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THE MECHANISMS OF ACID–BASE AND IONOREGULATION IN THE FRESHWATER RAINBOW TROUT DURING ENVIRONMENTAL HYPEROXIA AND SUBSEQUENT NORMOXIA. I. EXTRA- AND INTRACELLULAR ACID–BASE STATUS

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Abstract. The extracellular acid–base status of the freshwater rainbow trout *(Salmo gairdneri)* was continuously monitored during 24 h normoxia ($P_{I_{O_2}}$ = 120–150 torr; control), 72 h hyperoxia ($P_{I_{O_2}}$ = 500–600 torr) and 24 h return to normoxia. Hyperoxia induced a marked respiratory acidosis (ΔpHe = −0.23 unit) due to a 3-fold elevation in arterial CO_2 tension which was completely compensated over 72 h by a comparable rise in plasma bicarbonate, reflecting effective removal of acidic equivalents from the ECF. Upon return to normoxia, arterial CO_2 tension rapidly returned to normal against a background of high plasma bicarbonate, provoking a metabolic alkalosis which was largely compensated by 24 h. This effective restoration of acidic equivalents in the ECF occurred more rapidly than the original removal.

Intracellular acid–base status was measured during normoxia and after 72 h hyperoxia using the steady state distribution of ^{14}C-DMO. The rate of ^{14}C-DMO excretion was 0.479 ± 0.048 (% DMO lost per hour) during normoxia, and significantly decreased with hyperoxia. A considerable overestimate of mean whole body pHi would have resulted had this not been taken into account. Whole body and white expaxial muscle were similar with a pHe − pHi gradient of *ca.* 0.5 during normoxia, and underwent identical changes during hyperoxia. Intracellular pH was completely compensated by 72 h hyperoxia as intracellular bicarbonate increased 4-fold. The overall net removal of acidic equivalents from the ICFV was approximately one half that from the ECFV, but pHe regulation did not occur at the expense of pHi regulation. The ultimate restoration of both pHe and pHi during hyperoxia must have occurred *via* kidney or gills.

Acid–base balance | Hyperoxia
^{14}C-DMO excretion | Intracellular pH
Extracellular pH | *Salmo gairdneri*

The maintenance of acid–base homeostasis in vertebrates requires a continual balance between intake, endogenous production, and excretion of acidic equiva-

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lents. Air-breathing forms generally rely on ventilation to modulate internal carbon dioxide tension for short-term blood pH regulation. Water-breathers may be limited in this regard by the distinctive design of their respiratory gas-exchanger as well as the physico-chemical properties of the aqueous external medium. Instead, they appear to regulate blood pH mainly by plasma bicarbonate adjustment. The intrinsic and extrinsic mechanisms which are thought to be involved include intracellular buffering (*e.g.*, Heisler and Neumann, 1980), renal excretion (*e.g.*, Wood and Caldwell, 1978) and modulation of electroneutral cationic and anionic exchanges at the gill epithelium (*e.g.*, Maetz and García-Romeu, 1964; Cameron, 1976). However, the relative quantitative contribution of each of these processes has not been well studied. The aim of the present investigation was to make such an assessment using environmental hyperoxia as an experimental tool to provoke internal acid–base disequilibria.

The mechanisms of acid–base regulation in freshwater teleosts have commonly been investigated by examining their responses to various exogenous stimuli which cause internal acid–base disturbance – *e.g.* changes in water ionic composition (*e.g.*, DeRenzis and Maetz, 1973), environmental hypercapnia (*e.g.*, Cameron, 1976; Perry *et al.*, 1981), low ambient pH (*e.g.*, McDonald *et al.*, 1980), temperature alterations (*e.g.*, Cameron and Kormanik, 1982a), as well as the infusion of acid or base loads (*e.g.*, Wood and Caldwell, 1978; Cameron and Kormanik, 1982b). These stimuli may themselves directly interfere with ion-exchange processes at the gills or cause other deleterious effects within the animal, thereby complicating interpretation. However, environmental hyperoxia appears unique in this regard as the ensuing 'respiratory' acidosis (P_{CO_2} elevation) is endogenously generated. This occurs both as a consequence of internal diffusive and/or perfusive limitation of branchial CO_2 excretion (Wood and Jackson, 1980), probably due to the vasoconstrictory influence of high P_{O_2} on the gills (Haswell *et al.*, 1978) and external convective limitation due to hypoventilation (Dejours, 1973; Truchot *et al.*, 1980). The hyperoxia-induced depression in blood pH is also well tolerated by the fish and a compensatory reaction (bicarbonate accumulation) is initiated within a few hours. At least in the rainbow trout, complete compensation in the blood is reached by *ca.* 48 h, regardless of the ensuing hyperoxia (Wood and Jackson, 1980). Return to normoxia promotes a marked 'metabolic' alkalosis, for P_{CO_2} levels quickly drop to normal, while the elevated HCO_3^- levels are reduced more slowly.

In this, the first of three papers, blood gases and extracellular acid–base status are described prior to, during, and following hyperoxic exposure (PI_{O_2} = 500–600 torr) in the freshwater rainbow trout *(Salmo gairdneri)* as a framework upon which the relative roles of the intracellular compartment, kidney and gills in the compensation could be assessed. The intracellular acid–base status was studied under normoxia and hyperoxia using the steady-state distribution of ^{14}C-DMO (5,5-dimethyl-2-4-oxazolidinedione), paying particular attention to the possibility of artifact induced by DMO excretion (*cf.* Cameron and Kormanik, 1982a). Subsequent papers de-

scribe renal function (Wheatly *et al.*, 1984) and branchial ionoregulatory mechanisms during these adjustments and assess the overall contribution of all three sites to the total acidic equivalent budget of the animal (Wood *et al.*, 1984).

Materials and methods

Experimental animals. One-to-two year old rainbow trout (*Salmo gairdneri;* 190–400 g) of both sexes were obtained from Spring Valley Trout Farm, Petersburg, Ontario, Canada, and held in fibreglass tanks for at least two weeks. Each tank received a continuous flow of aerated, dechlorinated tapwater (12–14 °C) with the following composition: $[Na^+] \approx 0.6$ mEq $\cdot L^{-1}$, $[Cl^-] \approx 0.8$ mEq $\cdot L^{-1}$, $(Ca^{2+}] \approx 1.6$ mEq $\cdot L^{-1}$, $[K^+] \approx 0.04$ mEq $\cdot L^{-1}$, titration alkalinity ≈ 2.0 mEq $\cdot L^{-1}$, total hardness ≈ 140 mg $\cdot L^{-1}$ as $CaCO_3$, pH ≈ 7.8. The fish were starved for 7 days prior to use to minimize any influence of feeding on ion or acid–base metabolism.

Three experimental series were performed, of which I and II are reported in the present paper (see Wheatly *et al.*, 1984 and Wood *et al.*, 1984, for Series III). For this study, trout were fitted with a dorsal aortic catheter (Smith and Bell, 1964) while under MS-222 anaesthesia and subsequently allowed to recover in normoxic water for 72 h. Cannulated fish were kept individually in 2 L lucite boxes which were contained within covered, 10 L, rectangular chambers (see diagram in McDonald, 1983). Glass marbles were used to reduce effective closed-circuit volume to 4–6 L. The exact volume was measured at the end of the experiment by means of radioisotope dilution (^{22}Na). Water was continually aerated, mixed, and circulated through the inner fish box at *ca.* 0.5 L $\cdot$ min^{-1} by an airlift pump at the rear. The outer boxes were surrounded with a water jacket which maintained temperature at 12–14 °C. By switching the airlift gas from humidified air to humidified O_2, the $P_{I_{O_2}}$ could be rapidly (< 10 min) changed from normoxic (120–150 torr) to hyperoxic levels (500–600 torr) without measurable alteration of $P_{I_{CO_2}}$ (< 0.3 torr as determined with a Radiometer P_{CO_2} electrode). The experimental water was renewed by thorough flushing (30 L/chamber) with water at the appropriate $P_{I_{O_2}}$ and temperature at 12–24 h intervals so as to ensure ambient ammonia levels remained < 500 μM $\cdot L^{-1}$.

Experimental protocol. *Series I* examined extracellular blood gas, acid–base, ionic and hematological status of trout during 24 h normoxia (control), 72 h hyperoxia, and 24 h return to normoxia. Blood samples (600 μl) were sequentially drawn from individual trout (mean wt = 294 ± 16 g; n = 12) prior to (control, 'C' in figures), at 5, 24, 48, 72 h of hyperoxia and after 5 h and 24 h of recovery in normoxia. Whole blood oxygen tension, hematocrit, hemoglobin, plasma pH, total carbon dioxide content, and protein concentration were measured. The remaining plasma was analyzed for ionic content as described by Wheatly *et al.* (1984). An equal volume of Cortland saline (Wolf, 1963) was injected to replace blood volume lost.

Series II studied the effects of hyperoxia on intracellular acid–base status. Following post-operative recovery, fish were either maintained in normoxic water (control; mean wt = 280 ± 17 g, n = 9) or exposed to 72 h hyperoxia and then sampled (experimental; mean wt = 298 ± 18 g, n = 7). For the analysis of mean whole body and white muscle intracellular pH, a dose of ^{14}C-labelled DMO was infused via the DA catheter over 1 min. Allowing for a DMO-equilibration time of 4–6 h (preliminary results), 3–5 blood samples (300 μl) were then removed with saline replacement at intervals up until 12–13 h post-injection and analyzed for hematocrit, plasma pH, total carbon dioxide content and ^{14}C-labelled DMO radioactivity. Water samples were collected simultaneously and ^{14}C-radioactivity measured to account for possible isotope excretion. At 12–13 h, a terminal blood sample was drawn. The fish was then rapidly killed by a blow on the head, weighed, and epaxial white muscle samples excised from a site just caudal to the dorsal fin for ^{14}C-radioactivity analysis.

Analytical techniques and calculations. Blood samples were drawn anaerobically using gastight Hamilton syringes. Hemoglobin concentration (Hb) was measured using the cyanmethemoglobin technique (Blaxhall and Daisley, 1973; Sigma reagents). Hematocrit (Ht) was determined by centrifugation (5000 g for 4 min). Mean corpuscular hemoglobin concentration (MCHC) was calculated as the ratio of [Hb]/fractional Ht. Total protein in plasma (Pr^-) was determined using a Goldberg refractometer (American Optical).

Arterial blood oxygen tension (Pa_{O_2}), pH (pHa) and plasma total carbon dioxide content (Ca_{CO_2}) were measured immediately upon collection using Radiometer microelectrodes and methodology outlined in McDonald *et al.* (1980) and Wood and Jackson (1980). Arterial plasma bicarbonate concentration ($[HCO_3^-]a$) and carbon dioxide tension (Pa_{CO_2}) were then calculated by the Henderson–Hasselbalch equation using values of pK^1 and α_{CO_2} tabulated in Severinghaus (1965). The change in the concentration of acidic equivalents (formerly termed 'metabolic acid or base load') in the blood plasma over a given time interval was calculated according to the formula of McDonald *et al.* (1980):

$$\Delta H_p^+ = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta(pH_1 - pH_2) \quad (1)$$

where β is the slope of the true plasma non-bicarbonate buffer line (*i.e.*, $-\Delta HCO_3^- / \Delta pH$) and subscripts 1 and 2 refer to successive sampling times. The 'β' value was calculated from the [Hb] (g/100 ml) at each time 2 by means of the regression relationship:

$$\beta = -1.271\,[Hb] - 2.31 \quad (2)$$

determined by Wood, McDonald and McMahon (1982) for rainbow trout (true) plasma *in vitro* at this temperature. Variations in plasma protein had a negligible effect relative to [Hb] on β. The net load of acidic equivalents in blood plasma at any

sample time was calculated by summing the ΔH_p^+ values, signs considered, for each period from the control sample onwards.

Intracellular pH(pHi) was estimated using the weak acid DMO (5,5-dimethyl-2,4-oxazolidinedione) distribution method of Waddell and Butler (1959). An infusion dose of 7 μCi ^{14}C-labelled DMO (New England Nuclear) per ml saline per kg fish was washed in with an additional 2 ml saline per kg. For mean whole body pHi estimates, aliquots of injection stock (20 μl in triplicate), plasma (40 μl in duplicate) and water (5 ml) were added to scintillation fluid (10 ml PCS; Amersham) and ^{14}C-radioactivity measured on a Beckman LS-250 scintillation counter. For epaxial white muscle pHi analyses, duplicate muscle (0.5–0.6 g fresh wt) and plasma samples (50 μl) were digested in tissue solubilizer (5 ml NCS; Amersham) for 8–10 days. The clear digest was then neutralized (*ca.* 150 μl glacial acetic acid), diluted with scintillation fluid (10 ml OCS; Amersham) stored in the dark for 48 h to reduce chemiluminescence, and ^{14}C-radioactivity measured as described above. The channels ratio method was used to correct for quenching.

Mean whole body pHi at each time or terminal tissue pHi was calculated according to the equation (Cameron, 1980):

$$\mathrm{pHi} = \mathrm{pK_{DMO}} + \log\left\{\frac{\mathrm{DMOi}}{\mathrm{DMOe}}\left(1 + 10^{(\mathrm{pHe} - \mathrm{pK_{DMO}})} - 1\right)\right\} \tag{3}$$

where, pHe is the plasma pHa (measured in duplicate) and $\mathrm{pK_{DMO}}$, the dissociation constant for DMO calculated as 6.38 (14 °C) using the empirical relationship between $\mathrm{pK_{DMO}}$ and temperature at an ionic strength of 0.125, given in Malan *et al.* (1976). DMOe (cpm · ml^{-1}) and DMOi represent the concentrations of DMO in the extracellular (ECF) and intracellular (ICF) fluid compartments, respectively. The compartmental volumes needed for calculation of DMOe and DMOi were measured with ^{14}C-mannitol under identical normoxic conditions in a separate study (Milligan and Wood, 1982). The values (means ± SEM, n) were:

	Whole body (ml · kg^{-1} fish weight)	Epaxial white muscle (ml · kg^{-1} muscle weight)
ECFV	273.5 ± 8.8 (12)	73.2 ± 2.6 (8)
ICFV	459.1 ± 9.4 (11)	698.0 ± 3.5 (8)

The pHi calculation assumes (1) that the dissociation constant ($\mathrm{pK_{DMO}}$) is the same in the aqueous phases of the intra- and extracellular compartments, (2) that the plasma DMO concentration is representative of the ECF, (3) that the distribution of DMO in ICF is uniform, and (4) that the arterial plasma pH is a reasonable approximation of interstitial fluid pH (Waddell and Bates, 1969; Roos and Boron, 1981). The plasma DMO data obtained in the present study exhibited irregular fluctuation over the first few hours but stabilized by 4–6 h post-infusion,

thereafter slowly declining. Calculated values of pHi did not change significantly after this time. Therefore we concluded that the distribution of the undissociated acid (DMO) between ECFV and ICFV had reached a steady state by 4–6 h.

Significant amounts of ^{14}C-DMO were lost from the fish to the medium (see Results). Therefore, for the mean whole body pHi determinations, DMOi (cpm · ml^{-1}) was calculated as:

$$\text{DMOi} = \frac{(\text{total dose injected}) - (\text{loss to water}) - (\text{DMOe} \cdot \text{ECFV})}{\text{ICFV}} \quad (4)$$

Mean whole body pHi was estimated for each blood and water sample pair between 6–13 h post-infusion and then values averaged for individual fish (grand means presented in table 2). After calculation of pHi and measurement of pHa and Ca_{CO_2}, intracellular bicarbonate levels were calculated by the Henderson–Hasselbalch equation using constants at the appropriate pH from Severinghaus (1965) assuming a uniform Pi_{CO_2} equal to Pa_{CO_2}. While $P\bar{v}_{CO_2}$ would be a more realistic estimate of Pi_{CO_2}, this quantity was not measured. However, it is unlikely that any large systematic error resulted, as the resulting bicarbonate data were used only for comparative purposes.

Statistical analyses. All measured and calculated values throughout the text are presented as means ± SEM (n) unless otherwise stated. The reduction in n in Series I at 72–96 h reflects cannula failure. Time-dependent responses to hyperoxia or re-institution of normoxia were tested relative to the normoxic controls (labelled 'C' in figures) using a Student's two-tailed *t*-test (paired design, Series I; unpaired design, Series II). Possible relationships between some variables were assessed by computing simple Pearson's correlation coefficients. A 5% level of significance was chosen.

Results

Extracellular – Series I. Both Ht and [Hb] significantly decreased from normoxic controls by 52% and 55%, respectively, over the entire experimental period, probably as a consequence of repetitive sampling since MCHC remained virtually unchanged at ≈29 g · 100 ml^{-1} RBC (table 1). The non-bicarbonate buffering capacity of blood plasma (β) decreased in parallel with [Hb]. The gradual drop in plasma [Pr$^-$] was not significant which, together with the constancy of MCHC, indicated that major ECF-ICF compartmental water shifts were probably not occurring during hyperoxia.

Sequential changes in blood gases and extracellular acid–base status are illustrated in fig. 1. Over the initial 5 h of hyperoxia, Pa_{O_2} increased from 105 ± 3 (12) torr to 372 ± 15 (12) torr in parallel with a 3.5 fold rise in Pi_{O_2}; Pa_{O_2} remained elevated for the entire 72 h (fig. 1A). The initial 3-fold elevation in Pa_{CO_2} from

TABLE 1

Hematological indices and blood plasma non-bicarbonate buffer capacity in rainbow trout prior to, during, and following exposure to hyperoxia in Series I. Mean values (± SEM) significantly different from normoxic controls ($P < 0.05$) are designated by asterisks. Symbols have been defined in the text.

Variable	Treatment:	Normoxia	Hyperoxia				Normoxia	
		Control	5 h	24 h	48 h	72 h	5 h	24 h
	n:	(12)	(12)	(12)	(12)	(10)	(10)	(7)
Ht (%)		25.4 ± 2.2	25.5 ± 2.2	22.6* ± 1.8	19.5* ± 2.0	15.2* ± 1.8	12.7* ± 2.4	12.1* ± 2.3
[Hb] ($g \cdot 100\ ml^{-1}$)		8.15 ± 0.79	7.49 ± 0.73	6.92* ± 0.73	5.23* ± 0.62	3.72* ± 0.42	3.63* ± 0.47	3.66* ± 0.75
MCHC ($g \cdot 100\ ml^{-1}$ RBC)		31.5 ± 1.0	28.9 ± 1.5	29.9 ± 2.2	26.1* ± 0.9	28.3 ± 1.8	29.9 ± 2.0	29.8 ± 2.4
[Pr^-] ($g \cdot 100\ ml^{-1}$)		2.33 ± 0.14	2.47 ± 0.12	2.33 ± 0.12	2.14 ± 0.10	2.22 ± 0.10	2.07 ± 0.11	2.05 ± 0.11
β (slyke)		12.67 ± 1.01	11.82 ± 0.92	11.11* ± 0.92	8.74* ± 0.85	7.61* ± 0.54	6.93* ± 0.60	6.96* ± 0.73

2.29 ± 0.12 (12) torr to 6.71 ± 1.51 (12) torr over 5 h (fig. 1B) was accompanied by a marked depression in pHa from 7.909 ± 0.017 (12) to 7.677 ± 0.018 (12) (fig. 1C). Pa_{CO_2} continued to increase gradually to 8.20 ± 0.43 (10) torr by 72 h. However, in face of the maintained elevation in Pa_{CO_2}, pHa returned completely to normoxic levels by 72 h of hyperoxia. This compensation was effected by a steady increase in plasma [HCO_3^-]a from 7.16 ± 0.51 (12) mEq $\cdot L^{-1}$ to 23.82 ± 1.52 (10) mEq $\cdot L^{-1}$ (fig. 1D). With the re-institution of normoxia, Pa_{O_2} and Pa_{CO_2} rapidly declined, reaching control normoxic values by 5 h (fig. 1A) and 24 h (fig. 1B) respectively. While Pa_{CO_2} was already close to control levels by 5 h, plasma [HCO_3^-]a declined much more slowly (fig. 1D). This marked drop in Pa_{CO_2} against a background of continued high plasma [HCO_3^-]a provoked a substantial elevation of pHa to 8.084 ± 0.038 (10). By 24 h return to normoxia, both pHa and [HCO_3^-]a had reached values only slightly above normoxic controls.

The time course and extent of this compensation were assessed on a pH–bicarbonate diagram (fig. 2). The non-bicarbonate buffer lines were plotted for control and 72 h points respectively using the β values given in table 1. The initial effects of hyperoxia were rapid and characterized by a respiratory acidosis which was already partially compensated by 5 h, as shown by the small shift in [HCO_3^-]a above the normoxic buffer line. More prolonged exposure led to a large build-up of HCO_3^-, restoring pHa to control levels. Upon re-institution of normoxia, Pa_{CO_2} fell rapidly so that a metabolic alkalosis developed due to the high [HCO_3^-]a. Readjustment ($\downarrow$[HCO_3^-]a) was even more rapid than the original compensation ($\uparrow$[HCO_3^-]a) and almost complete by 24 h.

The cumulative changes in ΔH_p^+ have been plotted in fig. 3. A positive deviation

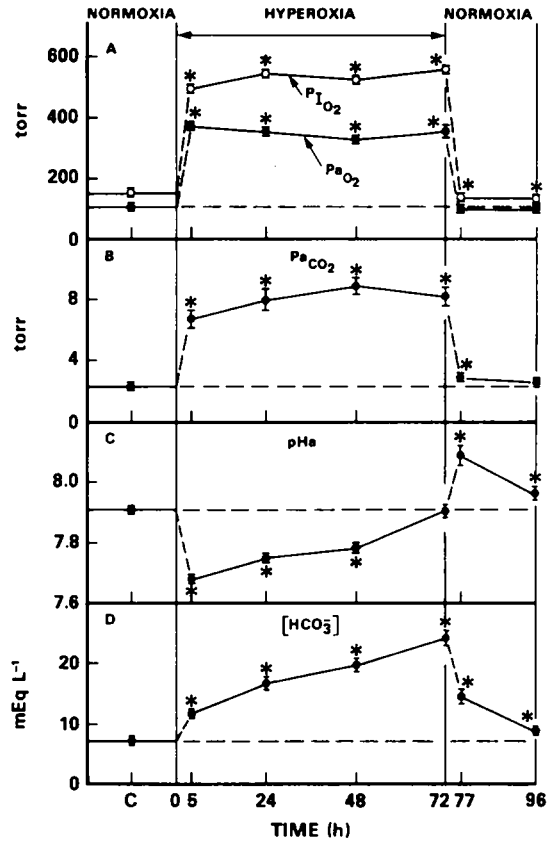

Fig. 1. Time-dependent changes in arterial blood gases and plasma acid–base status of rainbow trout prior to (normoxic control; 'C'), during, and following (normoxic recovery) hyperoxic exposure at 12–14 °C in Series I. Asterisks represent significant differences ($P < 0.05$) from normoxic control values indicated by broken lines. Values are means ± SEM. n = 12 at C – 48 h, 10 at 72 h and 77 h, and 7 at 96 h.

of ΔH_p^+ from zero signifies metabolic acid load (or base deficit) whereas a negative deviation represents a metabolic base load (or acid deficit). Over the 72 h of hyperoxia, compensation of the respiratory acidosis was effected by a gradual removal of acidic equivalents from the plasma (accumulation of base) totalling 16.08 ± 1.72 (10) mEq · L^{-1}. During normoxic recovery all but 2.26 ± 0.70 (7) mEq · L^{-1} were replaced over 24 h to compensate for the induced alkalosis. Note the very rapid replacement over the first 5 h.

Intracellular – Series II. The intracellular acid–base status (whole body and white muscle) of rainbow trout during normoxia and after 72 h hyperoxia is summarized in table 2. The arterial plasma acid–base status has been assumed to represent the ECFV compartment (pHe = pHa). Note that the magnitude and direction of changes in pHe, Pe_{CO_2} and [HCO_3^-]e substantiated those found in

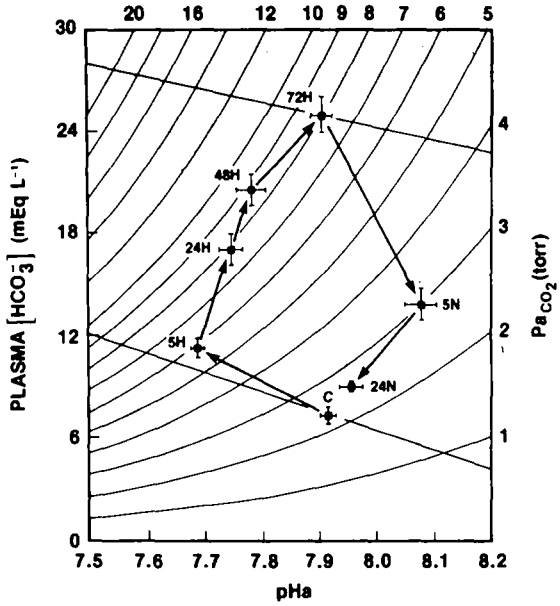

Fig. 2. A graphical representation of the simultaneous changes in pHa, $[HCO_3^-]a$ and Pa_{CO_2} in rainbow trout at rest (C), during hyperoxia (H) and upon re-institution of normoxia (N) in Series I. The non-bicarbonate buffer curves (diagonal lines) were plotted using the slopes (β) given in table 1. See legend of fig. 1 for other details.

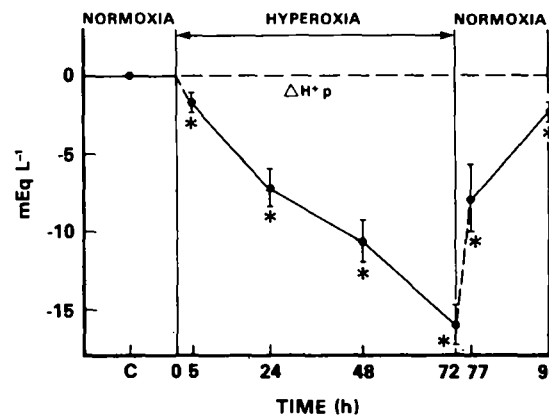

Fig. 3. The cumulative load of acidic equivalents in blood plasma (ΔH_p^+) during hyperoxia and normoxic recovery in rainbow trout in Series I. Negative values signify a metabolic base load (or acid deficit). See legend of fig. 1 for other details.

TABLE 2

A comparison of extra- and intracellular acid–base status in rainbow trout during normoxia (control) and after 72 h of hyperoxia. Values are means ± SEM. Symbols have been defined in the text.

Compartment	Variable	Normoxia (n = 9)	Hyperoxia (n = 7)	*P*
ECF*	pHe	7.852 ± 0.027	7.813 ± 0.027	n.s.
	Pe_{CO_2} (torr)	2.64 ± 0.14	11.02 ± 0.98	<0.001
	$[HCO_3^-]e$ (mEq · L^{-1})	7.00 ± 0.58	25.77 ± 0.98	<0.001
ICF – whole body	pHi	7.317 ± 0.044	7.322 ± 0.050	n.s.
	$[HCO_3^-]i$ (mEq · L^{-1})	1.89 ± 0.21	7.76 ± 0.78	<0.001
ICF – white muscle	pHi	7.296 ± 0.025	7.317 ± 0.023	n.s.
	$[HCO_3^-]i$ (mEq · L^{-1})	1.59 ± 0.08	7.10 ± 0.45	<0.001

* Arterial values.

Series I (fig. 1); the cause of the slightly higher Pe_{CO_2} in hyperoxia in Series II is unknown. Both the absolute magnitudes of pHi and $[HCO_3^-]i$, and the effects of 72 h hyperoxia on them, were the same for mean whole body and epaxial white muscle.

The mean pHe – pHi gradient in normoxic trout was ≈0.5 pH units; hyperoxic exposure did not modify the magnitude of this gradient. The four-fold rise in $[HCO_3^-]i$ paralleled the increase in $[HCO_3^-]e$. Compensation of pHi was therefore just as complete by 72 h as seen for pHe.

White muscle pHi was significantly correlated both with pHe ($r = 0.50$; $P < 0.05$; fig. 4A) and whole body pHi ($r = 0.66$; $P < 0.01$; fig. 4B). The slope of the regression of white muscle pHi on pHe (ΔpHi/ΔpHe) was 0.390, indicating much

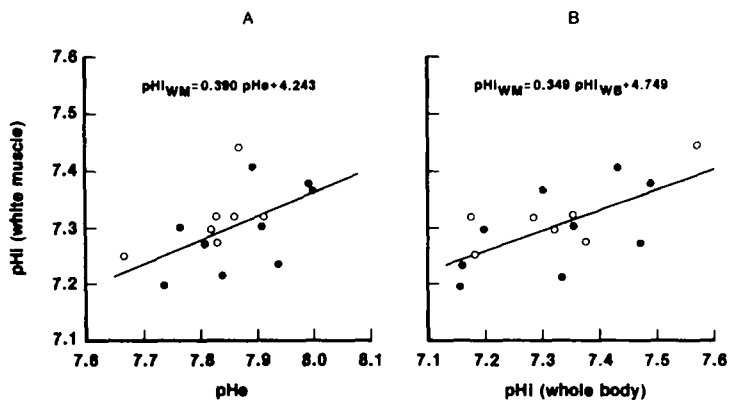

Fig. 4. The relationships between white epaxial muscle intracellular pH (pHi) and both extracellular pHe (A) and whole body pHi (B) for normoxic (●) and hyperoxic (○) rainbow trout of Series II. Regression equations are listed in the figure. Correlation coefficients and significance levels are given in the text.

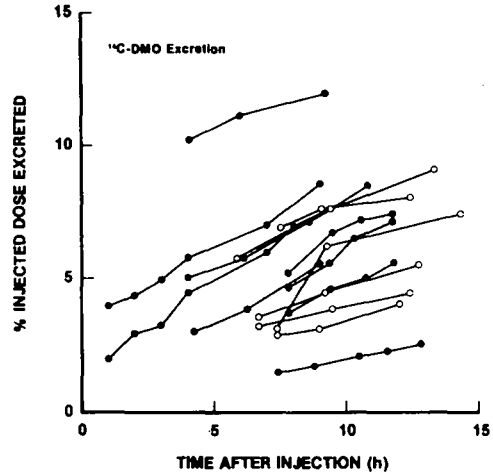

Fig. 5. The percentage of infused ^{14}C-DMO radioactivity excreted over time in individual normoxic (●) and hyperoxic (O) rainbow trout of Series II.

tighter regulation of pHi than pHe. This probably also reflects the much greater buffer capacity of muscle cells relative to extracellular fluids. Interestingly, despite the fact that mean white muscle pHi was equal to mean whole body pHi in both groups (table 2), the slope of the regression of white muscle pHi on whole body pHi was very low (0.349). This suggests that other intracellular compartments may have more variable pHi values than white muscle.

The percentages of infused ^{14}C-labelled DMO lost with time by individual trout in normoxia and hyperoxia are plotted in fig. 5. DMO loss was detected as early as 1 h post-infusion, increasing thereafter in a more or less linear fashion. This trend was exhibited by each trout with total losses ranging from 2 to 12% over the post-infusion period monitored. Extrapolation of the lines back to time zero produced positive intercepts. This suggests that elevated and highly variable 'surge' loss rates occurred shortly after injection, followed thereafter by more stable rates. By averaging the slopes of each line in fig. 5, mean DMO excretion rates (% DMO lost per hour) for the more stable phase of 0.479 ± 0.048 ($n = 9$) and 0.320 ± 0.055 ($n = 7$) were calculated for normoxic and hyperoxic trout, respectively. Notably, the rates were significantly lower (by 33%) in the latter ($P < 0.05$).

Discussion

Intracellular pH measurement

The measurement of mean whole body pHi by the weak-acid DMO distribution method is still a relatively novel exercise in fish physiology and thus warrants

comment. Indeed, direct validation of the DMO technique itself has not been performed in intact teleosts. However, it is evident from the recent body of work of Cameron and co-workers on channel catfish that the DMO method yields pHi data which are reproducible and internally consistent for both individual tissues and whole body (Cameron, 1980; Cameron and Kormanik, 1982a; Burggren and Cameron, 1980). In the present study, 4–6 h was required to attain a steady-state distribution of DMO within the trout, as recently reported by Walsh and Moon (1982) for the American eel. This may be compared with the 2–4 h period for the channel catfish (Cameron, 1980; Cameron and Kormanik, 1982a). Since plasma and ECF labels take 2–3 h and 3–4 h respectively to equilibrate in trout (Milligan and Wood, 1982), it is likely that most of this DMO equilibration time reflects convective mixing in the ECFV. Diffusive equilibration of DMO across the ECF/ICF boundary is probably quite rapid (Hinke and Menard, 1978), but must be properly evaluated before DMO can be employed to measure dynamic changes in pHi in fish. For this reason, only the stable situations of normoxia and 72 h hyperoxia were assessed in the present study.

A large number of assumptions underlie the actual calculation of pHi_{DMO} (see Methods) which in itself requires 5 independent measurements plus the pK_{DMO}. Model calculations illustrate that the two factors having by far the greatest influence on calculated pHi are pHe and the DMOi/DMOe ratio (Malan *et al.*, 1976; Hinke and Menard, 1978). Fortunately both can be measured quite accurately as long as DMO excretion is taken into account (see below). It is also helpful that calculated pHi is fairly insensitive to variations in the size of ECF and ICF compartments, at least when ECFV is low relative to ICFV (Hinke and Menard, 1978). In the present study, it has been assumed that the ECFV/ICFV ratio was not significantly changed from the normoxic value after 72 h of hyperoxia. An extreme example illustrates the possible magnitude of error in this assumption. A severe fluid volume disturbance, (27% decrease in total ECFV by shift into ICFV), accompanied by marked hematological and ionic changes, was found in trout during 72 h exposure to acidic water (pH $\approx$4.3; Milligan and Wood, 1982). In the very unlikely event this also occurred during hyperoxia, underestimates of white muscle pHi by only 0.008 pH units, and whole body pHi by 0.117 units, would result. However, both the hematological (table 1) and ion data (Wheatly *et al.*, 1984) support our contention that the ECFV/ICFV ratio of normoxia was not affected by 72 h of hyperoxia.

The present whole body pHi measurements appear quite reasonable. Firstly, the observed mean whole body ECF–ICF pH gradient of 0.54 pH units in rainbow trout is comparable with that reported in humans (Manfredi, 1963) and other vertebrates (see Roos and Boron, 1981). Secondly, it is encouraging that mean whole body pHi values were not significantly different from white epaxial muscle pHi in normoxic or hyperoxic trout (table 2; fig. 4) because the bulk of fish body mass is comprised of white muscle. In the channel catfish (Cameron and Kormanik, 1982a), whole body pHi estimates were 0.1 pH units higher than white muscle

pHi. Part of this discrepancy may be related to the fact that DMO excretion was not taken into consideration in their study (see below).

To our knowledge, the significant excretion of ^{14}C-labelled DMO radioactivity found in the rainbow trout (fig. 5) has not been measured in any other fish species. Without an account of this, an error in mean whole body pHi estimates as high as 0.4 pH units could have resulted in individual fish. The rate of DMO excretion should also not be considered a constant factor, for there were highly variable initial losses followed by more stable effluxes ranging from 0.179 to 0.621% DMO per hour in individual fish. The rate of DMO loss averaged 33% lower in the hyperoxic group, suggesting a branchial origin in view of the inhibitory effect of hyperoxia on gill transfer factors (see Introduction). Freshwater fish gills have been shown to be permeable to other non-electrolytes of high molecular weight such as inulin and mannitol (Masoni and Payan, 1974; Isaia *et al.*, 1978; Kirschner, 1980).

Acid–base status during hyperoxia

The overall modifications of extracellular acid–base status which occurred during 72 h hyperoxia and re-institution of normoxia in rainbow trout in the present study qualitatively confirmed those previously reported for this species (Wood and Jackson, 1980). However, the slightly higher level of $P_{I_{O_2}}$ employed in the present study provoked a more significant rise in Pa_{CO_2} and depression of pHa (fig. 1) which in turn probably accounted for the more delayed but larger compensatory accumulation of plasma bicarbonate (*i.e.*, 72 h *vs* 48 h; Wood and Jackson, 1980). Moreover, 24 h normoxia was insufficient to completely reverse the acid–base changes produced by hyperoxia whereas 20 h was adequate in Wood and Jackson's study.

The effects of hyperoxia on intracellular acid–base balance have not been previously reported in any other fish species. The complete compensation of both mean whole body and white epaxial muscle pHi by 72 h hyperoxia clearly indicated that the rainbow trout has an excellent capacity for pHi regulation. Cameron (1980) found similar evidence for mean whole body pHi regulation during exposure of channel catfish *(Ictalurus punctatus)* to environmental hypercapnia. The intracellular pH of muscle in the seawater elasmobranch *Scyliorhinus stellaris* (Heisler *et al.*, 1976) and the tropical freshwater teleost *Synbranchus marmoratus* (Heisler, 1982) were also largely compensated during environmental and endogenously generated hypercapnia respectively.

As the time-course of pHi adjustment during hyperoxia was not addressed it is uncertain whether pHi adjustment preceded, followed, or paralleled pHe compensation, or if an intracellular acidosis ever occurred in the first place. However, in general terms it seems likely that the extracellular CO_2 retention provoked by hyperoxia would reduce the gradient for CO_2 removal from the intracellular compartment resulting in a build-up of CO_2 in the ICF and a subsequent reduction in pHi. At the same time, the extracellular acidosis may also have played a role

TABLE 3
An analysis of the compartmental changes in the pool of acidic equivalents (ΔH^+) in the rainbow trout after 72 h of hyperoxia.

Compartment	Volume ($ml \cdot kg^{-1}$)	ΔH^+ ($\mu Eq \cdot ml^{-1}$)	ΔH^+ pool ($\mu Eq \cdot kg^{-1}$)	%
ECFV	273.5	−16.66[a]	−4557	62.8
ICFV	459.1	− 5.87[b]	−2695	37.2
Total			−7252	100

[a] From Series I.
[b] From Series II.

in the development of an intracellular acidosis. By 5 h hyperoxia a marked rise in plasma potassium levels occurred (Wheatly *et al.*, 1984) which could have resulted from an ICF–ECF K^+/H^+ shift (Ladé and Brown, 1963). The size of the initial excursion in pHi would ultimately be determined by the intrinsic buffering power of the ICF (Burton, 1978) which is probably several times that of the ECF (Castellini and Somero, 1981). Cameron (1980) found that the fall in whole body pHi during hypercapnia in the catfish was *ca.* 60% of that in pHe, and that adjustment of pHe and pHi thereafter occurred more or less in parallel.

The present data allowed construction of an acidic equivalent budget for the two compartments of the whole animal at the end of 72 h hyperoxia (table 3), by which time compensation was complete. By employing eq. (1) (see Materials and Methods) and multiplying by the respective compartmental volumes, the change in the total acidic equivalent pool was estimated for ECFV (Series I Results) and ICFV (Series II Results). Use of eq. (1) entailed knowledge of β which was predicted from eq. (2) for extracellular fluids. While β for the whole body ICFV was unknown, the term $\beta \cdot (pH_1 - pH_2)$ in eq. (1) became zero because whole body pHi compensation was complete (table 2).

The analysis illustrated two important points (table 3). Firstly, extracellular compensation under these steady-state conditions was certainly not attributable to intracellular sequestering of acidic equivalents. In fact, acidic equivalents were also removed from the ICFV. Therefore, there must have been a large excretion of acidic equivalents (or uptake of base) to the external environment through kidney and/or gills, a problem which is addressed in the subsequent papers (Wheatly *et al.*, 1984; Wood *et al.*, 1984). Secondly, despite the much larger volume of the ICF (167% of ECFV), the total pool changes were only about one half of those in the ECFV, reflecting the fact that the necessary changes in $[HCO_3^-]$ for pHi restoration were much less than for pHe restoration because of the lower initial pH and $[HCO_3^-]$ levels in the ICFV. Cameron (1980), employing a slightly different theoretical analysis, reached very similar conclusions about the compensation of environmental hypercapnia in the channel catfish.

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