

where Q^*out_o and Q^*out_t were the total amounts of radioactivity in the water at the beginning and end of the flux period, Q_{out} was the mean ion concentration in the medium during the flux period, 't' was the duration of the flux period, and 'W' was body weight. Frequent analysis during the critical first 12 h of recovery minimized variations in Q_{out}; necessary because this equation assumes no net flux (i.e. that Q_{out} remains constant). This equation also assumes no significant backflux of radioisotope. During the initial flux periods of 4 h duration or less this backflux was assumed to be negligible (McDonald *et al.*, 1983). For 12 h flux periods the backflux was determined by replacement of radioactive water with non-radioactive water and measuring the appearance of isotopes from the frog into the water. These values were then used to correct the flux of isotopes into the animal when it was in radioactive water. J_{out} (efflux) for Na^+ and Cl^- was calculated by subtracting J_{net} from J_{in} (signs considered).

(ii) When radioisotope is injected into the animal, efflux (J_{out}) is measured by the appearance of radioactivity into the water according to the following equation (modified from equation 1 of Kirschner, 1970):

$$J_{out} = \frac{Q^*out_t \cdot V_t}{X_{in} \cdot t \cdot W} \qquad (2)$$

where V_t is the volume of water remaining in the flux box at the end of the flux period, X_{in} is the specific activity (Kirschner, 1970) of radio-ion in the plasma of the bullfrog, and the other parameters are as described above. Flux of radioisotope back into the animal is assumed to be negligible since the concentration of radioactive ion in the bathing medium was kept low by periodic replacement of the water. J_{in} for Na^+ and Cl^- was calculated from the sum of J_{net} plus J_{out} (signs considered).

Net cutaneous acid fluxes (in μequiv/kg·h) were determined as the difference between the apparent H^+ uptake or loss (apparent base loss or uptake) and the ammonia excretion by procedures described by McDonald and Wood (1981) and McDonald (1983a). By this technique the apparent H^+ flux is determined by the change in titratable alkalinity of the water. Titratable alkalinity was determined within 12 h of collection by titrating continuously aerated 10 ml samples to a pH below 4.0 with 0.02 N-HCl.

It is important to note that a net cutaneous H^+ flux would be the result of the combined movements across the skin of H^+, NH_4^+, HCO_3^- and OH^-. While it is impossible to distinguish between these forms all are equivalent in terms of the animal's acid-base status (McDonald, 1983b).

5. Calculations

a) Arterial HCO_3^- and PCO_2

The partial pressure of CO_2 in plasma (PCO_2) was calculated

from the following equation:

$$PCO_2 = \frac{TCO_2}{(1 + 10^{(pH - pk_1')}) \cdot \alpha CO_2} \quad (3)$$

where TCO_2 and pH of plasma and blood, respectively, are measured, and pk_1' (negative log of the apparent first dissociation constant for CO_2) and αCO_2 (solubility coefficient for CO_2) corrected to experimental temperature and blood pH were obtained from Severinghaus (1965). Plasma $[HCO_3^-]$ was calculated using the equation:

$$[HCO_3^-] = TCO_2 - \alpha CO_2 \cdot PCO_2 \quad (4)$$

Although values for pK_1' and αCO_2 for amphibians at 25°C are available (Boutilier *et al.*, 1979a; Boutilier and Toews, 1981a), accurate extrapolation of these values could not be made and also may differ from bullfrog plasma values. Differences between Boutilier's experimentally derived values and those of Severinghaus (1965) are small so that any error inherent in the use of mammalian values of pK_1' and αCO_2 should be negligible.

b) Na^+ and Cl^- Spaces

The Na^+ or Cl^- space represents that volume occupied by the ion assuming that its concentration in the plasma remained unchanged. The space is estimated by infusing a known amount of radioactive Na^+ and/or Cl^- into the animal and periodically sampling the blood, urine and water for analysis of radioactivity. Once isotope equilibration is reached the ion space is calculated by using the equation:

$$\text{Ion space (ml)} = \frac{\text{total ion radioactivity in body (cpm)}}{\text{concentration of ion radioactivity in plasma (cpm/ml)}} \quad (5)$$

where the numerator is determined from the difference between the quantity of radioisotope infused (in cpm) and the quantity of radioisotope lost *via* the urine and across the skin (in cpm). The ion space value can then be used to estimate the total amount of mobilizable ion in the animal (i.e. space (ml) x plasma [ion] (μequiv/ml) = total amount of ion (μequiv)).

c) Respiratory and non-respiratory acids in whole blood

Increase in PCO_2 and acid metabolites (i.e. lactic acid) following forced submergence, and non-respiratory acid following acid infusions, will increase the quantity of H^+ buffered by the blood non-bicarbonate (mainly protein) buffer systems. The quantity of buffered respiratory acid (ΔH^+r) was estimated by graphical interpretation of the pH-bicarbonate diagram (Davenport, 1974) as described by Woodbury (1974) and Wood *et al.* (1977). This respiratory component is expressed as a percentage of the total $[H^+]$ change at 't_n' attributable to the change in PCO_2.

The non-respiratory and/or metabolic acids (ΔH^+_m) may be estimated from the following formula based on graphical analysis of the pH-bicarbonate diagram (McDonald *et al.*, 1980; 1982):

$$\Delta H^+_m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2) \qquad (6)$$

where β is the slope of the non-bicarbonate buffer line (ΔHCO_3^-/ pH) expressed as mequiv/l·pH unit, i.e. slykes, and the subscripts 1 and 2 refer to whole blood $[HCO_3^-]$ and plasma pH measurements made prior to and following induction of a blood acidosis. The value of β employed in the analysis was taken from Lenfant and Johansen (1967):

$$\beta = -16.4 \text{ slykes} \qquad (7)$$

at a blood hemtocrit of 23.5%. It is known that β varies linearly with hematocrit (McDonald *et al.*, 1980). Since the mean hematocrit of bullfrog blood under control conditions (Table 3) was not very different from 23.5%, the β value of -16.4 slykes was used consistently in all of the analyses. Additionally, this value is midway between that found in other anuran species (range from -11.5 to -23.6 slykes; cf. Toews and Boutilier, 1983) and is thus considered to be representative for bullfrogs. Any resultant error due to the use of the assumed values for hematocrit and β are small (< ± 5%) and therefore should not qualitatively affect the conclusions drawn concerning non-respiratory acids buffered by the blood (ΔH^+_m).

d) Quantity of H^+ buffered by the ECF (ΔH^+_{ECFV})

The H^+ load to the ECF (ΔH^+_{ECFV}; units = μequiv/kg body weight) was estimated according to equation (3) of McDonald *et al.* (1982):

$$\Delta H^+_{ECFV} = BV \cdot \Delta H^+_m + [IS - BV(1 - hct)] \cdot \Delta H^+_{IF} \qquad (8)$$

where BV is blood volume, IS is the inulin space, and ΔH^+_m and ΔH^+_{IF} (interstitial fluid) were calculated from (7). For ΔH^+_{IF} a β value of separated plasma (β = -3.29; McDonald *et al.*, 1980) was used with the assumption that it was similar to the interstitial fluid. This assumption is reasonable because blood acid-base and ion status of *Bufo marinus* are very similar to that of the bullfrog (unpublished observations, this study) and are similar to values published for fish (-2.06; cf McDonald *et al.*, 1982) and urodeles (-3.99; Boutilier and Toews, 1981a). The values used for inulin space (i.e. interstitial fluid volume plus

plasma volume) and blood volume for fully hydrated bullfrogs were, respectively, 270 ml/kg and 65 ml/kg (Thorson, 1964). If values at the lower end of the given ranges for IS and BV (i.e. ∿200 and ∿50 ml/kg, respectively; Thorson, 1964) were used, the estimated ΔH^+_{ECFV} would consistently be about 13% less than the results presented below. However, this small change, again, should not affect the qualitative interpretation of the data.

e) Renal clearances and clearance ratios

Quantification of the mechanisms of renal electrolyte handling using the concept of clearance was introduced by Smith (1951). In frogs, urine flow rate (UFR) is largely determined by glomerular filtration, such that UFR and glomerular filtration rate (GFR) are linearly related (Forster, 1938). In this study GFR has been estimated as 1.52 x UFR from the data of Forster (1938). This estimate is similar to that obtained from the data of Long (1973) and Deeds *et al.* (1977). Renal clearance (C) of an electrolyte x is defined (after Koch, 1974; Wheatly *et al.*, 1983):

$$C_x = \frac{[x]_u \times UFR}{[x]_p} \qquad (9)$$

where u and p refer respectively to urine and plasma concentrations. The clearance value (C) expresses the degree to which the electrolyte (or metabolite) is removed from blood by excretion into urine. Net tubular electrolyte reabsorptive or secretive processes can be quantified by the clearance ratio:

$$\frac{C_x}{GFR} = \frac{x \text{ excretion}}{x \text{ filtration}} \qquad (10)$$

If the clearance ratio is < 1, then net reabsorption has occurred; if > 1, then net secretive processes have occurred (Koch, 1974; Wheatly *et al.*, 1983).

RESULTS

1. Control Measurements

Control values for blood chemistry, urinary excretion rates, and cutaneous flux rates are shown for each experimental series in Tables 3 to 5. These data are similar to values reported in the literature for this, and other species of Rana (Tables 3 to 5 for references). On this basis, these bullfrogs are considered to have been in healthy condition prior to the induction of the blood acid-base disturbance.

2. General Observations

Infusions of HCl resulted in immediate stimulatory effects upon locomotory activity and upon ventilation; the less concentrated acid had slightly less effect. No immediate mortalities resulted from the acid infusions. However, all frogs infused with 1.0 N-HCl died within 72 h. Infusion with 0.1 N-HCl resulted in the death of the small bullfrogs (Series 7) within 24 h despite the reduced load. The large bullfrogs infused with the less concentrated acid all survived beyond 72 h but had died by 102 h. These differences in time course to mortality may be related to differences in body size. Necropsies revealed tissue damage at the femoral artery catheter site. Renal failure or damage was evident in some bullfrogs infused with HCl (see below).

Frogs infused with NH_4Cl or forcibly submerged showed no lasting detrimental effects. All bullfrogs were alive for at least 2 weeks

Table 3. References. Blood and plasma acid-base, ions and metabolite characteristics of cannulated bullfrogs at control conditions.

1. MacKenzie and Jackson, 1978. (20°C)
2. Yoshimura *et al.*, 1961.
3. Mullen and Alvarado, 1976. (*R. pipiens*)
4. Carmena-Suero *et al.*, 1980. (22°C)
5. Lenfant and Johansen, 1967.
6. Howell *et al.*, 1970. (20°C)
7. Erasmus *et al.*, 1970/71. (20°C)
8. Howell, 1970. (20°C)
9. Mullen and Alvarado, 1976. (*R. pipiens*)
10. Jungreis, 1970. (*R. pipiens*; 25°C)
11. Roberston, 1972. (*R. pipiens*; 25°C)
12. Deeds *et al.*, 1977. (24-29°C)
13. Long, 1973 (uncatheterized; 25°C)
14. Lillo, 1978 (21-25°C)
15. Hutchison and Turney, 1975. (*R. pipiens*; 15°C)
16. Putnam, 1979. (*R. pipiens*; 16°C)
17. Scheer and Mumbach, 1982. (*R. esculenta*; 15°C)
18. Pitts, 1974.

Table 3. Blood and plasma acid-base, ions and metabolite characteristics of cannulated bullfrogs at control conditions.

Rana catesbiana (T = 21°C); values are means ± 1 S.E.M.

Blood Parameter	Series 2 N = (3)	3 (4)	4 (4)	5 (4)	7 (2)	8 (3)	Literature Values
pH	7.963 ±0.024	7.885 ±0.012	7.843 ±0.017	7.850 ±0.047 0.042	7.812 ±0.050	7.934 ±0.014	7.92 ±0.03[1] 7.87±0.08[6] 7.92±0.07[14]
PCO_2 (mmHg)	10.92 ± 0.73	10.77 ± 0.44	12.52 ± 0.73	12.22 ± 1.75	11.57 ± 1.09	12.51 ± 0.49	8.2±0.6[5] 11.1±0.5[1] 12.7±3.8[6]
$[HCO_3^-]$ (mmol/l)	33.42 ± 2.06	25.11 ± 1.59	28.04 ± 0.44	29.81 ± 2.95	22.21 ± 0.71	36.64 ± 1.46	30.2±4.8[6] 21±3[12]
hct (%)	25.36 ± 3.33	19.92 ± 1.15	19.05 ± 1.18	22.9 ± 2.3	12.72 ± 1.15	25.72 ± 1.94	40.44±1.2 23.5[5] 21.61±23.7[10]
$[Na^+]$ (mequiv/l)	119.12 ± 5.23	128.25 ± 1.64	105.78 ± 1.51	131.96 ± 1.31	114.50 ± 3.96	119.07 ± 1.41	97[2] 97±8[13] 112±3[9] 113±4[12]
$[Cl^-]$ (mequiv/l)	57.42 ± 3.08	66.65 ± 0.83	60.95 ± 1.05	61.71 ± 2.24	76.54 ± 1.30	57.45 ± 1.70	68±3[9] 81±8[11]
$[K^+]$ (mequiv/l)	3.74 ± 0.21	2.54 ± 0.37	4.44 ± 0.10	5.21 (n=1)	2.61 ± 0.12	4.36 ± 0.16	2.8±0.8[12]
$[Ca^{2+}]$ (mequiv/l)	3.89 ± 0.28	2.38 ± 0.16	3.29 ± 0.04	4.51 ± 0.86	2.53 ± 0.06	4.41 ± 0.27	4.6[11]
$[NH_4^+]$ (μequiv/l)	299.31 ±21.27	258.92 ±15.63	187.96 ± 8.60	142.09 ± 8.67	228.22 ±29.62	255.05 ±26.99	100[13]
$[P_i]$ (mmol/l)	1.096 ±0.119	1.345 ±0.046	1.423 ±0.234	0.978 ±0.031		1.234 ±0.064	similar to mammals[18]
[UREA] (mmol/l)	0.264 ±0.225	2.258 ±0.331	1.063 ±0.130	0.265 ±0.125		0.356 ±0.136	0.77±0.12[13]
[Lactate] (mmol/l)	1.247 ±0.395	0.010 ±0.008	0.491 ±0.057	1.76 ±0.11	1.12 ±0.17	0.605 ±0.083	
[glucose] (mmol/l)	undetectable	2.412 ±0.237	1.168 ±0.077	2.429 ±0.746			
protein (g/100 ml)		2.52 ±0.14	2.73 ±0.07	3.02 ±0.48			
Na^+ space (ml/kg)			483.8 ±18.70	453.9 ±15.2			
Cl^- space (ml/kg)			393.0 ±20.6	425.9 ±18.6			

Table 4. Urine acid-base and ion characteristics of bullfrogs during control periods. Values are means ± 1 S.E.M.

Urine Parameter	Series 2 N = (3)	3 (4)	4 (4)	5 (4)	7 (2)	8 (5)	Literature Values
Flowrate (ml/kg·h)	4.09 ± 0.36	.433 ± 0.63	4.61 ± 0.47	9.16 ± 1.44	8.64 ± 1.09	3.77 ± 0.31	6.6±0.9[1] 14.2±1.6[2]
pH	7.56 ± 0.06	8.02 ± 0.13	7.40 ± 0.08	7.80 ± 0.04	7.61 ± 0.10	6.78 ± 0.14	7.30±0.12[1]
NH_4^+ (μeq/kg·h)	36.86 ± 3.23	31.04 ± 5.62	28.48 ± 3.81	67.20 ±11.79	97.18 ± 1.85	29.08 ± 1.85	32.8±9.4[1]
TA-HCO_3^- excretion (μeq/kg/h)	-19.51 ± 3.11	-16.16 ± 2.97	-11.34 ± 2.94	-25.40 ± 7.37	-4.47 ±10.49	-3.28 ± 1.56	
Total H^+ excretion (μeq/kg/h)	17.37 ± 2.42	18.74 ± 9.85	18.89 ± 2.18	41.79 ± 9.15	92.71 ±21.74	24.64 ± 2.10	
Na^+ (μeq/kg·h)	5.29 ± 0.67	5.76 ± 1.12	1.38 ± 0.12	71.47 ±38.74	4.61 ± 2.45	7.39 ± 1.58	27.3±7.7[1] 2.5[3] ± 9.2[4]
Cl^-	0.16 ± 0.04	1.31 ± 0.060	1.14 ± 0.19	39.45 ±28.94	4.21 ± 1.22	0.53 ± 0.10	20.1±6.1
K^+	9.95 ± 1.14	0.08 ± 0.02	7.56 ± 0.95	13.77 ± 4.06	26.52 ± 5.72	5.33 ± 0.81	6.7±5.9[1] 2.4±1.0[6]
Ca^{++}	3.70 ± 0.54	0.27 ± 0.11	1.62 ± 0.25	3.61 ± 1.05		3.10 ± 0.35	0.22±0.06[5]
P_i μmol/kg·h	21.49 ± 2.00		11.85 ± 1.27	8.79 ± 2.16		28.85 ± 2.54	4.4±4.2[1]
UREA (μmol/kg·h)	135.97 ± 6.65	90.30 ±14.72	58.76 ± 8.09	277.19 ±96.68	25.30 ± 4.09	118.84 ± 8.40	167±22[2]

References

1. Yoshimura *et al.*, 1961 (mean ± S.D.)

2. Long, 1973

3. Scheer *et al.*, 1974 (R. pipiens; water $[Na^+]$ = 0.5 mmol/1)

4. Ibid (R. pipiens; water $[Na^+]$ = 1.3 mmol/1)

5. Baldwin and Bentley, 1981 (R. pipiens, 18°C; 0.01 mM Ca^{++})

6. Frazier and Vanatta, 1981 (R. pipiens, room temp., 2 mM NaCl solution)

Table 5. Whole body (*) or cutaneous ion and acid fluxes during control periods. Values are mean ± 1 S.E.M.

Ion flux	Series 1 N = (3)*	2 (3)	3 (4)	4 (4)	6 *(2)	7 (2)	8 (5)	Literature values μeq/kg·h
H_2O ml/kg·h	0.29 ± 0.83	3.96 ± 0.21	2.98 ± 0.25	3.03 ± 0.29	-0.78 ± 0.66	7.21 ± 1.07	3.16 ± 0.15	
$J_{net}^{Na^+}$ μeq/kg·h	-18.70 ± 5.56	-7.39 ± 2.17	-29.97 ± 5.25	-35.96 ± 7.00	-41.95 ± 5.88	-80.37 ±45.31	-18.29 ± 5.17	-100[3] 93±50[8]
$J_{in}^{Na^+}$	31.10 ± 2.78	27.91 ± 2.03		33.03 ±10.39		150.57 ±24.77	52.75 ± 4.08	190±10[3] 150[5] 181±50[8] 230[6]
$J_{out}^{Na^+}$	49.80 ± 6.47	32.96 ± 3.05		69.41 ±36.15		230.94 ±67.76	74.04 ± 7.29	88±29[8] 481[2] 295±25[8][7] 378[1] 200±30[3] 125
$J_{net}^{Cl^-}$	-13.41 ± 3.58	-10.74 ± 4.10	-29.45 ± 3.85	-42.10 ± 3.13	-40.58 ± 6.37	-67.58 ±28.03	-7.33 ± 2.75	-60[3] 53±20[8]
$J_{in}^{Cl^-}$	20.39 ± 1.75	15.04 ± 1.33				57.77 ±16.26	32.25 ± 2.43	180±20[3] 150±17[8]
$J_{out}^{Cl^-}$	29.53 ± 2.58	22.64 ± 2.46				125.33 ±43.86	41.39 ± 4.68	240±200[3] 97±16[8]
$J_{net}^{K^+}$	-7.55 ± 2.01	-12.31 ± 2.26	-16.86 ± 2.78	-24.09 ± 4.88	-25.98 ± 4.03	-31.25 ±15.96	-20.81 ± 2.52	-20±10[3] -15.4±3.6[11] -11.8±2.8[11]
$J_{net}^{Ca^{++}}$	-1.18 ± 0.37	+0.11 ± 0.04	-0.47 ± 0.66	-1.45 ± 0.75	+1.09 ± 0.34		+1.39 ± 0.27	-0.32±0.04[9] -022±0.08[10]
J_{net}^{Pi} μmol/kg·h	-6.94 ± 1.51	-0.95 ± 0.16	-0.41 ± 0.11	2.53 ± 0.45	-12.17 ± 4.19		-2.85 ± 0.59	
J_{net}^{UREA}	-61.20 ±18.65	-5.54 ± 1.00	-0.47 ± 0.22	-2.33 ± 1.29	-42.75 ± 5.23	-3.90 ± 1.00	-10.35 ± 2.75	
$J_{net}^{NH_4^+}$	-67.70 ±12.39	-4.33 ± 1.07	-11.15 ± 1.53	-22.78 ± 4.03	-48.16 ± 7.80	-19.06 ± 9.69	-17.06 ± 1.96	-70±10[3]
$J_{net}^{H^+}$	-14.21 ±11.65	+8.32 ± 6.77	+2.56 ± 6.01	+10.86 ± 2.91	+6.64 ± 4.86	+27.11 ±49.01	+15.14 ± 5.29	

* net fluxes include renal contributions

1. Scheer *et al.*, 1974 (R. pipiens, from 0.2 mM-NaCl)
2. Ibid (R. pipiens, from 1.3 mM-NaCl)
3. Alvarado and Moody, 1970 (newly transformed bullfrogs, wt = 8.0 g)
4. Mayer, 1969. Ehrenfeld, 1972 (R. esculenta, 20°C)
5. Brown, 1962 (R. pipiens, 20°C)
6. Greenwald, 1971 (R. pipiens, 20°C)
7. Scheer and Mumbach, 1982 (R. esculenta, 15°C, from 0.1 M-NaCl)
8. Garcia-Romeu and Ehrenfeld, 1975a(R. esculenta from 1.8 meq/l NaCl buffered pH 7.0)
9. Baldwin and Bentley, 1982 (R. pipiens, 18°C, from 0.01 mM-Ca^{++})
10. Ibid (R. pipiens, 18°C, 0.12 mM-Ca^{++})
11. Frazier and Vanatta, 1981 (R. pipiens, room temp. from 2 mM-NaCl)

after termination of the experimental series.

3. Blood Acid-Base and Ion Balance

All acid infusion procedures, and forced submergence, produced a marked blood acidosis. In contrast to the HCl infusions, the blood acidosis produced by NH_4Cl infusion or forced submergence was rapidly and fully corrected, causing no apparent long-lasting ill effects.

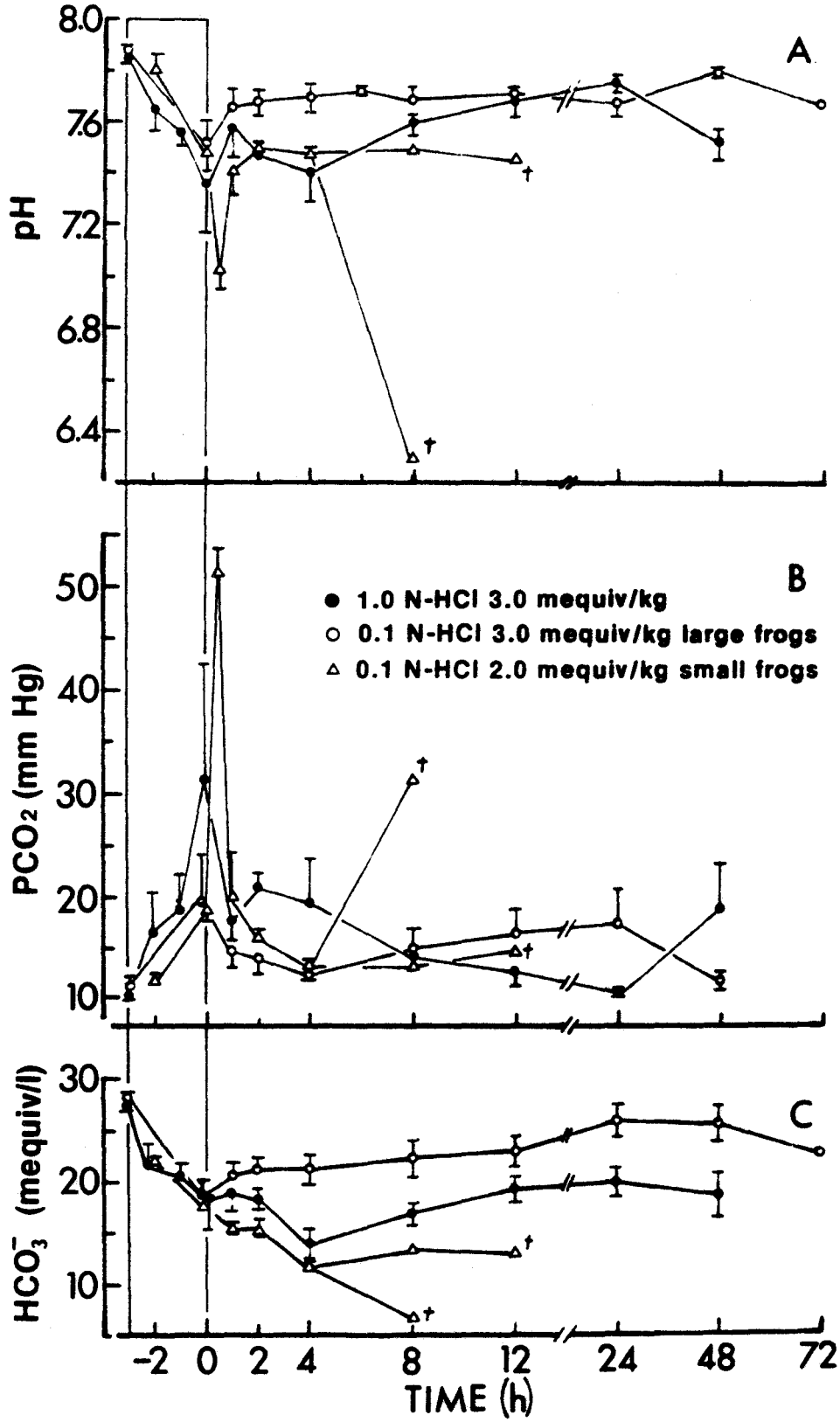
Acid-Base Disturbances

(a) HCl Infusions (Series 3, 4 & 7)

Infusion of HCl produced a blood pH depression of at least 0.2 units in all bullfrogs. This was accompanied by a depression of plasma $[HCO_3^-]$ and an elevation of PCO_2 (Fig. 2), indicating that the acidosis had both respiratory (i.e. elevated PCO_2) and non-respiratory (i.e. infused acid plus endogenous acids) components.

Blood acid-base parameters in the infusions using large bullfrogs showed a trend towards recovery of normal acid-base status. The small bullfrogs, although infused with less acid per unit body weight (Series 7), showed only partial correction (Fig. 2). A mixed respiratory/ metabolic acid persisted throughout the post-infusion periods despite trends toward recovery. The respiratory contribution to the pH change (ΔH^+r, see Methods) immediately post-infusion was estimated to be about 70% in frogs infused with 1.0 N-HCl and small frogs infused with 0.1 N-HCl. By 8 h and 2 h, respectively, the majority of the acidosis was of the fixed (i.e. non-respiratory) type. In the large bullfrogs infused with 0.1 N-HCl the persistent elevation of PCO_2 was responsible for about 30% of the pH depression.

Fig. 2. (A) Plasma pH, (B) PCO_2 and (C) bicarbonate concentration in bullfrogs prior to (t=-3 or -2 h), during (t=-3 to 0 h) and following intravascular infusion of HCl. Closed circles (N=4) bullfrogs (394 ± 14 g) infused with 3,000 μequiv/kg of 1.0 N-HCl. Open circles (N=4) bullfrogs (354 ± 8 g) infused with 3,000 μequiv/kg of 0.1 N-HCl. Open triangles (N=2) bullfrogs (118 ± 5 g) infused with 2,000 μequiv/kg of 0.1 N-HCl. Vertical bars from t=-3 h to t=0 h represents the 3 h infusion period. The '†' indicates the final sample obtained before death.


8.0
7.6
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A
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40
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20
10
PCO₂ (mm Hg)
B
● 1.0 N-HCl 3.0 mequiv/kg
○ 0.1 N-HCl 3.0 mequiv/kg large frogs
△ 0.1 N-HCl 2.0 mequiv/kg small frogs
30
20
10
HCO₃⁻ (mequiv/l)
C
-2
0
2
4
8
12
24
48
72
TIME (h)

Fig. 3. Changes in the non-respiratory acid load buffered by the blood (ΔH^+_m) (closed circles) and blood lactate concentration (open triangles) during and following HCl infusions. (A) 1.0 N-HCl, 3,000 µequiv/kg. (B) 0.1 N-HCl, 3,000 µequiv/kg - large frogs. (C) 0.1 N-HCl, 2,000 µequiv/kg - small frogs. For other details see legend of Fig. 2.

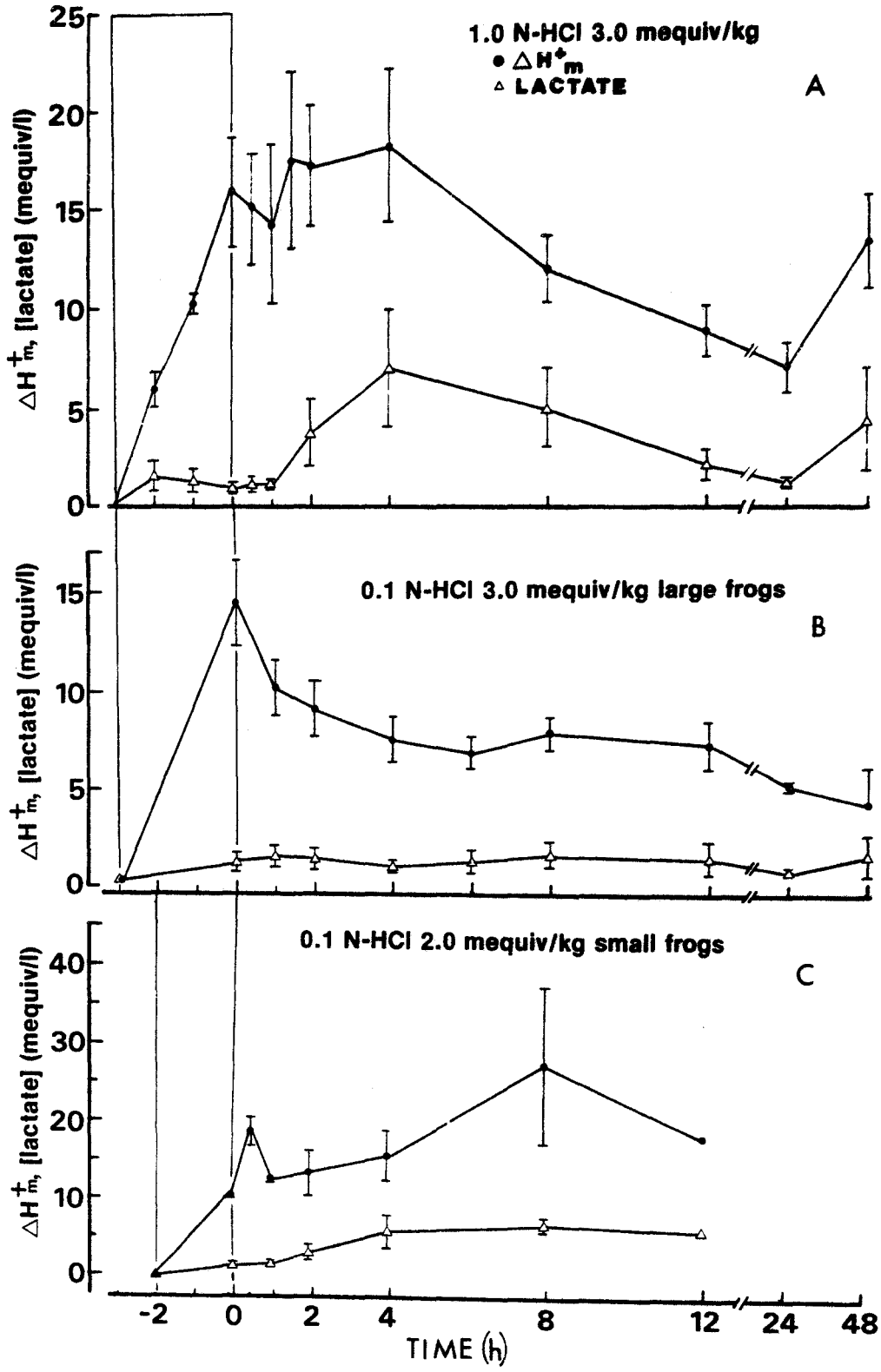
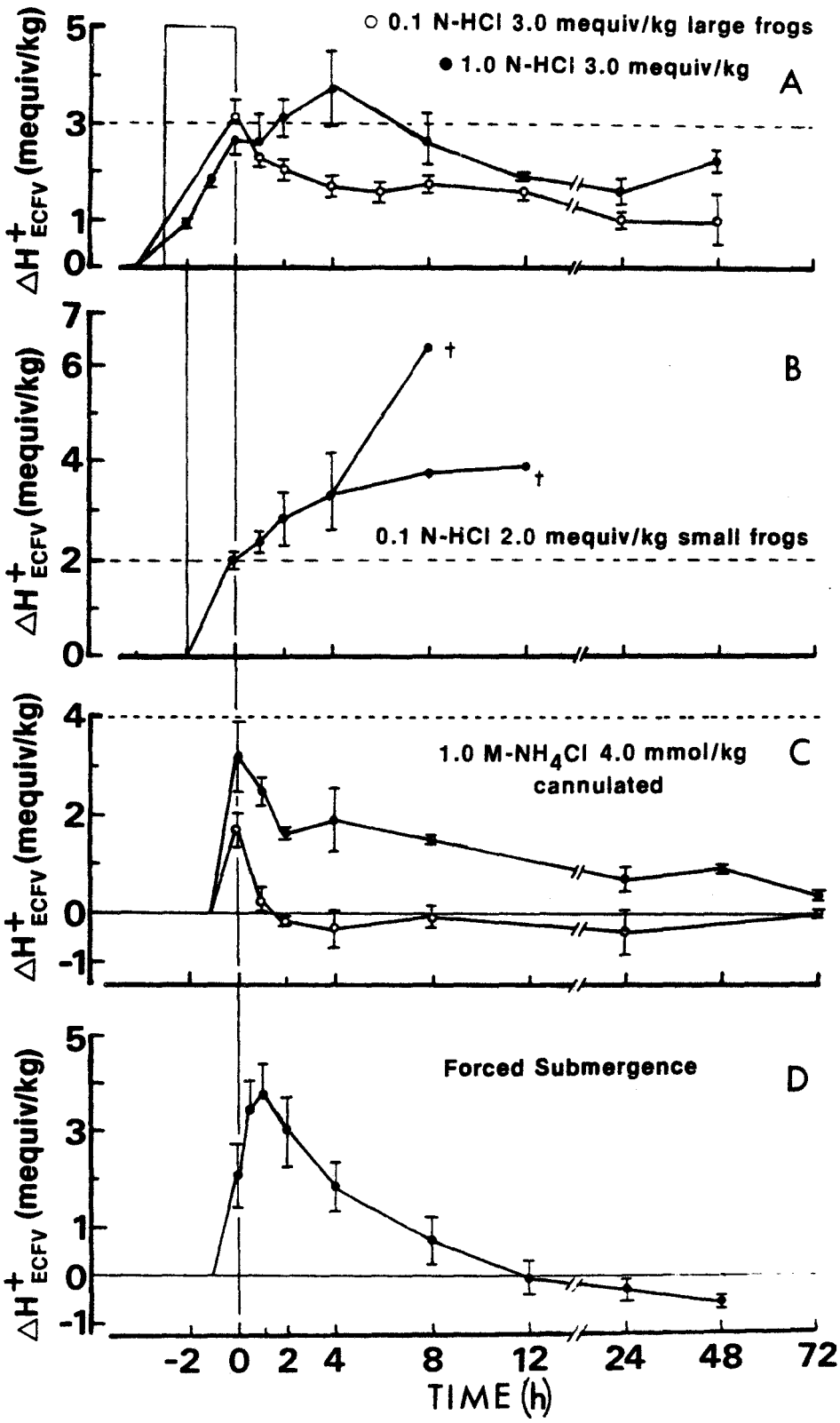

1.0 N-HCl 3.0 mequiv/kg
● △ H^+_m
△ LACTATE
A
0.1 N-HCl 3.0 mequiv/kg large frogs
B
0.1 N-HCl 2.0 mequiv/kg small frogs
C
ΔH^+_m, [lactate] (mequiv/l)
TIME (h)
-2
0
2
4
8
12
24
48

Fig. 4. The non-respiratory acid load estimated to be buffered by the ECF during and following induction of the acidosis. (A) Open circles - large bullfrogs infused with 0.1 N-HCl. Closed circles - 1.0 N-HCl. (B) Small bullfrogs infused with 0.1N-HCl. Data for the 2 frogs plotted separately after t=4 h. (C) Bullfrogs infused with NH_4Cl. Open circles, N=2; closed circles, N=2. Dashed lines in A-C represent the amount of H^+ or NH_4^+ infused. (D) Forced submergence. For other details see legend of Fig. 2.


○ 0.1 N-HCl 3.0 mequiv/kg large frogs
● 1.0 N-HCl 3.0 mequiv/kg
A
B
0.1 N-HCl 2.0 mequiv/kg small frogs
1.0 M-NH4Cl 4.0 mmol/kg
cannulated
C
Forced Submergence
D
ΔH+ ECFV (mequiv/kg)
TIME (h)
-2 0 2 4 8 12 24 48 72

The fixed acid (ΔH_m^+) component of the acidosis is shown in Fig. 3. As predicted, HCl infusion resulted in a sustained non-repiratory acidosis which was never fully corrected. A major response to acid infusion was an elevation in blood lactate (Fig. 3). The lactic acid contribution was large and paralleled the ΔH_m^+ time course in frogs infused with 1.0 N-HCl (Fig. 3a). A similar trend was apparent in small frogs (113-122g) loaded with 0.1 N-HCl (Fig. 3c) but there was no appreciable lactacidosis in the large frogs (340-410g) infused with 0.1 N-HCl (Fig. 3b). Estimation of the quantity of fixed acid in the ECF (ΔH^+_{ECFV}) again shows the persistent nature of the acidosis (Fig. 4). In large bullfrogs 50% of the expected fixed acid load remained in the ECF after 24 h (Fig. 4A). However the small bullfrogs showed a progressive increase in acid loading of the ECF in excess of that infused (Fig. 4B). Thus at no time was there a net clearance of the infused acid load from the ECF.

(b) NH_4Cl Infusions (Series 5)

Intravascular infusion of 4.0 μmol/kg of 1.0 M-NH_4Cl resulted in a mixed respiratory/fixed acidosis which was corrected within 1 h post-infusion (Fig. 5). The initial respiratory acidosis component (40 ± 15% of the total pH change) rapidly diminished to a compensatory alkalosis. This was evident by the depressed PCO_2 and plasma [HCO_3] at 2 h (Fig. 5B, C). All blood acid-base parameters were normal by 8 h post-infusion.

Unlike HCl infusions, NH_4Cl loading was followed by a rapid clearance of fixed acid (ΔH^+_m) from the blood (Fig. 6A). Two types of response were seen. Two bullfrogs had corrected the fixed acidosis

Fig. 5. (A) Plasma pH, (B) PCO_2 and (C) bicarbonate concentration in bullfrogs before and after infusion with 4,000 µmol/kg of 1.0 M-NH_4Cl (N=4, open circles) or 45 min enforced submergence (N=3, closed circles). See legend of Fig. 2 for time scale details.

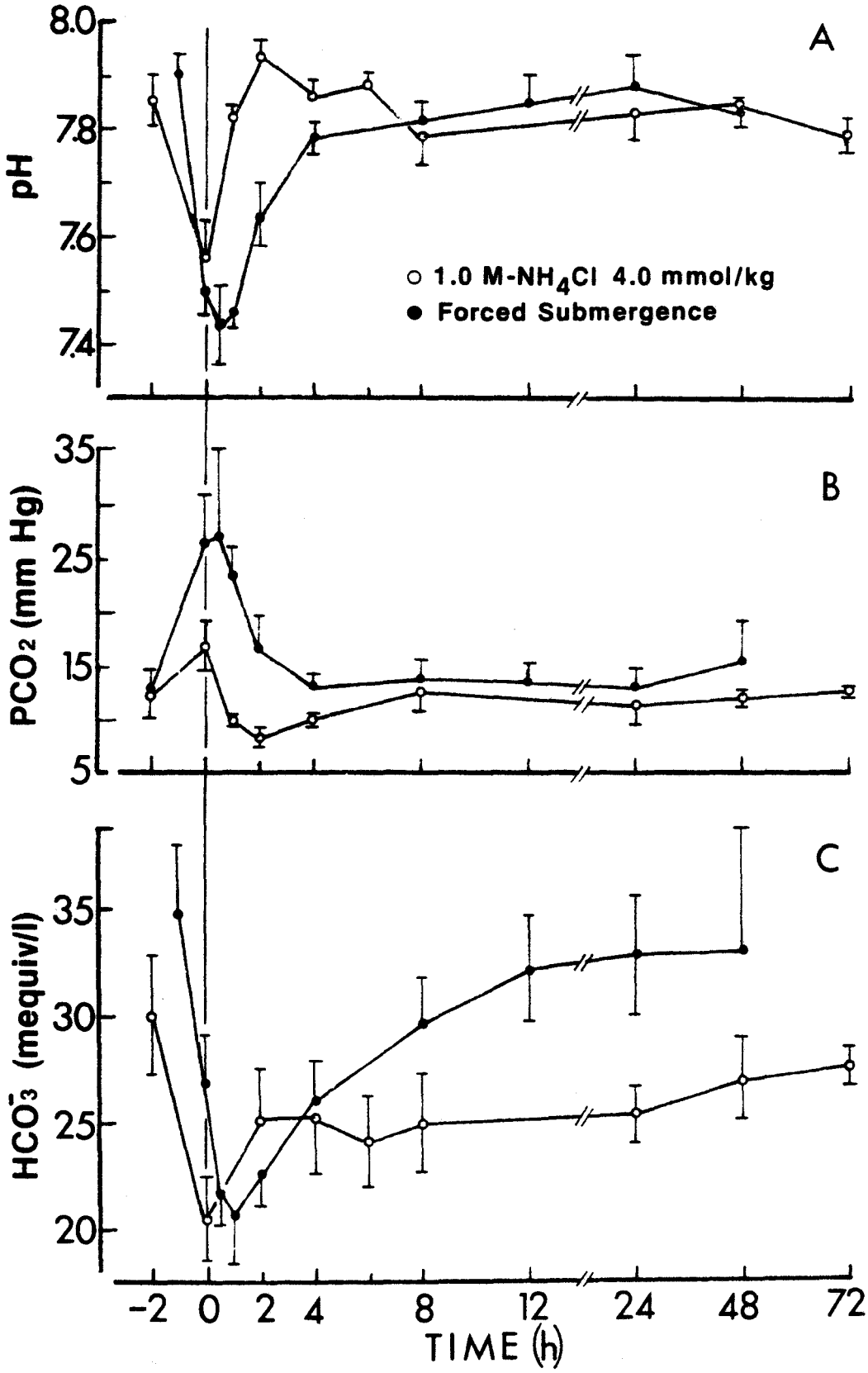
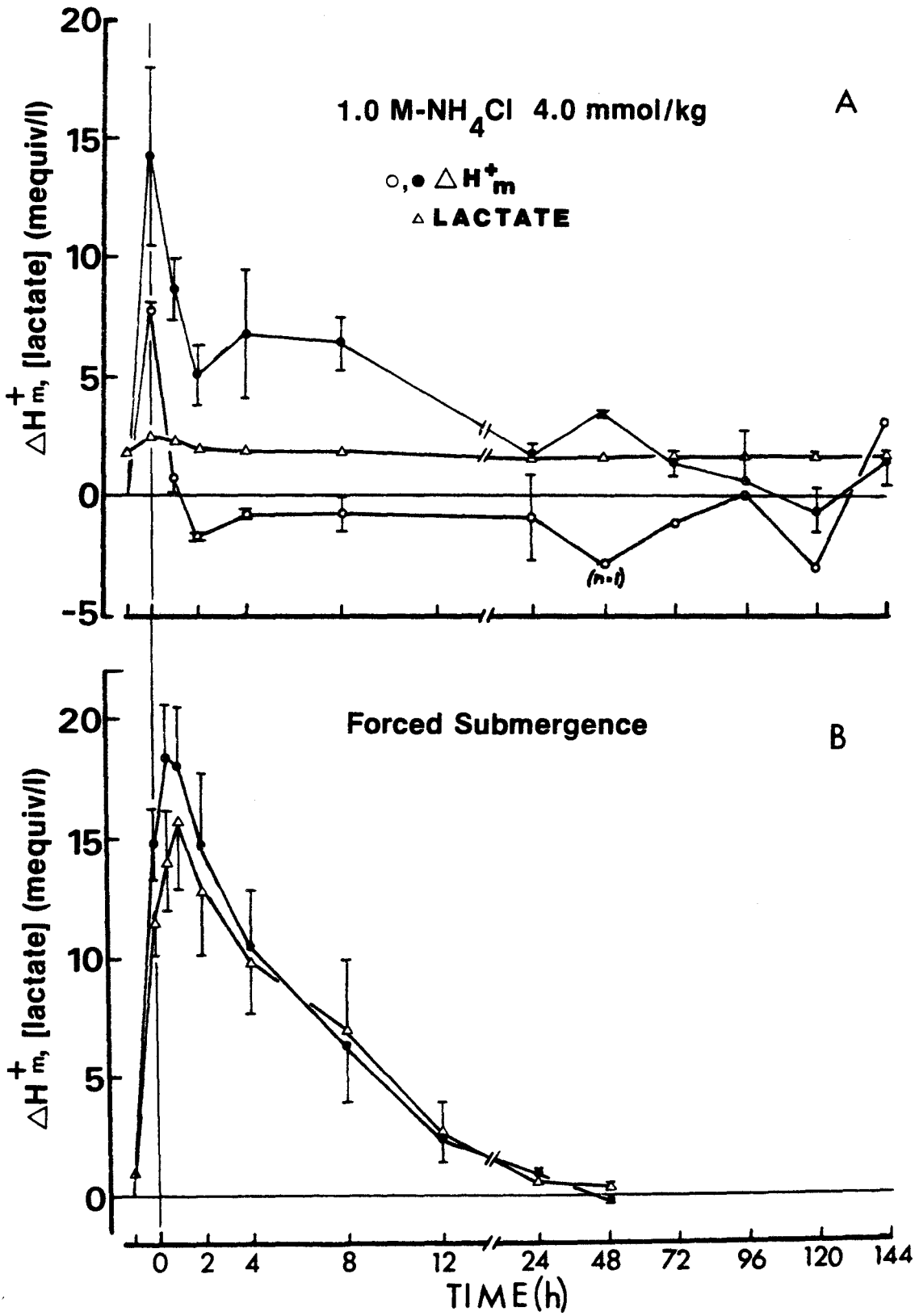

8.0
7.8
7.6
7.4
pH
A
○ 1.0 M-NH4Cl 4.0 mmol/kg
● Forced Submergence
35
25
15
5
PCO2 (mm Hg)
B
35
30
25
20
HCO3- (mequiv/l)
C
-2
0
2
4
8
12
24
48
72
TIME (h)

Fig. 6. Non-respiratory acid load buffered by the blood (ΔH^+_m) and lactate concentration before and following NH_4Cl infusions (A), and forced submergence (B). (A) Open circles - ΔH^+_m, N=2; closed circles - ΔH^+_m, N=2; open triangles - lactate, N=4. (B) Open triangles - lactate; closed circles ΔH^+_m. See legend of Fig. 5 for other details.


1.0 M-NH4Cl 4.0 mmol/kg
A
○,● △ H+m
△ LACTATE
ΔH+m, [lactate] (mequiv/l)
20
15
10
5
0
-5
(n-1)
Forced Submergence
B
0 2 4 8 12 24 48 72 96 120 144
TIME(h)

within 2 h whereas in the other frogs correction was not complete until 96 h. As shown in Fig. 6A, there was a negligible contribution by lactic acid to ΔH^+_m. Immediately post-infusion the estimated ΔH^+_{ECFV} was 3.17 ± 0.75 mequiv/kg and 1.70 ± 0.33 mequiv/kg, respectively, in these 2 groups (Fig. 4C). The virtually complete clearance of fixed acid in these frogs is in marked contrast to acid clearance following HCl infusions.

(c) Forced Submergence (Series 8)

As in the previous treatments, prolonged forced submergence resulted in a marked blood acidosis of greater severity than NH_4Cl infusions (Fig. 5) but of smaller magnitude than HCl infusions (cf. Fig. 2). Nevertheless, a normal blood acid-base status was fully restored within 12 h, which while longer than NH_4Cl infusions was of much shorter duration than HCl infusions.

The immediate post-dive respiratory component of the acidosis contributed 61 ± 12% of observed pH depression (-0.5 units). However, this respiratory contribution was very short-lived such that by 4 h post-dive the acidosis was greater than 95% of metabolic origin. Throughout the recovery period the amount of acid estimated to be buffered in the blood (ΔH^+_m) was virtually equal to the blood [lactate] (Fig. 6B). By t=4 h the analysis revealed that blood lactate was directly proportional to ΔH^+_m. The maximum proton load buffered by the ECF was estimated to be 3.75 ± 0.65 meq/kg at 1 h (Fig. 4D). The ECF proton load returned to zero by 12 h, concurrent with the restoration of normal blood acid-base status.

The theoretical blood lactate concentration at t=0 h was estimated from the exponential decline in blood lactate after the initial peak (Fig. 6B). Therefore the total effective lactate load in equilibrium with the blood upon termination of the dive was calculated using the formula of Kobayashi and Wood (1980):

$$\text{lactate load} = \text{lactate}_{t_o}\ (\mu\text{equiv/ml}) \times \text{lactate space (ml/kg)},$$

where lactate space was assumed equal to the ECF volume (Yudkin and Cohen, 1975; Kobayashi and Wood, 1980). Thus the effective lactate load was estimated to be 5,270±820 μequiv/kg (N=3), an acid load potentially larger than any infused with the possible exception of NH_4Cl - see Introduction.

Ionic Disturbances

The major ionic changes related to disturbance of acid-base status were marked elevations in plasma concentrations of K^+, Ca^{++}, and NH_4^+. Changes in plasma $[Na^+]$ and $[Cl^-]$ also occurred and reflect the effects of volume expansion of the ECF and the subsequent changes in ECF composition.

(a) HCl Infusions

Plasma $[Na^+]$ decreased about 9% during dilute acid infusions (Fig. 7A), reflecting the approximately 10% increase in ECF volume (taken as 270 ml/kg, Thorson, 1964). Since Cl^- was in the infusate, plasma $[Cl^-]$ didn't change during the acid-loading period (Fig. 7B). The post-infusion periods were characterized by steady-state levels of Cl^-. However, plasma $[Na^+]$ progressively decreased following 1.0 N-HCl infusion and dilute HCl infusion in the small frogs.

Dilute acid infusions in large bullfrogs didn't result in change of plasma $[K^+]$ and $[Ca^{++}]$. In contrast, infusion of concentrated HCl induced a 3-fold increase in plasma $[K^+]$ during the infusion period (Fig. 7C). This was rapidly corrected by 1 h post-infusion and plasma $[K^+]$ subsequently decreased to below control values. The small frogs exhibited a post-infusion doubling of plasma $[K^+]$ over 12 h (Fig. 7C). In these latter 2 experiments slight elevations in plasma $[Ca^{++}]$ occurred during acid-loading (Fig. 7D). Following 1.0 N-HCl infusion, plasma $[Ca^{++}]$ decreased to below control values by 1 h, similar to the trend shown in plasma $[K^+]$.

Plasma concentrations of glucose, and P_i rose dramatically with

Fig. 7. Plasma $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Ca^{++}]$ prior to, during, and following HCl infusions. See legend of Fig. 2 for more details.

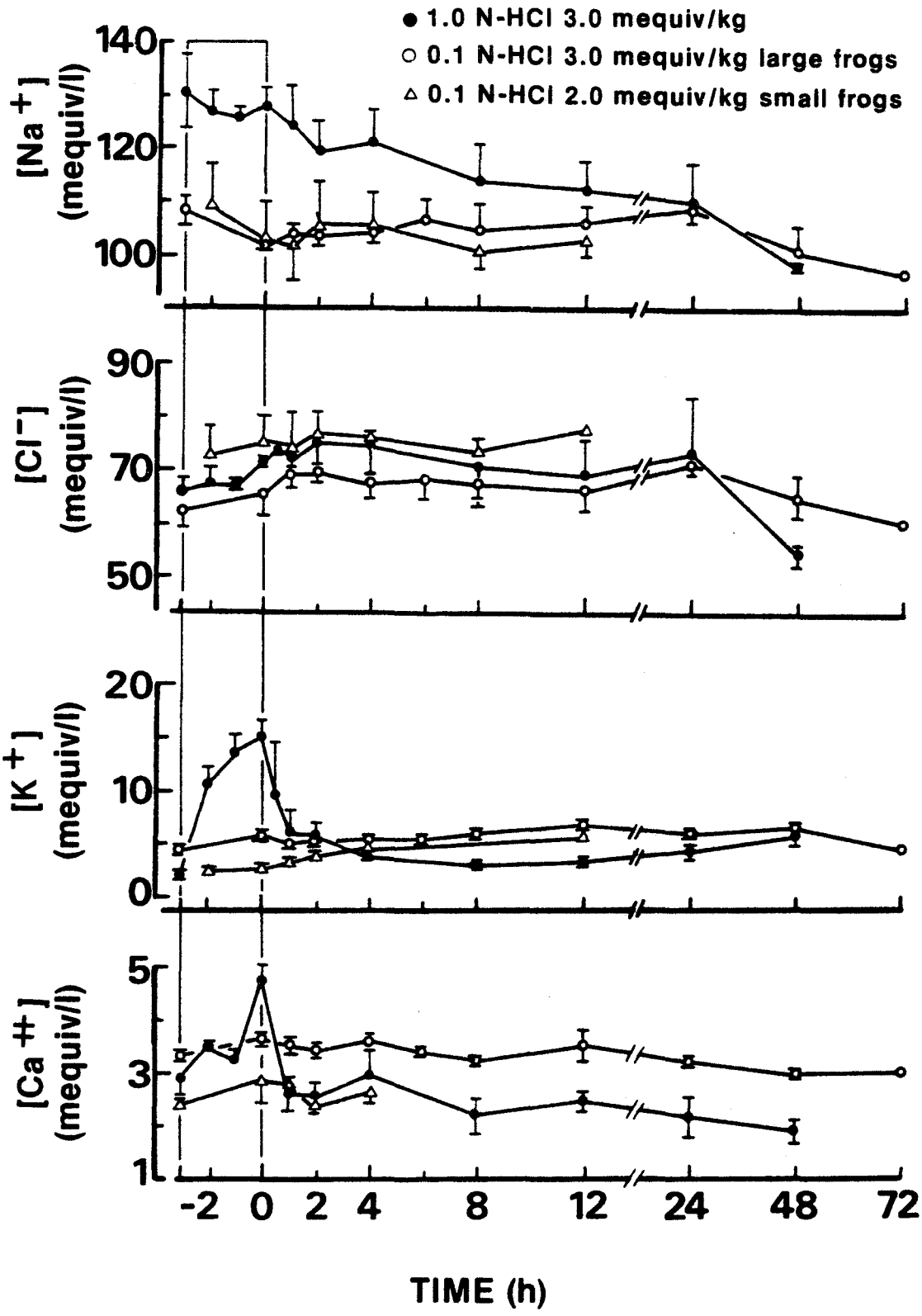

● 1.0 N-HCl 3.0 mequiv/kg
○ 0.1 N-HCl 3.0 mequiv/kg large frogs
△ 0.1 N-HCl 2.0 mequiv/kg small frogs
[Na+] (mequiv/l)
140
120
100
[Cl−] (mequiv/l)
90
70
50
[K+] (mequiv/l)
20
10
0
[Ca++] (mequiv/l)
5
3
1
-2
0
2
4
8
12
24
48
72
TIME (h)

Fig. 8. Plasma [glucose], [P_i] and [protein] prior to, during, and following HCl infusions. See legend of Fig. 2 for other details.

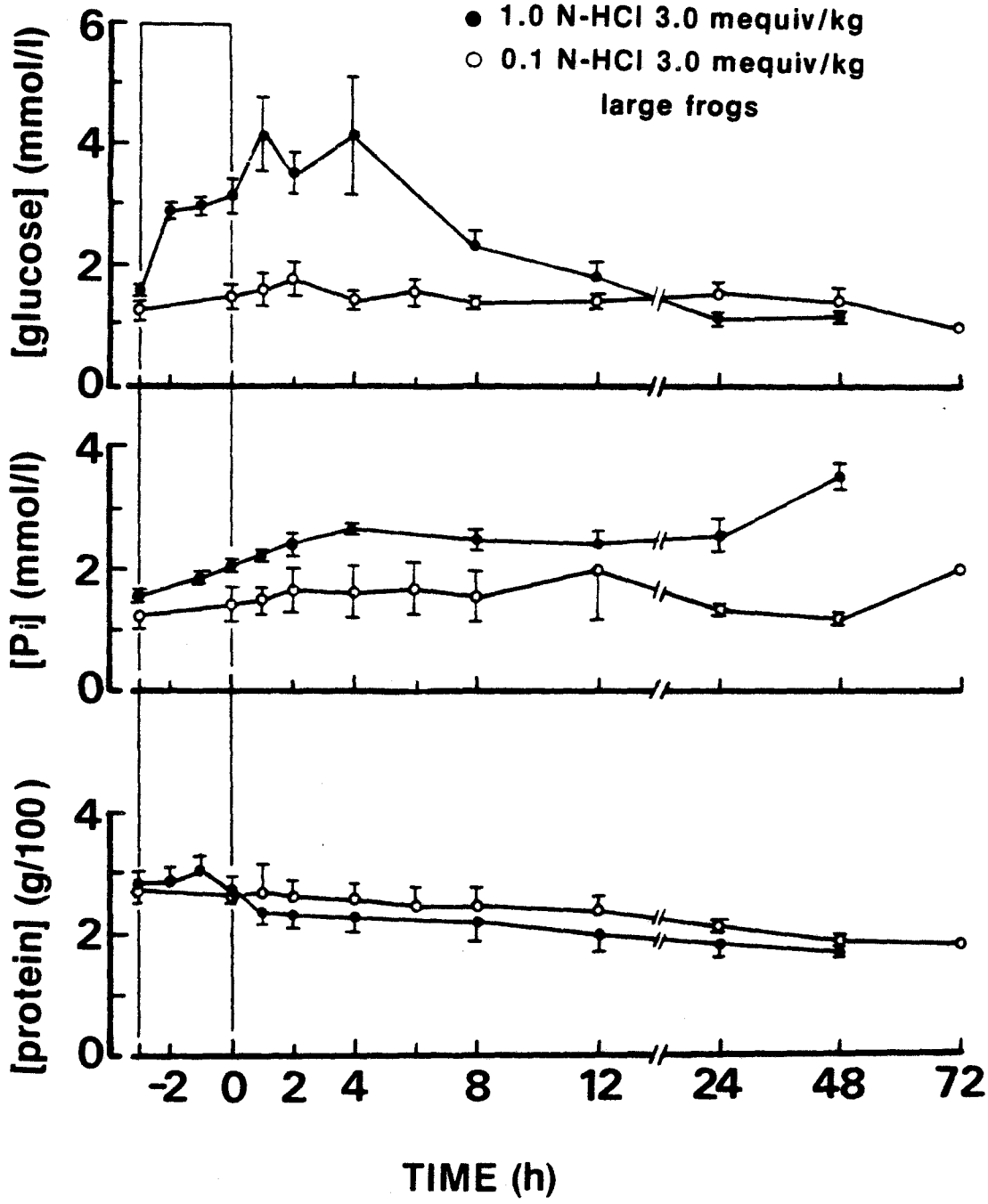
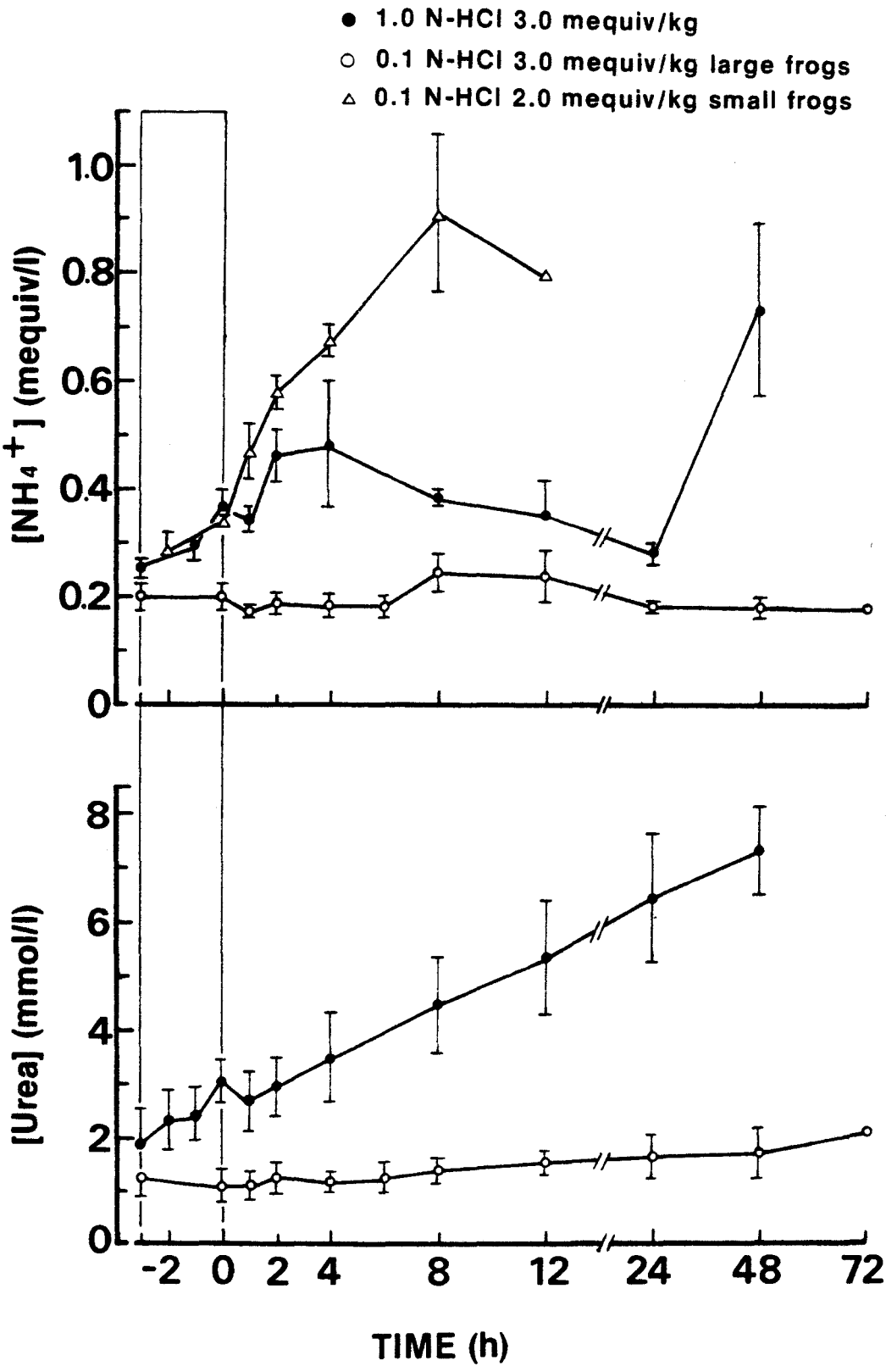

● 1.0 N-HCl 3.0 mequiv/kg
○ 0.1 N-HCl 3.0 mequiv/kg
large frogs
[glucose] (mmol/l)
[Pi] (mmol/l)
[protein] (g/100)
0
2
4
6
-2
0
2
4
8
12
24
48
72
TIME (h)

Fig. 9. Plasma [NH_4^+] and [urea] prior to, during, and following HCl infusions. See legend of Fig. 2 for other details.


● 1.0 N-HCl 3.0 mequiv/kg
○ 0.1 N-HCl 3.0 mequiv/kg large frogs
△ 0.1 N-HCl 2.0 mequiv/kg small frogs
[NH4+] (mequiv/l)
1.0
0.8
0.6
0.4
0.2
0
[Urea] (mmol/l)
8
6
4
2
0
-2
0
2
4
8
12
24
48
72
TIME (h)

infusion of 1.0 N-HCl, while dilute acid-infusion resulted in only small changes (Fig. 8A, B). Both treatments also resulted in similar depression of plasma protein levels (Fig. 8C), possibly related to repetitive blood sampling. These parameters were not measured in the small bullfrogs due to the limited amount of blood available. Concurrent with renal failure in large bullfrogs infused with 1.0 N-HCl, and in the small bullfrogs, both plasma urea and ammonia increased 3-fold above controls (Fig. 9). No apparent changes occurred in plasma $[NH_4^+]$ and [urea] in large bullfrogs infused with dilute acid.

(b) NH_4Cl Infusions

As in HCl infusion a volume expansion induced depression of plasma $[Na^+]$ was evident (Fig. 10A). This was rapidly corrected and plasma Na^+ was maintained at constant levels. Plasma $[Cl^-]$ (Fig. 10B) increased dramatically with loading, again this was restored within 1 h post-infusion. This transient in plasma Cl^- is similar to that seen by plasma NH_4^+ (Fig. 11A) as may be expected following NH_4Cl loading.

Plasma $[K^+]$ and $[Ca^{++}]$ more than doubled within 1 h post-infusion (Fig. 10C, D). While Ca^{++} levels were restored within 2 h, plasma K^+ maintained a 2-fold elevation for at least 24 h into the recovery period. While plasma $[NH_4^+]$ was rapidly corrected (Fig. 11A), plasma [urea] increased post-infusion and remained elevated (Fig. 11B). No other changes were apparent.

(c) Forced Submergence

Unlike acid infusions, enforced submergence produced no notable

Fig. 10. Plasma $[Na^+]$, $[Cl^-]$, $[K^+]$ and $[Ca^{++}]$ prior to and following NH_4Cl infusion (open circles) or forced submergence (closed circles). See legend of Fig. 5 for more details.

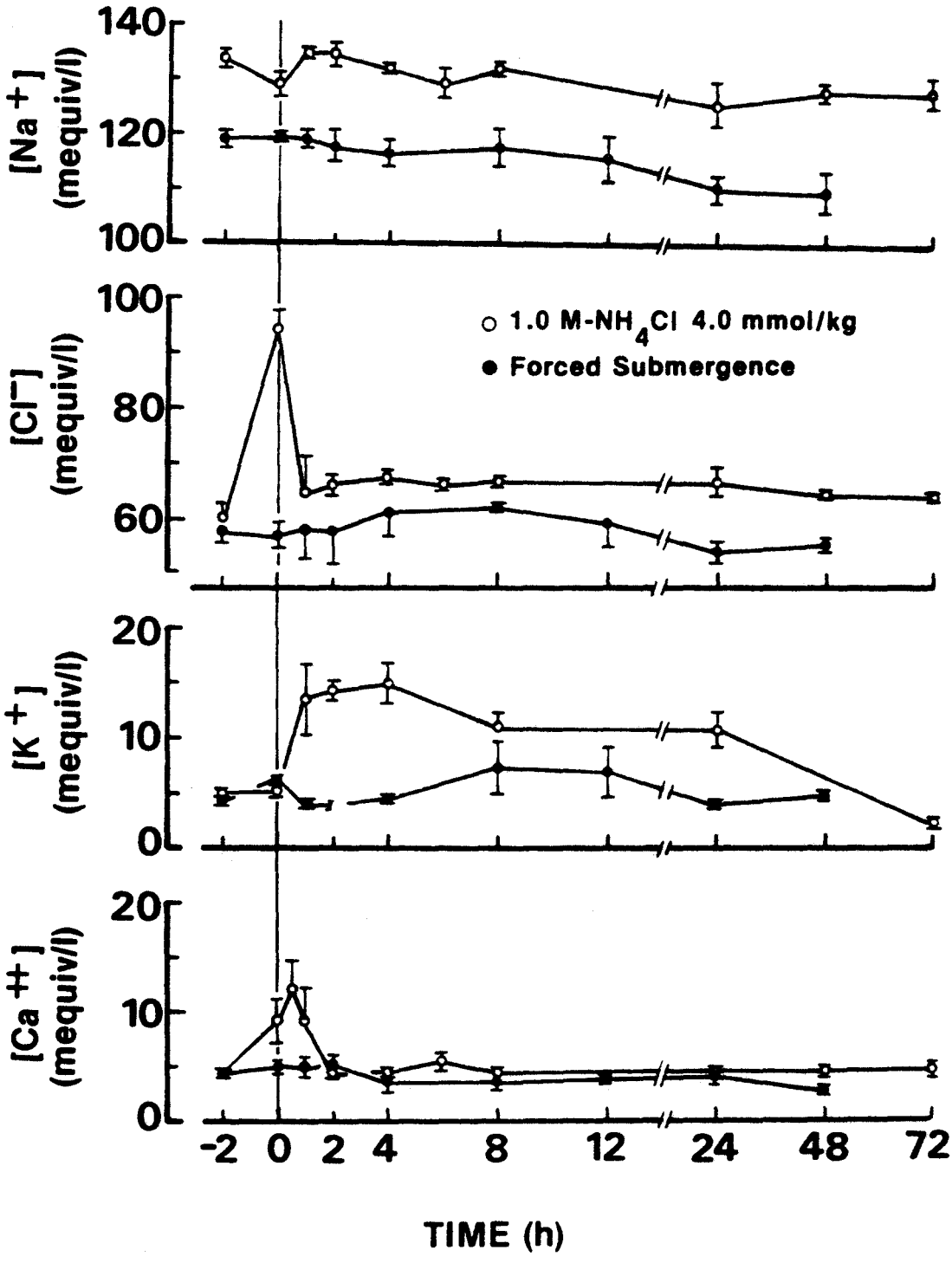
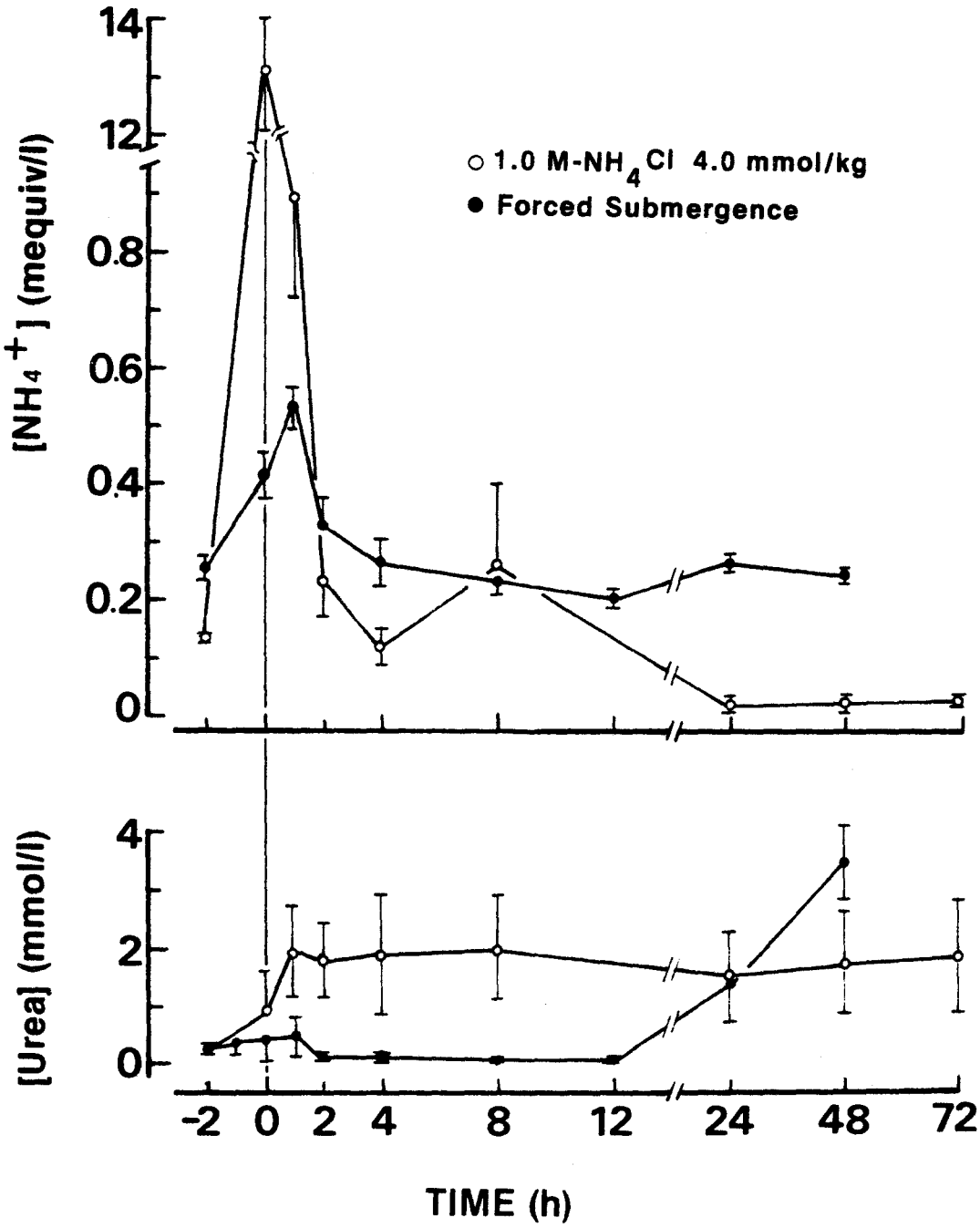

[Na+] (mequiv/l)
140
120
100
[Cl−] (mequiv/l)
100
80
60
○ 1.0 M-NH4Cl 4.0 mmol/kg
● Forced Submergence
[K+] (mequiv/l)
20
10
0
[Ca++] (mequiv/l)
20
10
0
-2 0 2 4 8 12 24 48 72
TIME (h)

Fig. 11. Plasma [NH_4^+] and [urea] prior to and following NH_4Cl infusions (open circles) or forced submergence (closed circles). See legend of Fig. 5 for other details.


14
12
0.8
0.6
0.4
0.2
0
[NH4+] (mequiv/l)
○ 1.0 M-NH4Cl 4.0 mmol/kg
● Forced Submergence
4
2
0
[Urea] (mmol/l)
-2
0
2
4
8
12
24
48
72
TIME (h)

changes in plasma [Na^+] or [Cl^-] (Fig. 10A, B). A 2-fold increase in plasma [K^+] to 6.3 ± 0.4 meq/l was restored to normal by 1 h into recovery, but large transient increases occurred until 24 h post-emergence (Fig. 10C). Plasma Ca^{++} showed similar, though diminished, changes, as in NH_4Cl infused frogs. A large transient increase in plasma [NH_4^+] occurred during and immediately following the dive, peaking at 528 ± 42 μequiv/l by 1 h post-emergence (Fig. 11A) and subsequently returning to normal by 8 h. Plasma urea did not change until after 12 h, [urea] increased 3-fold by 48 h (Fig. 11B). There were no evident changes in plasma P_i concentrations. Plasma proteins and glucose were not measured.

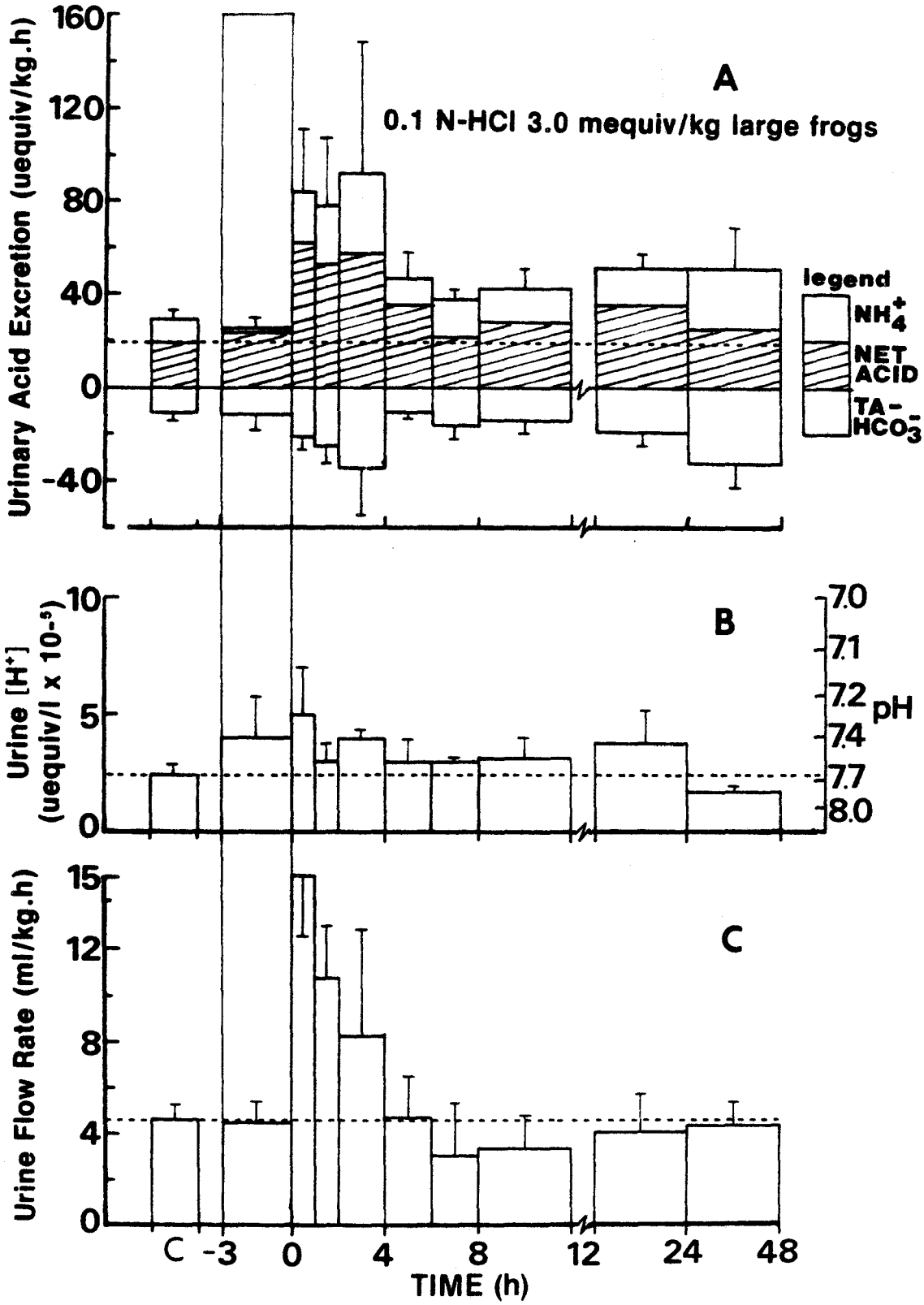
4. Renal Acid Excretion

Infusions of 1.0 N-HCl into large bullfrogs and of 0.1 N-HCl into small bullfrogs resulted in kidney failure. In frogs which maintained renal function the total acid (i.e. NH_4^+ + TA-HCO_3^-) excretion by the kidneys increased during acidosis. Urine pH was depressed slightly during periods of peak acid excretion. All treatments in which renal function was maintained resulted in a large increase in UFR immediately following infusions or forced submergence.

(a) HCL Infusions

Infusion of 1.0 N-HCl resulted in increased renal acid excretion during the first 7 h from the onset of acid infusions. During this period 60 μequiv/kg of acid, after correction for controls, was excreted. This represented a 45% increase in renal acid excretion but only accounted for 2% of the amount of acid infused. Renal failure was

Fig. 12. (A) Total urinary acid excretion, $NH_4^+ + TA - HCO_3^-$, (B) Urine pH, and (C) Urine flow rate prior to, during, and following infusion of 0.1 N-HCl into large bullfrogs. See legend of Fig. 2 for other details.


A
0.1 N-HCl 3.0 mequiv/kg large frogs
Urinary Acid Excretion (uequiv/kg.h)
160
120
80
40
0
-40
legend
NH_4^+
NET ACID
$TA-HCO_3^-$
B
Urine $[H^+]$ (uequiv/l x 10^{-5})
10
5
0
7.0
7.1
7.2
7.4
7.7
8.0
pH
C
Urine Flow Rate (ml/kg.h)
15
12
8
4
0
C
-3
0
4
8
12
24
48
TIME (h)

evident for the remainder of the experiment.

Large frogs infused with 0.1 N-HCl exhibited a 3-fold increase in NH_4^+ excretion immediately post-infusion (Fig. 12A). However, because of a concurrent decrease in TA-HCO_3^-, the total acid excretion increased by 2-fold only. Total acid excretion remained slightly elevated for 48 h post-infusion, accounting for the excretion of 660 μequiv/kg of H^+, after correction for controls. This accounted for about 22% of the infused load. After 48 h, net "base" excretion by the kidneys occurred. Mean urine pH (Fig. 12B) was maintained slightly acid relative to controls during the period of acid excretion, and became relatively alkaline by 48 h. UFR tripled immediately post-infusion and declined exponentially to control levels by 6 h (Fig. 12C).

(b) NH_4Cl Infusions

Similar to HCl infusions, this treatment stimulated urinary NH_4^+ and total acid excretion (Fig. 13A). By 6 h post-infusion a substantial increase in TA-HCO_3^- also contributed to the total acid excretion. Over the first 5 days of recovery (not shown) there occurred, on average, a 110% increase in total acid excretion over control values. This acid excretion amounted to about 2,300 μequiv/kg of "H^+", or about 95% of the estimated acid load to the ECF (cf. Fig. 4C). Urinary pH increased when TA-HCO_3 was lowest (i.e. immediately post-infusion) but thereafter decreased gradually as TA-HCO_3^- increased (Fig. 13B). UFR was initially 2-fold higher than controls during the infusion period, but was variable and not dissimilar to control values after infusions were complete (Fig. 13C).

Fig. 13. (A) Total urinary acid excretion, $NH_4^+ + TA - HCO_3^-$, (B) Urine pH and (C) Urine flow rate of the bullfrog prior to, during, and following infusion of NH_4Cl. See legend of Fig. 5 for other details.

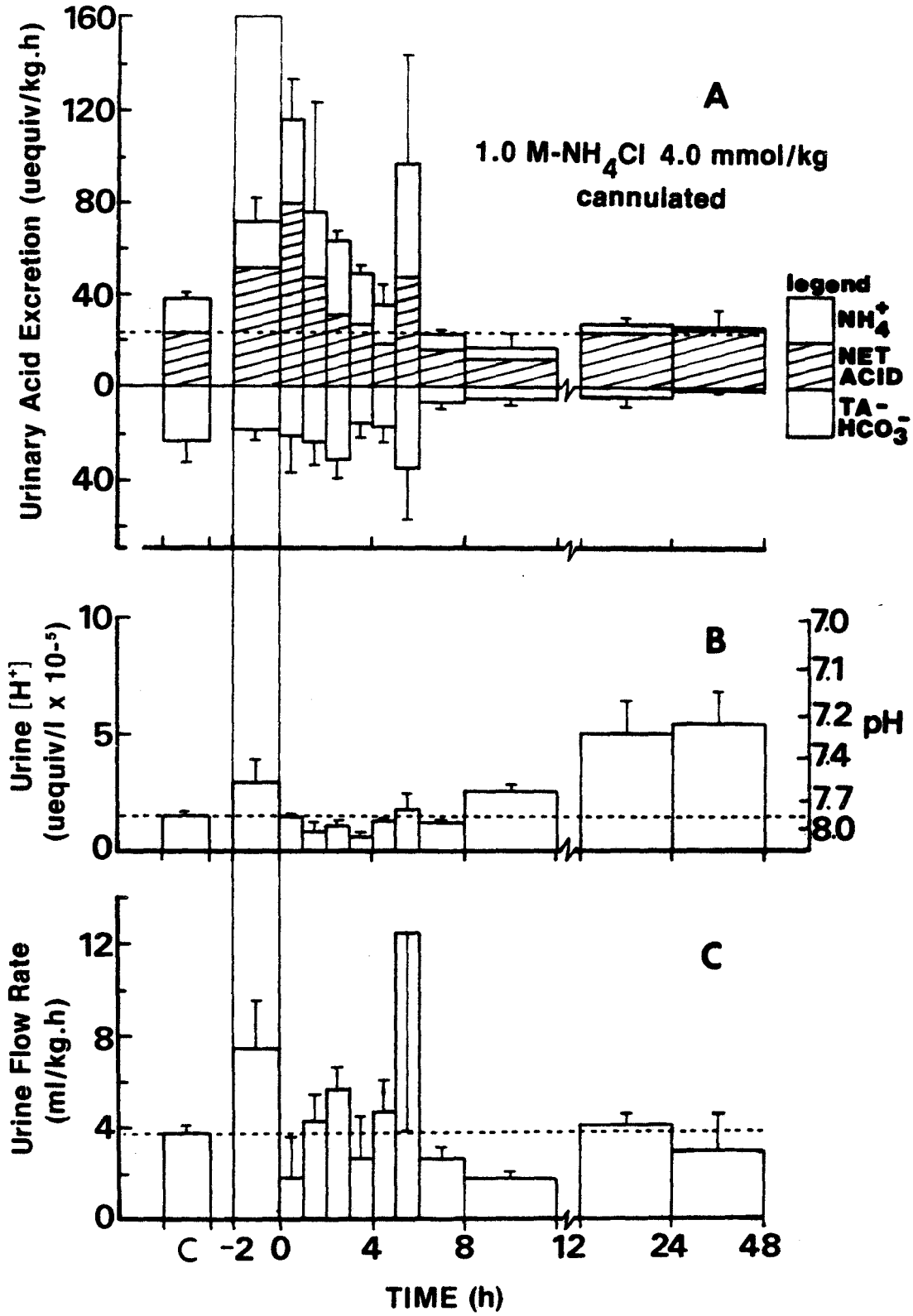
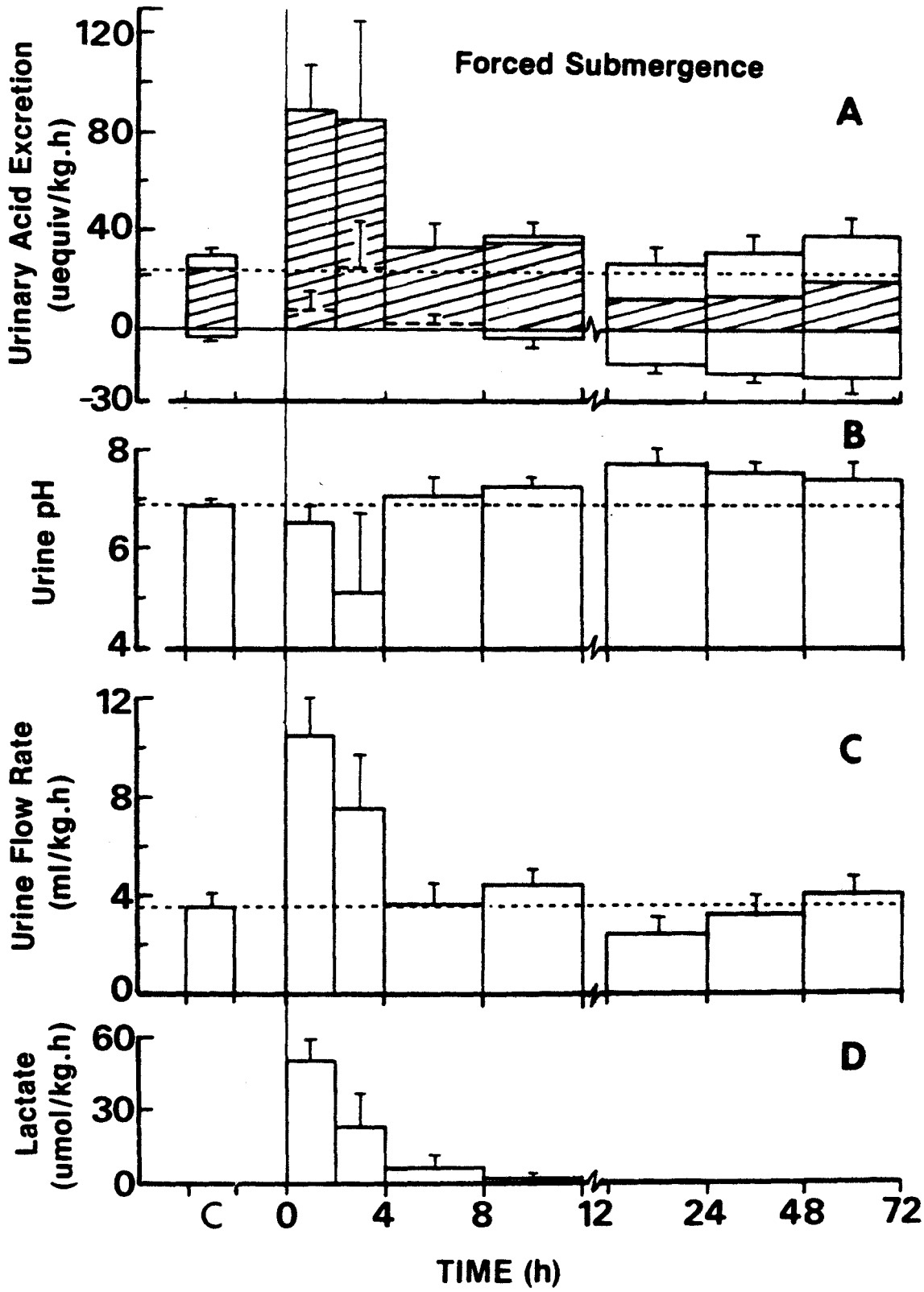

A
1.0 M-NH4Cl 4.0 mmol/kg
cannulated
legend
NH4+
NET ACID
TA-HCO3-
Urinary Acid Excretion (uequiv/kg.h)
160
120
80
40
0
40
B
Urine [H+] (uequiv/l x 10-5)
10
5
0
7.0
7.1
7.2
7.4
7.7
8.0
pH
C
Urine Flow Rate (ml/kg.h)
12
8
4
0
C
-2
0
4
8
12
24
48
TIME (h)

Fig. 14. (A) Total urinary acid excretion, $NH_4^+ + TA - HCO_3^-$, (B) Urine pH, (C) Urine flow rate, and (D) Urinary lactate excretion before and following 45 min enforced submergence. See legend of Fig. 5 for other details.


Forced Submergence
A
B
C
D
Urinary Acid Excretion (uequiv/kg.h)
120
80
40
0
-30
Urine pH
8
6
4
Urine Flow Rate (ml/kg.h)
12
8
4
0
Lactate (umol/kg.h)
60
30
0
C
0
4
8
12
24
48
72
TIME (h)

(c) Forced Submergence

The acidosis induced by forced submergence resulted in rapid renal compensatory responses. NH_4^+ + TA-HCO_3^- (total acid excretion) increased 2 to 4-fold in the first 4 h of post-dive recovery (Fig. 14A). After correction for controls, renal acid excretion over the first 12 h (i.e. the duration of the ECF disturbance) was 325 μequiv/kg, only a small part of the estimated acid load to the ECF at t=0 h (cf. Fig. 4D). This increase in acid excretion was accompanied by a decrease in urine pH (Fig. 14B). Then urine pH became alkaline relative to controls, concurrent with the decline in total acid excretion. UFR increased 3-fold immediately upon emergence and then decreased to control values by 8 h (Fig. 14C). A large lactate efflux was observed during this period of elevated UFR (Fig. 14D) but represented less than 4% of the estimated lactate load to the ECF.

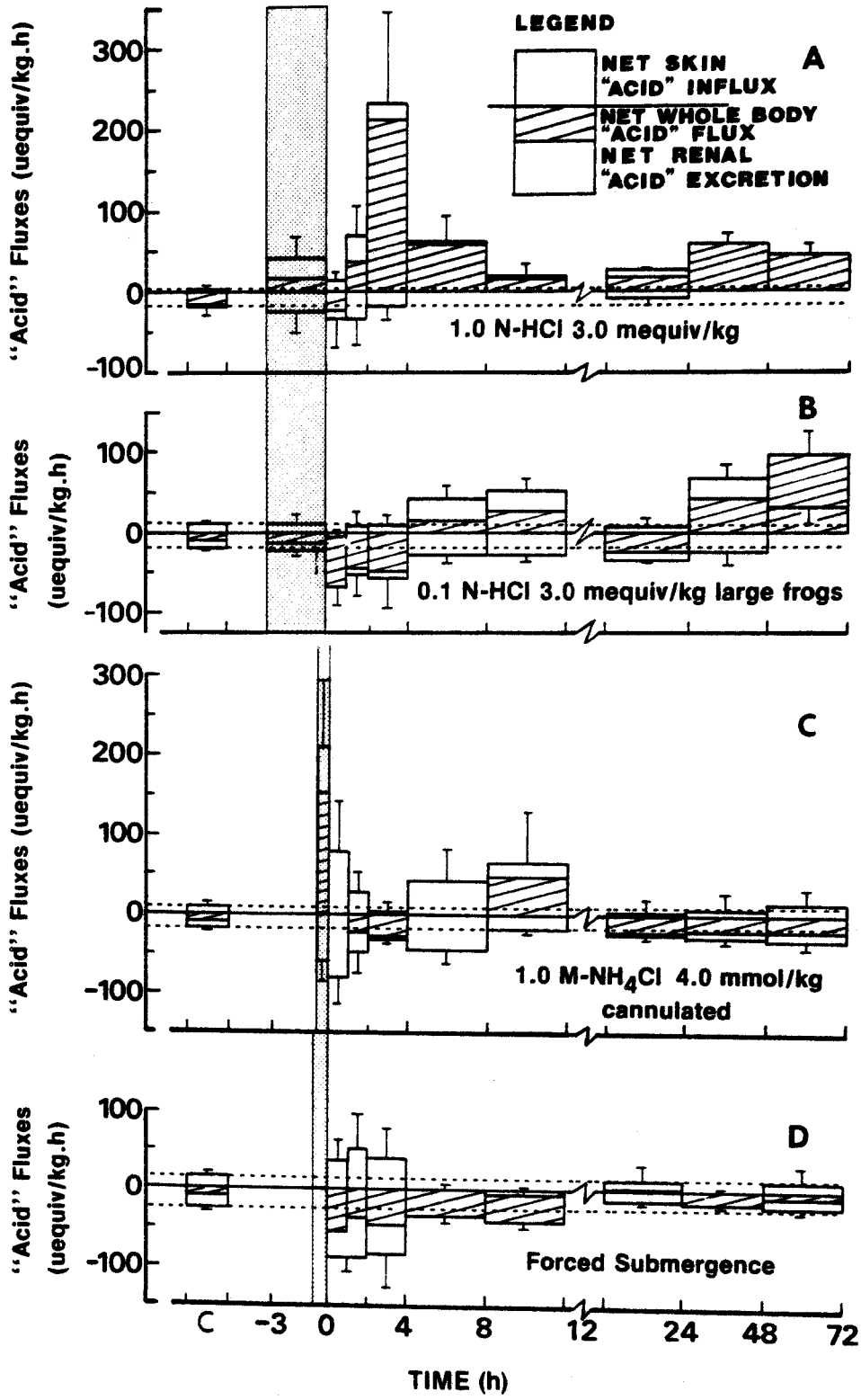
5. Total Acid Efflux

In this section the net cutaneous "acid" flux is considered together with the acid excretion by the kidneys (Fig. 15). Usually there was a persistent "base" loss across the skin. This has been plotted as an "acid" uptake (its equivalent) in Fig. 15. The sum of the skin plus renal components thus yields the net "H^+/OH^-" flux of the whole animal, i.e. shaded bars in Fig. 15.

Under control conditions the rate of "acid" uptake by the skin averaged 12.8 ± 4.1 μequiv/kg·h while the average renal acid excretion was -35.7 ± 12.0 μequiv/kg·h.

Following all treatments, despite the large extracellular

Fig. 15. Cutaneous, renal and whole body net "acid" fluxes prior to, during and following induction of the acidosis in bullfrogs. (A) 1.0 N-HCl; (B) 0.1 N-HCl, large frogs; (C) NH_4Cl infusion; (D) forced submergence. Stippled area represents duration of acid infusion or forced submergence.


LEGEND
NET SKIN "ACID" INFLUX
NET WHOLE BODY "ACID" FLUX
NET RENAL "ACID" EXCRETION
A
B
C
D
"Acid" Fluxes (uequiv/kg.h)
300
200
100
0
-100
1.0 N-HCl 3.0 mequiv/kg
0.1 N-HCl 3.0 mequiv/kg large frogs
1.0 M-NH4Cl 4.0 mmol/kg
cannulated
Forced Submergence
C
-3
0
4
8
12
24
48
72
TIME (h)

acidosis, there was no significant net excretion of acid across the skin. Individual frogs occasionally exhibited a net cutaneous acid loss for brief periods (i.e. t=1 h, Fig. 15B; t=12 and 48 h, Fig. 15D) but the averages were never significantly different from zero. Indeed, all treatments led to a stimulation of cutaneous "base" efflux which persisted for 2 to 8 h depending on the treatment. Therefore the skin appeared to be incapable of a large and rapid response to an acid challenge.

As a result of the persistent base loss across the skin and cessation of renal function in bullfrogs infused with 1.0 N-HCl, a continuous uptake of acid by the whole animal was seen (Fig. 15A) until the frogs died. The total acid uptake (i.e. skin plus renal "base" efflux) in these animals over the 75 h post-infusion period amounted to 5,830 μequiv/kg which was additional to the 3,000 μequiv/kg infused (Table 6). Of this total H^+ load to the body it was estimated (cf. Fig. 4A) that only 28% was buffered in the ECF, therefore the remainder must have been buffered intracellularly (Table 6).

Infusion of dilute HCl into large bullfrogs, NH_4Cl infusions, and forced submergence all resulted in periods of elevated rates of whole body acid excretion. In these treatments this was due primarily to stimulated renal acid excretion. Despite the fact that a net acid excretion by the skin was not seen, there was, however, a decrease in the cutaneous net "base" efflux during these periods of elevated acid excretion (Fig. 15B, C, D).

Indeed, the slight decrease in cutaneous "base" efflux by frogs infused with 0.1 N-HCl seen during the initial 7 h from the onset of

Table 6. Total acid and ion fluxes after correction for control (μeq/kg)

Series	Time	Skin	Renal	Total	Estimated ECF load or loss	Estimated ICF load or loss
1.0N HCl	$^{1}H^{+}$ 75 h	+4,700	+1,130	+5,830	+2,440	+6,390
	Na^{+}	-2,780	-420	-3,200	-8,045	+4,845
	$^{1}Cl^{-}$	-3,440	-710	-4,150	-3,375	+2,225
	K^{+}	-4,990	0	-4,990	+920	-4,070
	Ca^{++}	-270	-25	-295	-135	-160
net charge		+100	+1,395	+1,495	-1,445	-1,610
1. 3,000 μeq/kg infused						
0.1N HCl	$^{1}H^{+}$ 99 h	+3,160	-1,100	+2,060	+2,030	+3,030
	Na^{+}	-3,910	-690	-4,600	-1,350	-3,250
	$^{1}Cl^{-}$	-5,060	-1,100	-6,160	+1,110	-2,050
	K^{+}	-4,270	-1,880	-6,150	+515	-6,665
	Ca^{++}	-340	-130	-470	-80	-390
net charge		-300	-2,620	-2,920	+5	-5,225
0.1N HCl	$^{2}H^{+}$ 24 h	+1,700	0	+1,700	+3,890	+810
	Na^{+}	-1,840	0	-1,840	-3,080	+1,240
	$^{2}Cl^{-}$	-1,780	0	-1,780	+380	-160
	K^{+}	-1,650	0	-1,650	+920	-2,570
	Ca^{++}	--	0	--	+55	-55
net charge		-10	0	-10	+1,405	-1,225
2. 2,000 μeq/kg infused						
1.0 M NH_4Cl	H^{+} 5 days	+290	-1,170	-876	-340	-535
	Na^{+} (120 H)	-2,590	-685	-3,280	-1,135	-2,200
	$^{3}Cl^{-}$	-2,460	-860	-3,320	+160	+640
	K^{+}	-415	+135	-280	-810	+530
	Ca^{++}	-170	+170	0	-80	+80
net charge		-425	-590	-1,120	-2,525	-2,765
3. 4,000 μmol/kg infused						
F.S.	H^{+} 72 h	-625	+210	-415	-600	+185
	Na^{+}	+150	+20	+170	-2,590	+2,760
	Cl^{-}	0	-20	-20	-675	+655
	K^{+}	-170	0	-170	+80	-250
	Ca^{++}	0	-40	-40	-460	+420
net charge		-645	+210	-435	-2,895	+2,460

infusion (i.e. t=-3 to +4 h; Fig. 15B) may be considered as an acid excretion relative to controls. This reduction in base efflux, assuming that endogenous base efflux remained constant at control values, amounted to only 0.7% of the total acid infused, by 7 h. Thereafter the cutaneous "base" efflux exceeded control values and prevailed over the renal excretion of H^+. Therefore, despite the elevated renal acid excretion, a net uptake of 2,060 μequiv/kg by the body occurred over the 96 h (4 days) post-infusion period (Table 6). Since about 40% of the total H^+ load (5,060 μequiv/kg) was estimated to be buffered by the ECF (cf. Fig. 4B) the remainder must have been buffered intracellularly (Table 6).

The effect of NH_4Cl infusions is shown in Fig. 15C. Relative to HCl-infused frogs only an insignificant amount of acid was taken up across the skin over the first 5 days of recovery. The result was a net loss of acid by the whole animal of about 875 μequiv/kg, when corrected for control levels of endogenous "base" efflux, over this 5-day period. Because the cutaneous acid efflux was negligible, the kidneys were the major source of the acid excretion. Since the actual H^+ load to the body induced by NH_4Cl infusions is unknown, estimations of the relative amount of H^+ excreted could not be made. However estimates of ΔH^+_{ECFV} indicated that virtually no excess acid remained in the ECF after 5 days (cf. Fig. 4C).

Following forced submergence, as in NH_4Cl infusions, the cutaneous response to acid excretion was slow. However, there was an average 19% decrease in the rate of cutaneous "base" efflux (relative to controls) over the first 12 h of post-dive recovery. This contributed

10% to the whole body acid excretion of 360 μequiv/kg relative to controls. This would have accounted for only about 7% of the estimated acid load (cf. Fig. 4D, curve extrapolated back to t=0 h). The remaining acid was not excreted over the remaining 2½ days of the observed recovery period. Also, since no net H^+ load to the body was apparent after 72 h of recovery (Fig. 4D; Table 6), the excess acid must have been removed metabolically.

6. Cutaneous Unidirectional Na^+ and Cl^- Fluxes

Since only the abdomino-lateral surfaces of the frogs were immersed in the water during flux periods, the measured transcutaneous ion and acid fluxes must therefore have occurred predominantly across the ventral skin.

During the control periods the bullfrogs were close to, but not quite in, ionic equilibrium (Table 5; Fig. 16, 17). There were slight net cutaneous losses of Na^+ and Cl^- which varied with external [NaCl] and body size (Fig. 7). On average, these losses represented only about 0.04%/h and 0.09%/h, respectively, of the exchangeable pools of Na^+ and Cl^- (see Methods). The unidirectional fluxes of Na^+ and Cl^-, under control conditions, increased with increasing ionic strength of the water and decreasing body weight (Table 7). The data for large bullfrogs (first two rows of Table 7) show a less than 2-fold increase for J_{in} with a 3-fold or greater increase in ionic strength of the water, which suggests that Na^+ and Cl^- uptake mechanisms were close to saturation at the lower external ion levels.

All treatments, with the exception of acid-loaded small

Table 7. Cutaneous Na^+ and Cl^- fluxes as a function of external [Na^+, Cl^-] (μequiv/l ± 1 S.E.M.) and mean body weight (g). Na^+ and Cl^- pool sizes in μequiv/kg (see Methods). Fluxes expressed as μequiv/kg·h.

$[Na^+]_{ext}$;$[Cl^-]_{ext}$	exchangeable Na^+ pool	exchangeable Cl^- pool	$J_{in}Na^+$	$J_{out}Na^+$	$J_{net}Na^+$	$J_{in}Cl^-$	$J_{out}Cl^-$	$J_{net}Cl^-$
210 ± 2;245 ± 25	56,100	26,000	30.7	50.7	-24.4	17.7	26.1	-27.4
(N=18; 390 g)			±1.5	±10.5	±8.7	±2.7	±3.4	±9.1
592 ± 6;839 ± 5	--	--	52.8	74.0	-18.3	32.3	41.4	7.3
(N=5; 366 g)			±4.1	±7.3	±5.2	±2.4	±4.7	±2.8
815 ± 3;890 ± 10	--	--	150.6	230.9	-80.4	57.8	125.3	-67.6
(N=2; 118 g)			±24.8	±67.8	±45.3	±16.3	±43.9	±28.0

Fig. 16. Cutaneous unidirectional (J_{in} and J_{out}) and net fluxes (shaded bars) of Na^+ and of Cl^- following HCl infusions. Stippled area indicates period of acid infusion.

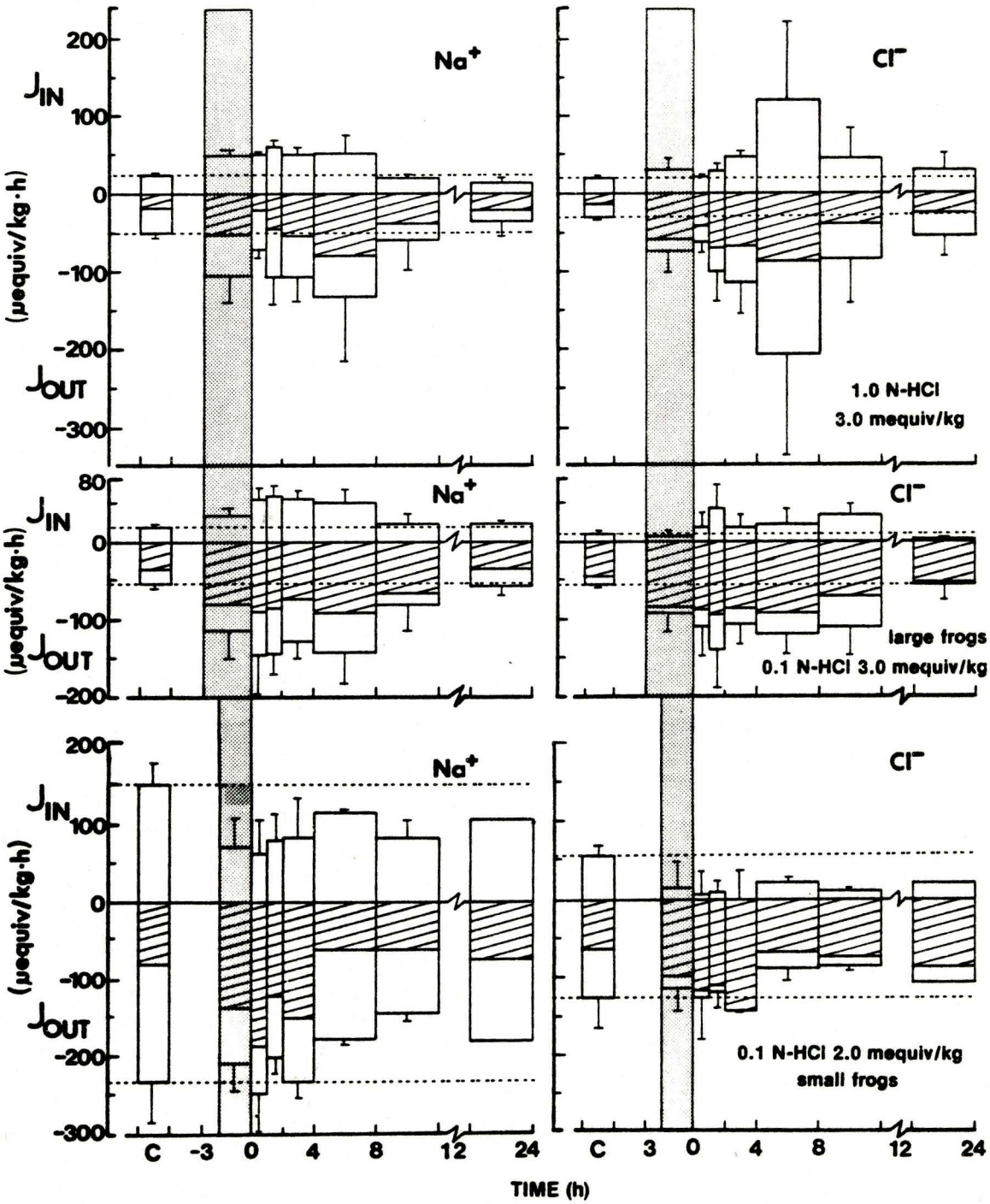
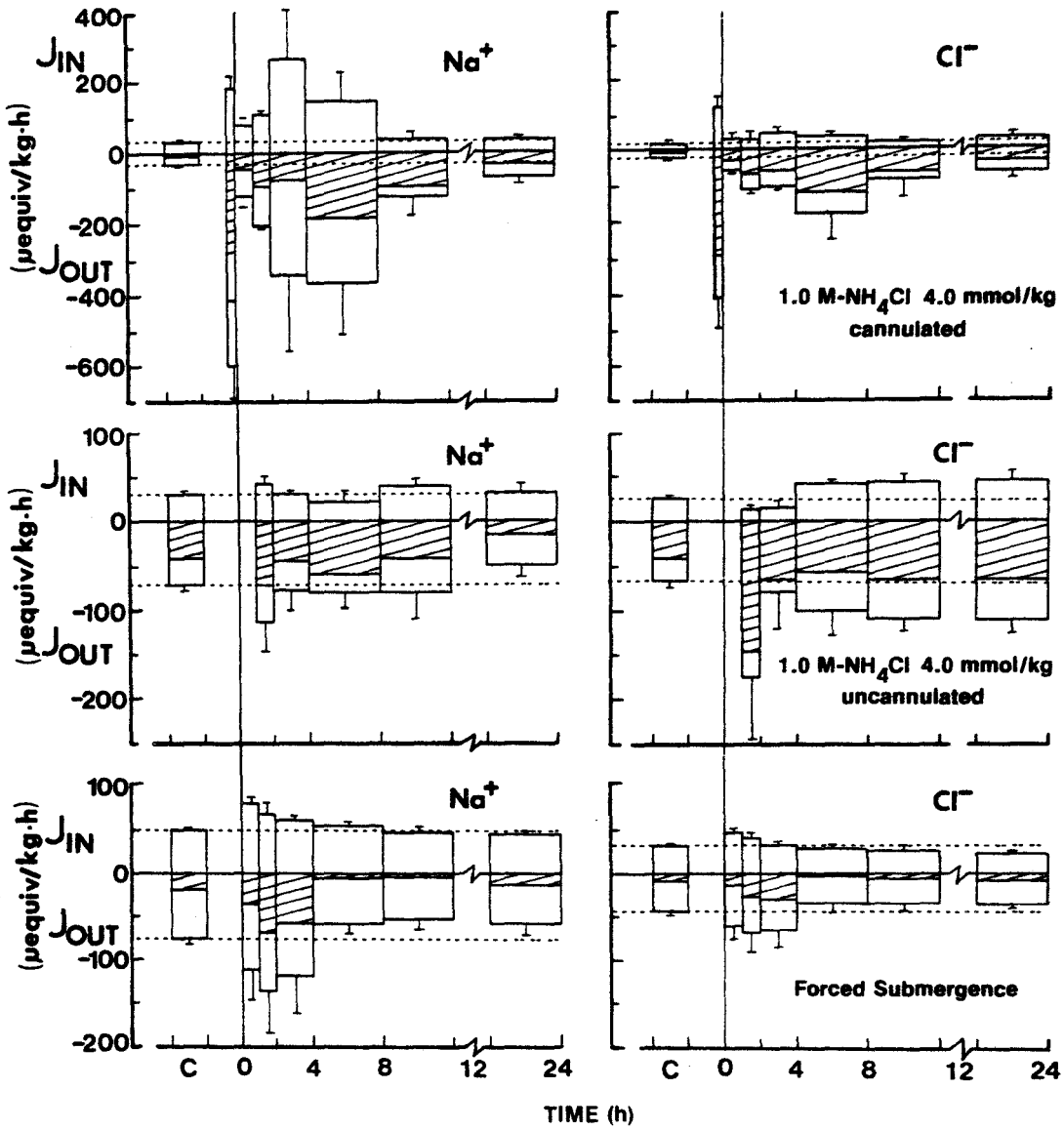

Na+
Cl−
J_IN
J_OUT
(μequiv/kg·h)
1.0 N-HCl
3.0 mequiv/kg
large frogs
0.1 N-HCl 3.0 mequiv/kg
0.1 N-HCl 2.0 mequiv/kg
small frogs
C -3 0 4 8 12 24
C 3 0 4 8 12 24
TIME (h)

Fig. 17. Cutaneous unidirectional (J_{in} and J_{out}) and net fluxes (shaded bars) of Na^+ and of Cl^- following NH_4Cl infusions and enforced submergence.


400
200
0
-200
-400
-600
100
0
-100
-200
J_{IN}
J_{OUT}
(μequiv/kg·h)
Na^+
Cl^-
1.0 M-NH_4Cl 4.0 mmol/kg
cannulated
1.0 M-NH_4Cl 4.0 mmol/kg
uncannulated
Forced Submergence
C
0
4
8
12
24
TIME (h)

bullfrogs, resulted in a stimulation of both influx and efflux of Na^+ and Cl^- (Fig. 16, 17). Intravascular NH_4Cl-loading was by far the most potent stimulator of Na^+ and Cl^- turnover (Fig. 17A): 4 and 8-fold increases in influx and efflux, respectively, were observed. HCl infusions into large bullfrogs resulted in 2 to 3-fold elevations in Na^+ and Cl^- turnover, but less than 2-fold increases were evident in uncannulated frogs loaded with NH_4Cl (Fig. 17B) and in forcibly submerged frogs (Fig. 17C). Also, except for frogs infused with 1.0 N-HCl (Fig. 16A), the increase in Na^+ uptake always exceeded the elevation in Cl^- influx. The Na^+ and Cl^- turnovers returned to near-control values by 24 h into the recovery periods (Fig. 16, 17), but thereafter all of the HCl-loaded frogs exhibited further large and progressive increases of both Na^+ and Cl^- influx and efflux. The efflux component always exceeded the influx and this resulted in continued ion losses until the animals died (not shown).

No correlations could be found between Na^+/Cl^- uptake and changes in acid/base excretion in acid-loaded frogs. However, following enforced submergence, a very strong correlation was found between Na^+ influx and cutaneous ammonia excretion (Fig. 18); this indicated that Na^+/NH_4^+ exchange occurred at a 1:1 ratio.

Direct correlations were evident between the efflux and influx of Na^+, and the efflux and influx of Cl^- as well (Table 8). These data suggest the further possibility that the acidosis may also have stimulated Na^+/Na^+ and Cl^-/Cl^- exchanges.

Fig. 18. The relationship between Na^+ influx (J_{in} Na^+) and the net ammonia ($NH_3 + NH_4^+$) flux (J_{net} ammonia) across the skin of the bullfrog following enforced submergence:

J_{in} $Na^+ = 0.98 \cdot J_{net}$ ammonia + 30.33

($r = 0.94$; $n = 11$. $P < 0.01$).

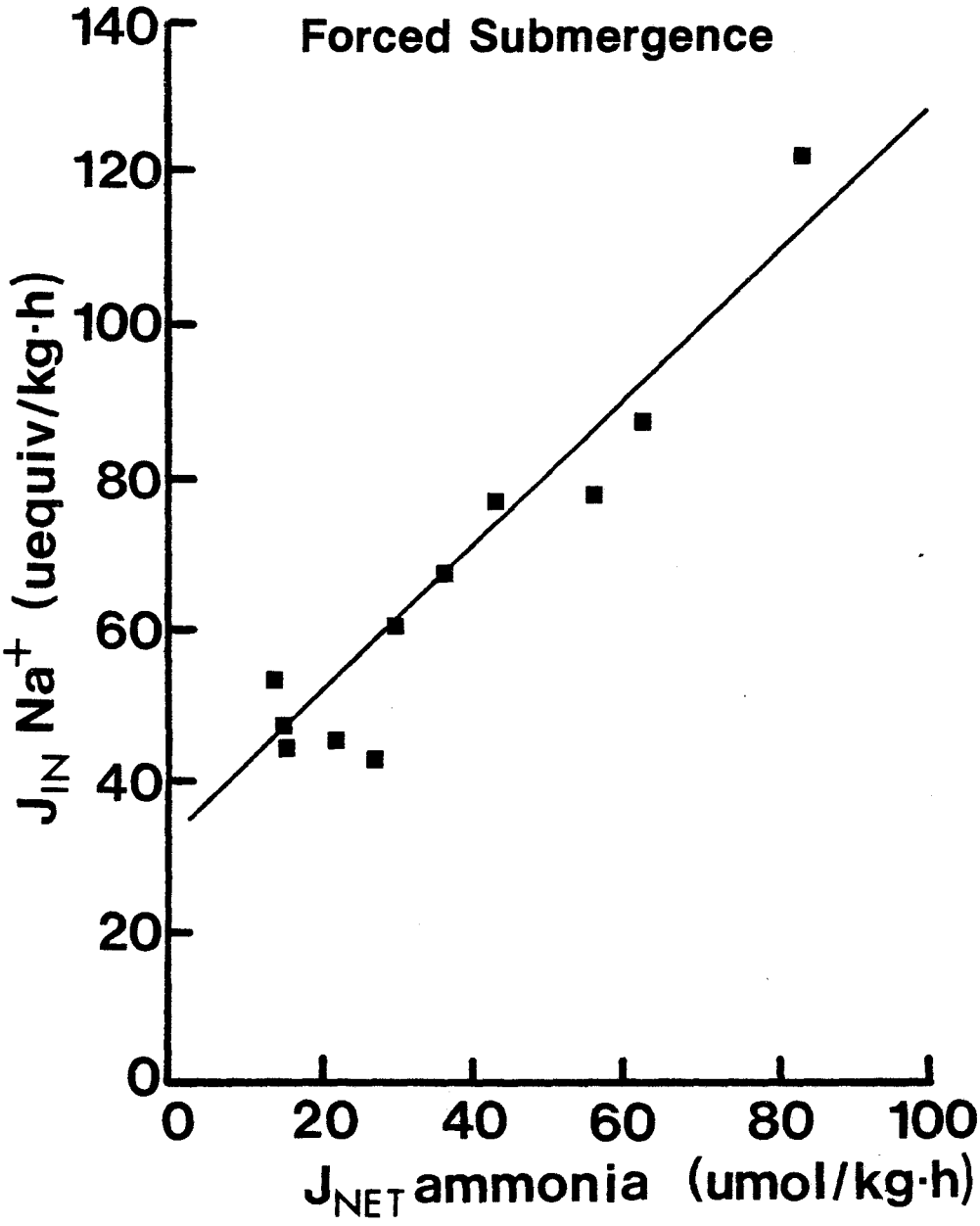

Forced Submergence
140
120
100
80
60
40
20
0
J_{IN} Na^+ (uequiv/kg·h)
0
20
40
60
80
100
J_{NET} ammonia (umol/kg·h)

Table 8. Correlations between J_{out} Na^+ *vs.* J_{in} Na^+ and $J_{out}Cl^-$ *vs.* J_{in} Cl^- during the first 24 h of recovery from induction of the acidosis. n = number of observations. * indicates statistical significance: * $P \leq 0.01$; ** $P \leq 0.05$.

Experiment	Na^+	Cl^-
1.0 N-HCl (n=13)	r = 0.817* $J_{in} = 0.29\ J_{out} + 21.11$	r = 0.961* $J_{in} = 0.62\ J_{out} - 17.68$*
0.1 N-HCl large frogs (n=9)	r = 0.920* $J_{in} = 0.39\ J_{out} - 1.42$	r = 0.771** $J_{in} = 0.56\ J_{out} - 37.18$
1.0 M-NH_4Cl cannulated frogs (n=10)	r = 0.817* $J_{in} = 0.22\ J_{out} + 45.17$	r = 0.838* $J_{in} = 0.19\ J_{out} - 18.67$
1.0 M-NH_4Cl uncannulated frogs (n=8)	r = 0.602 $J_{in} = 0.28\ J_{out} + 8.31$	r = 0.630 $J_{in} = 0.13\ J_{out} + 8.04$
Forced submergence (n=6)	r = 0.831** $J_{in} = 0.28\ J_{out} + 33.66$	r = 0.806** $J_{in} = 0.40\ J_{out} + 15.74$

7. Total Ion and Acid Fluxes

The net ion and acid movements across the ventral skin and by the kidneys (i.e. total ion and acid flux) are shown in Fig. 19 and 20. These figures illustrate that the charge balance between cation and anion movement was maintained and also show the relative contributions by the skin and kidneys to the total ion movement. For clarity all net movements of ions and acid/base have been plotted as losses. For example an uptake of Ca^{++} by the skin would be plotted as an equivalent loss of anion. Similarly an uptake of "H^{+}" is indicated as a loss of "OH^{-}", its equivalent. In Fig. 19 and 20 the differences between the total cation fluxes and the total anion fluxes are due to the flux of unmeasured ion(s) and measurement error. Also, the P_i fluxes were plotted in units of μmol/kg·h and thus results in the under-estimation of the total anion flux by an unknown but likely small amount.

Losses of ions both across the skin and by the kidneys predominanted during the control periods as well as following the acid challenge. However it was the skin, and not the kidneys, which proved to be the major route for ion losses. The exception is inorganic phosphate, the cutaneous efflux of which was always negligible during the control periods.

Concomitant with the acidosis was a large increase in the cutaneous net losses of all ions. The substantial nature of the increase in these fluxes, and the fact that cation and anion fluxes were affected by similar amounts, suggests an overall increase in permeability of the

Fig. 19. Cutaneous and renal ion and acid net fluxes before, during and following HCl infusions in the bullfrog. (A) 1.0 N-HCl, 3,000 equiv/kg. (B) 0.2 N-HCl, 3,000 μequiv/kg - large bullfrogs. (C) 0.2 N-HCl, 2,000 μequiv/kg - small bullfrogs: Ca^{++} and P_i not measured; urinary component is absent from t=-2 h onwards due to cessation of renal function. Note that inorganic phosphate (P_i) is plotted as $\mu mol/kg \cdot h^{-1}$ and is thus underestimated (see text for further details).

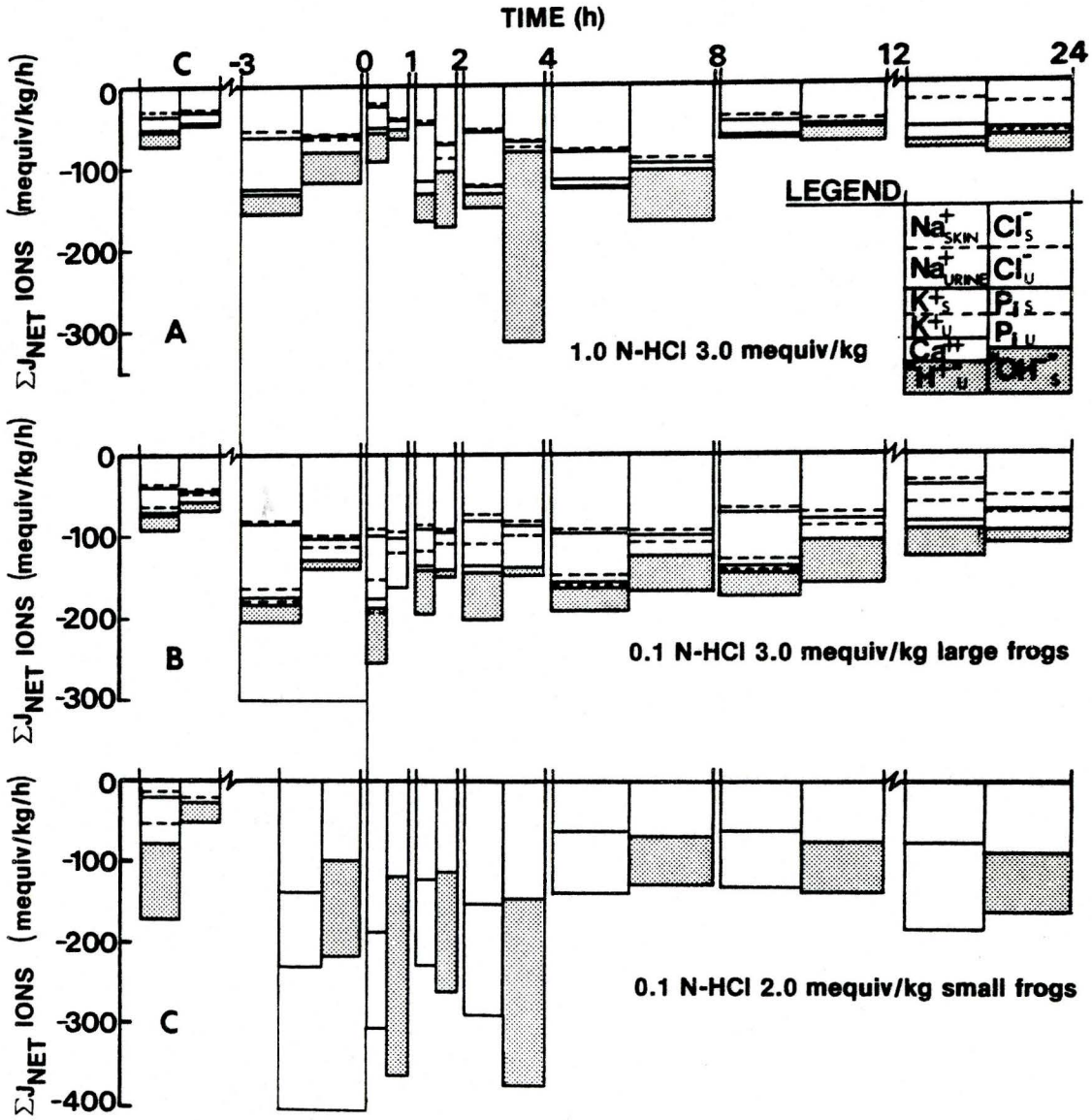
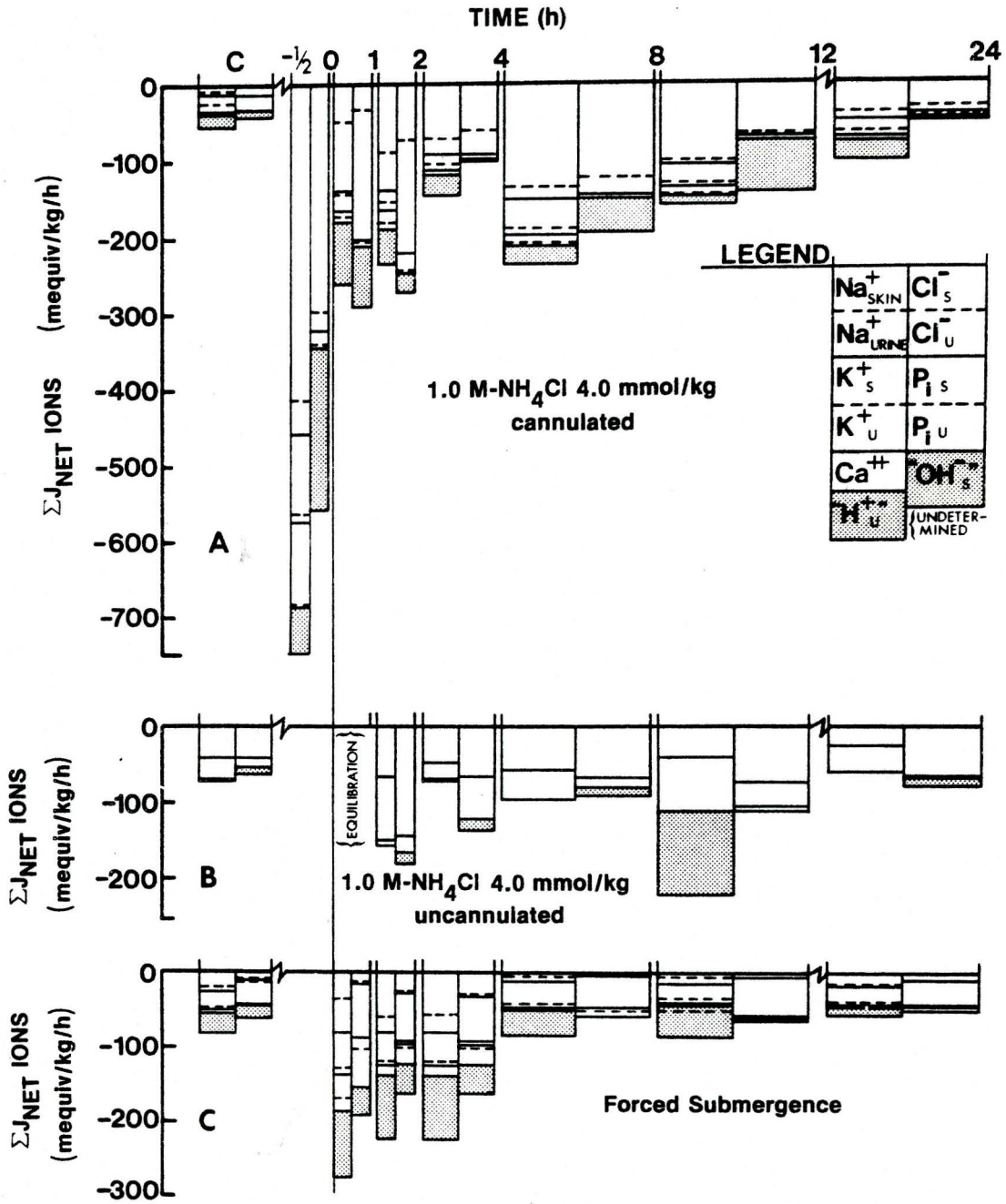

TIME (h)
C
-3
0
1
2
4
8
12
24
0
-100
-200
-300
-400
A
B
C
ΣJNET IONS (mequiv/kg/h)
LEGEND
Na+SKIN
Cl-S
Na+URINE
Cl-U
K+S
PJ S
K+U
PJ U
Ca++
H+U
OH-S
1.0 N-HCl 3.0 mequiv/kg
0.1 N-HCl 3.0 mequiv/kg large frogs
0.1 N-HCl 2.0 mequiv/kg small frogs

Fig. 20. Cutaneous and renal ion and acid net fluxes before, during and following (A) intravascular infusion of 4,000 μmol/kg of 1.0 M-NH_4Cl, (B) dorsal lymph sac infusion of 4,000 μmol/kg of 1.0 M-NH_4Cl: the whole body net fluxes are shown because the renal component was not separated from the cutaneous component, (C) 45 min enforced submergence: net flux of lactate anion is also indicated.


TIME (h)
C
-½
0
1
2
4
8
12
24
0
-100
-200
-300
-400
-500
-600
-700
ΣJNET IONS (mequiv/kg/h)
A
LEGEND
Na+SKIN
Cl-S
Na+URINE
Cl-U
K+S
Pi S
K+U
Pi U
Ca++
"OH-"S
"H+"U
UNDETER-MINED
1.0 M-NH4Cl 4.0 mmol/kg
cannulated
EQUILIBRATION
B
1.0 M-NH4Cl 4.0 mmol/kg
uncannulated
C
Forced Submergence

skin. This change in cutaneous permeability to ions was usually corrected within 24 h. Although the renal contribution to the total ion losses remained relatively small during acidosis, the increases in renal ion excretion were proportionately similar to that seen at the skin.

Infusions of HCl all resulted in an approximate 2-fold increase in total ion losses which were maintained for 8 to 24 h depending on the treatment (Fig. 19). The most persistent losses were exhibited by large frogs infused with 0.1 N-HCl (Fig. 19B). Transcutaneous losses of Na^+, K^+ and Cl^- were similar in magnitude and thus an increase in the 'strong ion difference'(SID) occurred. To maintain electroneutrality an increase in the efflux of weak base across the skin would be required, and this, in fact, was apparent (Fig. 19). After the first 24 h of post-infusion recovery all bullfrogs loaded with HCl exhibited a further and progressive stimulation of both cutaneous and renal (if renal failure hadn't occurred) ion losses which persisted until the animals died (not shown).

Similar to HCl loading, bullfrogs subjected to NH_4Cl infusions or forced submergence exhibited a large initial stimulation of cutaneous and renal ion losses. But, in contrast to HCl-loaded animals, the magnitude of these losses declined in approximately exponential fashion. Ion losses had fallen to control values within 24 h for NH_4Cl-loaded frogs (Fig. 20A, B) and within 8 h following forced submergence (Fig. 20C). Unlike HCl infused frogs, renal and cutaneous ion losses were maintained close to control values following the initial 24 h of recovery (not shown).

Intravascular infusion of NH_4Cl resulted in the largest initial loss of ions (Fig. 20A). The massive losses of strong cations resulted in a considerable SID which was made up, in large part, by elevated losses of weak base across the skin. In the first 2 h post-infusion, renal losses of Na^+ and Cl^- exceeded cutaneous losses by 2 to 3-fold.

Dorsal lymph sac infusions of NH_4Cl resulted in total Na^+ plus K^+ losses of similar magnitude to Cl^- losses and thus "base" losses remained low (Fig. 20B). These large Cl^- losses accounted for about 84% of the total infused by 5 days of recovery. A substantial, but electrically unbalanced, net excretion of "acid" occurred during the t=8 to 12 h flux period. These frogs also exhibited a net uptake of Ca^{++} across the skin during the later part of the recovery period.

The initial large cation losses induced by lactacidosis were balanced by elevated excretions of Cl^-, P_i, lactate$^-$ and weak bases (Fig. 20C). Cutaneous phosphate losses remained negligible throughout the recovery period. After the initial hour of post-dive recovery, the cutaneous uptake of Ca^{++} continued at pre-acidosis values. Unlike the other treatments ion losses were relatively rapidly restored. Also in contrast to the other treatments, with the exception of 0.1 N-HCl loading of large bullfrogs, renal acid excretion exceeded cutaneous base losses, and thus contributed to the whole body net excretion of acid.

8. Renal Clearances of Ions

Clearance ratios for Na^+, Cl^-, K^+ and Ca^{++} were calculated for three acidosis experiments (Table 9). Under control conditions clearance

Table 9. Renal clearance ratios for bullfrogs following acid infusions or forced submergence. Values are means ± 1 S.E.M.

Experiment	Time (h)	Na^+	K^+	Ca^{++}	Cl^-
0.1 N-HCl large frogs (N=3)	C	0.003 ±0	0.377 ±0.042	0.092 ±0.018	0.003 ±0.001
	t=0	0.005 ±0.001	0.294 ±0.104	0.130 ±0.045	0.005 ±0.001
	24	0.006 ±0.002	0.379 ±0.089	0.284 ±0.113	0.025 ±0.016
	48	0.005 ±0.003	0.787 ±0.189	0.183 ±0.088	0.013 ±0.003
1.0 M-NH_4Cl (N=3)	C	0.015 ±0.002	0.189 ±0.040	0.026 ±0.008	0.007 ±0.005
	t=0	0.062 ±0.022	0.182 ±0.078	0.036 ±0.012	0.113 ±0.048
	24	0.013 ±0.002	--	0.042 ±0.004	0.018 ±0.013
	48	0.010 ±0.001	--	0.103 ±0.006	0.005 ±0.003
Forced Submergence (N=2)	C	0.008 ±0.001	0.104 ±0.027	0.103 ±0.038	0.002 ±0.001
	t=0	0.024 ±0.007	0.040 ±0.017	0.298 ±0.128	0.002 ±0.001
	24	0.008 ±0.001	0.038 ±0.014	0.173 ±0.114	0.002 ±0.001
	48	0.008 ±0	0.028 ±0.028		0.002 ±0.001

ratios for Na^+ and Cl^- were very low, indicating very strong reabsorptive processes for these ions. Net reabsorption of K^+ was variable between experimental series, ranging from 0.104 to 0.377 (i.e. 62.3% to 89.6% reabsorption). Similarly Ca^{++} reabsorption ranged from 89.7% to 97.4% under control conditions.

In the three experiments shown (Table 9) the acidosis resulted in decreased tubular reabsorption of ions, as indicated by the increase in clearance ratios. The exceptions were a decrease in K^+ clearance ratios following forced submergence, i.e. increased K^+ reabsorption; Cl^- reabsorption didn't change in these frogs.

Clearance ratios for frogs infused with HCl remained elevated even after 48 h, showing no trend to recovery of control values. Following NH_4Cl infusions reabsorption of Na^+ and Cl^- were normal by 24 and 48 h respectively; Ca^{++} reabsorption continued to decrease.

9. Total Ammonia and Urea-N Fluxes

The net losses of ammonia ($NH_3 + NH_4^+$) and urea-N (i.e. 1 mmol of urea ≡ 2 mequiv of urea-N) by both the skin and the kidneys are shown in Fig. 21 and 22. During the control period the ratio urea-N:ammonia-N was normally greater than unity, reflecting the ureotelic metabolism of the bullfrog. An exception was the two small bullfrogs (Fig. 21C) in which ammonia excretion exceeded urea excretion. In all control periods the renal excretion of ammonia and urea always exceeded cutaneous efflux.

The major trend seen following induction of the acidosis was a marked increase in renal and/or cutaneous ammonia excretion. Infusions

Fig. 21. Cutaneous and renal net fluxes of ammonia ($NH_3 + NH_4^+$) and urea-N prior to and following HCl infusions in the bullfrog. (A) 1.0 N-HCl; (B) 0.1 N-HCl - large bullfrogs; (C) 0.1 N-HCl - small bullfrogs. See legend of Fig. 2 for other details.

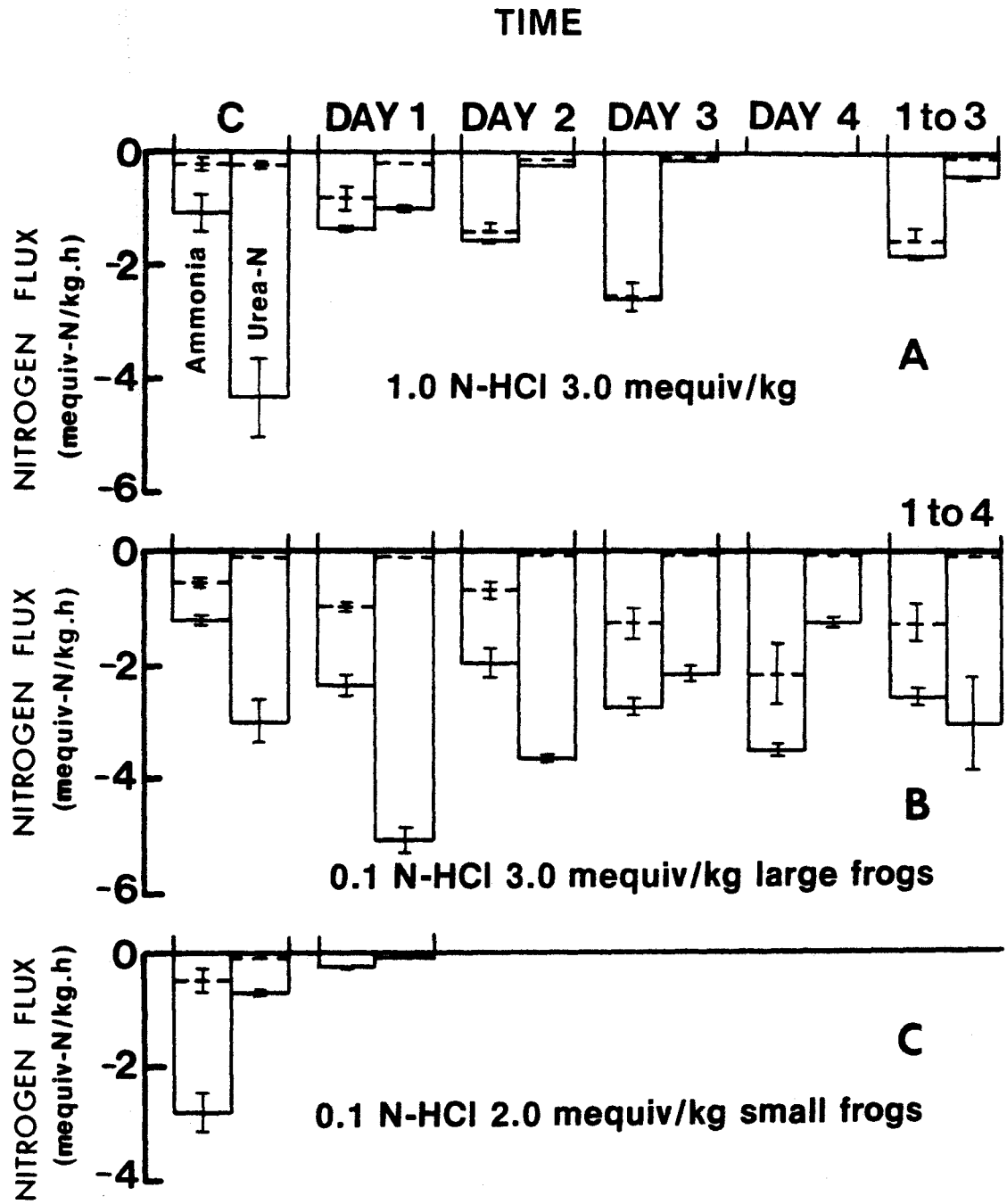
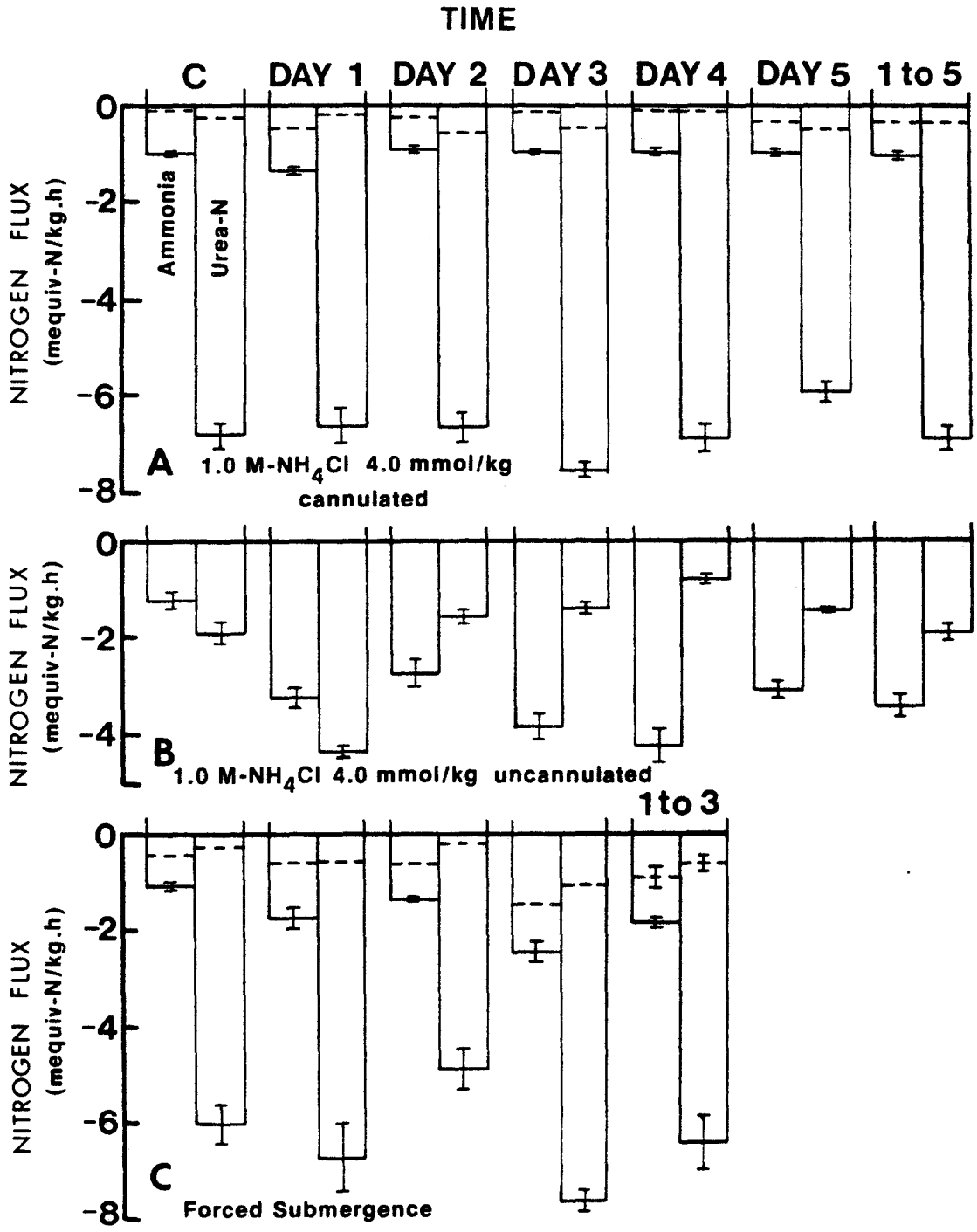

TIME
C
DAY 1
DAY 2
DAY 3
DAY 4
1 to 3
1 to 4
NITROGEN FLUX
(mequiv-N/kg.h)
0
-2
-4
-6
Ammonia
Urea-N
A
1.0 N-HCl 3.0 mequiv/kg
B
0.1 N-HCl 3.0 mequiv/kg large frogs
C
0.1 N-HCl 2.0 mequiv/kg small frogs

Fig. 22. Cutaneous and renal net fluxes of ammonia ($NH_3 + NH_4^+$) and urea-N prior to and following intravascular NH_4Cl infusion (A); dorsal lymph sac NH_4Cl infusion - whole body only (B); and forced submergence (C) in the bullfrog. See legend of Fig. 5 for other details.


TIME
C
DAY 1
DAY 2
DAY 3
DAY 4
DAY 5
1 to 5
NITROGEN FLUX (mequiv-N/kg.h)
0
-2
-4
-6
-8
Ammonia
Urea-N
A
1.0 M-NH4Cl 4.0 mmol/kg
cannulated
B
1.0 M-NH4Cl 4.0 mmol/kg uncannulated
1 to 3
C
Forced Submergence

of HCl resulted in a change to predominantly cutaneous ammonia excretion by 4 days post-infusion (Fig. 21). This changeover occurred rapidly in frogs in which renal failure occurred, and resulted in a 57% reduction in nitrogen excretion over 3 days despite a 5-fold elevation in cutaneous ammonia efflux (Fig. 21A). In large frogs infused with 0.1 N-HCl marked increases in both ammonia and urea excretion occurred during the first day of recovery (Fig. 21B). Thereafter renal urea excretion was gradually superseded by cutaneous ammonia efflux such that by Day 4 total ammonia excretion exceeded total urea-N excretion by almost 3-fold. Thus the overall trend was an initial stimulation of both ammoniogenesis and ureogenesis, followed by a change to ammoniotelic nitrogen metabolism occurring in the second day of recovery. The result, over 4 days, was a 30% increase in nitrogen excretion.

Intravascular infusion of NH_4Cl resulted in no significant increase in whole body nitrogen excretion over 5 days of recovery (Fig. 22A). However the average cutaneous efflux of both ammonia and urea was slightly elevated during these five days. Presumably the infused nitrogen was only slowly released and thus was undetectable. In contrast, dorsal lymph sac infusion of NH_4Cl resulted in a change to ammoniotelic excretion (Fig. 22B) similar to that occurring following HCl infusions. In the first day of recovery both total ammonia and total urea excretion doubled. Thereafter ammonia excretion progressively increased while urea excretion was reduced, such that after 4 days of recovery the ratio of urea N:ammonia-N had decreased to 0.19 from the control value of 1.8. The result over 5 days was a 175% increase in ammonia excretion accounting

for 69% of the total elevation in nitrogenous efflux. This elevation in ammonia excretion amounted to about 7,500 μequiv/kg, or almost double that infused (i.e. 4,000 μequiv NH_4^+/kg).

During three days of recovery from forced submergence the cutaneous efflux of ammonia and urea increased 2 to 3-fold while renal excretion of ammonia increased by about 30% and urea excretion remained relatively constant (Fig. 22C). This increase in ammoniogenesis/excretion amounted to an efflux of about 2,100 μmol/kg over three days.

10. Water Fluxes

The net cutaneous flux of water has been plotted together with the renal water excretion (i.e. urine flow rate) in Fig. 23 and 24 to show their contributions to the water balance of the bullfrog. During the control periods the net whole animal water loss (shaded bars in Fig. 23 and 24) to the medium was low and represented less than 0.3%/h of the total body water (based on ∿86% water content, Thorson, 1964).

During acid infusions there was an initial stimulation of the net cutaneous water uptake but UFR remained relatively constant at control values (Fig. 23, 24A). However, immediately following HCl infusions (Fig. 23) and forced submergence (Fig. 24C) there was a marked reduction in the net cutaneous uptake of water. Since UFR was also elevated during this period (except in renal failure, Fig. 23A and C) a large net loss of water by the whole animal occurred during the first 4 to 8 h. In these frogs this period of water loss was followed by an extended period of net water uptake (Fig. 23B, 24C).

The most potent stimulator of cutaneous water influx was the intravascular infusion of NH_4Cl (Fig. 24A); second was dorsal lymph sac infusion of NH_4Cl (Fig. 24B). In the cannulated frogs UFR remained constant, such that a large and continuous whole animal water uptake persisted for the first 12 h of recovery (Fig. 24A). A persistent 8 h whole body water uptake in dorsal lymph sac infused frogs (Fig. 24B) was reversed within 12 h post-infusion. All frogs infused with NH_4Cl appeared to be in net water balance within 24 h.

Fig. 23. Cutaneous, renal and whole body net water fluxes prior to, during and following HCl infusion in the bullfrog. (A) 1.0 N-HCl; (B) 0.1 N-HCl - large bullfrogs; (C) 0.1 N-HCl - small bullfrogs. See legend of Fig. 2 for other details.

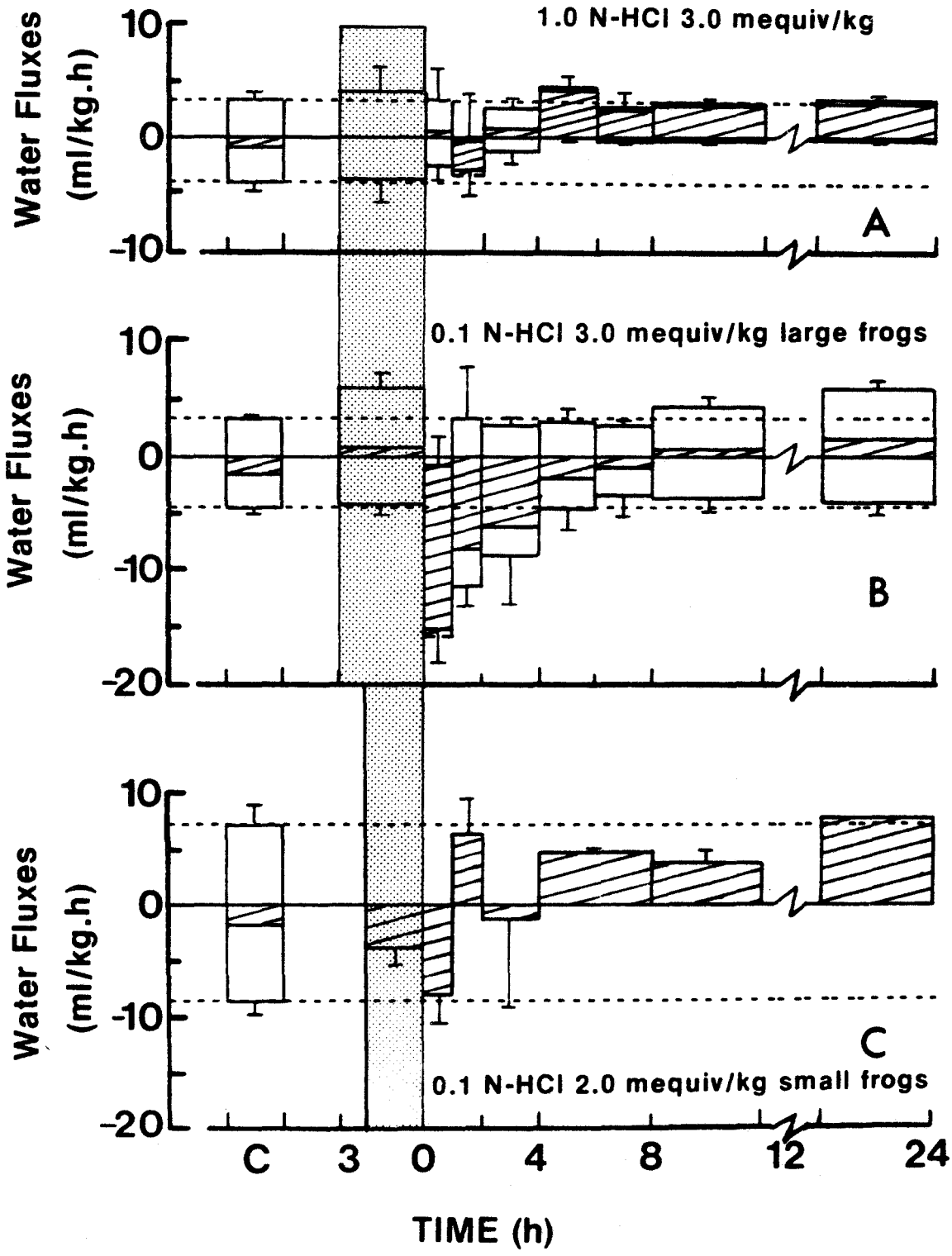
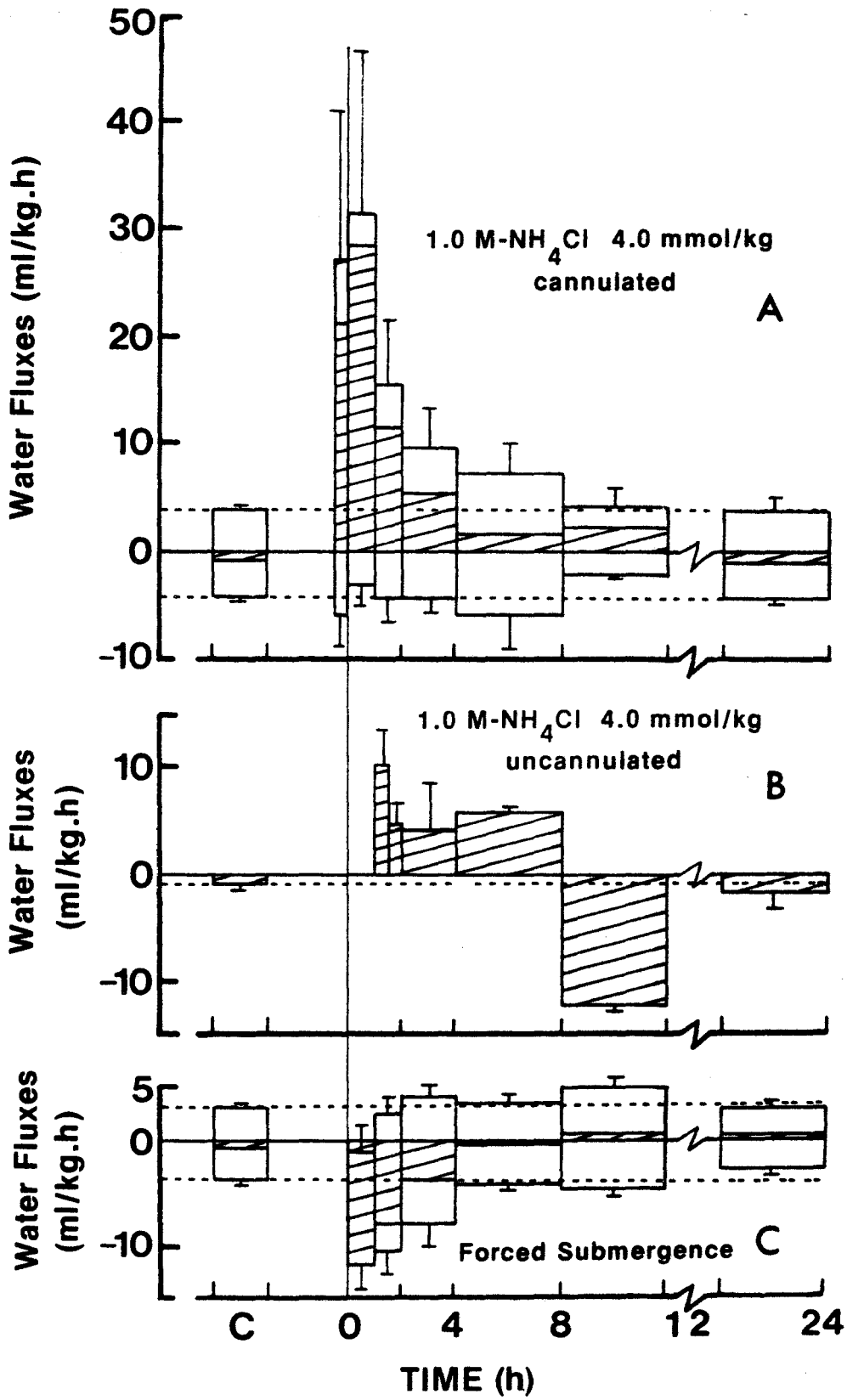

1.0 N-HCl 3.0 mequiv/kg
A
0.1 N-HCl 3.0 mequiv/kg large frogs
B
0.1 N-HCl 2.0 mequiv/kg small frogs
C
Water Fluxes (ml/kg.h)
10
0
-10
-20
C
3
0
4
8
12
24
TIME (h)

Fig. 24. Cutaneous, renal and whole body net water fluxes prior to, during, and following intra-vascular NH_4Cl infusion (A); dorsal lymph sac NH_4Cl infusion, whole body only (B); and forced submergence (C) in the bullfrog.


Water Fluxes (ml/kg.h)
50
40
30
20
10
0
-10
1.0 M-NH4Cl 4.0 mmol/kg
cannulated
A
Water Fluxes (ml/kg.h)
10
0
-10
1.0 M-NH4Cl 4.0 mmol/kg
uncannulated
B
Water Fluxes (ml/kg.h)
5
0
-10
Forced Submergence
C
C
0
4
8
12
24
TIME (h)

DISCUSSION

1. Methodology

(a) Induction of the acidosis

A major question that arises from this type of study concerns the physiological significance of acid infusions and enforced submergence in relation to the natural habits and physiology of the frog. For example, the naturally diving aquatic toad *Xenopus laevis* has been shown to incur a much milder acidosis than that occurring in forcibly submerged animals (Boutilier, 1981). Blood lactate remains low and acid-base status is restored within 10 min of breathing at the surface, even following voluntary dives of 40 min or greater (Boutilier, 1981). While it can be argued that this is the more natural acid-base disturbance, its small magnitude does not readily allow an examination of the various mechanisms of acid-base regulation. Given present techniques a marked and prolonged disturbance must be induced. So while acid infusions and forced submergence are 'unnatural' they do provide useful techniques for the study of vertebrate acid-base regulation.

The major advantage of HCl (i.e. fixed acid) infusions is its quantification of a substantial acid-base disturbance when loads in excess of 500 μequiv/kg are used. A major disadvantage is that loading in excess of 3,000 μequiv/kg constitutes a severe challenge to acid-base regulation in lower vertebrates and can result in extensive tissue damage and kidney necrosis in fish (Cameron and Kormanik, 1982) and amphibians (present study). NH_4Cl infusions and hypoxia/forced

submergence, on the other hand, are well tolerated by fish (Kobayashi and Wood, 1980; Cameron and Kormanik, 1982) and amphibians (Vanatta and Frazier, 1981; Emilio and Shelton, 1980; Boutilier, 1981; Jones, 1972b). However, while producing large and prolonged pH disturbances, they do not permit the accurate quantification of the acid loading to the animal.

(b) Bladder catheterization

In order to determine the renal contribution to acid-base regulation urine was collected by bladder catheterization. This method has the advantages that (1) it eliminates handling stresses which affect blood parameters (Mbangkolo and De Roos, 1983) and cutaneous ion fluxes (Garcia-Romeu *et al.*, 1969); urine flow rate remains relatively unchanged compared to occlusive ureteral cannulation which results in elevated UFR (Sawyer, 1957; Yoshimura *et al.*, 1961; Jard and Morel, 1963; Long, 1973). It has the major disadvantage that bladder urine is continuously siphoned rather than periodically voided, thereby greatly reducing the residence time in the bladder. Thus the urine was not representative of true bladder urine and hence was not a measure of the response of the entire urinary system (i.e. kidneys plus bladder). The amphibian urinary bladder is known to possess reabsorptive and secretory functions which may be important in acid-base regulation (Bentley, 1966; Middler *et al.*, 1968; Frazier and Vanatta, 1973; Skadhauge, 1977; Al-Awqati, 1978; Brodsky *et al.*, 1980; Finn, 1980, Fitzgerrel and Vanatta, 1980; Hill and Frazier, 1983; Satake *et al.*, 1983). However, it has been shown that under normal hydrated conditions bladder urine collected by

continuous siphoning is very similar in composition to ureteral urine (Yoshimura *et al.*, 1961). Therefore the urinary excretions measured in this study are considered to be similar to true tubular urine and the results are discussed as a renal response.

It should be noted that if the amphibian urinary bladder is indeed important in acid-base regulation, as some recent studies indicate (Brodsky *et al.*, 1980; Fitzgerrel and Vanatta, 1980; Frazier and Vanatta, 1971; Frazier and Zachariah,1979; Hill and Frazier, 1983) then the effective removal of the urinary bladder by chronic catheterization put additional emphasis on cutaneous and renal acid-base regulation in the present study.

(c) Water ionic strength, buffering, and volume to frog surface area.

In experimental series 1 through 6 water of low ionic strength and buffering capacity (see Table 1) was used for bathing the frogs in order to amplify changes in water ion concentrations. It is known that the ionic strength and buffering of water can affect the kinetics of ion exchange processes at the skin (Greenwald, 1971, 1972; Garcia-Romeu and Ehrenfeld, 1975b; Bentley and Yorio, 1976). To test this, water of higher ionic strength and buffering was used in the last two series of experiments (see Table 1). It was found that under control conditions the large bullfrogs showed no marked difference in the cutaneous net fluxes of water and electrolytes when in soft or hard water; however the unidirectional fluxes (i.e. J_{in} and J_{out} both) of Na^+ and Cl^- were about 20% greater in hard water frogs (see Table 5 and 7). These results support the earlier findings (cited above) that J_{in} will increase with

increasing ionic strength of the external water, but since J_{out} is similarly stimulated the net effect appears to be the same. Therefore the ionic strength and buffering of the water were of little or no importance in these experiments.

The volume of water used to bathe the frogs within the flux chambers was small and varied with the duration of the flux period. The volumes used (50 ml for ½-1 h flux periods; 100 ml for 2-4 h; 250 ml for 12 h) were low enough so that even small changes in cutaneous water and electrolyte fluxes could be detected. The disadvantage of using these small volumes is that all flux measurements shown represent only the contribution by the ventral cutaneous epithelium and not the ventral and dorsal skin both. However, this volume was always sufficient to bathe fully that part of the integument known to be important in electrolyte and water uptake from the environment. The cutaneous surfaces most active in water and ion transfers in anurans are the abdominal skin and ventral limb skin (Krogh, 1939; Shoemaker, 1964; Bentley and Yorio, 1976; Shoemaker and Nagy, 1977). For the short-duration flux periods when only 50 or 100 ml was used, the frog adopted a posture of pressing its abdomen to the bottom of the flux chamber, permitting maximal exposure of skin surface area to the available water. At no time during the shorter flux periods were the frogs observed to be elevated over the water surface. Therefore fluxes in 50 ml or 500 ml (during control experiments) of water were similar, since in both cases dorsal skin was not bathed by the water in the flux chamber.

2. Blood, ECF, and Non-ECF Contributions to Acid-base and Ion Balance.

a) Comparative aspects

In all bullfrogs acid infusions or forced submergence produced a marked blood acidosis. The common pattern of blood acid-base disturbance, a combined respiratory/non-respiratory acidosis, was qualitatively similar to that seen in other vertebrates following enforced dives, hypoxia/apnoea, or exercise (Andersen, 1966; Kobayashi and Wood, 1980; Boutilier, 1981; Heisler, 1982; Toews and Boutilier, 1983). This mixed acidosis was due to a combination of CO_2 production/retention plus infused and/or endogenously produced H^+. Typically, a short time into the recovery period, this mixed acidosis developed into a pure non-respiratory acidosis. The magnitude and duration of blood pH depression in vertebrates subjected to various short-term acid stresses are presented in Table 10 for comparison with the present study.

In the present study HCl infusions produced an initial acidosis that was of about 2-fold greater magnitude than previously reported for HCl-infused fish (Cameron, 1980; Cameron and Kormanik, 1982; McDonald *et al.*, 1982). This, however, is largely attributable to the 2-3 fold larger loads employed both in the present study and by Yoshimura *et al.* (1961). When *Bufo marinus* was infused with an HCl load comparable to that employed in some fish studies (Cameron, 1980, McDonald *et al.*, 1982) a virtually identical initial pH disturbance was produced (Table 10; Lindinger and McDonald, unpublished observations). However, in *Bufo*, this disturbance was corrected in less than half the time.

Table 10. Magnitude and duration of blood pH disturbance following induction of short-term non-respiratory acidoses in vertebrates.

Animal	Treatment	ΔpH	Duration	Reference
-teleosts-				
Platichthys stellatus	exhausting activity	-0.38	6 h	Wood *et al.*, 1977
Parophrys vetulus	1.0 N-Hcl; 1 mequiv/kg	-0.1 to -0.7	1-2 h	McDonald *et al.*, 1982
Ictalurus punctatus	1.0 N-HCl; 1 mequiv/kg	-0.2	2 h	Cameron, 1980
	2.0 M-NH_4Cl; 2 mmol/kg	-0.3	3 h	Cameron & Kormanik, 1982
	2.0 N-HCl; 1 mequiv/kg	-	4-6 h	Cameron & Kormanik, 1982
Salmo gairdneri	6 min severe exercise	-0.5	8 h	Wood *et al.*, 1983 Turner *et al.*, 1983
	5 min strenuous exercise	-0.5	4 h	Holeton *et al.*, 1983
	0.02 N-HCl; 0.1 mequiv/kg	-	2 h	Wood & Caldwell, 1978
	20-35 min hypoxia	-0.3	4 h	Kobayashi & Wood, 1980
	$(NH_4)_2SO_4$; 2.5 mmol/kg	-0.45	>22 h	Cameron & Heisler, 1983
Tinca tinca	5 min severe exercise	-0.5	4-24 h	Jensen *et al.*, 1983
-anurans-				
Xenopus laevis	30 min enforced activity	-0.4	10 h	Boutilier *et al.*, 1980
	30 min voluntary dive	-0.05	10 min	Boutilier, 1981
	30 min forced submergence	-0.25	8 h	Boutilier, 1981
	30 min forced submergence	-0.2	4 h	Emilio & Shelton, 1980
	9 min manual stimulation	-0.7	-	Putnam, 1979
Bufo boreas	15 min manual stimulation	-0.65	-	Putnam, 1979
Bufo marinus	30 min mechanical stimulation	-0.39	4 h	McDonald *et al.*, 1980
	1.0 N-HCl; 1 mequiv/kg	-0.2	<1 h	Lindinger & McDonald, unpub.
	1.0 M-Na_2SO_4; 10 mmol/kg	no change	no change	Long, 1982a
Rana pipiens	5 min manual stimulation	-0.75	-	Putnam, 1979
Rana esculenta	1 h apnoea in air	-0.3	0.7 h	Jones, 1972a
Rana catesbiana	0.1 N-HCl; 3 mequiv/kg	-0.3	24 h	Yoshimura *et al.*, 1961
	large animals 0.1 N-HCl; 3 mequiv/kg	-0.38	48 h	this study
	small animals 0.1 N-HCl; 2 mequiv/kg	-0.8	didn't recover	this study
	1.0 N-HCl; 3 mequiv/kg	-0.5	24 h	this study
	1.0 M-NH_4Cl; 4 mmol/kg	-0.3	0.5 h	this study
	45 min enforced submergence	-0.46	12 h	this study
-reptiles-				
Pseudemys scripta elegans	2-4 h enforced submergence	-0.8	2 h	Jackson & Silverblatt, 1974
	6 h enforced submergence	-0.9	15 h	Robin *et al.*, 1981
Alligator mississippiensis	45 min forced submergence	-0.6	1 h	Andersen, 1961
-birds-				
domestic duck	13 min forced submersion	-0.36	~1 h	Andersen *et al.*, 1965
-mammals-				
Castor fiber	10 min forced submersion	-0.2	-	Clausen & Ersland, 1970/71
white rat	0.37 M-NH_4Cl; 3.7 mmol/kg	-0.13	-	Guntupalli *et al.*, 1982
	0.37 M-NH_4Cl; 7.5 mmol/kg	-0.65	-	Guntupalli *et al.*, 1982
	0.123 M-HCl; 3.7 mequiv/kg	-0.14	-	Guntupalli *et al.*, 1982
	0.5 M-lactate; 3.7 mmol/kg	-0.09	-	Guntupalli *et al.*, 1982

Similarly, NH_4Cl infusions in amphibians (present study) employed loads double that previously used in fish (Cameron and Kormanik, 1982). Nevertheless, the resulting acid-base disturbances were identical (Table 10) and, more importantly, more rapidly corrected (0.5 h *vs.* 3 h) in amphibians. Furthermore, rainbow trout infused with a similar NH_4^+ load as $(NH_4)_2SO_4$ (Cameron and Heisler, 1983) required more than 22 h to correct the disturbance. Therefore, both HCl and NH_4Cl infusions in fish and amphibians clearly suggest that amphibians have a greater capacity for buffering infused acid loads.

Strenuous or exhaustive exercise, hypoxia or enforced submergence, all of which lead to lactic acid production, generally produce more severe blood acid-base disturbances than infused HCl or NH_4Cl (Table 10). The reason for this is that animals can tolerate much larger loads of endogenously produced acid than can be infused directly into the blood. For example, severe exercise in trout (Turner *et al.*, 1983) and enforced submergence in bullfrogs (present study) produced estimated lactate, and hence acid, loads of 3.6 and 5.3 mequiv/kg respectively. Typically, infused fixed acid (i.e. HCl) loads in excess of 2.0 mequiv/kg will, on the other hand, cause death in both fish (Cameron and Kormanik, 1982) and amphibians (Yoshimura *et al.*, 1961; present study).

The magnitude of the acid-base disturbance resulting from lactacidosis in both fish and amphibians has generally been similar (Table 10). For example, hypoxia (Kobayashi and Wood, 1980) and strenuous exercise (Holeton *et al.*, 1983) in trout produced a nearly identical estimated lactate load ($\sim$ 5.0 mequiv/kg) to that estimated

in the present study following forced submergence in bullfrogs. In these circumstances the trout corrected the blood acid-base disturbance more rapidly (i.e. 4 h *vs.* 12 h in bullfrogs - Fig. 5), although for both species blood lactate concentration remained elevated for a similar length of time ($\sim$ 12 h post-disturbance). This suggests that the trout and the frog both metabolized the lactate load at the same rate but that the trout more rapidly excreted or buffered the H^+ load. This is in contrast to their response to infused loads where the frogs were more effective at buffering.

In contrast to fishes and amphibians, mammals infused with even larger loads of HCl or NH_4Cl, or forcibly submerged, had relatively small blood acid-base disturbances which were rapidly corrected (Table 10). Forced submergence in reptiles, on the other hand, produced larger pH disturance, but these were still corrected rapdily compared to fishes and amphibians (Table 10).

Despite the apparent differences in experimental regimes among the vertebrate studies (Table 10) two main factors can account for the blood acid-base responses between mammals and lower vertebrates (Table 11). Firstly, the rapidity of correction of acid-base status appears to be related to differences in metabolic rate. For example mammals of 0.4 to 1 kg body mass have a metabolic rate about 80-fold higher than amphibians of similar body mass at 20°C (Prosser, 1973). Furthermore, metabolic rate in fish and amphibians is proportional to ambient temperature (Prosser, 1973) and this may partially account for the differences among lower vertebrates (Table 11). Secondly, the severity of the acidosis appears to be proportional to the buffering

Table 11. Blood buffer capacity with transition from water-breathing to air-breathing in vertebrates.

Animal	Mode of Respiration	Ambient Temperature (°C)	$\frac{HCO_3^-}{pH}$	Reference
FISHES				
Platichthys stellatus	water-breather	9	-5.2	Wood *et al.*, 1977
Hippoglossoides elassodon	water-breather	11	-6.5	Turner *et al.*, 1983
Tinca tinca	water-breather	15	-7.7	Jensen *et al.*, 1983
Salmo gairdneri	water-breather	15	-8.5	Wood *et al.*, 1982
AMPHIBIANS				
Rana catesbiana	bimodal	20	-16.4	Lenfant & Johansen, 1967
Bufo marinus	bimodal	25	-20.4	Boutilier *et al.*, 1979a
TURTLE				
Chrysemys picta belli	diving air-breather	3	-12.4	Ultsch & Jackson, 1982
MAMMALS				
beaver	diving air-breather	35*	-27	Clausen & Ersland, 1968
water vole	diving air-breather	35*	-34	Clausen & Ersland, 1968
man	nondiving air-breather	37*	-29	Davenport, 1974

* body temperature (°C)

capacity of the blood . Among vertebrates, differences in blood buffer capacity correspond closely to hemoglobin content of the blood; and in general, the buffer capacity increases with the phylogenetic transition from water- to air-breathing (Table 11).

The present study also suggests that, within any one species, body size may be important in the animals' ability to regulate acid-base balance. When small bullfrogs (∿120 g) were infused with two-thirds the load of 0.1 N-HCl as large bullfrogs (∿400 g) a much more severe acid-base disturbance was incurred (Table 1). The specific reason for these allometric effects is not clear; however it may be that ECF buffering capacity varies with body mass in a different fashion than ECF volume. Similar size effects, also unexplained, have been reported in the dogfish shark, *Scyliorhinus stellaris* (Heisler *et al.*, 1976; Heisler *et al.*, 1980) and in the lemon sole, *Parophrys vetulus* (McDonald *et al.*, 1982).

b) Respiratory contributions

The typical response to induction of an acid stress to the blood in vertebrates is an initial respiratory acidosis component to the total blood pH depression (Andersen, 1966; McDonald *et al.*, 1980; Holeton *et al.*, 1983; Jensen *et al.*, 1983). This respiratory component probably results from both a stimulation of aerobic metabolism related to the stress of acid infusions, enforced submergence, or strenuous activity (i.e. elevated CO_2 output related to increased muscular activity) and acid titration of blood HCO_3^- (Dejours, 1981).

These respiratory contributions to the acidosis (ΔH^+_r)

declined relatively rapdily in NH_4Cl-loaded and forcibly submerged frogs. But ΔH^+_r persisted following HCl infusions, contributing 20-30% to the maintenace of the blood pH depression. In these frogs plasma $[HCO_3^-]$ remained low during recovery, reflecting the sustained cutaneous "acid" uptake (Table 6, Figure 15).

The restoration of elevated blood PCO_2 to normal values occurred within 4 h, most likely by a combination of pulmonary and cutaneous excretion of CO_2 (Jackson, 1978; Mackenzie and Jackson, 1978; McDonald *et al.*, 1980; Emilio and Shelton, 1980). Similarly slow kinetics of CO_2 washout have been reported in amphibians subjected to strenuous exercise (Boutilier *et al.*, 1980; McDonald *et al.*, 1980) or hypercapnic exposure (Boutilier *et al.*, 1979a; Boutilier and Toews, 1981b), and in exercised (Wood *et al.*, 1977; Turner *et al.*, 1983; Jensen *et al.*, 1983; Holeton *et al.*, 1983) and acid-infused fish (McDonald *et al.*, 1982). Since ventilatory adjustment of blood pH occurs rapidly in air-breathers (Comroe, 1974; Dejours, 1981) the amphibian might be expected to display a more rapid CO_2 excretion than fish. However the apparent similarity is not surprising given that the amphibian lung is poorly developed and that the cutaneous surface area for gas exchange is considerably less than that of the fish gill (cf. Czopek, 1965; Hughes and Morgan, 1973).

c) Mechanisms of ECF and non-ECF buffering

Infused and endogenously produced acid rapidly titrated ECF bicarbonate and non-bicarbonate buffers. This was evident by the initial depression of plasma pH and $[HCO_3^-]$, and elevation of PCO_2, ΔH^+m and ΔH^+_{ECFV}. The total (cutaneous plus renal) acid excretion was always

only a small, sometimes negligible, portion of the estimated acid load to the animal (Table 6). Therefore much of the acid load was either retained indefinitely and buffered (i.e. HCl-loading) or retained and eventually metabolized (i.e. NH_4Cl-loading and forced submergence). Although ECF buffering by bicarbonate, phosphate and ammonia are easily detected and measured, the major component of ECF buffering is performed by proteins in blood and interstitial fluid (Woodbury, 1974).

Estimates of the total amount of excess acid buffered by the ECF (Table 6) indicated that most of this excess was buffered within the cells, i.e. by the intracellular fluid compartment. This is supported by the observation that the quantity of excess acid removed from the ECF during the recovery period was greater than that determined to have been excreted (Table 6). The large amount of acid estimated to have been buffered by the ICF in HCl-loaded frogs indicates the potential for an intracellular acidosis which may have been the proximate cause of death in these animals. A similar mechanism has been postulated to be the proximate cause of death in severely exercised trout (Wood, Turner and Graham, 1983).

The occurrence and magnitude of ICF buffering was also indicated by the elevation of plasma $[K^+]$ during HCl infusions and forced submergence, and following NH_4Cl infusions. This rise in plasma $[K^+]$ may result from a decrease in cell membrane integrity and/or inhibition of the Na/K^+-ATPase of cell membranes (Woodbury, 1974; Levitt, 1980) resulting from the effects of elevated ECF $[H^+]$.

Further evidence for the role of the intracellular compartment

in acid-base regulation is indicated by the elevation in blood [P_i] and [ammonia] following HCl infusions and enforced submergence. Phosphate and ammonia buffers are believed to be mobilized from the soft tissues (i.e. muscle) during acid stresses (Simkiss, 1968; Woodbury, 1974; Pitts, 1974). The disappearance of phosphate and ammonia from the blood was coincident with the correction of blood pH.

Inorganic buffering by $CaCO_3$ stored in the paravertebral lime sacs and ductus endolymphaticus (Guardabassi, 1960; Brown, 1964; Simkiss, 1968; Barlet, 1982) may have provided an additional source of buffer for the ECF. In the adult anuran these lime sacs are believed to serve as a buffering system during respiratory acidosis (Simkiss, 1968; Robertson, 1972); however a role for such stores of $CaCO_3$ in non-respiratory acidosis has not previously been reported (see also Barlet, 1982).

The role for $CaCO_3$ buffering in the present study is indicated by the elevation of plasma [Ca^{++}] following acid infusions and forced submergence. That the rise in plasma [Ca^{++}] was not due to the release of calcium phosphates from bone or the soft tissues (Pitts, 1974) is supported by the following observations: 1) plasma P_i was always much lower than plasma [Ca^{++}] and did not rise concurrent with elevations in plasma [Ca^{++}]; 2) Bone calcium phosphates and carbonates represent only 1.5% of bone ash Ca^{++} in anurans as compared to 5.3% in man, 13.1% in turtles, and are negligible in fish (Robertson, 1972; Istin, 1975; Barlet, 1982); 3) Amphibians do not 'store' calcium in bone since they lack osseous trabeculae (Romer, 1962). 4) The release of Ca^{++} and phosphate from bone (Pitts, 1974) by the action of

parathormone (PTH) as occurs in mammals (Arruda *et al.*, 1982) is limited in amphibians (Simkiss, 1968). Instead, current evidence indicates the actions of PTH and calcitonin in anurans modulates plasma Ca^{++} levels by mechanisms involving $CaCO_3$ stored in the paravertebral lime sacs (Simkiss, 1968; Robertson, 1972; Robertson, 1974; Boschwitz and Bern, 1971; Barlet, 1982).

The stored $CaCO_3$ is believed to be in dynamic equilibrium with HCO_3^- in the ECF (Simkiss, 1968; Istin, 1975; Barlet, 1982). Thus $CaCO_3$ may be mobilized during non-respiratory acidosis (i.e. decreased blood $[HCO_3^-]$) by the following reactions (after Simkiss, 1968; Robertson, 1972):

$$\underset{\text{(solid)}}{CaCO_3} \rightleftharpoons Ca^{++} + CO_3^{=}$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{=}$$

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3 \overset{\text{c.a.}}{\rightleftharpoons} H_2O + \underset{\text{(gas)}}{CO_2}$$

Thus the release of 1 mol of $CaCO_3$ has the potential of buffering 2 moles of H^+ to produce CO_2 plus water. Therefore stored $CaCO_3$ may provide a very powerful ECF buffering system under conditions of acute or chronic blood acidosis. This would be functionally similar to the role of bone phosphates and carbonates in the compensation of acidosis in mammals (Arruda *et al.*, 1982; Barlet, 1982).

3. Renal Mechanisms of Acid-Base Regulation

In vertebrates the kidneys play an important role in acid excretion. Active secretion of H^+ by the kidney tubules, together with

Table 12. Characteristics of renal acid excretion in freshwater teleost fishes and anuran amphibians during non-respiratory acidosis (all rates of excretion expressed as μquiv/kg/time).

Animal	Acid Load	Time	Increase in urinary excretion ΣH^+	$TA\text{-}HCO_3^-$	NH_4^+	minimum urine pH	% of acid load excreted	Reference
Salmo gairdneri	0.02 N-HCl 100 μequiv/kg	72 h	149	∿70	∿80	6.88	149	Wood & Caldwell, 1978
Ictalurus punctatus	1.0 N-HCl 1,000 μequiv/kg	36 h	490 (non-significant)	-	-	decreased	49	Cameron, 1980
	2.0 N-HCl 1,000 μequiv/kg		extensive tissue damage					Cameron & Kormanik, 1982
Rana catesbiana	0.1 N-HCl 2,000 μequiv/kg	24 h	cessation of renal function in small (120 g) frogs					this study
	0.1 N-HCl ∿1,700 μequiv/kg	5 days	∿1,700	no change	1,700	7.1	100	Yoshimura *et al.*, 1961
	0.1 N-HCl 3,000 μequiv/kg	99 h	1,100	-720	1,820	7.13	36.7	this study
	1.0 N-HCl 3,000 μequiv/kg	75 h	renal failure					this study
Ictalurus punctatus	2.0 M-NH_4Cl 2,000 μmol/kg	24 h	∿375	no change	∿375	5.35	14 ∿19% of ammonia load	Cameron & Kormanik, 1982
Rana catesbiana	1.0 M-NH_4Cl 4,000 μmol/kg	24 h	∿165	no change	∿165	7.2	∿4% of ammonia load	this study
Salmo gairdneri	lactacidosis: 20-35 min hypoxia	72 h	∿520	∿430	∿90	6.8	12-25	Kobayashi & Wood, 1980
Rana catesbiana	lactacidosis: 45 min forced submergence	12 h	340	120	240	5.2	6% of estimated	this study

renal ammoniogenesis, appears to be the primary mechanism underlying the excretion of, respectively, titratable (i.e. TA-HCO_3^-) and non-titratable (i.e. NH_4^+) buffer acids in all vertebrates so far studied (Yoshimura *et al.*, 1961; Pitts, 1974; Wood and Caldwell, 1978; McDonald and Wood, 1981; Long, 1982b; Buerkert *et al.*, 1983; Wheatly *et al.*, 1983). In lower vertebrates, however, there appear to be major differences in the capacities of these two mechanisms. Table 12 summarizes these renal mechanisms in freshwater fish and amphibians possessing glomerular kidneys.

In response to HCl infusions, bullfrogs (Yoshimura *et al.*, 1961; this study) did not acidify the urine and hence TA-HCO_3^- remained unchanged (Table 12). Furthermore, Yoshimura *et al.* (1961) found that bullfrogs infused with as much as 12 mmol HCl/kg (as 0.1 N-HCl) showed no significant change in urinary pH and TA-HCO_3^- excretion. HCl-loaded bullfrogs do, however, exhibit large increases in urinary NH_4^+ excretion, accounting for 100% (Yoshimura *et al.*, 1961) or less (37%; this study) of the infused acid load within 5 days in those animals which maintained renal fuction (Fig. 12, 21).

The bullfrogs' apparent inability to acidify the urine during HCl-induced acidosis is not typical of lower vertebrates. For example, fish infused with HCl (Table 12) or chronically exposed to pH 4.2 water (McDonald and Wood, 1981) increase total urine acid efflux (ΣH^+) by elevations in both NH_4^+ and TA-HCO_3^- excretion rates, concurrent with a decrease in urine pH.

Similarly, when fish were infused with NH_4Cl (Cameron and Kormanik, 1982) urine pH declined; however, this was not accompanied by

an increase in TA-HCO_3^- excretion (Table 12). In amphibians, on the other hand, both urine pH and TA-HCO_3^- excretion remained unchanged (this study). Furthermore, fish evidently cleared a greater proportion of the total ammonia load (19% *vs.* 4%) in 24 h compared to bullfrogs (Table 12).

It may be argued that the most representative renal responses to acidosis in lower vertebrates are induced by the endogenous production and release of lactic acid; this produces a true metabolic acidosis. Lactacidosis in fish (Kobayashi and Wood, 1980) and frogs (this study) generally produced renal responses which were qualitatively similar but quantitatively different (Table 12). Elevation in renal excretion of NH_4^+ and TA-HCO_3^- was accompanied by increases in urinary acidification in both species, such that 5-6% of the estimated H^+ load had been excreted in 24 h (Kobayashi and Wood, 1980; Fig. 14). This renal response in bullfrogs was rapid but not sustained beyond 12 h (Fig. 14). Trout, on the other hand, maintained elevated rates of acid excretion for at least 3 days post-disturbance, resulting in the renal excretion of 12-25% of the proton load (Table 12 - Kobayashi and Wood, 1980).

Restoration of normal acid-base status during lactacidosis occurs largely by metabolism.[1] Therefore an evaluation of the relative competence of the fish and amphibian kidneys for acid excretion under these conditions may not be as appropriate as the previous comparisons employing HCl or NH_4Cl-induced acidosis. It is thus apparent, on the basis of the latter, that the kidney of the freshwater teleost may have a higher capacity for acid excretion than the anuran kidney.

[1] *See addendum, pp. 119-120.*

Renal handling of lactate by bullfrogs was similar to that reported for fish (Kobayashi and Wood, 1980), and mammals (Yudkin and Cohen, 1975), with less than 4% of the total estimated lactate loads being excreted. Renal lactate threshold for bullfrogs was estimated to be 2.5-5.0 mequiv/l of blood lactate. This value is similar to the estimate of 4-10 mequiv/l for trout (Kobayashi and Wood, 1980) but is lower than that reported for mammals (6.7 mequiv/l; Pitts, 1974).

Among vertebrates, mammals have the greatest capacity to acidify the urine and thereby can excrete large amounts of titratable and non-titratable buffer acids relatively rapdily (Pitts, 1974). In mammals the important tubular ion exchanges affecting H^+ secretion/HCO_3^- reabsorption are Na^+/H^+ and/or Cl^-/HCO_3^- (Pitts, 1974). Therefore increased tubular reabsorption of Na^+ and decreased Cl^- reabsorption is seen in acidotic mammals (Pitts, 1974). That these trends are not evident in lower vertebrates may be due to a reduced capacity for ion and water reabsorption resulting from the lack of renal zonation, loop of Henle, and osmotic gradients as well as the 100-200-fold decrease in numbers of nephrons (cf. Deyrup, 1964; Hickman and Trump, 1969; Riegel, 1971, Hoar, 1975). Evidence in support of this conclusion is scant. However, hyperoxia-induced acidosis in trout (Wheatly *et al.*, 1983) and acidosis in bullfrogs (this study) resulted in decreased tubular reabsorption of all strong ions (Table 9) concurrent with an apparent acidosis-induced diuresis (Wood and Caldwell, 1978; Cameron and Kormanik, 1982; present study Fig. 12, 13, 14). Thus it appears that acid excretion in lower vertebrates may not be as tightly coupled to renal ionoregulatory mechanisms (Yoshimura *et al.*, 1961; Wood and Caldwell,

1978; Cameron, 1980; Kobayashi and Wood, 1980; Long, 1982a; Wheatly *et al.*, 1983) such as that seen in mammals (Hills, 1973; Pitts, 1974).

4. Cutaneous Mechanisms of Acid-Base Regulation

As pointed out in the Introduction, current evidence indicates that the branchial epithelium of fishes and the cutaneous epithelium of anurans possess the same mechanisms for independent and electroneutral Na^+ and Cl^- absorption from dilute aquatic environments. Therefore by manipulation of the relative intensities of these two ion uptake mechanisms (Na^+/H^+ or NH_4^+ and Cl^-/HCO_3^- exchanges) these animals have potentially the same extra-renal mechanisms for acid-base regulation (Garcia-Romeu *et al.*, 1969; Evans *et al.*, 1982; Heisler, 1982; McDonald, 1983b, Toews and Boutilier, 1983).

In the severely acidotic fish the branchial epithelium is the predominant site of excretion of excess protons (Cameron, 1978; Heisler, 1982). For example, in fish infused with large ($\geq$ 1 mequiv/kg) acid loads, 80 to 100% of the acid excretion was by the gills (Cameron, 1980; Cameron and Kormanik, 1982; McDonald *et al.*, 1982). Similar branchial contributions have been seen in fish during hypoxia- or exercise-induced lactacidosis (Heisler, 1982; Holeton *et al.*, 1983). While it is known that frogs are capable of acidifying the external medium via the skin (Fleming, 1957; Friedman *et al.*, 1967; Vanatta and Frazier, 1981), a definite role for the skin in acid-base regulation has been difficult to demonstrate *in vivo*.

A potential role for the frog skin in acid-base regulation has indicated by the following two studies. Frazier and Vanatta (1980)

have shown that the *in vitro* skin of *Rana pipiens* excreted increased amounts of ammonia ($NH_3 + NH_4^+$) when isolated skin preparations from acidotic frogs were examined. Furthermore, most of these skins also acidified the external solution. More recently Vanatta and Frazier (1981) have shown that the *in vivo* frog skin is also capable of excreting both H^+ and ammonia at elevated rates following an NH_4Cl-induced acidosis.

The present study has confirmed that the frog skin excretes ammonia at elevated rates during acidosis, and may continue doing so for up to 5 days following correction of the blood pH disturbance. However, in contrast to the studies of Vanatta and Frazier (1981), the external medium was made alkaline by all animals; i.e. there was no net excretion of acid by the skin (Fig. 15, 16). This suggests that either most of the ammonia was excreted as NH_3, or was cleared as NH_4^+ accompanied by an equivalent amount of base (i.e. HCO_3^-/OH^-).

The frogs' inability to acidify the external medium during severe blood acidoses indicates cutaneous Na^+ and Cl^- absorptive mechanisms are ineffective at acid-base regulation. In fact, cutaneous Na^+ uptake was consistently elevated over Cl^- uptake (Fig. 16, 17), suggesting that Na^+/H^+, NH_4^+ exchanges may have been greater than Cl^-/HCO_3^- exchanges. However, the difference between the two exchanges could not be correlated with a net excretion of acid under any circumstance. Only following forced submergence was a highly significant correlation ($r = 0.941$; $P < 0.01$) found between Na^+ influx and ammonia efflux (Fig. 18). This does suggest a 1:1 exchange of external Na^+ for endogenous NH_4^+, assuming that all of the excreted ammonia was NH_4^+.

However, if Cl^-/HCO_3^- exchange was occurring at the same, or greater, rate in these animals then, of course, no net acid excretion across the skin would result (Fig. 15D).

Thus it can be concluded that Na^+/H^+, Cl^-/HCO_3^- exchange was either not occurring to any marked extent, was occurring at the same rate, or was manipulated in the appropriate manner but masked by the substantial increase in net cutaneous ion losses which accompanied the acid-base disturbances (Fig. 19, 20).

These large net losses of ions (Fig. 19, 20) together with the elevated cutaneous efflux of urea (Fig. 21, 22) and the accompanying changes in net cutaneous water flux (Fig. 23, 24) strongly suggest that the overall permeability of the cutaneous epithelium increased in response to blood pH disturbances. The cause of this apparent increase in cutaneous permeability seems to be an effect of the acid-base disturbance intself, since volume-loaded controls (Series 2; - see Methods) did not produce significant changes in cutaneous fluxes (Table 5). The largest initial increase in permeability occurred during intravascular infusion of NH_4Cl (Fig. 20) while the most persistent elevation of cutaneous permeability occurred following HCl infusions (Fig. 19). The response to lactacidosis (Fig. 20) was initially of equal magnitude to that of HCl-induced acidosis (Fig. 19) but the former was much more rapidly corrected.

Thus it appears that the duration of the increase in permeability is proportional to the duration of the extracellular acidosis, since the latter was most prolonged in the case of HCl infusion. If indeed it is the elevated ECF $[H^+]$ which is responsible for the permeability

increase, then questions arise regarding the specific locus of this effect. Theoretically, at least, there are two potential sites of action: the baso-lateral plus apical membranes which together constitute the transcellular ion diffusion path, and the intercellular tight junctions which constitutes the paracellular diffusion path.

In vitro studies using frog skin epithelium (Erlij and Martinez-Palomo, 1972; Gonzalez *et al.*, 1976) indicate that the paracellular pathway is normally "tight', such that less than 10% of the observed ion efflux is estimated to occur *via* this route (Ussing and Windhager, 1964; Erlij and Martinez-Palomo, 1978). These tight junctions, however, may be rendered "leaky" by various chemical treatments on the external surface (Gonzalez *et al.*, 1976) or internal surface (Whittembury *et al.*, 1976; Erlij and Martinez-Palomo, 1978), and furthermore are normally leaky in some epithelia, i.e. intestine, gall bladder (Erlij and Martinez-Palomo, 1978; Ussing *et al.*, 1974).

Current evidence from *in vitro* skins indicate that the tight junctions constitute a negatively charged environment for ion diffusion at physiological pH, and are thus cation selective (Gonzalez *et al.*, 1976; Erlij and Martinez-Palomo, 1978; Bullivant, 1981). This suggests that they would favour diffusion of strong cations (i.e. Na^+, K^+, Ca^{++}) over that of strong anions (i.e. Cl^-, lactate anion). The constraints of electroneutrality would then dictate that to maintain charge balance there must be a gradient driven influx of H^+, or loss of weak base (its functional equivalent) across the skin. This hypothesis is supported by the fact that cutaneous net losses of Na^+ and K^+ were indeed greater than Cl^- losses in all cases (Fig. 19,20). These ion losses

would, in effect, obscure any attempt at acid-base regulation at the skin, masking any acid excretion which potentially could occur across the apical membrane. An interesting possibility raised by the apparent forced base loss/acid uptake seen during acidosis in bullfrogs is that these animals may, in fact, be better at correcting a base load (i.e. alkalosis) to the ECF. This subject warrants further investigation.

Low pH solutions bathing the internal surface of isolated frog skins do not produce an increase in paracellular permeability (Gonzalez *et al.*, 1976). Therefore the cutaneous response observed in the present study may not be due to a direct pH effect but rather to secondary effects perhaps mediated by humoral factors.

5. Summary and Conclusions

Acid infusions or forced submergence in bullfrogs resulted in a marked blood acidosis of mixed respiratory/'metabolic' origin. Correction of the respiratory component was rapid and led to a predominantly non-respiratory acidosis. Initially excess acid was buffered by the ECF. ECF acid-base status was restored primarily by ICF buffering and/or metabolism, rather than by excretion of excess acid.

Infusions of HCl produced the most severe blood acid-base disturbances. While large bullfrogs (400 g) infused with 3,000 μequiv/kg of 0.1 N or 1.0 N-HCl slowly (by 24 h post-infusion) corrected blood acid-base status, small bullfrogs (120 g) never recovered normal acid-base status. The persistent cuteneous net base efflux (acid uptake), concurrent with renal failure, dictated that restoration of blood

acid-base status occurred primarily by means of intracellular buffering. Thus it is proposed that development of a sustained intracellular acidosis may have been the proximate cause of death in these animals.

While NH_4Cl infusions in bullfrogs produced a marked pH depression, blood acid-base status was most rapidly corrected in these animals. Rapid stimulation of cutaneous and renal ammonia excretion appears to have been the primary mechanism responsible.

Lactacidosis in bullfrogs, induced by 45 min forced submergence, resulted in a pronounced metabolic acidosis which was fully corrected within 12 h. Excretion of excess acid was minimal under these circumstances despite the large ($\sim$5.3 mmol/kg) estimated acid load.

In addition to the initial ECF buffering following all treatments there were indications of non-ECF buffering of the acid load. These included mobilization of buffers from the soft tissues and possible utilization of $CaCO_3$ stores.

Despite the limited ability of the bullfrog to acid-base regulate, the present study demonstrates that the kidney may be the major organ (excluding a potential role by the urinary bladder) of acid-base regulation in anuran amphibians: the renal response is rapid, more complete and appears to be under better control than that of the skin. Nevertheless the bullfrog kidney, in comparison to the kidney of the freshwater teleost and particularly that of mammals, appears to be less capable of urinary acidification and concurrent excretion of titratable buffer acid under conditions of exogenously produced (i.e. HCl and NH_4Cl infusion) blood acidosis. Although there appeared to be a marked improvement in the bullfrog kidneys' potential

for acid-base regulation following forced submergence, the actual acid excretion was minimal.

In contrast to the fish gill, the bullfrog skin appears to be incapable of net acid excretion, although, it should be noted that the apparent large increase in cutaneous permeability may have masked the occurrence of cutaneous acid-base regulation. As a result, renal contributions to acid clearance were usually counteracted to various degrees by the persistent "base" losses ("acid" uptake) at the skin.

Thus the question arises as to why the bullfrog skin and kidneys are not at least as competent at acid-base regulation as the gills and kidneys of the freshwater teleost. While it is evident that the bullfrog skin and kidneys have a limited capacity for acid-base regulation, the results demonstrate that the bullfrog is nonetheless very tolerant of, and able to recover quite adequately from, large and prolonged disturbances of acid-base and ion balance.

It is interesting to speculate on the physiological significance of this considerable tolerance. Anuran amphibians exist in the transition zone between dilute aquatic and terrestrial habitats and thus face periodic challenges to their water balance along with changes in modes of breathing. This affects the relative ease with which they can regulate their ionic state (or need to) and excrete toxic body wastes such as ammonia. It can thus be argued that they must possess physiologically 'flexible' mechanisms for respiration, osmoregulation, and even acid-base regulation to meet the challenges to their diverse environment. The development of precisely regulated control mechanisms for operation in either water or on land would presumably occur at

the expense of adequate function in the other. Therefore it appears that bullfrogs, and perhaps anuran amphibians in general, have evolved the strategy of being very tolerant to environmental change, this being the most appropriate solution to life in a semi-aquatic environment.

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ADDENDUM

Anaerobiosis induced by strenuous exercise and prolonged apneoa in vertebrates results in an accumulation of lactate ion and H^+ within the muscle cells (Hutchison and Turney, 1975; Putnam, 1979; Hultman and Sahlin, 1980; Hutchison *et al.*, 1981; Quinn and Burggren, 1983; Turner *et al.*, 1983). These end products result primarily from the incomplete oxidation of stored glycogen by the Embden-Meyerhof pathway of glycolysis (Lehninger, 1975; Hochachka and Mommsen, 1983). This buildup of lactate and H^+ within muscle is inhibitory to normal cellular function at high concentrations and is believed to be a major factor affecting muscular activity (Hultman and Sahlin, 1980; Quinn and Burggren, 1983). Excessive intracellular acidification is prevented by the presence of imidazale-based buffers in vertebrate muscles (Davey, 1980; Hochachka and Mommsen, 1983). Furthermore, there also appears to be a close relationship between the degree of intramuscular buffering and the catalytic potential of M_4 lactate dehydrogenase isozyme in a variety of vertebrates (Castellini and Somero, 1981). Thus in the exercised bullfrog (Quinn and Burggren, 1983), elevated blood and muscle lactate levels were corrected primarily by increased conversion of lactate to pyruvate by lactate dehydrogenase in the muscles; the ultimate fate of lactate was probably the reconversion to glycogen by gluconeogenesis within muscles, kidneys and liver (Quinn and Burggren, 1983) and oxidation of pyruvate.

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