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RESPIRATORY, VENTILATORY, ACID–BASE AND IONOREGULATORY PHYSIOLOGY OF THE WHITE SUCKER *CATOSTOMUS COMMERSONI*: THE INFLUENCE OF HYPEROXIA

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SUMMARY

Blood gases, acid–base status, plasma ions, respiration, ventilation and cardiovascular function were measured in white suckers, using standard cannulation methods. Basic respiratory parameters under normoxia were compared to those in the active, pelagic rainbow trout and in other benthic teleosts. Sustained environmental hyperoxia (350–550 torr) increased arterial O_2 (102–392 torr) and venous O_2 (17–80 torr) tensions so that blood O_2 transport occurred entirely via physical solution. Dorsal aortic blood pressure and heart rate fell, the latter due to an increase in vagal tone. Ventilation volume declined markedly (by 50%) due to a decrease in ventilatory stroke volume, but absolute O_2 extraction rose so that O_2 consumption was unaffected. While the preceding effects were stable with time, arterial and venous CO_2 tensions approximately doubled within 4 h, and continued to increase gradually thereafter. This CO_2 retention caused an acidosis (7·993–7·814) which was gradually compensated by an accumulation of plasma $[HCO_3^-]$. However, even after 72 h, arterial pH remained significantly depressed by 0·10 units. The gradual rise in plasma $[HCO_3^-]$ was accompanied by a progressive fall in both $[Na^+]$ and $[Cl^-]$; $[K^+]$ and $[Ca^{2+}]$ remained unchanged. The responses of the sucker to hyperoxia are compared to those of the rainbow trout.

INTRODUCTION

Environmental hyperoxia, though perhaps less common than hypoxia, is a natural phenomenon to which aquatic animals are sometimes exposed in the wild (e.g. Garey & Rahn, 1970; Dejours, 1973). The experimental use of hyperoxia has proved to be a valuable tool in understanding the respiratory physiology of water-breathers. Several studies have shown that hyperoxia markedly depresses ventilation volume (Peyraud & Serfaty, 1964; Dejours, 1973; Randall & Jones, 1973; Truchot, 1975; Dejours &

Beekenkamp, 1977; Dejours, Toulmond & Truchot, 1977; Jouve & Truchot, 1978 Wood & Jackson, 1980). This occurs despite an accompanying CO_2 retention which raises blood P_{CO_2} and depresses blood pH (i.e. respiratory acidosis: Dejours, 1973; Randall & Jones, 1973; Truchot, 1975; Dejours & Beekenkamp, 1977; Dejours *et al.* 1977; Jouve & Truchot, 1978; Wood & Jackson, 1980). This finding demonstrates the dominance of O_2 over CO_2 and/or $[H^+]$ in setting the ventilatory drive of water-breathers, in direct contrast to air-breathers (Dejours, 1973).

The extent to which the respiratory acidosis of hyperoxia is compensated by $[HCO_3^-]$ accumulation in the plasma appears highly variable. For example, Dejours & Beekenkamp (1977) reported no deviation from the normal buffer curve in the crayfish *Astacus leptodactylus* during 44 days of hyperoxia, while Dejours (1973) found a partial compensation of respiratory acidosis which became stable after 24 h in the carp *Cyprinus carpio*. Nevertheless, arterial pH remained about 0·2 units below normal for the following 5+ days. A similar pattern of stable but incomplete compensation was seen in the crab *Carcinus maenas* (Truchot, 1975) and the eel *Anguilla anguilla* (Bornancin, DeRenzis & Maetz, 1977). Complete compensation has been observed in only one species – the trout *Salmo gairdneri* (Wood & Jackson, 1980), where it occurred within 24–48 h.

One possible explanation of this variation is that it reflects differences in the normal habitat and life style of the animals. The trout is an active, pelagic species in which accurate control of blood pH may be critical to blood O_2 transport and exercise performance. The other animals tend to be more benthic, less active species where blood pH control may be less important. Furthermore, such organisms are more likely to encounter fluctuations in P_{O_2} and P_{CO_2} levels in their benthic environments; continual readjustment of blood pH by HCO_3^- mobilization or removal may not be advantageous under such circumstances.

Therefore the primary aim of the present study was to examine the responses to hyperoxia in the white sucker *Catostomus commersoni*, a benthic or shallow-water bottom feeder tolerant of a wide variety of conditions (Carl, Clemens & Lindsey, 1959). As far as possible, the methodology duplicated that of Wood & Jackson (1980) so as to facilitate comparison with the trout. A second objective was to look for changes in plasma ions during hyperoxic exposure. Acid–base regulation in freshwater teleosts is traditionally attributed to Na^+ *vs* acid (H^+, NH_4^+) and Cl^- *vs* base (HCO_3^-, OH^-) exchanges at the gills (Cameron, 1978). Any readjustment of plasma HCO_3^- levels by elevated H^+ efflux or reduced HCO_3^- efflux might perturb normal ionoregulation. Such a disturbance has been seen in the trout during adaptation to high environmental P_{CO_2}, where plasma $[HCO_3^-]$ accumulation was accompanied by plasma $[Cl^-]$ depression (Lloyd & White, 1967). As the white sucker has not previously been examined by cannulation methodology of this sort, a final aim was simply to document normal respiratory, ventilatory, cardiovascular and ionoregulatory parameters in resting animals and to assess the influence of the experimental procedures on the measured values.

MATERIALS AND METHODS

White suckers *Catostomus commersoni* Lacépède were obtained from commercial suppliers in the metropolitan Toronto area. The fish, though small (75–240 g), were

almost all sexually mature. At McMaster, they were held in dechlorinated tap water ($[Na^+]$ = 1·0, $[Cl^-]$ = 1·0, $[K^+]$ = 0·05, $[Ca^{2+}]$ = 2·0 m-equiv l^{-1}) at 12–16 °C for several days prior to experimentation. They were not fed during this time nor during the subsequent experimental period.

The fish were anaesthetized in 1:10000 MS-222 (Sigma) on an operating table (Smith & Bell, 1964) and fitted with either blood catheters or ventilation masks. Caudal artery and caudal vein catheters were constructed from Clay-Adams PE 50 polyethylene tubing pulled to a fine tip and threaded into the haemal arch. The tubing was inserted through an incision between the epaxial and hypaxial muscle masses and anchored in place along the lateral surface after closure of the wound with silk suture (Hickman, 1965; Mackay & Beatty, 1968; Wood, McMahon & McDonald, 1977). Arterial and venous catheters could not usually be implanted in the same animal. The cannulae were filled with Cortland saline (Wolf, 1963) heparinized at 20 i.u. ml^{-1}. Ventilation masks were constructed by suturing a sheet of latex dental dam around the mouth just posterior to the oral sucker in such a manner as not to impede normal ventilatory movements. Fine wires were implanted subcutaneously on both sides of one opercular opening to monitor ventilation rate via changes in impedance. Following the operation, the animals were allowed to recover for 24–48 h.

The catheterized fish were placed in individual plexiglass boxes (40 × 6 × 7 cm deep) covered with black plastic to reduce visual disturbance. The chambers were flushed with a water flow of at least 250 ml.min^{-1} from a gas exchange column which could be aerated with either air or pure O_2 to produce normoxic (P_{I,O_2} = 135–160 torr) or hyperoxic (P_{I,O_2} = 350–550 torr) conditions. P_{I,CO_2} was 1·2 torr (absolute range 0·8–1·6 torr) and unaffected by normoxia or hyperoxia.

Four experimental series were performed. In the first (n = 14), a control blood sample under normoxia was drawn (time 0) and then the animals were subjected to 3 days of hyperoxia, with additional blood samples being drawn at 4, 24, 48 and 72 h. The second series (n = 12) served as a control for the experimental procedures, with blood samples at 0, 24, 48 and 72 h during continuous normoxia. The third and fourth series were performed to check if repetitive blood sampling influenced the measured values. In these series, animals were sampled only at 0 and 72 h under either hyperoxia (n = 7) or normoxia (n = 6). In addition to these series, samples were drawn by blind cardiac puncture from 12 unoperated fish in order to assess the effects of cannulation on blood ion levels.

Blood sample volumes were 600 μl or 350 μl, of which 200 μl were returned to the fish following measurement of blood P_{O_2} and P_{CO_2} levels. The missing volume was replaced with Cortland saline. All samples were analysed for pH, P_{O_2}, P_{CO_2}, total CO_2 content (C_{CO_2}), haematocrit, and plasma Na^+, Cl^- and protein concentrations. The 0 and 72 h samples were also assayed for plasma K^+ and Ca^{2+} levels. The analytical techniques are detailed in McDonald, Höbe & Wood (1980) and Wood & Jackson (1980). In brief, P_{O_2}, P_{CO_2} and pH levels were measured using Radiometer microelectrodes thermostatted to the experimental temperature and connected to a Radiometer PHM 71 MK2 acid–base analyser. C_{CO_2} was determined by the Cameron (1971) technique and plasma $[HCO_3^-]$ was calculated as the difference between C_{CO_2} and $\alpha CO_2.P_{CO_2}$ where αCO_2 is the solubility coefficient for CO_2 in plasma (Severinghaus,

1965). Plasma protein concentration was measured by refractometry (American Optical TS meter), $[Cl^-]$ by coulometric titration (Radiometer CMT-10), and $[Na^+]$, $[K^+]$, and $[Ca^{2+}]$ by flame photometry (Eel or Coleman 20) employing appropriate swamping to eliminate interference effects.

In fish fitted with arterial catheters, dorsal aortic blood pressure (BP_a) and heart rate (f_H) were determined prior to each blood sampling period by connecting the catheter to a Hewlett-Packard 267 BC pressure transformer system. Mean BP_a was calculated after Burton (1972):

$$BP_a = \frac{1 \text{ systolic} + 2 \text{ diastolic}}{3}.$$

Approximately 4 h after the final blood sample, 1 μmole.kg^{-1} of atropine sulphate in 1·5 ml.kg^{-1} Cortland saline was infused via the arterial catheter to assess the influence of vagal tone on heart rate (cf. Wood *et al.* 1979*a*).

The relationship between blood haematocrit and β, the slope of the non-bicarbonate buffer line ($\Delta[HCO_3^-]/\Delta pH$) was determined for whole blood and true plasma by *in vitro* CO_2 titration (Wood *et al.* 1977). Blood samples ($n = 7$) covering a wide range of haematocrits (3–43%) for the analysis were drawn by cardiac puncture as described above.

Animals fitted with oral membranes ($n = 9$) were placed in ventilation collection boxes covered with black plastic. The methodology was identical to that developed by Davis & Cameron (1970) and employed by Wood & Jackson (1980) in their study on hyperoxia in the trout. After control ventilatory measurements under normoxia (time 0), 3 days of hyperoxia were imposed with observations at 4 h, 24 h, 48 h and 72 h. Each set of measurements consisted of several determinations of P_{I,O_2} (from directly in front of the fish's mouth), P_{E,O_2} (from the rear of the mixing chamber), ventilation volume ($\dot{V}_W$) (by overflow), and respiration frequency (f_R). The latter was recorded by connecting the opercular wires to a Biocom impedance converter. Ventilatory stroke volume ($V_{s,R}$) was estimated as

$$V_{s,R} = \frac{\dot{V}_W}{f_R}$$

and O_2 consumption ($\dot{M}_{O_2}$) was calculated by the Fick principle:

$$\dot{M}_{O_2} = (P_{I,O_2} - P_{E,O_2}) \cdot \beta_{WO_2} \cdot \dot{V}_W,$$

where β_{W,O_2} is the solubility coefficient for O_2 in fresh water at the experimental temperature (Dejours, 1975). The percentage utilization of O_2 from the water by the fish (U_{W,O_2}) was estimated as:

$$U_{W,O_2} = \frac{P_{I,O_2} - P_{E,O_2}}{P_{I,O_2}} \times 100\%.$$

All data have been expressed as means ± 1 standard error (n). Arterial and venous data have been presented separately, except for plasma ion levels, plasma protein and haematocrit, in which there were no consistent arterial–venous differences. During the course of each experiment, n numbers tended to decline due to cannula failure, tearing

Table 1. *A comparison of plasma ion levels, protein concentrations and haematocrit in cannulated suckers and suckers sampled by cardiac puncture (means* ± 1 S.E.; *n)*

	Cannulation	Cardiac puncture	*P*
Na^+ (m-equiv l^{-1})	114·8 ± 1·5 (37)	119·1 ± 4·3 (12)	N.S.
Cl^- (m-equiv l^{-1})	89·0 ± 1·8 (37)	90·7 ± 3·2 (12)	N.S.
K^+ (m-equiv l^{-1})	1·90 ± 0·09 (36)	3·46 ± 0·17 (10)	0·001
Ca^{2+} (m-equiv l^{-1})	3·67 ± 0·11 (37)	3·80 ± 0·33 (12)	N.S.
Plasma protein (g/100 ml)	2·10 ± 0·13 (37)	2·40 ± 0·29 (12)	N.S.
Haematocrit (%)	13·4 ± 1·4 (37)	27·8 ± 2·3 (12)	0·001

of the oral membrane, fish death or other misfortune. Cannula failure was especially prevalent on the venous side, and therefore venous data are presented only up to 24 h. The actual starting and finishing *n* numbers for the three major series on which the Figures are based were as follows:

(i) hyperoxia blood series: arterial, 8 at time 0, 4 at 72 h; venous, 6 at time 0, 4 at 24 h;

(ii) normoxia control blood series: arterial, 8 at time 0, 6 at 72 h; venous, 4 at time 0, 2 at 72 h;

(iii) hyperoxia ventilation series: 9 at time 0, 4 at 72 h.

Within each experimental series, the significance of differences was evaluated by means of the paired Student's two-tailed *t* test, using the time 0 value for each fish as its own control. Difference between groups was assessed by the 2-tailed unpaired *t* test. Significance was assumed at $P < 0{\cdot}05$.

RESULTS

Normal resting values for a variety of ionic, respiratory, ventilatory and cardiovascular parameters in white suckers under normoxia are summarized in Tables 1 and 2. As all cannulated animals were treated identically until time 0, the means in these tables were compiled using the time 0 values from all experimental series. Table 1 also compares certain blood characteristics in the cannulated fish with those sampled by cardiac puncture. From this it is clear that the operative procedures had no effect on plasma $[Na^+]$, $[Cl^-]$ or $[Ca^{2+}]$. $[K^+]$ was significantly lower, but this may have been an artifact of cardiac puncture. Blood samples taken by cardiac puncture sometimes exhibited slight haemolysis. Such samples were rejected, but very low-level, visually undetectable haemolysis could cause plasma $[K^+]$ elevation of the magnitude seen in Table 1. Cannulation caused a marked reduction in haematocrit, probably due to blood loss from the caudal incision. Plasma protein concentration was not significantly affected.

Table 3 presents the regression relationships between blood haematocrit and the non-bicarbonate buffer capacities (β). At the normal pre-operative haematocrit of

Table 2. *Respiratory, ventilatory and cardiovascular parameters in resting white suckers (means ± 1 S.E.; n)*

Weight (g)	140·8 ± 4·8 (48)	pH_a	7·957 ± 0·016 (20)
P_{I,O_2} (torr)	142·7 ± 1·0 (48)	pH_v	7·861 ± 0·027 (17)
P_{a,O_2} (torr)	108·7 ± 4·3 (30)	BP_a (cm H_2O)	$\frac{29·9 \pm 1·8}{23·0 \pm 1·6} = 25·3 \pm 1·7$ (19)
P_{v,O_2} (torr)	22·4 ± 3·2 (15)	f_H (no. min^{-1})	41·5 ± 3·3 (19)
P_{I,CO_2} (torr)	1·2 ± 0·19 (4)	f_R (no. min^{-1})	56·8 ± 2·1 (20)
P_{a,CO_2} (torr)	2·37 ± 0·09 (20)	$\dot{V}_W$ (ml.kg^{-1}.min^{-1})	212·7 ± 29·9 (8)
P_{v,CO_2} (torr)	3·41 ± 0·17 (15)	$V_{s,R}$ (ml.kg^{-1}.$stroke^{-1}$)	3·87 ± 0·57 (8)
C_{a,CO_2} (mM.l)	9·80 ± 0·59 (20)	U_{W,O_2} (%)	46·2 ± 6·0 (7)
C_{v,CO_2} (mM.l)	12·38 ± 0·65 (17)	$\dot{M}_{O_2}$ (μm O_2.kg^{-1}.min^{-1})	29·6 ± 2·9 (7)

Table 3. *The regression relationships between blood haematocrit (Ht)* and the non-bicarbonate buffer capacity (β)† of whole blood and true plasma in the white sucker*

Whole blood ($n = 7$)	β† $= -25·96 \pm 6·70$‡ $r = 0·866$	$(Ht^*) - 1·32 \pm 0·80$‡ $P < 0·05$
True plasma ($n = 7$)	β† $= -23·44 \pm 7·02$‡ $r = 0·831$	$(Ht^*) - 2·28 \pm 0·84$‡ $P < 0·05$

* Ht expressed as a decimal.
† β expressed in slykes (= mmol.l^{-1}.pH^{-1}).
‡ Standard error.

27·8% (Table 1), β would be 8·54 slykes for whole blood and 8·80 slykes for true plasma.

Sequential changes occurring over the 72 h experimental period in the hyperoxia series and the accompanying normoxic control series are illustrated in Figs. 1, 2 and 3. There were no significant differences between the two series in any time 0 values except for f_H (Fig. 1C) and pH_v (Fig. 2C). The reasons for these differences are unknown; they were most probably random effects due to the small sample sizes. Apart from the expected fall in haematocrit (Fig. 1E), plasma protein concentration (Fig. 3B), and the small (but not significant) rise in control plasma [HCO_3^-] there was no significant changes in any parameter in the normoxic control series over the experimental period (Figs. 1, 2 and 3). Thus repetitive blood sampling had a negligible effect on the measured values. This conclusion is strengthened by the two series in which blood samples were drawn only at 0 and 72 h. Without exception, the results from these series during both normoxia and hyperoxia were essentially identical to those obtained from animals subjected to daily sampling.

The nearly fourfold increase in P_{I,O_2} with the onset of hyperoxia (Fig. 1A) caused a similar increase in P_{a,O_2} from 102·3 ± 9·0 (8) to 392·7 ± 24·2 (7) torr at 4 h (Fig. 1B). P_{a,O_2} remained approximately stable for the remainder of the experimental period.

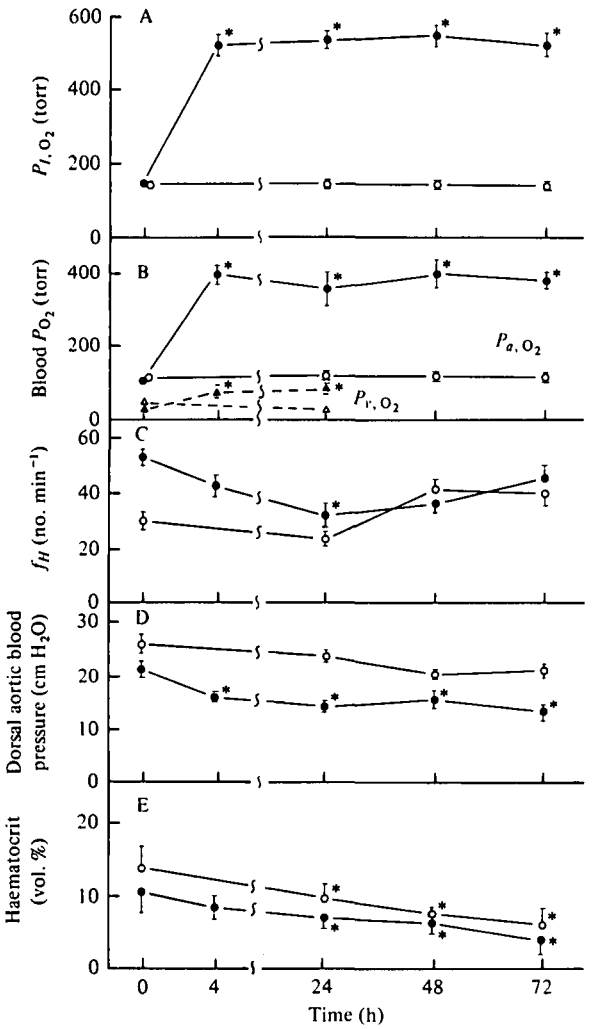

Fig. 1. Changes in (A) P_{I,O_2}; (B) P_{a,O_2} and P_{v,O_2}; (C) f_H; (D) BP_a; and (E) haematocrit in white suckers during 72 h of environmental hyperoxia (●, ▲ = experimental group) or normoxia (○, △ = control group). The time 0 values were taken under normoxia. Means ± 1 S.E. * = significantly different ($P < 0.05$) from time 0 value.

P_{v,O_2} was also significantly elevated from 17·3 ± 2·9 (6) to 80·8 ± 12·0 (4) torr at 24 h. Although the O_2 dissociation curve of the blood was not determined, it seems almost certain that the P_{O_2} levels would saturate the haemoglobin in both arterial and venous blood. Thus, blood O_2 transport occurred totally via physical solution during hyperoxia.

Arterial blood pressure fell by about 33% during the first 24 h of hyperoxia and thereafter remained stable (Fig. 1 D). This depressor effect was due to an equal decrease in both systolic and diastolic pressures. Interpretation of the cardiac response was complicated by the difference in initial values of f_H between the experimental and control groups (Fig. 1 C). Heart rate declined significantly after 24 h of hyperoxia, but

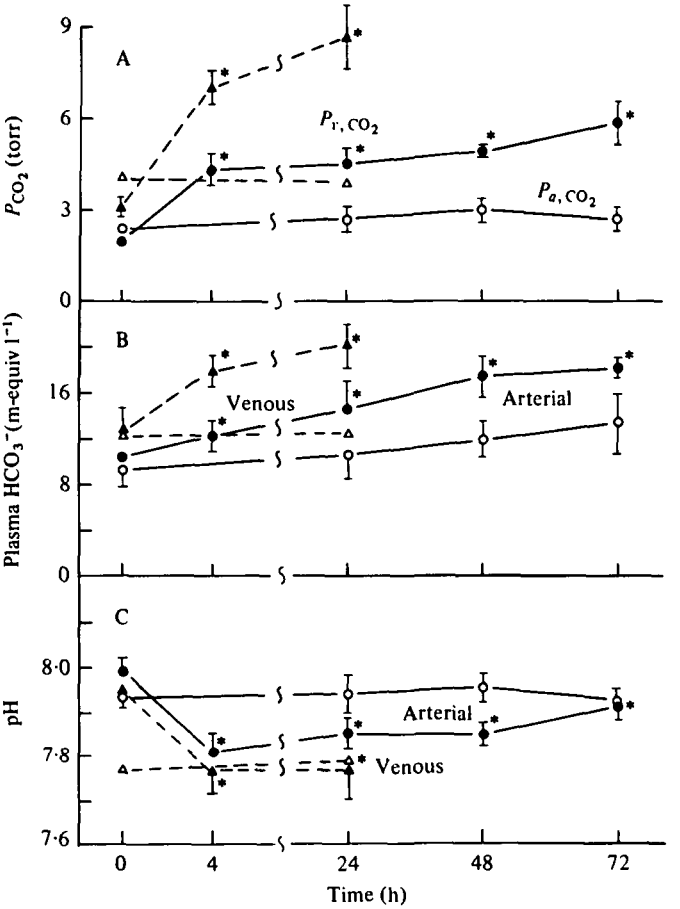

Fig. 2. Changes in (A) P_{a,CO_2} and P_{v,CO_2}; (B) arterial and venous plasma [HCO_3^-]; and (C) pH_a and pH_v in white suckers under 72 h of environmental hyperoxia (●, ▲ = experimental group) or normoxia (○, △ = control group). The time 0 values were taken under normoxia. Mean ± 1 S.E. * = significantly different ($P < 0.05$) from time 0 value.

Table 4. *The influence of atropine (1 μmole.kg^{-1}) on heart rate (no. min^{-1}) in white suckers during normoxia and hyperoxia (means ± 1 S.E.)*

	f_H before atropine	f_H after atropine	Δf_H
Normoxia* ($n = 9$)	50·0 ± 5·7	69·7 ± 2·3	19·7 ± 3·4
Hyperoxia* ($n = 6$)	43·3 ± 3·6	75·1 ± 2·9	31·8 ± 3·0
P	N.S.	N.S.	< 0·05

* Data combined from the larger and smaller series.

only to levels typical of the control fish. Thereafter f_H tended to rise back towards the initial level; a similar, though non-significant, trend was seen in the control group. However, administration of atropine (1 μmole.kg^{-1}) at the end of the experiment caused a significantly greater increase in f_H in the hyperoxic fish than in the normoxic

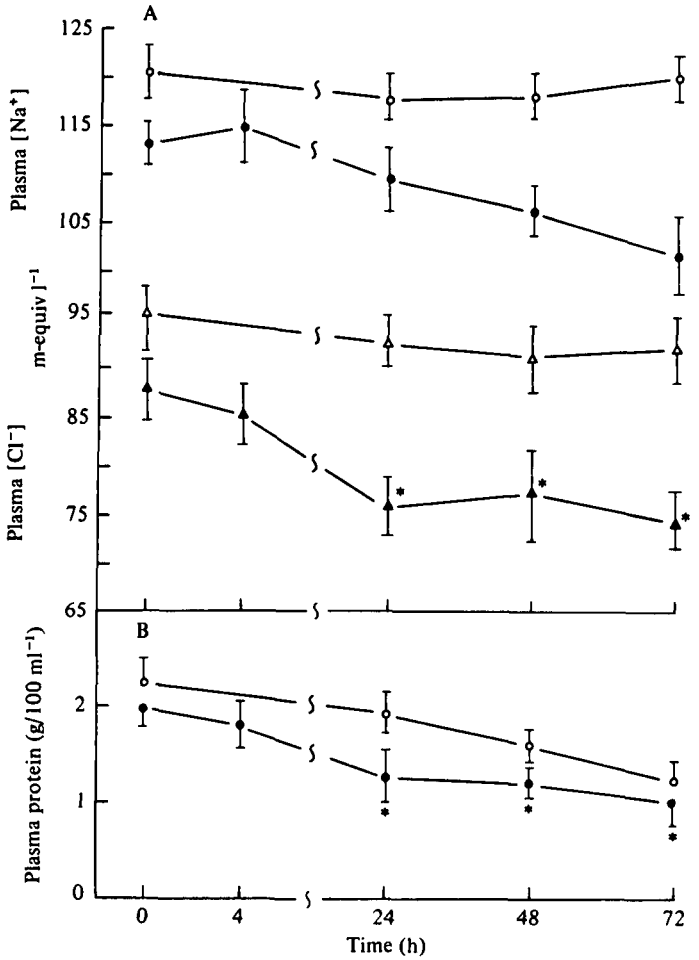

Fig. 3. Changes in (A) plasma [Na^+] and [Cl^-] and (B) plasma protein concentration in white suckers during 72 h of environmental hyperoxia (▲, ● = experimental group) or normoxia (○, △ = control group). The time 0 value were taken under normoxia. Means ± 1 S.E. * = significantly different ($P < 0.05$) from time 0 value.

controls (Table 4). This tends to confirm that a real decrease in f_H occurred during hyperoxia due to an increase in vagal tone.

Arterial P_{CO_2} doubled by 4 h hyperoxia and continued to increase gradually thereafter (Fig. 2A). Much larger increases were seen in P_{v,CO_2} (Fig. 2A). This CO_2 retention caused a significant decrease in both pH_a and pH_v by 4 h (Fig. 2C). A definite slow re-adjustment of pH_a occurred, but even at 72 h the value remained significantly depressed by $\simeq$ 0·1 unit below the initial level (Fig. 2C). This partial pH_a compensation was due to a progressive rise in plasma [HCO_3^-] (Fig. 2B). A similar trend was apparent on the venous side (Fig. 2B, C).

Hyperoxia caused a marked depression in [Cl^-] which became significant after 24 h (Fig. 3A). Plasma [Na^+] also tended to fall (Fig. 3A) but the change over 72 h just failed to be significant ($0.1 > P > 0.05$) because of one aberrant fish in which [Na^+]

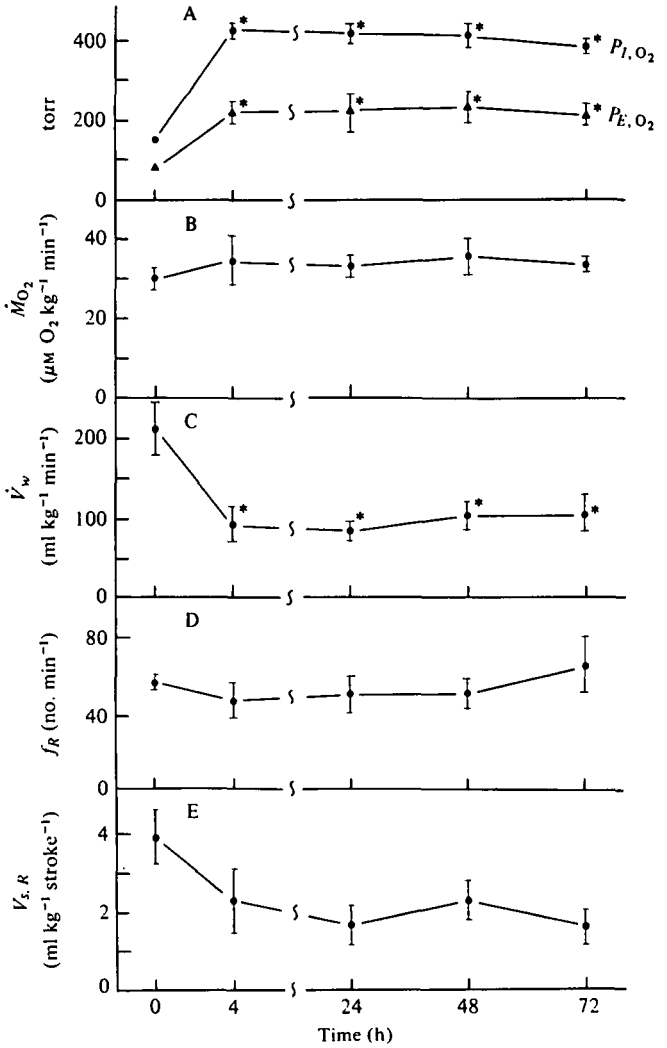

Fig. 4. Changes in (A) P_{I,O_2} and P_{E,O_2}; (B) $\dot{M}_{O_2}$; (C) $\dot{V}_W$; (D) f_R; and (E) $V_{s,R}$ in white suckers during 72 h of environmental hyperoxia. The time 0 values were taken under normoxia. * = significantly different ($P < 0.05$) from time 0 value.

actually increased markedly. However, with the inclusion of the data from the series sampled only at 0 and 72 h, the depression of plasma [Na^+] became highly significant (Table 5). The paired comparisons in Table 5 show that [Na^+] fell by 10·1 m-equiv l^{-1}, [Cl^-] by 13·0 m-equiv l^{-1}, while arterial [HCO_3^-] increased by 5·4 m-equiv l^{-1}. There was no significant change in [Ca^{2+}] or [K^+]. Calculation of the 'anion gap' from mean ion values in Table 5 (i.e. $[Na^+]+[K^+]+[Ca^{2+}]-[Cl^-]-[HCO_3^-]$) showed that it declined by about 2 m-equiv l^{-1} over the experimental period in both normoxic and hyperoxic fish. This probably reflected the decrease in plasma protein concentration (Fig. 3B, Table 5) which would be expected to carry a significant portion of the unmeasured negative charge. However, relative to the normoxia

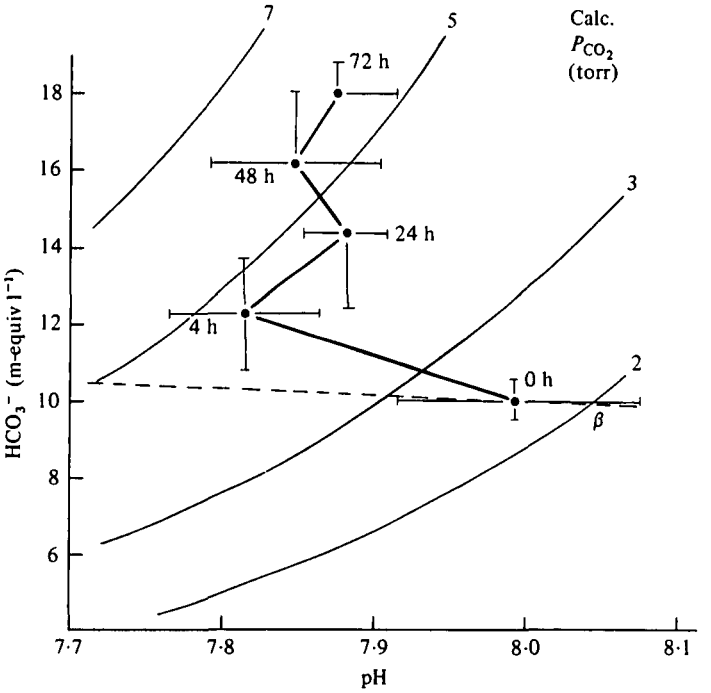

Fig. 5. Davenport diagram display of sequential changes in plasma acid–base status during 72 h of environmental hyperoxia in the white sucker. The time o values were taken under normoxia. The slope (−3·91) of the true plasma line through the time o point was calculated from the mean haematocrit (7·0%) and the regression equation of Table 3. Means ± 1 S.E.

Table 5. *Paired comparisons of plasma ion levels, protein concentration, and pH_a in white suckers before and after 72 h of normoxia or hyperoxia*

	Normoxia*			Hyperoxia		
	o h	72 h	*P*	o h	72 h	*P*
$[Na^+]$ (m-equiv l^{-1})	122·1 ± 3·7 (12)	120·7 ± 3·6	N.S.	113·3 ± 2·3 (11)	103·2 ± 3·4	< 0·01
$[Cl^-]$ (m-equiv l^{-1})	94·0 ± 2·2 (12)	92·0 ± 2·9	N.S.	87·0 ± 3·4 (11)	74·0 ± 3·4	< 0·001
$[K^+]$ (m-equiv l^{-1})	1·80 ± 0·17 (12)	1·58 ± 0·15	N.S.	1·92 ± 0·11 (10)	2·23 ± 0·17	N.S.
$[Ca^{2+}]$ (m-equiv l^{-1})	3·63 ± 0·22 (12)	3·68 ± 0·14	N.S.	3·65 ± 0·14 (10)	3·51 ± 0·23	N.S.
Arterial $[HCO_3^-]$ (m-equiv l^{-1})	9·41 ± 0·82 (8)	11·96 ± 2·12	N.S.	10·72 ± 0·91 (10)	16·12 ± 1·36	< 0·01
Anion gap† (m-equiv l^{-1})	24·1	22·0		21·2	18·8	
[Plasma protein] (g. 100 ml^{-1})	2·49 ± 0·21 (11)	1·30 ± 0·20	< 0·001	2·21 ± 0·30 (11)	0·97 ± 0·14	< 0·001
pH_a	7·926 ± 0·025 (8)	7·868 ± 0·038	N.S.	7·966 ± 0·024 (7)	7·872 ± 0·039	< 0·05

* Data combined from the larger and smaller series.
† $[Na^+] + [K^+] + [Ca^{2+}] - [Cl^-] - [HCO_3^-]$.

controls, there was no real change in the anion gap after 3 days hyperoxia, indicating that the marked decrease in [Cl^-] was balanced both by a decrease in [Na^+] and an increase in [HCO_3^-].

Ventilation rates measured by impedance conversion in animals fitted with oral membranes (f_R = 56·4 ± 3·2 (8) beats.min^{-1}; Fig. 4D) were identical to those recorded visually from resting animals without membranes (57·1 ± 2·9 (12) beats.min^{-1}). A threefold increase in P_{I,O_2} (Fig. 4A) had no effect on f_R (Fig. 4D) but caused a profound and significant drop in $\dot{V}_W$ from 212·7 ± 29·9 (8) to 91·9 ± 22·4 (7) ml.kg^{-1}.min^{-1} at 4 h hyperoxia (Fig. 4C); thereafter $\dot{V}_W$ remained stable at this level. The decrease in $\dot{V}_W$ during hyperoxia was almost compensated by an increase in absolute O_2 extraction (i.e. $P_{I,O_2}-P_{E,O_2}$; Fig. 4A). Consequently, relative extraction (i.e. U_{W,O_2}) was unaffected and $\dot{M}_{O_2}$ remained unchanged (Fig. 4B). In view of the stability of f_R, the drop in $\dot{V}_W$ was attributed to a large fall in $V_{s,R}$ (Fig. 4E). However, the latter change was not statistically significant because of the large variability and small n number in the data.

DISCUSSION

The results of the various internal controls performed in this study clearly indicate that cannulation methodology had a negligible effect on most measured parameters. The only significant exceptions were haematocrit and plasma protein concentration, which were rather seriously reduced by cannulation (Table 1) and/or repetitive blood sampling (Fig. 1E, 3B; Table 5). Fortunately this species appears very tolerant of anaemia. In any case, the present study dealt with hyperoxia, a condition under which any influence of anaemia should be minimized. In future studies, this problem could be overcome simply by employing larger animals, which commonly occur in the wild (Cărl *et al.* 1959).

Normal respiratory and ventilatory parameters in resting white suckers (Table 2) under normoxia were surprisingly similar to those reported for the active, pelagic rainbow trout *Salmo gairdneri* (see Wood *et al.* 1979*a* for a recent summary of trout values). Furthermore the sucker values were somewhat dissimilar to those reported for other less active, more benthic, teleosts such as the flounder *Platichthys stellatus* (Wood *et al.* 1979*a*), the tench *Tinca tinca* (Eddy, 1974) and the carp *Cyprinus carpio* (Itazawa & Takeda, 1978). These features included relatively high blood O_2 tensions, high $\dot{V}_W$, high $\dot{M}_{O_2}$ and low U_{W,O_2} (Table 2). Calculations based on the data in Table 2 also indicate a relatively low P_{O_2} gradient (ΔP_{O_2}) across the gills (= 44 torr) and high transfer factor for O_2 (T_{O_2} = 0·0150 ml O_2.kg^{-1}.min^{-1}.torr^{-1}), again as in the trout (see Wood *et al.* 1979*a*, for formulae). It would be interesting to see whether this difference was substantiated in other areas such as blood O_2 affinity, the magnitude of the Bohr effect, resting cardiac output and exercise performance. If so, the white sucker, despite its bottom-feeding habits, would resemble a pelagic teleost in its respiratory physiology.

Plasma ion levels in the white sucker (Tables 1, 5) were unusual in their abnormally low [Na^+] and [Cl^-] relative to most other fresh-water teleosts, benthic or pelagic (Holmes & Donaldson, 1969). The only previous study in this species (Hickman, 1965) reports almost identical values in much larger specimens. The difference,

Therefore, appears real. The reason is unknown, though of course by tolerating lower plasma ion levels, the animal minimizes the cost of osmoregulation and ionoregulation in fresh water.

The initial respiratory responses of the white sucker to hyperoxia were typical of other freshwater animals which have been studied (see Introduction) and almost identical to those described in the rainbow trout (Wood & Jackson, 1980). These effects included a marked reduction in $\dot{V}_W$ (Fig. 4C) due to a depression of $V_{s,R}$ (Fig. 4E) at constant f_R (Fig. 4D), a maintenance of normal $\dot{M}_{O_2}$ (Fig. 4B) by increase of absolute O_2 extraction (Fig. 4A), a rise in P_{a,O_2} in proportion to P_{I,O_2}, (Fig. 1A, B), and an elevation of P_{a,CO_2} (Fig. 2A) causing a drop in pH_a (Fig. 2B). New findings were the accompanying rise in P_{v,CO_2} (Fig. 2A), fall in pH_v (Fig. 2C), and marked increase in P_{v,O_2} (Fig. 1B), the latter indicating blood O_2 transport entirely by physical solution during hyperoxia.

The CO_2 retention during hyperoxia has been routinely ascribed to convective limitation at the gills – i.e. a consequence of the reduced $\dot{V}_W$ (Randall & Jones, 1973; Dejours, 1973, 1975; Truchot, 1975; Dejours & Beekenkamp, 1977; Bornancin *et al.* 1977). However, Wood & Jackson (1980) showed in the trout that diffusive limitation due to branchial vasoconstriction (Haswell, Perry & Randall, 1978) played a much more important role in raising P_{a,CO_2}. In the present study there is no direct evidence of the mechanism involved. However, calculations based on the data in Figs. 1B, 4A and 4B show that the branchial transfer factor for O_2 (T_{O_2}) decreased about 50% during hyperoxia, from 0·0150 ml $O_2 . kg^{-1} . min^{-1} . torr^{-1}$ at 24 h hyperoxia. This certainly points to a serious diffusive limitation at the gills during hyperoxia.

A Davenport (1974) diagram has been constructed to illustrate the time course of compensation of the hyperoxic acidosis (Fig. 5). The slope of the plasma buffer line ($\beta = -3{\cdot}91$) was calculated from the mean haematocrit (7·0) using the regression equation of Table 3. This plot clearly shows that compensation had been implemented within 4 h, there being a significant rise in plasma $[HCO_3^-]$ above the buffer line by this time. Thereafter, P_{a,CO_2} continued to increase gradually while a simultaneous accumulation of $[HCO_3^-]$ prevented any further fall in pH_a. However, even after 72 h, no obvious steady state was attained, and pH_a remained significantly depressed. In this regard, the sucker differed from the trout (Wood & Jackson, 1980), where complete compensation was seen in 24–48 h. Nevertheless, the extent of compensation in the sucker was better than in all other benthic animals which have been studied (see Introduction).

Cardiovascular responses to hyperoxia have not previously been reported. The present results show a definite decrease in BP_a (Fig. 1D), and apparent fall in f_H (Fig. 1C) due to an increase in vagal tone. Thus both hypoxia (e.g. Holeton & Randall, 1967) and hyperoxia seem to lower heart rate via the same efferent pathway, the parasympathetic vagus. Possibly different peripheral receptors are involved, the proved external ones for hypoxia (Daxboeck & Holeton, 1978; Smith & Jones, 1978) and the postulated internal ones (Holeton, 1977) for hyperoxia. The latter apparently cause vagal tachycardia when stimulated by hypoxaemia (Holeton, 1977; Wood *et al.* 1979*b*) and so could cause vagal bradycardia when stimulated by hyperoxaemia (Fig. 1B) during environmental hyperoxia. The involvement of such blood-based receptors

would explain the secondary increase in f_H (Fig. 1 C) seen in both experimental and control groups as the fish became anaemic from repetitive blood sampling (Fig. 1 E). The reduction in BP_a (Fig. 1 D) could be a direct result of this bradycardia or could come from either a branchial vasoconstriction (Haswell *et al.* 1978) or from systemic vasodilation.

Perhaps the most interesting feature of the current data was the change in blood ionic status during hyperoxia (Fig. 3; Table 5). The only other study of ionoregulation during hyperoxia (in the eel, Bornancin *et al.* 1977) reported no significant change, but did not employ paired comparisons. Such comparisons may be needed to detect small ionic changes. In the sucker, the increase in plasma $[HCO_3^-]$ (Fig. 2 B) during the partial compensation of hyperoxic acidosis (Fig. 2 C) was accompanied by a fall in both $[Na^+]$ and $[Cl^-]$ (Fig. 3 A; Table 5). This phenomenon was probably not due to plasma dilution by water influx or retention since plasma $[K^+]$ and $[Ca^{2+}]$ were unaffected (Table 5). A reciprocal fall in $[Cl^-]$ for a rise in $[HCO_3^-]$ would be expected if the pH_a compensation occurred via a reduction of branchial Cl^- *vs* HCO_3^- exchange (Cameron, 1978). However, this should not cause a decrease in $[Na^+]$. Indeed, plasma Na^+ levels should remain unchanged or increase, if, alternatively, an elevation of branchial Na^+ *vs* H^+ exchange was responsible for the accumulation of HCO_3^- (Cameron, 1978).

The reason for the depression in plasma Na^+ may lie in the kidney. While the actual mechanism responsible for $[HCO_3^-]$ accumulation is unknown, it is clear, in the trout at least (Wood & Jackson, 1980), that the kidney increases its rate of HCO_3^- reabsorption so as to prevent excretion of the greater filtered HCO_3^- load during hyperoxic compensation. Thus HCO_3^- accumulated in the blood by the unknown mechanism is not lost in the urine. On the other hand it is possible that the sucker kidney does not increase renal H^+ secretion (= HCO_3^- reabsorption) to the same extent, and therefore some of the HCO_3^- added to the blood is lost in the urine. By analogy with mammals (Hills, 1973) the renal H^+ pump would be an H^+ *vs* Na^+ exchange, and therefore this HCO_3^- would be lost in association with Na^+. Such a reduced renal response would explain the depression of plasma $[Na^+]$, the slower rate of $[HCO_3^-]$ accumulation, and the incomplete compensation of pH_a in the sucker relative to the trout. The trout, on the other hand, shows a depression of plasma $[Cl^-]$, a rise in $[HCO_3^-]$, but no change in $[Na^+]$ (Lloyd & White, 1967) during the compensation of a rather similar acidosis due to high environmental P_{CO_2} (Janssen & Randall, 1975; Eddy *et al.* 1977).

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