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THE EFFECT OF TEMPERATURE ON THE OXYGEN DISSOCIATION CURVES OF WHOLE BLOOD OF LARVAL AND ADULT LAMPREYS (*GEOTRIA AUSTRALIS*)

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SUMMARY

1. Oxygen dissociation curves of the whole blood of larvae and adults of the Southern Hemisphere lamprey *Geotria australis* have been determined between pH 6·8 and 8·2 at 5, 15 and 25 °C.

2. The P_{50}'s at temperatures of 5, 15 and 25 °C and a pH of 7·75 were respectively 0·57, 0·92 and 1·19 mmHg in larvae and 6·9, 10·3 and 19·0 mmHg in adults.

3. The relatively very high affinity of larval blood for oxygen may reflect an adaptation to low environmental oxygen tensions.

4. The Bohr shift was not significantly affected by either temperature or life-cycle stage.

5. The slope (n) in Hill plots increased with temperature and oxygen saturation, and was greater in adults than in larvae.

INTRODUCTION

The lampreys and hagfishes (Cyclostomes) are the sole survivors of the agnathan stage in vertebrate evolution (Bardack & Zangerl, 1971). Both groups contain, however, many highly specialized features, such as those associated with their feeding mechanisms (Hubbs & Potter, 1971). Yet there is also little doubt that the lampreys and hagfishes have retained some primitive features. In this context, it is almost certainly of significance that their haemoglobins differ from those of all other vertebrates. Thus, while the haemoglobins of all gnathostomes are tetrameric (Perutz, 1969; Lehninger, 1975), those of the hagfishes are predominantly monomeric in the oxygenated state (Bannai, Sugita & Yoneyama, 1972; Bauer, Engels & Paleus, 1975), as they also are to a large degree in dilute solutions and at neutral pH in the case of lampreys (Riggs, 1972). Under various conditions, the haemoglobins of lampreys aggregate in solutions to form dimers and tetramers (Riggs, 1972). Although Riggs postulated in 1972 that 'it is very doubtful that lamprey haemoglobin ever dissociates to any significant extent at the high protein concentrations which must exist in the red cell', he now believes that this view may be incorrect (A. Riggs, pers. comm.).

Most of the studies of oxygen dissociation curves of lampreys have been performed

on haemoglobin solutions (e.g. Wald & Riggs, 1951; Gibson, 1955; Briehl, 1963; Love & Rumen, 1963; Antonini *et al.* 1964; Behlke & Scheler, 1970*a–c*; Dohi, Sugita & Yoneyama, 1973; Johansen, Lenfant & Hanson, 1973). While Manwell (1963) and Potter, Hill & Gentleman (1970) provided data on the oxygen dissociation curves of erythrocyte suspensions, the only comparable study on the whole blood of lampreys was the work of Bird, Lutz & Potter (1976) on *Lampetra fluviatilis*. Although the latter study provided data on blood from both the larvae and adults, the experiments were restricted to a single temperature.

The current study was undertaken to acclimate animals at three very different physiologically realistic temperatures and then record at the same respective temperatures the oxygen dissociation curves and the Bohr effect produced by the unique lamprey haemoglobins in whole blood. The investigation, which was performed on the Southern Hemisphere lamprey *Geotria australis* (Gray), also concentrated on evaluating the differences between the curves of both the larval and adult stages of this species. The data were compared with those recorded at a single temperature for ammocoetes and adults of the Northern Hemisphere lamprey *L. fluviatilis* (Bird *et al.* 1976). Such comparisons were considered particularly relevant in view of the unusually high haemotocrits found in larval *G. australis*, a feature that may have evolved as an adaptation for life in an oxygen-depleted environment (Macey & Potter, 1981).

MATERIALS AND METHODS

The ammocoetes of *Geotria australis* were obtained using an electric fish shocker in tributaries of the Donnelly and Warren rivers in south-western Australia (lat. 34° S, long. 116° E). The adults of this species were collected early in their spawning run using a dip net to remove them from in front of a weir which was impeding their migration upstream. The animals were taken to the laboratory, where they were held for at least 2 days under temperature and light conditions paralleling those existing in the field at the time of capture. Separate groups of animals were acclimated to each of the temperatures at which the subsequent blood experiments were to be performed by either raising or lowering the temperature by 1 °C every 2 days. They were held for 2–3 weeks at each of the three experimental temperatures (5, 15 and 25 °C) before they were used to provide blood. The larvae were always maintained in laboratory aquaria supplied with food and a natural silt substrate into which they would readily burrow. The adults, which do not feed during their upstream migration, were kept in large tanks containing large stones and other structures to provide cover and a water supply that was well aerated and filtered.

All animals were anaesthetized in MS 222 (Calbiochem). Blood was extracted from the caudal vessels of larvae (Bird *et al.* 1976; Macey & Potter, 1981) and by cardiac puncture from adults (Macey & Potter, 1981). In order to obtain sufficient blood for each oxygen dissociation curve in the ammocoete, it was necessary to pool the blood from approximately 15–20 animals to obtain the 0·25 ml required.

Oxygen dissociation curves were determined using the 'electrolytic' method of Longmuir & Chow (1970) in the manner described in the study of the blood of *Lampetra fluviatilis* (Bird *et al.* 1976). The blood was maintained within the apparatu

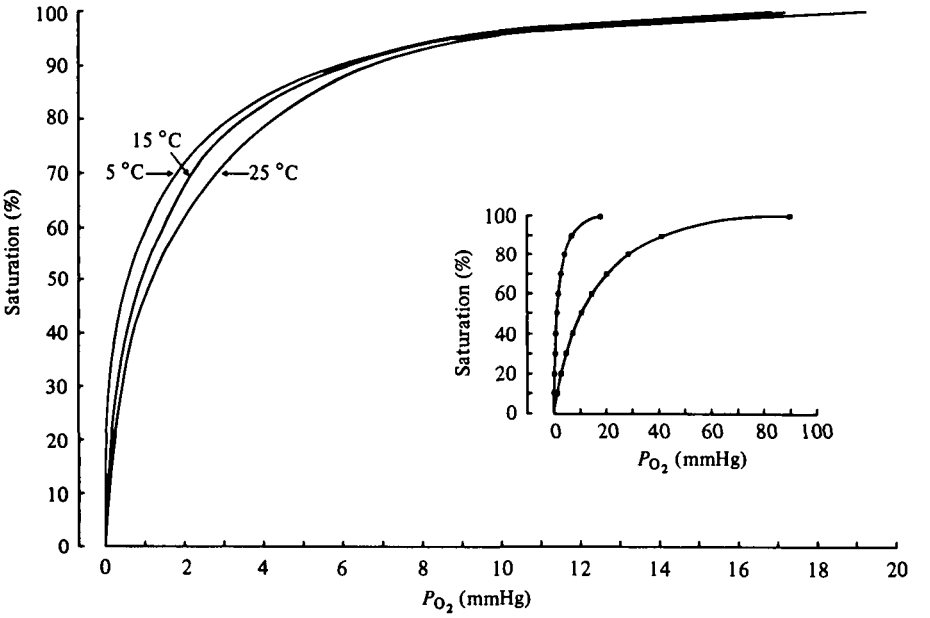

Fig. 1. Oxygen dissociation curves for the blood of larval *Geotria australis* at a pH of 7·75 and temperatures of 5, 15 and 25 °C. Inset: a comparison of larval (●) and adult blood (■) at a pH of 7·75 and 15 °C.

at the temperature to which the animals had been acclimated, i.e. 5, 15 or 25 °C. The relationship between the P_{50} and pH at each of the experimental temperatures was expressed in the form of regression equations for the blood of both larval and adult lampreys. The slope in these equations corresponds to the Bohr effect (Table 1, Fig. 3). Regression equations were also calculated in the same manner as those given in Table 1 at successive 10% intervals in saturation. This enabled the P_{10}, P_{20}, P_{30}, etc. at each experimental temperature to be calculated and thus provide composite oxygen dissociation curves for larval (Fig. 1) and adult blood (Fig. 2) at a pH of 7·75. This latter pH was chosen to enable interspecific comparisons to be made between the composite curves of *G. australis* and the single curves of larval and adult blood of *L. fluviatilis* recorded at this pH by Bird *et al.* (1976). The total number of curves plotted for larval and adult *G. australis* at the three temperatures and within the pH range 6·8–8·2 are given in Table 1.

The Hill plots given by $\log (Y/100-Y)$ *v.* $\log P_{O_2}$, where Y = percentage saturation of the blood and P_{O_2} = partial pressure of oxygen, yielded curved lines (Fig. 4). The lines were found to be best represented by a cubic equation, from which the slope (n) at 35, 50 and 80% levels of oxygen saturation were then calculated (Table 2).

RESULTS AND DISCUSSION

Values for the P_{50} of larval blood of *Geotria australis* at a pH of 7·75 and temperatures of 5, 15 and 25 °C were 0·57, 0·92 and 1·19 mmHg, which demonstrates that the oxygen affinity decreases with an increase in temperature (Fig. 1). All the

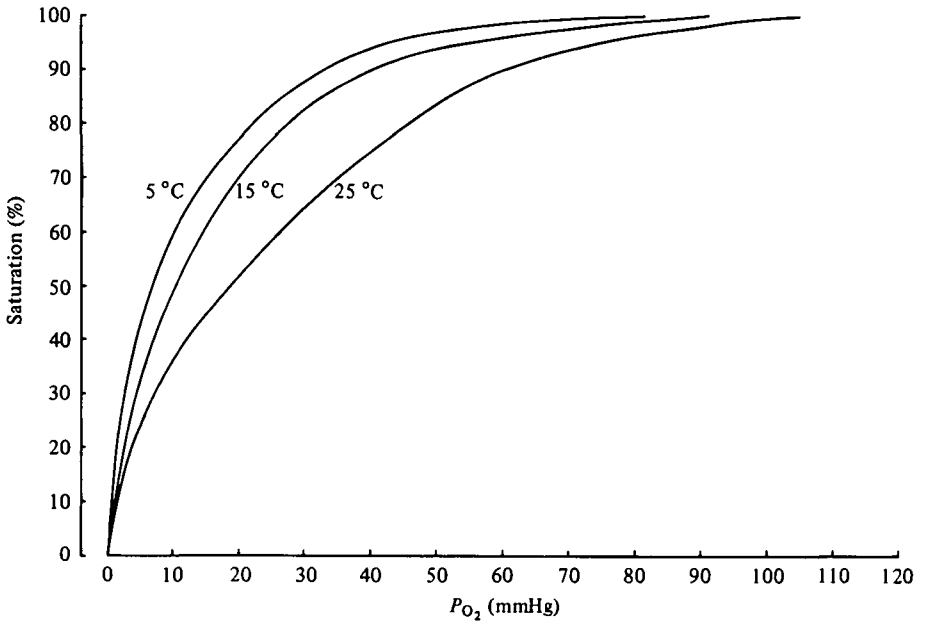

Fig. 2. Oxygen dissociation curves for the blood of adult *Geotria australis* at a pH of 7·75 and temperatures of 5, 15 and 25 °C.

above values are considerably lower than the P_{50} of 1·95 mmHg recorded at 10 °C and the same pH for the blood of larval *L. fluviatilis* (Bird *et al.* 1976). Comparisons between the above values for *G. australis* at 5 and 15° C and that of *L. fluviatilis* at 10 °C suggest that at 10 °C and a pH of 7·75 the oxygen affinity of the blood of ammocoetes of the Southern Hemisphere lamprey is approximately 2½ times greater than that of the holarctic species. It is also evident from the Bohr effect graph that this statement is true over a range of pH values (Fig. 3). Moreover, a comparison of the larval oxygen dissociation curves for *G. australis* at 5 and 15 °C with that given by Bird *et al.* (1976) for *L. fluviatilis* at 10 °C provide every indication that the oxygen affinity is much greater in the Southern Hemisphere lamprey over a wide range of P_{O_2} values.

The exceptionally high oxygen affinity of blood from larval *G. australis* parallels the situation found with the haematocrit and haemoglobin concentration. For example, a haematocrit of 41·5% and a haemoglobin concentration of 11·1 g 100 ml^{-1} in ammocoetes of *G. australis* (Macey & Potter, 1981) may be compared with haematocrits of 22–29% and haemoglobin concentrations of 5·6–7·4 g 100 ml^{-1} in various other species of lampreys (Potter, Hill & Gentleman, 1970; Potter & Beamish, 1978; Macey & Potter, 1981). A high affinity of the blood for oxygen, allied with a very high haemoglobin concentration, which increases the oxygen capacity, are features that would be of advantage to an animal living in an oxygen-depleted environment. Such a situation may well exist in the ammocoete habitats found in the rivers of south-western Australia where flow virtually ceases during the summer to produce on occasions isolated pools in which ammocoetes are sometimes found.

Values for the P_{50} of adult blood of *Geotria australis* at a pH of 7·75 and te

peratures of 5, 15 and 25 °C were 6·9, 10·3 and 19·0 mmHg (Fig. 2). The P_{50} of 10·7 mmHg recorded at the same pH for the blood of adult *L. fluviatilis* at 10 °C (Bird *et al.* 1976) is thus much more comparable to that of *G. australis* at 15 than 5 °C. That a similar situation is present over a wide range of pH values is borne out by the data in Fig. 3 showing the Bohr effect in adult *L. fluviatilis* and adult *G. australis*. Since the data on the blood of adult *G. australis* shows that the curve shifts to the right with increasing temperature (Fig. 2), the oxygen affinity of the blood of adult *G. australis* is greater than that of adult *L. fluviatilis*, but the differences are much less pronounced than in the ammocoetes of these two species.

The oxygen dissociation curve of the blood of the larvae at a given pH lay well to the left of that of the adult at each of the three experimental temperatures (cf. Figs. 1 and 2). While oxygen dissociation curves can be shifted to the left by exposure of the animal to experimentally induced hypoxia (see Wood, 1980), it should be emphasized that our larvae were maintained in burrowed conditions approximating those normally found in the field. Moreover the difference in the oxygen affinity, which reflects a change from larval to adult haemoglobins (Potter & Nicol, 1968), is almost certainly related to differences in the environment and the modes of life of the two life-cycle stages. Thus, the ammocoete is a relatively sedentary burrowing microphagous animal living in the soft deposits of rivers (Potter, 1980), whereas the adult is more active, attacking marine teleosts and undertaking long migrations (Hardisty & Potter, 1971). The high oxygen affinity of larval blood can be thus considered as an adaptation to enable ammocoetes to obtain oxygen from a surrounding environment which may be rather depleted in oxygen. By contrast, the much lower oxygen affinity of adult blood may be an adaptation to the higher environmental oxygen tensions that are found in the open waters of the sea and the faster-flowing regions of river systems frequented by the adults of anadromous parasitic species. It also has the effect of producing a greater oxygen delivery pressure which would be of particular advantage during the upstream migration when the animal has to overcome the effects of downstream flow and any natural or artificial barriers.

The shift to the right of the oxygen dissociation curve of both larval and adult blood with increasing temperature parallels the situation found in many other vertebrates, including fishes (see, for example, Grigg, 1969). This shift represents a weakening of the bond between haemoglobin and oxygen, with the result that oxygen is given up more readily. Such a feature would be of physiological significance to lampreys since, as with poikilotherms in general (Schmidt-Nielsen, 1979), the metabolic rate increases with a rise in temperature (Hill & Potter, 1970; Johansen, Lenfent & Hanson, 1973; Claridge & Potter, 1975). In other words, it is of advantage to lampreys to have a more rapid delivery of oxygen to the tissues at higher temperatures.

In the context of the change in position of the oxygen dissociation curve with life-cycle stage and temperature, it is relevant to calculate the heat of combination of oxygen with haemoglobin (ΔH) between 5 and 15 °C and 15 and 25 °C. The respective values were $-34{\cdot}41$ and $-18{\cdot}37$ kJ mole^{-1} in the larvae and $-27{\cdot}26$ and $-42{\cdot}32$ kJ mole^{-1} in the adult. Thus, in contrast to the situation in adult blood, ΔH in ammocoetes is therefore lower between 15 and 25 °C than between 5 and 15 °C. This implies that, while in general a shift to the right with increasing temperature

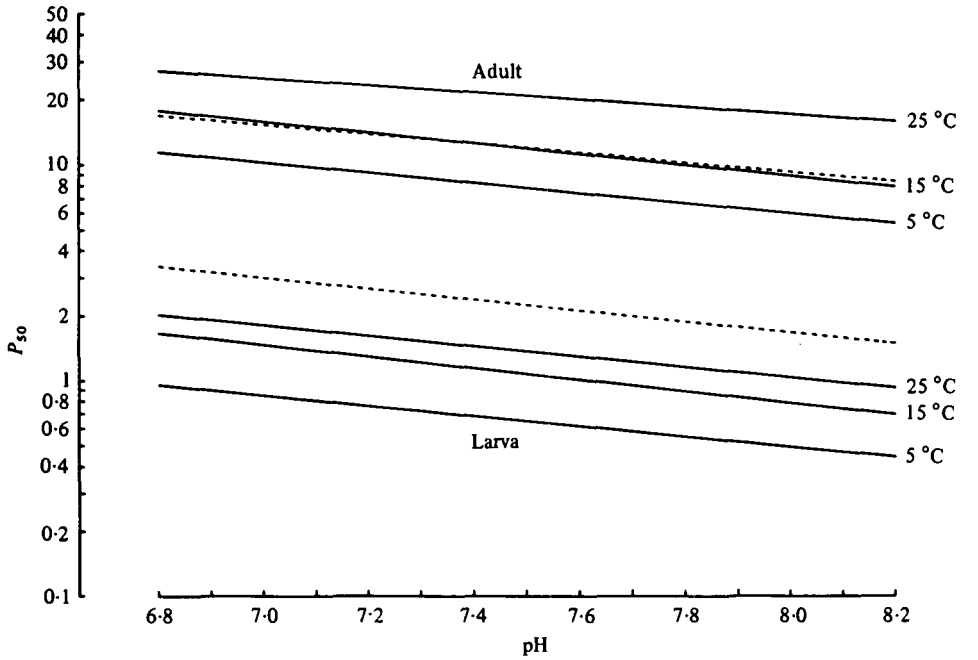

Fig. 3. The Bohr effect in larval and adult blood of *Geotria australis* at 5, 15 and 25 °C. The Bohr effect for larval and adult blood of *Lampetra fluviatilis* at 10 °C is represented by dashed lines.

Table 1. *The Bohr effect equations for the blood of larval and adult* Geotria australis *at temperatures of* 5, 15 *and* 25 °C *and over the pH range of* 6·8 *to* 8·2

Stage	°C	Bohr effect equations
Larva	5	Log P_{50} = 1·6117 − 0·2396 pH (n = 18, r = 0·6377)
	15	Log P_{50} = 2·0407 − 0·2681 pH (n = 31, r = 0·7089)
	25	Log P_{50} = 1·9090 − 0·2364 pH (n = 16, r = 0·6004)
Adult	5	Log P_{50} = 2·6338 − 0·2319 pH (n = 17, r = 0·7861)
	15	Log P_{50} = 2·9532 − 0·2502 pH (n = 29, r = 0·6542)
	25	Log P_{50} = 2·5571 − 0·1649 pH (n = 13, r = 0·7600)

is usually of value to poikilotherms, it may be advantageous at higher temperatures to restrict the degree to which this shift occurs in burrowed ammocoetes which may be extracting oxygen from an environment depleted in oxygen.

The Bohr effect, calculated over the pH range 6·8–8·2, did not differ significantly ($P > 0·05$) between the blood of ammocoetes at each of the three temperatures (Fig. 3, Table 1). The respective values for ammocoetes at 5, 15 and 25 °C were −0·24, −0·27 and −0·24. While the Bohr effect in adults changed from −0·23 and −0·25 at 5 and 15 °C respectively to −0·16 at 25 °C (Fig. 3, Table 1), these values also did not differ significantly ($P > 0·05$). The Bohr effect recorded by Bird *et al.* (1976) for larval (−0·25) and adult blood (−0·22) of *L. fluviatilis* at 10 °C did not differ significantly ($P > 0·05$) from that of the comparable life-cycle stage of *G. australis* measured at 5, 15 and 25 °C (Fig. 3).

A comparison by Schmidt-Nielsen (1979) of the Bohr shift in a wide range

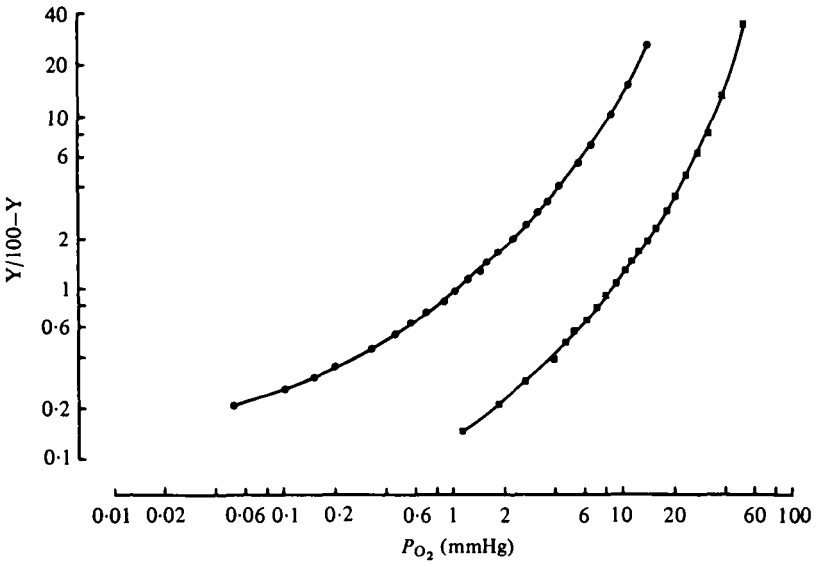

Fig. 4. Hill plots calculated from single oxygen dissociation curves for larval (●) and adult (■) blood of *Geotria australis* at a pH of 7·75 and 15 °C.

mammals showed that this effect exhibited a pronounced tendency to decrease in magnitude with increasing body weight. Despite the fact that the adults of *L. fluviatilis* and *G. australis* attain weights which are respectively about 30 and 250 times greater than those of their larvae, the above data show that the Bohr effect in the blood of the two life-cycle stages is very similar in both species. This similarity is also striking in view of differences in the degree of activity of ammocoetes and adults and in the oxygenation properties of their respective haemoglobins.

The data now collected for lamprey blood indicate that, at least in the larvae and adults of *G. australis* and *L. fluviatilis*, the Bohr effect is less than −0·30. The values are therefore markedly different from the following Bohr effects found with haemoglobin solutions in other species: −0·70 in larval and adult *Petromyzon marinus* (Wald & Riggs, 1951; Manwell, 1963), −0·45 for adult *Ichthyomyzon unicuspis* (Manwell, 1963) and −0·41 for adult *Lampetra tridentata* (Johansen *et al.* 1973).

The curvilinear nature of the Hill plots obtained for both the major life-cycle stages of *G. australis* is illustrated by the data shown for larval and adult blood at a pH of 7·75 and a temperature of 15 °C (Fig. 4). Determination of the slope (n) in the Hill plots made at 35, 50 and 80% oxygen saturation from oxygen dissociation curves representing a range of pH values showed that at these three saturations the n values were independent of pH in both larval and adult blood at 5, 15 and 25 °C. In blood from both ammocoetes and early upstream migrants, n increased with the degree of oxygen saturation (Table 2), paralleling the situation found in the blood of *L. fluviatilis* (Bird *et al.* 1976). For example, at 15 °C, the respective values for 35, 50 and 80% saturation were 0·65, 0·86 and 1·37 in larval blood and 0·90, 1·22 and 1·97 in adult blood. Furthermore, n increased with temperature. Thus, the respective values at 50% saturation and temperatures of 5, 15 and 25 °C were 0·63, 0·86 and

Table 2. *The mean values* ± 1 *standard error for the slopes* (*n*) *in Hill plots for the blood of larval and adult* Geotria australis *at three oxygen saturations* (35, 50 *and* 80%) *and three temperatures* (5, 15 *and* 25 °C)

Stage	°C	Saturation (%)	Mean	± 1 S.E.	*n*
Larva	5	35	0·4445	0·0307	18
		50	0·6286	0·0411	18
		80	1·2053	0·0334	18
Larva	15	35	0·6456	0·0311	31
		50	0·8601	0·0273	31
		80	1·3677	0·0387	31
	25	35	0·7138	0·0647	16
		50	0·9896	0·0607	16
		80	1·5842	0·0710	16
Adult	5	35	0·8506	0·0393	17
		50	1·0759	0·0282	17
		80	1·6079	0·0380	17
	15	35	0·9005	0·0409	29
		50	1·2248	0·0346	29
		80	1·9708	0·0615	29
	25	35	0·8196	0·0417	13
		50	1·3639	0·0431	13
		80	2·4564	0·1047	13

0·99 in larval blood and 1·07, 1·22 and 1·36 in adult blood. As in *L. fluviatilis* (Bird *et al.* 1976), the values at a given saturation and temperature were always lower in larvae than in upstream migrants. For example, at 80% and 25 °C, the *n* value was 1·58 in ammocoetes and 2·46 in adults.

It is evident from this study and from that of Bird *et al.* (1976) that, in *G. australis* at all temperatures and *L. fluviatilis* at 10 °C, values for *n* of less than 1·0 are typically found in larval blood at oxygen saturations below 50% and the same generalization applies to adult blood below 35%. While values of *n* less than 1·0 could indicate negative or hindering haem–haem interactions (Manwell, 1963), A. Riggs (pers. comm.) has suggested that it could be the result of functional heterogeneity amongst the haemoglobins.

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REFERENCES

ANTONINI, E., WYMAN, J., BELLELLI, L., RUMEN, N. & SINISCALCO, M. (1964). The oxygen equilibrium of some lamprey hemoglobins. *Archs Biochem. Biophys.* **105**, 404–408.

BANNAI, S., SUGITA, Y. & YONEYAMA, Y. (1972). Studies on haemoglobin from the hagfish *Eptatretus burgeri*. *J. biol. Chem.* **247**, 505–510.

BARDACK, D. & ZANGERL, R. (1971). Lampreys in the fossil record. In *The Biology of Lampreys*, vol. 1 (ed. M. W. Hardisty and I. C. Potter), pp. 67–84. London: Academic Press.

BAUER, C., ENGELS, U. & PALEUS, S. (1975). Oxygen binding to haemoglobins of the primitive vertebrate *Myxine glutinosa* L. *Nature, Lond.* **256**, 66–68.

BEHLKE, J. & SCHELER, W. (1970*a*). Der Einfluss von Liganden auf den Assoziationsgrad des Desoxy-Hämoglobins der Flussneunaugen (*Lampetra fluviatilis* L.). *FEBS Lett.* **7**, 177–179.

BEHLKE, J. & SCHELER, W. (1970*b*). Ligandeninduzierte konformationsstudien am Neunaugen-Methb. *Studia Biophys, Berlin* **24**, 61–68.

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**, 245–249.

BIRD, D. J., LUTZ, P. L. & POTTER, I. C. (1976). Oxygen dissociation curves of the blood of larval and adult lampreys (*Lampetra fluviatilis*). *J. exp. Biol.* **65**, 449–458.

BRIEHL, R. W. (1963). The relation between the oxygen equilibrium and aggregation of subunits in lamprey haemoglobin. *J. biol. Chem.* **238**, 2361–2366.

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.

DOHI, Y., SUGITA, Y. & YONEYAMA, Y. (1973). The self-association and oxygen equilibrium of hemoglobin from the lamprey, *Entosphenus japonicus*. *J. biol. Chem.* **248**, 2354–2363.

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

GRIGG, G. C. (1969). Temperature-induced changes in the oxygen equilibrium curve of the brown bullhead, *Ictalurus nebulosus*. *Comp. Biochem. Physiol.* **28** A, 1203–1223.

HARDISTY, M. W. & POTTER, I. C. (1971). 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.

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.

JOHANSEN, K., LENFANT, C. & HANSON, D. (1973). Gas exchange in the lamprey *Entosphenus tridentatus*. *Comp. Biochem. Physiol.* **44** A, 107–119.

LEHNINGER, A. L. (1975). *Biochemistry*. London: Worth.

LONGMUIR, I. S. & CHOW, J. (1970). Rapid method for determining effect of agents on oxyhemoglobin dissociation curves. *J. appl. Physiol.* **28**, 343–345.

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

MACEY, D. J. & POTTER, I. C. (1981). Measurements on various blood cell parameters during the life cycle of the Southern Hemisphere lamprey, *Geotria australis* Gray. *Comp. Biochem. Physiol.* **69** A, 815–823.

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.

PERUTZ, M. F. (1969). The haemoglobin molecule. *Proc. R. Soc. Lond.* B **173**, 113–140.

POTTER, I. C. (1980). The ecology of larval and metamorphosing lampreys. *Can. J. Fish. Aquat. Sci.* **37**, 1641–1657.

POTTER, I. C. & BEAMISH, F. W. H. (1978). Changes in haematocrit and haemoglobin concentration during the life cycle of the anadromous sea lamprey, *Petromyzon marinus* L. *Comp. Biochem. Physiol.* 60 A, 431–434.

POTTER, I. C. & NICOL, P. I. (1968). Electrophoretic studies on the haemoglobins of Australian lampreys. *Aust. J. exp. Biol. med. Sci.* **46**, 639–641.

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

RIGGS, A. (1972). The haemoglobins. In *The Biology of Lampreys*, vol. 1 (ed. M. W. Hardisty and I. C. Potter), pp. 261–286. London: Academic Press.

SCHMIDT-NIELSEN, K. (1979). *Animal Physiology*. Cambridge: Cambridge University Press.

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

WOOD, S. C. (1980). Adaptation of red blood cell function to hypoxia and temperature in ectothermic vertebrates. *Am. Zool.* **20**, 163–172.