

THE HEMOGLOBIN OF THE SEA LAMPREY,
PETROMYZON MARINUS

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Cyclostomes are the most primitive living vertebrates. Together with the extinct ostracoderms, the most ancient fossil vertebrates, they form the class Agnatha, which has pursued an independent evolution since earliest vertebrate origins. All biologists are familiar with the peculiar anatomical organization of these animals. It is becoming evident that this is matched by an equally peculiar chemical constitution, which presents on the molecular level a curious mixture of vertebrate and invertebrate characteristics.

So for example the hagfishes, *Myxine* and *Bdellostoma*, are the only vertebrates known to possess, like typical marine invertebrates, the same salinities in their blood as in sea water, varying as the concentration of the medium varies (cf. Smith, 1932; Bond, Cary, and Hutchinson, 1932). Lampreys, on the other hand, have the much lower blood salinities characteristic of other vertebrates.¹

The retina of the sea lamprey, *Petromyzon marinus*, an anadromous animal which spawns in fresh water and can live as an adult either in fresh water or in the sea, contains the porphyropsin-vitamin A₂ system characteristic of anadromous and fresh water vertebrates. All invertebrates so far examined possess instead visual systems derived from vitamin A₁ (Wald, 1941-42; 1945-46).

The hemoglobins of cyclostomes have unique properties. Vertebrate hemoglobins typically have isoelectric points in the region of neutrality, at pH 6.7-7.0. Invertebrate hemoglobins are more acid, with isoelectric points of 4.5-6.0. The isoelectric point of the hemoglobin of the brook lamprey (*Lampetra*) is at pH 5.6 (Pedersen, 1940), that of sea lamprey hemoglobin at pH 5.7 (Roche and Fontaine, 1940), like those of typical invertebrates. A partial analysis of the amino acid composition of sea lamprey hemoglobin reveals arginine and lysine in proportions typical of vertebrate hemoglobins, while histidine and

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¹ Lampreys have serum osmotic pressures like those of fresh water teleost fishes. The freezing point depressions of fresh water fish sera range from about 0.45 to 0.55°C. Those reported for *Petromyzon* are 0.473-0.500 (Dekhuyzen, 1905); and 0.586 (Burian, 1910). Measurements on *P. marinus* serum by one of us yielded the average value 0.53°C.

cystine are present in proportions characteristic of invertebrate hemoglobins (Roche and Fontaine, 1940).

The blood hemoglobins of all other classes of vertebrate have molecular weights about 68,000, and contain four hemes. In sea and fresh water lampreys the blood hemoglobin has only about 1/4 this weight, about 17,000, like vertebrate muscle hemoglobin (Pedersen, 1940; Roche and Fontaine, 1940).

This low molecular weight, if associated with the usual iron content, implies that lamprey hemoglobin, like muscle hemoglobin, contains a single heme. In this case its equilibrium with oxygen is represented by the simple equation, $\text{Hb} + \text{O}_2 = \text{HbO}_2$, and by the mass action formula

$$K = \frac{(\text{HbO}_2)}{(\text{Hb})(p\text{O}_2)}$$

K here has the force of an affinity constant. The relation between the percentage of hemoglobin oxygenated (y) and the oxygen pressure (p) should then be described exactly by Hill's equation,

$$\frac{y}{100} = \frac{Kp^n}{1 + Kp^n},$$

in which K is the same affinity constant, and $n = 1$. This is the equation of a rectangular hyperbola. It represents correctly the oxygen equilibria of muscle hemoglobin and certain invertebrate hemoglobins. In the blood hemoglobins of other vertebrates, however, in which four hemes interact with one another, the oxygen equilibrium function is always S-shaped, and n in the Hill equation is considerably greater than 1.²

The possibility that lamprey blood hemoglobin has a hyperbolic oxygen equilibrium curve raises curious physiological problems. Such hyperbolic curves have been found heretofore associated with high oxygen affinities, a combination of properties that fits the hemoglobin more for oxygen storage than for oxygen transport. In the lamprey, the blood hemoglobin certainly serves to transport oxygen, apparently with high efficiency, for the lamprey, particularly during its spawning migration, is a vigorous animal. The present measurements were undertaken to explore this situation further.

EXPERIMENTS

Mature lampreys were taken from the Exeter River at Exeter, New Hampshire, in the early stages of their spawning migration. They were brought back to the laboratory, and kept alive in cold running water.

The procedures used in these experiments have already been described (Riggs, 1950-51 *a*). Blood was drawn from the ventricle of the heart with a syringe contain-

² It has been alleged on the basis of measurements by Kawamoto (1929) that the hemoglobin of the fresh water eel has a hyperbolic oxygen dissociation curve. This is mistaken; n in the Hill equation for eel hemoglobin is about 1.8 (Riggs, 1950-51 *b*).

ing citrate. The erythrocytes were centrifuged, washed repeatedly with saline, and were laked by adding distilled water at 0°C. All these operations were carried out under carbon monoxide, to protect the hemoglobin from oxidation. To determine the oxygen equilibrium, the hemoglobin solution, contained in a specially designed cell of large volume, was first reduced by evacuation. It was then equilibrated repeatedly at 20°C. with various pressures of oxygen, obtained by injecting measured amounts of air into the vessel. The degree of oxygenation was measured in the Beckman spectrophotometer.

TABLE I

Oxygen Equilibrium Data for Lamprey Hemoglobin, Measured at 20°C. at Various pH's

Buffer, 0.1 M K₂HPO₄ - KH₂PO₄. *n* and *K* are constants in the Hill equation; and *K* = 1/*p*₅₀, the pressure of oxygen at which the hemoglobin is half saturated.

pH.....	6.2	6.8	7.4
<i>n</i> (Hill).....	1.0	1.2	1.0
<i>p</i> ₅₀ , mm. Hg.....	68.0	19.0	10.0
<i>K</i> (affinity).....	0.015	0.052	0.10
O ₂ capacity.....	1.65	1.77	3.03
(moles O ₂ /ml. × 10 ⁶)			

pH 6.2		pH 6.8		pH 7.4	
<i>p</i> O ₂	HbO ₂	<i>p</i> O ₂	HbO ₂	<i>p</i> O ₂	HbO ₂
mm. Hg	per cent	mm. Hg	per cent	mm. Hg	per cent
3.36	6.5	0.67	4.5	3.34	23.0
6.7	8.3	1.33	4.9	6.68	35.0
13.5	19.0	2.67	9.0	10.0	51.0
23.6	25.0	4.34	16.1	16.7	64.0
40.4	37.5	6.34	20.7		
57.2	45.0	8.35	27.9		
74.0	53.7	11.7	33.7		
159.0	71.0	16.7	46.3		
760.0	100.0	20.0	51.1		
		26.7	62.6		
		33.4	68.7		
		46.7	80.7		

The oxygen equilibrium function was determined at pH 6.2, 6.8, and 7.4. The results of these measurements are summarized in Table I. In Fig. 1 these data are fitted with curves computed from Hill's equation. At pH 6.2 and 7.4, the best fit is obtained when *n* in the equation is 1.0. At pH 6.8, the *n* for best fit is 1.2; this departure from unity probably is not significant. The oxygen equilibrium curve is therefore, as expected, a rectangular hyperbola.

Compared with other hemoglobins which possess this type of equilibrium function, lamprey hemoglobin has a low affinity for oxygen. The affinity constant, *K*, is the reciprocal of *p*₅₀, the oxygen pressure at which the hemoglobin is half saturated; for in the equilibrium equation

$$K = \frac{(\text{HbO}_2)}{(\text{Hb})(p\text{O}_2)}$$

at half saturation

$$\frac{(\text{HbO}_2)}{(\text{Hb})} = 1, \quad \text{and} \quad K = 1/p\text{O}_2 = 1/p_{50}.$$

Values of K and p_{50} are shown in Table I.

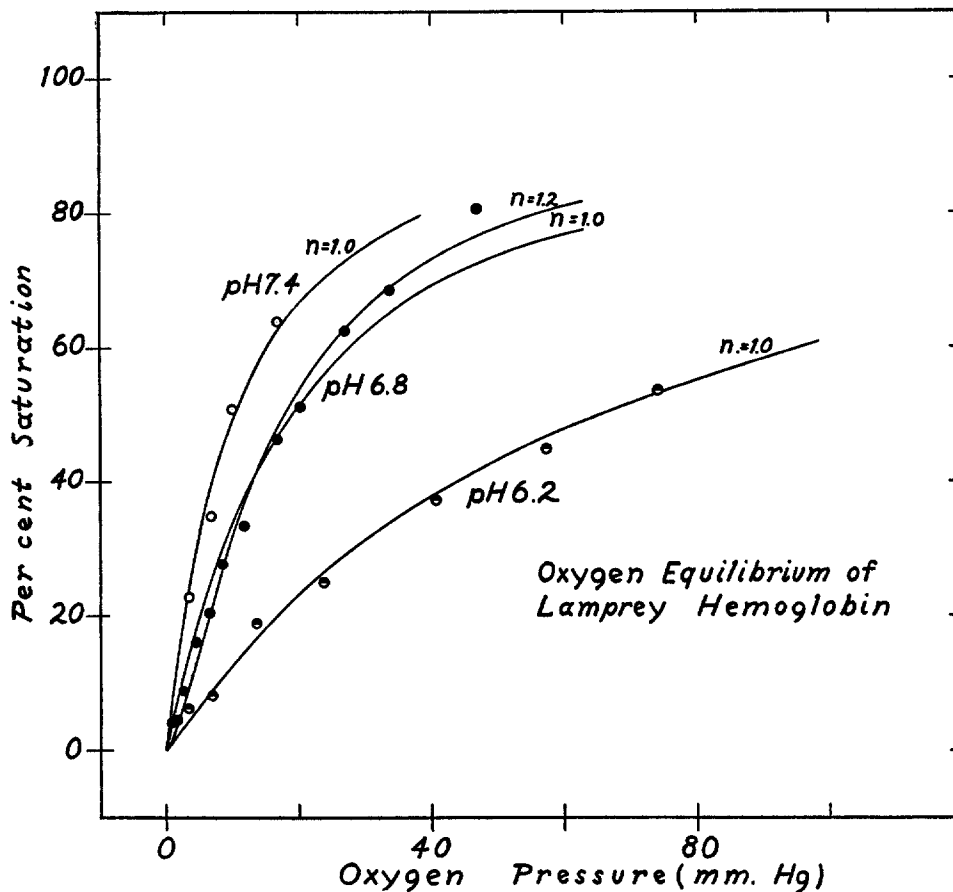


FIG. 1. Oxygen equilibrium curves of lamprey hemoglobin at 20°C. This hemoglobin has a relatively low oxygen affinity and displays a very large Bohr effect. The curves are computed from Hill's equation

$$\frac{y}{100} = \frac{Kp^n}{1 + Kp^n},$$

in which y is the per cent HbO_2 (ordinate), p is the oxygen pressure (abscissa), and K and n are constants. At pH 7.4 and 6.2, the data are fitted best when $n = 1$; the curve is a rectangular hyperbola. At pH 6.8 the best fit is obtained when $n = 1.2$; this departure from unity probably is not significant.

Lamprey hemoglobin displays a very large Bohr effect, the oxygen equilibrium curve moving far toward the right as the acidity increases (Fig. 1). The affinity constant K decreases about 7 times— p_{50} rising by this amount—as the pH falls from 7.4 to 6.2. The entry of acids into lamprey blood therefore tends strongly to discharge oxygen; conversely lamprey hemoglobin becomes a much stronger acid on oxygenation.

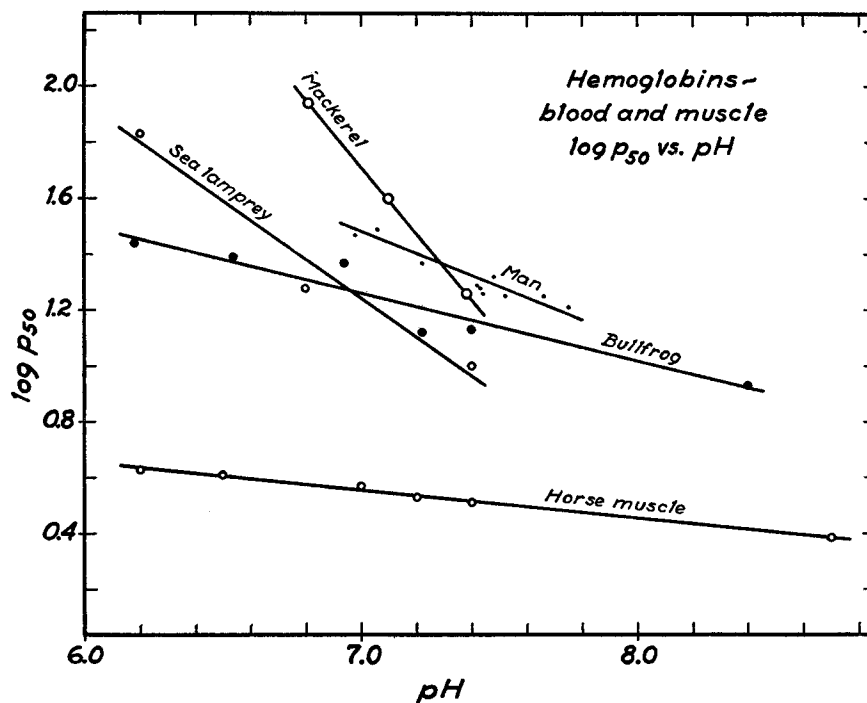


FIG. 2. The Bohr effect in vertebrate blood and muscle hemoglobins. Ordinates, logarithm of p_{50} , the oxygen pressure in mm. Hg at which the hemoglobin is half saturated. Blood hemoglobins of man (37.5°C.?), Adair (1925); mackerel (25°C.), Hall and McCutcheon (1938); lamprey (20°C.); and bullfrog (20°C.), Riggs (1950-51 a); and horse muscle hemoglobin (37°C.), Theorell (1934).

In many vertebrate blood and muscle hemoglobins, $\log p_{50}$ or $\log K$ is linear with pH over a considerable range. This relation holds approximately also for lamprey hemoglobin over the range investigated (Fig. 2). In a single measurement we found the pH of lamprey serum to be 6.6, considerably more acid therefore than most sera heretofore examined. From Fig. 2 this is seen to correspond to a $\log p_{50}$ at 20°C. of 1.53, or a p_{50} of 34 mm. Hg. This probably approximates the average physiological condition.

The slopes of the lines in Fig. 2, $\Delta \log p_{50} / \Delta \text{pH}$, yield a convenient measure

of the Bohr effect. This ratio is about 0.7 for lamprey hemoglobin at 20°C., as compared with 0.4 for human blood hemoglobin (37.5°C?), 1.2 for mackerel hemoglobin (25°C.), 0.24 for bullfrog hemoglobin (20°C.), and 0.1 for horse muscle hemoglobin (37°C.). The Bohr effect in the lamprey is among the largest found in vertebrates.

The Bohr effect reflects the fact that one or more groups on the hemoglobin molecule become more strongly acidic on oxygenation. The slopes of the lines in Fig. 2 can be used to estimate this increase in acidity. Assuming one oxygen-linked acid group per heme, Wyman has derived an expression relating its minimum decrease in pK on oxygenation to the ratio $\Delta \log p_{50}/\Delta pH$ (Wyman, 1948, p. 463).³ In lamprey hemoglobin this minimum change in pK is 1.5, compared with 0.7 for human blood hemoglobin and 0.2 for horse muscle hemoglobin.

DISCUSSION

At the close of a tortuous discussion involving whether the oxygen equilibrium curve of mammalian hemoglobin is hyperbolic or S-shaped, Joseph Barcroft, who had at times represented both sides of the argument, concluded not only that it is S-shaped, but that this is of central importance for hemoglobin function (Barcroft, 1924). The point is that a blood hemoglobin must not only combine with oxygen in the lungs or gills, but must release it in the tissues. It is important also, particularly in an active animal, that oxygen be discharged from the blood at relatively high tensions; for the rate of penetration of oxygen into the tissues is proportional to its gradient, and this is larger the higher the tension at which oxygen leaves the blood.

A hemoglobin possessing a hyperbolic equilibrium curve tends to retain its oxygen down to low pressures. The corresponding S-shaped curve has the effect that oxygen is discharged at higher tensions; conversely, for a given decrease in tension between the arterial and venous circulations, more oxygen is discharged. The force of these considerations may be judged from J. S. Haldane's remark: "A man would die on the spot of asphyxia if the oxygen dissociation curve of his blood were suddenly altered so as to assume the form which Hüfner supposed it to have in the living body;" *i.e.*, the hyperbolic form (Haldane, 1922).

Haldane, however, conceded too much here to the form of the oxygen equilibrium curve, apart from its other properties. What he said is certainly true of the hemoglobins known heretofore to have hyperbolic oxygen equilibria—those of vertebrate muscle, the nematode worm *Nippostrongylus* (Davenport, 1949–50), and the red organ of the bot-fly larva *Gastrophilus* (Keilin and Wang,

³ The expression is $\Delta pK = 2 \log \frac{1+r}{1-r}$, in which $r = \Delta \log p_{50}/\Delta pH$ (Wyman, 1948, p. 463).

1946). These hemoglobins unite the hyperbolic equilibrium function with very high oxygen affinities and—in muscle hemoglobin, in which alone this relation has been examined—with a very small Bohr effect. They surrender their oxygen only under highly anaerobic conditions. They are useful for limited oxygen storage, not for oxygen transport.

The lamprey is the only vertebrate known to have a blood hemoglobin with a hyperbolic oxygen equilibrium curve. That it transports oxygen efficiently can scarcely be doubted by anyone who knows this animal. Lamprey hemoglobin escapes Haldane's dictum through two devices: (1) a relatively low oxygen affinity; its oxygen equilibrium is described by a very slow hyperbola. Such a slow hyperbola, as is evident in Fig. 1, can result in a considerable oxygen exchange at relatively high arterial and venous tensions. In a sense

TABLE II
Properties of the Blood Hemoglobin of the Sea Lamprey Compared with Muscle and Blood Hemoglobins of Mammals

	Lamprey blood hemoglobin	Mammalian muscle hemoglobin	Mammalian blood hemoglobin
Molecular weight	17,000	17,000	68,000
Hemes per molecule	1	1	4
<i>n</i> in Hill equation	1	1	2.8
<i>p</i> ₅₀ , mm. Hg.	34 (20°C., pH 6.6)	3.7 (37°C., pH 7.0)	22-27 (37-38°C., pH 7.4)
Bohr effect: $\frac{\Delta \log p_{50}}{\Delta \text{pH}}$	0.7	0.1	0.4-0.5

lamprey hemoglobin sacrifices the capacity to saturate with oxygen in the gills in order more readily to surrender it in the tissues. (2) It has one of the largest Bohr effects known among vertebrates. A relatively small discharge of carbon dioxide or other acidic metabolites from the tissues into the blood therefore helps greatly to release oxygen.

These properties are summarized in Fig. 2 and in Table II. They distinguish lamprey hemoglobin sharply from muscle hemoglobin, and bring it together functionally with the most effective of the vertebrate blood hemoglobins. They furnish a renewed demonstration on the molecular level of the peculiar mixture of archaic traits and special adaptations that characterize the cyclostomes.

SUMMARY

The blood hemoglobin of the sea lamprey presents a curious mixture of primitive and highly specialized properties. Like muscle hemoglobin, it has a molecular weight of about 17,000, and apparently contains a single heme. Its isoelectric point is like that of a typical invertebrate hemoglobin. Its amino

acid composition is partly characteristic of invertebrate, partly of vertebrate hemoglobins (Pedersen; Roche and Fontaine).

In the present experiments, the oxygen equilibrium curve of this pigment was measured at several pH's. As expected, it is a rectangular hyperbola, the first such function to be observed in a vertebrate blood hemoglobin.

Other hemoglobins known to possess this type of oxygen dissociation curve—those of vertebrate muscle, the worm *Nippostrongylus*, and the bot-fly larva—appear to serve primarily the function of oxygen storage rather than transport. Lamprey hemoglobin on the contrary is an efficient oxygen-transporting agent. It achieves this status by having, unlike muscle hemoglobin, a relatively low oxygen affinity, and a very large Bohr effect. In these properties it rivals the most effective vertebrate blood hemoglobins.

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