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ACID–BASE REGULATION IN THE ATLANTIC HAGFISH *MYXINE GLUTINOSA*

BY D. G. McDONALD*, V. CAVDEK*, L. CALVERT* AND C. L. MILLIGAN†

Huntsman Marine Science Centre, St Andrews, New Brunswick, Canada

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Summary

Blood acid–base status and net transfers of acidic equivalents to the external environment were studied in hagfish, *Myxine glutinosa*, infused with ammonium sulphate (4 mequiv kg^{-1} NH_4^+) or with sulphuric acid (3 mequiv kg^{-1} H^+). Hagfish extracellular fluids (ECF) play a greater role in acid–base regulation than in teleosts. This is because hagfish have a much larger blood volume relative to teleosts, despite a relatively low blood buffering capacity. Consequently, infusion of ammonium sulphate produced only half of the acidosis produced in marine teleosts in comparable studies, and hagfish readily tolerated a threefold greater direct H^+ load. Furthermore, the H^+ load was largely retained and buffered in the extracellular space. Despite smaller acid–base disturbances, rates of net H^+ excretion to the external environment were, nonetheless, comparable to those of marine teleosts, and net acid excretion persisted until blood acid–base disturbances were corrected. We conclude that the gills of the hagfish are at least as competent for acid–base regulation as those of marine teleosts. The nature of the H^+ excretion mechanism is discussed.

Introduction

In 1984, Evans found evidence for Na^+/H^+ and Cl^-/HCO_3^- exchanges in the primitive osmoconforming hagfish *Myxine glutinosa* L., and was the first to suggest that acid–base regulation by the gills preceded osmoregulation in the evolution of the vertebrates. The evidence for these exchanges came from ion substitution experiments where transfer of hagfish to Na^+-free sea water sharply reduced net H^+ efflux while transfer to Cl^--free sea water reduced base efflux. Both effects were reversible. However, hagfish seemed to be unable to respond rapidly to an internal acidosis, for Evans (1984) found that branchial net H^+ fluxes failed to respond to hypercapnic exposure. More recently, histological and biochemical

*Present address: Department of Biology, McMaster University, 1280 Main St West, Hamilton, Ontario, Canada, L8S 4K1.

† Present address: Department of Zoology, University of Western Ontario, London, Ontario, Canada.

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studies (Mallatt *et al.* 1987; Bartels, 1988) have demonstrated the presence of mitochondria-rich cells in the gills (i.e. 'chloride cells') containing Na^+/K^+-ATPase and carbonic anhydrase, features consistent with a cell type specialized for ion/acid–base exchanges. However, the tissue has a relatively low microsomal ATPase activity, only half to one-third of that of marine teleosts (Mallatt *et al.* 1987).

These observations led us to question the competence of the gills of the hagfish for acid–base regulation. Our objective, therefore, was to establish whether the gills of the hagfish can, in fact, respond rapidly to an internal acid–base disturbance and, if so, whether the capacity of the gills for acid–base regulation is comparable to that of marine teleosts. To this end, we have purposely chosen experimental acid–base disturbances directly comparable to our previous work on freshwater and marine species; rainbow trout *Onchorhynchus mykiss* (McDonald and Prior, 1988), coho salmon *Onchorhychus kisutch* and starry flounder *Platichthys stellatus* (Milligan *et al.* 1991) and lemon sole *Parophyrs vetulus* (McDonald *et al.* 1982).

Experimental acid–base disturbances were produced by infusion with ammonium sulphate and with sulphuric acid. Ammonium sulphate produces an acid–base disturbance by virtue of a rapid clearance of NH_3 from plasma, leaving H_2SO_4 behind. The advantage of ammonium sulphate as a treatment is that it can be infused as a neutral solution and thus large loads are readily tolerated. Sulphuric acid is less well tolerated but its advantage is that its removal from the blood, either to the water or to the intracellular compartment, can be quantified more readily.

Materials and methods

Experimental animals

Atlantic hagfish (*Myxine glutinosa*, 29–85 g, $N=91$) were obtained from baited traps in Passamaquoddy Bay and transported to the Huntsman Marine Sciences Centre, St Andrews, NB. After transportation, the animals were allowed to acclimatize for up to 1 week in a large tank supplied with aerated sea water at 13 °C. Animals were not fed while in the holding facility and all fish were used within 2 weeks of capture.

Experimental protocol

Ammonium and acid infusions

Ammonium sulphate and sulphuric acid loads, radioactively labelled with $^{35}SO_4^{2-}$, were administered by injection, from the ventral surface, into the subcutaneous sinus at the midpoint of the animal. The whole procedure was accomplished within 30 s. Following injection, fish were returned to their holding

chambers and examined either for effects upon blood acid–base status or for net fluxes to the external environment of ammonia, H^+ and $^{35}SO_4^{2-}$. The ammonium sulphate load consisted of 0.6 ml 100 g^{-1} of a 0.33 mmol ml^{-1} solution of $(NH_4)_2{}^{35}SO_4$ neutralized to pH 7.5 (New England Nuclear; specific activity: 10 μCi $mmol^{-1}$), yielding a final load of 4 mequiv kg^{-1} NH_4^+. The sulphuric acid load consisted of 1.0 ml 100 g^{-1} of a 0.15 mmol ml^{-1} solution of $H_2{}^{35}SO_4$, pH<1.0 (specific activity: 20 μCi $mmol^{-1}$), yielding a final load of 3 mequiv kg^{-1} H^+.

For measurements of blood acid–base status, hagfish were held individually in darkened 10 l rectangular containers supplied with flowing aerated sea water at the experimental temperature. For blood collection, a method similar to that described by Germain and Gagnon (1968) was employed, although anaesthesia was found to be unnecessary. Animals were taken from the water and briefly suspended by the head, which caused blood to pool in the caudal subcutaneous sinus. The sinus was then penetrated with a 1.0 ml heparinized Hamilton gas-tight syringe. Volumes of up to 1 ml could routinely be withdrawn by this method, within 30 s and with little apparent disturbance to the animal. Nonetheless, to limit any trauma associated with this method of blood collection, each hagfish was sampled only once. Hagfish infused with ammonium or acid loads were sampled at 0.25, 0.5, 1, 2 or 6 h post-infusion (N=6 for each treatment at each time period). An additional 12 fish were sampled without any infusion to provide data for resting blood acid–base status.

For flux measurements, hagfish were transferred to individual cylindrical flux chambers (length 30 cm, internal diameter, 5 cm) supplied with an airline that provided aeration and mixing of chamber contents and, prior to the start of flux measurements, with flowing sea water at the experimental temperature. Animals were typically allowed to acclimate to the experimental chamber for at least 4 h prior to experimentation. For flux measurements, the flow to the chambers was stopped, about 100 ml was removed to adjust the chamber volume to 400 ml, and the chambers were bathed in flowing sea water to maintain the experimental temperature. On each animal a 1 h flux measurement was made prior to injection of the acid or ammonium load. Water samples (15 ml) were taken at the beginning and end of each flux period for analysis of ammonia, titratable alkalinity and $^{35}SO_4^{2-}$. Each flux period was preceded by a 10 min flush period, during which the boxes were flushed with fresh sea water and any mucus produced by the animal was removed. Mucus production typically occurred for only a brief period (<10 min) immediately following the injection of the loads.

Blood buffering capacity in vitro

Blood was collected from 12 fish (0.3–1.0 ml per fish, total volume=9.6 ml), pooled and then centrifuged. 3.5 ml of plasma was withdrawn and the remainder reconstituted to give haematocrits of 17, 24 and 33 %. Plasma and blood samples (1.5–2.7 ml) were placed in tonometer flasks and equilibrated with humidified CO_2 mixtures (0.25, 0.5, 1 or 2 %) in air. Each sample was allowed to equilibrate

for 0.5 h with each CO_2 mixture. Following equilibration, 150 μl samples were withdrawn for measurements of pH, total CO_2 and haemoglobin concentration.

Analytical techniques

Blood pH was measured on 40 μl samples injected into a Radiometer pH microelectrode (type G297/G7) maintained at experimental temperature and linked to a Radiometer PHM 72 acid–base analyzer. Total CO_2 (in mmol l^{-1}) was determined on 40–60 μl blood samples injected into a Corning 965 CO_2 analyzer. Haemoglobin was measured on 10 μl samples added to 2.5 ml of Drabkin's reagent (Sigma Chemical Co.). Haematocrit was determined on 50 μl of blood transferred to heparinized capillary tubes and centrifuged at 3000 *g* for 5 min. Plasma $^{35}SO_4^{2-}$ activity was determined on 50 μl samples mixed with 5 ml of water and 10 ml of fluor (ACS; Amersham) and counted on an LKB Rackbeta liquid scintillation counter. Plasma ammonia concentration was measured enzymatically using the glutamate dehydrogenase reaction (Kun and Kearney, 1971) with Sigma reagents.

Activity of $^{35}SO_4^{2-}$ in water was determined on 5 ml samples added to 10 ml of fluor and counted as described above. Water ammonia was measured with a micromodification of the salicylate–hypochlorite method (Verdouw *et al.* 1978). Titratable alkalinity was determined by titration of water samples (5 ml) to a fixed end point (pH 4.0) with 0.02 mol l^{-1} HCl, using methods detailed in McDonald and Wood (1981). The samples were aerated during titration to mix the contents and remove CO_2. As McDonald *et al.* (1982) point out, any mucus secreted by the fish can lead to overestimates of the apparent base loss. This is potentially a serious problem with hagfish as they produce copious amounts of a viscous mucus when handled or stressed. Fortunately, we found that after an initial secretion (almost always evoked by handling and/or injection) the hagfish did not again produce any mucus over the course of the experiment.

Calculations

Blood P_{CO_2} and $[HCO_3^-]$ were calculated from pH and total CO_2 measurements using the Henderson–Hasselbalch equation with αCO_2 and pK′ values calculated from equations given by Heisler (1984).

The quantity of fixed acid added to the blood, the mineral acid load, ΔH_m^+ in mequiv l^{-1}, was calculated from the equation of McDonald *et al.* (1980):

$$\Delta H_m^+ = [HCO_3^-]_i - [HCO_3^-]_f - \beta(pHi - pHf), \quad (1)$$

where 'i' and 'f' refer to plasma pH and whole-blood $[HCO_3^-]$ measured at the beginning and end of the sample interval. The bicarbonate buffer capacity, β in mequiv l^{-1} pH unit^{-1}, of hagfish blood was determined from the following equation:

$$\beta = -4.65 - 0.71[Hb], \quad r^2 = 0.873, \quad (2)$$

determined from the *in vitro* CO_2 titration of hagfish blood described above [Hb] is haemoglobin concentration. For the calculation of ΔH_m^+, a β value of

6.51 was used, calculated from the average [Hb] in hagfish of $2.64\,g\,dl^{-1}$ (see Table 1).

Total mineral acid load to the extracellular fluid (ECF) was calculated as:

$$\Delta H_m^+ ECF = \frac{BV \times \Delta H_m^+ WB + [(ECFV - PV)\Delta H_m^+ ISF]}{\text{Body mass}}, \quad (3)$$

where BV and PV are blood and plasma volumes of 0.187 and $0.164\,l\,kg^{-1}$, respectively (from Forster *et al.* 1989), ECFV is the extracellular fluid volume of $0.27\,l\,kg^{-1}$ (from Hardisty, 1979) and $\Delta H_m^+ WB$ (whole blood) is calculated from equation 1. To estimate the mineral acid load to the interstitial fluid ($\Delta H_m^+ ISF$) the β value of ISF was taken to be the same as that for separated plasma, $4.94\,mequiv\,l^{-1}\,pH\,unit^{-1}$ (Table 1) and interstitial [HCO_3^-] was assumed to be the same as that of whole blood.

Net fluxes of ammonia and titratable alkalinity were calculated from changes in their respective concentrations in water with time and were expressed as $\mu equiv\,kg^{-1}\,h^{-1}$, with negative values indicating net loss or efflux and positive values indicating net gain or influx. The rate of net acid–base transfer (J_{net}^{H}) was calculated from the difference between ammonia (J_{net}^{Amm}) and titratable alkalinity fluxes, signs considered. Excretion of $^{35}SO_4^{2-}$ was determined from the appearance of $^{35}SO_4^{2-}$ in the water. Plasma [$^{35}SO_4^{2-}$] was determined from plasma counts and the specific activity of the infusate (in $cts\,min^{-1}\,mmol^{-1}$).

Statistics

Means±1 S.E.M. are reported throughout. Within-group comparisons were analyzed by repeated-measures analysis of variance (ANOVA) ($P<0.05$). If significant, pairwise comparisons were made using Fisher's PLSD (protected least significant difference).

Results

Suitability of blood sampling procedure

In this study, blood samples were obtained by an acute procedure not usually considered adequate for measurements of blood acid–base status (Holeton *et al.* 1980). Consequently, it is necessary first to consider the validity of our blood sampling technique.

Routine acid–base status and haematology for *Myxine* are shown in Table 1 and, for comparative purposes, for the Pacific hagfish *Eptatretus cirrhatus* (Wells *et al.* 1986) and for a benthic marine teleost, the starry flounder *Platichthys stellatus* (Milligan and Wood, 1987). For both the latter, the blood acid–base status was determined on blood collected from indwelling catheters in well-rested fish. Despite the differences in sampling methodology, there is a close similarity in pH and [HCO_3^-] amongst the three groups. This tends to confirm that the sampling

Table 1. *Blood acid–base status and haematology in resting Atlantic hagfish (*Myxine glutinosa, *present study), Pacific hagish (*Eptatretus stellatus, *Wells* et al. *1986*, Wells and Forster, 1989†) and starry flounder (*Platichthys stellatus, *Milligan and Wood, 1987)*

	Atlantic hagfish	Pacific hagfish	Starry flounder
Temperature (°C)	13	16	12
pH	7.727±0.031 (12)	7.77±0.14 (3)*	7.824±0.054 (8)
$[HCO_3^-]$ (mequiv l^{-1})	8.3±0.6 (12)	–	7.81±0.96 (8)
P_{CO_2} (kPa)	0.45±0.04 (12)	0.16±0.05 (3)*	0.39±0.002 (8)
[Haemoglobin] (g dl^{-1})	2.64±0.2 (11)	3.04±0.13 (7)†	3.79±0.33 (8)
Haematocrit (%)	17.7±1.5 (11)	12.6±0.4 (7)†	14.9±1.75 (7)
Mean cell [Hb] (MCHC, g 100 ml^{-1} RBC)	11.9±0.8 (11)	24.2±0.1 (7)†	26.9±2.2 (7)
Buffer capacity, whole blood (mequiv l^{-1} pH $unit^{-1}$)	6.51	3.5*	8.17
Buffer capacity per gram Hb, (mequiv l^{-1} pH $unit^{-1}$ g^{-1} Hb)	0.71	–	1.46
[Plasma protein] (g dl^{-1})	2.76	2.38±0.4 (7)†	2.35±0.5
Buffer capacity, separated plasma (mequiv l^{-1} pH $unit^{-1}$)	−4.94	–	−3.04

In *Myxine*, blood was withdrawn by acute penetration of the caudal sinus. For the other two species, samples were drawn from indwelling catheters in the ventral aorta (*Eptatretus*) and caudal artery (flounder) from well-rested fish.

Buffer capacity of *Myxine* blood was calculated from the equation: $\beta = -4.65 - 0.71[Hb]$ (equation 2, Materials and methods).

MCHC was calculated from [Hb] and haematocrit.

Values are mean±S.E.M. (*N*).

protocol for hagfish in the present study had relatively little immediate disturbing effect upon blood acid–base status. Any longer-term effect was eliminated by sampling each animal only once.

Forster *et al.* (1989) describe another potential problem with blood sampling in hagfish, especially from the subcutaneous sinuses. These authors showed that sinus blood had a much lower haematocrit than central vessel blood (approx. 4.0% *vs* 13.5%) suggesting that the two compartments were slow to equilibrate with one another and that red blood cells (RBCs) are restricted from entering the subcutaneous sinuses. However, the haematocrits we report are, in fact, higher than that of central blood reported by Forster *et al.* 1989 (Table 1). This suggests that our sampling method, where blood was forced by gravity into the caudal sinus, was responsible for expressing plasma with RBCs into the sinus much more rapidly than it would normally exchange. Furthermore, the very rapid development of acid–base disturbances following the infusion of the H_2SO_4 load (see Fig. 2A) suggests that our subcutaneous injection rapidly circulated with central vascular fluids.

Ammonium sulphate infusion

Ammonium sulphate levels rose in the blood following infusion, but the levels fluctuated with time (Fig. 1E). The peak plasma ammonium concentration was 1.2 mequiv l^{-1} greater than the resting level and was reached at 0.5 h, whereas the peak plasma [$^{35}SO_4^{2-}$] was 2.9 mequiv l^{-1} and was reached at 1 h. The greater elevation of [$^{35}SO_4^{2-}$] than of [NH_4^+] reflects the more rapid removal of the latter from the extracellular compartment. The fluctuations suggest that ammonium sulphate was relatively slow to equilibrate in hagfish blood.

Although slow to equilibrate, ammonium sulphate still produced a typical 'mineral acidosis' characterized by a depression of pH and [HCO_3^-] (Fig. 1A,B). There was no significant change in P_{CO_2} until 6 h post-infusion (Fig. 1C). The acidosis (ΔH_m^+) developed slowly (Fig. 1D), did not reach a peak until 2 h post-infusion, and thereafter slowly abated until fully corrected at 6 h post-infusion.

Despite the fact that the acidosis developed slowly, the branchial response was immediate (see Fig. 3A) with a sevenfold increase in ammonium excretion and an immediate reversal of J_{net}^H, from a net H^+ uptake under resting conditions to a net H^+ excretion. The net acid excretion persisted for 6 h post-infusion but gradually declined. By 2 h the hagfish had cleared 39 % of the ammonium load and by 6 h, 61 % of the load. The sulphate load was cleared much more slowly, 7.1 % by 2 h and 11.2 % by 6 h.

Sulphuric acid infusion

The 1.5 mmol kg^{-1} H_2SO_4 load (3 mequiv kg^{-1} H^+) was tolerated well by hagfish and no mortalities occurred. With this load there was a significant titration of bicarbonate stores (Fig. 2B), compared to the $(NH_4)_2SO_4$ load, as indicated by the pronounced elevation in P_{CO_2} (Fig. 2C). P_{CO_2} increased by about fivefold and remained elevated for at least 2 h. Compared to ammonium sulphate infusion, the peak acidosis was about 50 % larger and developed much more rapidly (Fig. 2D *vs* Fig. 1D). Furthermore, the sulphate component appeared to distribute quite differently. While blood sulphate levels fluctuated as before (compare Fig. 2E to Fig. 1E), the peak [$^{35}SO_4^{2-}$] in blood was about three times higher, despite the fact that the $^{35}SO_4^{2-}$ load was lower (3 *vs* 4 mequiv kg^{-1}). The blood acidosis remained relatively stable for the first 2 h post-infusion at about 6.5 mequiv l^{-1}, but by 6 h had nearly disappeared (Fig. 2D).

Again, the branchial response to the acid load (3 mequiv kg^{-1} H^+) was immediate; an immediate reversal of J_{net}^H from net uptake to net excretion, which persisted relatively unchanged for the 6 h post-infusion period. By 2 h the fish had excreted 0.9±0.2 mequiv kg^{-1} or 30 % of the load, by 6 h the fish had excreted 2.1±0.7 mequiv kg^{-1} H^+ or 70 % of the load. In contrast, the $^{35}SO_4^{2-}$ clearance was quite low, only 1.4 % was cleared by 2 h [*vs* 7 % for $(NH_4)_2SO_4$] and only 4.4 % by 6 h.

As the amount of acid infused is known, it is possible to calculate the distribution of the load between extracellular and intracellular compartments.

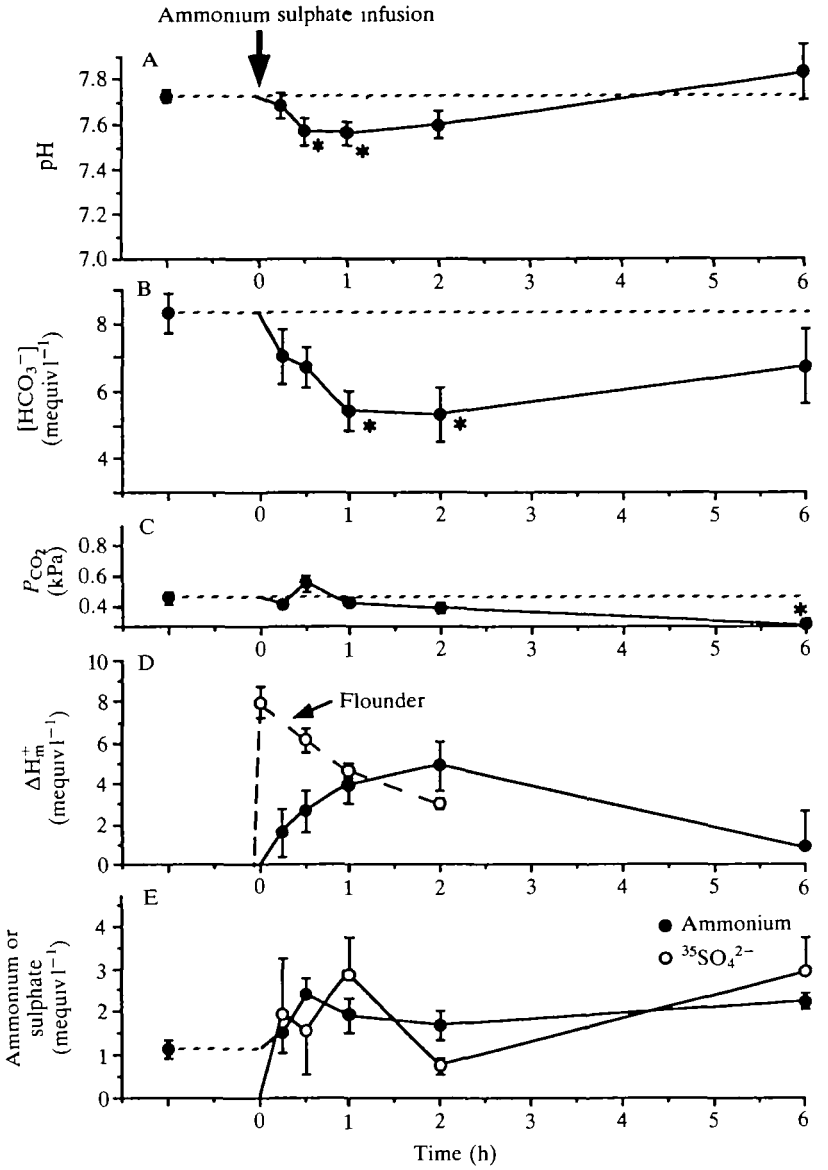

Fig. 1. Blood acid–base status prior to and following $(NH_4)_2SO_4$ infusion (2 mmol kg^{-1} injected into the subcutaneous sinus) in the hagfish *Myxine glutinosa* at 13°C. Blood was withdrawn from the caudal subcutaneous sinus. Each animal was sampled only once, total N=42. Data are means (N=12 for controls, N=6 for post-infusion samples) ±1 S.E.M. (A) Plasma pH; (B) whole-blood $[HCO_3^-]$; (C) plasma P_{CO_2}; (D) mineral acid load buffered in plasma (ΔH_m^+); (E) plasma total ammonium and $^{35}SO_4^{2-}$ concentrations. Asterisks indicate means significantly different from control values (P<0.05). For comparison, data from starry flounder, *Platichthys stellatus*, are shown in D. Flounder were infused with 2 mmol kg^{-1} $(NH_4)_2SO_4$ *via* a catheter implanted in the caudal artery, N=6, data from Milligan *et al.* (1991).

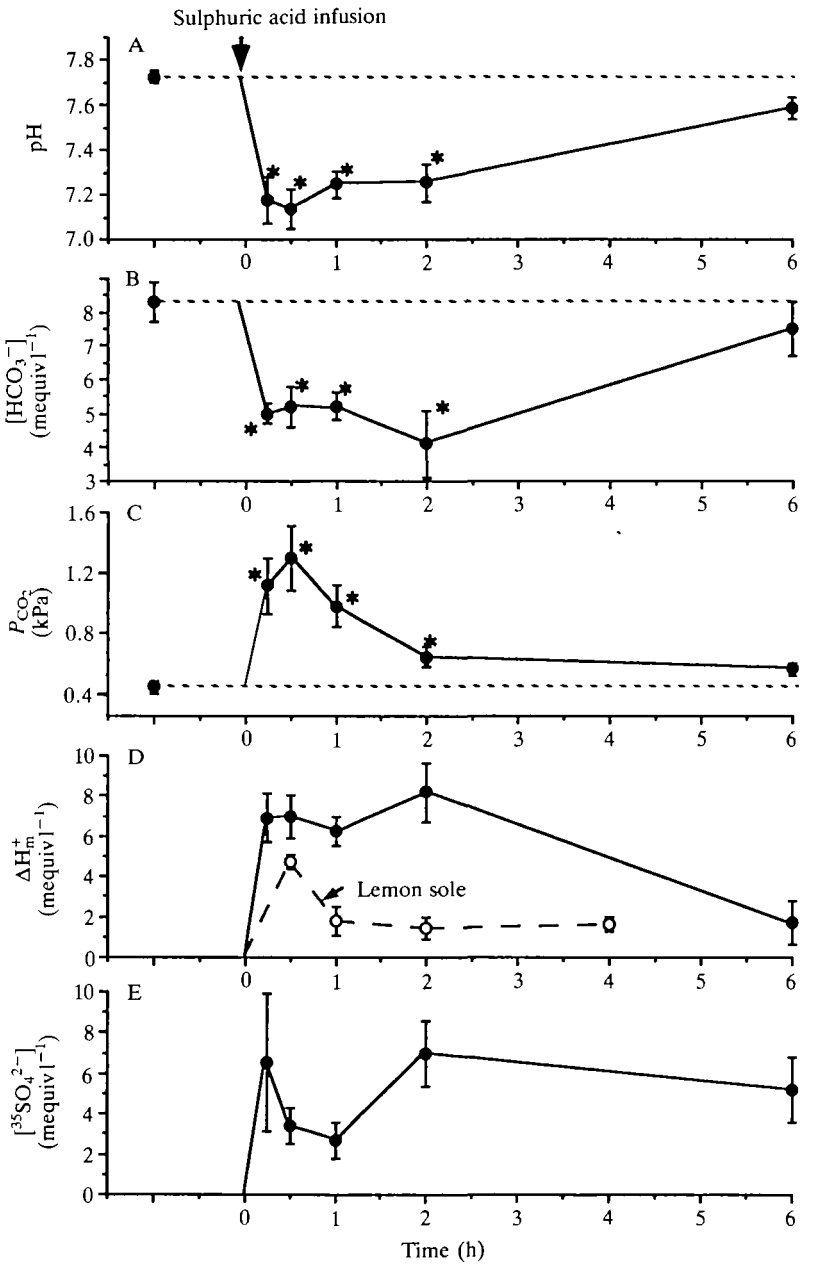

Fig. 2. Blood acid–base status prior to and following H_2SO_4 infusion (1.5 mmol kg^{-1} injected into the subcutaneous sinus) in the hagfish *Myxine glutinosa* at 13°C. Blood was withdrawn from the caudal subcutaneous sinus. Each animal was sampled only once, total N=42. Data are means (N=12 for controls, N=6 for post-infusion samples) ±1 S.E.M. (A) Plasma pH; (B) whole-blood $[HCO_3^-]$; (C) plasma P_{CO_2}; (D) mineral acid load buffered in plasma (ΔH_m^+); (E) plasma $^{35}SO_4^{2-}$ concentration. For comparison, data from lemon sole, *Parophyrs vetulus*, is shown in D. Sole were infused with 1 mmol kg^{-1} HCl *via* a catheter implanted in the caudal vein, N=6, data from McDonald *et al.* (1982). Asterisks indicate means significantly different from control values (P<0.05).

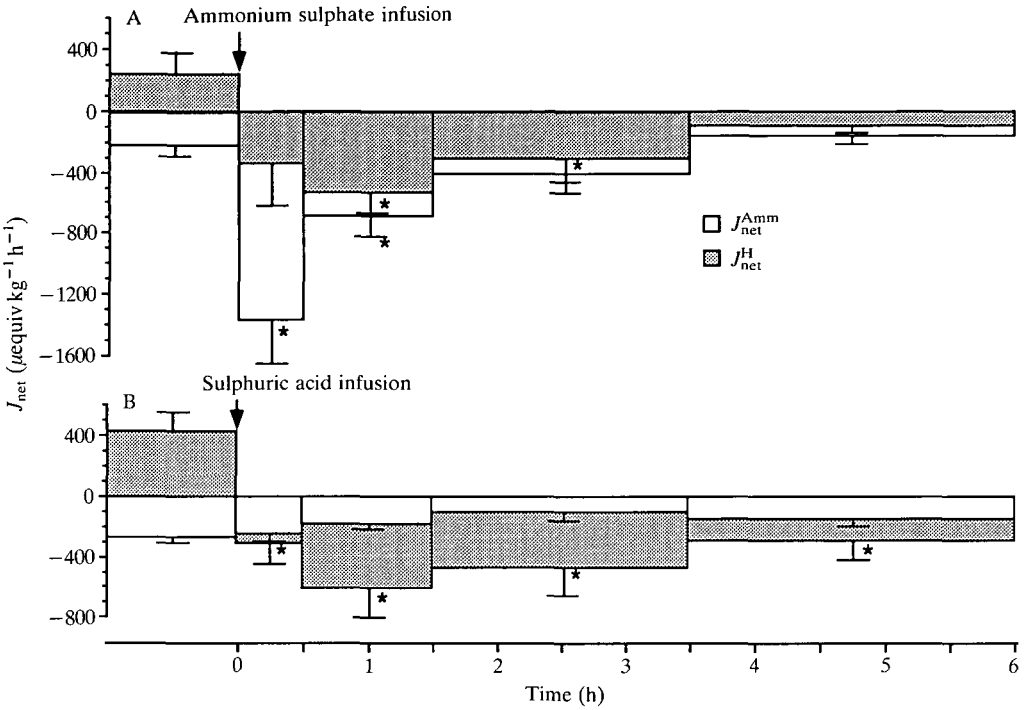

Fig. 3. Effect of a 2 mmol kg^{-1} ammonium sulphate infusion (A) and a 1.5 mmol kg^{-1} sulphuric acid infusion (B) on net ammonium flux (J^{Amm}_{net}) and net H^+ flux (J^{H}_{net}) in hagfish. Data are means±1 S.E.M. (N=10 or 9). A negative value is a net excretion, a positive value is a net uptake. Asterisks indicate means significantly different from control values (P<0.05).

This involves a calculation of the load to extracellular fluids; that portion not accounted for must either have entered the intracellular fluid (ICF) or have been excreted (equation 3, Materials and methods). At 2 h post-infusion, the amount buffered in extracellular fluids is estimated to be 2.2 mequiv kg^{-1}. Since 0.9 mequiv kg^{-1} had been excreted by this time (and 3 mequiv kg^{-1} infused), no H^+ would have entered the intracellular compartment. By 6 h, 0.44 mequiv kg^{-1} was buffered in blood and 2.1 mequiv kg^{-1} had been been excreted. This means that only 15.3 % of the infused acid load had entered the intracellular compartment. These calculations suggest that intracellular buffering played a relatively minor role in dealing with this acidosis.

Discussion

In this study we have demonstrated that hagfish, the most primitive of vertebrates, have, in fact, a substantial capacity to regulate the acid–base balance of their body fluids. They corrected rather severe experimental acid–base disturbances within 6 h of induction, largely by the transfer of acidic equivalents to

the external medium. Given their low urine flow rates (0.1–1.0 ml $100\,g^{-1}\,day^{-1}$; Hardisty, 1979) and therefore little, if any, renal capacity for acid–base regulation, the transfer probably took place at the gills. This is assuming that the skin with its mucous secretion plays no role in this transfer. While mucous secretion has been implicated in some ionoregulatory function (mostly divalent cation secretion, McFarland and Munz, 1965) hagfish typically do not release mucus unless disturbed. In the present study, hagfish produced mucus only when handled (a large-volume viscous mass) but this did not occur during the periods when net H^+ transfers were being measured.

One of the main objectives of this study was to compare the capacity for acid–base regulation of the hagfish with that of more advanced fishes and for that reason we infused, into extracellular fluids, acid loads similar or identical to that employed in our previous studies on marine teleosts. However, this comparison is complicated by the fact that hagfish have a much larger blood volume than other fishes (187 *vs* 20–60 ml kg^{-1}; Forster *et al.* 1989). This means that infused acid loads will be distributed in a much larger space and therefore produce smaller acid–base disturbances. While this effect is, in part, diminished by the lower buffering capacity of hagfish haemoglobin (less than half that of flounder, Table 1), the larger blood volume together with the greater contribution of plasma protein to buffering (4.94 *vs* 3.04 mequiv l^{-1} pH $unit^{-1}$, Table 1) means that the buffering capacity of the blood space in hagfish is still about 2.5 times greater than that of the flounder (Table 1). The effect of this greater buffering is best illustrated by the sulphuric acid infusion, where 3 mequiv kg^{-1} was well tolerated by hagfish. In comparison, the maximum direct acid load routinely tolerated by the lemon sole (McDonald *et al.* 1982) was only 1 mequiv kg^{-1} and this only if infused over about a 1 h period. Higher loads or more rapid infusion rates produced acid–base disturbances from which lemon sole could not recover. Indeed, the peak acid–base disturbance in the hagfish was only slightly greater than that of lemon sole despite the three times greater H^+ load (see Fig. 2D).

Despite smaller acid–base disturbances in hagfish, the peak J^{H}_{net} values are well within the range reported for various 'mineral' acidoses in various species (Table 2). Overall, there is a trend of higher acid excretion rates in marine species compared to freshwater species. Particularly noteworthy are the salmonids, where, in freshwater-adapted forms, peak acid excretion rates (in adult animals) rarely exceed 300 μequiv $kg^{-1}\,h^{-1}$ while, in marine species, peak rates of up to 1600 μequiv $kg^{-1}\,h^{-1}$ have been reported. Nonetheless, within marine species there are important interspecific differences in peak J^{H}_{net}. In bottom-dwelling, sluggish flatfish J^{H}_{net} values are generally lower than in active pelagic salmonids; a phenomenon we have related to the less active lifestyle of the former (lower $\dot{V}_{O_2max}$, greater hypoxic tolerance; Milligan *et al.* 1991).

Acid excretion in hagfish following infusion of $(NH_4)_2SO_4$ (4 mequiv kg^{-1} NH_4^+) approaches that of starry flounder similarly treated (−523 *vs* −757 μequiv $kg^{-1}\,h^{-1}$; Table 2), even though the infusion produced a much smaller acute acidosis in hagfish; ΔH^{+}_{m} was less than one-third of that produced in

Table 2. *Peak branchial net H^+ excretion rates, J^H_{net}, in marine and freshwater-adapted fish following various treatments*

Species	Treatment	J^H_{net} (μequiv l^{-1} kg^{-1} h^{-1})	Reference
Marine, pelagic			
Coho salmon *Oncorhynchus kisutch*	$(NH_4)_2SO_4$ infusion,	−1638±682 (5)	Milligan *et al.* (1991)
Rainbow trout *Oncorhynchus mykiss*	10 min enforced exhaustive exercise	−1137±80 (7)	Tang *et al.* (1989)
Dogfish *Scyliorhinus stellarus*	10 min enforced exhaustive exercise	−942 (9)	Holeton and Heisler (1983)
Marine, benthic			
Starry flounder *Platichthys stellatus*	$(NH_4)_2SO_4$ infusion, 2 mmol kg^{-1}	−757±156 (6)	Milligan *et al.* (1991)
Hagfish *Myxine glutinosa*	$(NH_4)_2SO_4$ infusion, 2 mmol kg^{-1}	−523±121 (9)	Present study
Hagfish *Mxyine glutinosa*	H_2SO_4 infusion, 1.5 mmol kg^{-1}	−608±168 (9)	Present study
Starry flounder *Platichthys stellatus*	10 min enforced exhaustive exercise	−480±153 (8)	Milligan and Wood (1987)
Lemon sole *Parophyrs vetulus*	HCl infusion, 1 mmol kg^{-1}	−350±60 (4)	McDonald *et al.* (1982)
Freshwater			
Rainbow trout *Oncorhynchus mykiss*	10 min enforced exhaustive exercise	−200−−300	Tang *et al.* (1989) McDonald *et al.* (1989); Wood (1988)
Rainbow trout *Onchorychus mykiss*	$(NH_4)_2SO_4$ infusion, 1.5 mmol kg^{-1}	−196±42 (5)	D. G. McDonald (unpublished results)

Values are mean±S.E.M. (*N*).
Data are means determined for either 0.5 or 1 h immediately following treatment.
Ammonium sulphate or acid loads were administered by bolus injection over 10 min or less.

the flounder (Fig. 1D). Infusion of H_2SO_4, in contrast, produced an H^+ excretion rate almost double that of lemon sole infused with HCl (608 *vs* 350 μequiv $kg\,h^{-1}$, Table 2). In part, the higher rate of excretion can be attributed to the higher load (3 *vs* 1 mequiv kg^{-1} H^+), but the acute acidosis in lemon sole was, nonetheless, almost two-thirds of that in the hagfish (Fig. 2D). These observations suggest that the hagfish is at least as competent at acid–base regulation as are benthic marine teleosts, assuming that flatfish (flounder and sole) are typical of the latter.

Nature of the H^+ excretion mechanism in the hagfish

How are hagfish able to match or surpass benthic teleosts in H^+ excretion with an epithelium that is apparently incapable of performing an ionoregulatory function and has a much lower Na^+/K^+-ATPase activity? The simplest expla-

nation is that the gill ATPase activity in the hagfish is devoted solely to H^+ transfers (as suggested by Mallatt *et al.* 1987) either *via* Na^+/H^+ exchange or directly as an H^+-ATPase, while in hypo-osmoregulating marine teleosts much of the ATPase activity must be devoted to NaCl excretion. Thus, according to Evans (1984), the branchial ATPase in the hagfish would operate similarly to that in freshwater teleosts by producing the electrochemical gradients necessary for apical Na^+/H^+ exchange and/or Cl^-/HCO_3^- exchange. While the operation of the former in H^+ excretion would lead to the accumulation of Na^+, the gain in Na^+, even at maximum H^+ excretion rates (Table 2), would be inconsequential, adding only about 0.2 % h^{-1} to the total body Na^+ pool. It is worth noting in this regard that branchial ATPase levels of hagfish are similar to those of freshwater salmonids (Mallatt *et al.* 1987), but the peak net H^+ excretion rates in hagfish are 2–3 times higher (Table 2). Again, the simple explanation may be the abundance of counterions (i.e. Na^+ and Cl^-) in sea water. In freshwater teleosts, recent studies have shown that the rate of branchial H^+ transfer is limited by external NaCl, with elevation of external [Na^+] promoting H^+ excretion and elevation of [Cl^-] promoting base excretion (McDonald *et al.* 1989; Goss and Wood, 1990). Certainly, the very much higher J^{H}_{net} in seawater- compared to freshwater-adapted rainbow trout for a similar acid–base disturbance (exhaustive exercise, Table 2) could be explained on this basis.

An additional possibility is that the gills of hagfish are simply more permeable to acid–base equivalents than are the gills of benthic teleosts. It is possible that passive diffusion of HCO_3^-, OH^-, NH_4^+ or H^+ through fairly non-selective pathways, such as paracellular channels, could play an important role in acid–base regulation. Indeed, we recently proposed that transfers *via* the paracellular pathway in freshwater rainbow trout could explain up to one-third of the total net H^+ transfer following an acidosis induced by ammonium sulphate infusion (McDonald and Prior, 1988). While we have no direct evidence for this pathway in hagfish, one highly suggestive observation is the much greater apparent sulphate permeability of the gills in hagfish compared to that in flounder. This is based on the observation that hagfish cleared over 7 % of the $^{35}SO_4^{2-}$ load by 2 h compared to only 0.3 % in the flounder, even though the peak blood $^{35}SO_4^{2-}$ levels were much lower in the hagfish (2.9 *vs* 6.9 mequiv l^{-1}). Furthermore, the even higher H^+ excretion rate in the coho salmon (Table 2) in response to a 2 mmol kg^{-1} $(NH_4)_2SO_4$ load, compared to hagfish, is accompanied by an even higher sulphate clearance rate, 15.3 % by 2 h (Milligan *et al.* 1991). Sulphate probably crosses the gills *via* a paracellular route. Therefore, these observations from three different marine species tend to argue for a close correlation between paracellular permeability, as measured by sulphate loss, and branchial capacity for net H^+ transfer.

Whether the mechanism for H^+ excretion in the hagfish is apical Na^+/H^+ exchange, paracellular H^+ diffusion or a combination of both, it is clear that a substantial capacity for acid–base regulation exists in this primitive osmoconformer, supporting the conclusion of Evans (1984) that acid–base regulation developed before osmoregulation in the evolution of the vertebrates.

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