J. exp. Biol. (1976), 65, 449–458
With 3 figures
Printed in Great Britain

OXYGEN DISSOCIATION CURVES OF THE BLOOD OF LARVAL AND ADULT LAMPREYS (*LAMPETRA FLUVIATILIS*)

BY D. J. BIRD,* P. L. LUTZ† AND I. C. POTTER*

School of Biological Sciences, University of Bath, Claverton Down, Bath BA2 7AY

(*Received* 22 *March* 1976)

SUMMARY

1. An electrolytic method was used to plot the oxygen dissociation curves of whole blood from both the larva and adult of the lamprey *Lampetra fluviatilis* at a temperature of 10 °C and over a pH range of 6·5–8·1.

2. Larval blood has a far higher affinity for oxygen than that of adults, the respective calculated P_{50}'s at a pH of 7·75 being 1·9 and 10·7 mmHg.

3. The high affinity of larval blood is of use to a relatively sedentary animal living in burrows, and the increased oxygen delivery pressure brought about by the shift of the curve to the right in the adult is of advantage to an animal exhibiting greater activity.

4. The n value obtained from the Hill plots increased with increasing saturation and were lower in larvae than adults at the same level of blood saturation.

5. The Bohr effect in larvae at 10 °C over the pH range 6·5–8·1 was −0·25, a value which did not differ significantly from the −0·22 found in adults.

INTRODUCTION

Lampreys and hagfishes are the sole representatives of the Agnatha, the most primitive group of vertebrates. Although they are also in many ways highly specialized (Hubbs & Potter, 1971), their possession of a monomeric haemoglobin with a molecular weight of approximately 17000–18000 (Roche & Fontaine, 1938, 1940; Allison *et al.* 1960; Rumen & Love, 1963; Behlke & Scheler, 1970*a*) almost certainly represents the retention of a primitive character. Using work on the lamprey haemoglobin as part of the basis for a phylogenetic discussion of the genealogy of haemoglobin, Goodman, Moore & Matsuda (1975) have considered the selective advantages that may have been involved in the evolution of the heterotetrameric type of structure found in all other vertebrates (Wald & Riggs, 1951). It should be noted, however, that although lamprey haemoglobin is monomeric when oxygenated,

* Present address: School of Environmental and Life Sciences, Murdoch University, Murdoch, Western Australia 6153.

† Present address: School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149.

it does form homodimers and transitory tetramers in its deoxygenated state (Behlke & Scheler, 1970*a*; Anderson & Gibson, 1971).

Much of the work on the affinity of lamprey haemoglobins for oxygen has concentrated on solutions rather than on the characteristics of the whole blood (e.g. Wald & Riggs, 1951; Gibson, 1955; Briehl, 1963; Love & Rumen, 1963; Antonini *et al.* 1964; Anderson, 1970; Behlke & Scheler, 1970*a*, *b*, *c*; Dohi, Sugita & Yoneyama, 1973). By contrast, Manwell (1963) has examined both haemoglobin solutions and erythrocyte suspensions. He found that in each case the oxygen affinity was greater in the larval (= ammocoete) components than in those of the adult, a feature which he suggested possibly reflected the 'mud dwelling habits' of the larva in its river environment. There can be little doubt that the adult is more active than the larva since both in its search for food, which usually consists of teleost fishes, and during its upstream spawning run, it often travels considerable distances and sometimes also has to overcome obstacles to its migration (Hardisty & Potter, 1971*a*, *b*).

Although both the studies on the affinity of larval erythrocyte suspensions for oxygen have yielded what have been termed sigmoidal oxygen dissociation curves (Manwell, 1963; Potter, Hill & Gentleman, 1970), the curve in the adult has been described both as sigmoidal (Johansen, Lenfant & Hanson, 1973) and as approximately hyperbolic (Manwell, 1963). With respect to the curves, Manwell (1963) has stated that when the n value (the slope) in Hill plots is below 1·5 the curve is essentially hyperbolic, whereas at higher values it is sigmoidal. The position in lampreys is complicated, however, by the fact that n changes markedly with oxygenation, a feature apparently related to changes in the degree of aggregation of the haemoglobin molecules (see Johansen & Lenfant, 1972; Riggs, 1972).

This study was undertaken in order to provide oxygen dissociation curves of the blood of the larva and adult of the anadromous parasitic lamprey, *Lampetra fluviatilis* (L.). The electrolytic technique described by Longmuir & Chow (1970) was used since, compared with other techniques, it reduces the time lag between the equilibration and measurement of the blood (Lutz *et al.* 1973) and because it is particularly good at ascertaining the shape of the curve at low P_{O_2}'s (Hughes, Palacios & Palomeque, 1975). The results are related to the difference between larvae and adults in terms of their mode of life, metabolic rate and ability to survive in reduced oxygen tensions.

MATERIALS AND METHODS

Larvae of *L. fluviatilis* were collected by electric fish shocker from a site on the River Teme approximately 15 km from its junction with the River Severn. The adults of the river lamprey came from in front of the weir at Tewkesbury on the River Severn soon after they had re-entered fresh water on their upstream spawning migration. The samples were taken in the autumn and early winter at a time when the water temperatures lay between 6 and 12 °C. After the lampreys had been brought back to the laboratory, they were held for at least 3 weeks in well aerated tanks maintained at 10 ± 1 °C and exposed to a light/dark regime paralleling field conditions. Larvae were provided with a natural soft substrate in which they remained burrowed for the duration of the acclimation period.

After the lampreys had been anaesthetized in MS 222 (Sandoz), they were im-

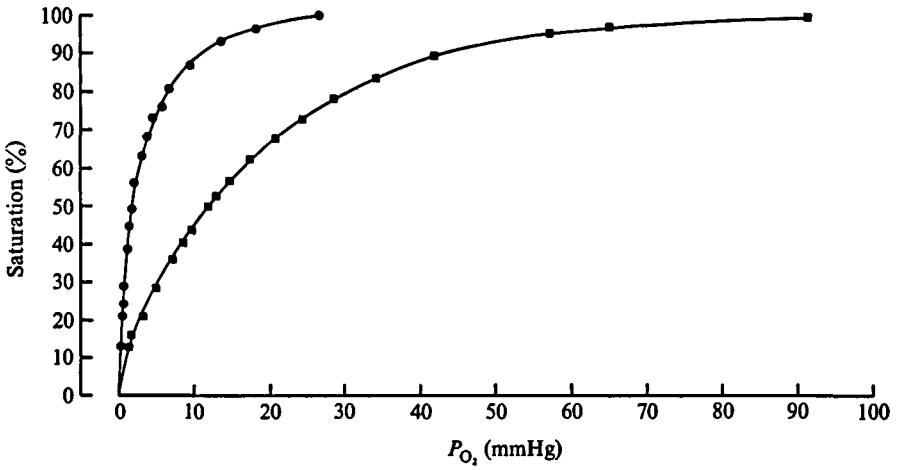

Fig. 1. Oxygen dissociation curves for the blood of a larva (●) and adult (■) of *L. fluviatilis* at a temperature of 10 °C and a pH of 7·75.

mediately cut in half just behind the cloaca. Care was taken to place the heparinized collecting syringe next to the point at which the caudal vein had been severed so that the blood was uncontaminated with other body fluids. The volume of blood used in the experiments was 0·5 ml. While this volume could always be obtained from a single adult lamprey, it was necessary in the case of larvae to pool blood from between eight and twelve separate individuals. The oxygen dissociation curves of larval and adult blood were determined by the electrolytic method described by Longmuir & Chow (1970). The apparatus contains a 30 ml temperature-controlled chamber (10 ± 0·1 °C) into which 0·1 M phosphate buffer containing 0·003 M sodium succinate is introduced before being deoxygenated by the addition of 0·5 ml of heart muscle preparation (Keilin & Hartree, 1938). The blood was then added and, after the recorder had again registered zero oxygen, the action of the heart muscle preparation was inhibited by the addition of 0·3 ml of 0·5 M oxaloacetic acid. Oxygen was generated at a constant rate by a platinum electrode and the subsequent changes in P_{O_2} followed by a Clarke oxygen electrode. The oxygen dissociation curves were plotted on a Watanabe multicorder. A total of 39 larvae and 16 adult curves over the pH range 6·5–8·1 were produced, each using blood from different individuals, or in the case of ammocoetes, groups of individuals.

RESULTS

Typical oxygen dissociation curves of larval and adult blood of *L. fluviatilis* recorded at 10 °C and at a pH of 7·75 illustrate the marked difference that exists in the oxygen affinity of the blood of the two different stages in the life cycle (Fig. 1). For example, in Fig. 1 the P_{50} for larval blood is 1·8 mmHg whereas in adults it is 11·8 mmHg. These values correspond closely to the respective values of 1·9 and 10·7 calculated from the equation given later for the relationship between pH and P_{50}. Another way of demonstrating the difference between larvae and adult is by comparing in Fig. 1 the values which correspond to the range 70 to 100% saturation.

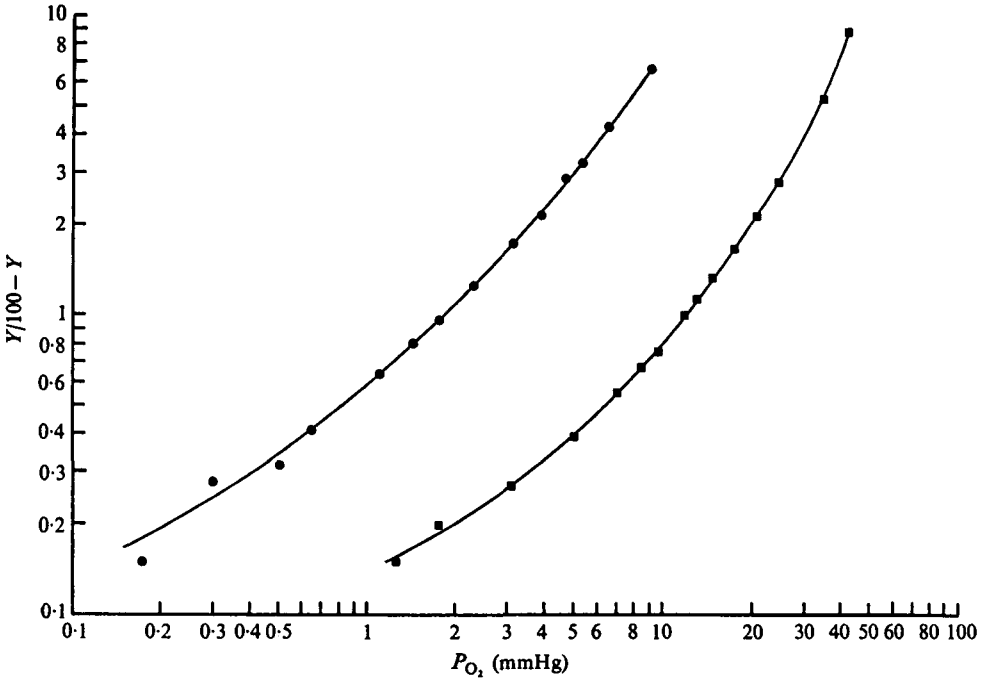

Fig. 2. Hill plots of the blood of the larva (●) and adult (■) used for Fig. 1.

In the larval blood this is represented by a P_{O_2} ranging from 4·0 to 26·4 mmHg, whereas in the adult the corresponding values are 21·5 to 91·9 mmHg. The marked affinity of larval blood for oxygen is obviously of advantage to an animal living in burrows formed in silt deposits where the water flow is slow and oxygen tensions are probably reduced (Hardisty & Potter, 1971*a*). By contrast, the adult rarely burrows and is much more active, undergoing a downstream movement after the completion of metamorphosis and later an upstream spawning migration during which it has to travel considerable distances and overcome regions of fast water and obstacles such as dams and weirs (Hardisty & Potter, 1971*b*). During the marine trophic phase which separates these two movements, the adult lamprey also has to be able to swim sufficiently rapidly to reach potential teleost hosts. Clearly, there will therefore have been strong selection pressures to shift the oxygen dissociation curve in the adult to the right to facilitate an increase in oxygen delivery pressure. In terms of metabolism, these views are also consistent with the fact that the adult lampreys have a higher metabolic rate (Hill & Potter, 1970; Potter & Rogers, 1972; Beamish, 1973; Claridge & Potter, 1975).

Hill plots of log $(y/100-y)$ vs log P_{O_2}, where y = percentage saturation of the blood and P_{O_2} = partial pressure of oxygen, yielded curved lines (Fig. 2). To provide an approximate measure of this change, the n value, i.e. the slope in these plots, was calculated over restricted saturation ranges of 30–40%, 45–55% and 75–85%, subsequently referred to as 35, 50 and 80% respectively. The values of n at each of these saturation levels were clearly seen to be independent of pH over the pH range used in this study. In larval blood, the mean (±95% confidence limits) were

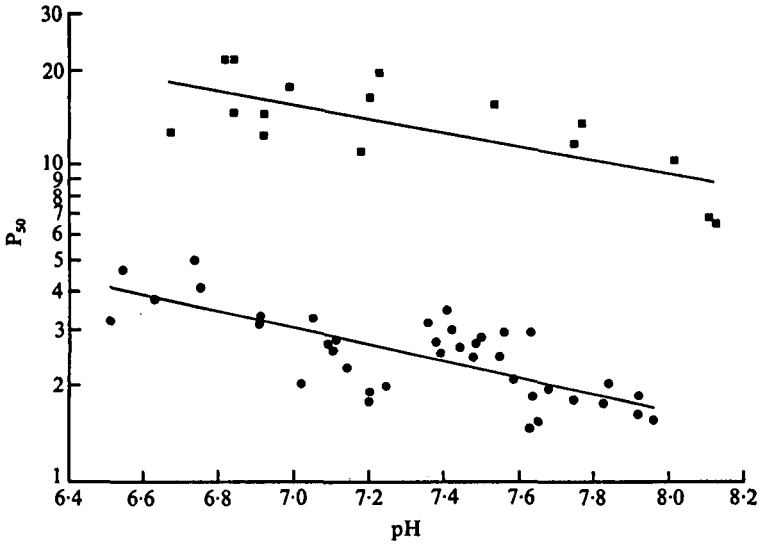

Fig. 3. The Bohr effect in larval (●) and adult (■) blood of *L. fluviatilis*.

$0{\cdot}85 \pm 0{\cdot}055$, $1{\cdot}02 \pm 0{\cdot}093$ and $1{\cdot}49 \pm 0{\cdot}093$, whereas in adult blood the respective values were $1{\cdot}00 \pm 0{\cdot}071$, $1{\cdot}21 \pm 0{\cdot}077$ and $2{\cdot}32 \pm 0{\cdot}12$. These data show that the rate of change in n with increasing saturation occurs more rapidly in adults than larvae. Since an $n > 1$ has been taken as implying some kind of subunit interaction (Riggs, 1972) and an $n < 1$ has been said to indicate negative or hindering haem–haem interactions (Manwell, 1963), it appears that some inhibition might be occurring in larval blood at low saturation levels. While, however, an $n < 1$ could mean negative cooperativity, Riggs (personal communication) has suggested that it might be better explained in terms of functional heterogeneity. As he has pointed out, in some vertebrate haemoglobins an $n < 1$ is associated with large differences between the α and β chain subunits which may behave electrophoretically as a single unit.

The Bohr effect over the pH range 6·5–8·1 was calculated as $-0{\cdot}25$ in larvae and $-0{\cdot}22$ in adults (Fig. 3). The full equations were:

$$\text{larvae} \quad \log P_{50} = 2{\cdot}2579 - 0{\cdot}2538\,\text{pH} \quad (n = 39,\ r = 0{\cdot}740),$$

$$\text{adults} \quad \log P_{50} = 2{\cdot}7007 - 0{\cdot}2158\,\text{pH} \quad (n = 16,\ r = 0{\cdot}714).$$

The relatively small Bohr shift does not conflict markedly with the findings for erythrocyte suspensions of larval *Ichthyomyzon hubbsi* in which there was a negligible effect over the pH range of 6·7–7·7 (Potter *et al.* 1970). It does differ greatly, however, from the $-0{\cdot}70$ given by Manwell (1963) for haemoglobin solutions of larval and adult *Petromyzon marinus*, a value identical to that recorded for haemoglobin solutions of adult sea lampreys by Wald & Riggs (1951). At the same time these values are higher than the $-0{\cdot}45$ given for solutions of *Ichthyomyzon unicuspis* by Manwell (1963), using exactly the same method as he had employed for *P. marinus*, and also the $-0{\cdot}41$ recorded by Johansen *et al.* (1973) for *Lampreta* (*Entosphenus*) *tridentata*. It would therefore appear that the Bohr effect in haemoglobin solutions of adult *P. marinus* may be greater than that of other species. Furthermore, since all the

values for the Bohr effect in solutions are greater than those we found in either larva or adult, the Bohr effect in solutions may be higher in haemolysates than in whole blood. In this context, Riggs (1972) has pointed out that in dilute solutions all lamprey haemoglobins exhibit a marked Bohr effect. At the same time caution must be exercised as our experimental temperature was 10 °C lower than the minimum employed in the studies on solutions. In agreement with the similarity in the Bohr effect found in larval and adult haemoglobin solutions of *P. marinus* by Manwell (1963) was the lack of a significant difference ($P > 0{\cdot}05$) between the Bohr effect in the blood of larval and adult *L. fluviatilis*.

DISCUSSION

In this study, an attempt has been made to produce oxygen dissociation curves which are as physiologically realistic as possible within the limits of the experimental regime. Particular attention was paid to acclimation and experimental temperatures, to the choice of pH, and to the provision of sufficient data to permit confidence limits to be placed on n values and correlation coefficients for the Bohr effect. Thus, the animals were acclimated at a temperature similar to the mean field temperature at the time of capture, the experiments later also being performed at this temperature. The importance of this approach was illustrated by the increased variability of oxygen dissociation curves for blood from a few animals acclimated at just over 20 °C and run at 10 °C compared with those in which both the acclimation and experimental temperatures were the same. While recognizing the quality of Manwell's (1963) excellent attempt to relate the characteristics of lamprey blood to the biology of the whole animal, it should be noted that the experimental temperature he used (25–26 °C) for the determination of oxygen dissociation curves of erythrocyte suspensions was very high and probably lies outside the range normally experienced by the species he used. Furthermore, it is only 5 °C below the ultimate incipient lethal level for larval *P. marinus* (Potter & Beamish, 1975), a value which may be even lower in the case of adults. With respect to pH, the range employed in our experiments was chosen on the basis that it covered the values we recorded for five adult *L. fluviatilis* (7·3–7·5) acclimated at 10 °C and those given for the pH of blood of larval *I. hubbsi* by Potter *et al.* (1970).

In studies carried out at three different temperatures on the behaviour and tolerance of larval *I. hubbsi* to hypoxia, the animals emerged from the substrate at oxygen tensions just above the lethal level and exhibited a greatly elevated rate of branchial pumping (Potter *et al.* 1970). The level of blood saturation that corresponds to the minimal P_{O_2} required to survive for 96 h lay in the range 51–61 %. A similar investigation of the tolerance of adult lampreys at 9·5 °C has shown that at this stage in the life cycle the animal changes its behaviour pattern at a P_{O_2} of approximately 31 mmHg, when it ceases to remain attached for long periods by means of its oral disc (Claridge & Potter, 1975). Below this level, the animal exhibits frequent vigorous swimming movements and, during the intermittent periods of rest, displays a markedly increased ventilatory frequency compared with values recorded during inactivity at higher oxygen tensions. The maximum ventilation rate is reached at 19 mmHg. At 12 mmHg, death occurred within 5–8 h. From

calculations for the oxygen dissociation curve of the blood of adults at a pH of 7·4, the mean value for adult *L. fluviatilis* blood, behaviour would have started to change at a blood saturation of approximately 77 %. The ventilatory frequency would have reached a peak at about 62 % and the saturation levels just above the 96 h lethal tensions would lie in the region of 53 %. These studies therefore suggest that there is a similar minimal level of blood saturation (*ca.* 50–60 %) in both larva and adult required to provide sufficient oxygen both for the tissues and for the costly high rate of ventilatory pumping at reduced oxygen tensions.

Because of the differences in the experimental methods used for obtaining the oxygen dissociation curves of larval *I. hubbsi* and adult *L. fluviatilis*, care must be taken in comparing the results of the two studies. It has been shown, for example, that the electrolytic method employed in this investigation tends in fishes to produce lower P_{50}'s than is the case with other techniques (Hughes *et al.* 1975). This finding is in agreement with the fact that in this investigation of larval *L. fluviatilis* the P_{50} was less than in the studies on larval *I. hubbsi*, where a spectrophotometric technique was employed. However, the correlation in both larva and adult between differing levels of saturation of the blood and oxygen tensions which induce changes in behaviour, very rapid branchial pumping and death, implies that haemoglobin is of importance at both stages in the life cycle. Such a view is consistent with the presence in the blood of a large number of erythrocytes and an appreciable amount of haemoglobin. For example, the mean haematocrits recorded for larval *I. hubbsi* and *P. marinus* were 24·7 and 28·7 respectively (Potter *et al.* 1970; Beamish & Potter, 1972), with the former of these species having a corresponding haemoglobin concentration of 7·42 g %. In the case of adults, haematocrits as high as 64 have been recorded (Korzhuev & Glazova, 1967) and means in excess of 30 have been found in populations of *L. fluviatilis* by Ivanova-Berg & Sokolova (1959) and in *P. marinus* by Beamish & Potter (1972).

The differences in the oxygen affinity of larval and adult blood are related to differences in the chemical properties of their haemoglobins, a feature illustrated by the lack of a close correspondence between the haemoglobin electropherograms of larvae and adults in species of *Lampetra* (Adinolfi, Chieffi & Siniscalco, 1959; Uthe & Tsuyuki, 1967; Potter & Brown, 1975), *Petromyzon* (Manwell, 1963; Uthe & Tsuyuki, 1967; Beamish & Potter, 1972), *Ichthyomyzon* (Manwell, 1963; Uthe & Tsuyuki, 1967) and *Mordacia* (Potter & Nicol, 1968). In the river lamprey, *L. fluviatilis*, the two major components of the adult haemoglobins have been shown to differ in the presence or absence of a blocked NH_2 terminal group, a feature which does not apparently lead to differences in their oxygen equilibria (Braunitzer, 1966; Braunitzer & Fujika, 1969; Fujika, Braunitzer & Rudloff, 1970; Riggs, 1972). Differences between the chemical properties of the haemoglobins of larval and adult river lampreys are paralleled by differences between the nuclear/cytoplasmic ratio in the erythrocytes of the two different stages (Potter, Robinson & Brown, 1974). Moreover, the location of the principal haemopoietic sites has been shown to change during the metamorphosis of the river lamprey, the site of blood cell formation no longer being the typhlosole and nephric fold but the cells that accumulate in the intercellular spaces of the fat column (Percy & Potter, 1976). It is possible, therefore, that the first two sites produce erythrocytes containing only the larval

type of haemoglobin, and the formation of blood cells with adult haemoglobins is restricted to the fat column.

The values recorded for n in larval lampreys are somewhat inconsistent. For example, Manwell (1963) has recorded an n of 1·8–1·9 in erythrocyte suspensions of *I. unicuspis* whereas an n of this magnitude was only approached at very high saturation levels in larvae of *L. fluviatilis* and *I. hubbsi*. With respect to both these latter species, a marked similarity in n at P_{50} was also found even though it was calculated from data obtained by two entirely different experimental methods. Furthermore, the increase in n in the blood of larval *L. fluviatilis* from a value of approximately 1 at P_{50} to higher values at greater levels of saturation is consistent with the findings for erythrocyte suspensions from larval *I. hubbsi* (Potter *et al.* 1970; Riggs, 1972). In both the two major haemoglobin components and in the whole haemolyzate, Antonini *et al.* (1964) have recorded an n of 1·25 for the larval brook lamprey *Lampetra planeri*. Since haemoglobin electropherograms of the larvae of this species are identical with those of *L. fluviatilis*, its presumed ancestral form (Hardisty & Potter, 1971*c*; Potter & Brown, 1975), the n values for the larvae of these two species may also be the same.

In adult stages there is no variation in the values calculated for n. Manwell (1963) has given an n of 1·2–1·4 for erythrocyte suspensions and haemoglobin solutions of *P. marinus*, values which are essentially the same as the 1·2 in *L. fluviatilis* recorded both for solutions by Antonini *et al.* (1964) and by ourselves for blood. By contrast an n of 1·5–1·7 has been reported for haemoglobin solutions of *P. marinus* by Antonini *et al.* (1964) whereas Johansen *et al.* (1973) have given a value of 1·9 for *L. tridentata*.

The fact that Antonini *et al.* (1964) obtained markedly different values for haemoglobin solutions of adults of *P. marinus* and *L. fluviatilis* at identical concentrations (4–5 mg ml^{-1}) and at the same pH shows that there are interspecific differences in this parameter. It may also be of significance that the highest n values in adults were obtained in species which attain a large size and therefore presumably live longer and travel further during this stage in the life cycle (Hardisty & Potter, 1971*b*; Hubbs & Potter, 1971). However, not all the differences can apparently be explained in terms of variation between species and it seems likely that other factors contribute to the variability. That some of the variation is the result of differences between solutions of different concentration is supported by the fact that n is very clearly related to the concentration of the haemoglobin (Riggs, 1972; Dohi *et al.* 1973). Another variable is experimental temperature, our studies for example being carried out at values 10–15 °C lower than those of most other workers. In view of the change in n with saturation, it is possible that the range over which n has been calculated might also have differed slightly and have thus contributed to the variation.

Despite differences between the pattern observed in Hill plots for the blood of lampreys and most gnathostomes, there are a number of parallels that can be drawn between ontogenetic and physiological aspects of the haemoglobins of these two vertebrate groups. Thus, the change in the chemical properties of the haemoglobins occurring in lampreys during the transition from larvae to adult is similar to the change that takes place in mammals at about the time of birth and in amphibians

during their metamorphosis (Maclean & Jurd, 1972). The great affinity for oxygen of the haemoglobins of the larva is also present in the blood of teleosts living in a poorly oxygenated environment and a shift of the oxygen dissociation curve to the right, as seen in the adult lamprey, is also observed in teleosts living in well aerated water (Krogh & Leitch, 1919; Johansen & Lenfant, 1972). The displacement of the curve to the right is also apparently related to increased oxygen consumption, a feature paralleling the situation within groups of vertebrates exhibiting different metabolic rates (Dejours, 1975).

Financial assistance was provided by the Science Research Council. Our gratitude is expressed to Professor A. Riggs for his helpful criticism of this paper.

REFERENCES

ADINOLFI, M., CHIEFFI, G. & SINISCALCO, M. (1959). Haemoglobin pattern of the cyclostome *Petromyzon planeri* during the course of development. *Nature, Lond.* **184**, 1235–6.

ALLISON, A. C., CECIL, R., CHARLWOOD, P. A., GRATZER, W. B., JACOBS, S. & SNOW, N. S. (1960). Hemoglobin of the lamprey, *Lampetra fluviatilis*. *Biochim. biophys. Acta* **42**, 43–8.

ANDERSEN, M. E. (1970). Ligand binding reactions of lamprey haemoglobin. *Fedn Proc. Fedn Am Socs exp. Biol.* **29**, 855 Abs.

ANDERSEN, M. E. & GIBSON, Q. H. (1971). A kinetic analysis of the binding of oxygen and carbon monoxide to lamprey hemoglobin. *J. biol. Chem.* **246**, 4790–9.

ANTONINI, E., WYMAN, J., BELLELLI, L., RUMEN, N. & SINISCALCO, M. (1964). The oxygen equilibrium of some lamprey haemoglobins. *Archs Biochem. Biophys.* **105**, 404–8.

BEAMISH, F. W. H. (1973). Oxygen consumption of adult *Petromyzon marinus* in relation to body weight and temperature. *J. Fish. Res. Bd Can.* **30**, 1367–70.

BEAMISH, F. W. H. & POTTER, I. C. (1972). Timing of changes in the blood, morphology and behaviour of *Petromyzon marinus* during metamorphosis. *J. Fish. Res. Bd Can.* **29**, 1277–82.

BEHLKE, J. & SCHELER, W. (1970*a*). Der Einfluss von Liganden auf den Assoziationsgrad des Desoxy-Hämoglobins der Flussneunaugen (*Lampetra fluviatilis* L.). *FEBS Lett.* **7**, 177–9.

BEHLKE, J. & SCHELER, W. (1970*b*). Ligandeninduzierte konformationsstudien am Neunaugen-Methb. *Studia biophys, Berlin* **24**, 61–8.

BEHLKE, J. & SCHELER, W. (1970*c*). Wirkung von Liganden auf des Assoziations-Dissoziations-Gleichgewicht des Methämoglobins der Flussneunaugen (*Lampetra fluviatilis* L.). *Eur. J. Biochem.* **15**, 245–9.

BRAUNITZER, G. (1966). Phylogenetic variation in the primary structure of haemoglobins. *J. cell comp. Physiol.* **67**, Supp. 1, 1–20.

BRAUNITZER, G. & FUJIKI, H. (1969). Zur evolution der Vertebraten. Die Konstitution und Tertiarstruktur des Hämoglobins des Flussneunauges. *Naturwissenschaften* **56**, 322–3.

BRIEHL, R. W. (1963). The relation between the oxygen equilibrium and aggregation of subunits in lamprey hemoglobin. *J. biol. Chem.* **238**, 2361–6.

CLARIDGE, P. N. & POTTER, I. C. (1975). Oxygen consumption, ventilatory frequency and heart rate of lampreys (*Lampetra fluviatilis*) during their spawning run. *J. exp. Biol.* **63**, 193–206.

DEJOURS, P. (1975). *Principles of Comparative Respiratory Physiology*. Amsterdam: North Holland.

DOHI, Y., SUGITA, Y. & YONEYAMA, Y. (1973). The self-association and oxygen equilibrium of hemoglobin from the lamprey, *Entosphenus japonicus*. *J. biol. Chem.* **248**, 2354–63.

FUJIKI, H., BRAUNITZER, G. & RUDLOFF, V. (1970). N-formylproline as N-terminal amino acid of lamprey haemoglobin. *Hoppe-Seyler's Z. physiol. Chem.* **351**, 901.

GIBSON, Q. H. (1955). Reactions of oxygen and carbon monoxide with the haemoglobin of the lamprey. *J. Physiol. Lond.* **128**, 70.

GOODMAN, M., MOORE, G. W. & MATSUDA, G. (1975). Darwinian evolution in the geneaology of haemoglobin. *Nature, Lond.* **253**, 603–8.

HARDISTY, M. W. & POTTER, I. C. (1971*a*). The behaviour, ecology and growth of larval lampreys. In *The Biology of Lampreys*, vol. 1. (ed. M. W. Hardisty and I. C. Potter), pp. 85–125. London: Academic Press.

HARDISTY, M. W. & POTTER, I. C. (1971*b*). The general biology of adult lampreys. In *The Biology of Lampreys*, vol. 1 (ed. M. W. Hardisty and I. C. Potter), pp. 127–206. London: Academic Press.

HARDISTY, M. W. & POTTER, I. C. (1971*c*). In *The Biology of Lampreys*, vol. 1 (ed. M. W. Hardisty and I. C. Potter), pp. 249–77. London: Academic Press.

HILL, B. J. & POTTER, I. C. (1970). Oxygen consumption in ammocoetes of the lamprey *Ichthyomyzon hubbsi* Raney. *J. exp. Biol.* **53**, 47–57.

HUBBS, C. L. & POTTER, I. C. (1971). Distribution, phylogeny and taxonomy. In *The Biology of Lampreys*, vol. 1 (ed. M. W. Hardisty and I. C. Potter), pp. 1–65. London: Academic Press.

HUGHES, G. M., PALACIOS, L. & PALOMEQUE, J. (1975). A comparison of some methods for determining oxygen dissociation curves of fish blood. *Revta esp. Fisiol.* **31**, 83–90.

IVANOVA-BERG, M. M. & SOKOLOVA, M. M. (1959). Seasonal changes in the blood composition of the River Lamprey (*Lampetra fluviatilis* L.). *Vop. Ikhtiol.* **13**, 156–162.

JOHANSEN, K. & LENFANT, C. (1972). A comparative approach to the adaptability of O_2-Hb affinity. In *Oxygen Affinity of Haemoglobin and Red Cell Acid Base Status* (ed. M. Rørth and P. Astrup), pp. 750–83. Copenhagen: Munksgaard.

JOHANSEN, K., LENFANT, C. & HANSON, D. (1973). Gas exchange in the lamprey, *Entophenus tridentatus*. *Comp. Biochem Physiol.* **44**A, 107–19.

KEILIN, O. & HARTREE, E. F. (1938). Cytochrome oxidase. *Proc. R. Soc.* B **125**, 171–86.

KORZHUEV, P. A. & GLAZOVA, T. N. (1967). Ecological and physiological adaptations of the blood in Cyclostoma. *Dokl. Akad. Nauk. SSSR* **172**, 236–8.

KROGH, A. & LEITCH, I. (1919). The respiratory function of the blood in fishes. *J. Physiol., Lond.* **52**, 288–300.

LONGMUIR, I. S. & CHOW, J. (1970). Rapid method for determining effect of agents on oxyhemoglobin dissociation curves. *J. appl. Physiol.* **28**, 343–5.

LOVE, W. & RUMEN, N. (1963). Heme–heme interaction in lamprey haemoglobin – an explanation. *Biol. Bull. mar. biol. Lab., Woods Hole* **125**, 353–8.

LUTZ, P. L., LONGMUIR, I. S., TUTTLE, J. V. & SCHMIDT-NIELSON, K. (1973). Dissociation curve of bird blood and effect of red cell oxygen consumption. *Respir. Physiol.* **17**, 269–75.

MACLEAN, N. & JURD, R. D. (1972). The control of haemoglobin synthesis. *Biol. Rev.* **47**, 393–437.

MANWELL, C. (1963). The blood proteins of Cyclostomes: a study in phylogenetic and ontogenetic biochemistry. In *The Biology of Myxine* (ed. A. Brodal and R. Fänge), pp. 372–455. Oslo: Universitetsforlaget.

PEDERSEN, K. O. (1940). Proteins. In *The Ultracentrifuge* (ed. T. Svedberg and K. O. Pedersen), pp. 355–415. Oxford: Clarendon Press.

PERCY, R. C. & POTTER, I. C. (1976). Blood cell formation in the River lamprey, *Lampetra fluviatilis*. *J. Zool., Lond.* **178**, 319–40.

POTTER, I. C. & BEAMISH, F. W. H. (1975). Lethal temperatures in ammocoetes of four species of lampreys. *Acta zool., Stockh.* **56**, 85–91.

POTTER, I. C. & BROWN, I. D. (1975). Changes in haemoglobin electropherograms during the life cycle of two closely related lampreys. *Comp. Biochem. Physiol.* **51**B, 517–19.

POTTER, I. C., HILL, B. J. & GENTLEMAN, S. (1970). Survival and behaviour of ammocoetes at low oxygen tensions. *J. exp. Biol.* **53**, 59–73.

POTTER, I. C. & NICOL, P. I. (1968). Electrophoretic studies on haemoglobins of Australian lampreys. *Aust. J. exp. Biol. med. Sci.* **46**, 639–41.

POTTER, I. C. & ROGERS, M. J. (1972). Oxygen consumption in burrowed and unburrowed ammocoetes of *Lampetra planeri*. *Comp. Biochem. Physiol.* **41**A, 427–32.

POTTER, I. C., ROBINSON, E. S. & BROWN, I. D. (1974). Studies on the erythrocytes of larval and adult lampreys (*Lampetra fluviatilis*). *Acta zool., Stockh.* **55**, 173–7.

RIGGS, A. (1972). The haemoglobins. In *The Biology of Lampreys*, vol. 2 (ed. M. W. Hardisty and I. C. Potter), pp. 261–86. London: Academic Press.

ROCHE, J. & FONTAINE, M. (1938). Sur le pigment respiratoire de la lamproie marine (*Petromyzon marinus* L.) et sur la répartition zoologique des pigments respiratoires protohématiniques (hémoglobins et érythrocruorines). *C. r. hebd. Seanc. Acad. Sci., Paris* **206**, 626–8.

ROCHE, J. & FONTAINE, M. (1940). Le pigment respiratoire de la lamproie marine (*Petromyzon marinus* L.) et la répartition zoologique des erythrocruorines et des hémoglobines. Contribution à l'etude biochemique des Cyclostomes. *Annals Inst. océanogr., Monaco* **20**, 77–86.

RUMEN, N. M. & LOVE, W. E. (1963). The six haemoglobins of the sea lamprey (*Petromyzon marinus*). *Archs Biochem. Biophys.* **103**, 24–35.

UTHE, J. F. & TSUYUKI, H. (1967). Comparative zone electropherograms and muscle myogens and blood proteins of adult and ammocoete lamprey. *J. Fish. Res. Bd Can.* **24**, 1269–73.

WALD, G. & RIGGS, A. (1951). The hemoglobin of the sea lamprey. *J. gen. Physiol.* **35**, 45–53.