THE JOURNAL OF EXPERIMENTAL ZOOLOGY 242:273-281 (1987)

Control of Red Cell Volume and pH in Trout: Effects of Isoproterenol, Transport Inhibitors, and Extracellular pH in Bicarbonate/Carbon Dioxide-Buffered Media

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ABSTRACT The effects of extracellular pH and beta-adrenergic stimulation on the volume and pH of rainbow trout red cells were studied in HCO_3^-/CO_2 buffered media. A decrease in extracellular pH caused an increase in red cell volume and a decrease in intracellular pH. The pH-induced changes in cell volume were inhibited by 4,4'-Diisothiocyanostilbene-2,2'-Disulfonic acid, (DIDS), an inhibitor of the anion exchange pathway, but not by amiloride, an inhibitor of Na^+/H^+ exchange, indicating that these volume changes are mainly associated with movements of chloride across the red cell membrane, and that the Na^+/H^+ exchanger is not activated by changes in intracellular pH alone. The adrenergic drug, isoproterenol, promoted cell swelling and proton extrusion even in the presence of 10 mM HCO_3^-, showing that the adrenergic response plays a significant role in the control of cytoplasmic pH. These responses were enhanced by a decrease in extracellular pH, showing that the adrenergic response is of benefit to stressed animals. DIDS markedly enhanced the effect of isoproterenol on the pH_i, but abolished the increase in red cell volume. The effects of furosemide were similar to those of DIDS, suggesting that these transport inhibitors have a similar mode of action. Amiloride, on the other hand, inhibited both the volume and the pH changes associated with adrenergic stimulation. These observations support the double Na^+/H^+ and HCO_3^-/Cl^- exchange model of adrenergic swelling in fish red cells.

Adrenergic stimulation of trout red cells in vitro causes an increase in cell volume and a decrease in the concentration gradient of protons across the cell membrane (Nikinmaa, '82a; Nikinmaa and Huestis, '84; Cossins and Richardson, '85). The increase in intracellular pH appears to be due to beta-adrenergic activation of Na^+/H^+ exchange (Baroin et al., '84b; Nikinmaa and Huestis, '84; Cossins and Richardson, '85), whereas the swelling involves uptake of water following the accumulation of Na^+ and Cl^- in the cell, most likely by a double Na^+/H^+ and HCO_3^-/Cl^- exchange (Nikinmaa and Huestis, '84; Borgese et al., '86).

In all of the above studies, the cells were in nominally bicarbonate-free media. Nikinmaa ('82a, '83) used TRIS-buffered media, Nikinmaa and Huestis ('84) and Baroin et al. ('84a,b) used HEPES-buffered solutions, and Cossins and Richardson ('85) used imidazole-buffered medium. Baroin et al. ('84b) question the significance of the Na^+/H^+ exchange in the control of red cell pH because in the presence of a high-capacity anion exchange pathway (Romano and Passow, '84; Baroin et al., '84a; Cossins and Richardson, '85; Heming et al., '87), the control of pH by Na^+/H^+ exchange may be redundant.

In contrast, Nikinmaa ('83) and Cossins and Richardson ('85) suggest that the activation of Na^+/H^+ exchange might influence oxygen binding of rainbow trout red cells by increasing intracellular pH. Thus, both in rainbow trout (Primmett et al., '86) and in striped bass (Nikinmaa et al., '84), catecholamine

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release during strenuous exercise affects red cell pH and, most likely, helps maintain the oxygen affinity of red cells in the face of extracellular acidification. Furthermore, adrenalin increases the intracellular pH of red cells suspended in plasma, when the extracellular pH is adjusted by changing the carbon dioxide tension of the medium (Heming et al., '87). Thus, the adrenergic responses affect the intracellular pH even in the presence of an HCO_3^-/CO_2 buffering system. Also, Cossins and Richardson ('85) have shown that the capacity of Na^+/H^+ exchange system approaches that of the anion exchanger.

In vitro, the red cell volume is also increased by extracellular acidification (e.g. Van Slyke et al., '23; Irving et al., '41). Owing to the rapid movements of Cl^- and HCO_3^- across the red cell membrane, an extracellular acidification also leads to a decrease in intracellular pH. This decreases the negative charge on hemoglobin. To maintain electroneutrality, the intracellular concentration of Cl^- and HCO_3^- increases, and water follows osmotically; (for review, see e.g. Hladky and Rink, '77). Intracellular acidification as such activates the Na^+/H^+ exchanger in lymphocytes (Grinstein et al., '85). Consecutively, the cell volume increases. It is not clear if the Na^+/H^+ exchanger of fish red cells can be activated by pH changes alone. Furthermore, the interactions between pH and adrenergic stimulation have been seldom studied.

In the present paper, we have studied the effects of pH and isoproterenol, a beta-adrenergic agonist, on the volume and pH of trout red cells in HCO_3^-/CO_2 buffered media. By the use of the transport inhibitors, DIDS, furosemide, and amiloride, we have examined the roles of Na^+/H^+ and HCO_3^-/Cl^- exchange in the volume and pH changes. The effects of temperature on the adrenergic response were also studied, since changes in trout red cell volume in vivo after adrenalin injection appear to be different at different temperatures (Nikinmaa, '82b).

MATERIALS AND METHODS

Rainbow trout of both sexes (wt 200–300 g; N = 70) were obtained from Sun Valley Hatchery, Mission, British Columbia, and held outdoors at 13–15°C. Blood was taken into heparinized syringes from stunned fish via caudal or cardiac puncture, and the red cells washed twice in the Ringer's solution used. The Ringer's solution had the following composition: 145 mM NaCl, 4 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5 mM glucose, and 5 or 10 mM $NaHCO_3$. The pH of the extracellular medium was adjusted by varying the CO_2 tension, which ranged from atmospheric (pH 8.2; in this case, 10 mM HEPES was added to the buffer to stabilize the pH) to 2% CO_2 (pH 7.1) when the HCO_3^- concentration was 5 mM; in 10 mM HCO_3^- buffer, the pH 7.1 was obtained by using 4% CO_2.

After the washes, the sample was divided into four portions. Two of the subsamples were incubated for 30 min in a shaking tonometer in the Ringer's alone; in another, 0.1 mM DIDS (final concentration) was added; and in the fourth, 1 mM amiloride was added to investigate the effects of these drugs in the absence of adrenergic stimulation. The cells were incubated at different extracellular pH values (7.1–8.2), bicarbonate concentrations (5 and 10 mM), and temperatures (10 and 18°C). After the 30-min incubation, 0.4-ml samples were taken for extra- and intracellular pH determinations and determinations of cellular water content. Immediately after sampling, 10^{-5} M (final concentration) isoproterenol, freshly made for each experiment, was added to the DIDS and amiloride incubations and one of the incubations with Ringer's alone, and the cells incubated for another 30 min. Thereafter, another set of samples was taken for pH and cell water determinations. Similar incubations were also carried out using Ringer's in which either choline was substituted for sodium (pH 7.1), or 0.1 mM 2,4-dinitrophenol (a protonophore; see McLaughlin and Dilger '80) (pH 7.1) or 0.1 mM furosemide (pH 7.2) was added to the Ringer's.

Immediately after sampling, the cells and plasma were separated by 1 min centrifugation, and plasma pH measured using a Radiometer PHM 72 pH meter. The intracellular pH was determined from the distribution of the weak acid, ^{14}C-dimethyl oxazolidine-2,4-dione (DMO), between incubation medium and red cells as described by Nikinmaa and Huestis ('84), except that the cells were hemolyzed and deproteinized in 0.6 M perchloric acid to minimize quenching. The cellular water content (given as % = mg H_2O/100 mg wet cells) was determined from the dry and wet weight of the samples as described by Nikinmaa and Huestis ('84). Trapped extracellular water (2–3%; e.g. Houston, '85) was

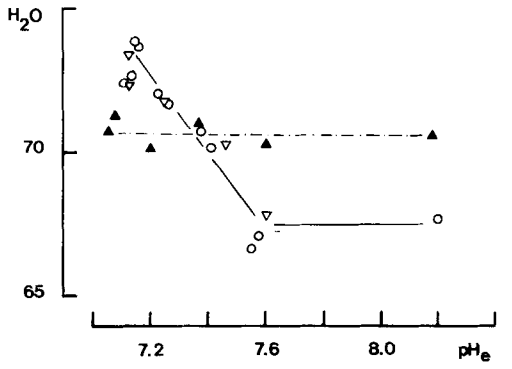

Fig. 1. Effect of external pH on the cellular water content of rainbow trout red cells. ○, control cells, [HCO_3^-] 5 mM; ▽, amiloride-treated cells (1 mM); ▲, DIDS-treated cells (0.1 mM). Each point represents mean of eight determinations. The cellular water content of control and amiloride-treated cells increased significantly below pH 7.6 (Mann-Whitney U-test, $P < 0.05$; the values below 7.6 compared to those at 7.6 and 8.2). No changes were observed in DIDS-treated cells.

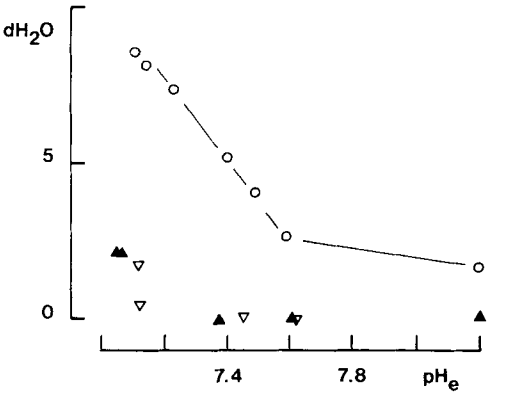

Fig. 2. Isoproterenol-induced increase in cellular water content of rainbow trout red cells. Cells were initially incubated for 30 min and their water content determined. Thereafter, 10^{-5} M isoproterenol was added to the medium and the water content determined after a further 30 min incubation. dH_2O (%) = $(H_2O_{isoproterenol} - H_2O)/H_2O$. Each point represents eight determinations. ○, control cells (10^{-5} M isoproterenol only); ▽, amiloride-treated cells (1 mM amiloride + 10^{-5} M isoproterenol); ▲, DIDS-treated cells (0.1 mM DIDS + 10^{-5} M isoproterenol. At each pH studied, treatment with isoproterenol caused a significant (Mann-Whitney U-test, $P < 0.05$) increase in cellular water content. This response was enhanced by a decrease in the pH. DIDS and amiloride drastically reduced the adrenergic response; the volume change was significant only in cells treated with DIDS at pH 7.1.

not taken into account. The chloride concentrations were determined using Radiometer CMT10 coulometric chloride titrator, and chloride ratios (Cl^-_{in}/Cl^-_{out}) calculated from the internal and external concentrations (in mmol/l water).

RESULTS

Cellular water content

Below extracellular pH value 7.6, the cell water increased linearly with decreasing extracellular pH, from 67–68% at pH 7.6 to 72–73% at pH 7.1 (Fig. 1). The volume of amiloride-treated cells changed as did that of control cells, whereas DIDS effectively abolished the pH-dependent changes in cell volume.

At each pH value studied, isoproterenol caused an increase in the cell volume. Again, this effect was pH-dependent, being ca. 1.5% at pH 8.2 and ca. 8% at pH 7.1 (Fig. 2). The isoproterenol-induced increase in cell volume was similar in both 5 and 10 mM HCO_3^-; the cell water content at extracellular pH 7.10–7.15 was 72.4 ± 0.4% in 5 mM HCO_3^- and 72.3 ± 0.5% in 10 mM HCO_3^- before the addition of isoproterenol, and increased to 78.2 ± 0.3 in 5 mM HCO_3^- and to 78.4 ± 0.3% in 10 mM HCO_3^- after the addition of isoproterenol (N = 8 in each case). Together, the beta-adrenergic and pH-induced changes represent a 15% increase in cellular water content between pH 8.2 and 7.1. The beta-adrenergic cell swelling was abolished by DIDS, amiloride (Fig. 2), and furosemide (furosemide control = 72.7 ± 1.3%; furosemide + isoproterenol = 73.1 ± 1.1%, N = 5 in each case).

Red cell pH

The red cell pH of untreated cells, measured by the DMO method and given from the Cl^- distribution supposing that both Cl^- and H^+ are passively distributed, were not significantly different at pH values above 7.3 (Fig. 3); at lower extracellular pH values, the DMO method gave consistently higher values for pH_i. The pH_i of nontreated cells at low extracellular pH was not affected by amiloride or by removing Na^+ from the incubation medium (Table 1), suggesting that Na^+/H^+ exchange does not occur under these conditions.

Above pH_e 7.4, DIDS did not affect the pH gradient across the red cell membrane but, at low extracellular pH values, caused a marked intracellular alkalinization as compared to control cells (Fig. 4). Simultane-

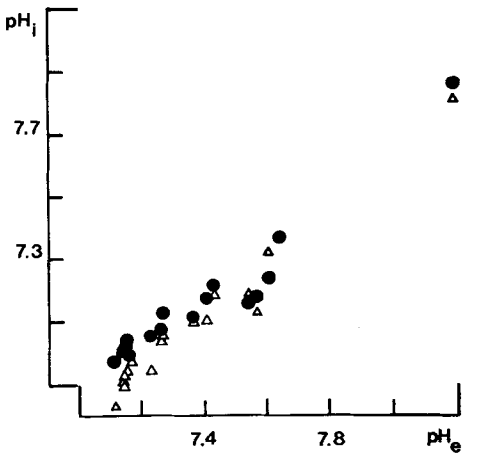

Fig. 3. Intracellular (pH_i) vs. extracellular pH (pH_e) of rainbow trout red cell suspensions. Each point represents a mean of three to eight determinations. ●, values measured using DMO method; △, values calculated from the chloride distribution across red cell membrane. The dependence of intracellular pH from extracellular pH, calculated for individual data points, is given by the following regression lines. DMO method: $pH_i = 0.725 \times pH_e + 1.80$, r = 0.93, n = 116; Cl distribution: $pH_i = 0.821 \times pH_e + 1.05$, r = 0.95, n = 77. The slope of regression line for Cl^- distribution is significantly ($P < 0.001$) steeper than the regression line for DMO method.

ously, a marked discrepancy between the proton (H^+_{out}/H^+_{in}) and chloride (Cl^-_{in}/Cl^-_{out}) distribution ratio across the red cell membrane developed; at extracellular pH 7.077 ± 0.007, the proton distribution ratio was 1.41 and chloride distribution ratio 0.47 (means of eight determinations). The alkalinization was independent of amiloride or Na^+, indicating that Na^+/H^+ exchange is not involved. It was prevented by treating the cells with 2,4-DNP (Table 2), which increases the proton permeability of biological membranes. At extracellular pH 7.1, the DIDS-induced alkalinization was dependent on the HCO_3^- concentration of the medium; it did not occur in HEPES-buffered medium and was significantly smaller in 5 mM than in 10 mM HCO_3^- concentration at constant pH.

Isoproterenol caused a significant reduction in the pH gradient across the red cell membrane at every pH studied except pH 8.2 (Fig. 5). This reduction was inhibited by amiloride (Table 3). This probably reflects inhibition of Na^+/H^+ exchange. The adrenergic response was enhanced by either DIDS or

TABLE 1. Extracellular and intracellular pH and the pH gradient across the red cell membrane in control cells, in amiloride (1 mM)-treated cells, and in cells incubated in the absence of sodium at low extracellular pH value

Treatment	pH_e	pH_i	dpH
Control (N = 4)	7.231 ± 0.018	7.051 ± 0.030	0.180
Amiloride (N = 4)	7.262 ± 0.008	7.070 ± 0.012	0.192
No sodium (N = 8)	7.207 ± 0.009	7.037 ± 0.012	0.170

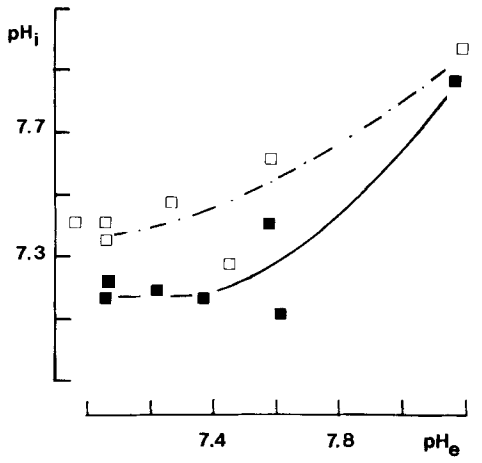

Fig. 4. Extracellular (pH_e) vs. intracellular (pH_i) pH in DIDS-treated cells before (■) and after (□) the addition of 10^{-5} M isoproterenol. Each point represents the mean of four to eight determinations.

furosemide (Table 3, Fig. 4). In DIDS-treated cells, at 5 mM HCO_3^- concentration, the pH gradient was reversed (pH_i higher than pH_e) at extracellular pH values below 7.4. The combined effect of DIDS and isoproterenol depends on the HCO_3^- concentration; in 10 mM HCO_3^-, the degree of cellular alkalinization was only half of that at 5 mM HCO_3^- (Table 3).

The adrenergic response was independent of temperature. The volume change observed at 10°C was similar to that observed at 18°C, as was the change in pH gradient (Table 4).

DISCUSSION

The red cell volume increased as a result of both extracellular acidification and adrenergic stimulation. The volume changes associated with changes in extracellular pH are due to the movements of bicarbonate and chloride across the red cell membrane as outlined in the introduction. A decrease in extracellular pH decreased the intracellular pH, but the observed changes in the intracellular pH did not activate the amiloride-sensitive Na^+/H^+ exchange. Thus, the Na^+/H^+ exchanger of trout red cells may differ from that of lymphocytes (Grinstein et al., '85) and lamprey red cells (Nikinmaa et al., '86), in which acidification of the intracellular compartment activates the exchanger. Notably, the sodium-dependent acid extrusion of lam-

*TABLE 2. Extracellular and intracellular pH and the pH gradient across the cell membrane in DIDS-treated red cells under different experimental conditions**

Experiment	pH_e	pH_i	dpH
A. 10 mM HCO_3/4% CO_2 N = 8	7.077 ± 0.007	7.226 ± 0.017	−0.149
B. 5 mM HCO_3/2% CO_2 N = 8	7.063 ± 0.012	7.173 ± 0.019	−0.111
C. 5 mM HCO_3/1% CO_2 N = 8	7.198 ± 0.008	7.207 ± 0.031	−0.009
D. 10 mM HEPES N = 4	7.153 ± 0.006	6.940 ± 0.014	0.213
E. 5 mM HCO_3/2% CO_2 0 mM Na; N = 8	7.023 ± 0.006	7.206 ± 0.019	−0.183
F. 5 mM HCO_3/2% CO_2 0.1 mM DNP; N = 8	7.146 ± 0.029	7.042 ± 0.040	0.104

*Means ± SEM, N are given. Intracellular pH (pH_i) was significantly higher than extracellular pH (pH_e) in Experiments A, B, and E ($P < 0.005$ Wilcoxon matched-pairs signed-ranks test.

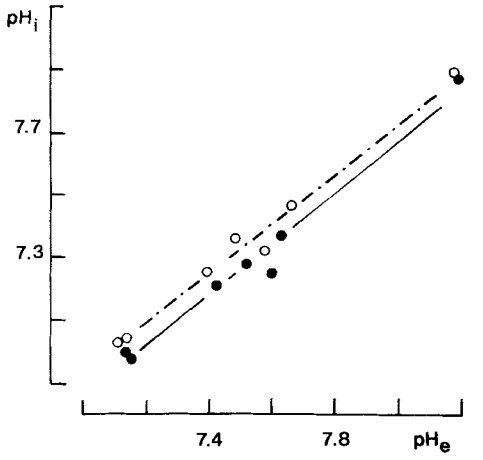

Fig. 5. Effects of isoproterenol on the intracellular vs. extracellular pH of rainbow trout red cells. Cells were first incubated for 30 min and their pH measured (●, control). Thereafter, 10^{-5} M isoproterenol was added and determinations repeated after 30 min (○, control + isoproterenol). Each point represents the mean of eight determinations. Apart from pH 8.2, isoproterenol caused a significant decrease in the pH gradient across red cell membrane at every extracellular pH studied ($P < 0.05$, Mann-Whitney U-test). The relation between extra- and intracellular pH for control cells was $pH_i = 0.802 \times pH_e + 1.24$; $r = 0.97$; $N = 60$; and for isoproterenol-treated cells, $pH_i = 0.792 \times pH_e + 1.38$; $r = 0.98$; $N = 59$.

prey red cells is insensitive to catecholamines (M. Nikinmaa, personal communication).

The volume changes associated with changes in pH could be prevented by blocking the anion exchange pathway by DIDS. In control red cells, chloride (and bicarbonate) rapidly enter or exit the cell in response to the changes in the charge of hemoglobin, induced by the cellular pH changes; and osmotic water follows, causing volume changes (see e.g. Hladky and Rink, '77). With decreasing pH, the chloride ratio (Cl^-_{in}/Cl^-_{out}) across the red cell membrane increases in rainbow trout blood (e.g. Heming, '84). When the movements of anions are inhibited using DIDS, the net influxes (at low pH) or effluxes (at high pH) of Cl^- and water do not occur. Thus, at low pH, the volume of DIDS-treated cells is smaller and at high pH, greater than that of control cells. Cala ('83,'85) has pointed out that the net movements of Cl^- are important in cell volume changes. It appears that bicarbonate, being in equilibrium with CO_2 and water, does not exert an osmotic influence.

*TABLE 3. Changes in the pH gradient across red cell membrane induced by isoproterenol**

	5 mM HCO_3^-			10 mM HCO_3^-		
	dpH0	dpHISO	Change	dpH0	dpHISO	Change
pH_e 7.1–7.2						
ISO only	0.172	0.102	−0.070	0.131	0.088	−0.043
Amiloride (1 mM)	0.155	0.126	−0.029	0.121	0.112	−0.009
DIDS (0.1 mM)	−0.111	−0.393	−0.282	−0.149	−0.307	−0.158
Furosemide (0.1 mM)	0.186	0.028	−0.158			
pH_e 7.3–7.4						
ISO only	0.220	0.150	−0.070			
Amiloride (1 mM)	0.247	0.243	−0.006			
DIDS (0.1 mM)	0.203	−0.100	−0.303			
pH_e 7.5–7.6						
ISO only	0.377	0.267	−0.110			
Amiloride (1 mM)	0.299	0.228	−0.071			
DIDS (0.1 mM)	0.505	0.174	−0.331	0.159	0.013	−0.146

*The cells were initially incubated for 30 min in the Ringer's solution or in the presence of the transport inhibitors (0). Thereafter, 10^{-5} M isoproterenol was added to the incubations and the cells incubated for 30 min in the presence of isoproterenol (ISO). At both times, the extracellular and intracellular pH were determined, and the pH gradient ($dpH = pH_e - pH_i$) calculated. The change in pH gradient (Change = dpHISO − dpH0) shows the effect of isoproterenol in the different treatments. Each value is a mean of eight determinations.

*TABLE 4. Effects of temperature and treatment with furosemide on the adrenergic response of rainbow trout erythrocytes**

Treatment	pH_e	pH_i	dpH	H_2O
A. 10°C Control	7.539 ± 0.019	7.286 ± 0.027	0.216	70.4 ± 0.3
Isoproterenol	7.491 ± 0.025	7.364 ± 0.019	0.127	73.3 ± 0.2
18°C Control	7.428 ± 0.005	7.208 ± 0.013	0.220	70.0 ± 0.3
Isoproterenol	7.402 ± 0.011	7.253 ± 0.041	0.150	73.6 ± 0.23
B. Control	7.281 ± 0.011	7.095 ± 0.035	0.186	72.7 ± 1.3
Furosemide	7.223 ± 0.012	7.195 ± 0.031	0.028	73.1 ± 1.1

*N = 8, Mean ± SEM given. The pH gradient (dpH) decreased significantly ($P < 0.05$ Wilcoxon matched pairs signed-ranks test) in every isoproterenol treatment, and isoproterenol treatment caused an increase ($P < 0.05$) in the red cell volume both at 10 and 18°C but not when cells were simultaneously treated with furosemide.

In DIDS-treated cells, a marked discrepancy between the chloride and proton distribution ratio across the red cell membrane develops at low extracellular pH values. Since this is not due to the function of Na^+/H^+ exchange, since the effect is greater at high than at low HCO_3^- concentration and since the intracellular pH of DIDS-treated cells decreases, if 2,4-dinitrophenol is added to the medium, it is possible that the discrepancy between the proton and chloride distribution ratios is a result of bicarbonate accumulation within the cell. Thus, the inhibition of the anion exchange pathway appears to be effective enough to prevent the equilibration of chloride and bicarbonate across the membrane during the time periods used in this study. HCO_3^- may be accumulated as the movements of CO_2, and its consecutive hydration to HCO_3^- and H^+ are rapid, but the bicarbonate ion formed cannot be exchanged for chloride and remains in the cell. If the protons formed in the hydration of CO_2 can be buffered by intracellular buffers, notably hemoglobin, and the permeability of the membrane for acid equivalents is low in the absence of anion exchange, intracellular pH changes little in spite of a decrease in extracellular pH.

Adrenergic drugs activate an Na^+/H^+ exchange in trout red cells in vitro (Nikinmaa and Huestis, '84; Baroin et al., '84b; Cossins and Richardson, '85). In nominally bicarbonate-free media, this activation increases the intracellular pH by 0.1–0.2 units (Nikinmaa and Huestis, '84; Cossins and Richardson, '85). Heming et al. ('87) showed that an increase in intracellular pH also occurs in red cells incubated in plasma (bicarbonate concentration approximately 10 mM). Our study confirms that intracellular pH increases even if bicarbonate/carbon dioxide system is used as a buffer and that the intracellular alkalinization can be inhibited by amiloride. Together, these data strongly suggest that adrenaline-activated Na^+/H^+ exchange plays a significant role in the control of cytoplasmic pH in trout red cells.

After the initial activation of Na^+/H^+ exchange and net efflux of protons from the red cells, a net efflux of HCO_3^- and a net influx of Cl^- occur (Nikinmaa and Huestis, '84; Cossins and Richardson, '85; Borgese et al., '86). Thereby, after the initial minutes, the intracellular pH reaches a new steady state, and no net movements of protons occur. However, owing to the continuing accumulation

of Na^+ and Cl^-, the cell volume continues to increase. The present results show that both DIDS and furosemide affect the cell volume and intracellular pH of isoproterenol-treated cells in a similar manner, inhibiting the volume changes but causing a marked intracellular alkalinization. This finding suggests that furosemide in this case inhibits the anion exchange (see e.g. Palfrey and Greengard, '81) and is in full agreement with the double Na^+/H^+ and HCO_3^-/Cl^- exchange model for adrenergic swelling. When the bicarbonate/chloride exchange is prevented, the sodium/proton exchange is capable of increasing intracellular pH to a higher level than when the anion exchange is operative.

Despite the role of HCO_3^-/Cl^- exchange in the adrenergic swelling, HCO_3^- concentration does not affect the volume increase at a constant pH (see also Baroin et al., '84b). Also, the effects of an increase in bicarbonate concentration on the adrenergically induced decrease in pH gradient across the red cell membrane are small. However, in DIDS-treated cells, the adrenergic pH response was smaller in 10 mM HCO_3^-/4% CO_2 than in 5 mM HCO_3^-/2% CO_2. Possibly the DIDS-induced alkalinization is so much greater in 10 than in 5 mM HCO_3^- that additional factors increasing pH_i, e.g. isoproterenol, can have less of an effect. Also, DIDS-insensitive movement of HCO_3^- across the red cell membrane may play a role. Up to 30% of HCO_3^- movements occur via such a pathway in SITS-treated cells (Heming et al., '87). With increasing concentrations of HCO_3^- (and total CO_2), HCO_3^- movements via this pathway would increase and, depending on the direction of net HCO_3^- movement, could counteract the effect of proton extrusion on the extra- and intracellular pH to a greater extent.

The adrenergic responses of red cells are more pronounced at low extracellular pH, indicating that the adrenergically activated sodium/proton transporter is sensitive to protons. These findings are consistent with the results of Nikinmaa ('83) and Heming et al. ('87) for cells incubated in TRIS-HCl buffered saline or plasma, respectively. However, the present data do not allow any conclusions to be made on the sidedness of proton action or on the conformational effects of protons on the transporter. However, since stress is invariably associated with a decrease in plasma pH in fish (Holeton and Randall, '67; Soivio and Nikinmaa, '81; Holeton et al., '83; Jensen et al., '83; Nikinmaa et al., '84; Primmett et al., '86), the finding indicates that the adrenergic response is an adaptation that partially offsets the detrimental effects that severe stress and low pH would otherwise have on the red cell oxygen transport.

The adrenergic responses are not affected by temperature in the range of 10–18°C (or in the wider range of 4–22°C; Salama and Nikinmaa, personal communication, 1986). This finding shows that the in vivo differences in the red cell responses to adrenalin or strenuous exercise between different temperatures (Nikinmaa, '82b) or different seasons (see Nikinmaa and Jensen, '86) are not caused by a simple temperature dependence of the adrenergic response.

ACKNOWLEDGMENTS

This study was supported by a grant from the Finnish National Research Council for Science (M.N.), by a NATO postdoctoral fellowship (J.F.S.), and by National Science and Engineering Research Council of Canada (B.L.T., D.J.R).

LITERATURE CITED

Baroin, A., F. Garcia-Romeu, T. Lamarre, and R. Motais (1984a) Hormone-induced co-transport with specific pharmacological properties in erythrocytes of rainbow trout, *Salmo gairdneri*. J. Physiol. (London), *350*:137–157.

Baroin, A., F. Garcia-Romeu, T. Lamarre, and R. Motais (1984b) A transient sodium-hydrogen exchange system induced by catecholamines in erythrocytes of rainbow trout, *Salmo gairdneri*. J. Physiol. (London), *356*:21–31.

Borgese, F., F. Garcia-Romeu, and R. Motais (1986) Catecholamine-induced transport system in trout erythrocyte. J. Gen. Physiol., *87*:551–566.

Cala, P.M. (1983) Volume regulation by red blood cells: Mechanisms of ion transport. Mol. Physiol., *4*:33–52.

Cala, P.M. (1985) Volume regulation by *Amphiuma* red blood cells: Characteristics of volume-sensitive K/H and Na/H exchange. Mol. Physiol., *8*:199–214.

Cossins, A.R., and P.A. Richardson (1985) Adrenalin-induced Na^+/H^+ exchange in trout erythrocytes and its effects upon oxygen-carrying capacity. J. Exp. Biol., *118*:229–246.

Grinstein, S., J.D. Goetz, S. Cohen, A. Rothstein, and E.W. Gelfand (1985) Regulation of Na^+/H^+ exchange in lymphocytes. Ann. N.Y. Acad. Sci., *456*:207–219.

Heming, T.A. (1984) The role of fish erythrocytes in transport and excretion of carbon dioxide. Ph.D. Thesis, University of British Columbia, Vancouver, 177 pp.

Heming, T.A., D.J. Randall, and M.M. Mazeaud (1987) Effects of adrenaline on ionic equilibria in red blood cells of rainbow trout (*Salmo gairdneri*). Fish Physiol. Biochem., *3*:83–90.

Hladky, S.B., and T.J. Rink (1977) pH equilibrium across the red cell membrane. In: Membrane Transport in Red Cells. J.C. Ellory and V.L. Lew, eds. Academic Press, London, pp. 115–135.

Holeton, G.F., and D.J. Randall (1967) The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of rainbow trout. J. Exp. Biol., *46:*317–327.

Holeton, G.F., P. Neumann, and N. Heisler (1983) Branchial ion exchange and acidbase regulation after strenuous exercises in rainbow trout (*Salmo gairdneri*). Respir. Physiol., *51:*303–318.

Houston, A.H. (1985) Erythrocytic magnesium in freshwater fishes. Magnesium, *4:*106–128.

Irving, L., E.C. Black, and V. Safford (1941) The influence of temperature upon the combination of oxygen with blood of trout. Biol. Bull., *80:*1–17.

Jensen, F.B., M. Nikinmaa, and R.E. Weber (1983) Effects of exercise stress on acidbase balance and respiratory function in blood of the teleost *Tinca tinca*. Respir. Physiol., *51:*291–301.

McLaughlin, S.G.A., and J.P. Dilger (1980) Transport of protons across membranes by weak acids. Physiol. Rev., *60:*825–863.

Nikinmaa, M. (1982a) Effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout, *Salmo gairdneri*. Mol. Physiol., *2:*287–297.

Nikinmaa, M. (1982b) The effects of adrenaline on the oxygen transport properties of *Salmo gairdneri* blood. Comp. Biochem. Physiol., *71A:*353–356.

Nikinmaa, M. (1983) Adrenergic regulation of haemoglobin oxygen affinity in rainbow trout red cells. J. Comp. Physiol., *152:*67–72.

Nikinmaa, M., and W.H. Huestis (1984) Adrenergic swelling in nucleated erythrocytes: Cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. J. Exp. Biol., *113:*215–224.

Nikinmaa, M., and F.B. Jensen (1986) Blood oxygen transport and acidbase status of stressed trout (*Salmo gairdnerii*): Pre- and postbranchial values in winter fish. Comp. Biochem. Physiol., *84A:*391–396.

Nikinmaa, M., J.J. Cech, Jr., and M. McEnroe (1984) Blood oxygen transport in stressed striped bass (*Morone saxatilis*): Role of beta-adrenergic responses. J. Comp. Physiol., *154:*365–369.

Nikinmaa, M., T. Kunnamo-Ojala, and E. Railo (1986) Mechanisms of pH regulation in lamprey (*Lampetra fluviatilis*) red blood cells. J. Exp. Biol., *122:*355–367.

Palfrey, H.C., and P. Greengard (1981) Hormone-sensitive ion transport systems in erythrocytes as models for epithelial ion pathways. Ann. N.Y. Acad. Sci., *372:*291–308.

Primmett, D.R.N., D.J. Randall, M.M. Mazeaud, and R.G. Boutilier (1986) The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*). J. Exp. Biol., *123:*139–148.

Romano, L., and H. Passow (1984) Characterization of anion transport system in trout red blood cell. Am. J. Physiol., *246:*C330–C338.

Soivio, A., and M. Nikinmaa (1981) The swelling of erythrocytes in relation to the oxygen affinity of the blood of the rainbow trout, *Salmo gairdneri* Richardson. In: Stress and Fish. A.D. Pickering, ed. Academic Press, London, pp. 103–119.

Van Slyke, D.D., H. Wu, and F.C. McLean (1923) Studies of gas and electrolyte equilibria in the blood. V. Factors controlling the electrolyte and water distribution in the blood. J. Biol. Chem., *56:*765–849.