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THE EFFECTS OF HYPERSALINE EXPOSURE ON OXYGEN-AFFINITY OF THE BLOOD OF THE FRESHWATER TELEOST *CATOSTOMUS COMMERSONI*

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Summary

After 10 days' exposure to an environmental salinity of 300 mosmol kg^{-1} NaCl, the freshwater stenohaline teleost *Catostomus commersoni* exhibited an increase in plasma osmolality and a reduction in plasma strong ion difference (SID). There were reductions in plasma pH (pHe), red blood cell (RBC) pH (pHi), plasma total CO_2 and erythrocyte nucleoside triphosphate (NTP) concentration, and increases in mean erythrocyte volume and plasma catecholamine levels. Despite the acidosis, the *in vitro* haemoglobin oxygen-affinity of blood from saline-acclimated fish was not significantly different from that of control fish (held in fresh water) which had higher pHe and pHi values at the P_{CO_2} tensions used. *In vitro* adjustment of SID of blood from control fish to approximate that of the saline-acclimated fish by the addition of NaOH and HCl significantly reduced pHe, pHi and the haemoglobin oxygen-affinity. Adjustment of the plasma osmolality of blood from control fish to values identical to those of the saline-acclimated fish by the addition of NaCl *in vitro* did not alter the haemoglobin oxygen-affinity. An increase in catecholamine concentration and a decrease in red blood cell NTP concentration in the saline-acclimated fish may have been compensatory mechanisms to maintain haemoglobin oxygen-affinity against acidosis-induced Bohr and Root effects during saline exposure.

Introduction

There is a growing body of literature concerned with the effects of salinity on stenohaline freshwater fish. Recently, it has been reported that the stenohaline freshwater teleost (*Catostomus commersoni*, the white sucker) can remain hyperosmotic to a 300 mosmol kg^{-1} saline environment (0·9 % NaCl) by increasing extracellular and intracellular sodium and chloride concentrations (Wilkes & McMahon, 1986*a*,*b*). However, it was also noted that chloride concentration

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increased more than sodium concentration, resulting in a decrease in the plasma strong ion difference calculated according to Stewart (1981) ($Na^+ + K^+ + Mg^{2+} + Ca^{2+} - Cl^-$-anions of metabolic acids, e.g. lactate). Concomitant with the reduction in SID, plasma bicarbonate. concentration and pH fell precipitously. Similar electrolyte disturbances have been reported for the grass carp (Maceina *et al.* 1980), the carp (Hegab & Hanke, 1982) and the spotted gar (Smatresk & Cameron, 1982). The dramatic decrease in plasma pH should have reduced blood oxygen-affinity and content owing to the Bohr shift and Root effect, and therefore interfered with oxygen loading at the gills. However, a further study (Wilkes, 1984) has demonstrated no change in the rate of ventilation or oxygen consumption, and no increase in plasma lactate concentration, which would be indicative of anaerobic metabolism.

Studies on the effects of hypoxia, exercise and temperature in fish (Wood *et al.* 1975; Wood, 1980; Soivio & Nikinmaa, 1981; Nikinmaa *et al.* 1984; Weber & Lykkeboe, 1978) have attributed increases in haemoglobin oxygen-affinity to a decrease in red blood cell (RBC) nucleoside triphosphate concentration (ATP and GTP). The decrease in RBC ATP or GTP concentration can occur as a result of either RBC swelling and/or an absolute reduction in RBC content of nucleoside triphosphates, perhaps due to a fall in red cell pyruvate kinase activity (Nikinmaa, 1983). Erythrocyte swelling has been attributed to circulating catecholamines acting on β-adrenergic receptors present in the red cell membrane which, in turn, stimulate sodium and chloride influx and associated water entry (Nikinmaa *et al.* 1984; Nikinmaa, 1982, 1983; Nikinmaa & Huestis, 1984; Bourne & Cossins, 1982; Cossins & Richardson, 1985). The net effect of these ion fluxes appears to be an increase in RBC intracellular pH (pHi) through the resultant swelling which dilutes organic polyanions (NTP, haemoglobin) and by Na^+/H^+ exchange. The increase in pHi and decrease in NTP concentration contribute to an increase in haemoglobin oxygen-affinity.

The purpose of the present study was to test the hypothesis that the consequences of a chronic reduction of plasma SID and pH on haemoglobin oxygen-affinity are offset by catecholamine-induced RBC swelling and reduction in erythrocyte NTP concentration. Haemoglobin oxygen-affinity was tested *in vitro* using blood from saline-acclimated fish and control fish. To test the effects of alteration of plasma SID and osmolality on oxygen affinity, blood from control fish was adjusted *in vitro* to match the ionic composition and osmotic strength of the blood of saline-acclimated animals.

Materials and methods

Experimental procedures

White suckers, *Catostomus commersoni* (100–200 g), were obtained from a commercial source in Sarnia, Ontario and transported to McMaster University, Hamilton, Ontario. Fish were acclimated at 10°C in dechlorinated tap water for at

least 1 week prior to experimentation. The acclimation tap water had the following composition (in mequiv l^{-1}): $Na^+ = 0{\cdot}6$, $Cl^- = 0{\cdot}8$, $Ca^{2+} = 1{\cdot}9$, $Mg^{2+} = 0{\cdot}3$, $K^+ = 0{\cdot}05$, and total hardness = 140 mg l^{-1} as $CaCO_3$. Following acclimation, fish were placed in individual 2-l darkened acrylic boxes supplied with a continuous flow of water (1 l min^{-1}) at 10°C. After 3 days, half the fish were transferred, in their fish boxes (to avoid any stress associated with handling), to the experimental condition and the other half were maintained in dechlorinated tap water as control animals. The experimental condition consisted of dechlorinated tap water adjusted to 300 mosmol kg^{-1} with sodium chloride (approx. 0·9%). The water in the experimental tank was recirculated through a biological filter to prevent the build-up of nitrogenous waste products. Animals were fed every other day with Silver Cup trout pellets. All experiments were performed at the acclimation temperature of 10°C.

Experimental fish were acclimated to saline conditions for 10 days prior to sampling. Preliminary studies demonstrated that this was sufficient time to achieve constant levels of plasma electrolytes, pH and total CO_2. Blood samples from saline-acclimated and control fish were obtained as follows. Each fish was anaesthetized in its box by pumping water from the appropriate acclimation condition, with added MS-222, through the box. Upon anaesthesia, fish were immediately transferred to a sling on an operating table, and the gills were ventilated through the buccal chamber with the same anaesthetic solution. Arterial and/or venous blood samples were drawn into a heparinized glass syringe *via* blind puncture of the haemal arch within 3 min of anaesthetization. To obtain sufficient blood for all subsequent analyses (approximately 5 ml) it was sometimes necessary to pool samples from two or three fish. This method allowed fish to be anaesthetized and sampled with minimum stress and proved more satisfactory than caudal artery/vein cannulation, which often results in altered haematological parameters (decreases in haematocrit and plasma protein concentration; Wilkes *et al.* 1981). Although effects of MS-222 on red cell pH and volume have been reported (Soivio *et al.* 1977; Houston *et al.* 1971*a*,*b*) it was felt that any errors incurred would be consistent for both control and experimental groups.

To determine the independent effects of increased osmotic strength and decreased plasma SID on P_{50}, Bohr value and RBC nucleoside triphosphate levels, blood from control fish was divided into three parts and subjected to the following treatments. The osmolality of one sample was raised to approximate that of the saline-exposed fish (320 mosmol kg^{-1}) by adding 10 μl of 4 mol l^{-1} NaCl to 1 ml of whole blood. A second sample was adjusted to the approximate SID of the saline fish (based upon ion data from a preliminary study) by adding 10 μl of 4·4 mol l^{-1} NaOH and 10 μl of 5·7 mol l^{-1} HCl to 1 ml of whole blood. The third sample remained unaltered. Specimens of blood were removed from each sample for determination of NTP, electrolyte, lactate, protein and haemoglobin concentrations, and osmolality. The remainder of the blood was used in the determination of the Bohr value as outlined below. Similar measurements were obtained from unaltered blood of saline-acclimated fish.

Analytical techniques

The Bohr value was determined from the slope of a $\log P_{50}$/pHi regression as suggested by Albers (1985), where P_{50} (oxygen affinity) is the P_{O_2} at which blood is half-saturated with oxygen. The P_{50} was determined using a modification of the mixing technique (Scheid & Meyer, 1978). Briefly, a single sample of whole blood (1–2 ml) was divided between two glass tonometers attached to a wrist-arm shaker. The samples were sequentially equilibrated to humidified 0·20, 0·40 and 0·75 kPa P_{CO_2} (1 kPa = 7·519 mmHg) balanced with either air (oxygenated blood) or nitrogen (deoxygenated blood), delivered by Wöstoff gas-mixing pumps. These P_{CO_2} levels bracket the normal range of occurrence *in vivo* (Wilkes *et al.* 1981; Höbe *et al.* 1984). The samples were equilibrated at each of the three P_{CO_2} levels for at least 1 h. Following equilibration, 200 μl of deoxygenated and 200 μl of oxygenated blood were drawn anaerobically into a 500 μl Hamilton glass syringe in which the dead space in the needle had been displaced with mercury. The syringe was inverted several times to ensure complete mixing of the two bloods by the mercury bead prior to analysis of P_{O_2}, true plasma pH (pHe), and RBC intracellular pH (pHi).

Whole-blood P_{O_2} was measured with a Radiometer E5046 oxygen electrode, and pHe and pHi with a Radiometer G297/G2 capillary pH electrode. Both electrodes were maintained at 10°C. RBC pHi measurements were based on the red cell lysate method of Zeidler & Kim (1977), using the freeze–thaw technique described by Milligan & Wood (1986) to prepare the haemolysate. True plasma total CO_2 was measured with a Corning 960 CO_2 analyser. The plasma was obtained from sealed haematocrit tubes of the blood mixture following centrifugation for 2–3 min. The haematocrit (Hct) was recorded prior to removal of the plasma.

The oxygen content of whole blood was determined at full saturation, at a P_{CO_2} of 0·2 kPa, using a Lex-O_2 Con (Lexington Instruments Corporation) oxygen analyser. A sample volume of 20 μl of whole blood was used. After conversion of the oxygen content from vol% to $\mu mol\,l^{-1}$, the haemoglobin-bound oxygen ($\mu mol\,l^{-1}$) was calculated by subtracting the dissolved component, using the solubility of oxygen in human plasma (Boutilier *et al.* 1984). To express the content in terms of haemoglobin (Hb) concentration ($\mu mol\,O_2\,g^{-1}\,Hb$), the haemoglobin-bound O_2 ($\mu mol\,l^{-1}$) was divided by the haemoglobin concentration ($g\,l^{-1}$). Haemoglobin concentration was determined by the cyanmethaemoglobin method (Sigma kit no. 525).

Red blood cell nucleoside triphosphate (NTP) concentration in 250 μl samples of whole blood was determined enzymatically by the phosphoglycerate phosphokinase/glyceraldehyde phosphate dehydrogenase system (Sigma kit no. 366-UV). The blood was mixed with an equal volume of ice-cold 12 % (w/v) trichloroacetic acid and allowed to stand for 5 min in an ice-bath. After centrifugation, the supernatant was removed and stored frozen for no more than 12 h prior to assay. Although NTP was measured as $\mu mol\,NTP\,l^{-1}$ whole blood, results have been expressed as cellular concentrations in the RBC ([NTP]/Hct, i.e. $\mu mol\,ml^{-1}$ RBC)

and as contents per gram of haemoglobin (Hb) (i.e. μmol NTP g^{-1} Hb) since NTP is almost entirely intracellular in fish blood (Wood *et al.* 1975). Mean cellular haemoglobin concentration (MCHC) was calculated as the [Hb]/[Hct] ratio (i.e. g Hb ml^{-1} RBC).

Total plasma protein concentration was measured using Biuret reagent (Sigma). Lactate concentration was determined enzymatically by the L-lactate dehydrogenase method using Sigma kit no. 826-UV.

Concentrations of the plasma cations Na^+, K^+, Ca^{2+} and Mg^{2+} were measured using a Varian AA-1275 atomic absorption spectrophotometer and chloride was measured coulometrically using a Radiometer CMT 10 chloridometer. Plasma osmolality was determined by the use of a Wescor 5100C vapour pressure osmometer. Strong ion difference (SID, mequiv l^{-1}) was estimated by subtracting the sum of chloride and lactate concentrations from the sum of the concentrations of the four major strong cations (Na^+, K^+, Ca^{2+} and Mg^{2+}). All plasma samples were separated from the whole blood which had been equilibrated with room air.

RBC intracellular ion (Na^+, K^+ and Cl^-) concentrations were determined upon samples of haemolysed red cells. After centrifugation, the plasma layer was removed and distilled water added to haemolyse the packed cells. The exact dilution factor was determined gravimetrically. Samples were removed for Na^+ and K^+ analysis using an EEL flame photometer and Cl^- analysis using a Radiometer CMT 10 chloridometer. RBC intracellular electrolyte concentrations were expressed per litre of packed cells.

Separate blood samples for plasma catecholamine analysis were taken from both saline-acclimated and control groups. The blood was immediately centrifuged, approximately 200 μl of plasma drawn off, 10 μl of preservative added (90 mg ml^{-1} EDTA and 60 mg ml^{-1} glutathione; Sigma Chemical Co.), and then the samples were immediately frozen in liquid nitrogen. Plasma samples were stored at −80°C for not more than 60 days before analysis. Concentrations of epinephrine and norepinephrine were measured in duplicate using a ^{3}H-labelled radioenzymatic assay (Cat-A-Kit, Upjohn Diagnostics).

Significance among the means was tested by a one-way analysis of variance, followed by the Student–Newman–Keuls test. Multiple comparisons among linear regressions were performed using an analysis of covariance and Student-Newman-Keuls test (Zar, 1984).

Results

Table 1 illustrates the effect of the various treatments on the plasma ionic composition, plasma SID, plasma osmolality and protein concentration. In saline-acclimated fish, there were significant increases above control values ($P < 0{\cdot}001$) in the concentration of sodium, from 120 to 154 mequiv l^{-1}, and chloride, from 93 to 151 mequiv l^{-1}. There were no significant changes in the concentrations of potassium, calcium and magnesium. Lactate concentration fell from 1·3 to 0·5 mequiv l^{-1}. Thus the changes in sodium and chloride concentrations were

Table 1. *Plasma electrolyte, lactate and protein concentrations, osmolality and calculated SID from the control (CON), saline-acclimated (SAL), SID-adjusted (SID) and osmolality-adjusted (OSM) groups*

Group	$[Na^+]$	$[K^+]$	$[Ca^{2+}]$	$[Mg^{2+}]$	$[Cl^-]$	[lactate]	[SID]	Osmolality	[Protein]	RBC $[Na^+]$	RBC $[K^+]$	RBC $[Cl^-]$
Control												
Mean	119·9	3·3	6·4	1·9	93·1	1·3	37·0	252	36	24·1	80·8	54·6
S.E.M.	1·7	0·4	0·5	0·1	2·4	0·2	1·8	3	2	3·2	3·2	3·1
N	14	14	14	14	14	14	14	14	16	10	10	10
Saline-acclimated												
Mean	154·2	2·5	5·3	2·0	150·5	0·5	13·0	312	26	25·4	98·2	85·5
S.E.M.	2	0·2	0·1	0·2	3·1	0·1	2·8	5	3	3·1	5·1	2·8
N	8	8	8	8	8	8	8	8	7	6	6	6
SID-adjusted												
Mean	158·9	2·4	6·4	1·9	140·8	1·0	27·8	321	39	41·8	88·7	81·8
S.E.M.	2·9	0·5	0·7	0·1	4·0	0·1	2·3	6	3	5·5	6·2	7·1
N	9	9	9	9	9	9	9	9	8	7	7	7
Osmolality-adjusted												
Mean	155·4	3·2	7·0	1·8	132·8	1·3	33·4	317	42	30·6	99·7	63·7
S.E.M.	2·6	0·7	0·7	0·1	3·4	0·2	2·5	5	1	2	2·8	8·3
N	8	8	8	8	8	8	8	8	8	8	8	8

Student–Newman–Keuls multiple range test: underline indicates no significant difference ($P > 0{\cdot}05$) among means. Groups are ranked low to high.

Plasma $[Na^+]$	CON SAL OSM SID	Plasma [lactate]	SAL SID CON OSM	RBC $[Na^+]$	CON SAL OSM SID
$[K^+]$	SID SAL OSM CON	SID	SAL SID OSM CON	$[K^+]$	CON SID SAL OSM
$[Ca^{2+}]$	SAL SID CON OSM	Osmolality	CON SAL OSM SID	$[Cl^-]$	CON OSM SID SAL
$[Mg^{2+}]$	OSM SID CON SAL	[Protein]	SAL CON SID OSM		
$[Cl^-]$	CON OSM SID SAL				

Plasma electrolytes, lactate and SID are expressed in mequiv l^{-1}, protein in g l^{-1} and osmolality in mosmol kg^{-1}. RBC ion concentrations are expressed in mequiv l^{-1} of packed cells.

chiefly responsible for the significant decrease in SID ($P < 0{\cdot}001$), from 37 to 13 mequiv l^{-1}, and the increase ($P < 0{\cdot}001$) in plasma osmolality (from 252 to 312 mosmol kg^{-1}). Plasma protein concentration fell ($P < 0{\cdot}05$) from 36 in control fish to 26 g l^{-1} in the saline-acclimated group.

The concentrations of sodium, potassium, calcium, magnesium and chloride, and the osmolality, of SID-adjusted plasma were not significantly different from corresponding values in plasma of saline-acclimated fish. The only difference between these two groups was a significantly lower ($P < 0{\cdot}05$) plasma protein concentration (26 *vs* 39 g l^{-1}) in the saline-acclimated group. However, in spite of the similarities in ionic composition, the SID was only lowered to 27·8 mequiv l^{-1} in the plasma of SID-adjusted blood. Although this value was significantly below that of control fish (37·0 mequiv l^{-1}; $P < 0{\cdot}05$), it was also significantly above that of the saline-acclimated fish (13·0 mequiv l^{-1}; $P < 0{\cdot}05$). Two possible explanations for this difference are: (1) accumulated measurement error of the electrolytes, and (2) the inability to control compartmentalization of the added ions, for chloride may have entered the RBCs to a greater extent than sodium in the SID-adjusted blood, as indicated below and in Table 1.

Addition of equimolar amounts of sodium and chloride to blood from control fish increased plasma osmolality significantly ($P < 0{\cdot}001$) to match that of saline-acclimated animals, 317 mosmol kg^{-1}. Since the relative difference between sodium and chloride remained constant in the osmolality-adjusted blood, SID was 33·4 mequiv l^{-1}, a value not significantly different from that in control fish. With the exception of plasma osmolality, and concentrations of sodium and chloride, there were no other significant differences between control and osmolality-adjusted blood samples in terms of plasma ionic composition.

Red cell intracellular levels of sodium, potassium and chloride are also reported in Table 1. Although sodium concentration remained constant, potassium and chloride concentrations increased significantly ($P < 0{\cdot}05$) in red cells of saline-acclimated fish. In contrast, concentrations of intracellular sodium and chloride, but not of potassium, increased significantly ($P < 0{\cdot}05$) in SID-adjusted blood over control values. The absolute increase in chloride concentration was greater than that for sodium. However, there was no significant increase in either sodium or chloride concentration in the red cells of the osmolality-adjusted blood, although potassium concentration was elevated.

Haemoglobin (Hb) concentration, haematocrit (Hct), and both red blood cell NTP concentration and NTP content per gram Hb were significantly lower ($P < 0{\cdot}005$) in saline-acclimated than in the control animals (Table 2). However, the corresponding values in SID-adjusted and osmolality-adjusted blood were not significantly different from the control values. The mean cell haemoglobin concentration (MCHC) of the saline-acclimated group decreased significantly ($P < 0{\cdot}05$) from a control value of 0·253 to 0·229 g Hb ml^{-1}, suggesting RBC swelling. This, in turn, accounted for the greater fall in RBC NTP concentration (31 %) than in the [NTP]/[Hb] ratio (24 %). MCHC of the SID-adjusted blood was not different from the control value, whereas that of the osmolality-adjusted

Table 2. *Haematocrit (Hct), haemoglobin (Hb), mean cell haemoglobin concentration (MCHC) and nucleoside triphosphate concentration in blood from control (CON), saline-acclimated (SAL), SID-adjusted (SID) and osmolality-adjusted (OSM) groups*

Group	Hct (%)	Hb ($g l^{-1}$)	MCHC ($g ml^{-1}$ RBC)	NTP ($\mu mol ml^{-1}$ RBC)	NTP ($\mu mol g^{-1}$ Hb)	Norepinephrine ($nmol l^{-1}$)	Epinephrine ($nmol l^{-1}$)
Control							
Mean	32·0	80	0·253	11·3	45·1	4·6	12·7
S.E.M.	1·9	6	0·003	0·3	1·7	0·4	1·8
N	14	13	13	14	14	10	10
Saline-acclimated							
Mean	20·6	47	0·229	7·9	34·5	46·5*	44·7*
S.E.M.	3·0	8	0·006	0·6	2·2	14·3	13·4
N	7	7	7	7	7	9	9
SID-adjusted							
Mean	33·2	82	0·246	10·9	44·6		
S.E.M.	2·4	6	0·004	0·4	2·0		
N	5	5	5	5	5		
Osmolality-adjusted							
Mean	32·6	91	0·278	12·0	42·9		
S.E.M.	1·0	3	0·005	0·3	1·1		
N	8	8	8	8	8		

Student–Newman–Keuls multiple range test: underline indicates no significant difference ($P > 0.05$) among means. Groups are ranked low to high.

Hct SAL <u>CON OSM SID</u>

Hb SAL <u>CON SID OSM</u>

MCHC SAL <u>SID CON</u> OSM

$NTP\,ml^{-1}$ RBC SAL <u>SID CON OSM</u>

$NTP\,g^{-1}$ Hb SAL <u>OSM SID CON</u>

Plasma norepinephrine and epinephrine concentrations in control and saline-acclimated groups are also shown, and an asterisk indicates values significantly different from control ($P < 0.05$).

blood was significantly elevated ($P < 0{\cdot}001$) over the other three groups, suggesting cell shrinkage.

In the plasma of saline-acclimated fish, there were significant increases in concentration ($P < 0{\cdot}05$) of epinephrine, 3·5-fold over the control value of $12{\cdot}7\,\text{nmol}\,l^{-1}$, and of norepinephrine, 10 times greater than the value of $4{\cdot}6\,\text{nmol}\,l^{-1}$ (Table 2).

The effect of saline-acclimation, or artificially adjusting the plasma SID, was to lower significantly the plasma pH and total CO_2 at any given P_{CO_2} (Table 3). In contrast, the plasma pH in osmolality-adjusted plasma was not significantly different from the control level.

Changes in RBC intracellular pH (pHi) were qualitatively similar to those in pHe (Table 3). Thus osmolality adjustment had no significant effect on pHi at any given P_{CO_2}. In contrast, pHi fell significantly in both saline-acclimated and SID-adjusted blood at all P_{CO_2} values.

When pHi was plotted against pHe, it was clear that the absolute ranges for both values were shifted downwards by both saline-acclimation and SID-adjustment (Fig. 1). Overall there were no significant differences between the slopes of the four regression lines representing each of the treatments; however, the regression line for the osmolality-adjusted blood was significantly elevated ($P < 0{\cdot}05$) above that of the control blood.

Regardless of experimental treatment, oxygen affinity decreased (*i.e.* P_{50} increased) with increasing P_{CO_2} (Fig. 2). Despite the much lower pHe and pHi values in saline-acclimated fish, P_{50} of the blood was not significantly different from control values at P_{CO_2} values of either 0·20 or 0·40 kPa. At $P_{CO_2} = 0{\cdot}75$ kPa, the P_{50} was statistically greater ($P < 0{\cdot}05$) than that of the control. Although there was no significant difference in the oxygen affinity between control blood and osmolality-adjusted blood, *in vitro* reduction of SID resulted in a significant ($P < 0{\cdot}05$) elevation of P_{50} (decrease in oxygen affinity) at all three P_{CO_2} levels. At 0·75 kPa (a value representative of the maximum region of venous values; Wilkes *et al.* 1981) haemoglobin oxygen-affinity of SID-adjusted blood was statistically equivalent to that found in the saline-acclimated fish.

The oxygen affinity showed strong pH dependency (Fig. 3). As indicated by the regression lines, $\log P_{50}$ increased with a reduction in pHi in all four treatments. The average Bohr values ($\Delta \log P_{50}/\Delta$pHi) were −0·742, −0·688, −0·608 and −0·996 for the control, saline-acclimated, SID-adjusted and osmolality-adjusted blood, respectively. There were no significant differences among the slopes or y-intercepts of the regression lines for the SID-adjusted, osmolality-adjusted and control groups. However, the elevation of the regression line for the saline-acclimated group was significantly reduced ($P < 0{\cdot}05$) indicating that, at any given pHi, blood from saline-acclimated fish had a higher oxygen affinity (lower P_{50}).

In terms of oxygen-carrying capacity, there was a reduction in the amount of oxygen carried at full saturation (at $P_{CO_2} = 0{\cdot}20$ kPa) in the saline-acclimated fish (Table 4). The decrease, however, was due to the significant reduction ($P < 0{\cdot}05$) in haemoglobin concentration of the saline-acclimated fish compared with that of

Table 3. *Plasma pH (pHe), RBC pH (pHi) and plasma total CO_2 (C_{CO_2}) in blood from control (CON), saline-acclimated (SAL), SID-adjusted (SID) and osmolality-adjusted (OSM) groups at 0·20, 0·40 and 0·75 kPa P_{CO_2}* in vitro

	pHe			pHi			C_{CO_2} (mmol l^{-1})		
Group	at 0·20	at 0·40	at 0·75	at 0·20	at 0·40	at 0·75	at 0·20	at 0·40	at 0·75
Control									
Mean	8·09	7·92	7·75	7·52	7·43	7·34	9·1	10·9	12·0
S.E.M.	0·03	0·03	0·03	0·02	0·02	0·02	0·7	0·9	0·8
N	14	14	14	11	12	11	12	12	12
Saline-acclimated									
Mean	7·64	7·50	7·35	7·35	7·26	7·13	3·3	4·2	5·2
S.E.M.	0·04	0·03	0·03	0·03	0·03	0·03	0·4	0·5	0·3
N	7	7	7	7	6	7	7	7	7
SID-adjusted									
Mean	7·59	7·47	7·38	7·38	7·25	7·17	3·0	4·1	5·4
S.E.M.	0·06	0·06	0·04	0·03	0·02	0·02	0·4	0·9	0·8
N	5	4	5	5	4	5	5	3	4
Osmolality-adjusted									
Mean	7·98	7·83	7·67	7·52	7·46	7·36	8·0	9·4	10·7
S.E.M.	0·03	0·04	0·04	0·02	0·02	0·03	0·7	0·9	0·7
N	8	8	8	8	7	6	8	8	8

Student–Newman–Keuls multiple range test: underline indicates no significant difference ($P > 0·05$) among means. Groups are ranked low to high.

pHe 0·20 kPa	SID	SAL	OSM	CON	pHi 0·20 kPa	SAL	SID	OSM	CON	C_{CO_2} 0·20 kPa	SID	SAL	OSM	CON
0·40 kPa	SID	SAL	OSM	CON	0·40 kPa	SID	SAL	CON	OSM	0·40 kPa	SID	SAL	OSM	CON
0·75 kPa	SAL	SID	OSM	CON	0·75 kPa	SAL	SID	CON	OSM	0·75 kPa	SAL	SID	OSM	CON

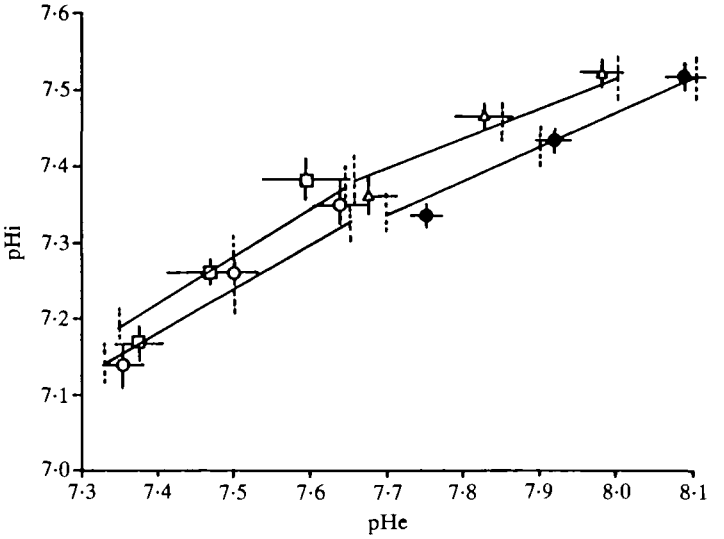

Fig. 1. Linear regression of RBC pH (pHi) *vs* plasma pH (pHe) of blood from control (●), saline-acclimated (○), SID-adjusted (□) and osmolality-adjusted (△) groups equilibrated *in vitro* to 0·20, 0·40 and 0·75 kPa P_{CO_2}. The regression lines were calculated from raw data using the least-squares method. For purposes of clarity, points represent the mean ± 1 S.E.M. for each group, at each of the three P_{CO_2} tensions used, and the dotted lines represent the 95 % confidence limits of each regression. Except for a significant difference ($P < 0{\cdot}05$) between the elevation (y-intercept) of the regression line for the osmolality-adjusted group compared with the control, there were no statistical differences ($P > 0{\cdot}05$) among the groups in terms of the slopes or elevations. Regression lines are described by the following equations:

control	($N = 34$)	pHi = 3·943 + 0·441pHe ($r = 0{\cdot}75$),
saline-acclimated	($N = 20$)	pHi = 3·054 + 0·558pHe ($r = 0{\cdot}83$),
SID-adjusted	($N = 14$)	pHi = 2·990 + 0·572pHe ($r = 0{\cdot}76$),
osmolality-adjusted	($N = 20$)	pHi = 4·395 + 0·390pHe ($r = 0{\cdot}74$).

All r values are statistically significant at $P < 0{\cdot}05$.

the other three groups. When expressed in terms of haemoglobin concentration (i.e. μmol O_2 g^{-1} Hb), the amount of oxygen carried at full saturation in these fish was not significantly different from the control or osmolality-adjusted groups. However, in the SID-adjusted group, there was a significant reduction ($P < 0{\cdot}05$).

Discussion

The ionic, osmotic, haematological and acid–base disturbances observed in the plasma of saline-acclimated *C. commersoni* are similar to those previously reported (Wilkes & McMahon, 1986*a*) and to those for grass carp (Maceina *et al.* 1980), spotted gar (Smatresk & Cameron, 1982) and carp (Hegab & Hanke, 1982). The increase in plasma osmolality was primarily due to an increase in sodium and chloride concentrations. Because the plasma concentrations of potassium, calcium

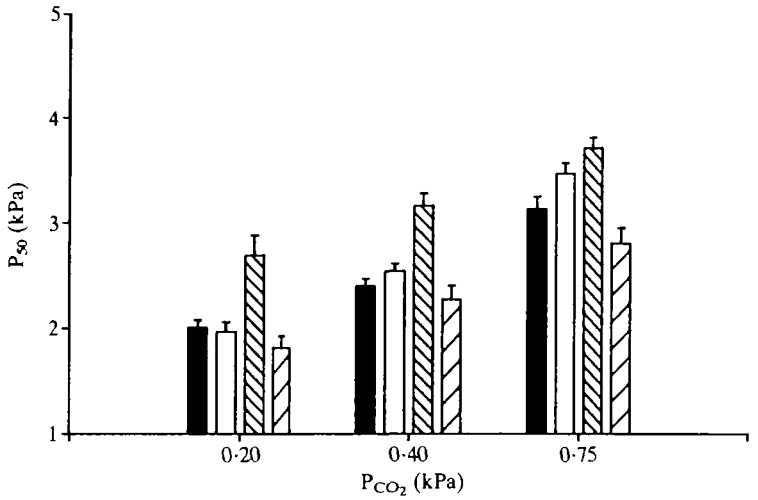

Fig. 2. LogP_{50} *vs* P_{CO_2} of blood from control (■), saline-acclimated (□), SID-adjusted (▦) and osmolality-adjusted (▨) groups equilibrated *in vitro* to 0·20, 0·40 and 0·75 kPa P_{CO_2}. Bars represent the mean+1S.E.M. for each group. Lines underscore subsets of means among which there were no statistical differences ($P > 0{\cdot}05$) in logP_{50} at each of the three values of P_{CO_2} as determined by Student–Newman–Keuls test.

0·20 kPa P_{CO_2} OSM CON SAL SID
0·40 kPa P_{CO_2} OSM CON SAL SID
0·75 kPa P_{CO_2} OSM CON SAL SID.

and magnesium remained constant, it is reasonable to assume that the increases in sodium and chloride concentration were due to a net influx during the acclimation process rather than an osmotic loss of water. Indeed, the decreases in haematocrit and plasma protein and haemoglobin concentrations suggest that plasma volume was expanded during saline acclimation. The decrease in plasma SID was attributable to the greater increase in chloride concentration over that of sodium. Wilkes & McMahon (1986*b*) have suggested that the mechanism by which the net influx of chloride exceeds that of sodium is probably related to the approximately neutral electrochemical gradient for the latter and an inwardly directed gradient for the former during saline exposure.

Coincidental with the decrease in plasma SID in the saline-acclimated fish were the decreases in plasma pH and total CO_2 observed *in vitro* at any given P_{CO_2}. Lactacidosis was not involved. The decrease in plasma pH might be expected to reduce the oxygen affinity of haemoglobin, owing to the Bohr shift, and impair oxygen loading at the gills, owing to the Root effect. However, under identical conditions Wilkes (1984) found that ventilation and respiration rates remained at control levels during saline exposure, which suggests that oxygen uptake and delivery to the tissues were unaffected. Results from the present study demonstrate that oxygen affinity and capacity of haemoglobin measured *in vitro* were maintained close to control values during saline exposure. The P_{50} values at P_{CO_2}

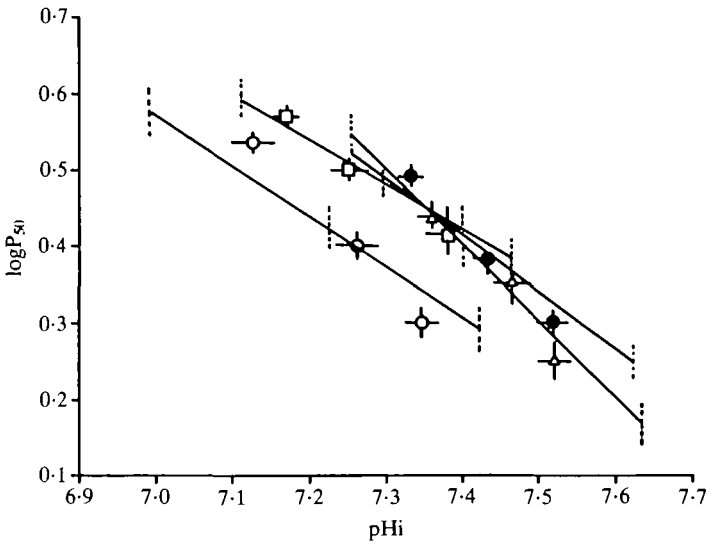

Fig. 3. Linear regression of $\log P_{50}$ *vs* RBC pH (pHi) for blood from control (●), saline-acclimated (○), SID-adjusted (□) and osmolality-adjusted (△) groups. The regression lines were calculated from raw data using the least-squares method. For purposes of clarity, points represent the mean ± 1 S.E.M. for each group, at each of the three P_{CO_2} tensions used in the *in vitro* determination of blood oxygen affinity, and the dotted lines represent the 95 % confidence limits of each regression. As determined by analysis of covariance, there were no significant differences ($P > 0{\cdot}05$) among the slopes (Bohr values) of the regression lines; however, there were differences ($P < 0{\cdot}05$) among the elevations (y-intercepts). Lines underscore subsets of means among which there were no statistical differences ($P > 0{\cdot}05$) in elevation of the regression lines as determined by Student–Newman–Keuls test.

SAL SID CON OSM

Regression lines are described by the following equations:

control ($N = 34$)	$\log P_{50} = 5{\cdot}899 - 0{\cdot}742\text{pHi}$	($r = -0{\cdot}75$),
saline-acclimated ($N = 20$)	$\log P_{50} = 5{\cdot}399 - 0{\cdot}688\text{pHi}$	($r = -0{\cdot}75$),
SID-adjusted ($N = 14$)	$\log P_{50} = 4{\cdot}917 - 0{\cdot}608\text{pHi}$	($r = -0{\cdot}83$),
osmolality-adjusted ($N = 20$)	$\log P_{50} = 7{\cdot}769 - 0{\cdot}996\text{pHi}$	($r = -0{\cdot}88$).

All r values are statistically significant at $P < 0{\cdot}05$.

levels representative of arterial blood (0·2–0·4 kPa), and the Hb-O_2 content at saturation (at $P_{CO_2} = 0{\cdot}2$ kPa) were the same as those measured under control conditions (Fig. 2; Table 4) despite markedly lower pHe and pHi values at these P_{CO_2} tensions. P_{50} was slightly elevated at a P_{CO_2} of 0·75 kPa, which is at the high end of the venous range (Wilkes *et al.* 1981). When the SID of blood from control fish was adjusted *in vitro* to match that of the saline-acclimated fish, the pHe and RBC pHi values matched those of the saline-acclimated group (Table 3). However, strong positive Bohr and Root effects were noted (Fig. 3, Table 4). Absolute confirmation requires *in vivo* measurements of arterial and venous blood

Table 4. *Haemoglobin oxygen content (vol% and μmol g^{-1} Hb) at full saturation and 0·20 kPa P_{CO_2} for control (CON), saline-acclimated (SAL), SID-adjusted (SID) and osmolality-adjusted groups (OSM)*

Group	Oxygen content (vol%)	Oxygen content (μmol g^{-1} Hb)
Control		
Mean	11·9	63·7
S.E.M.	0·6	1·4
N	10	10
Saline-acclimated		
Mean	6·7	68·0
S.E.M.	1·0	1·4
N	7	6
SID-adjusted		
Mean	9·8	54·7
S.E.M.	0·7	2·2
N	7	7
Osmolality-adjusted		
Mean	12·8	66·5
S.E.M.	0·6	1·4
N	6	6

Student–Newman–Keuls multiple range test: underline indicates no significant difference ($P > 0·05$) among means. Groups are ranked low to high.

O_2 content (vol%)	SAL SID CON OSM
O_2 content (μmol g^{-1} Hb)	SID CON OSM SAL

gases, but the available evidence is highly supportive of the hypothesis that regulation of haemoglobin oxygen-affinity helps protect blood oxygen delivery during saline exposure in the white sucker.

That haemoglobin oxygen-affinity was defended in the saline-acclimated fish is also illustrated in Fig. 3. There was no significant difference in the $\log P_{50}$ *vs* pHi regression between osmolality-adjusted, control and SID-adjusted blood. However, despite the lower RBC pHi in saline-acclimated fish, haemoglobin oxygen-affinity was actually increased, as indicated by the position of the $\log P_{50}$/pHi regression.

Coincidental with these findings were observations of a swelling of erythrocytes and a reduction in [NTP]. It is well known that a decrease in cellular NTP concentration increases oxygen affinity (Wood *et al.* 1975; Wood, 1980; Weber & Lykkeboe, 1978). Reduction in fish red cell NTP concentration may be due to a catecholamine-induced swelling of the cells, or reduction in cellular nucleotide synthesis (Nikinmaa, 1983). Both mechanisms may have exerted an influence on

red cell NTP levels in the current experiment, with the metabolic change exerting the quantitatively larger effect. The [NTP]/[Hb] ratio fell by about 24 % in saline-acclimated fish (Table 2), suggesting reduced nucleotide synthesis and/or enhanced catabolism. In addition, an RBC swelling of about 10 % was indicated by the fall in MCHC in saline-acclimated fish, resulting in an overall reduction of about 31 % in the concentration of NTP in the intracellular milieu (Table 2). Coincidental with the reduction in MCHC and cellular NTP concentration were increases in plasma norepinephrine and epinephrine concentrations (Table 2). The catecholamine effect on the RBC involves both an inhibition of pyruvate kinase, resulting in decreased NTP synthesis, and a stimulation of cell swelling (Nikinmaa, 1982, 1983; Nikinmaa *et al.* 1984). The swelling occurs through stimulation of β-adrenergic receptors on the surface of the erythrocyte which activates ion uptake and osmotic influx of water. Recently, it has been shown that β-adrenergic receptors are present on white sucker erythrocytes: swelling occurs in the presence of epinephrine, and the response can be blocked in the presence of propranolol (R. L. Walker & D. E. Chiles, unpublished observations).

The manipulations of SID and osmolality *in vitro* were performed to determine what direct role, if any, the plasma strong ion difference and osmotic changes had on the observed protection of O_2 affinity and associated intracellular changes. The answer appears to be very little. Although both treatments tended to raise pHi slightly at a given pHe (Fig. 1), neither had any acute effect in lowering NTP levels or MCHC (i.e. causing cell swelling, Table 2) or in lowering P_{50} at a given P_{CO_2} (Fig. 2) or pHi (Fig. 3). Indeed, osmolality-adjusted blood demonstrated a significant increase in MCHC (cell shrinkage, Table 2), whereas SID-adjusted blood exhibited a significant elevation in P_{50} (i.e. reduced O_2 affinity) at the two lower values of P_{CO_2} (Fig. 2).

In rainbow trout, an increase in RBC pHi associated with catecholamine-mediated RBC swelling and ion fluxes appears to be a major factor in the protection of haemoglobin oxygen-affinity during extracellular acidosis (Nikinmaa, 1982, 1983; Cossins & Richardson, 1985; Primmett *et al.* 1986; Milligan & Wood, 1986; Perry & Vermette, 1987). Although pHi regulation may have made some contribution to the maintenance of haemoglobin oxygen-affinity in the present study, it does not appear to have been a major factor. RBC pHi fell markedly in concert with the extracellular acidosis associated with plasma SID reduction during chronic saline exposure of the white sucker (Fig. 1; Table 3). The changes in pHi of the saline-acclimated fish erythrocytes were equivalent to those associated with acute plasma SID reduction *in vitro*, where catecholamines were not elevated, and the slopes of all four pHi/pHe regressions were not different. Therefore, despite the increase in plasma catecholamines in the saline-acclimated group, there was not the markedly reduced effect of pHe on pHi reported by Nikinmaa (1983) for rainbow trout.

Although the positions of the pHi *versus* pHe relationships were slightly elevated in both saline-acclimated and SID-adjusted blood relative to that in the control (Fig. 1), the differences were not significant and the comparisons were

made over a different range of pHe. Over a comparable range, a similar but significant elevation was seen in osmolality-adjusted blood. This implies that increase in blood osmotic concentration, to the extent seen in this study, alters the blood pHi/pHe relationship in favour of a slightly greater pHi at any given pHe.

With regard to the red blood cell ion concentrations, the control values are similar to those reported for carp and rainbow trout (Houston & Smeda, 1979). However, the increases in sodium and/or potassium ion concentrations in the red cells of osmolality-adjusted and SID-adjusted blood, as well as in the erythrocytes from saline-acclimated fish, are difficult to explain. The increases in ion concentration in the red cells of the osmolality-adjusted blood are likely to be due in part to cell shrinkage, although MCHC decreased by only 10% whereas Na^+ and K^+ concentrations were elevated by about 25%. Surprisingly, the sodium and potassium concentrations were elevated in the SID-adjusted RBCs despite no change in cell volume.

Of major interest, owing to a potential influence on haemoglobin oxygen-affinity, is the rise in the red cell chloride concentrations in the saline-acclimated and SID-adjusted blood. These changes are not unexpected as trout red cells are readily permeable to chloride (Heming *et al.* 1986). A rise in plasma chloride concentration seen in these two groups probably resulted in an increase in RBC chloride, presumably due to diffusion. Such an increase may have a negative influence on haemoglobin oxygen-affinity (Houston & Smeda, 1979). This, along with the reduction in pHi, may explain the reduced affinity seen in the SID-adjusted blood samples. However, similar values for pHi and chloride concentration in red blood cells from saline-acclimated fish did not coincide with reduced oxygen-affinity. Again, the lower NTP concentration in the blood of the saline-acclimated fish may be the important difference in this regard.

In summary, plasma osmolality increases and SID decreases in the freshwater stenohaline teleost *C. commersoni* acclimated to an environmental salinity of 300 mosmol kg^{-1} (NaCl). Although the mechanism underlying the observed changes in pH and total CO_2 can be described qualitatively by the reduction in SID, quantitative prediction depends on the empirical determination of the weak acid concentration (equivalents per litre), the dissociation constants pertinent to the experimental species and temperatures and, finally, strong ion activities rather than molar concentrations. Substantial decreases in plasma pHe, RBC pHi, plasma total CO_2 and haemoglobin oxygen-affinity were observed when plasma SID was artificially reduced *in vitro*. However, the effects on oxygen affinity were significantly attenuated in saline-acclimated fish, largely as a result of reduction in red cell NTP concentration (due perhaps to metabolic degradation and cell swelling). Coincident, and perhaps responsible for the reduction in red cell NTP and the swelling, was an elevation in plasma catecholamines.

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