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HYPOXIC ACCLIMATION IN THE LAMPREY, *LAMPETRA FLUVIATILIS*: ORGANISMIC AND ERYTHROCYTIC RESPONSES

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SUMMARY

Acute exposure of *Lampetra fluviatilis* to hypoxia (P_{O_2} = 40–50 mmHg) resulted in a large increase in ventilation frequency and a significant increase in O_2 consumption (from 40 to 60 mg kg^{-1} h^{-1} at 8 °C). After 1 week's hypoxia, the O_2 consumption decreased (from 60 to 50 mg kg^{-1} h^{-1}), indicating the existence of slow, acclimatory changes that remove some of the strain from the ventilatory response. The hypoxic animals had a higher blood O_2 affinity than the normoxic controls. This acclimatory response is not the result of a decreased allosteric interaction between the haemoglobin and erythrocytic organic phosphates, as in teleost fish, but is attributable partly to dilution of haemoglobin within the red cells and partly to an increase in the intracellular pH. The intraerythrocytic pH of hypoxic animals, measured with a freeze-thaw method, was higher than the plasma pH, suggesting that protons are not passively distributed.

INTRODUCTION

In acute hypoxia, the ventilatory frequency of lampreys increases markedly (Johansen, Lenfant & Hanson, 1973; Claridge & Potter, 1975). The resulting increase in ventilation cost raises the standard O_2 consumption in *Lampetra fluviatilis* (Claridge & Potter, 1975). This response to acute hypoxia is similar to that seen in some teleosts, for example rainbow trout (Hughes & Saunders, 1970).

In teleosts, prolonged hypoxia additionally evokes energetically less costly adaptive mechanisms which involve the enhancement of blood O_2 loading in gills (cf. Nikinmaa, 1981; Weber, 1982). In eel (Wood & Johansen, 1972), plaice (Wood, Johansen & Weber, 1975), carp (Weber & Lykkeboe, 1978) and rainbow trout (Soivio, Nikinmaa & Westman, 1980; Tetens & Lykkeboe, 1981; Nikinmaa & Soivio, 1982) blood oxygen affinity increases because of large decreases in the erythrocytic concentrations of nucleotide triphosphates (NTP) and resultant increases in the red cell pH.

It is not known whether the respiratory properties of the blood of cyclostomes adapt to prolonged hypoxia. The O_2 affinity of *in vitro* preparations of lamprey haemoglobin

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is not influenced by the organophosphates which depress haemoglobin O_2 affinity in teleost and mammalian red cells (Johansen *et al.* 1973; R. E. Weber & M. Nikinmaa, in preparation), suggesting that a hypoxic response, if present, will involve other cellular and molecular mechanisms. In the present study we have investigated the whole blood O_2-binding properties in *Lampetra fluviatilis*, their modification in hypoxia, and the cellular mechanisms underlying these modifications. The oxygenation reactions of the erythrocytes and haemoglobin of this species will be the subject of a separate report (R. E. Weber & M. Nikinmaa, in preparation).

MATERIALS AND METHODS

The lampreys, *Lampetra fluviatilis*, were caught in Simojoki river in northern Finland during their spawning run (August–November), and transferred either to Helsinki or Odense, where they were allowed to acclimate to laboratory conditions for at least 1 month. During this period, the P_{O_2} of the water was in excess of 130 mmHg and P_{CO_2} was 0·5–1·5 mmHg. The pH varied between 7·2 and 7·6 in Helsinki, and 7·7 and 8·0 in Odense. Altogether 37 animals (36·7 ± 1·5 g, $\bar{x} \pm$ S.E.M.) were used.

Oxygen consumption studies

Eight animals were transferred from the holding tanks into 2-1 glass respirometers (kept at 8 °C). The animals were allowed to acclimate for 2 h, after which the O_2 concentration in the respirometer was determined. The flow of water into the respirometer was then stopped for 1 h, and the O_2 concentration measured at the end of this period. The O_2 consumption rate was calculated from the difference in O_2 concentrations, the water volume and the weight of the fish. The measurements were repeated three times in normoxic water; between the measurements water was allowed to flow through the respirometers for 30 min. After the third normoxic measurement the respirometer was flushed with hypoxic water (P_{O_2}, 40–50 mmHg) for 30 min, and the O_2 consumption measured three times as above, giving the values for acute hypoxic stress. The fish were then kept in the hypoxic water for a week, after which the O_2 consumption rates under chronic hypoxia were measured as above.

Studies on the respiratory properties of blood

These experiments were carried out at 15 and 2 °C. In the 15 °C-experiments, eight animals were transferred into a 60-1 aquarium where they were allowed to consume part of the dissolved oxygen and gradually induce environmental hypoxia. The animals were prevented from contact with air by a perforated Plexiglass sheet positioned about 2 cm below the water surface. The P_{O_2} of the water was kept at 40–50 mmHg by a flow of water through the aquarium and mixing of the water in the aquarium; if the O_2 tension increased or decreased beyond these limits, the flow or mixing rates were adjusted. Although the flow-through system prevented excessive accumulation of ammonia or CO_2, water P_{CO_2} increased from about 1 mmHg to 3 mmHg in the course of the experiment. The normoxic control animals (nine in number) were kept in the original, aerated, holding tank throughout the experiment.

The procedure in the 2 °C-experiments was the same as at 15 °C, except that th

water P_{O_2} was decreased by bubbling N_2 into the aquarium. Seven hypoxic and five normoxic (control) animals were used at this temperature.

After 7 days' acclimation the fish were anaesthetized in MS 222 solution ($2\,g\,l^{-1}$) and blood samples taken from caudal vessels into heparinized syringes. The following were then measured:

- plasma pH (immediately after sampling, using Radiometer's BMS 3 and PHM 71 & 72);
- red cell pH (after separating plasma and red cells, and freezing and thawing the red cell mass twice, as described by Jensen & Weber, 1982);
- blood P_{50} values (at P_{CO_2} values of 0·8 and 3·7 mmHg, with a mixing method after 30 min equilibration in Radiometer BMS 2 tonometers);
- blood haemoglobin concentration (Hb; with the cyanmethaemoglobin method);
- mean cellular Hb concentration (MCHC; calculated from blood Hb and haematocrit values);
- plasma and red cell lactate concentrations (with Boehringer test kit no. 124842);
- red cell nucleoside triphosphate (NTP) concentration (with Boehringer test kit no. 123897);
- ATP and guanosine triphosphate (GTP) (by thin layer chromatography, after Johansen, Lykkeboe, Weber & Maloiy, 1976);
- plasma glucose concentration (with Boehringer test kit no. 123896);
- plasma and red cell Na^+ and K^+ concentrations (with a Perkin Elmer atomic absorption spectrophotometer, or an EEL flame spectrophotometer);
- plasma and red cell Cl^- concentration (with a Radiometer CMT 10 chloride titrator);
- plasma and red cell Mg^{2+} concentration (with Wako test kit no. 273-32809);
- plasma Ca^{2+} concentration (with Wako test kit no. 276-21809).

RESULTS

Ventilatory frequency and oxygen consumption

The ventilation frequency at 15 °C (Fig. 1) increased drastically ($P < 0{\cdot}001$) from 99 ± 14 (8) in normoxia to 241 ± 37 (8) at 40–50 mmHg P_{O_2} in acute hypoxia. Below 40 mmHg P_{O_2} the animals were not able to maintain the high ventilation frequency, but started to show apnoeic periods of varying length, resulting in a decreased number of ventilatory movements per minute. After 7 days of hypoxic exposure the ventilation frequency at 40–50 mmHg had decreased slightly to 224 ± 40 (8). The O_2 consumption of the animals increased significantly ($P < 0{\cdot}01$) from $40 \pm 3{\cdot}7\,mg\,kg^{-1}\,h^{-1}$ ($x \pm$ S.E.M.; number of fish 8) in normoxia to $60 \pm 5{\cdot}2\,mg\,kg^{-1}\,h^{-1}$ in acute hypoxia. In prolonged hypoxia, the O_2 consumption of the animals decreased significantly ($P < 0{\cdot}05$) from that in acute hypoxia, to $53 \pm 3{\cdot}5\,mg\,kg^{-1}\,h^{-1}$, presumably reflecting the decrease in ventilatory activity compared to that in the non-acclimated specimens.

Blood oxygen transport

Blood Hb concentration and MCHC

At both 15 and 2 °C the blood Hb concentration was practically the same in the hypoxic as in the normoxic lampreys (Tables 1, 2), indicating that, unlike some

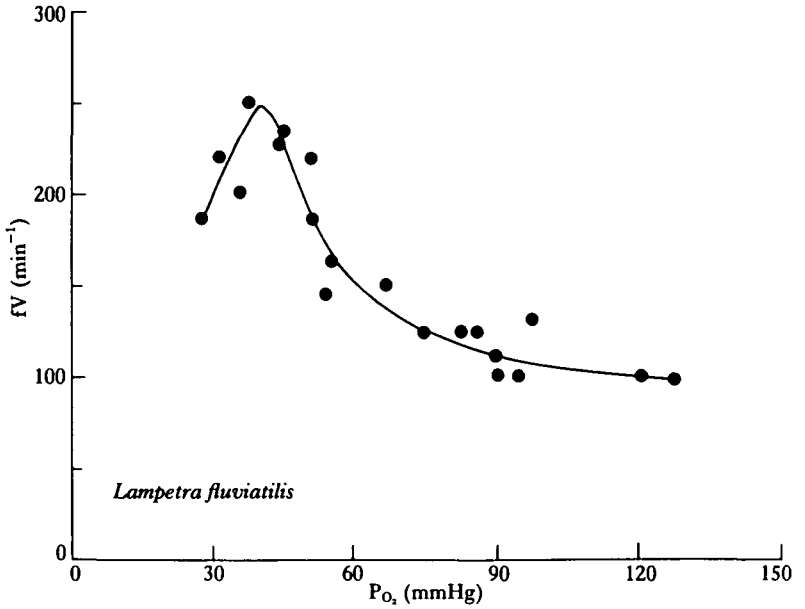

Fig. 1. The ventilatory frequency (fV) of lampreys as a function of P_{O_2} of the water at 15 °C. The frequency was counted visually for 1 min. Each point is a mean of determinations for four fish. The curve was fitted by eye. The water P_{O_2} was decreased from 130 mmHg to 30 mmHg in 8 h. For further details see text.

teleosts such as killifish (Greaney & Powers, 1978) and eel (Wood & Johansen, 1972), the lamprey is unable to modify the blood O_2-carrying capacity in response to hypoxia. On the other hand, MCHC fell by more than 20 % at 15 °C from approximately 17 to 14 mmol monomeric Hb l^{-1} packed cells; the decrease was most probably due to cellular swelling. At 2 °C the slight decrease in MCHC was not statistically significant.

Plasma and red cell pH

There was practically no pH gradient across the red cell membrane in normoxia at either temperature (Tables 1, 2). In hypoxia, however, the measured red cell pH was approximately 0·2 units higher than the plasma pH both at 15 and at 2 °C (Fig. 2). In the normoxic specimens, plasma pH was inversely related to acclimation temperature (between 2 and 15 °C, $\Delta pH/\Delta$ °C values were −0·016 and −0·011, for the cells and the plasma respectively), reflecting conformity with the relative alkalinity concept applicable to ectotherm vertebrates (Howell, Baumgardner, Bondi & Rahn, 1970). No similar pH regulation is seen in the hypoxic specimens (Fig. 2).

Oxygen equilibria

The log P_{50} *vs* pH diagrams are given in Fig. 3. The Bohr factor in the blood of the hypoxic animals (−0·14) was significantly ($P < 0{\cdot}02$) lower than that in the blood of normoxic animals (−0·34) in the pH range from 7·3 to 8·2. These regression coefficients were compared using the *t*-test as described by Goldstein (1964). The regression equations were:

Table 1. *Blood values of hypoxic and normoxic lampreys at 15 °C*

Parameter	Hypoxic	*P*	Normoxic
Hb ($g\,l^{-1}$)	86·5 ± 8·2 (8)	NS	95·5 ± 3·3 (8)
MCHC ($g\,l^{-1}$)	227·6 ± 12·9 (8)	0·01	283·6 ± 15·9 (8)
plasma pH	7·605 ± 0·056 (7)	NS	7·596 ± 0·015 (5)
red cell pH	7·751 ± 0·030 (7)	0·01	7·561 ± 0·030 (8)
plasma lactate ($mg\,l^{-1}$)	854 ± 304 (7)	0·01	235 ± 35 (8)
red cell lactate ($mg\,l^{-1}$)	573 ± 273 (7)	0·05	96 ± 11 (8)
red cell NTP ($mmol\,l^{-1}$)	1·36 ± 0·06 (7)	NS	1·91 ± 0·35 (8)
plasma K^+ ($mmol\,l^{-1}$)	3·64 ± 0·06 (5)	NS	3·32 ± 0·04 (5)
red cell K^+ ($mmol\,l^{-1}$)	71·2 ± 2·5 (5)	0·05	76·8 ± 1·6 (5)
plasma Na^+ ($mmol\,l^{-1}$)	128·0 ± 3·1 (5)	NS	131·8 ± 5·7 (5)
red cell Na^+ ($mmol\,l^{-1}$)	40·4 ± 2·9 (5)	NS	32·8 ± 2·5 (5)

The means, standard errors of the mean with the number of animals (in brackets) are given.
Student's *t*-test was used for statistical comparisons of the groups.
In the red cell determinations, values are per litre of packed red cells.
NS = not significant.

Table 2. *Blood values of hypoxic and normoxic lampreys at 2 °C*

Parameter	Hypoxic	*P*	Normoxic
Hb ($g\,l^{-1}$)	61·8 ± 2·1 (7)	NS	60·7 ± 2·5 (5)
MCHC ($g\,l^{-1}$)	185·4 ± 8·7 (7)	NS	194·2 ± 9·3 (5)
plasma pH	7·558 ± 0·040 (6)	NS	7·740 ± 0·047 (5)
red cell pH	7·730 ± 0·077 (6)	NS	7·774 ± 0·092 (4)
plasma lactate ($mg\,l^{-1}$)	65·4 ± 7·1 (7)	NS	68·0 ± 16·5 (5)
red cell lactate ($mg\,l^{-1}$)	32·2 ± 3·7 (6)	0·01	25·3 ± 2·4 (5)
red cell NTP ($mmol\,l^{-1}$)	1·04 ± 0·07 (4)	0·1	1·33 ± 0·10 (4)
plasma glucose ($g\,l^{-1}$)	0·54 ± 0·05 (7)	NS	0·56 ± 0·05 (4)
plasma Ca^{2+} ($mmol\,l^{-1}$)	1·94 ± 0·15 (5)	NS	2·00 ± 0·11 (5)
plasma Mg^{2+} ($mmol\,l^{-1}$)	0·52 ± 0·04 (7)	0·05	0·71 ± 0·07 (7)
red cell Mg^{2+} ($mmol\,l^{-1}$)	3·70 ± 0·19 (6)	NS	3·44 ± 0·15 (4)
plasma K^+ ($mmol\,l^{-1}$)	3·54 ± 0·40 (7)	NS	3·62 ± 0·40 (5)
red cell K^+ ($mmol\,l^{-1}$)	60·4 ± 2·1 (6)	0·001	73·4 ± 2·4 (4)
plasma Na^+ ($mmol\,l^{-1}$)	108·4 ± 2·0 (6)	NS	110·8 ± 3·3 (5)
red cell Na^+ ($mmol\,l^{-1}$)	41·1 ± 5·8 (6)	0·1	28·2 ± 1·8 (4)
plasma Cl^- ($mmol\,l^{-1}$)	109·8 ± 3·2 (5)	NS	115·8 ± 3·4 (5)
red cell Cl^- ($mmol\,l^{-1}$)	66·3 ± 6·8 (6)	NS	72·7 ± 5·9 (4)

For details see legend to Table 1.

$$\log P_{50} = -0{\cdot}14\,\mathrm{pH} + 2{\cdot}29,\ r = 0{\cdot}64,\ N = 19 \text{ and}$$

$$\log P_{50} = -0{\cdot}34\,\mathrm{pH} + 3{\cdot}88,\ r = 0{\cdot}88,\ N = 14, \text{ respectively.}$$

Calculating the P_{50} values for *in vivo* plasma pH at 15 °C (7·6 both in normoxia and hypoxia) yields values of 20 mmHg in normoxia and 16 mmHg in hypoxia, indicating a higher O_2 affinity in the hypoxia-acclimated specimens. The P_{50} and Bohr factor values of the hypoxic and normoxic specimens are lower and higher, respectively, than those obtained by Bird, Lutz & Potter (1976) for *L. fluviatilis* blood at a slightly lower temperature: ($\log P_{50} = -0{\cdot}22\,\mathrm{pH} + 2{\cdot}70$; $r = 0{\cdot}71$, $N = 16$ at 10 °C).

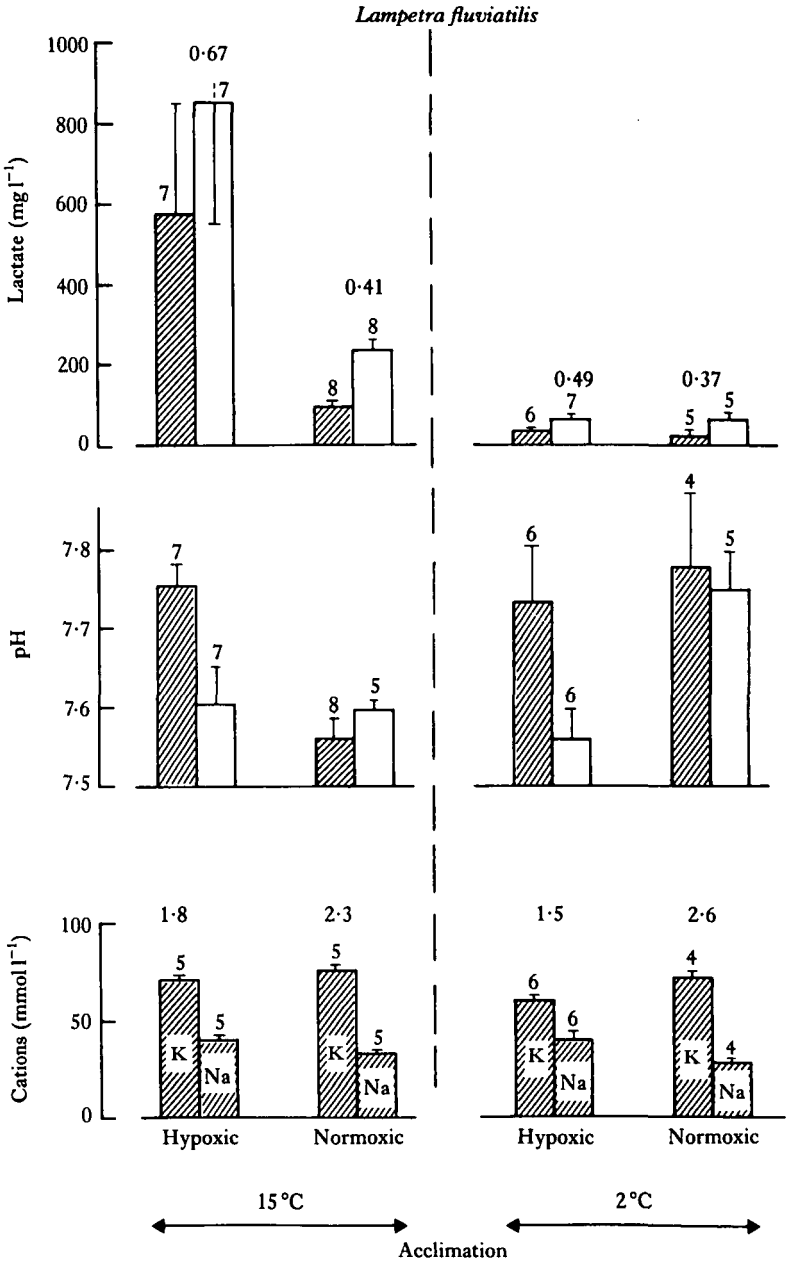

Fig. 2. Lactate, K^+ and Na^+ concentrations and pH in erythrocytes (hatched histograms) and plasma (open histograms) of lampreys acclimated to hypoxic and normoxic water at 15 °C (left) and 2 °C (right). Numbers on the individual columns refer to the number of specimens. E/P, erythrocyte/plasma ratio; K^+/Na^+, concentration ratios in the erythrocytes. The values above column pairs show the erythrocyte/plasma lactate concentration ratios (uppermost panels) and the erythrocytic K^+/Na^+ concentration ratios (lowermost panels).

Organic phosphate and lactate concentrations

At both temperatures, the erythrocytic NTP concentration tended to decrea

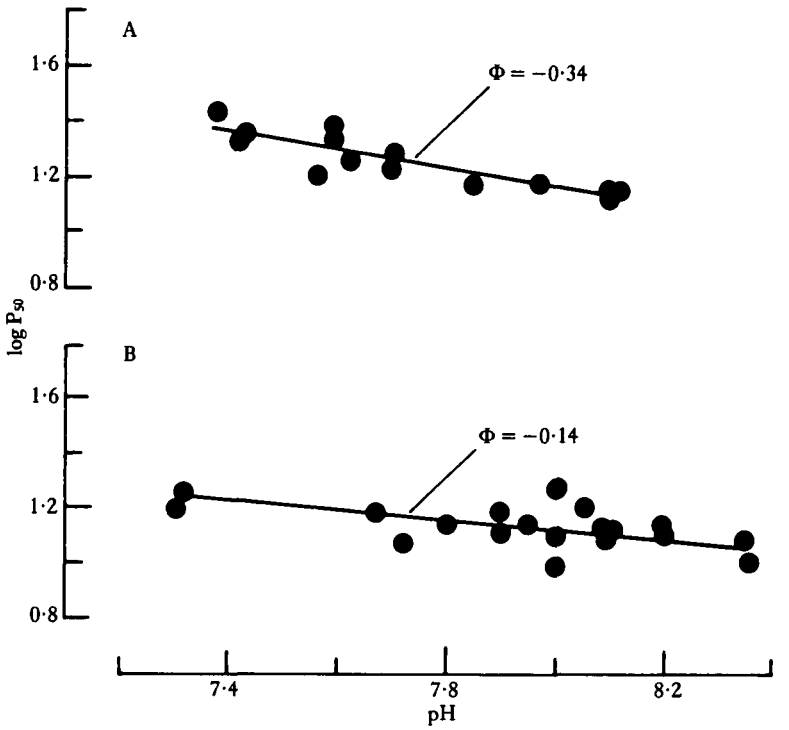

Fig. 3. Log P_{50} *vs* pH plot for the blood of (A) normoxic and (B) hypoxic lampreys at 15 °C, Φ = Bohr factor. The regression equations are given in the text.

during hypoxic exposure (Tables 1, 2). The decrease was mostly due to red cell swelling, as indicated by the fact that the NTP/Hb (monomer) molar ratio remained largely unaltered. In normoxia and hypoxia, the ratio was 0·11 and 0·10, respectively, at 15 °C, and 0·10 and 0·12, respectively, at 2 °C. Thin layer chromatography experiments showed that the erythrocytic NTPs consist largely of ATP but also contain some GTP (respective concentrations were 0·70 ± 0·02 S.D. and 0·33 ± 0·05 mmol l^{-} cells, $N = 3$). Bartlett (1982) similarly demonstrated the presence of GTP (at concentrations of 6–66 % of that of ATP) in red cells of individuals of the lamprey *Entospherus tridentatus*. That organophosphates other than NTPs may occur in lampreys follows from the observations (Johansen *et al.* 1973; Bartlett, 1982) that *E. tridentatus* red cells contain 2,3-diphosphoglycerate (DPG) at concentrations of 9–40 % of the ATP + GTP pool.

At 15 °C, both the red cell and plasma lactate concentrations increased markedly in hypoxia (Table 1; Fig. 2). The increase in the cellular concentration was proportionally greater than that in plasma; the red cell/plasma lactate ratio thus increased from 0·41 to 0·67. At 2 °C the plasma lactate concentration remained unchanged although the red cell lactate concentration increased significantly (Table 2). As a result the red cell/plasma ratio increased from 0·37 in normoxia to 0·49 in hypoxia.

Inorganic ion concentrations

Hypoxic exposure at 15 °C slightly increased the plasma concentration of K^+ while the red cell K^+ level showed a pronounced decrease. On the other hand, a slight

decrease in plasma Na^+ concentration was concomitant with a marked increase in red cell Na^+ (Table 1). This resulted in a distinct fall in red cell K^+/Na^+ ratio from 2·3 to 1·8 (Fig. 2). A similar decrease in this ratio, from 2·6 to 1·5, took place in hypoxia at 2 °C as red cell K^+ concentration decreased and the Na^+ concentration increased without significant changes in plasma concentrations (Table 2; Fig. 2). Also, the plasma Mg^{2+} concentration was lower in hypoxia than in normoxia at 2 °C (Table 2).

DISCUSSION

The blood responses were remarkably similar at 15 and at 2 °C. At both temperatures hypoxic exposure induced lower red cell K^+/Na^+ ratios, higher intraerythrocytic than plasma pH, and increased red cell/plasma lactate ratios. However, the changes in MCHC, and in plasma and red cell lactate concentrations were more pronounced at 15 °C than at 2 °C, demonstrating that the same degree of environmental hypoxia is more stressful at the higher temperature, which is ascribable to the higher metabolic rate under these conditions (see Claridge & Potter, 1975).

As in teleosts, the initial response of *Lampetra fluviatilis* to hypoxia is hyperventilation (see also Claridge & Potter, 1975). The resulting increase in the cost of ventilation is reflected in higher O_2 consumption rates. If in prolonged hypoxia the high cost of ventilation had to be maintained, the energy stores of the fish could be seriously depleted. However, in 7-day hypoxia the O_2 consumption was about 15 % lower than in acute hypoxia, showing that some other adaptive responses had occurred, reducing the energy-demanding contribution from the ventilatory response.

The increased blood O_2 affinity which will enhance O_2 loading in gills of the hypoxic animals appears to be part of this adaptation. The oxygen-binding properties of the haemoglobin (R. E. Weber & M. Nikinmaa, in preparation) suggest involvement of two mechanisms: a decrease in cellular haemoglobin concentration, as would result from red cell swelling, and an increase in the intracellular pH. A third factor that favours a higher blood O_2 affinity in hypoxic animals at low pH is that the Bohr effect is lower in hypoxic than in normoxic animals.

Although no data are available for the physiological concentration range, the O_2 affinity of lamprey haemoglobin seems to be strongly dependent on its concentration. Briehl (1963) showed that an increase in Hb concentration from 0·25 to 15·8 mmol l^{-1} (haem basis) decreased the O_2 affinity 10-fold in *Petromyzon marinus*. In *Entosphenus japonicus*, Dohi, Sugita & Yoneyama (1973) observed that the P_{50} value increased from about 15 mmHg to 35 mmHg as Hb concentration increased from 1 to 8 mmol l^{-1} at pH 6·9. The increase in the O_2 affinity with dilution may be attributable to dissociation of haemoglobin to monomers, which have a higher O_2 affinity than dimers or tetramers (Riggs, 1972). R. E. Weber & M. Nikinmaa (in preparation) will show that dilution of *L. fluviatilis* haemoglobin in solution not only increases its O_2 affinity but also decreases the Bohr effect, supporting the view that the corresponding changes observed in the whole blood may result from erythrocytic swelling.

The increase in the red cell pH in the hyperventilating hypoxic specimens at 15 °C will increase the O_2 affinity of the blood *via* the Bohr effect. The parallel reduction in the Bohr effect will favour maintenance of a high O_2 affinity. Dilution increases the proportion of monomeric haemoglobins which lack heterotropic interactions basic

the Bohr effect. The low pH sensitivity of dilute *L. fluviatilis* haemoglobin in solution (R. E. Weber & M. Nikinmaa, in preparation) supports this view.

An intriguing finding was that red cell pH was higher than plasma pH in the hypoxic animals. Some methodological factors may affect red cell pH measurements and deductions about the pH gradient. Firstly there was a time lag between plasma and red cell pH measurement. Secondly, the samples were taken from anaesthetized fish, and this may influence the pH gradient across the red cell membrane. Thirdly, according to Roos & Boron (1981) the freeze-thaw method for intracellular pH is reliable only for mammalian red cells. However, the intracellular pH values estimated from the freeze-thaw measurements are generally lower than those obtained with other methods (see Roos & Boron, 1981). Our finding of a higher pH in the red cells than in the plasma is furthermore in accordance with the calculations of Riggs (1972) [based on Manwell's (1963) and Briehl's (1963) data from *P. marinus*] which reflect an intracellular pH that is 0·7 units higher than the extracellular one. Such a difference could exist if the majority of impermeable polyions in the cell were positively charged (cf. Riggs, 1972). This is, however, unlikely, as the chloride ratio ($Cl^-_{cell}/Cl^-_{plasma}$) is less than one, and the same for both normoxic (0·60) and hypoxic (0·63) animals at 2 °C. At anion and volume equilibrium, the anion ratio (for those anions that can exchange across red cell membrane) can be calculated from the relation (cf. Hladky & Rink, 1977):

$$r = \frac{2(B + ZP)}{2B + (Z + 1)P},$$

where r = anion ratio (for exchangeable anions), B = number of cations in the cell (mostly Na^+ and K^+), P = number of impermeable polyions in the cell (mostly haemoglobin and organic phosphates) and Z = the charge on impermeable polyions. Z can be solved by substituting data from Table 2 into the above equation (anion ratio taken as 0·6; all other values converted into number of particles/cell). According to these calculations the charge of impermeable polyions is −3.

Red cell pH could also be higher than plasma pH, if protons were not passively distributed. A sodium/proton exchange mechanism operates in *Amphiuma* red cells (Kregenow, 1981). If such an exchange was functional in lamprey, the existing sodium gradient across the red cell membrane could drive the exchange and raise extracellular H^+ concentration above the intracellular level.

Red cell swelling and changes in intracellular pH may be achieved by related mechanisms. In rainbow trout red cell swelling and increase in intracellular pH seem to occur simultaneously as a result of beta-adrenergic stimulation (Nikinmaa, 1982). In rainbow trout (Nikinmaa & Soivio, 1982; Nikinmaa, 1982) as well as in lamprey (this study), the change in intracellular H^+ concentration seems to be twice the change in cell volume.

The red cell swelling could, however, also be explained by decreased activity of the K^+/Na^+ pump, which would explain the observed changes in K^+ and Na^+ concentrations (Tables 1, 2). Often a decreased pump activity is the result of anoxia, and a resultant depletion of ATP stores (cf. Macknight & Leaf, 1980). It is, however, questionable if the P_{O_2} in hypoxic blood is low enough to cause decreased ATP synthesis and impairment of the pump function. Greaney & Powers (1978) showed

that the ATP concentration of killifish red cells decreased only in complete anoxia and Tetens & Lykkeboe (1981) concluded that hypoxia does not impair ATP synthesis by rainbow trout red cells, suggesting that a hormonal mechanism could regulate the NTP concentration and O_2 affinity of these cells. Hormonal control by adrenalin seems, indeed, to be implied at least in the rapid O_2 affinity changes of rainbow trout blood (Nikinmaa, 1982). Adrenalin moreover influences the cation concentrations of nucleated red cells by changing the cation permeability of the red cell membrane (see Palfrey, Alper & Greengard, 1980).

We conclude that hypoxic exposure in the lamprey does increase blood O_2 affinity, despite insensitivity of the Hb-O_2 affinity to organic phosphate concentration and ionic strength, and that the change in blood O_2 affinity appears to be mediated by changes in the red cell membranes resulting in lower cellular Hb concentration and increased cellular pH.

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