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Intracellular acid-base responses to environmental hyperoxia and normoxic recovery in rainbow trout

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Abstract. Exposure of rainbow trout to environmental hyperoxia (P_{IO_2} ~ 530 Torr) resulted in an extracellular respiratory acidosis which was fully compensated by 72 h; return to normoxia (P_{IO_2} ~ 145 Torr) at this time induced a metabolic alkalosis which was corrected by 24 h. Intracellular pHi ([^{14}C]DMO method), fluid volumes [^{3}H]PEG-4000 method), and electrolytes were monitored. Environmental hypercapnia (P_{ICO_2} ~ 6.5 Torr) was employed to confirm that intracellular responses were specific to respiratory acidosis. Gill pHi did not change during respiratory acidosis despite a very low non-HCO_3^- buffer capacity, but gill ICFV decreased markedly. A large loss of gill intracellular [Cl^-]i in excess of [Na^+]i, combined with a substantial gain in [K^+]i, contributed to gill pHi regulation by raising branchial [SID]i. In weakly buffered brain tissue, active adjustment of pHi started within 3 h, but two well buffered tissues, RBC and white muscle, exhibited compounding metabolic acidoses during the first 12–24 h. The muscle response was associated with small increases in ICFV and [Cl^-]i, and a large decrease in [K^+]i which reduced muscle [SID]i. We hypothesize that this initial export of K^+ and basic equivalents served to regulate pH in more critical compartments (*e.g.* gills, brain) at the expense of muscle acidosis. By 48 h, pHi restoration in all tissues was complete, in advance of pHe regulation (72 h). Return to normoxia at 72 h elevated muscle, brain, and gill pHi, but there was no evidence of a comparable 'altruistic' role of muscle during this metabolic alkalosis. Regulation of pHi was complete by 24 h recovery, accompanied by partial or complete restoration of intracellular ions and fluid volumes.

Acid-base status, intracellular, hyperoxia (trout); Electrolytes, intracellular Na^+, K^+; Fish, rainbow trout; Hyperoxia, acid-base status (trout)

There is now an extensive literature on the extracellular acid-base and ionoregulatory responses of fish to respiratory acidosis (see Truchot, 1987 for review). In brief, the initial decrease in extracellular pHe associated with P_{CO_2} elevation is later compensated by a rise in HCO_3^- concentration, resulting from a net excretion of acidic equivalents into, or base uptake from, the external water. These fluxes occur largely at the gills by manipulation of the rates of exchange of Na^+ and Cl^-, the two major strong electrolytes in the extracellular fluid. The net result is usually a small gain of Na^+, a large loss of

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Cl^-, and a rise in plasma HCO_3^- approximately equal to the resultant increase in the 'strong ion difference' (SID). Upon removal of the high P_{CO_2} by return to control conditions, a metabolic alkalosis results from the elevated extracellular HCO_3^-. This is then corrected by opposite changes in gill fluxes – a small loss of Na^+, a large gain of Cl^-, and excretion of basic equivalents into, or acid uptake from, the external water.

Only a few studies have examined the accompanying acid-base responses of the various intracellular compartments (Hobe *et al.*, 1984; Cameron, 1985; Perry *et al.*, 1988; Heisler, 1989; Wood *et al.*, 1990). In general, these have indicated that compensatory adjustments of pHi occur, but with considerable heterogeneity amongst tissues and species in the extent of pHi depression, and the rate of compensation during respiratory acidosis. Nothing is known about pHi changes during the metabolic alkalosis phase. The only information on intracellular ionic responses is the work of Cameron and Iwama (1987) showing small increases in muscle $[Na^+]i$, $[Cl^-]i$, and $[K^+]i$ without a marked change in intracellular SID in catfish exposed to environmental hypercapnia for several days.

Therefore, the goal of the present study was to characterize changes in intracellular acid-base status, ions, and associated fluid volume shifts during respiratory acidosis and metabolic alkalosis. Rainbow trout were subjected to a standard normoxia – 72 h environmental hyperoxia – 24 h normoxic recovery regime. Hyperoxia induces CO_2 retention, and therefore respiratory acidosis, because of reductions in both ventilation and perfusion, whereas the excess CO_2 quickly washes out upon return to normoxia, resulting in metabolic alkalosis (Wood and Jackson, 1980). Extracellular acid-base and ionic status, and branchial and renal acid-base and ion fluxes during this experimental regime have all been well characterized in previous studies (Hōbe *et al.*, 1984; Wheatly *et al.*, 1984; Wood *et al.*, 1984; Goss and Wood, 1990). Some additional experiments were performed with environmental hypercapnia to confirm that observed intracellular responses with hyperoxia were associated with respiratory acidosis, and were not extraneous effects of high environmental P_{O_2}.

In addition to white muscle, nervous tissue (brain), and red blood cells, we were particularly interested in the intracellular compartment of the major regulatory site, the gills. Recently, rapid changes in gill surface morphology have been observed in fish subjected to either environmental hyperoxia or hypercapnia (Cameron and Iwama, 1987; Laurent and Perry, 1991). Furthermore, changes in pHi, intracellular ions, and cell volume are now recognized as important cues or consequences of altered Na^+, Cl^-, and acid-base fluxes and cell morphology in many transporting epithelia (Roos and Boron, 1981; Busa, 1986; Schultz and Hudson, 1986). We suspected that such changes might well occur in the cells of the gills during the compensation of respiratory acidosis and metabolic alkalosis.

Materials and methods

Experimental animals and regime. Experiments were performed on 195 rainbow trout (*Oncorhynchus mykiss* Walbaum; 268 ± 4 g) obtained from Spring Valley Trout Farm,

Petersburg, Ontario, and held in flowing dechlorinated Hamilton tapwater at seasonal temperature (8–18 °C). Fish were acclimated to the experimental temperature (15 ± 1 °C) in the same water quality for 2 weeks prior to surgery, during which time *ad libitum* feeding with commercial trout pellets was suspended. Composition of the acclimation/experimental water was $[Na^+] \sim 0.6$, $[Cl^-] \sim 0.8$, $[K^+] \sim 0.04$, $[Ca^{2+}] \sim 2.0$, $[Mg^{2+}] \sim 0.6$, titration alkalinity ~ 1.9 mEq · L^{-1}, total hardness ~ 140 mg · L^{-1} as $CaCO_3$, pH 7.9–8.3.

The experimental protocol was very similar to that of Hōbe *et al.* (1984), to which the reader is referred for additional details. Each fish was fitted with an indwelling dorsal aortic catheter, placed in the inner compartment of an individual recirculating 12 L flux box, and allowed to recover for 72 h in normoxic water at 15 ± 1 °C. Water was renewed at 12 h intervals throughout the recovery and subsequent experimental period by thorough flushing (60 L per box) with water of the correct P_{O_2}, P_{CO_2}, and temperature. These exchanges kept water ammonia levels below 75 μmol · L^{-1}. Environmental conditions could be changed from normoxia (P_{O_2} = 130–160 Torr, P_{CO_2} < 0.3 Torr) to either hyperoxia (P_{O_2} = 480–580 Torr, P_{CO_2} < 0.3 Torr) or hypercapnia (P_{O_2} = 130–160 Torr, P_{CO_2} = 5.8–7.2 Torr) within 10 min by changing the airlift gas from air to pure O_2 or 1% CO_2 in air. Each fish was sampled only once, terminally. Groups (n = 7–17) were killed under normoxia (control), after 3, 12, 24, 48, and 72 h hyperoxia, after 3, 12, and 24 h of post-hyperoxia recovery under normoxia (*i.e.* 75, 84, and 96 h of the experiment), and at 3 and 12 h of hypercapnia. A separate normocapnic control group was performed for the hypercapnia tests. However, as experimental conditions (P_{O_2} = 130–160 Torr, P_{CO_2} < 0.3 Torr) were identical to those of the normoxia control group, and none of the data were significantly different between the two control groups, the results were combined to yield single control means (n = 18–25).

Intracellular pH, fluid volume, ion, and buffer capacity determinations. These measurements are all dependent upon accurate determination of extracellular fluid volume (ECFV), total tissue water content, and by difference, intracellular fluid volume (ICFV). While total water content can be measured accurately, ECFV determinations are problematical. Following a critical comparison of three radiolabelled ECFV markers (mannitol, inulin, polyethylene glycol-MW 4000) and the Cl^-/K^+ space technique in trout tissues (Munger *et al.*, 1991), polyethylene glycol (PEG-4000) was selected as the marker of choice. PEG-4000 yielded the most conservative values of ECFV, and was the only one to provide reasonable estimates in gill tissue. However, since PEG-4000 does not appear to penetrate the blood-brain barrier, it underestimated brain ECFV. In a preliminary experiment using [^{14}C]mannitol, brain ECFV under normoxic control conditions was found to be 19.63 ± 1.94 (10) % of the total tissue water content, *versus* 3.69 ± 0.25 (8) % with [^{3}H]PEG-4000. This [^{14}C]mannitol value was therefore arbitrarily applied to brain water content measurements throughout the study to estimate ECFV for the purpose of brain intracellular pH (pHi) determinations. Model calculations demonstrated that the brain pHi calculation was robust, and relatively insensitive to reasonable fluctuations in brain ECFV. In contrast, brain intracellular ion

calculations were critically sensitive to the ECFV estimate; therefore only pHi determinations were performed for brain.

Intracellular pH in brain, white muscle, and gill tissue was measured by the DMO technique (5,5-dimethyl-2,4-oxazolidinedione); the method has been described in detail and critically evaluated previously (Milligan and Wood, 1985, 1986; Wright *et al.*, 1988). In brief, 12–13 h prior to the planned time of sacrifice, trout were injected, via the dorsal aortic catheter, with a 1 ml · kg^{-1} dose of 7 μCi · ml^{-1} [^{14}C]DMO (New England Nuclear; specific activity 50 mCi · mmol^{-1}) plus 28 μCi · ml^{-1} [^{3}H]PEG-4000 (New England Nuclear; specific activity 5 mCi · mmol^{-1}) in 140 mM NaCl, flushed in with an equal volume of Cortland saline. Tissue ECFV, ICFV, pHi, and intracellular ions were calculated from measurements of plasma and tissue [^{3}H]PEG-4000 and [^{14}C]DMO radioactivities, water contents, ion concentrations, and extracellular pH (pHa). All relevant equations are given by Wright *et al.* (1988).

Non-HCO_3^- buffer capacities (β_{NB}) for trout white muscle and brain were taken from Milligan and Wood (1986), while β_{NB} values for gill tissue and packed RBCs were measured by identical methods (acid titration of tissue homogenates under an N_2 atmosphere). The technique measures total physico-chemical buffering (*i.e.* non-HCO_3^- plus HCO_3 buffering). However, as the original HCO_3 content of the tissues is low, and the P_{CO_2} kept close to zero during titration, the contribution of the HCO_3^- component is negligible. β_{NB} values were corrected for the buffering of trapped ECF and expressed on an ICF basis.

All estimates of pHi, intracellular ions, and β_{NB} in gill tissue were also corrected for the influence of trapped RBCs. Calculations were based on the measured hemoglobin concentrations of whole blood and gill tissue homogenate, the measured whole blood hematocrit, the measured RBC pHi, the measured RBC β_{NB}, and estimated ion concentrations in RBC ICF, as described by Munger *et al.* (1991). In practice, the correction had negligible influence on the results, reflecting the fact that the trapped RBC ICFV in gill tissue amounted to only about 0.04 ml · g^{-1}. Therefore, values in white muscle and brain, which had much lower trapped RBC contents, were not corrected.

Sampling protocol. Immediately prior to killing of each fish, water samples were taken from the entrance of the inner chamber and analysed for P_{O_2} and P_{CO_2}. Blood samples (2.0 ml) were then drawn via the catheter into ice-cold gas-tight syringes. Blood samples were analysed for Pa_{O_2}, true plasma Ca_{CO_2}, whole blood pHa, RBC pHi, hematocrit (Hct), hemoglobin (Hb), plasma protein and water content, Na^+, Cl^-, K^+, cortisol, and [^{14}C]DMO and [^{3}H]PEG-4000 radioactivities. The fish was then rapidly killed by a blow on the head, and tissue sampling completed within 2 min. Gill arches were removed immediately, rinsed briefly with water, and blotted with tissue paper. Surficial tissue (*i.e.* mainly lamellae and filament surface) was obtained by scraping the epithelium off the filament cartilage onto a glass plate using a microscope slide, after which the tissue was homogenized with a spatula. The whole brain and a sample of epaxial white muscle from a site just caudal to the dorsal fin were removed and blotted thoroughly. Tissue samples were then subdivided, weighed, and processed for water

content, Hb content (gill only), Na^+, Cl^-, and K^+ concentrations (gill and white muscle only), and [^{14}C]DMO and [^{3}H]PEG-4000 radioactivities.

Analytical techniques. The measurement of water and blood gases, Hb, Hct, and acid-base status, and the calculation of plasma Pa_{CO_2}, HCO_3^-, mean cell hemoglobin concentration (MCHC), and true plasma β_{NB} were performed as described by Hōbe *et al.* (1984). RBC pHi was determined by the freeze-thaw lysate method on a red cell pellet obtained by centrifugation of whole blood at 9000 × *g* for 2 min. Plasma was removed, analyzed for protein and water content by refractometry (American Optical TS meter), aliquotted for radioactivity counting, and the remainder frozen at − 70 °C for later analysis of electrolytes and cortisol. Cortisol was determined with a commercial ^{125}I-radioimmunoassay kit (Corning).

[^{14}C]DMO and [^{3}H]PEG-4000 radioactivities were determined in duplicate samples (50–150 mg) of brain, white muscle, gill tissue homogenate, and blood plasma (100 μl). Samples were digested in glass scintillation vials with 2 ml NCS (Amersham) for less than 12 h at 40 °C, neutralized with 60 μl of glacial acetic acid, and then diluted with 10 ml organic scintillant (OCS, Amersham). The vials were dark-adapted overnight to reduce chemoluminescence, and then counted on an LKB Wallac 1217 liquid scintillation counter. ^{14}C and ^{3}H dpm were separated by means of an on-board dual label quench correction program using the external standard method. Quench standards were made from the tissues of interest in the same NCS/OCS system.

Hb content of the gill homogenate was determined by emulsifying 100 mg in 5 ml of Drabkin's reagent with a Janke and Kunkle Ultra-Turrax, centrifuging at 9000 × *g* for 15 min, and then measuring the absorbance of the supernatant against appropriate cyanmethemoglobin standards at 540 nm. A correction for background absorbance was applied using homogenate obtained from gills perfused free of RBCs.

Subsamples of gill homogenate, brain and white muscle were dried to a constant weight at 85 °C for water content. Gill and muscle tissue were then ground to a fine powder with a mortar and pestle. The powder was incubated for 2 weeks in 10–100 volumes 8% perchloric acid, and the acid extract then analyzed for ions. Na^+ and K^+ in tissue extracts and plasma were determined by atomic absorption (Varian 1275-AA) and Cl^- by coulometric titration (Radiometer CMT10).

Data have been expressed as means ± 1 SE (*N*). Comparisons between two independent means were performed by Student's unpaired two-tailed *t*-test. Comparisons amongst multiple independent means were performed by a one-way analysis of variance followed by Duncan's new multiple range test in cases where the *F*-value indicated significance. A significance level of $P \leq 0.05$ was used throughout. For the sake of clarity, only those values significantly different from the normoxic control are marked with asterisks in figures.

Results

Extracellular responses to hyperoxia. Upon exposure of trout to hyperoxia, Pa_{O_2} rose about 3.5-fold in concert with the 3.5-fold elevation in PI_{O_2}, resulting in a marked increase in the PI_{O_2}–Pa_{O_2} gradient (fig. 1A). These changes were approximately stable

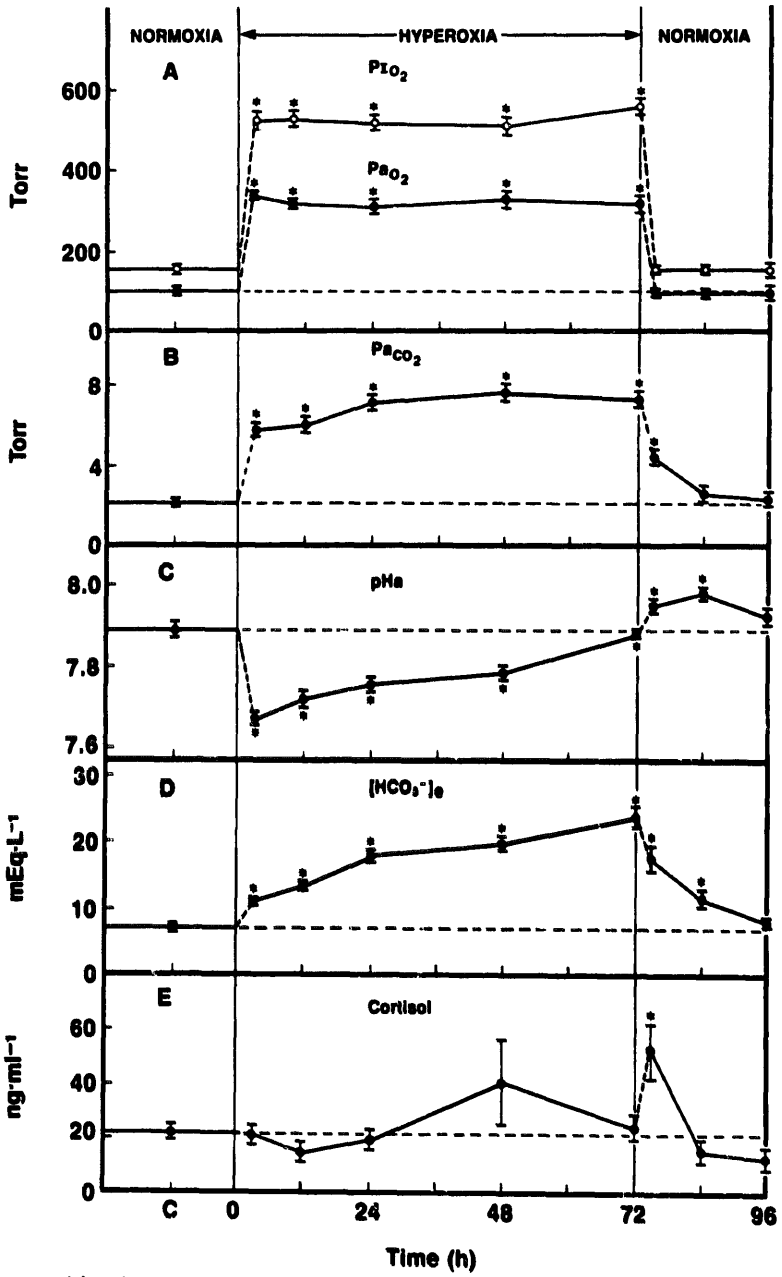

Fig. 1. The effects on blood gases, extracellular acid-base status, and cortisol in rainbow trout caused by exposure to environmental hyperoxia for 72 h followed by return to normoxia for 24 h at 15 °C. (A) Inspired (PI_{O_2}) and arterial (Pa_{O_2}) O_2 tensions; (B) arterial CO_2 tension (Pa_{CO_2}); (C) arterial plasma pH (pHa); (D) arterial plasma bicarbonate concentrations ($[HCO_3^-]$); and (E) plasma cortisol concentration. Means ± 1 SE (N = 18–25 under control normoxic conditions at C and 7–17 at all other sample times). Asterisks indicate experimental means significantly different ($P \leq 0.05$) from the normoxic control mean at C.

throughout 72 h of hyperoxia. In contrast, Pa_{CO_2} rose from 2.0 to 5.8 Torr within 3 h, and thereafter gradually increased further to about 7.5 Torr at 48–72 h (fig. 1B). The increase in Pa_{CO_2} resulted in an immediate 0.25 unit depression in pHa (fig. 1C). By 72 h, pHa had been restored to control levels, accompanied by a progressive increase in plasma $[HCO_3^-]e$ from 7.0 mEq · L^{-1} to 23.2 mEq · L^{-1} (fig. 1D). With the reinstitution of normoxia, Pa_{O_2} and Pa_{CO_2} declined quickly, reaching control levels within 3 h and 12 h, respectively. Plasma $[HCO_3^-]e$ fell more slowly, returning to resting levels only at 24 h recovery (fig. 1D). The drop in Pa_{CO_2} against a background of continued high $[HCO_3^-]e$ resulted in a significant elevation in pHa at 3 h and 12 h recovery.

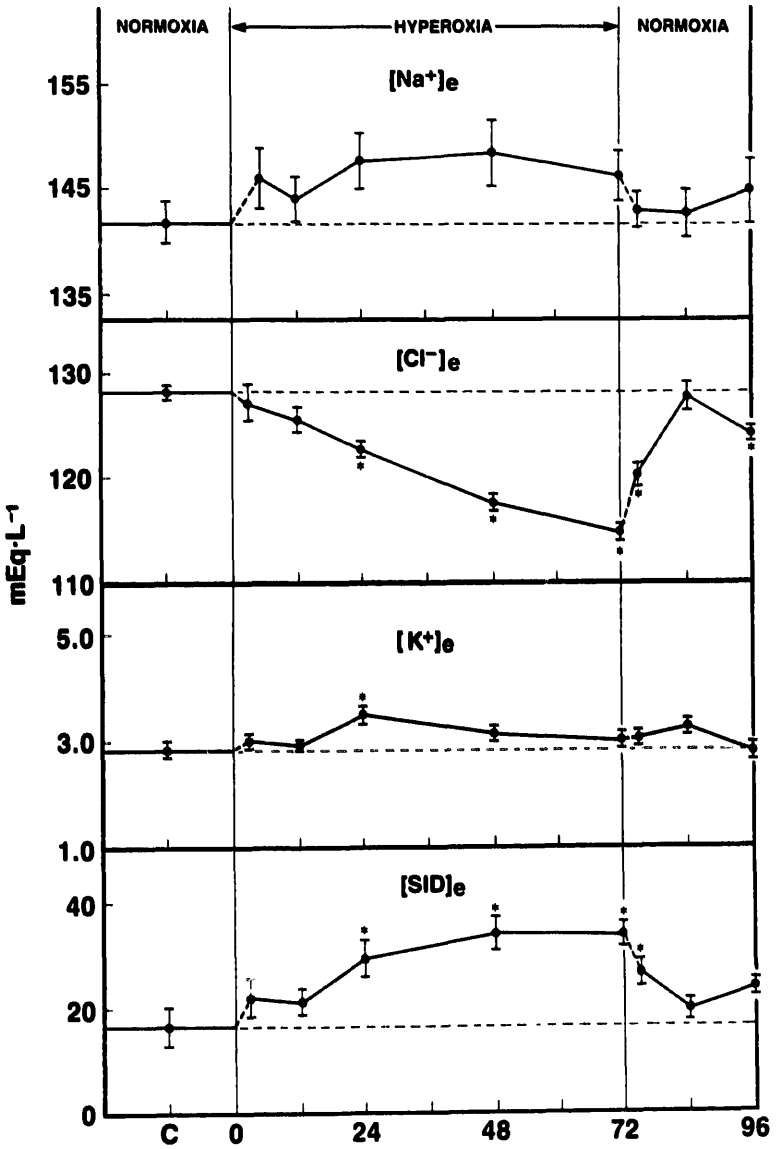

Fig. 2. The effects in rainbow trout on extracellular electrolytes caused by exposure to environmental hyperoxia for 72 h followed by return to normoxia for 24 h at 15 °C. (A) Plasma sodium ($[Na^+]e$); (B) plasma chloride ($[Cl^-]e$); (C) plasma potassium ($[K^+]e$); and (D) plasma strong ion difference ([SID]e) calculated as $[Na^+]e + [K^+]e - [Cl^-]e$. See legend of fig. 1 for other details.

Cortisol measurements, taken as a general index of stress, showed a significant increase only at 3 h after return to normoxia (fig. 1E). In general, the blood gas and acid-base responses were very similar to those reported in an earlier study employing an identical hyperoxic protocol, but using trout sampled repetitively rather than singly as in the present investigation (Hōbe *et al.*, 1984).

Plasma $[Na^+]e$ rose by about 4.5 $mEq \cdot L^{-1}$ during hyperoxia, but none of the increases were significant (fig. 2A). However, $[Cl^-]e$ progressively declined, an effect which became significant by 24 h, and reached -13.5 $mEq \cdot L^{-1}$ by 72 h (fig. 2B). Plasma $[K^+]e$ also tended to increase slightly, though the effect was significant only at 24 h (fig. 2C). The decrease in $[Cl^-]e$ was the major contributor to the observed increase in plasma [SID]e (here calculated as $[Na^+]e + [K^+]e - [Cl^-]e$; fig. 2D), which paralleled the increase in $[HCO_3^-]e$ (fig. 1D). After 72 h exposure, when pHa was fully compensated, [SID] had increased by 17.3 $mEq \cdot L^{-1}$ and $[HCO_3^-]$ by 16.2 $mEq \cdot L^{-1}$. Upon return to normoxia, plasma ions generally returned to control levels, though $[Cl^-]e$ remained significantly depressed at 24 h recovery. There were no significant changes in either plasma protein or blood Hb concentrations, which remained stable at control levels of 2.70 ± 0.10 (25) $g \cdot 100\ ml^{-1}$ and 5.72 ± 0.40 (25) $g \cdot 100\ ml^{-1}$, respectively. Thus, observed changes in plasma electrolytes were not due to fluid volume shifts. As with acid-base status, these changes in extracellular electrolytes were similar to those of an earlier study using an identical hyperoxic protocol with repetitively sampled trout (Wheatly *et al.*, 1984).

Intracellular responses to hyperoxia. Of the various tissues examined, gill had by far the lowest intracellular β_{NB} value (table 1). Brain β_{NB} was also relatively low but almost twice as high as the gill value. In contrast, white muscle and red blood cells were both extremely well buffered, with similar β_{NB} values 5–6-fold higher than that of gill tissue.

Under control conditions at pHe (pHa) = 7.88, mean pHi values were 7.63 in brain, 7.43 in gill tissue, 7.32 in red blood cells, and 7.30 in white muscle, the latter two not significantly different from one another (fig. 3). Despite its very low β_{NB} value (table 1), gill pHi was very well regulated, with no significant changes relative to control at any time during hyperoxic exposure (fig. 3B). However, all other tissues exhibited significant

TABLE 1

Intracellular buffer capacities (β_{NB}) of several tissues in the rainbow trout.

Tissue	β_{NB}[a] (slykes)
Gill	13.71 ± 0.97 (8)
Brain	24.83 ± 0.39 (5)[b]
White muscle	73.59 ± 4.87 (7)[b]
Red blood cells	74.47 ± 9.21 (7)

[a] Expressed per unit intracellular water.

[b] From Milligan and Wood (1986).

declines in pHi which were greatest at 3 h (figs. 3C–E), the time of maximum depression of pHe (– 0.25 units; fig. 3A).Decreases in pHi were smaller than those in pHe and not directly related to the β_{NB} value of the tissue. The fall in pHi was greatest in well buffered white muscle (– 0.16 units), intermediate in brain and RBC (– 0.10 units), and least in gill tissue (negligible), the tissue with the lowest β_{NB} value. In all tissues, pHi was regulated more rapidly than pHe, returning to control levels at 24 h (RBC) to 48 h (brain and white muscle), whereas pHe remained significantly depressed at these times.

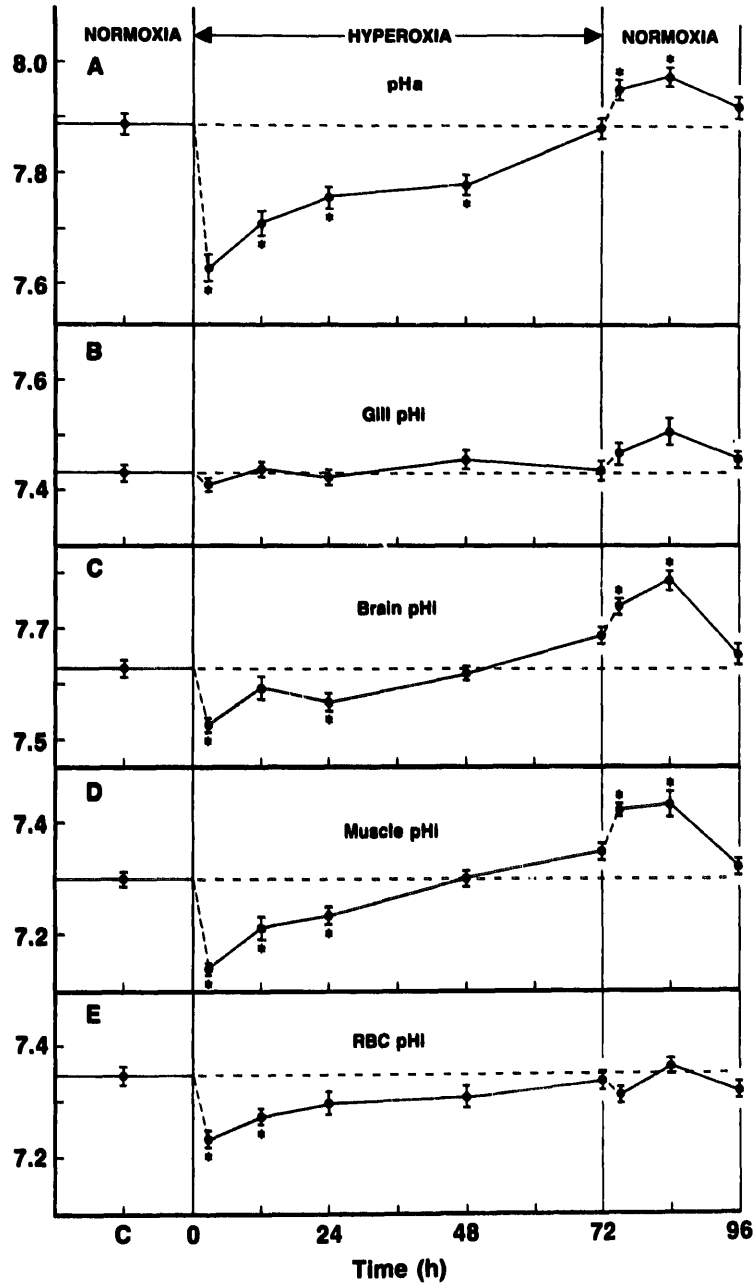

Fig. 3. The effects in rainbow trout on pH in various intracellular compartments caused by exposure to environmental hyperoxia for 72 h followed by return to normoxia for 24 h at 15 °C. (A) Extracellular pHe = arterial plasma pHa; (B) gill intracellular pHi; (C) brain intracellular pHi; (D) white muscle intracellular pHi; and (E) red blood cell intracellular pHi. See legend of fig. 1 for other details.

During normoxic recovery, rather different patterns occurred, with increases in brain and white muscle pHi approximately equal to those in pHe (*i.e.* about + 0.13 units at 12 h recovery relative to the 72 h hyperoxia values). Gill pHi tended to increase (fig. 3B), but the changes were not significant relative to either control or 72 h hyperoxia values (though the 12 h normoxic recovery value was elevated relative to the 3 h and 24 h hyperoxia values). There were also no significant changes in RBC pHi (fig. 3E). In all cases, control values were re-established by 24 h recovery.

Under control conditions, mean gill intracellular concentrations of Na^+ (55 mEq · L^{-1}) and Cl^- (75 mEq · L^{-1}) were much higher than normally seen in the

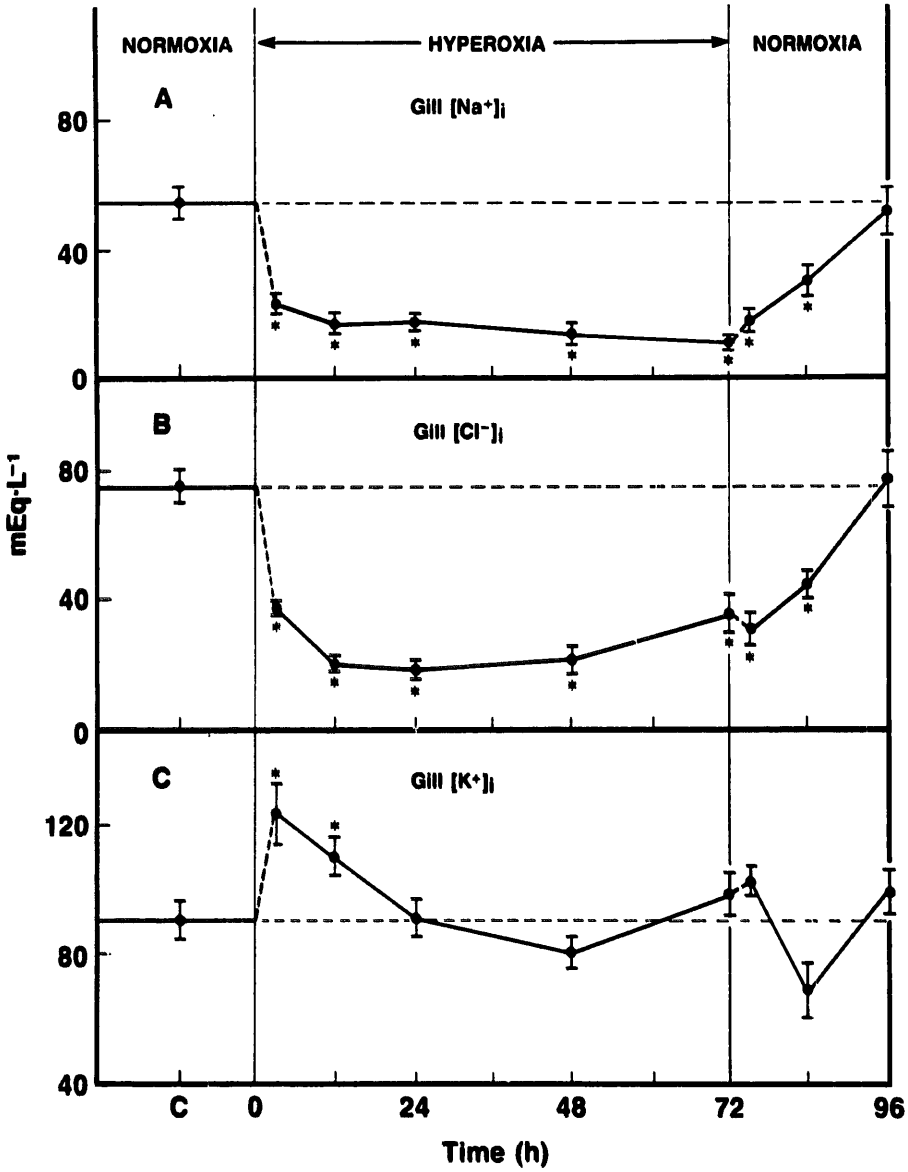

Fig. 4. The effects in rainbow trout on intracellular electrolytes (expressed as mEq · L^{-1} intracellular water) in gill tissue caused by exposure to environmental hyperoxia for 72 h followed by return to normoxia for 24 h at 15 °C. (A) Gill intracellular sodium ([Na^+]i); (B) gill intracellular chloride ([Cl^-]i); and (C) gill intracellular potassium ([K]i). See legend of fig. 1 for other details.

intracellular compartments of other tissues, while K^+ levels (90 mEq · L^{-1}) were not unusual (fig. 4). Both [Na^+]i and [Cl^-]i in gills declined dramatically upon hyperoxic exposure, a response which was largely complete after only 3 h (fig. 4A,B). Final levels maintained during hyperoxia were approximately [Na^+]i = 15 mEq · L^{-1}, [Cl^-]i = 20 mEq · L^{-1}. In contrast, branchial [K^+]i increased at 3 h, but had returned to control levels by 48 h with no further significant variations (fig. 4C). Upon reinstitution of normoxia, gill [Na^+]i and [Cl^-] slowly increased, returning to control levels by 24 h recovery (fig. 4A,B).

In white muscle (fig. 5), control intracellular levels of Na^+ (6 mEq · L^{-1}), Cl^- (8 mEq · L^{-1}), and K^+ (189 mEq · L^{-1}) were all very different from those in gill tissue.

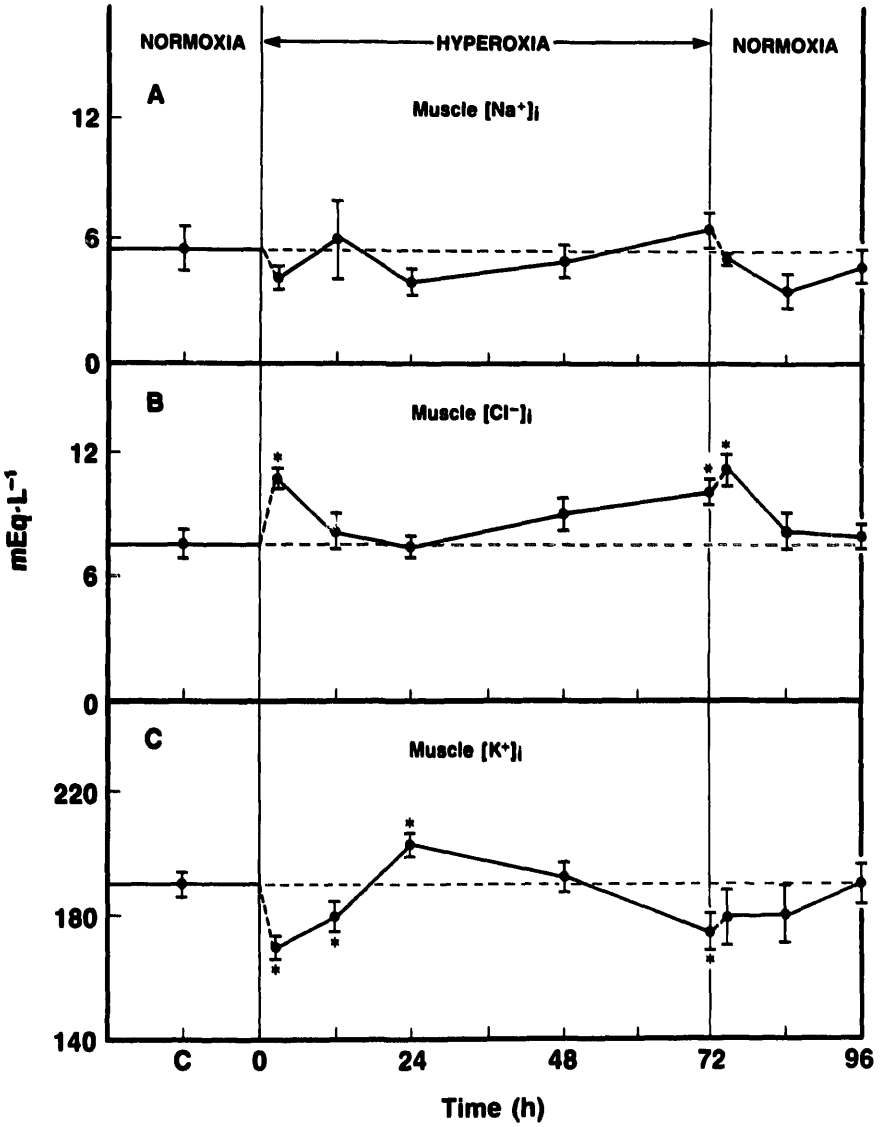

Fig. 5. The effects in rainbow trout on intracellular electrolytes (expressed as mEq · L^{-1} intracellular water) in white muscle tissue caused by exposure to environmental hyperoxia for 72 h followed by return to normoxia for 24 h at 15 °C. (A) muscle intracellular sodium ([Na^+]i); (B) muscle intracellular chloride ([Cl^-]i); and (C) muscle intracellular potassium ([K]i). See legend of fig. 1 for other details.

Muscle [Na^+]i exhibited no significant variation throughout the experiment (fig. 5A). However, muscle [Cl^-]i increased significantly at 3 h hyperoxia, and again at 72 h hyperoxia and 3 h recovery (fig. 5B). In contrast, muscle [K^+]i decreased at 3 h and 12 h hyperoxia, rebounded at 24 h, and then decreased again at 72 h hyperoxia (fig. 5C). Control levels were restored during normoxic recovery.

Under control conditions, gill ICFV and ECFV were 592 $ml \cdot kg^{-1}$ and 215 $ml \cdot kg^{-1}$, respectively; white muscle ICFV and ECFV were 724 and 49 $ml \cdot kg^{-1}$, respectively. Changes in intracellular ion levels during hyperoxia and normoxic recovery were accompanied by marked shifts in fluid volume distributions in both gill and white muscle tissue. Measurements of total tissue water content and ICFV are illustrated in fig. 6A,B; differences between the two represent ECFV.

Most notable was a progressive decline in gill ICFV which was significant after only 3 h (-32 $ml \cdot kg^{-1}$), and reached almost 20% (-108 $ml \cdot kg^{-1}$) by 48 h exposure (fig. 6A). This pattern coincided with the fall in total *measured* intracellular ion concentration ([Na^+]i + [K^+]i + [Cl^-]i) which amounted to 15% at 3 h, and stabilized at almost 50% by 48 h, due largely to the synchronous declines in gill [Na^+]i and [Cl^-]i (fig. 4). However, upon return to normoxia, gill ICFV recovered only partially by 24 h (fig. 6A), whereas intracellular electrolytes had recovered fully by this time (fig. 4). The decrease in gill ICFV during hyperoxia reflected both a reduction in total gill water content and a relative shift of fluid from ICFV to ECFV. The fall in gill water content was the smaller component, which stabilized at about -18 $ml \cdot kg^{-1}$ after 3 h (fig. 6A). Thus, gill ECFV increased significantly at all times from 12 h exposure through to the end of the experiment.

This shrinkage of the ICFV during hyperoxia was seen only in the gills. White muscle exhibited a small increase in ICFV of about 20 $ml \cdot kg^{-1}$ which became significant at 12 h exposure and persisted through the return to normoxia (fig. 6B). As total muscle water content did not change significantly, there was a reciprocal decrease in muscle ECFV which was significant at most sample times. There was no obvious relationship between measured intracellular ions (fig. 5) and these small changes in muscle fluid volumes. ICFV and ECFV were not measured in brain because PEG-4000 was not a reliable marker in this tissue, but there were no significant changes in brain total water content during hyperoxic exposure (fig. 6B).

Red blood cells also tended to swell during hyperoxia. ICFV was not measured directly, but MCHC, which provides an inverse index of RBC size, yields similar information. MCHC declined throughout hyperoxic exposure, though the response was significant only at 12 h and 48 h (fig. 6C). This effect represented a 5–10% swelling of the RBCs, and was sufficient to explain significant increases in Hct (not shown) observed at several sample times.

Responses to hypercapnia. Exposures to moderate hypercapnia ($P_{I_{CO_2}} \sim 6.5$ Torr) for 3 h or 12 h were performed principally to confirm that intracellular responses observed during the hyperoxia tests were specifically associated with respiratory acidosis, rather than with high $P_{I_{O_2}}$. Environmental hypercapnia induced changes in pHa (fig. 7B)

similar to those caused by environmental hyperoxia (fig. 1C). Slightly greater increases in Pa_{CO_2} (fig. 7A *vs* fig. 1B) were compensated by larger increases in $[HCO_3^-]e$ (not shown). Patterns of change in pHi during hypercapnia (fig. 7B) were virtually identical

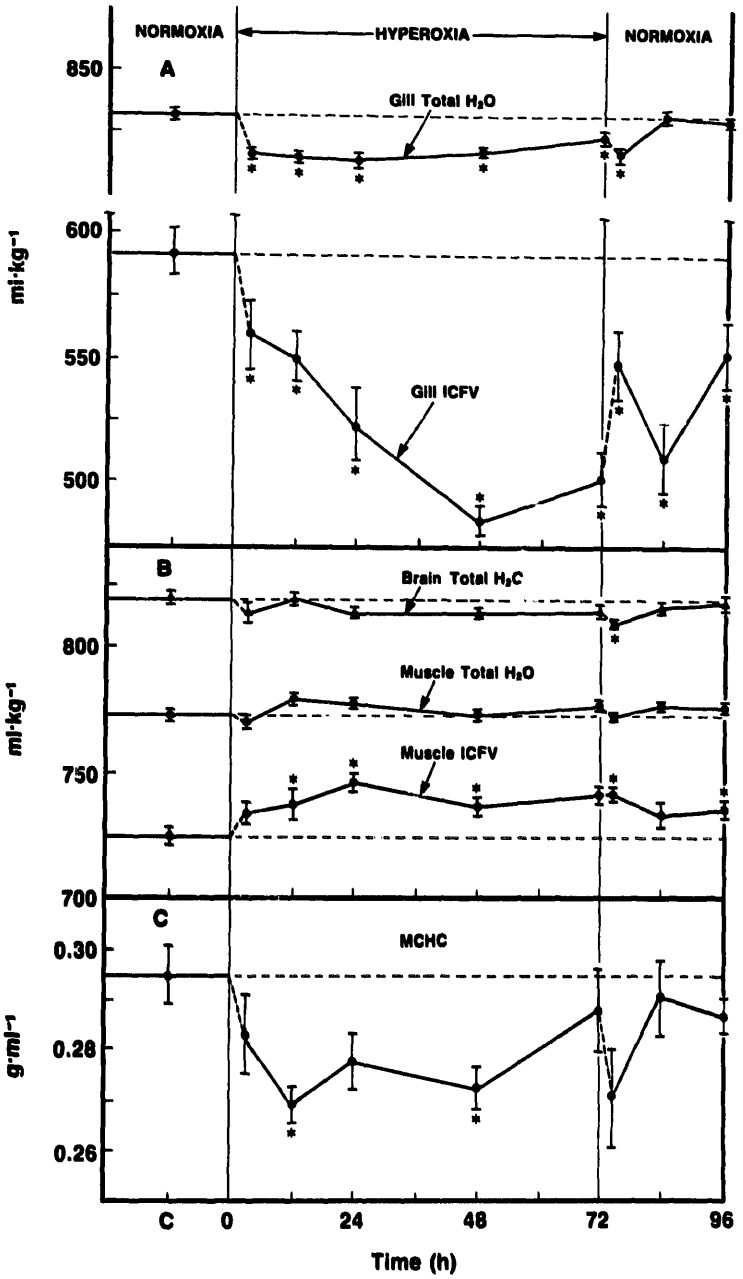

Fig. 6. The effects in rainbow trout on fluid volume distributions in various tissues (expressed as ml · kg $^{-1}$ tissue wet weight) caused by exposure to environmental hyperoxia for 72 h followed by return to normoxia for 24 h at 15 °C. (A) Gill total tissue water content (H_2O) and intracellular fluid volume, (ICFV), the difference between the two representing gill extracellular fluid volume (ECFV); (B) brain and white muscle total tissue water contents (H_2O) and white muscle intracellular fluid volume (ICFV), the difference between the latter two representing white muscle extracellular fluid volume (ECFV); brain ECFV and ICFV were not measured; and (C) mean red cell hemoglobin concentration (MCHC). See legend of fig. 1 for other details.

to those observed during hyperoxia (fig. 3). Thus, white muscle pHi dropped to the greatest extent, brain and RBC pHi exhibited intermediate depressions, while gill pHi did not change significantly. RBC pHi had returned to control levels by 12 h hypercapnia, while muscle pHi remained significantly depressed at this time. Unfortunately, the 12 h brain pHi samples were lost through mishap.

Changes in tissue fluid volumes (fig. 7C) were also very similar to those occurring during hyperoxia (fig. 6). Gill ICFV decreased markedly, whereas white muscle ICFV increased slightly. While there were no significant changes in intracellular electrolytes in white muscle (fig. 7E,F), the shrinkage of gill ICFV was again accompanied by large

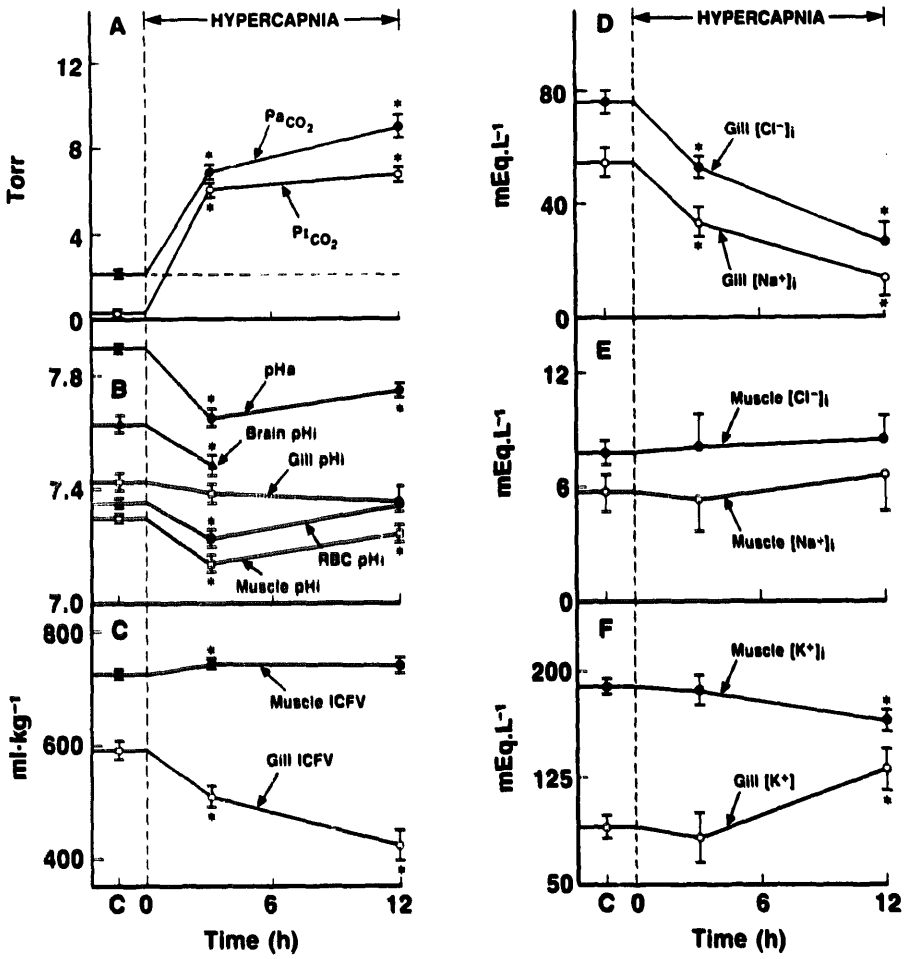

Fig. 7. The effects in rainbow trout caused by exposure to environmental hypercapnia for 3 h or 12 h at 15 °C. (A) Arterial (Pa_{CO_2}) and inspired (PI_{CO_2}) CO_2 tensions; (B) arterial plasma pHa = extracellular pHe, and intracellular pH (pHi) in brain, gill, red blood cells (RBC), and white muscle tissues; (C) intracellular fluid volumes (ICFV) in white muscle and gill tissues (expressed as ml · kg $^{-1}$ tissue wet weight); (D) gill intracellular chloride ([Cl $^-$]i) and sodium ([Na $^+$]i) concentrations (expressed as mEq · L $^{-1}$ intracellular water); (E) white muscle intracellular chloride ([Cl $^-$]i) and sodium ([Na $^+$]i) concentrations (expressed as mEq · L $^{-1}$ intracellular water); and (F) intracellular potassium concentrations ([K $^+$]i) in white muscle and gill tissues (expressed as mEq · L $^{-1}$ intracellular water). Means ± 1 SE (N = 18–25 under control normocapnic conditions at C, and 12–13 at 3 h and 12 h hypercapnia). Asterisks indicate experimental means significantly different ($P \leq 0.05$) from the normocapnic control mean at C.

decreases in gill [Na^+]i and [Cl^-]i (fig. 7D) and an increase in gill [K^+]i (fig. 7F). These intracellular ionic changes were qualitatively similar to the hyperoxia responses (fig. 4) but occurred more slowly, with the 12 h hypercapnia means approximating the 3 h hyperoxia means.

Discussion

Evaluation of methods. The extracellular data of the present study were based on direct measurements; in contrast, the intracellular data were largely derived values based on a number of different measurements and assumptions, and therefore subject to potentially greater error. Limitations specifically associated with the [^{14}C]DMO technique in trout have been evaluated previously (Milligan and Wood, 1985, 1986). These studies have shown that DMO re-equilibration time (< 15 min) in response to pH changes is rapid relative to the time scale of the present measurements, and that for most tissues, the technique yields reliable values for whole tissue pHi.

The largest potential source of error for all intracellular data is the derivation of ICFV from the distribution of the ECFV label. All ECFV measurements are relative rather than absolute values dependent on the characteristics of the label. Intracellular concentrations (of [^{14}C]DMO, ions) are calculated by subtracting the calculated extracellular content (product of measured extracellular concentration and estimated ECFV) from the measured total tissue content, and dividing this difference by the calculated ICFV, itself estimated as the difference between measured total tissue water and estimated ECFV. In general, the larger the true ECFV, and the greater the extracellular concentration of a particular substance relative to its true intracellular concentration, the greater is the potential for error. Recently, this error has been assessed in detail for trout tissues with particular reference to [^{3}H]PEG-4000 (Munger *et al.*, 1991).

This analysis concludes that reasonable variations in the ECFV measurement have negligible effect on the pHi value for tissues like white muscle where the true ECFV is low. However, for tissues like gills, where the true ECFV is high, the influence is greater, but not proportionately so, because of the complex nature of the pHi calculation. The pHi *versus* ECFV relationship exhibits a relatively shallow plateau in the range of gill ECFVs provided by PEG-4000, which are 35–50% lower than those provided by other markers. PEG-4000 is the only one to yield reasonable control gill pHi values (~ 7.4 *versus* 6.4–7.1 for other markers). To put the potential error in perspective, the observed changes in gill fluid volume distribution (increasing ECFV, decreasing ICFV; figs. 6A, 7C) during respiratory acidosis in the present study, *if totally artifactual*, would have *lowered* calculated pHi by up to 0.2 pH units. This would have artificially increased the anticipated acidifying effect of the elevated internal P_{CO_2}. However, in reality, pHi did not change (figs. 3B and 7B), and there is therefore no reason to believe that the observed excellent regulation of gill pHi was artifactual.

In contrast, for intracellular ion calculations, Munger *et al.* (1991) demonstrated that

even when true ECFV is low (*e.g.* white muscle), the potential for error is significant for $[Na^+]i$ and $[Cl^-]i$, though not for $[K^+]i$. At higher true ECFVs, the potential for error becomes highly significant for all three ions. Notably, in contrast to pHi, there is no moderating 'plateau effect'. Munger *et al.* (1991) concluded that for most intracellular ion measurements, relative trends would be much more reliable than absolute values. To put these potential errors in perspective, the observed changes in muscle fluid volume distribution (decreasing ECFV, increasing ICFV; figs. 6B and 7C) during respiratory acidosis, *if totally artifactual*, could have been responsible for all of the observed increase in muscle $[Cl^-]i$ (fig. 5B) but would have had negligible influence on the observed changes in muscle $[K^+]i$ (figs. 5C and 7F). The observed fluid shifts in the gills (fig. 6A), again *if totally artifactual*, could have accounted for 30–55% of the observed decreases in $[Na^+]i$ and $[Cl^-]i$ and increases in $[K^+]i$ in t$^-$he branchial tissue during respiratory acidosis (figs. 4 and 7F).

In this regard, Munger *et al.* (1991) recorded rather different control levels of intracellular elsectrolytes in the gills of trout. The stock of trout was different, but experimental conditions and methods were otherwise identical. In their fish, control normoxic $[Na^+]i$ (29 $mEq \cdot L^{-1}$ *vs* 55 $mEq \cdot L^{-1}$) and $[Cl^-]i$ (44 $mEq \cdot L^{-1}$ *vs* 75 $mEq \cdot L^{-1}$) were substantially lower and $[K^+]i$ (118 $mEq \cdot L^{-1}$ *vs* 90 $mEq \cdot L^{-1}$) higher than in the fish of the present study. We have no definite explanation for the difference between the two studies though it may relate to a slightly different fluid volume distribution in the gills of the trout studied by Munger *et al.* (1991). These lower control $[Na^+]$ and $[Cl^-]i$ values seem intrinsically more reasonable, though there are no other gill data available for reference. Nevertheless, even these lower control $[Na^+]i$ and $[Cl^-]i$ values are still significantly higher than the concentrations recorded during hyperoxia in the present study (fig. 4). We conclude that absolute measurements of intracellular ions are very difficult in a tissue such as the gill epithelium. Qualitatively, the gill intracellular ion responses during hyperoxia are real (fig. 4), but their magnitude may have been quantitatively over-estimated.

A further consideration is that intracellular acid-base parameters, ion concentrations and fluid volumes represent 'global averages' for all the different cell types in a particular tissue. For homogeneous tissues such as white muscle and RBCs, this is not a serious concern. However, brain tissue consists of glial cells and numerous different types of neurons. Gill tissue is particularly heterogeneous, and the present sampling method would have included pavement cells (likely the largest component), mitochondrial-rich 'chloride' cells, mucous cells, pillar cells, neuro-epithelial cells, non-differentiated cells, and capillary endothelium (Laurent and Perry, 1991). The various cells may exhibit different or even opposite intracellular responses which cannot be distinguished in the global average response.

Finally, several recent studies have suggested that acid titrations of tissue homogenates may overestimate tissue intracellular β_{NB} values (Wiseman and Ellington, 1989; Portner, 1990). The problem seems to be worse in invertebrates (Portner, 1990), and we are aware of no relevant studies in fish. However, in the present study, the RBC β_{NB} obtained by acid titration of homogenates (74.5 slykes; table 1) was very close to the

value (75.5 slykes) which could be calculated from CO_2 titrations of whole blood and the known relationship between pHe and RBC pHi changes during such titrations, both of which were determined in earlier studies (Wood *et al.*, 1982; Milligan and Wood, 1985).

Responses to hypercapnia. The hypercapnia experiments were performed to confirm that intracellular responses to hyperoxia were specifically associated with respiratory acidosis, and not with high P_{O_2} itself. For the great majority of responses, the hypercapnia results (fig. 7) provided this confirmation with only minor quantitative differences from the hyperoxia results. Therefore, these data will not be discussed separately. In the following discussion of hyperoxia responses, only those few cases where the hypercapnia data differed in a qualitative or a major quantitative fashion will be addressed.

Extracellular responses to hyperoxia. Changes in plasma acid-base status and ions during hyperoxia and normoxic recovery were generally very similar to those recorded in an earlier study using the same protocol with repetitively sampled trout (Hōbe *et al.*, 1984; Wheatly *et al.*, 1984). Relationships between these responses and accompanying changes in ventilation (Wood and Jackson, 1980) and the fluxes of ions and acidic equivalents at gills (Wood *et al.*, 1984; Goss and Wood, 1990) and kidney (Wheatly *et al.*, 1984) have been described in detail elsewhere (Truchot, 1987).

In brief, hyperoxia induces persistent hypoventilation and CO_2 retention. The resulting respiratory acidosis is corrected largely by the excretion of acidic equivalents across the gills, aided by a relatively minor excretion via the kidney. The branchial acid excretion is accompanied by a substantial net loss of Cl^- and small net uptake of Na^+ across the gills. In consequence, plasma [Cl^-]e falls, plasma [Na^+]e rises slightly, and plasma [HCO_3^-]e increases sufficiently to restore control extracellular pH by 72 h (figs. 1 and 2). Upon return to normoxia, there is an immediate increase in ventilation which quickly lowers Pa_{CO_2} while the elevated [HCO_3^-]e persists for some time. The resulting metabolic alkalosis is corrected more quickly than the original acidosis (fig. 1). This compensation is achieved largely by a reversal of branchial flux patterns, again with a minor renal contribution. Net basic equivalent excretion (or acid uptake) is accompanied by a net uptake of Cl^- and loss of Na^+ across the gills which return plasma [HCO_3^-]e, [Na^+]e, [Cl^-]e, and pHa to more or less normal values within 24 h (figs. 1 and 2). During both phases of the experiment, there are virtual 1 : 1 relationships between net Na^+-Cl^- (*i.e.* SID) fluxes and acidic equivalent fluxes in the opposite direction, and between changes in plasma [SID] and plasma metabolic base load.

Intracellular acid-base responses to hyperoxia. In all four tissues examined (gill, brain, white muscle, and RBC), pHi was completely regulated within 48 h during the respiratory acidosis of hyperoxia (fig. 3). Notably, pHi compensation preceded pHe compensation. This confirms the report of Hōbe *et al.* (1984) that both white muscle pHi and mean whole body pHi were not significantly different from control at 72 h hyper-

oxia. These findings are also in broad agreement with several studies employing hypercapnia to induce respiratory acidosis. Thus, Perry *et al.* (1988) reported complete regulation of liver and RBC pHi, but not pHe, after 24–48 h hypercapnia in trout. In catfish, pHi in red muscle, heart ventricle, and brain was completely compensated after 24 h hypercapnia, whereas pHe was still depressed (Cameron, 1985). In dogfish, heart ventricle pHi, but not pHe, was completely regulated after 5 days hypercapnia (Heisler, 1989). In another elasmobranch, the skate, pHi compensation in heart ventricle and brain, while incomplete, was faster than pHe compensation over the first 24 h of hypercapnia (Wood *et al.*, 1990). The general pattern which emerges is that of preferential regulation of intracellular acid-base status, at least in essential and metabolically active tissues. However, in three of the species (catfish, dogfish, skate), white muscle pHi was less well regulated.

A more detailed analysis of acid-base responses in the extracellular and four intracellular compartments is provided by the pH-HCO_3^- diagrams of fig. 8. Appropriate β_{NB} values for true plasma were calculated from measured blood [Hb] (see Wood *et al.*, 1982) and for tissues were taken from table 1. The active regulation of extracellular acid-base status during hyperoxia was evidenced by the clear elevation of plasma $[HCO_3^-]e$ above that predicted from the slope of the passive non-HCO_3^- buffer line from 12 h onwards (from 3 h onwards during hypercapnia), and its more precipitous decline during normoxic recovery (fig. 8A), in agreement with previous studies (Wood and Jackson, 1980; Höbe *et al.*, 1984).

The most precise regulation of pHi occurred in gill tissue, with no significant changes from the control value throughout the experimental regime (fig. 8b), despite the extremely low β_{NB} value (table 1). The slightly greater deviation during hypercapnia was still not significantly different from control. The clear divergence of these experimental responses from the large pHi changes predicted by the passive buffer line indicated that gill pHi was actively regulated during systemic respiratory acidosis (hyperoxia, hypercapnia) and metabolic alkalosis (normoxic recovery). We are aware of no gill pHi data on other species. However, in recent studies on trout using similar methods, gill pHi exhibited very small but significant changes in response to systemic metabolic acidosis and metabolic alkalosis induced by acid and base infusions (G.G. Goss and C.M. Wood, unpublished results). However again, the magnitude of these changes was far less than predicted by passive buffering. While whole gill pHi is clearly well regulated, it remains possible that small changes in the intracellular acid-base status of the branchial transport cells are responsible for the large changes in Na^+/'acid' and Cl^-/'base' exchanges occurring at the gills during these systemic acid-base disturbances (Wood *et al.*, 1984; Truchot, 1987; Goss and Wood, 1990). Conversely, it is also possible that modulation of these exchanges contributes to this precise regulation of gill pHi. K^+ exchange with the extracellular compartment also appears to play an important role (see below).

Brain was the other tissue exhibiting immediate regulation of pHi, with responses differing from those predicted by passive buffering within 3 h of hyperoxic exposure, and again within 3 h of return to normoxia (fig. 8C). Similar evidence of active pHi regu-

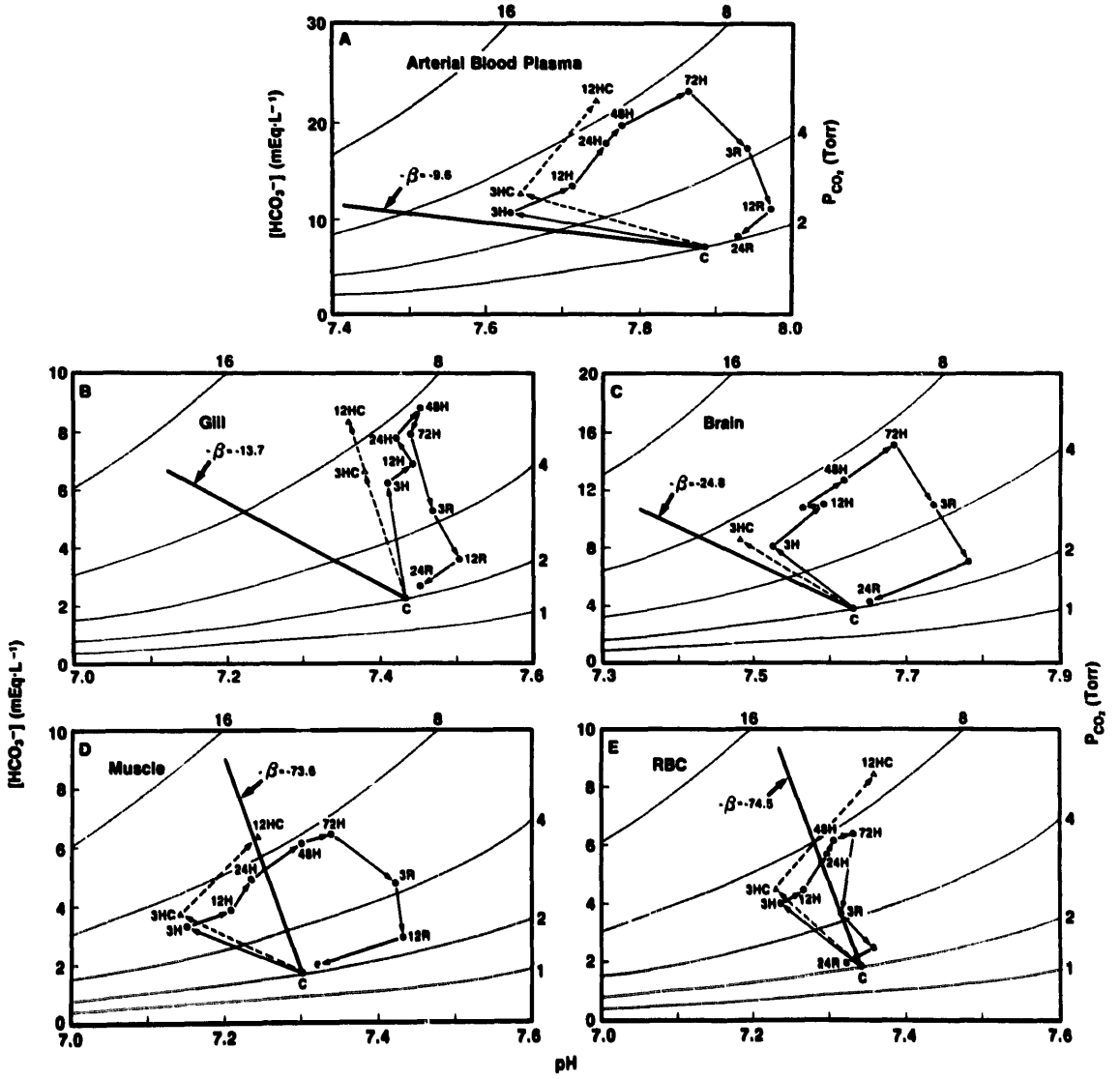

Fig. 8. Graphical representations of the simultaneous changes in pH, $[HCO_3^-]$, and P_{CO_2} levels caused by the experimental treatments in (A) arterial blood plasma (extracellular fluid) and the intracellular fluid compartments of (B) gill tissue; (C) brain tissue; (D) white muscle tissue, and (E) red blood cells (RBC) of rainbow trout at 15 °C. Equilibration of P_{CO_2} between arterial blood plasma and the intracellular compartments has been assumed. For each compartment, the appropriate non-HCO_3^- buffer relationship (heavy line with slope = $-\beta_{NB}$ in slykes) has been plotted. The solid dots (●) connected by solid lines and arrows represent mean data from normoxic control (C), 72 h of environmental hyperoxia (H), and 24 h of return to normoxia (R). The open triangles (△) connected by broken lines and arrows represent mean data from normocapnic control (C, same as normoxic control), 3 h, and 12 h of environmental hypercapnia (HC). Means only, *N* values as in legends of figs. 1 and 7 for hyperoxia and hypercapnia treatments, respectively.

lation in brain has been reported in both catfish (Cameron, 1985) and skate (Wood *et al.*, 1990) exposed to environmental hypercapnia. Control of intracellular acid-base status is presumably critical to the maintenance of normal function in this essential tissue.

In contrast to gill and brain tissue, white muscle exhibited a much greater depression

of pHi than predicted by passive buffering during the first 3 h of hyperoxia – *i.e.* a compounding metabolic acidosis (fig. 8D). However, active regulation had started within 12 h, and returned pHi to normal by 48 h. This pattern of an initial metabolic acidosis compounding the respiratory acidosis is very similar to that in the white muscle of skate exposed to environmental hypercapnia (Wood *et al.*, 1990). The pattern also agrees well with the model calculations of Heisler (1989) for dogfish exposed to environmental hypercapnia, and with the measurements of Cameron (1985) which suggest that muscle pHi regulation is slow in catfish under the same conditions. Presumably, the metabolic acidosis occurs because basic equivalents generated by passive buffering in muscle are initially exported to other compartments, an explanation which fits with measured intracellular ionic changes (see below). This flux would help regulate acid-base status in the extracellular (fig. 8A) and critical intracellular compartments such as gill (fig. 8B) and brain (fig. 8C). Only later, once branchial acidic equivalent excretion is fully activated (Wood *et al.*, 1984), is the muscle pHi regulation completed.

Interestingly, during the first 12 h of normoxic recovery, the muscle data did not deviate significantly from the pattern predicted by passive buffering alone (fig. 8D). Thus, white muscle did not reverse its 'altruistic' role to serve as a sink for basic equivalents during metabolic alkalosis. This may explain why pHi excursions were greater in gill and brain at this time (fig. 8B,C), despite a smaller pHe excursion (fig. 8A) and greater activation of acid-base exchange mechanisms with the environment (Wood *et al.*, 1984; Goss and Wood, 1990).

The other well buffered tissue, RBCs, exhibited a response which was qualitatively similar to that of white muscle (fig. 8D), with a compounding metabolic acidosis during the initial stages of hyperoxia or hypercapnia (fig. 8E). A similar pattern was seen in the RBCs of skate during the initial stages of exposure to hypercapnia (Wood *et al.*, 1990). However, RBCs are a special case, due to the presence of the Band 3 Cl^-/HCO_3^- exchanger in the cell membranes, which will rapidly export much of the HCO_3^- formed by passive buffering to the extracellular compartment (Nikinmaa and Tufts, 1989), thereby depressing pHi below the point predicted by passive buffering. RBC acid-base status would not be expected to follow the buffer line, let alone rise above it, unless active regulation mechanisms were brought into play. Therefore, it is notable that RBC pHi was returned to normal by 12 h during hypercapnia and by 24 h during hyperoxia (fig. 8E). Adrenergic activation of Na^+/H^+ exchange is well documented in trout RBCs (Nikinmaa and Tufts, 1989) and hypercapnic exposure is known to be a potent elicitor of catecholamine mobilization (Perry *et al.*, 1989). Therefore, the rapid regulation of RBC pHi during hypercapnia was not surprising. However, short term hyperoxic exposure (0.5 h) is reported to cause no catecholamine mobilization in trout (Perry *et al.*, 1989). Plasma catecholamine measurements during longer term exposures might prove informative.

Intracellular ionic and fluid volume responses to hyperoxia. The most remarkable intracellular responses were the dramatic fall in gill $[Na^+]i$, $[Cl^-]i$, and ICFV, and rise in $[K^+]i$ during hyperoxia (and hypercapnia), and the partial or complete reversal of these

changes following return to normoxia (figs. 4, 6A and 7C,D). As discussed previously, absolute values might be questioned, but the trends appear to be real. The shrinkage in gill ICFV is likely associated with the net reduction of intracellular electrolytes during respiratory acidosis. Three other major phenomena are occurring concurrently at the gills. The first is the precise regulation of gill pHi (figs. 3B and 8B). The second are the marked changes in the net branchial exchanges of Na^+, Cl^-, and acidic equivalents with the environment which were outlined earlier. The third are rapid morphological changes in the branchial epithelium, most notably alterations in the numbers, internal structure, and fractional exposure of chloride cells and overlying pavement cells (Laurent and Perry, 1991; G.G. Goss, C.M. Wood, S.F. Perry, and P. Laurent, unpublished results).

It seems probable that the morphological changes, the flux changes, and the intracellular ion, acid-base, and fluid volume adjustments are functionally linked together in some way, as in many other transporting epithelia (Roos and Boron, 1981; Busa, 1986; Schultz and Hudson, 1986). One obvious linkage is that the greater fall in gill $[Cl^-]i$ than gill $[Na^+]i$ (fig. 4A,B) might result from the greater fall in unidirectional Cl^- influx than in Na^+ influx across the gills during hyperoxia (Goss and Wood, 1990). Another is that this net loss of strong negative charge, coupled with the gain of strong positive charge from the elevation in gill $[K^+]i$ (fig. 4C), would greatly increase the intracellular SID, thereby contributing to the precise regulation of gill pHi in the face of respiratory acidosis (fig. 8B). It should be noted that while the directional influence of individual ion shifts can be assessed, the absolute intracellular SID cannot be quantified with the present data. In contrast to the extracellular compartment, where $[Na^+]e + [K^+]e - [Cl^-]e$ provides a reasonable estimate of absolute SID (fig. 2D), many other strong ions (Mg^{2+}, phosphate, organic anions) together with activity coefficients, must be considered in the intracellular compartment.

In contrast to gill tissue, white muscle ICFV increased significantly during hyperoxia and hypercapnia (figs. 6B and 7C). This swelling of muscle cells was reminiscent of the well known swelling of RBCs during respiratory acidosis (Nikinmaa and Tufts, 1989), evidenced by the significant fall in MCHC in the present study (fig. 6C). An increase in white muscle ICFV similarly occurred in the skate during exposure to hypercapnia (Wood *et al.*, 1990). The explanation could be a simple Donnan effect associated with high P_{CO_2}, but the fact that the swelling was not reversed during normoxic recovery (fig. 6B) suggests that a more complex explanation might also apply.

The increase in muscle $[Cl^-]i$ (fig. 5B) would concur with the Donnan effect explanation as the consequence of Cl^-/HCO_3^- exchange. Thus, some of the $[HCO_3^-]i$ generated by passive buffering in white muscle would be transferred to the extracellular compartment in exchange for $[Cl^-]e$, thereby contributing to the observed increase in plasma $[HCO_3^-]e$ (fig. 1D), fall in plasma [Cl]e (fig. 2B), and metabolic acidosis in white muscle during hyperoxia (fig. 8D). However, this result must be interpreted with caution because no change in muscle $[Cl^-]i$ was detected during hypercapnia (fig. 7E). The large potential for error in muscle $[Cl^-]i$ and $[Na^+]i$ measurements through ECFV error was noted earlier.

In contrast, muscle [K^+]i measurements were virtually insensitive to ECFV error and qualitatively consistent between the hyperoxia and hypercapnia exposures. The precipitous drop in muscle [K^+]i at the start of hyperoxia (fig. 5C), and slower decline during hypercapnia (fig. 7F), would substantially reduce the intracellular SID, and therefore greatly contribute to the metabolic acidosis in white muscle (fig. 8D) and the associated export of basic equivalents to other compartments. Elevations in plasma [K^+]e (fig. 2C; also Wheatly *et al.*, 1984), and gill [K^+]i (figs. 4C and 7F) occurred in concert with the decrease in muscle [K^+]i. In effect, an H^+ uptake (*i.e.* base efflux)/K^+ efflux 'exchange' appears to operate at the muscle cell membrane. Activation of this mechanism during the initial stages of respiratory acidosis serves to preferentially regulate acid-base status in other more critical compartments at the expense of increased muscle acidosis. An identical phenomenon has been observed in mammals, but the exact mechanism of the 'exchange' remains obscure (Williams and Epstein, 1989). The only previous examination of muscle intracellular ions in fish under respiratory acidosis (Cameron and Iwama, 1987) did not show this phenomenon. However, in their study, samples were taken from catfish after several days exposure to a progressively increasing environmental P_{CO_2}, so the conditions were not comparable. In the present study, the shift of K^+ and basic equivalents out of white muscle over the first 12 h amounted to 5–10 mEq · kg^{-1} on a whole fish basis, comparable to the approximately 11 mEq · kg^{-1} of net acidic equivalent excretion to the environment measured over the entire 72 h hyperoxic exposure (Wood *et al.*, 1984). Clearly, this short term 'buffering' role of the muscle compartment is quantitatively important, and deserves further attention in future studies.

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