

## REVIEW

# HAEMOGLOBIN FUNCTION IN INTACT LAMPREY ERYTHROCYTES: INTERACTIONS WITH MEMBRANE FUNCTION IN THE REGULATION OF GAS TRANSPORT AND ACID–BASE BALANCE

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### Summary

Haemoglobin function within lamprey erythrocytes offers a unique solution to gas transport among vertebrates. Lamprey haemoglobin within intact erythrocytes is in oligomer/monomer equilibrium and has an oxygen affinity similar to that of haemoglobin in other active fishes. The cooperativity of oxygen binding, which is reduced at low pH values, the effect of protons and the effect of the concentration of haemoglobin on its oxygen affinity are all due to dissociation/association reactions of the haemoglobin molecules. The permeability of the lamprey red cell membrane to acid and base equivalents is very low, and plasma bicarbonate cannot therefore be dehydrated to carbon dioxide to any significant extent during the residence time of blood in the gills. This potential limitation on carbon dioxide excretion is

overcome, however, by the high intraerythrocytic pH and the marked oxygenation-linked pH changes in the erythrocyte, which are due to the large Haldane effect of the haemoglobin. Owing to the relative impermeability of the erythrocyte membrane to acid equivalents, intraerythrocytic haemoglobin cannot take part in the acid–base buffering of the extracellular compartment. As a consequence, extracellular acid loads cause marked fluctuations in plasma pH.

Key words: oxygen transport, carbon dioxide transport, Haldane effect, Bohr effect, Agnatha, lamprey, erythrocyte, haemoglobin, acid–base balance, membrane function, *Petromyzon marinus*, *Lampetra fluviatilis*.

### Introduction

Haemoglobin function in lampreys has mainly been studied using (dilute) solutions (e.g. Wald and Riggs, 1951; Briehl, 1963; Antonini *et al.* 1964; Andersen and Gibson, 1971; Dohi *et al.* 1973; see also reviews by Riggs, 1972; Perutz, 1990). On the basis of these studies, it is clear that lamprey haemoglobins in solution show cooperativity as well as distinct Bohr and Haldane effects. Furthermore, the oxygen affinity of lamprey haemoglobin depends on its concentration in the solution. This finding, and the observation that the apparent molecular mass of deoxygenated haemoglobin is higher than that of oxygenated haemoglobin (Briehl, 1963; Andersen, 1971; Dohi *et al.* 1973), indicates that, in lampreys, the cooperativity of oxygen binding and the effect of protons on the haemoglobin oxygen-affinity are caused by reversible association/dissociation reactions of monomers/oligomers (for reviews, see Riggs, 1972; Perutz, 1990). This contrasts with the situation in all other vertebrate haemoglobins, in which these effects are the result of conformational changes of the tetrameric molecule.

The behaviour of lamprey haemoglobin in solution can be

explained by the following model (Perutz, 1990). The dimeric/oligomeric form of the molecule has a low oxygen affinity owing to constraints resulting from interactions between its subunits. In the dimer, binding of oxygen weakens these interactions, the molecule dissociates to its subunits and, consequently, the oxygen affinity of the other subunit is increased. It is likely that the distal histidine (E7) is involved in this allosteric transition (Perutz, 1990): in the monomeric form, it would be in an internal position within the haem pocket (where it could form a hydrogen bond with oxygen), and in the dimeric form it would be in an external position (where it could form a hydrogen bond with an aspartate or glutamate of the neighbouring molecule). The Bohr effect would, in this case, be accounted for by the differences in the pK values of the histidine in its internal position (5.5–6) and in its external position hydrogen-bonded to a carboxylate (approximately 8).

Until recently, it has been difficult to interpret the oxygen binding data of whole blood on the basis of the behaviour of haemoglobin in solution. For example, the oxygen affinity of lamprey blood was much higher than expected on the basis of

the behaviour of haemoglobin in solution (Riggs, 1972). Also, the numerical value of the Bohr factor estimated for whole blood was much smaller than expected on the basis of data from haemoglobin solutions (Bird *et al.* 1976; Nikinmaa and Weber, 1984).

The apparent disagreement between oxygen binding data for haemoglobin in solution and for haemoglobin within intact erythrocytes was resolved when it was observed that the properties of the lamprey erythrocyte membrane were markedly different from those of the erythrocyte membrane of other vertebrates. The permeability of lamprey erythrocytes to chloride and to acid or base equivalents is very low (Ohnishi and Asai, 1985; Nikinmaa and Railo, 1987; Tufts and Boutilier, 1989, 1990). As a consequence of the minute passive fluxes of acid equivalents across the erythrocyte membrane, intracellular pH is maintained at a high value mainly by sodium/proton exchange across the red cell membrane (Nikinmaa, 1986; Nikinmaa *et al.* 1986, 1993; Tufts and Boutilier, 1990; Tufts, 1992; Virkki and Nikinmaa, 1994).

In this review, we examine how these unique membrane properties affect haemoglobin function within intact lamprey erythrocytes and how the properties of haemoglobin and of the cell membrane affect not only oxygen transport but also carbon dioxide transport and acid-base regulation.

#### Haemoglobin oxygen-affinity in lamprey erythrocytes

The resting pH of lamprey *Lampetra fluviatilis* blood at 10 °C is 7.7–7.9 (Mattsoff and Nikinmaa, 1988). Since plasma pH decreases by 0.011 units with each 1 °C increase in temperature (Nikinmaa and Weber, 1984), at 18 °C the values would be approximately 7.6–7.8. At an extracellular pH of 7.67, well within the range of resting plasma pH values at 18 °C, the  $P_{50}$  value for lamprey erythrocytes is 2.9 kPa (Nikinmaa, 1993a). The resting plasma pH value is similar to, and the  $P_{50}$  value similar to or higher than, the values measured in rainbow trout (plasma pH at 19 °C is 7.67 and the  $P_{50}$  value is 3.4 kPa; Nikinmaa, 1981; plasma pH at 18 °C is 7.65 and the *in vivo*  $P_{50}$  value is 4.9 kPa in normoxia and 3.6 kPa in hypoxia; Nikinmaa and Soivio, 1982). In carp, *Cyprinus carpio*, however, the  $P_{50}$  value at an extracellular pH of 7.7 is clearly smaller, i.e. 1.1 kPa (a value calculated from the data of Salama and Nikinmaa, 1988) than in lamprey. Thus, lamprey erythrocytes show oxygen affinities of the range expected for an active animal.

When erythrocyte pH is related to the oxygen affinity of haemoglobin within intact erythrocytes (Fig. 1), it becomes obvious that, to have a blood oxygen-affinity that is similar to or higher than that of rainbow trout at rest, *Lampetra fluviatilis* must have a much higher intraerythrocytic pH (pHi). Indeed, at 10 °C, the intraerythrocytic pH of *Lampetra fluviatilis*, at a resting extracellular pH of 7.78, was 7.96 (Mattsoff and Nikinmaa, 1988). At a similar temperature, and at an extracellular pH of 7.8, the intraerythrocytic pH of rainbow trout was 7.45 (Perry *et al.* 1987).

When the lampreys *Lampetra fluviatilis* and *Petromyzon*

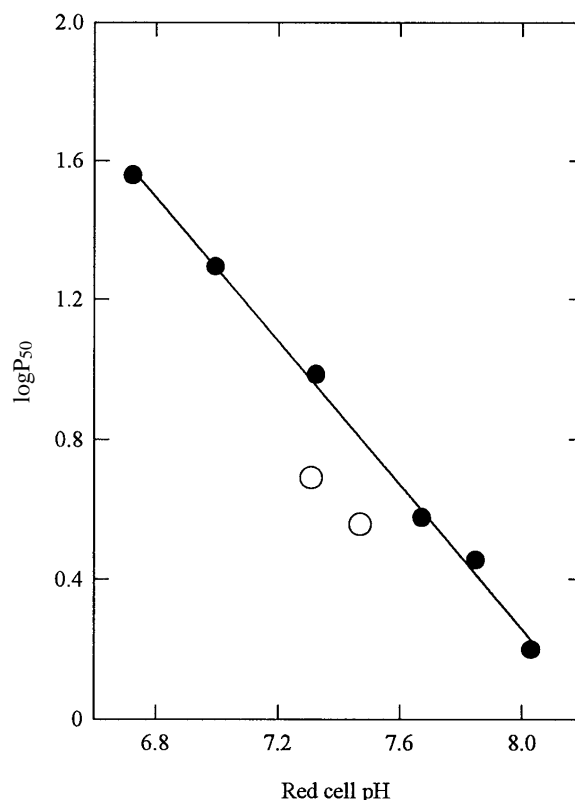


Fig. 1. The mean haemoglobin oxygen-affinity (given as  $\log P_{50}$ ; filled circles) in intact *Lampetra fluviatilis* erythrocytes as a function of red cell pH.  $P_{O_2}$  was measured in kPa;  $N=6$  for each point. Original data from Nikinmaa (1993a). For comparison, data on the *in vivo* value of mean  $\log P_{50}$  (open circles) for rainbow trout (*Oncorhynchus mykiss*) at a similar temperature and at two intracellular pH values are given.  $N=6$ ; original data from Nikinmaa and Soivio (1982).

*marinus* are compared, it is apparent that the intrinsic haemoglobin oxygen-affinity of *Petromyzon marinus* is higher than that of *Lampetra fluviatilis*, since the  $P_{50}$  value of *Petromyzon marinus* haemoglobin in dilute solutions is lower than that of *Lampetra fluviatilis* haemoglobin at similar pH values (Antonini *et al.* 1964). This difference is also seen in the whole blood; the  $P_{50}$  value of *Petromyzon marinus* haemoglobin at a pHi of 7.64 at 10 °C was 3.1 kPa (Ferguson *et al.* 1992), i.e. similar to that of *Lampetra fluviatilis* haemoglobin at a pHi of 7.67 and at 18 °C. Taking into account the exothermic reaction of oxygen binding and the dependence of intracellular pH on temperature (Nikinmaa and Weber, 1984), this result indicates clearly the higher oxygen affinity of *Petromyzon marinus* than of *Lampetra fluviatilis* haemoglobin in intact erythrocytes at a given pH. Since the intraerythrocytic pH of *Petromyzon marinus* is approximately 0.2 units lower than that of *Lampetra fluviatilis* (see Tufts, 1991; Ferguson *et al.* 1992; Mattsoff and Nikinmaa, 1988), it is likely that the resting *in vivo* whole-blood oxygen-affinities of the two species are similar.

Both lamprey species maintain their intraerythrocytic pH

using a sodium-dependent acid extrusion mechanism, most likely sodium/proton exchange (Nikinmaa, 1986; Nikinmaa *et al.* 1986; Tufts and Boutilier, 1990; Tufts, 1992). As stated in the Introduction, we have suggested that the maintenance of intracellular pH by sodium/proton exchange is possible because the permeability of the lamprey erythrocyte membrane to acid equivalents is low. This suggestion can be experimentally verified using, for example, tributyltin chloride, a hydroxyl ion/chloride exchanger. Tufts and Boutilier (1990) showed that treatment of lamprey erythrocytes with tripropyltin chloride caused a reduction in the steady-state intraerythrocytic pH of *Petromyzon marinus*. The effect of organotin compounds is immediate (Fig. 2; L. V. Virkki and M. Nikinmaa, unpublished data) showing that the effect is indeed caused by the ion-exchange action of the compound and not by its metabolic effects. On the basis of the data in Figs 1 and 2, a reduction of intracellular pH to the values observed in tributyltin-chloride-treated cells would increase the  $P_{50}$  value of *Lampetra fluviatilis* haemoglobin to about 10 kPa at 18 °C. Such a value would seriously impair oxygen loading at the gills, even in normoxic conditions.

In conclusion, the haemoglobin oxygen-affinity within intact erythrocytes of lampreys is similar to that in other active, aerobic fish. The maintenance of this oxygen affinity requires that the intraerythrocytic pH be maintained at a higher level than in teleosts. The maintenance of a high intraerythrocytic pH is possible because the passive permeability of red cell membrane to acid equivalents is low (Nikinmaa and Railo, 1987; Tufts and Boutilier, 1989, 1990) and because the cells possess a sodium/proton exchanger which influences the red cell pH even in resting conditions (Nikinmaa, 1986; Tufts, 1992); the exchanger is even further activated by acidification (Virkki and Nikinmaa, 1994).

### Cooperativity of oxygen binding

Bird *et al.* (1976) found that the Hill plots of oxygen binding by *Lampetra fluviatilis* blood were curvilinear. Whereas at low oxygen saturations, the calculated  $n$  values were close to 1, they increased with increasing oxygen saturation and the highest values obtained were above 2. Subsequent studies using intact erythrocytes over an intracellular pH range of 8–6.7 (Nikinmaa, 1993a) showed that the cooperativity was also markedly affected by pH. For example, at a pHi of 6.7, the Hill plot was linear and the  $n$  value was close to 1. The higher the intracellular pH, the more curvilinear the Hill plot became (Fig. 3). These findings are compatible with the suggestion that deoxygenated lamprey haemoglobin is in the low-affinity oligomeric form, which is further stabilized by low pH values, such that at the lowest pH values examined no dissociation occurs. As the pH increases, the interactions between the individual chains of the oligomers are weakened, and oxygenation causes dissociation of the low-affinity oligomers to the high-affinity monomers. Consequently, an oxygenation-dependent increase in Hill's  $n$  value is observed. The higher the pH, the lower the oxygen tension required for

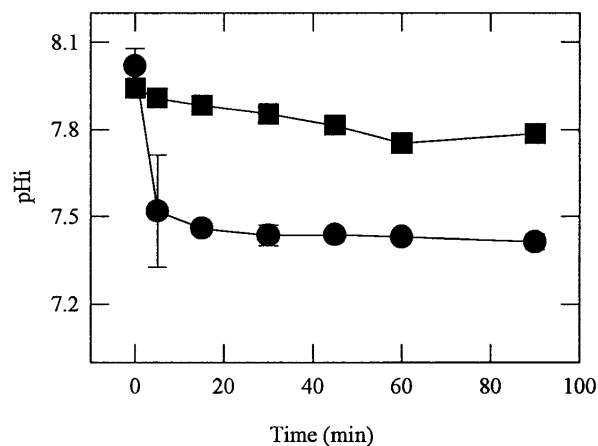


Fig. 2. The effect of  $10^{-6} \text{ mol l}^{-1}$  tributyltin chloride on the intraerythrocytic pH of *Lampetra fluviatilis* at an extracellular pH of 7.5 (at 20 °C). Squares, control cells, which were equilibrated under air in lamprey Ringer (for composition, see Virkki and Nikinmaa, 1994;  $N=2$ ); Circles, cells incubated in the presence of tributyltin chloride, added after the time 0 sample had been taken (means  $\pm$  S.E.M.;  $N=4-5$ ).

the dissociation of oligomers and for the consequent increase in Hill's  $n$  value.

### Effects of protons on haemoglobin function

Early studies on the effects of pH on the oxygen binding properties of lamprey blood suggested that the Bohr factor would be small, with values between  $-0.1$  and  $-0.3$  (Bird *et al.* 1976; Nikinmaa and Weber, 1984). However, in those studies, the haemoglobin oxygen-affinity was given as a function of extracellular pH, since at that time the unique regulation of intracellular pH in lamprey erythrocytes was not understood. Later studies (Ferguson *et al.* 1992; Nikinmaa, 1993a), in which the change in oxygen affinity was related to intracellular pH, showed that the Bohr factor was quite large. In *Petromyzon marinus* at 10 °C and at a pHi of 7.3–7.6, the Bohr factor ( $\Delta \log P_{50} / \Delta \text{pHi}$ ) was  $-0.63$  (Ferguson *et al.* 1992), and in *Lampetra fluviatilis* at 18 °C and at a pHi of 6.7–8.0, the Bohr factor was  $-1.03$  (Nikinmaa, 1993a; Fig. 1). For *Lampetra fluviatilis*, the value of  $-1.03$  therefore indicates the release of one proton per monomeric haemoglobin. A similar value has been obtained on the basis of the nonbicarbonate buffering capacity of the erythrocyte, the intraerythrocytic haemoglobin concentration and the measured intracellular pH changes upon oxygenation (Nikinmaa, 1993a). Notably, conclusions based on studies of haemoglobin solutions indicate that the Bohr effect of lamprey haemoglobin is due to the release of one proton per haemoglobin chain upon oxygenation (Andersen, 1971; Riggs, 1972; Perutz, 1990).

As is apparent from the concept of linked functions (e.g. Wyman, 1964), a large Bohr effect (the effect of protons on oxygen binding and dissociation) implies that oxygenation has

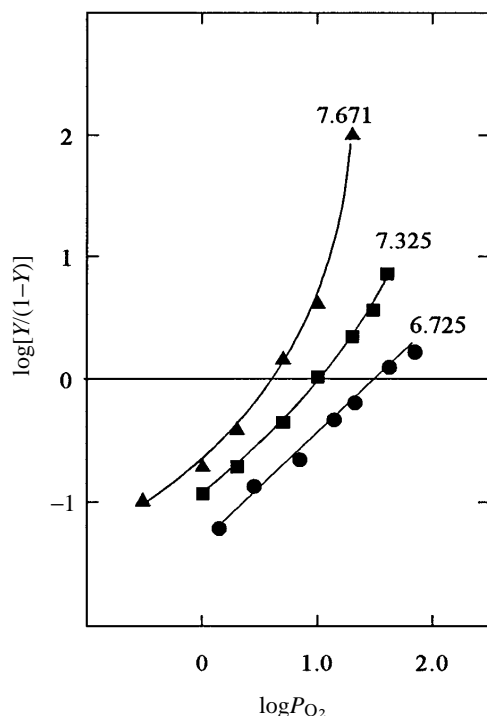


Fig. 3. Hill plots for haemoglobin within intact *Lampetra fluviatilis* erythrocytes at different red cell pH values. The values given on the figure indicate the red cell pH at the  $P_{50}$  value.  $Y$ , fractional oxygen saturation;  $P_{O_2}$  was measured in kPa; mean values;  $N=6$  for each point. Original data from Nikinmaa (1993a).

a large effect on proton uptake and release, i.e. the Haldane effect is large. Consequently, deoxygenation increases the red cell pH of *Lampetra fluviatilis* by up to 0.33 units and the red cell pH of *Petromyzon marinus* by up to 0.45 units (Nikinmaa and Mattsoff, 1992; Ferguson *et al.* 1992).

Lapennas (1983) has discussed the significance of the Bohr (and Haldane) factor in mammals. In his analysis, he used the 'physiological' oxygen equilibrium curve, i.e. the relationship between oxygen saturation and oxygen tension that pertains *in vivo*, where blood pH is not constant but changes as oxygen is exchanged for carbon dioxide. The analysis took into account the changes in blood pH caused by the liberation of carbon dioxide into the blood and the uptake of protons upon deoxygenation of haemoglobin (Haldane effect), and the effect of protons on haemoglobin oxygen-affinity (Bohr effect). Lapennas (1983) concluded that Bohr factors between  $-0.35$  and  $-0.5$ , i.e. half of the respiratory quotient, give maximal benefit in terms of oxygen delivery, since they produce the greatest possible rightward shift of the physiological oxygen equilibrium curve in working tissues (i.e. the net effect of the decrease in the pH of capillary blood and the pH-induced decrease in haemoglobin oxygen-affinity is maximal). However, large Bohr factors (i.e. those approaching the respiratory quotient) minimize arterio-venous pH changes and facilitate carbon dioxide transport. When applying this analysis to lamprey blood, and taking into account that intraerythrocytic

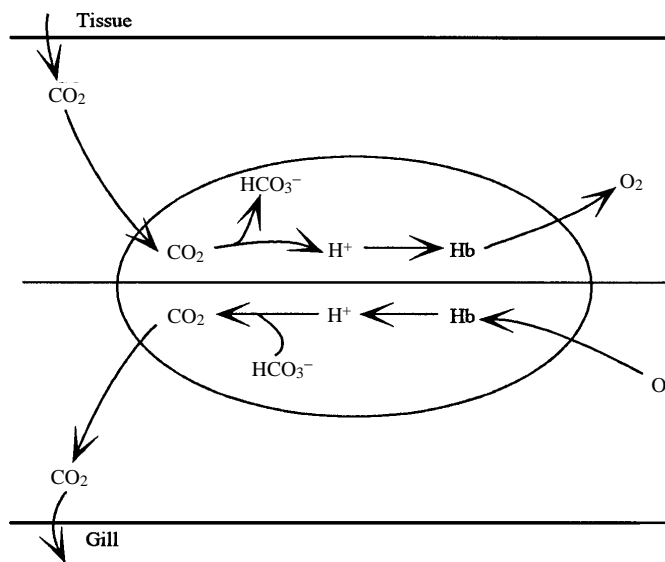


Fig. 4. Schematic representation of carbon dioxide transport in lamprey blood. In the tissue capillaries, carbon dioxide diffuses into the erythrocyte down its partial pressure gradient and is hydrated to bicarbonate and protons in a reaction catalysed by carbonic anhydrase. Protons are effectively taken up by haemoglobin upon deoxygenation, and intraerythrocytic pH increases. This increases the total amount of bicarbonate that can be loaded in the erythrocytes. In the gills, protons are released from haemoglobin upon oxygenation, the intraerythrocytic pH decreases, and bicarbonate is effectively dehydrated to carbon dioxide in a reaction catalysed by carbonic anhydrase. Carbon dioxide then diffuses down its partial pressure gradient across the gills. Modified from Tufts and Boutilier (1989) and Nikinmaa (1990).

pH must be used, Ferguson *et al.* (1992) concluded that the large Bohr/Haldane effect is probably oriented towards carbon dioxide transport. Indeed, the Haldane effect within intact lamprey erythrocytes is so large that the intraerythrocytic pH in venous blood is higher than that in arterial blood (Tufts *et al.* 1992).

The role played by the large Haldane effect of haemoglobin in carbon dioxide transport by lamprey blood becomes obvious when the mechanism of carbon dioxide transport by lamprey blood is examined. Tufts and Boutilier (1989, 1990) and Nikinmaa (1990) first presented the model for carbon dioxide transport by lamprey blood (Fig. 4). Owing to the negligible permeability of the lamprey red blood cell membrane to bicarbonate and other acid-base equivalents (Nikinmaa and Railo, 1987; Tufts and Boutilier, 1989; Nikinmaa and Mattsoff, 1992; Nikinmaa, 1993b; Cameron and Tufts, 1994), the carbonic anhydrase contained within the erythrocyte (Nikinmaa *et al.* 1986; Henry *et al.* 1993) is inaccessible to plasma bicarbonate. As a consequence, only a small proportion of plasma bicarbonate can be converted to carbon dioxide in the uncatalysed dehydration reaction during the passage of blood through the gills, because the half-time of the uncatalysed reaction is approximately 1 min at 15 °C (Heming, 1984) and the residence time of blood in the gills is at the most a few seconds (Bhargava *et al.* 1992). Thus, most of the carbon

dioxide excreted from the blood in the gills must come from the erythrocytic bicarbonate stores, and the efficiency of carbon dioxide transport by lamprey blood depends critically on how much of the total carbon dioxide the erythrocytes can load in the tissues and unload in the gills. The major factors influencing the loading and unloading of carbon dioxide within the erythrocytes are (1) the changes in carbon dioxide tension in the circulation and (2) the carbon dioxide dissociation curves of oxygenated and deoxygenated erythrocytes. On the basis of the carbon dioxide dissociation curves for deoxygenated and oxygenated *Lampetra fluviatilis* erythrocytes (Nikinmaa and Mattsoff, 1992; Fig. 5), more than 80 % of the maximal decrease in the total carbon dioxide content of the erythrocytes (*in vitro* conditions resembling the situation in gills) can be attributed to the oxygenation-dependent decrease in the total carbon dioxide content (at a constant carbon dioxide tension) and less than 20 % to the decrease in carbon dioxide tension. Ferguson *et al.* (1992) also observed a marked difference in the total carbon dioxide content of oxygenated and deoxygenated erythrocytes of *Petromyzon marinus*. Furthermore, they showed that oxygenation did not affect the plasma carbon dioxide content. The oxygenation-dependent decrease in the intraerythrocytic carbon dioxide content is fully accounted for by the oxygenation-dependent decrease in intraerythrocytic pH caused by the Haldane effect of haemoglobin (Nikinmaa and Mattsoff, 1992). Thus, on the basis of *in vitro* data, the Bohr/Haldane effect appears to play a critical role in carbon dioxide transport by lamprey blood.

Tufts *et al.* (1992) investigated the role of erythrocytes in carbon dioxide transport *in vivo*. According to their results, 62 % of the excreted carbon dioxide originates from the erythrocytes in resting *Petromyzon marinus*. After exercise, the contribution of erythrocytes is increased to 78 % of the total carbon dioxide content difference between venous and arterial blood. It is possible that these values underestimate the role of erythrocytes in carbon dioxide excretion because of the inherent time delays in the measurements of total carbon dioxide content (of true plasma in postbranchial blood). Nevertheless, the results indicate the major role played by erythrocytes in carbon dioxide excretion in lampreys *in vivo*. In comparison, only 15 % of the excreted carbon dioxide originates from the erythrocytes in rainbow trout (Heming, 1984).

In conclusion, the large Bohr effect of lamprey haemoglobin within intact erythrocytes plays an important role in facilitating carbon dioxide transport. As a consequence of the high venous intraerythrocytic pH (0.15–0.2 units higher than arterial intraerythrocytic pH) in *Petromyzon marinus* (Tufts *et al.* 1992), carbon dioxide is efficiently loaded into erythrocytic stores, mainly as bicarbonate. As a consequence of the decrease in carbon dioxide tension of blood at the gills, and especially the marked reduction in intraerythrocytic pH due to the release of protons upon oxygenation of haemoglobin, carbon dioxide is efficiently unloaded in the gills. Thus, the measured decrease in total carbon dioxide content during the passage of blood through the gills in the lamprey (Tufts *et al.*

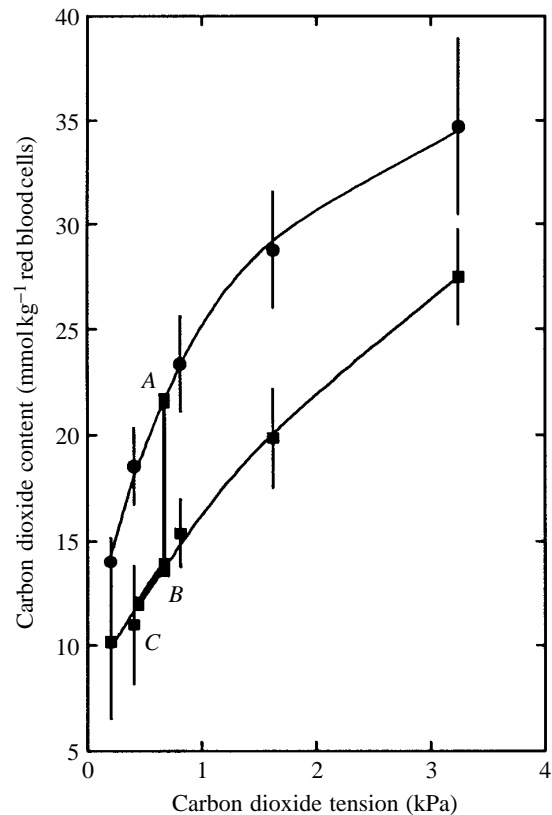


Fig. 5. The carbon dioxide dissociation curves of lamprey erythrocytes at low (circles; oxygen tension 1 kPa) and high (squares; oxygen tension 20 kPa) oxygen tension. The line from A to B indicates the decrease in total erythrocytic carbon dioxide content upon oxygenation of the erythrocytes (as takes place in gills). The distance from B to C indicates the further reduction of total carbon dioxide content that results from a 0.3 kPa decrease in carbon dioxide tension (as occurs in the gills of exercised lamprey). Values are means  $\pm$  S.E.M.,  $N=6$  for each point; original data from Nikinmaa and Mattsoff (1992).

1992) is similar to that observed for active teleosts such as rainbow trout (e.g. Nikinmaa and Jensen, 1986), despite the fact that, in lampreys, plasma bicarbonate plays only a limited role in carbon dioxide excretion owing to the slow permeation of bicarbonate through the membrane.

#### Effect of concentration on haemoglobin function in intact erythrocytes

In dilute solutions, the oxygen affinity of lamprey haemoglobin is markedly concentration-dependent (Wald and Riggs, 1951; Briehl, 1963; Antonini *et al.* 1964; Andersen and Gibson, 1971; Dohi *et al.* 1973; Nikinmaa and Weber, 1993). An increase in the concentration decreases haemoglobin oxygen-affinity by increasing the proportion of low-affinity oligomers. Since haemoglobin within intact erythrocytes shows both cooperativity and a large Bohr effect, it is probable that association/dissociation reactions of haemoglobin also occur within the erythrocyte. If this were the case, then the

haemoglobin oxygen-affinity should respond to variations in red cell volume (which affect intracellular haemoglobin concentration). This possibility could be tested experimentally by shrinking the cells osmotically and then measuring oxygen equilibrium curves, because the volume of osmotically shrunk *Lampetra fluviatilis* erythrocytes does not change after the initial decrease (Virkki and Nikinmaa, 1994). We have recently carried out these experiments (Airaksinen and Nikinmaa,

Table 1. *P*<sub>50</sub> values for lamprey erythrocytes at different mean cellular haemoglobin concentrations

<i>P</i> <sub>50</sub> value (kPa)	MCHC (g l <sup>-1</sup> red blood cells)
4.26±0.07	280.5±3.8
4.64±0.13	331.2±3.3
5.64±0.40	382.5±6.0

Values are means ± S.E.M., *N*=7–8.  
The mean cellular haemoglobin concentration (MCHC) was increased by shrinking the cells osmotically. The osmolality of the medium was increased by adding 90 or 180 mmol l<sup>-1</sup> sucrose to the lamprey Ringer.  
Data from Airaksinen and Nikinmaa (1995).

1995). As Table 1 indicates, there is a clear decrease in the haemoglobin oxygen-affinity (an increase in *P*<sub>50</sub>) within intact erythrocytes when the mean cellular haemoglobin concentration increases. Although this decrease occurs, there is a tendency for the shrunk erythrocytes to have a higher intracellular pH than that observed in erythrocytes of normal volume (Virkki and Nikinmaa, 1994).  
Thus, concentration changes occurring within intact erythrocytes appear to be adequate to cause changes in the aggregation state of the haemoglobin molecule and, as a consequence, in the haemoglobin oxygen-affinity. It is not yet clear whether such volume changes are part of the normal physiological regulation of haemoglobin function. However, data from hypoxia-acclimated *Lampetra fluviatilis* indicate that the mean cellular haemoglobin concentration is lower after a week's acclimation to hypoxia than during normoxia (Nikinmaa and Weber, 1984).

**Buffering by haemoglobin – limitations due to the low permeability of the erythrocyte membrane to acid equivalents**

The general vertebrate pattern of buffering extracellular acid loads involves the Jacobs–Stewart cycle: protons entering the

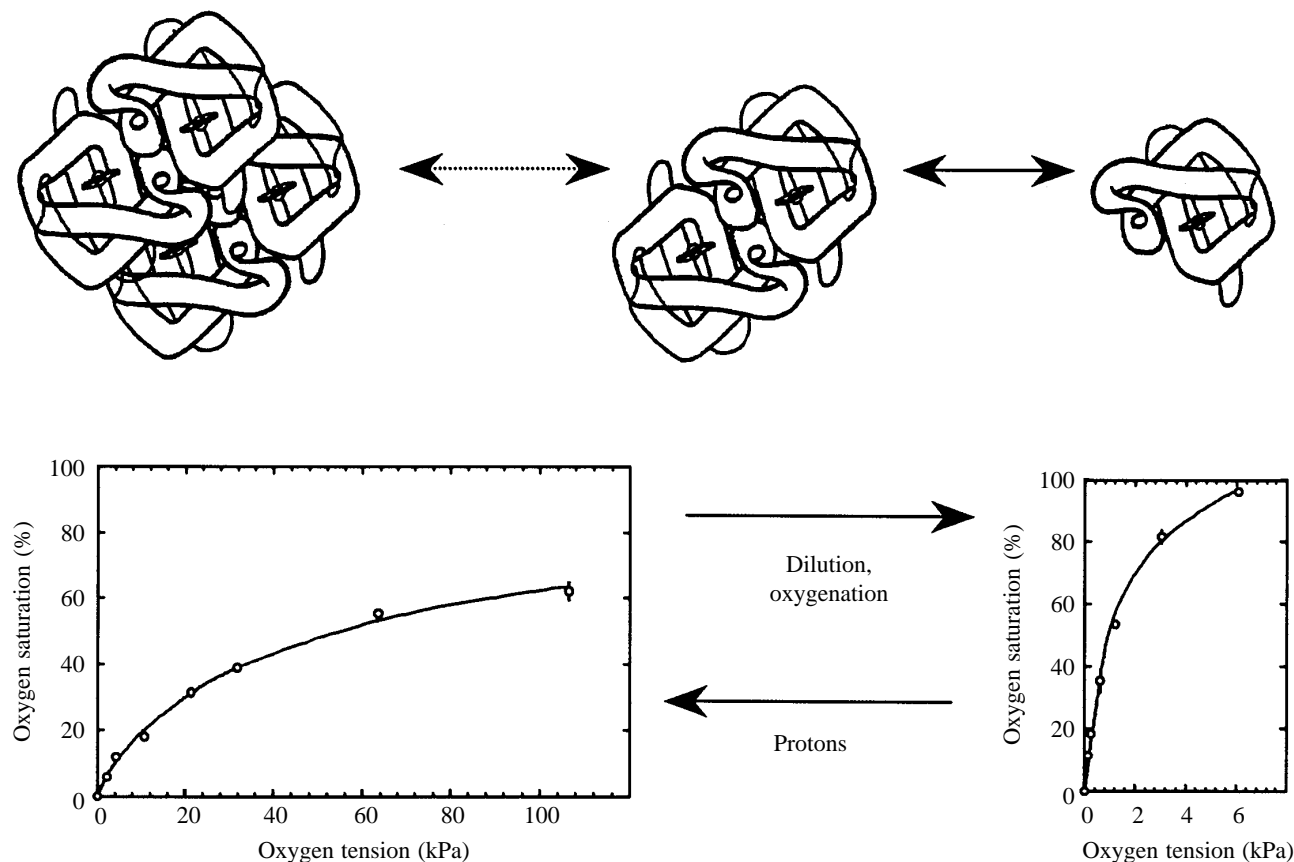


Fig. 6. Schematic representation of the regulation of haemoglobin function in intact lamprey erythrocytes. The haemoglobin molecule is in monomer/oligomer (tetra- and/or dimer) equilibrium. The equilibrium is shifted towards monomers by a reduction in cellular haemoglobin concentration, by an increase in oxygen partial pressure and by an increase in red cell pH. A shift from oligomers to monomers increases the haemoglobin oxygen-affinity markedly, since the oligomers have a much lower oxygen affinity than the monomers.

plasma react with bicarbonate ions to form carbon dioxide (and water). The carbon dioxide formed diffuses into the erythrocytes and is hydrated to bicarbonate and protons. The protons are taken up by intracellular buffers, notably haemoglobin, and bicarbonate is able to leave the cell *via* rapid anion exchange, thus replenishing the extracellular buffer store. Although the erythrocytic buffering capacity is substantial in lampreys ( $35\text{--}70\ \mu\text{mol pH unit}^{-1}\text{ g}^{-1}$ ; Tufts and Boutilier, 1989, 1990; Nikinmaa and Mattsoff, 1992), this scheme is not possible in lampreys because of the slow movement of bicarbonate across the red cell membrane. Consequently, extracellular acid loads can only be buffered by plasma buffers, and the nonbicarbonate buffering capacity of whole blood (or true plasma, i.e. plasma that is in contact with erythrocytes during the determination of the buffering capacity) is very low, i.e.  $3\ \mu\text{mol pH unit}^{-1}\text{ g}^{-1}$  in *Petromyzon marinus* (Tufts and Boutilier, 1990) and  $2.5\ \mu\text{mol pH unit}^{-1}\text{ g}^{-1}$  in *Lampetra fluviatilis* (Mattsoff and Nikinmaa, 1988). When the erythrocyte membrane is made permeable to acid equivalents using tripropyltin chloride, the nonbicarbonate buffering capacity of true plasma is significantly increased in *Petromyzon marinus* (Tufts and Boutilier, 1990), confirming that intraerythrocytic buffers do not take part in buffering extracellular acid loads in normal conditions.

Extracellular acid loads can thus cause marked fluctuations in the extracellular pH of lampreys. For example, exposure of *Lampetra fluviatilis* to pH 4 for 24 h caused a 0.9 unit decrease in the plasma pH (Mattsoff and Nikinmaa, 1988); a similar exposure in rainbow trout caused only a 0.1–0.3 unit decrease (Ultsch *et al.* 1981; Booth *et al.* 1982).

### Conclusions

Haemoglobin function within lamprey erythrocytes offers a unique solution to gas transport among vertebrates. A schematic representation of factors controlling haemoglobin function is given in Fig. 6. Both the cooperativity of oxygen binding and the effect of protons on haemoglobin oxygen-affinity are due to dissociation/association reactions of the haemoglobin molecules. In intact erythrocytes, dissociation/association reactions can occur, since the intracellular pH can be maintained at a relatively high value by sodium/proton exchange. This is possible because the permeability of the red cell membrane to acid and base equivalents is very low.

Owing to the low bicarbonate permeability of the red cell membrane, only a minor proportion of plasma bicarbonate can be dehydrated to carbon dioxide during the residence time of blood in the gills. This potential limitation on carbon dioxide excretion is overcome, however, by the high intracellular pH and the marked oxygenation-linked pH changes, which greatly increase the effectiveness of carbon dioxide excretion by the erythrocytes.

Although both oxygen and carbon dioxide transport are as effective in lamprey blood as in that of active teleosts, there is one significant disadvantage caused by the relative

impermeability of the lamprey erythrocyte membrane to acid equivalents: the buffering of extracellular acid loads becomes extremely ineffective. As a consequence, extracellular acid loads cause marked fluctuations in lamprey plasma pH. Thus, the major advantage gained by the evolution of rapid anion exchange appears not to be related to gas transport but to extracellular buffering.

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