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ACID–BASE AND IONIC REGULATION IN THE AMERICAN EEL (*ANGUILLA ROSTRATA*) DURING AND AFTER PROLONGED AERIAL EXPOSURE: BRANCHIAL AND RENAL ADJUSTMENTS

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SUMMARY

The involvement of the gill and kidney in acid–base regulation was examined in the American eel, *Anguilla rostrata*, during 36 h of continuous air-exposure and subsequent return to water. While in air, eels developed a severe mixed respiratory/-metabolic acidosis. Renal acid excretion increased only slightly during the latter stages of air-exposure. A pronounced reduction in urine flow rate was important to minimize dehydration but essentially eliminated the kidney as a route for excess acid excretion. Upon return to the water, eels had accrued an extracellular metabolic acid load of 9·53 mmol l^{-1}. The metabolic acid was cleared from the extracellular compartment at an exceptionally low rate (approximately 70 μmol kg^{-1} h^{-1}) and about 50 % of the acid load remained after 18 h of recovery in water. The clearance of metabolic acid was accounted for by enhanced branchial acid excretion which was related primarily to adjustments of unidirectional Na^+ fluxes. Unidirectional Cl^- fluxes were undetectable using radiotracer methods. We speculate that the inefficiency of acid–base regulation in the eel compared to other teleosts is, in part, related to the absence of significant branchial Cl^-/HCO_3^- exchange.

INTRODUCTION

An interest in the physiological consequences of aerial exposure in eels has developed because of their habit of making routine excursions onto land to migrate between ponds. Unlike true amphibious air-breathing fishes (e.g. *Synbranchus marmoratus*), however, the eel is not particularly well-adapted for aerial gas transfer. Consequently, during prolonged air-exposure, the eel sustains a severe extracellular acidosis of respiratory and metabolic origin (Berg & Steen, 1965; Hyde, Moon & 1987) that is compensated gradually upon return to water (Hyde *et al.* 1987). The two major sites of acid excretion in fishes are the gill and kidney (see

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review by Heisler, 1984). During air-exposure, the gill is eliminated as a potential site of acid–base regulation and the importance of the kidney is questionable as there is the possibility of reduced urine flow to counteract dehydration. Upon return to water, both locations are available for transfer of excess acidic equivalents into the external medium. There is reason to suspect, however, that the eel has a limited capacity to modify branchial acid excretion compared to other fishes because of the extremely low rates of Cl^-/HCO_3^- exchange (Kirsch, 1972; Bornancin, De Renzis & Maetz, 1977). There is ample evidence to suggest that modulation of this pathway is the prevalent mechanism for regulating internal acidosis in fish (Wood, Wheatly & Hõbe, 1984; Wood & Perry, 1985; Claiborne & Heisler, 1986; Perry, Malone & Ewing, 1987). Thus, prolonged air-exposure followed by immediate return to water presents a unique opportunity to study the partitioning of acid–base regulation between the gill and kidney as a function of water availability, and to examine the effectiveness of the gill in acid excretion in the apparent absence of significant Cl^-/HCO_3^- exchange.

In the present study, we assessed the branchial and renal adjustments in the American eel (*Anguilla rostrata*) during and following 36 h of continuous air-exposure. The results demonstrate that the eel possesses a severely limited ability for acid–base regulation whether in air or water.

MATERIALS AND METHODS

Experimental animals

Immature American eels (*Anguilla rostrata*) weighing between 50·5 and 156·5 g [mean mass = 84·3 ± 13·8 g (±S.E.); $N = 90$] were obtained in August 1985 from an eel ladder associated with the Saunder's hydroelectric dam (Ontario Hydro) in Cornwall, Ontario. Eels were transferred on ice to the University of Ottawa where they were kept unfed in large aquaria supplied with aerated, dechlorinated City of Ottawa tap water ($[Na^+]$, 0·10 mmol l^{-1}; $[Cl^-]$, 0·10 mmol l^{-1}; $[Ca^{2+}]$, 0·35–0·40 mmol l^{-1}; $[K^+]$, 0·03 mmol l^{-1}; pH 7·5–8·0). Water temperature ranged between 4 and 9°C during the course of the experiments and both control and experimental fish were subjected to the same temperature variations. Eels were kept on a 12 h:12 h light:dark photoperiod and allowed to acclimate to laboratory conditions for at least 2 weeks prior to experimentation.

Surgical procedures

Eels were anaesthetized in a solution of ethyl-*m*-aminobenzoate (2 g l^{-1}; MS 222, Sigma) adjusted to pH 7·5–8·0 with Tris buffer (Trizma Base, Sigma). To permit periodic blood sampling, indwelling cannulae were implanted in the caudal artery. An incision was made approximately 10 cm caudal to the anus and slightly below the lateral line. The haemal arch was then exposed and polyethylene tubing (Clay Adam PE 50; i.d. = 0·58 mm; o.d. = 0·96 mm) was inserted into the caudal artery after

puncturing the vessel wall with a 26 gauge hypodermic needle. The wound was sutured and the cannula secured to the dorsal fin.

To allow continuous urine collection, urinary bladder catheters were implanted into eels lacking caudal artery cannulae. A polyethylene tube (Clay Adams PE 60; i.d. = 0·76 mm; o.d. = 1·22 mm) was inserted through the urinary papilla and into the urinary bladder a distance of approximately 1 cm. To prevent the occurrence of kinks, a more flexible length of tubing (Tygon) was attached to the catheter and sutured to the anal fin.

Following surgery, eels were transferred to an experimental holding box where they were allowed to recover for at least 48 h before experimentation commenced. Two different types of holding boxes were employed depending on the nature of the experiment. In the experiments requiring blood sampling, the holding box consisted of an opaque plastic tube (4 cm internal diameter) perforated with small holes (0·75 cm diameter; density 15 dm^{-2}) that permitted adequate water or air convection but prevented excessive movement of the eels. Additionally, the tube was continuously flushed with flowing water except during periods of air-exposure. The tube was stoppered at both ends and suspended in an opaque acrylic chamber (volume 6 l) supplied with flowing water. The holding chamber was aerated vigorously with compressed air to enhance air/water convection. The caudal artery cannula was secured outside the holding box to allow easy access and was flushed at least once daily with freshwater teleost saline (Wolf, 1963) containing 10 units ml^{-1} of heparin (ammonium salt, Sigma). Exposure to air was achieved by draining the water from the holding chamber to a level below that of the suspended tube. The remaining water served to humidify the air surrounding the eel and ensured that the skin remained moist. In the experiments requiring urine collection and branchial ion flux measurements, the holding box was an opaque acrylic chamber (volume 6 l) equipped with a false bottom that was perforated with small holes. Air-exposure was accomplished by draining the water to a level approximately 1 cm below the false bottom. Vigorous aeration of the remaining water in the flux box (about 1 l) humidified the air surrounding the eel and allowed sufficient air convection. Continuous urine collection for the duration of the experiment was achieved by allowing the urinary catheters to drain, by gravity, into plastic collection vials below the holding boxes.

Protocol

Eels were exposed to air for 36 h and then subsequently returned to water for an 18 h period of recovery. To conserve blood, separate groups of fish were used for blood acid–base measurements and blood solute determinations. Blood samples (0·7 ml) were withdrawn from the caudal artery before air-exposure, periodically during air-exposure and after return to water. Control eels were kept in water for 54 h.

Blood samples were either analysed immediately for pH and total carbon dioxide content (C_{CO_2}) or centrifuged and the plasma stored (−70 °C) for subsequent analysis

of plasma solutes [Na^+, K^+, Ca^{2+}, Cl^-, ammonium and inorganic phosphate (Pi)] and osmolality.

Branchial solute fluxes

Branchial unidirectional Na^+ and Cl^- fluxes were determined on separate groups of bladder-catheterized fish by monitoring the disappearance of ^{22}Na (as NaCl; Amersham) or ^{36}Cl (as HCl; ICN) in a 6-l holding box (see above). Approximately 1·0 μCi of ^{22}Na or 1·5 μCi of ^{36}Cl was added to each box and allowed to mix for 15 min. An initial water sample (20 ml) was removed following the mixing period and another after 6 h. Activity of ^{22}Na or ^{36}Cl was determined immediately on 5·0-ml samples while the remaining water was frozen and stored (−20°C) for subsequent ionic analysis (Na^+, Cl^-, K^+, NH_4^+). Time periods chosen for the branchial flux determinations were 0–6 h before air-exposure, and 0–6 h and 6–12 h following 36 h of continuous air-exposure.

Branchial net acid fluxes were determined according to the method of McDonald & Wood (1981) from measurements of titratable alkalinity and ammonia concentrations (see below) on initial and final water samples. The method does not discern between the excretion of acid and uptake of base but this is inconsequential with respect to acid–base regulation.

Renal solute fluxes

Urine was collected continuously in plastic vials for 6- or 12-h periods before air-exposure (0–6, 6–12 h), during air-exposure (0–12, 12–24, 24–36 h) and after air-exposure (0–6, 6–12 h). Evaporation was minimized by covering the plastic collection vials with parafilm. Urine samples were analysed immediately following collection for pH and volume. 100 μl of urine was diluted 20 times, acidified (1 % v/v) with 1 mol l^{-1} nitric acid and left frozen at −20°C until further assays could be performed. Samples then were thawed and total ammonia, Pi, Na^+, Cl^-, K^+ and Ca^{2+} concentrations were determined. The remaining urine was stored at −70°C for later determination of osmolality.

Urine from a separate group of fish ($N = 16$) was used immediately following collection to determine total urinary acid excretion. Total urinary acid excretion was calculated as the sum of the titratable component ($TA - HCO_3^-$) and the non-titratable component (total ammonia) multiplied by the urine flow rate (UFR).

Blood/water/urine analysis

Blood pH was determined with a microcapillary pH electrode (Radiometer G299A) maintained at ambient water temperatures in conjunction with a Radiometer PHM-71 acid–base analyser and BMS3-MK2 blood microsystem. C_{CO_2} of the blood was determined on 100-μl samples using a carbon dioxide analyser (Corning Model 905). Blood P_{CO_2} and bicarbonate concentration ($[HCO_3^-]$) were calculated from the measured C_{CO_2} and pH values according to the Henderson–Hasselbalch equation. Values for the appropriate dissociation constants of carbonic acid and the solubility coefficients of CO_2 at various temperatures and pH values were obtained from

Boutilier, Heming & Iwama (1984). Metabolic acid load, in mmol l^{-1}, was calculated according to the following equation:

$$\text{Metabolic acid load} = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2), \quad (1)$$

where β is the *in vitro* non-carbonic acid buffer value of eel blood at a haematocrit of 20 % ($-10{\cdot}12$ mmol l^{-1} whole blood; from Hyde *et al.* 1987).

Plasma and urine osmolalities were determined using a Wescor 5100C vapour pressure osmometer recalibrated with 100 mmol kg^{-1} standard to compensate for low urine osmolality. Plasma, urine and water concentrations of Na^+, K^+ and Ca^{2+} were determined by flame emission spectrophotometry (Varian model Spectra AA-10). Cl^- levels were determined by amperometric titration (Buchler–Cotlove Chloridometer). [Pi] was determined spectrophotometrically using a commercial assay kit (Sigma), and total ammonia was measured by a micromodification of the salicylate–hypochlorite reaction (Verdouw, van Echteld & Dekkers, 1968). Pi and ammonia determinations were performed on plasma samples deproteinized with 12 % trichloracetic acid.

^{22}Na and ^{36}Cl activities were determined on 5 ml water samples by liquid scintillation counting (LKB 1215 Rackbeta). Branchial net fluxes (J_{net}) for these electrolytes and unidirectional fluxes (i.e. J_{in}, J_{out}) for Na^+ and Cl^- were determined according to Maetz (1956). Accordingly, influxes (J_{in}) and net gains by the animal have positive signs while effluxes (J_{out}) and net losses have negative signs. Titratable alkalinity was determined on 10-ml water samples by titrating to pH 4·00 with 0·02 mol l^{-1} HCl, as described by McDonald & Wood (1981). The net branchial acid flux ($J_{net}^{H^+}$) was calculated as the sum of the titratable acidity flux (J^{TA}; i.e. the negative of the titratable alkalinity) and ammonia flux (J^{amm}).

Urinary [$TA-HCO_3^-$] was measured by adding a known volume of 0·02 mol l^{-1} HCl to 200 μl of urine to lower the pH below 5·0, then vigorously aerating and agitating the sample for 15 min to remove CO_2. NaOH (0·02 mol l^{-1}) was then gradually added using a microburette (Gilmont) to restore urine pH to the blood pH representative of the particular sampling period. The difference between the quantities of acid and base added to the urine yielded the titratable component of urinary acid excretion.

Statistical analysis

Data shown in figures and tables are means ± 1 S.E. Where appropriate, a paired or unpaired Student's *t*-test was used to compare sample means and 5 % was taken as the fiducial limit of significance.

RESULTS

Blood acid–base status and plasma solutes

Air-exposure caused extracellular acidosis, the severity of which increased as the duration of air-exposure increased (Table 1). After 36 h, whole blood pH was reduced by approximately 0·6 pH units. The acidosis was composed of both

Table 1. *The effects of prolonged air-exposure on blood acid–base status in the American eel,* Anguilla rostrata

Time (h)	N	pH	P_{CO_2} (mmHg)	$[HCO_3^-]$ (mmol l^{-1})	Metabolic acid load (mmol l^{-1})
0	4	8·15 ± 0·04	2·68 ± 0·29	11·38 ± 1·30	0
Air-exposure					
1	5	8·08 ± 0·05	3·08 ± 0·76	10·54 ± 1·57	1·55
2	5	7·99 ± 0·06*	3·87 ± 1·08	9·88 ± 1·77	3·14
4	7	8·04 ± 0·06*	3·26 ± 0·89	9·86 ± 1·55	2·68
6	7	7·89 ± 0·05*	5·06 ± 1·07*	11·10 ± 1·04	1·92
12	7	7·88 ± 0·07*	5·27 ± 1·12*	10·70 ± 0·87	3·44
24	7	7·63 ± 0·10*	6·71 ± 1·32*	9·34 ± 1·24	7·34
36	7	7·51 ± 0·13*	8·26 ± 1·58*	8·35 ± 0·89*	9·53
Post air-exposure					
1	5	7·72 ± 0·14*	3·17 ± 0·41	6·75 ± 0·90*	8·94
18	4	7·92 ± 0·05	2·67 ± 0·35	8·48 ± 1·04*	5·22

Means ± 1 S.E.
N refers to the sample size at the indicated time. * indicates significant difference from the pre-exposure value.
1 mmHg = 133·3 Pa.

respiratory and metabolic components, as indicated by the elevation of arterial P_{CO_2} and the substantial metabolic acid load. Upon return to water, arterial P_{CO_2} was restored rapidly to initial levels thereby eliminating the respiratory portion of the acidosis. However, blood pH remained depressed (although this was not statistically significant) after 18 h of recovery in water due to the persistent metabolic acid load. The data summarized in Table 1 are presented elsewhere (Hyde *et al.* 1987) as a pH–HCO_3^- diagram.

Plasma osmolality increased markedly throughout the air-exposure period (Fig. 1A) suggesting significant dehydration. Osmolality was restored rapidly to initial values upon re-immersion in water. Ammonia and Pi concentrations in the plasma changed in a similar manner to osmolality during and following air-exposure (Fig. 1B,C). The effects of air-exposure on the major plasma electrolytes are illustrated in Fig. 2. A gradual elevation of $[K^+]$ occurred in the absence of haemolysis, which was reversed following air-exposure during the 12 h period of recovery in water (Fig. 2C). However, plasma levels of Na^+, Cl^- and Ca^{2+} did not parallel the changes in plasma osmolality, although $[Na^+]$ tended to increase during air-exposure. Although plasma $[Cl^-]$ actually decreased while eels were in air, this observation may not be significant because $[Cl^-]$ remained low even after return to water (Fig. 2B). Plasma $[Ca^{2+}]$ was stable throughout the experiment.

Branchial adjustments

Using standard radiotracer methods, it was not possible to detect branchial influx of Cl^- in either control or experimental animals over 6-h measuring periods. For this

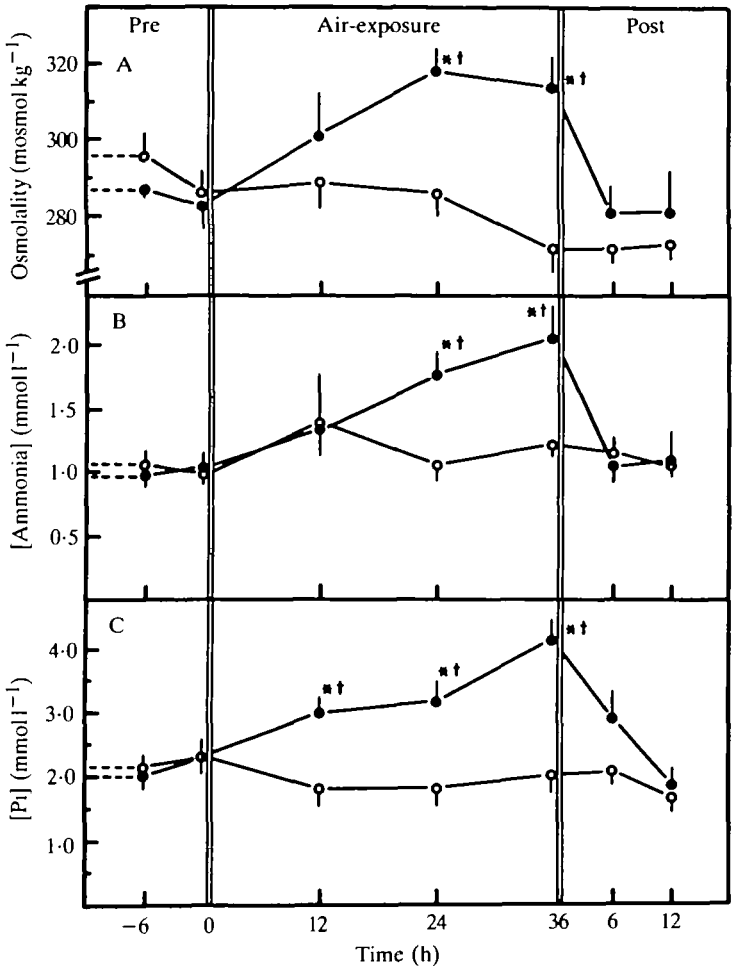

Fig. 1. The effects of prolonged air-exposure (●——●, $N = 7$) on selected plasma variables in the American eel. (A) Osmolality, (B) ammonia concentrations and (C) inorganic phosphate concentrations. Control eels (○——○, $N = 7$) were left in water for the entire experiment (54 h). * represents significant difference from the control value at the corresponding time; † represents significant difference from pre-exposure values.

reason, net Cl^- fluxes rather than unidirectional Cl^- fluxes have been presented in Fig. 3.

As a consequence of stimulated $J_{in}^{Na^+}$ and to a lesser extent reduced $J_{out}^{Na^+}$ (not significantly different at 0–6 h post air-exposure), $J_{net}^{Na^+}$ was significantly elevated immediately upon returning the eels to water (Fig. 3A). At 6–12 h post air-exposure, $J_{net}^{Na^+}$ was not significantly different from the pre-air-exposed values, although it remained significantly elevated above the control eels. In contrast, $J_{net}^{Cl^-}$ was reduced (i.e. more negative) to the same extent in both control and experimental animals, presumably as a result of increased J_{out}^{Cl} since J_{in}^{Cl} was undetectable.

Due to the differential adjustments of branchial net fluxes of Na^+, K^+ and Cl^- (Fig. 3), a significant pattern emerged with respect to the difference between cation and anion net fluxes ($J_{net}^{Na^+} + J_{net}^{K^+} - J_{net}^{Cl^-}$, Fig. 3D). $J_{net}^{Na^+} + J_{net}^{K^+} - J_{net}^{Cl^-}$ was elevated during the initial 6 h following air-exposure and remained elevated (although not significantly different from initial values) during the 6- to 12-h flux period. Based on empirical evidence (Wood *et al.* 1984; Wood, Boutilier & Randall, 1986; Vermette & Perry, 1987), the magnitude of $J_{net}^{Na^+} + J_{net}^{K^+} - J_{net}^{Cl^-}$ is the primary factor influencing branchial net acid excretion ($J_{net}^{H^+}$). Consequently, one would predict an increase in branchial $J_{net}^{H^+}$ following air-exposure. Indeed, branchial $J_{net}^{H^+}$ did increase following air-exposure (Fig. 4) and was similar in magnitude to the increase in $J_{net}^{Na^+} + J_{net}^{K^+} - J_{net}^{Cl}$ (approximately 100 μmol kg^{-1} h^{-1} of additional $J_{net}^{H^+}$). The rise in $J_{net}^{H^+}$ was

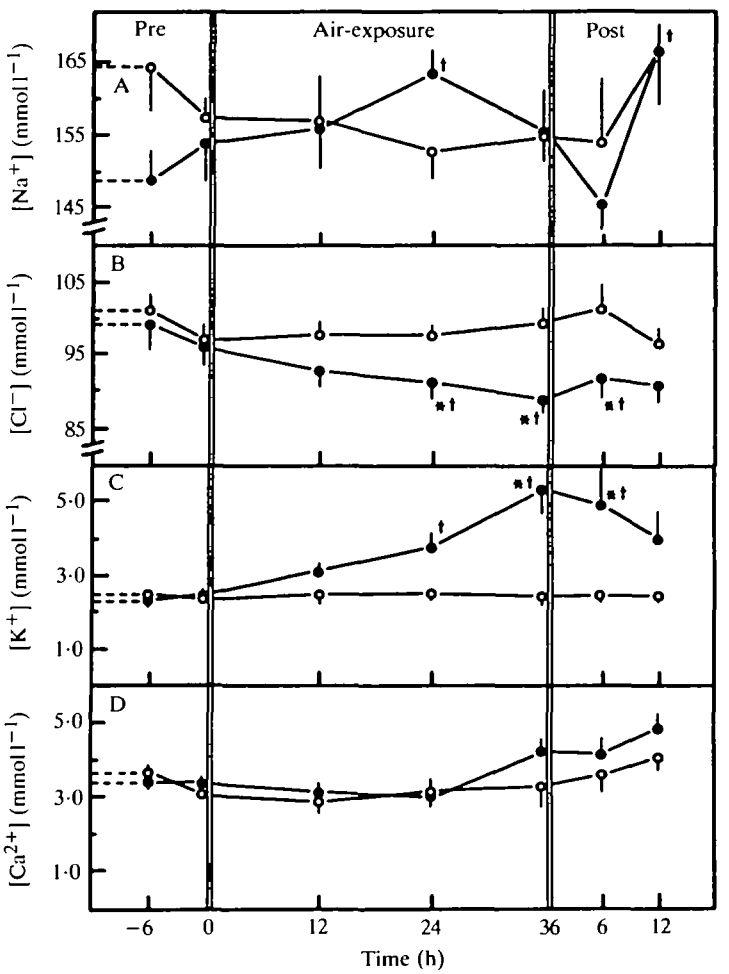

Fig. 2. The effects of prolonged air-exposure (●——●) on the concentrations of selected plasma ions. (A) Na^+, (B) Cl^-, (C) K^+ and (D) Ca^{2+}. * represents significant difference from the control value (○——○) at the corresponding time; † represents significant difference from pre-exposure values. Other details as in Fig. 1.

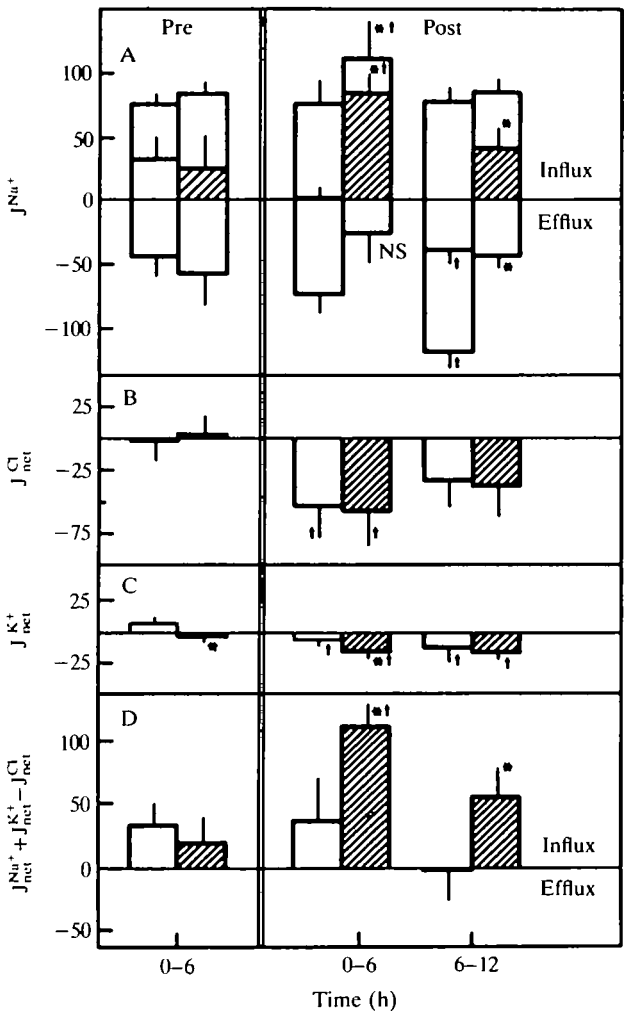

Fig. 3. The effects of prolonged air-exposure in the American eel on branchial solute fluxes. (A) Na^+ influx ($J_{in}^{Na^+}$, positive values), Na^+ efflux ($J_{out}^{Na^+}$, negative values) and Na^+ net flux ($J_{net}^{Na^+}$, cross-hatched bars; (B) Cl^- net flux (J_{net}^{Cl}); (C) K^+ net flux ($J_{net}^{K^+}$); (D) the net strong ion difference flux ($J_{net}^{Na^+} + J_{net}^{K^+} - J_{net}^{Cl^-}$; J_{net}^{SID}). Flux studies were performed at 0–6 h before air-exposure (Pre) and at 0–6 and 6–12 h following air-exposure (Post). All fluxes are expressed as $\mu mol\,kg^{-1}\,h^{-1}$. N numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; † represents significant difference from pre-exposure values. NS, not significant.

attributable to ammonia excretion increasing to a greater extent than titratable acid uptake (Fig. 4A).

Renal adjustments

Urine flow rate (UFR) decreased during air-exposure and was associated with a pronounced increase in urine osmolality (Fig. 5). Consequently, concentrations of

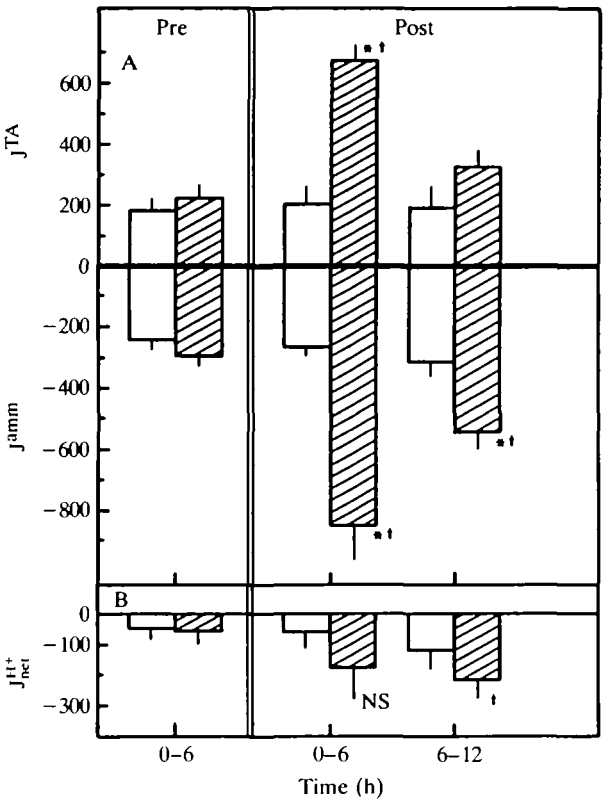

Fig. 4. Branchial excretion of acidic equivalents in the American eel before and after prolonged air-exposure. Shown are (A) the components of branchial acid excretion, titratable acidity flux (J^{TA}) and ammonia excretion (J^{amm}) expressed as μmol kg^{-1} h^{-1} and (B) the sum of these which represents net acid excretion ($J^{H^+}_{net}$). Flux rates are expressed as μmol kg^{-1} h^{-1}. All other details as in Fig. 3. NS, not significant.

the various urinary solutes (Na^+, Cl^-, K^+, Pi, NH_4^+), with the notable exception of Ca^{2+}, increased throughout the air-exposure period (Figs 6, 7). Urinary excretion rates of the various solutes, however, remained unchanged in air due to the depressed UFR (Figs 6, 8). Again, the exception was renal Ca^{2+} excretion, which actually decreased during air-exposure (Fig. 8D). Upon returning the eels to water, UFR was stimulated tremendously and osmolality was returned to control values. Urinary solute excretion rates were increased during the recovery phase in water due to the stimulation of UFR and/or elevated urinary solute concentrations (Figs 6, 8).

The involvement of the kidney in acid–base regulation during and following air-exposure in the American eel is illustrated in Fig. 9. The reduction in UFR during air-exposure precluded any substantial contribution of the kidney to acid–base regulation, although there was a statistically significant increase in urinary $J^{H^+}_{net}$ in the final 18 h of exposure. Renal $J^{H^+}_{net}$ was stimulated markedly during the first 6 h following return to water due to the combined effects of elevated UFR (Fig. 5), $J^{TA-HCO_3^-}$ (Fig. 9A) and J^{amm} (Fig. 9B).

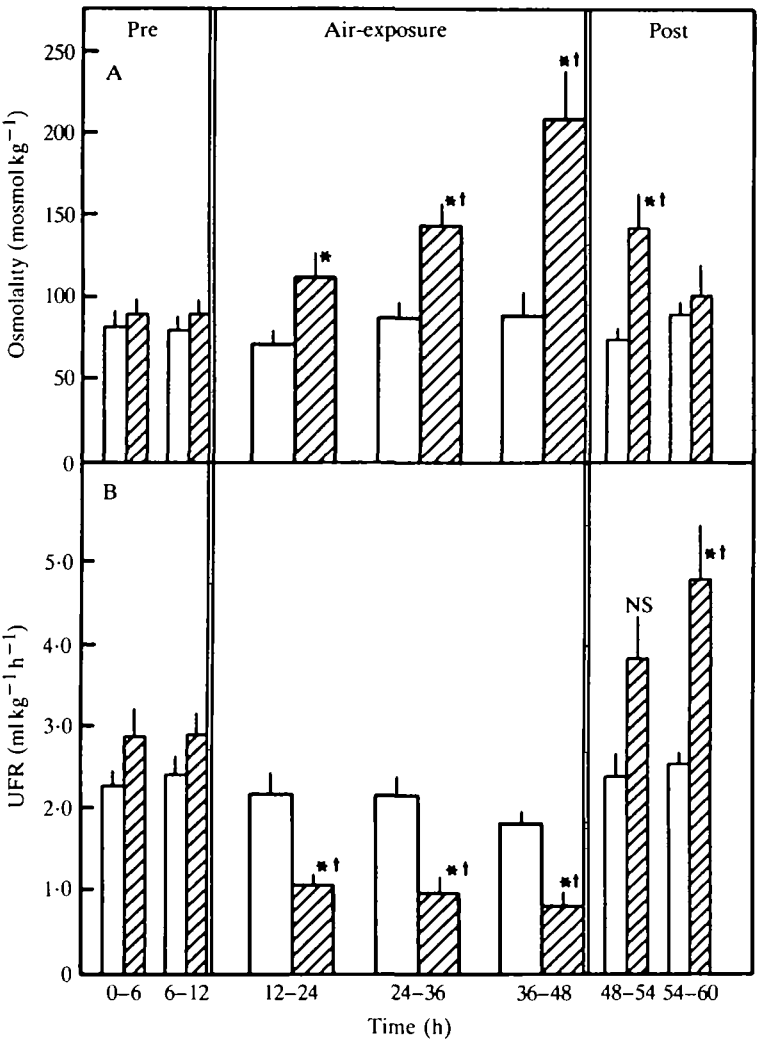

Fig. 5. The changes in (A) urine osmolality and (B) urine flow rate (UFR) associated with prolonged air-exposure in the American eel. Collection periods were increased to 12 h during air-exposure. *N* numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; † represents difference from pre-exposure values. NS, not significant.

DISCUSSION

Blood acid–base status

The results of the present study demonstrate that the eel, whether in air or water, possesses a severely limited capacity for acid–base regulation. The origin of the extracellular acidosis accompanying air-exposure is discussed in detail elsewhere (Hyde *et al.* 1987). Briefly, blood pH is reduced because of retention of respiratory CO_2 and increased anaerobic metabolism. As such, the acid–base disturbance

associated with prolonged air-exposure is similar to the mixed extracellular respiratory/metabolic acidosis frequently observed in fish after exhausting exercise (see review by Wood & Perry, 1985). Indeed, the accumulation of metabolic acid (base deficit) in the blood after 36 h in air ($9{\cdot}53\ mmol\ l^{-1}$) is approximately equal to the extracellular fluid (ECF) metabolic acid load in a variety of fish species immediately post-exercise (*Scyliorhinus stellaris*, Piiper, Meyer & Drees, 1962; *Salmo gairdneri*, Milligan & Wood, 1986; *Katsuwonus pelamis*, Perry *et al.* 1985; *Raja ocellata*, Wood & Perry, 1985). Although the recovery from exhausting exercise is a distinctly different physiological process from the recovery after air-exposure, a comparison of

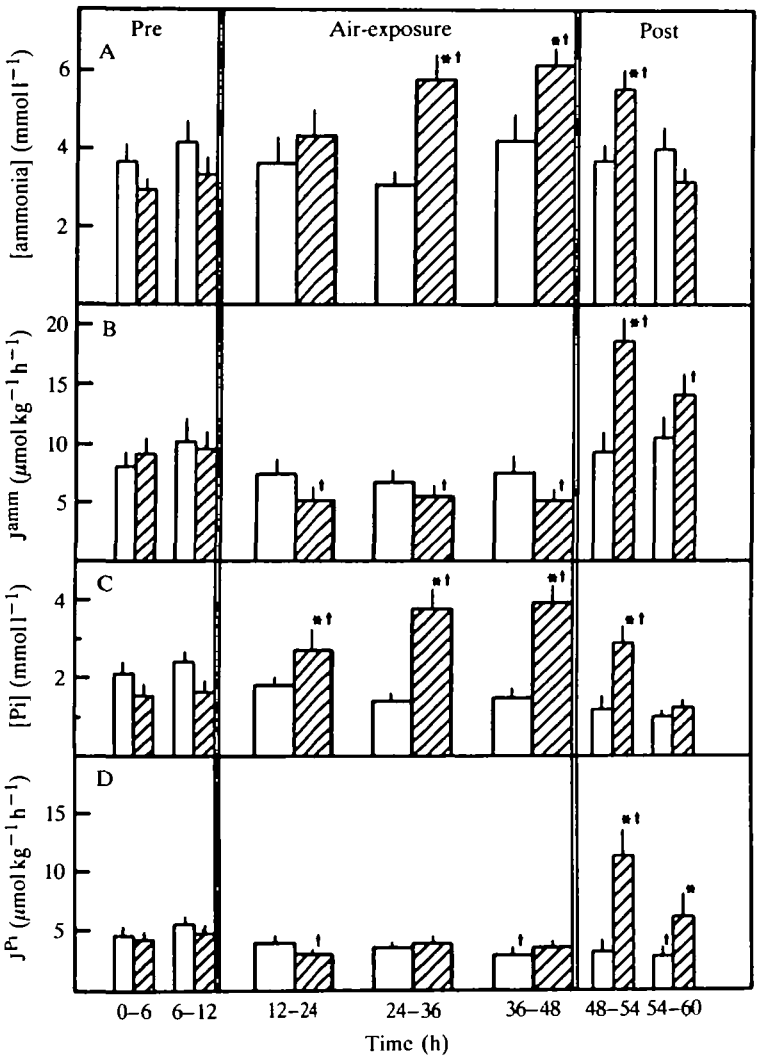

Fig. 6. The effects of prolonged air-exposure in the American eel on the urinary buffers, ammonia and inorganic phosphate (Pi). Represented are (A) ammonia concentrations, (B) ammonia excretion rate (J^{amm}), (C) inorganic phosphate concentrations [Pi] and (D) Pi excretion rate (J^{Pi}). All other details as in Fig. 5.

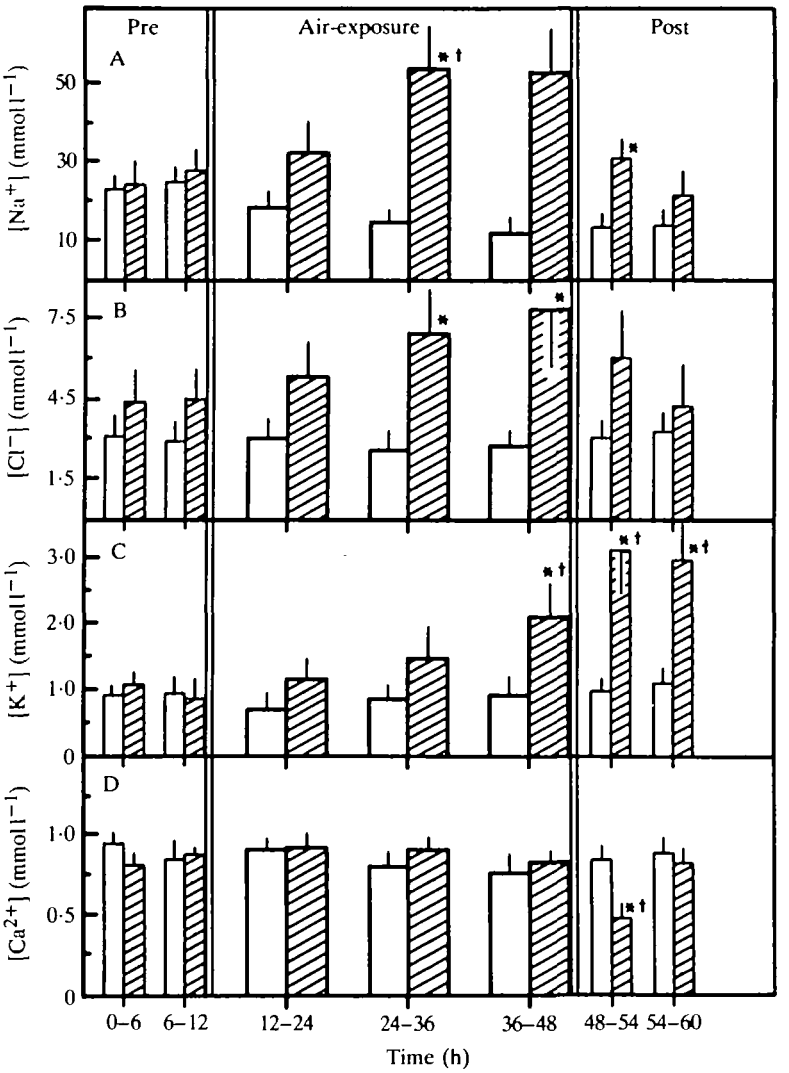

Fig. 7. The effects of prolonged air-exposure in the American eel on urine ion concentrations. (A) Na^+, (B) Cl^-, (C) K^+ and (D) Ca^{2+}. N numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; † represents significant difference from pre-exposure values.

our results with those obtained from exercising fish nonetheless does reveal the inefficiency of acid–base regulation in the eel. Faced with similar extracellular metabolic acid loads, fishes recovering from exhausting exercise are capable of restoring blood acid–base status usually within 12 h whereas the eel recovering from air-exposure manages to clear just 50 % of the ECF acid load after 18 h recovery in water. We speculate that the inefficiency of acid–base regulation in the eel compared with other fishes is related to fundamental differences in branchial ionic exchange

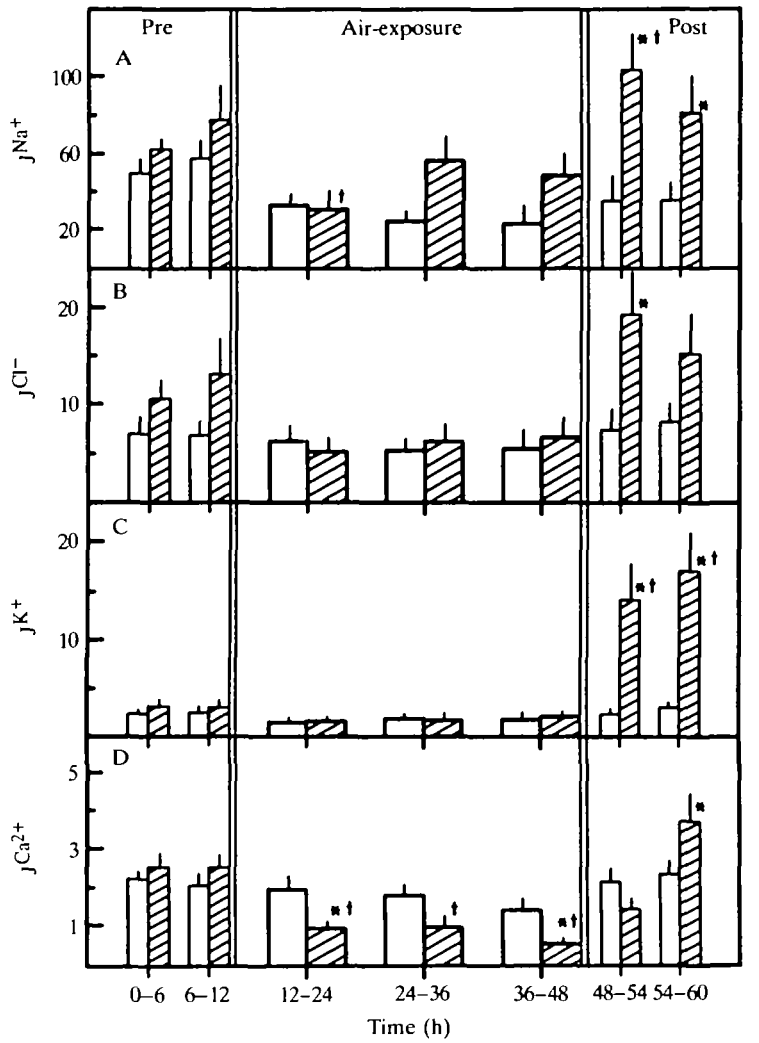

Fig. 8. The effects of prolonged air-exposure in the American eel on renal excretion rates (μmol kg^{-1} h^{-1}) of various ions. (A) Na^+ (J^{Na^+}), (B) Cl^- (J^{Cl^-}), (C) K^+ (J^{K^+}) and(D) Ca^{2+} ($J^{Ca^{2+}}$). N numbers are 16 for control fish (clear bars) and 15 for experimental fish (cross-hatched bars). * represents significant difference from the control value at the corresponding time; † represents significant difference from pre-exposure values.

mechanisms, although the possibility of slow metabolic H^+ removal leading to delayed recovery cannot be excluded.

Branchial adjustments

It is well established that branchial Cl^- influx in the eel is exceptionally low compared to other teleosts (1·2 μmol kg^{-1} h^{-1}; Kirsch, 1972; 3·6 μmol kg^{-1} h^{-1} Bornancin *et al.* 1977). Given the low rate of J_{in}^{Cl}, it is not surprising that we were

unable to detect unidirectional ^{36}Cl movements over 6-h flux periods using standard radiotracer methods [in the study of Kirsch (1972), 24 h was required to quantify $J_{in}^{Cl^-}$ accurately]. Because of the coupling of Cl^- uptake and HCO_3^- excretion in fishes (see reviews by Evans, 1984; Heisler, 1984), the low rate of $J_{in}^{Cl^-}$ in the eel is reflected by uniquely low plasma $[Cl^-]$ and relatively high $[HCO_3^-]$ and pH (e.g. Farrell & Lutz, 1975; Schmidt-Nielsen & Renfro, 1975; Bornancin *et al.* 1977; see also Table 1). Moreover, inhibition of Cl^-/HCO_3^- exchange and concomitant base retention during periods of internal acidosis is eliminated as an acid–base regulatory mechanism. Of course, it is the net flux of Cl^- rather than the unidirectional Cl^-

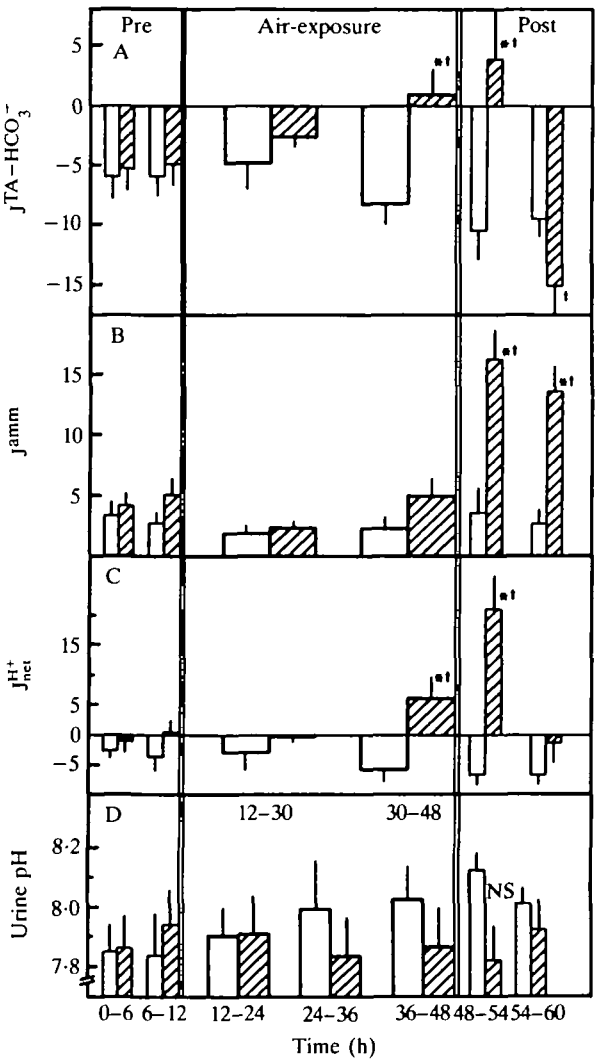

Fig. 9. The effects of prolonged air-exposure in the American eel on the renal efflux rates of (A) titratable acid minus bicarbonate ($J^{TA-HCO_3^-}$; measured as a single component), (B) ammonia (J^{amm}), (C) the net excretion of acidic equivalents ($J_{net}^{H^+}$) and (D) urine pH. Fluxes expressed as $\mu mol\,kg^{-1}\,h^{-1}$. All other details as in Fig. 5. NS, not significant.

fluxes that ultimately determines the contribution of Cl^- movements to the net transfer of acidic equivalents across the gill. Therefore, modulation of Cl^- efflux could potentially contribute to acid–base regulation. This prospect also appears to be eliminated in the eel because the passive efflux of Cl^- must be kept low to counteract partially the reduced Cl^- influx (Kirsch, 1972). Although the changes in Cl^- net flux reported here following air-exposure are consistent with the regulation of acidosis, we doubt their significance because the changes were relatively minor and control eels displayed a similar trend. The apparent inability of the eel to modulate branchial Cl^- movements is significant because the modification of J_{net}^{Cl} during and/or following a variety of acid–base disturbances has been reported to be the dominant mechanism of acid–base regulation in fishes (hyperoxic acidosis, Wood *et al.* 1984; following exhaustive exercise, Wood & Perry, 1985; hypercapnia, Claiborne & Heisler, 1986; Perry *et al.* 1987).

According to strong ion difference (SID) theory (Stewart, 1980) and experimental data (Wood *et al.* 1984, 1986; Vermette & Perry, 1987), branchial acid excretion is ultimately determined by the arithmetic difference between strong cation and strong anion net fluxes (net strong ion difference flux, J_{net}^{SID}). In the present study, we have estimated the J_{net}^{SID} as $J_{net}^{Na^+} + J_{net}^{K^+} - J_{net}^{Cl^-}$. The significant increase of J_{net}^{SID} observed after eels were returned to water is an appropriate response for compensating the acidosis accrued during air-exposure. The most significant response promoting the elevation of J_{net}^{SID} was stimulation of $J_{in}^{Na^+}$ in the initial 6 h following re-immersion in water. As expected, there was a reasonable correlation between the rate of the J_{net}^{SID} and the rate of branchial acid excretion. The absolute magnitude of the branchial net acid flux during recovery from air-exposure, albeit low compared to other fishes regulating equivalent acid loads, was sufficient to account for the slow decrease in the ECF metabolic acid load (approximately 70 μmol kg^{-1} h^{-1} over 18 h).

Renal adjustments

Under normal conditions, urine resides in the bladder for variable periods before being excreted. However, the bladder catheterization technique only enables the collection of continuously voided tubular urine. Thus, in the present study, we were unable to assess the contribution of the urinary bladder to acid–base or ionic regulation. Further problems associated with analysing urine collected continuously from gas-permeable urinary catheters include the inability to collect urine under anaerobic conditions, leading to CO_2 loss from the urine and pH fluctuations, and a considerable catheter dead space volume of approximately 0·35 ml resulting in catheter transit times which averaged 2·0 h in control fish.

Prolonged air-exposure was associated with ECF dehydration, as indicated by the pronounced rise in plasma osmolality, and was presumably equivalent to the volume of fluid excreted by the kidney and the amount lost by evaporation across the body surfaces. The increase in plasma osmolality (about 40 mosmol kg^{-1}) cannot be accounted for by elevated levels of the measured ions (Na^+, K^+, Cl^-, NH_4^+, Ca^{2+}), which suggests that there were considerable changes in some unmeasured osmolyte(s).

The kidney played a relatively minor role in acid–base regulation, both during air-exposure and during the recovery period in water. The primary adjustment of renal function while eels were in air was a marked reduction in UFR. This is clearly important for minimizing dehydration, but precludes a major role for the kidney in acid–base balance. Although UFR is reduced by the eel when in air, there is an obvious physiological limit in the capacity to concentrate the urine. Our results indicate that UFR in air-exposed eels approaches values reported for seawater-adapted eels (Schmidt-Nielsen & Renfro, 1975). We did not measure glomerular filtration rate (GFR) in the present study, thus the cause of the reduced UFR remains unclear. The pronounced increase in urine osmotic and ionic concentrations, however, suggests that increased tubular reabsorption of water or reduced tubular fluid secretion could be involved. Schmidt-Nielsen & Renfro (1975) reported that the reduced UFR in seawater-adapted eels was not due to adjustments of GFR but rather to changes in tubular fluid reabsorption and secretion.

Although renal acid excretion was significantly increased during the latter stages of air-exposure and the initial period of recovery, the magnitude of these changes was insufficient to alter substantially the ECF acid–base status during air-exposure or to assist in the recovery after air-exposure. Moreover, a significant component of the apparent increase in renal acid excretion after air-exposure must have been initiated during air-exposure but not measured during that period because of the reduced UFR and the catheter dead space volume. Hence, renal excretion of acidic equivalents accounted for approximately 6·5 % of the metabolic acid cleared after air-exposure, whereas branchial contributions accounted for 93·5 % of the acid cleared. The lack of involvement of the eel kidney in acid–base regulation is perhaps best illustrated by analysis of the urine between 6 and 12 h after air-exposure. During this period, a substantial ECF metabolic acid load existed, yet renal acid excretion was unaltered.

The reliance of other freshwater teleosts on the kidney for acid–base regulation is variable and is probably related to species differences and the nature of the acid–base disturbance. The simulation of metabolic acidosis by infusion of mineral acid in trout *Salmo gairdneri* (Wood & Caldwell, 1978) and catfish *Ictalurus punctatus* (Cameron & Kormanik, 1982) or external acidification in trout (McDonald & Wood, 1981) can stimulate renal acid-excreting mechanisms which can account for clearance of between 33 and 100 % of the accumulated acid load (33 %, Cameron & Kormanik, 1982; 50 %, McDonald & Wood, 1981; 100 %, Wood & Caldwell, 1978). Surprisingly, the injection of lactic acid into trout, which perhaps best mimics the elevation of ECF metabolic acid load in the eel at the conclusion of air-exposure, caused only minor changes in renal acid excretion and could account for only 6 % of total acid clearance (Kobayashi & Wood, 1980). Thus, the possibility that the eel kidney can respond effectively to other types of acid–base disturbances cannot be excluded.

In conclusion, the changes observed in J_{net}^{SID} during recovery from air-exposure were attributable primarily to modification of branchial Na^+/H^+ exchange. A correlation existed between the rate of J_{net}^{SID} and the rate of branchial acid excretion. Although the metabolic removal of acidic equivalents has been demonstrated in other

fishes, the increase in the rate of branchial net acid efflux was sufficient to account for the slow rate of recovery observed (70 μmol kg^{-1} h^{-1}). Net renal acid efflux was low (approximately 6·5% of total acid clearance) both during and after prolonged air-exposure. The predominant role of the kidney during air-exposure was to minimize dehydration. We suggest that the limited capacity for acid–base regulation displayed by the eel is, in part, a result of low rates of branchial Cl^-/HCO_3^- exchange.

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