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INTESTINAL BASE EXCRETION IN THE SEAWATER-ADAPTED RAINBOW TROUT: A ROLE IN ACID–BASE BALANCE?

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Summary

A potential role for the intestine of seawater-adapted teleosts in acid–base regulation was investigated following earlier reports of highly alkaline rectal fluids in the gulf toadfish *Opsanus beta*. Rectal samples taken from starved seawater-adapted rainbow trout had a high fluid pH (8.90±0.03; mean ± S.E.M., *N*=13) and base (HCO_3^-+2CO_3^{2-}) content of 157±26 mequiv kg^{-1} (*N*=11). In trout fitted with rectal catheters, rectal fluid was voided at a rate of 0.47±0.11 ml kg^{-1} h^{-1} (*N*=8), giving a net base excretion rate of 114±15 μequiv kg^{-1} h^{-1} (*N*=7). Drinking rates averaged 3.12±0.48 ml kg^{-1} h^{-1} (*N*=8), and accounted for only 6 % of the base excreted *via* the intestine, indicating substantial net transport of endogenously derived base into the intestine. Rectally excreted base was approximately balanced by an equivalent efflux of net acid from non-rectal sources (possibly as NH_4^+ excretion *via* the gills).

Samples taken from four sites along the intestine revealed that the most anterior region (the pyloric intestine) was responsible for the majority of HCO_3^-+2CO_3^{2-} accumulation. The pyloric intestine was subsequently perfused *in situ* to investigate possible mechanisms of base secretion. Net base fluxes were found to be dependent on luminal Cl^-, 76 % stimulated by amiloride, 20 % inhibited by 10^{-4} mol l^{-1} acetazolamide, but unaffected by either 10^{-4} mol l^{-1} SITS or 2×10^{-5} mol l^{-1} DIDS. This suggests that the mechanism of base secretion within the pyloric intestine may involve a Cl^-/HCO_3^--ATPase. It is speculated that intestinal base secretion may play a role in facilitating osmoregulation of seawater-adapted teleosts.

Key words: acid–base balance, marine teleost, rainbow trout, *Oncorhynchus mykiss*, rectal fluid, intestine, drinking, base secretion, carbonate, bicarbonate.

Introduction

Seawater-adapted teleosts are hypo-osmotic to the sea water in which they live and are constantly faced with an osmotic loss of water across their outer body surface. In order to compensate for this dehydration, they drink sea water (Smith, 1930; Shehadeh and Gordon, 1969; Evans, 1993). The intestine then plays an essential role in osmoregulation by actively absorbing NaCl which is followed osmotically by a volume of water, thereby compensating for the aforementioned fluid losses. This process inevitably results in a salt load which, in turn, must be excreted by the gills. Thus, the intestine of seawater-adapted teleosts has a vital role in osmoregulation in addition to its more conventional role in digestion and nutrient absorption.

Classically, the gills and kidney of teleost fish are considered to be the principal sites of acid–base regulation, with the gills normally dominating by contributing over 90 % to the transfer of acid–base-relevant ions to and from the external environment (Heisler, 1984, 1993; Wood, 1988). However, Walsh *et al.* (1991) recently reported intestinal carbonate deposits in the gulf toadfish (*Opsanus beta*) and found the intestinal fluids to be highly alkaline (pH 8.6) with an elevated total CO_2 concentration (68 mmol l^{-1}). Because the formation of intestinal carbonate was considered to be a feature of osmoregulation in marine teleosts in general (Walsh *et al.* 1991), it became apparent that the intestine might have an additional role in acid–base balance, at least in seawater-adapted teleosts. Nevertheless, these studies on toadfish lacked direct measurements of the rates of excretion (if any) of acid–base equivalents from the intestine. The initial aim of the present study was, therefore, to investigate the potential role of the intestine in acid–base regulation of a seawater-adapted teleost.

The euryhaline rainbow trout (*Oncorhynchus mykiss*), a species frequently used in studies of piscine ion and acid–base regulation (Wood, 1988; Goss *et al.* 1992), was selected for this study. Seawater-acclimated trout were found to excrete

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significant quantities of base *via* the intestine, and this secretion of base into the intestine arose endogenously. We then decided to investigate further (i) the relative importance of the intestine compared with the rest of the body for acid–base transfer, (ii) the site of base secretion within the intestine, and (iii) the possible mechanism(s) of base secretion into the intestinal lumen.

Materials and methods

Freshwater rainbow trout [*Oncorhynchus mykiss* (Walbaum)] were obtained from West Creek Trout Farms, Mission, BC, Canada, and held in 1000 l fibreglass tanks prior to seawater (SW) acclimation. For this, the salinity within the holding tanks was adjusted from that of fresh water to that of sea water (32±1 ‰) over a 24 h period. Ten days were then allowed for seawater acclimation prior to experimentation. During this time, fish were fed once daily with trout chow. The mean body mass of SW-acclimated trout used in the study was 249±14 g (N=53), and all experiments were carried out at 11 °C under natural photoperiod (May–June 1995).

Spot sampling of rectal fluid

To establish the acid–base content of fluid passing out of the rectum, SW-acclimated trout were netted individually from the holding tanks and anaesthetized in a 100 mg l^{-1} solution of tricaine methanesulphonate (MS222; Syndel) in sea water. Once anaesthetized, fish were held upside down in a damp sponge and the anus and surrounding area were washed with distilled water and lightly dried with tissues. A short length of polyethylene tubing (PE 160) attached to a 1 ml syringe and needle (18 gauge) was then inserted through the anal opening and the rectal contents were collected by gently withdrawing the syringe plunger. This ‘spot sampling’ procedure was carried out on eight fish that had been fed daily, and later on 14 fish that had been starved for the preceding 7 days. All the experiments described below were carried out using fish that had been starved for at least 7 days.

Continuous collection of rectal fluid

The dynamics of intestinal acid–base excretion were monitored in a second experiment in which voided intestinal contents were continuously collected *via* rectal catheters. For this procedure, fish were anaesthetized (100 mg l^{-1} MS222) and then transferred to a wet table where the gills were constantly irrigated with an aerated solution of 60 mg l^{-1} MS222 in sea water throughout surgery. Rectal catheters were modified Bard all-purpose urethral catheters (size 12 French, elastic rubber; Davol Inc.). The dilated end of urethral catheters was tied around a plastic sleeve fashioned from a 1 cm length of a 200 µl pipette tip heat-flared at both ends (o.d. 5–6 mm at the widest end). After filling the catheter with 150 mmol l^{-1} NaCl, the flared plastic sleeve was inserted about 0.5 cm into the anus and held in place by a purse-string ligature weaved through the skin in a ring around the anal opening. The catheter was additionally anchored with sutures to the anal and caudal fins. Once revived from the anaesthetic, fish were placed in individual chambers supplied with flow-through aerated sea water. Rectal output was then continuously collected into covered vials by maintaining catheter tips 3–5 cm below the water surface. Collections made over the first 24 h were discarded as these largely consisted of rectal fluid mixed with the saline used to fill the catheters. Subsequent collections were monitored every few hours, measured by change in mass of the collection vial, and then stored on ice until analysis of pH and HCO_3^-+2CO_3^{2-} base.

Drinking rates

After 2–4 days of rectal fluid collection, drinking rates were measured in eight of the trout fitted with rectal catheters, using a method similar to that described by Carrick and Balment (1983). Fish were transferred, without removal from their individual containers, to larger flux chambers which allowed recirculation of the total seawater volume (approximately 6 l) through the fish containers by means of an air-lift system (see McDonald, 1983, for a diagram of a larger version of this system). The sea water was spiked with approximately 1.3 MBq of [^{3}H]polyethylene glycol (PEG-4000, DuPont). Water samples were taken for radioactivity measurements at 0, 4 and 8 h. Fish were left for 8 h before being rapidly overdosed with buffered anaesthetic (1 g l^{-1} MS222), removed from the chambers and rinsed in clean sea water. A terminal blood sample was then taken by caudal puncture for measurement of plasma ion levels and radioactivity. The gastrointestinal tract (GIT) was then exposed by a ventral mid-line incision and ligated at both ends to prevent loss of contents. The entire GIT was then removed, weighed and homogenised in 100 ml of 10 % $HClO_4$ for 5 min in a commercial food processor. A subsample of the homogenate was then centrifuged at 500 ***g*** for 5 min, and the clear protein-free supernatant analysed for [^{3}H]PEG-4000 radioactivity by scintillation counting. There was never any radioactivity in the plasma samples, indicating that PEG-4000 was not absorbed but rather stayed within the GIT.

Whole-animal net acid–base fluxes

To establish whether the intestine plays a significant role in whole-animal acid–base balance, a set of experiments was conducted in which whole-animal net acid–base fluxes and ammonia excretion rates were measured in three groups of SW-acclimated trout: (i) unoperated control fish (N=9) only transferred to flux chambers, (ii) sham-catheterised fish (N=6) fitted with shortened catheters that were allowed to drain directly into the flux chamber water, and (iii) fish fitted with rectal catheters which were not allowed to drain into the flux chamber water (drainage into collection vials held outside the chamber or into an attached condom which collected and held the fluid whilst remaining inside the flux chamber; N=9). Surgery on catheterised and sham-operated fish was carried out as described above (see *Continuous collection of rectal fluid*), and all fish were subsequently transferred to either small (3.3–3.6 l) or large (6.4–7 l) flux chambers. All fish were

allowed to recover for 24 h following transfer, and during this recovery period (and between fluxes), chambers were continuously supplied with aerated sea water. All boxes also received independent aeration. The flux protocol consisted of removing the supply of sea water to the chamber and adjusting the water level to a known volume. Water samples (50 ml) were then taken at the beginning and end of each flux period, which lasted either 6 or 12 h depending on the chamber volume. A 10 ml aliquot of each subsample was stored at −20 °C for subsequent analysis of total ammonia concentration ($[NH_4^+]+[NH_3]$), and the remainder was stored at 10 °C in a screw-top plastic bottle for analysis of titratable alkalinity within 24 h of taking the first sample. At the end of experiments, catheterised fish were anaesthetized in the flux chamber, removed and the integrity of catheters checked by injecting saline and inspecting for leaks.

Sampling of gastrointestinal contents

Moving from anterior to posterior, the GIT of trout broadly consists of five sections; the oesophagus, stomach, pyloric intestine, mid intestine and posterior intestine. The three regions of the intestine are visibly distinct. The pyloric intestine is covered in numerous (30–80) blind-ended sacs called pyloric caeca, the mid intestine is narrower and free of caeca. The transition between the mid- and posterior intestine is marked by an abrupt increase in diameter and the appearance of externally visible annular folds. A one-way valve (the pyloric sphincter) separates the stomach contents from the beginning of the intestine. However, the lumen of the three intestinal segments forms a continuous tube up to the anal sphincter.

In order to locate the site of base excretion, ten SW-acclimated trout were killed with an overdose of MS222 to allow sampling of fluid from different positions along the GIT. Prior to intestinal sampling, a blood sample was taken (for measurement of plasma ions) by caudal puncture. Rectal fluid was first sampled from the tip of the anus (as for spot samples), and fish were opened *via* a ventral mid-line incision. The GIT was ligated *in situ* at the following five places: (i) the anterior end of the stomach, (ii) the pyloric sphincter, (iii) immediately posterior to the last pyloric caecum, (iv) at the beginning of the posterior intestine, and (v) immediately anterior to the anus. The tract was then cut at the oesophagus and anus and removed from the body cavity. The four areas isolated by the above ligatures (stomach, pyloric intestine, mid intestine and posterior intestine) were each opened with a longitudinal incision to allow sampling of fluid (and any solids) using a Pasteur pipette and forceps. In these starved trout, yellow or white strands of jelly-like material were usually found along the entire length of the intestine (from just posterior to the pyloric sphincter to the anus). These were termed ‘mucus tubes’ by Shehadeh and Gordon (1969) and will be described thus in the remainder of this paper. According to Shehadeh and Gordon (1969), they are 98 % water (w/w), consist of non-cellular material and are made up of a meshwork of fibres. In fed fish, faeces are always enclosed within these mucus tubes. Since they are excreted from the intestine even in starved fish, it is assumed that they originate in the pyloric intestine and move along the intestine lumen as new mucus tube is formed.

Tissue carbonic anhydrase activity

To assess the activity of carbonic anhydrase (typically present in acid–base-transporting epithelia), four trout were injected with 10 000 i.u. of heparin *via* the caudal artery/vein, left for 10 min for the heparin to circulate, anaesthetized and then placed on a surgery table where the gills were irrigated with a maintenance dose of MS222 (see above). The heart was exposed and a catheter inserted into the ventral aorta, which was then manually perfused with approximately 100 ml of heparinized (200 i.u. ml^{-1}) saline or until all the tissues had cleared of blood (which would otherwise cause contamination with carbonic anhydrase activity from red cells). Samples were taken from the following tissues: gill, oesophagus, stomach, pyloric, mid and posterior intestine and pyloric caeca. These sample were analysed for carbonic anhydrase activity by the method of Henry (1991).

In situ *perfusion of the pyloric intestine*

Having established that the pyloric intestine was the primary site of base accumulation in the GIT, it was considered pertinent to examine possible secretory mechanisms involved in this part of the intestine. For this, an *in situ* perfusion of the pyloric intestine was carried out on 11 trout. These trout were anaesthetised (see above), weighed and then transferred to a surgical table. A single ventral mid-line incision was made exposing the stomach, spleen, pyloric and mid intestine. Two ligatures were placed under the pyloric end of the stomach, and a small slit was made in the stomach wall just anterior to the pyloric sphincter. An inflow catheter (a length of PE 160 tubing flared at the tip) was inserted through this slit past the pyloric sphincter and just into the most anterior part of the intestine, where the caeca enter, and tied in place with one ligature. One end of a similar but shorter length (8 cm) of PE 160 tubing was implanted in the opposite direction (towards the oesophageal end of the stomach) and secured in place with the second ligature. The remainder of this length of tubing was led to the outside of the body. This allowed for external drainage of any imbibed sea water, as the stomach was effectively tied off. The ‘outflow’ catheter (a length of modified urethral catheter tubing; similar to that used for rectal catheters – see above) was then inserted into the beginning of the mid intestine and secured with a single ligature. This surgical preparation for *in vivo* perfusion of the pyloric intestine was similar to that used by Bogé *et al.* (1988), except that a stomach drain was included in the present study.

During all the above surgery, great care was taken not to damage the sub-intestinal vein, and immediately following catheter implantation 20 ml of ‘gut saline’ (see below) was slowly flushed through the inflow catheter by syringe to displace any mucus tubes. The three catheters were then anchored externally and the wound tightly closed with silk ligatures. Once revived from the anaesthetic, fish were placed

in individual chambers supplied with flow-through aerated sea water. The inflow catheter was immediately connected to an inflow reservoir and perfused with 'gut saline' at 0.33 ml min^{-1} using a peristaltic pump. Perfusion was continued and the outflow perfusate was collected into a covered plastic scintillation vial (using a siphon of 3 cm) for at least 2 h before beginning an experiment.

Each experiment consisted of five consecutive perfusion periods of 40 min each. The composition and exact flow rate of saline (measured by the decrease and increase in mass of the inflow and outflow vials, respectively) were measured in periods 1 (control), 3 (experimental) and 5 (recovery). Treatments were begun in period 2 and washed out in period 4, thus allowing 40 min of pre-treatment for the effects to develop or disappear. Experimental treatments consisted of: (i) control saline, (ii) 'Cl^--free' saline, (iii) 10^{-4} mol l^{-1} amiloride, a blocker of Na^+/H^+ exchange, (iv) 10^{-4} mol l^{-1} 4-acetamido-4′-isothiocyanostilbene-2,2′-disulphonic acid (SITS), a blocker of Cl^-/HCO_3^- exchange, (v) 2×10^{-5} mol l^{-1} 4,4′-diisothiocyanostilbene-2,2′disulphonic acid (DIDS), a blocker of Cl^-/HCO_3^- exchange, (vi) 10^{-4} mol l^{-1} acetazolamide, a blocker of carbonic anhydrase activity.

The standard saline used for gut perfusions ('gut saline') consisted of the following (in mmol l^{-1}): 100 NaCl, 50 $MgSO_4$, 35 $MgCl_2$, 5 $CaCl_2$, 5 KCl, 1 $KHCO_3$ (pH 7.2). The Cl^--free saline (in mmol l^{-1}) consisted of: 85 $MgSO_4$, 50 Na_2SO_4, 2.5 K_2SO_4, 5.0 $CaSO_4$, 1 $KHCO_3$ (pH 6.9). Thus, the latter had identical cation concentrations but Cl^- was essentially replaced with SO_4^{2-}. However, because sulphate is divalent, the total molarity (and therefore osmolality) was not matched in the two salines (432 and 339.5 mmol l^{-1}, respectively). No attempt was made to compensate for this difference.

Analytical techniques

The pH of gastrointestinal fluids was measured using a microcapillary pH electrode (Radiometer G279/G2) coupled with a PHM71 meter. The total base content of fluid samples was analysed by the double-titration procedure recommended by Hills (1973). This consisted of titrating a continuously aerated sample to below pH 4 with 0.02 mol l^{-1} HCl in order to remove all HCO_3^- and CO_3^{2-} as gaseous CO_2, and then titrating back to the starting pH using 0.02 mol l^{-1} NaOH. The differential in number of moles of HCl and NaOH required to return to the starting pH is then equivalent to the number of moles of HCO_3^- equivalents in the original sample. For these titrations, glass combination pH electrodes were used (Radiometer GK2401C or Cole-Palmer, in conjunction with a Radiometer PHM82 or Fisher 119 meter). Acid and base were added using digital microburettes (Gilmont). In the case of intestinal fluids, this double titration was carried out on 50 µl samples diluted in 10 ml of 40 mmol l^{-1} NaCl (to stabilise the pH electrode response). For intestinal solids (when present), a small amount (5–75 mg) was weighed into a 1.5 ml microcentrifuge tube and sonicated in 1 ml of 40 mmol l^{-1} NaCl. The resultant suspension was decanted into a titration vial and made up to a final volume of 10 ml with 40 mmol l^{-1} NaCl.

For comparison, the base content of rectal fluid spot samples was also estimated following measurement of total CO_2 by the method of Cameron (1971) using a P_{CO_2} electrode (Radiometer E5037) connected to a Radiometer PHM 72 meter. The quantity of basic equivalents [$HCO_3^-+2CO_3^{2-}$] was then calculated using the Henderson–Hasselbalch equation from the measured fluid pH and total CO_2 content, and the carbonic acid pK′ values for one-third-strength sea water at 11 °C derived from Skirrow (1975). This gave exactly the same mean base content as found using the double titration method described above. The latter has therefore been reported throughout the present study because it can be equally applied to the liquid and solid phases of the intestinal contents.

Terminal blood samples were centrifuged for 2 min at 13 000 ***g*** (Eppendorf microcentrifuge) to obtain plasma, which was stored on ice prior to Cl^- measurement and then stored frozen prior to dilution and Mg^{2+} analysis. The concentrations of Mg^{2+} and Cl^- in sea water, plasma and various gastrointestinal fluids were measured by colorimetric assay (Sigma kit no. 595-A) and by coulometric titration (Radiometer CMT-10), respectively. The concentration of total ammonia in sea water was analysed by colorimetric assay (Ivancic and Deggobis, 1984). ^3H radioactivity was determined by liquid scintillation counting of GIT homogenate supernatants (0.5–1.5 ml), plasma (0.20 ml) and sea water (5 ml) using an LKB 1217 Rackbeta scintillation counter. All samples were made up to a total seawater volume of 5 ml plus 10 ml of ACS fluor (Amersham), and quench correction was performed by the external standard ratio method and checked by internal standardization. The change in titratable alkalinity in seawater samples from the beginning to the end of whole-animal fluxes was measured by titrating 10 ml samples to pH 4.00 with 0.02 mol l^{-1} HCl, with continuous aeration of samples during titration to ensure mixing and removal of CO_2 (see McDonald and Wood, 1981).

Calculations and statistical analyses

Net acid or base excretion was calculated as the sum of the titratable alkalinity flux and the ammonia flux, signs considered, as described by McDonald and Wood (1981). Concentrations of HCO_3^- and CO_3^{2-} in GIT fluid samples were calculated from the measured pH and total [base] using a value of 9.57 for the pK'_2 of carbonic acid estimated for one-third-strength sea water at 11 °C from tables presented by Skirrow (1975).

Drinking rate was calculated from the total ^3H disints min^{-1} of the GIT and its contents, factored by time, mass of the fish and mean ^3H disints min^{-1} ml^{-1} of the external sea water.

Values are normally expressed as mean ± S.E.M. with the number of animals in parentheses. In the perfusion of pyloric intestine experiments, differences between experimental and control periods were assessed using one-tailed paired Student's *t*-tests. In the whole-body net acid–base and ammonia flux experiments, differences among control, catheterized and sham-catheterized trout were assessed using one-way analysis of variance (ANOVA) followed by Fisher's LSD. Differences were judged to be statistically significant when $P<0.05$.

Table 1. *Ion and acid–base variables in the rectal fluid of fed and starved rainbow trout acclimated to sea water*

	Fluid			Solids	Total sample
	pH	$[HCO_3^-+2CO_3^{2-}]$ (mequiv l^{-1})	$[Cl^-]$ (mequiv l^{-1})	$[HCO_3^-+2CO_3^{2-}]$ (mequiv kg^{-1})	$[HCO_3^-+2CO_3^{2-}]$ (mequiv kg^{-1})
Fed	8.61±0.04 (8)	68.0±8.3 (8)	64.4±11.1 (8)	266±70 (8)	78.8±8.2 (8)
Starved	8.90±0.03** (13)	111.8±8.7** (10)	40.2±4.6* (12)	676.4±123.7* (11)	156.8±25.5* (11)

Asterisks indicate statistically significant differences between fed and starved fish: *P<0.05, **P<0.005.
Values are means ± S.E.M. (N).

Results

Spot sampling of rectal fluid

The total quantities of rectal spot samples obtained from fed and starved trout were similar, averaging 0.240±0.034 and 0.237±0.029 g, respectively. This mass usually included both liquid and solids, of which the solids contributed 7.4±1.7 % to the total mass in fed fish and 6.2±1.7 % in starved fish. In fed fish, solids included variable amounts of faecal matter, whereas solids from starved fish consisted of white or yellow-coloured 'mucus tubes' with no obvious faecal contamination. In both fed and starved fish, the fluid was relatively clear but yellow-coloured, probably due to staining with bile. Table 1 shows the acid–base status of spot rectal samples from fed and starved trout. Rectal samples from starved fish had a higher pH and base content than those from fed fish. In particular, the base content of solids (mucus tubes) was 2.5-fold higher and the base content of the whole sample (fluid + solid) was twofold higher in starved fish. Although the base content of rectal solids was up to six times higher than that in rectal fluids (on a per gram basis), the majority of base resided in the fluid component of the spot samples.

Drinking rates, rectal fluid losses and base excretion rates in catheterised fish

Seawater-acclimated trout fitted with rectal catheters drank sea water at a rate of 3.12±0.48 ml kg^{-1} h^{-1} (N=8; Table 2), which falls in the middle of the range previously reported for SW-acclimated rainbow trout at the same temperature (Perrot *et al.* 1992; Shehadeh and Gordon, 1969). Collection of fluid (plus mucus tube solids) from rectal catheters averaged 0.47±0.11 ml kg^{-1} h^{-1} (N=8; assuming that 1 g is equivalent to 1 ml), although the appearance of this fluid in the collection vials was relatively sporadic, with periods of up to 5 h during which no fluid was voided. The contribution of mucus tube 'solids' to this rate varied from 0 to 17.5 %. From the drinking and excretion rates (Table 2), it can be seen that on average approximately 85 % of the imbibed sea water was absorbed from the GIT. From titration analysis of the fluid and solid components of voided rectal contents, the rate of base excretion *via* the intestine averaged 114.4±15.3 μequiv kg^{-1} h^{-1} (N=7).

Table 2. *Rates of seawater drinking, base intake, rectal fluid output and rectal base excretion in rainbow trout acclimated to sea water.*

Drinking rate (ml kg^{-1} h^{-1})	Base intake (μequiv kg^{-1} h^{-1})	Rectal fluid output (ml kg^{-1} h^{-1})	Base excretion (μequiv kg^{-1} h^{-1})
3.12±0.48 (8)	7.2±1.1 (8)	0.47±0.11 (8)	114.4±15.3 (7)

Base intake was calculated from the drinking rates and the measured base content of sea water.
Rectal base excretion was calculated from the rectal flow rates and base contents of all excreted components (both fluid and solid).
Values are means ± S.E.M. (N).

Whole animal acid–base fluxes

Ammonia excretion rates were similar in control, sham-catheterised and rectal-catheterised trout (Fig. 1). Net acid–base fluxes in control fish averaged 83±26 μequiv kg^{-1} h^{-1} (N=8), indicating significant net acid excretion under control conditions. In sham-operated fish (catheters draining directly into the flux chamber), the net acid flux was not significantly greater than in the controls. However, when rectal catheters were allowed to drain outside the flux chambers, net acid fluxes were substantially higher than those of both the control and sham-operated rates (Fig. 1). The differential between net acid excreted by catheterised and sham-operated trout was 168 μequiv kg^{-1} h^{-1}. This was similar to the net excretion of intestinal base in the two catheterised fish used in these acid–base flux experiments whose rectal catheters were drained externally (average 143.5±6.5 μequiv kg^{-1} h^{-1}), but was somewhat higher than the mean rate achieved for the previous experimental series where drinking rates were measured (114.4±15.3 μequiv kg^{-1} h^{-1}).

Composition of gastrointestinal fluids

Sea water from flux chambers contained approximately 2.3 mequiv l^{-1} of $HCO_3^-+2CO_3^{2-}$ base, whereas levels in fluid from the stomach were undetectable in the few samples obtained (Fig. 2). High concentrations of $HCO_3^-+2CO_3^{2-}$ base first appeared in the fluid of the pyloric intestine (Fig. 2) and

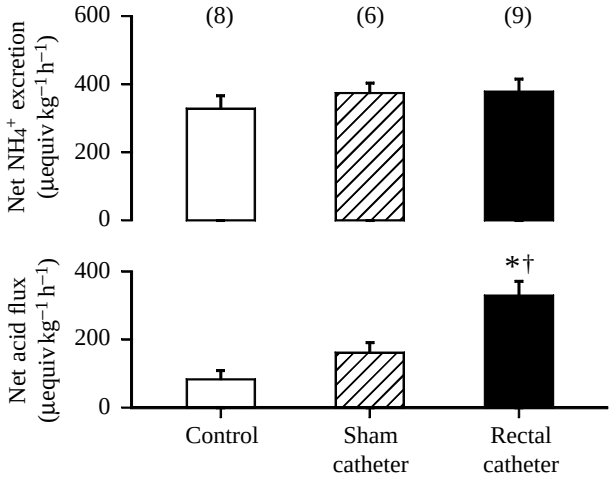

Fig. 1. Whole-body net ammonia excretion and net acid excretion rates in seawater-acclimated trout. Control trout (unoperated) were just transferred to flux boxes (open bars). Sham-operated trout were fitted with rectal catheters but these were cut short and allowed to drain into the flux chamber water (hatched bars). Catheters of 'Rectal catheter' trout were prevented from draining into the chamber water. Asterisks indicate a significant difference from the control fish; daggers indicate a significant difference from the sham-catheterised fish (P<0.05). Values are means + S.E.M., numbers of animals are indicated in parentheses.

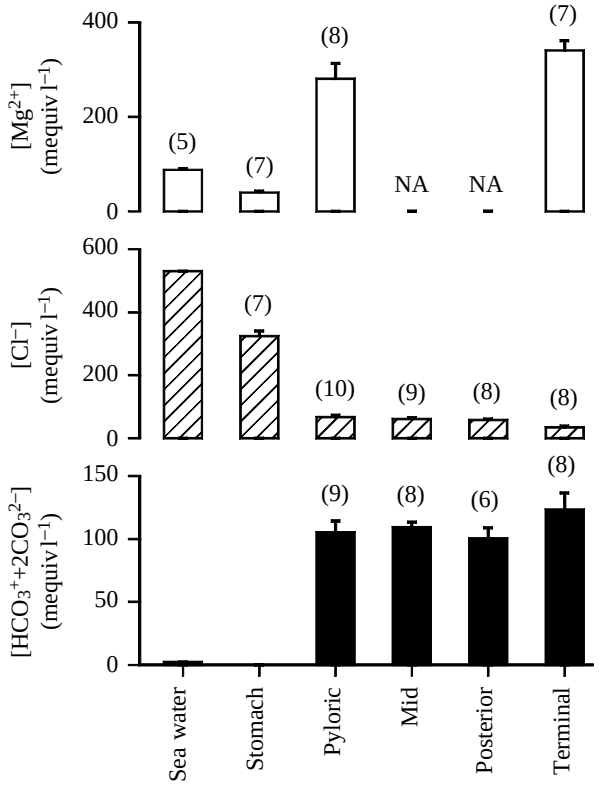

Fig. 2. Measured levels of Mg^{2+}, Cl^- and base ($HCO_3^- + 2CO_3^{2-}$) in fluid sampled from different positions along the gastrointestinal tract of seawater-acclimated rainbow trout. Values are means + S.E.M., numbers of samples are indicated in parentheses (NA means not applicable as analysis was not performed).

did not increase significantly further along the remaining intestine. Mucus tubes, stained yellow from bile, were found to be present along the entire length of the three intestinal segments. Bile sampled from three trout was found to be at pH 7.18±0.11, with a base content of less than 8 mequiv l^{-1} (excessive foaming and partial loss of samples during titration prevented a more accurate determination). Although there was a tendency for the base content of mucus tubes to increase along the intestine, this was not significant owing to large inter-individual variations. Levels of Cl^- were reduced by one-third between sea water and stomach fluid samples (Fig. 2). However, the most dramatic change was seen in the pyloric intestine, where $[Cl^-]$ was reduced to 67±6 mmol l^{-1}, almost one-fifth that of stomach fluid (Fig. 2). This tended to decline further along the intestine, although the only significant fall was seen in the terminal rectal samples, which reached 35±4 mmol l^{-1}, almost half the level found in the pyloric intestine. $[Mg^{2+}]$ in sea water was 87.9±2.6 mequiv l^{-1} (average of five measurements). This was reduced by twofold in the stomach fluid (Fig. 2), but $[Mg^{2+}]$ then increased dramatically to 280.5±32.9 mequiv l^{-1} (N=8) in the pyloric intestine and to 340.8±21.0 mequiv l^{-1} (N=7) in terminal rectal samples. Combining these fluid ion measurements with the seawater drinking and rectal fluid excretion rates measured in the previous experiments, it was calculated that 99 % of the ingested Cl^- and 41 % of the ingested Mg^{2+} were absorbed during passage through the gut.

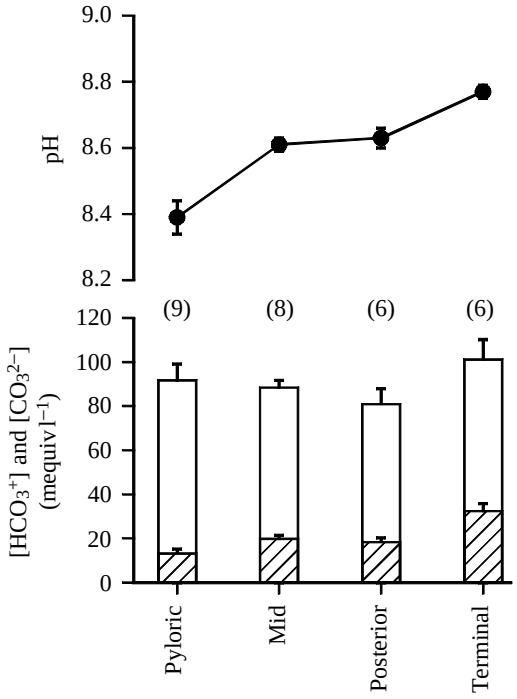

Fig. 3. Measured pH and calculated concentrations of HCO_3^- (open columns) and CO_3^{2-} (hatched columns) in fluid sampled from four positions along the intestine of seawater-acclimated rainbow trout. The *total* height of the column represents the HCO_3^- concentration. Values are means ± S.E.M., numbers of samples (i.e. animals) are indicated in parentheses.

Table 3. *Carbonic anhydrase activities in the gills and various tissues from the gastrointestinal tract*

Tissue	Carbonic anhydrase activity ($\mu mol\ CO_2\ g^{-1}\ min^{-1}$)
Gills	8408±3246
Stomach	7132±2355
Oesophagus	1789±651
Pyloric intestine	709±137
Mid intestine	574±169
Posterior intestine	676±175
Pyloric caecae	506±33

Values are means ± S.E.M., *N*=4.

Fig. 3 shows that the fluid pH was 8.39±0.05 (*N*=9) in the pyloric intestine and continued to rise distally along the mid and posterior sections, reaching its highest value in terminal rectal samples. Thus, the ratio of CO_3^{2-} to HCO_3^- increased, leading to a doubling of [CO_3^{2-}] between the pyloric intestine and rectum, while [HCO_3^-] only increased by 20 %, in line with the similar concentration of Mg^{2+}, and presumably absorption of water.

The plasma [Cl^-] of trout used in the drinking experiments and intestinal sampling averaged 168±4 mequiv l^{-1} (*N*=19), while plasma [Mg^{2+}] averaged 7.40±1.51 mequiv l^{-1} (*N*=11).

Carbonic anhydrase activity

Of the tissues sampled, gill and stomach had the highest levels of carbonic anhydrase activity, with means of 7132 and 8408 $\mu mol\ CO_2\ g^{-1}\ min^{-1}$ (Table 3). With the exception of the oesophagus, all other tissues sampled (pyloric, mid and posterior intestine and isolated pyloric caeca) had activities of less than 800 $\mu mol\ CO_2\ g^{-1}\ min^{-1}$.

Mechanism of base excretion in the pyloric intestine perfused in situ

The mean net base secretion rate in the perfused pyloric intestine for all the initial control periods (period 1) was 552±66 $\mu equiv\ kg^{-1}\ h^{-1}$ (*N*=11 fish, 29 determinations). The parallel net uptake of Cl^- was 2649±310 $\mu equiv\ kg^{-1}\ h^{-1}$, almost five times greater than the base secretion rate. During the same control periods, fluid was reabsorbed from the perfused preparation at 11.62±1.39 $ml\ kg^{-1}\ h^{-1}$.

Fig. 4 is a plot of net Cl^- uptake rate *versus* base secretion rate for all individual data points (*N*=57) for periods in which treatments had no significant effects, showing a significant correlation of 0.78. The slope (0.18) suggests a stoichiometry of one base equivalent secreted for every five chloride ions absorbed, on a net basis. Similarly, Fig. 5 shows a plot of net fluid absorption *versus* net Cl^- uptake, again for all individual data points (*N*=67) for periods in which treatments had no significant effects on either of these variables. This revealed a very strong regression relationship (*r*=0.93), with the slope suggesting a [Cl^-] in the absorbate of 228 mequiv l^{-1} (slightly higher than the luminal [Cl^-] of 185 mequiv l^{-1}).

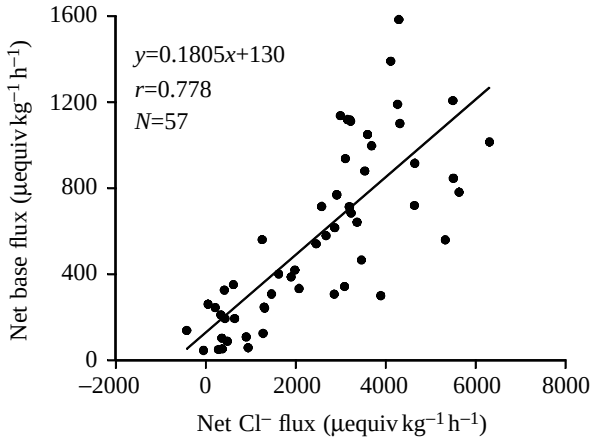

Fig. 4. Relationship between net base (HCO_3^-+$2CO_3^{2-}$) fluxes and net Cl^- fluxes in the *in situ* perfused pyloric intestine of seawater-acclimated rainbow trout. Positive numbers on the *x*- and *y*-axes represent net Cl^- absorption and net base secretion, respectively.

Fig. 6A shows that base secretion rate remained stable throughout the control series in which control saline was perfused during all five periods, indicating no deterioration in the base-secreting potential of the preparation over time. When Cl^--free saline was perfused, base secretion declined by 62 %, and this fall was only partially reversed in period 5 (Fig. 6A). The [Cl^-] measured in the outflowing saline during period 3 was 9.5±4.3 mequiv l^{-1} (*N*=5), indicating some net movement of Cl^- into the intestine lumen. The presence of 10^{-4} mol l^{-1} amiloride caused a 76 % stimulation of base secretion, whereas 10^{-4} mol l^{-1} SITS or 2×10^{-5} mol l^{-1} DIDS had no significant effect. (Fig. 6B). The presence of 10^{-4} mol l^{-1} acetazolamide in perfusates reduced base secretion by 20 %.

In the same perfusion experiments, Cl^--free saline was the only treatment to affect net Cl^- flux significantly, reducing it from absorption to net secretion in the experimental period

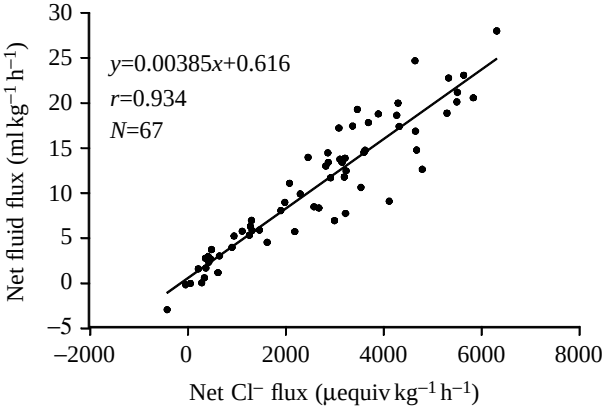

Fig. 5. Relationship between net fluid fluxes and net Cl^- fluxes in the *in vivo* perfused pyloric intestine of seawater-acclimated rainbow trout. Positive numbers on both axes represent net absorption of Cl^- and fluid.

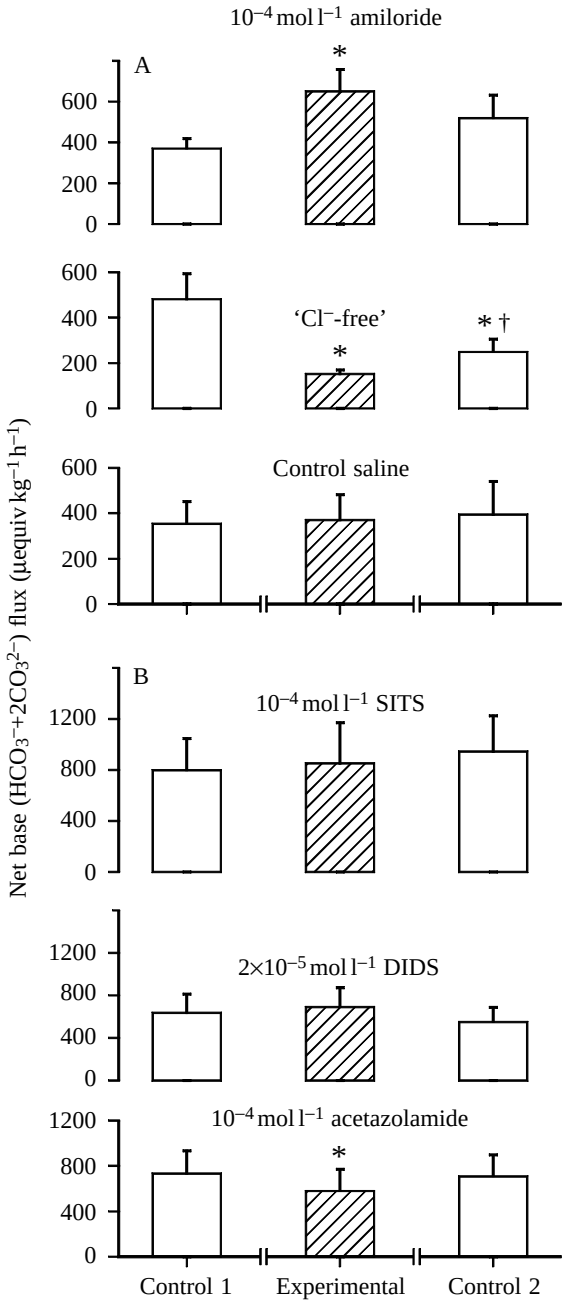

Fig. 6. (A,B) The effects of various treatments on net base ($HCO_3^-+2CO_3^{2-}$) fluxes in the *in vivo* perfused pyloric intestine of seawater-acclimated rainbow trout. Positive values represent net secretion of base (N=5 for all treatments except SITS where N=4). Asterisks indicate a significant difference from the control 1 period; daggers indicate that fluxes in the control 2 period were significantly different from those in the experimental period (P<0.05). Values are means + S.E.M.

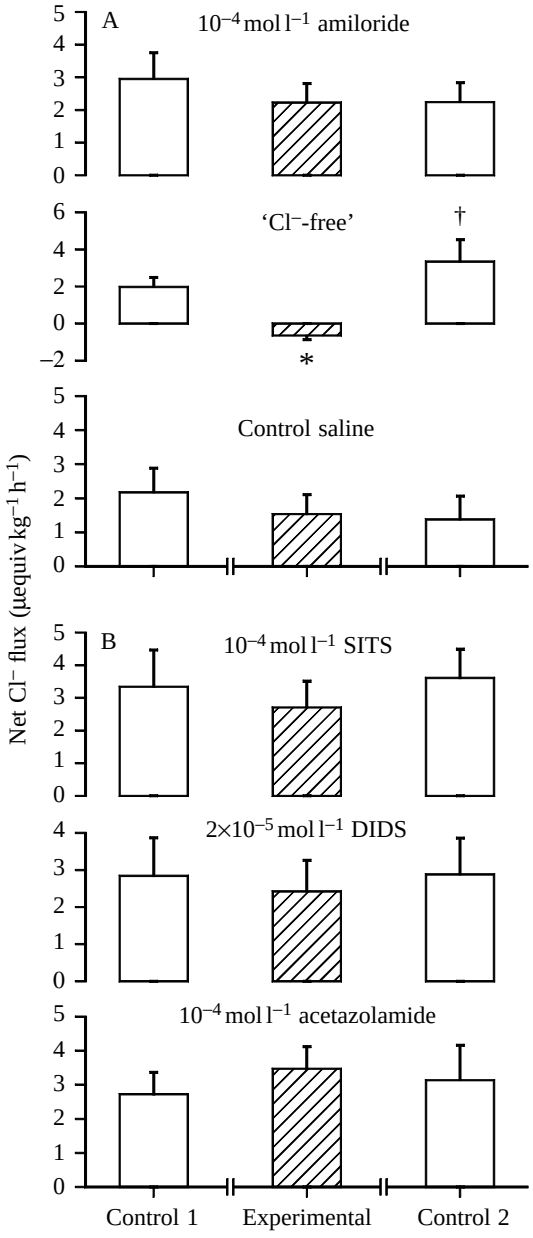

Fig. 7. (A,B) The effects of various treatments on net Cl^- fluxes in the *in vivo* perfused pyloric intestine of seawater-acclimated rainbow trout. Positive values represent net absorption of Cl^- (N=5 for all treatments except SITS where N=4). Asterisks indicate a significant difference from the control 1 period; daggers indicate that fluxes in the control 2 period were significantly different from those in the experimental period (P<0.05). Values are means + S.E.M.

(Fig. 7). Net fluid absorption was simultaneously reduced by Cl^--free saline (Fig. 8). Net fluid absorption was also significantly reduced in the presence of 10^{-4} mol l^{-1} amiloride (Fig. 8), in the absence of any significant change in net Cl^- flux. However, this effect was not reversible.

Discussion

Intestinal excretion of base from an endogenous source

Spot sampling of the rectal contents of SW-acclimated trout revealed fluid with a high pH and HCO_3^-/CO_3^{2-} content (Table 1), as previously found in the marine toadfish (Walsh *et al.* 1991; Wood *et al.* 1995). In a comprehensive study of salinity adaptations in rainbow trout, Shehadeh and Gordon (1969) reported a somewhat lower rectal fluid pH (8.1) for SW-

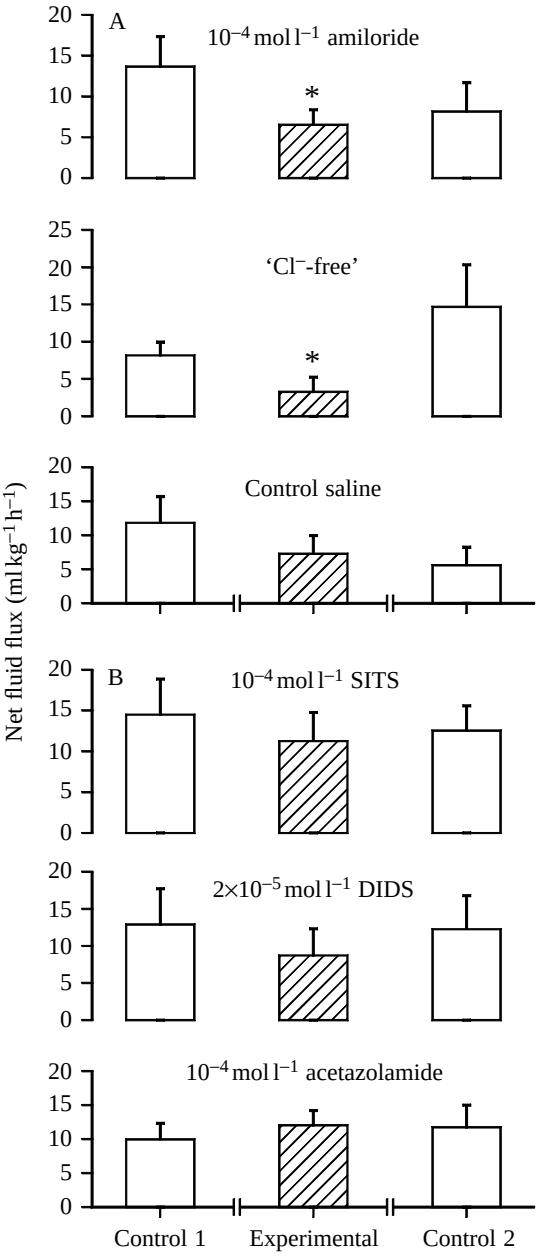

Fig. 8. (A,B) The effects of various treatments on net fluid fluxes in the *in vivo* perfused pyloric intestine of seawater-acclimated rainbow trout. Positive values represent net absorption of fluid (N=5 for all treatments except SITS where N=4). Asterisks indicate a significant difference from the control 1 period (P<0.05). Values are means + S.E.M.

acclimated rainbow trout, but made no measurements of rectal fluid HCO_3^-/CO_3^{2-} content. They did, however, report a high carbonate content in the intestinal mucus tubes and, in fact, made the first suggestion that the intestine may excrete a significant amount of carbonate from the body *via* these mucus tubes. Nevertheless, to our knowledge, this is the first time that excretion of acid–base equivalents *via* the intestine of fish has been quantified, revealing high rates of net base excretion at 114.4 μequiv kg^{-1} h^{-1} (Table 2). We can also now conclude that the HCO_3^-/CO_3^{2-} base excreted in rectal fluid and mucus tubes clearly arose from an endogenous source, because the amount of base being ingested in sea water accounted for only 6 % of that excreted rectally (Table 2). In addition, even this small amount of base taken up with imbibed sea water was reduced to zero during passage through the stomach, such that *all* base subsequently appearing in the intestine must have arisen endogenously.

Importance of the intestine relative to other routes of acid–base excretion

Having found that the intestine excretes significant net amounts of base, it was of interest to establish whether these SW-acclimated trout were in overall acid–base balance and the relative importance of the intestine as an acid–base excretory route. Control fish (non-catheterised) were found to have a significant net excretion of acidic equivalents (Fig. 1). Net acid excretion rates of this order (75–100 μequiv kg^{-1} h^{-1}) are not uncommon in non-feeding freshwater trout under laboratory conditions (e.g. Goss and Wood, 1990*a*,*b*; Tang *et al.* 1989; Wood, 1988), and rates as high as 360 μequiv kg^{-1} h^{-1} have been reported in resting marine teleosts such as toadfish (Evans, 1982). This probably reflects protein catabolism, which is a source of endogenous acidic equivalents (Wood, 1988; Goss and Wood, 1990*a*,*b*).

This net acid excretion was slightly higher in trout that were sham-catheterised (output *via* rectal catheters allowed to flow directly into the flux chamber water), suggesting that rectal catheters may impede intestinal base excretion to some degree and/or that the surgical manipulation elevates net acid excretion from other routes. However, of greater significance to the present study was the *difference* in net acid excretion between sham-catheterised and catheterised fish. In the catheterised animals, intestinal base excretion was effectively separated from the flux chamber water, which revealed that net acid is excreted *via* routes other than the intestine at a rate very similar to net base excretion measured from rectal catheters. In other words, when handling and surgical artefacts are taken into account, net base excretion *via* the intestine is approximately balanced by the net excretion of acid from other parts of the body. This is the only explanation for the differential in net acid excretion between the three groups as they all had very similar NH_4^+ excretion rates, suggesting a similar metabolic state throughout. Basic equivalent excretion *via* the intestine, in absolute terms, was equal to 32 % of the net ammonia excreted by the whole body under resting conditions (which exits primarily *via* the gills; see Wood, 1993, for a review). Thus, it would appear that the intestine of seawater-acclimated trout does have a considerable and hitherto unrecognised role in whole-animal acid–base balance and that, to some degree, there is an anatomical separation of net acid and base fluxes.

The possible sites of the net acid excretion which balances this intestinal base output are the gills, kidney and skin. Although the gills and kidney of teleost fish are considered to

be the principal sites of acid–base regulation (Heisler, 1984, 1993; Wood, 1988), the kidneys generally contribute less than 6 % to the net transfer of acid–base relevant ions in freshwater fishes (Wood, 1988). Furthermore, this proportion falls to less than 1 % in seawater-adapted teleosts, mainly as a result of the their notoriously low urine flow rates (Evans, 1982; McDonald *et al.* 1982). For an example of absolute rates, in freshwater trout the resting rate of net urinary acid excretion is only $6\,\mu equiv\,kg^{-1}\,h^{-1}$ (Wood, 1988) and it is likely to have been even lower in SW-acclimated trout, given the tenfold lower urinary flow rate (e.g. Bath and Eddy, 1979), even though urine acid content tends to be higher in seawater-adapted teleosts (Hickman and Trump, 1969; McDonald *et al.* 1982). However, a possible role for the skin in net acid excretion should not be ruled out, given that cutaneous ammonia excretion has been found to contribute up to 48 % of the total ammonia excreted by marine teleosts (Sayer and Davenport, 1987).

An interesting possibility is that the net acid excretion which balances intestinal net base excretion may be due, in part at least, to a greater proportion of NH_4^+ excreted across the gills relative to NH_3 in comparison with freshwater fish. In freshwater trout, diffusion of NH_3 may account for all ammonia excretion (Wilson *et al.* 1994) and is acid–base neutral (Wood, 1993). In contrast, branchial diffusion of NH_4^+ will be enhanced by the considerably higher ionic permeabilities (Payan *et al.* 1984) and the tendency for strongly positive (blood side) transepithelial potentials in seawater-adapted teleosts gills (Potts, 1984). In addition, while there is increasing evidence against the presence of a branchial Na^+/NH_4^+ exchange mechanism in the gills of freshwater fish (Avella and Bornancin, 1989; Wilson *et al.* 1994), it may well exist in seawater fishes and could potentially support high NH_4^+ transport rates given the favourable Na^+ gradient (Tang *et al.* 1989; Wilson and Taylor, 1992; Wood, 1993). Extrusion of NH_4^+ by either diffusion or electroneutral Na^+/NH_4^+ exchange would contribute to branchial acid extrusion, which is therefore likely to be higher in seawater- than in freshwater-acclimated trout.

Site of intestinal base secretion

The very dramatic increase in levels of HCO_3^-/CO_3^{2-} base between the stomach and the pyloric intestine (Fig. 2) suggests that this is the primary site of endogenous base secretion. The pyloric intestine also appears to be where the majority of fluid is absorbed, at least in rainbow trout (Bogé *et al.* 1988) and coho salmon *Oncorhynchus kisutch* (Kerstetter and White, 1994). This contrasts with the fact that the majority of *in vitro* studies on intestinal water absorption in seawater fish have focused almost exclusively on mid or posterior intestine (e.g. Veillette *et al.* 1993; Cornell *et al.* 1994), whole everted intestines of eel *Anguilla japonica* (e.g. Ando, 1990) or unidentified sheets of intestinal epithelium in an Ussing–Rehm chamber arrangement (e.g. Ando and Subramanyam, 1990).

A primary role of this most anterior section of the intestine is also supported to some degree by the sevenfold increase in $[Mg^{2+}]$ (relative to the stomach) measured in the present study (Fig. 2). Magnesium ions have often been considered to pass through the intestine of seawater-adapted teleosts with relatively little absorption, thus making them a potentially useful endogenous marker for net water movements (Hickman, 1968; Evans, 1993). In SW-acclimated rainbow trout this is not so obvious, as between 41 % (this study) and 53 % (Shehadeh and Gordon, 1969) of ingested Mg^{2+} is ultimately taken up from the gut. However, these calculations do not take into account any Mg^{2+} excreted *via* the mucus tube solids, which may be considerable (Walsh *et al.* 1991), so absorption of Mg^{2+} even in SW trout is likely to be small. Therefore, the 'relative' impermeability to Mg^{2+} together with the dramatic increase in $[Mg^{2+}]$ at the very beginning of the intestine add some support to the idea that the pyloric region is responsible for most of the fluid volume absorbed.

The continued concentration of Mg^{2+} distally along the intestine (Fig. 2; a 20 % increase between the pyloric intestine and terminal rectal fluid) suggests that some further net fluid absorption occurs in the mid and posterior intestine. In contrast, the similar relative increase in luminal $[HCO_3^-+CO_3^{2-}]$ implies that little or no net base secretion occurs in these more distal portions of the trout intestine and that changes in base concentration here are simply due to net water movements. However, the luminal fluid pH did become significantly more alkaline along the intestine and, concomitantly, $[CO_3^{2-}]$ doubled between the pyloric intestine and the terminal rectal samples (Fig. 3). This suggests that, although the pyloric intestine does perform the majority of base secretion, the more distal regions may have an alkalizing effect, but in the absence of increasing $[HCO_3^-]$. Possible explanations include changes in the partial pressure of CO_2 (not measured), secretion of OH^- or CO_3^{2-}, or a selective permeability to HCO_3^- in the posterior intestine.

Within the pyloric region of the intestine, there are at least three possible tissues that could be responsible for the secretion of basic equivalents; (i) the epithelium lining the pyloric intestine lumen itself, (ii) the numerous blind-ended pyloric caeca, and (iii) the diffuse exocrine pancreatic tissue in the fat surrounding the pyloric caeca and the hepatic portal vein (Kurokawa and Susuki, 1995). At present, it is unclear which of these three might be responsible for net base secretion into the pyloric intestine lumen. Interestingly, Buddington and Diamond (1987) found that pyloric caeca represented 70 % of the post-gastric surface area in rainbow trout and concluded that, unlike the distal intestinal caeca of birds and mammals which serve a fermentatory role, the caeca in fish are an adaptation for increasing the total surface available for absorption without adding length or thickness to the intestine itself. Although this was based on studies on the absorption of nutrients, it could equally apply to fluid absorption (and perhaps base secretion) in the seawater-acclimated trout. However, the function(s) of pyloric caeca still remains a disputed subject.

Given the obvious anatomical comparison with the duodenum of mammals, it is tempting to draw an analogy between bicarbonate secretion from the mammalian pancreas

and base secretion in the teleost pyloric intestine. However, one key difference is that fluid and HCO_3^- are both secreted in mammalian pancreatic ducts, but they move in opposite directions in the teleost pyloric intestine. In addition, the alkaline pancreatic secretions of mammals serve only to neutralize stomach acid, and duodenal contents rarely exceed pH 6–7 (Ganong, 1989). Why the intestinal contents of seawater trout are so highly alkaline (up to pH 8.9) remains unclear, but will undoubtedly have a number of significant and as yet unrecognised repercussions in teleosts adapted to sea water (not least on the digestive functions of the intestine), in addition to overall acid–base balance.

Mechanism of intestinal base secretion

Although the precise location of base secretion within the pyloric intestine has not yet been identified, the *in vivo* perfusion experiments have given us some insight into the possible mechanisms involved, whichever tissue might be the ultimate source.

The *in vivo* perfused pyloric intestine demonstrated high rates of net base secretion (Fig. 4). This is perhaps not surprising given that the gradient for bicarbonate transport was maximized by having only 1 mequiv l^{-1} HCO_3^- present in the inflowing luminal perfusate. Although this is much lower than the concentration of basic equivalents measured *in situ*, it will be similar to the concentration in the fluid entering the pyloric intestine from the stomach *in vivo*, which is practically HCO_3^--free (Fig. 2). Net fluid absorption was similarly high (Fig. 5), with the average rate being more than four times greater than the whole-animal fluid absorption rates (derived from the drinking and rectal fluid flow rates in Table 2). However, given that the rate of entry of fluid from the stomach to the intestine may be three times higher than the drinking rate (owing to fluid movement from the blood into the stomach; Evans, 1993), the net fluid absorption rate measured in the perfused pyloric intestine may be in line with *in situ* rates in this part of the intestine.

In theory, net bicarbonate secretion could be explained by primary movement of any one of the components of the CO_2/HCO_3^- buffer system (i.e. HCO_3^-, CO_3^{2-}, OH^-, H^+ or CO_2). However, studies on bicarbonate-secreting epithelia in mammals suggest that either proton uptake or bicarbonate secretion is the primary transport process (Case and Argent, 1993).

The combination of (i) reciprocal changes in [HCO_3^-] and [Cl^-] along the GIT *in situ* (Fig. 2), (ii) a correlation between net HCO_3^-/CO_3^{2-} and Cl^- fluxes in the perfused pyloric intestine (Fig. 4), and (iii) the large reduction in net base secretion when Cl^- was removed from the lumen (Fig. 6A) all implicate some form of apical Cl^-/HCO_3^- exchange mechanism in base secretion. However, the lack of effect of either DIDS or SITS (Fig. 6B) suggests that this may not be the band 3 anion exchanger typical of, for example, erythrocyte cell membranes.

The 5:1 stoichiometry of net Cl^- fluxes to HCO_3^- fluxes (Fig. 4) suggests that any Cl^-/HCO_3^- exchange only contributes about one-fifth of the overall net Cl^- absorption, the majority of which most probably proceeds *via* an apical $Na^+/K^+/2Cl^-$ cotransporter. However, the dramatic increase in net base secretion in the presence of mucosal amiloride (Fig. 6A) implies that unidirectional base secretion into the pyloric intestine may be much higher than the net fluxes measured here, and normally takes place in the face of a large background secretion of protons. Indeed, in the presence of amiloride, the stoichiometry of net Cl^- absorption to basic equivalent secretion fell to 3.4:1. Thus, base secretion may drive a larger proportion of the net Cl^- absorption than predicted under the control conditions (in the absence of amiloride).

Some *in vitro* studies have demonstrated a HCO_3^--dependent Cl^- transport system in the intestinal epithelium of the flounder (*Pseudopleuronectes americanus*; Field *et al.* 1978), the goby (*Gillichthys mirabilis*; Dixon and Loretz, 1986) and seawater Japanese eels (*Anguilla japonica*; Ando, 1990; Ando and Subramanyam, 1990). Ando (1990) proposed that this system may contribute to intracellular pH regulation, which in turn controls K^+ recycling across the apical membrane (*via* a pH-sensitive K^+ channel), a process required for effective Na^+ and Cl^- uptake by the apical $Na^+/K^+/2Cl^-$ cotransporter (which drives net water absorption). Ando and Subramanyam (1990) further proposed that, in the seawater eel, HCO_3^- is taken into the cell on a basolateral Na^+/HCO_3^- cotransporter and probably leaves the apical cell membrane on a DIDS-sensitive Cl^-/HCO_3^- exchanger. However, the insensitivity of base secretion to mucosal DIDS or SITS in the present study agrees with the results of Dixon and Loretz (1986), who concluded that there must be an active Cl^-/HCO_3^- exchange mechanism on the basolateral membrane and a conductive exit pathway for HCO_3^- or OH^- (or entry of H^+) on the apical membrane. Without direct measurements of membrane potential and intracellular ion concentrations, it is impossible to say with certainty whether an active Cl^-/HCO_3^--ATPase is required to explain base secretion in the SW trout. However, an earlier report of a HCO_3^--activated ATPase in the intestinal mucosa of the seawater eel (Morisawa and Utida, 1976) adds further support to this suggestion. In general, little is known about anion-translocating ATPases, with the exception of recent studies by Gerencser and colleagues on a Cl^-/HCO_3^--stimulated ATPase in the intestinal mucosa of the mollusc *Aplysia californica* (e.g. Gerencser and Zelezna, 1993).

The small effect of acetazolamide on net base secretion in the present study together with the relatively low levels of carbonic anhydrase activity within all portions of the intestine imply that the majority of secreted HCO_3^- is transported across the entire epithelium in this form and that intracellular production from CO_2 hydration is minor. This again is similar to the conclusions of Dixon and Loretz (1986) for the posterior intestine of the goby.

In summary, we can now say that base excreted *via* the intestine of seawater-acclimated trout arises endogenously and contributes significantly to the overall acid–base balance. In

addition, this net loss of HCO_3^-/CO_3^{2-} base is countered by a similar net loss of acid from other parts of the body, most likely the gills. The majority of this HCO_3^-/CO_3^{2-} base appears to be secreted from somewhere within the pyloric (most anterior) region of the intestine, although the precise location remains unknown. The mechanism of base secretion within the pyloric intestine appears to involve Cl^-/HCO_3^- exchange, which is insensitive to inhibitors of passive Cl^-/HCO_3^- exchangers, suggesting the presence of a primary active HCO_3^--transporting ATPase.

It seems likely that intestinal base excretion is a phenomenon specific to teleosts adapted to life in sea water, because carbonate-like deposits have been found in at least five truly marine species (Walsh *et al.* 1991), whereas they disappear in both trout and toadfish transferred to low salinity (Shehadeh and Gordon, 1969; Walsh *et al.* 1991). Further experiments are required to address the question of whether intestinal base excretion is modified in response to internal acid–base status and thus complements the gills as a site of true acid–base regulation. In addition, the possible function(s) of intestinal base excretion remains unknown. Given the high content of Ca^{2+} and Mg^{2+} in the mucus tube 'solids' found in the intestine of SW trout (Shehadeh and Gordon, 1969), it is interesting to speculate that base secretion may facilitate the osmoregulatory function of the intestine in seawater-adapted teleosts by precipitating these divalent ions as carbonates, thereby reducing the effective osmolality of fluid along the intestine and promoting further water absorption. The role of intestinal base excretion in acid–base balance and osmoregulation of seawater-adapted teleosts warrants further investigation.

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