

RESPIRATORY CHARACTERISTICS OF THE BLOOD OF *LABEO UMBRATUS* (SMITH) AND *LABEO CAPENSIS* (SMITH)

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ABSTRACT

Various respiratory characteristics of mudfish blood were investigated. Oxygen and carbon dioxide contents of mixed venous blood were $1,17 \pm 0,53$ and $11,35 \pm 1,65$ vol. % respectively. Blood oxygen dissociation curves showed P_{50} values of $11,84 \pm 3,1$; $13,77 \pm 2,0$ and $18,05 \pm 4,7$ mm Hg at CO_2 concentrations of 0–1%, 4–5% and 9–10%. A marked Bohr effect of $-0,59$ was observed and the Haldane effect was found to be relatively low. No differences could be observed between the characteristics of *L. umbratus* and *L. capensis* blood.

INTRODUCTION

The oxygen dissociation curves of fish blood have been fairly well documented up to date. Because the binding of oxygen to the haem groups alters the configuration of the haemoglobin molecule, the affinity of the remaining haem groups may be altered; it may either be enhanced or depressed (Satchell 1971). These interactions may thus serve to increase the affinity of the haemoglobin for oxygen when a certain critical level of saturation has been exceeded, and a sigmoid curve results. The absence of these interactions will cause the dissociation curve to be hyperbolic (Hill 1910). The dissociation curves of the hagfish, *Polistotrema stouti* and of the lamprey, *Petromyzon marinus*, were found to be hyperbolic (Manwell 1964 and Wald & Riggs 1951) with n values of 1 (Hill 1910). These fish have monomeric haemoglobins but hyperbolic dissociation curves have also been reported for the bloods of *Hydrolagus collei* (Manwell 1964) and *Squalus suchleyi* (Satchell 1971) which apparently have haemoglobins that are not monomeric (Satchell 1971). It therefore appears that interactions between the haem groups which result in a sigmoid curve do not necessarily occur in all fish with tetrameric haemoglobins (Satchell 1971).

The oxygen and carbon dioxide dissociation curves of local freshwater fish have been less well documented. It is known that the yellowfish (*Barbus holubi*), barbel (*Clarias gariepinus*) and mudfish (*Labeo capensis* and *Labeo umbratus*) all contain multiple haemoglobins (Hattingh & Du Toit 1973; Du Toit *et al* 1973) but nothing is known about the structure of haemoglobin in these fish. In this paper the oxygen and carbon dioxide dissociation curves of *Labeo umbratus* and *Labeo capensis* are reported, as well as the effects of various factors on these curves.

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MATERIALS AND METHODS

Adult, healthy fish of both species were seined in local water and acclimatized in the laboratory for two weeks. The animals were anaesthetized with MS 222 (75 mg/ℓ) and blood samples were obtained anaerobically by cardiac puncture using 5 ml syringes containing a film of heparin (5 000 units/ml).

The pH of the blood samples was measured immediately with a Radiometer model 25 pH meter to an accuracy of 0,02 pH units. Haemoglobin was determined as cyanmethaemoglobin according to the method of Kleihauer & Betke (1957). The oxygen and carbon dioxide contents in blood were determined with the manometric apparatus of Van Slyke & Plazin (1961).

For the dissociation curves, blood was equilibrated at 23°C with gases of known concentration and analyzed in the manometric apparatus. Oxygen dissociation curves were constructed as described by Bartels & Harms (1959). The Bohr factor, expressed as $\Delta \log P_{50} / \Delta \text{pH}$, was measured according to the procedure of Hilpert *et al* (1963). The active haemoglobin content was calculated from the oxygen capacity, assuming that 1 g Hb binds 1,36 ml O₂. The methaemoglobin concentration was determined according to the method of Evelyn & Mallory (1938). Carbon dioxide dissociation curves were constructed as described by Harms & Bartels (1961). The Haldane effect was evaluated from the curves of fully oxygenated and reduced blood.

RESULTS

1. Oxygen and carbon dioxide contents of mixed venous blood

The mean values and standard deviations obtained for the oxygen and carbon dioxide contents of blood (only *L. umbratus*) and the haemoglobin and methaemoglobin concentrations and pH values are shown in Table 1. The pH values were found to be very constant but the haemoglobin and methaemoglobin concentrations varied greatly, the latter more than the former. The low content of oxygen and the high content of carbon dioxide were constant observations and varied within very narrow limits.

2. Blood oxygen dissociation curves

Three sets of oxygen dissociation curves were determined at three different concentrations of carbon dioxide; 0–1%, 4–5% and 9–10%. No differences could be observed between the curves of *L. umbratus* and *L. capensis*. Figure 1 and Table 2 show the results obtained. The Bohr factor was found to be $-0,59$ and the n value 1,2 (0–1% CO₂). The oxygen capacity shown in Table 2 refers to conditions of 0–1% CO₂ and the “active” haemoglobin content; actual oxygen capacity determined and the oxygen capacity corrected for the methaemoglobin present in blood, are presented. It is clear that the theoretical or “active” haemoglobin content is higher than that actually determined and is also higher than that corrected for methaemoglobin.

Figure 1 also shows the magnitude of the Root effect in these fish and it can be seen that an increase in CO₂ concentration not only decreases the oxygen affinity but also decreases the oxygen capacity.

3. Carbon dioxide dissociation curves

The carbon dioxide dissociation curves showed the expected behaviour in that reduced blood carried more CO₂ than oxygenated blood. The Haldane effect was low; at 40 mm Hg pCO₂ deoxygenation of the blood increases the carbon dioxide binding capacity by 2 to 3 ml/100 ml blood, an effect which is about half that of human blood.

TABLE 1

Respiratory parameters of *L. umbratus* and *L. capensis* blood

Species	Mass g	Length cm	Hb g%	pH	Met Hb %	O ₂ vol %	CO ₂ vol %
<i>L. umbratus</i>	638,2 ± 222,5	37,8 ± 4,4	7,20 ± 2,60	7,47 ± 0,05	9,38 ± 6,07	1,17 ± 0,53	11,35 ± 1,65
<i>L. capensis</i>	629,1 ± 149,6	36,8 ± 2,7	7,38 ± 2,00	7,45 ± 0,12	8,23 ± 5,99	—	—

TABLE 2

Summary of blood characteristics of *L. umbratus* and *L. capensis* (Means ± S.D.)

<i>P</i> ₅₀ . mm Hg at pH 7,4 and 23°C			"Active" Hb	O ₂ capacity ml O ₂ /100 ml blood Met Hb corrected	Actual
0-1% CO ₂	4-5% CO ₂	9-10% CO ₂			
11,84 ± 3,1	13,77 ± 2,0	18,05 ± 4,7	10,35 ± 2,2	9,41 ± 1,7	5,91 ± 1,5

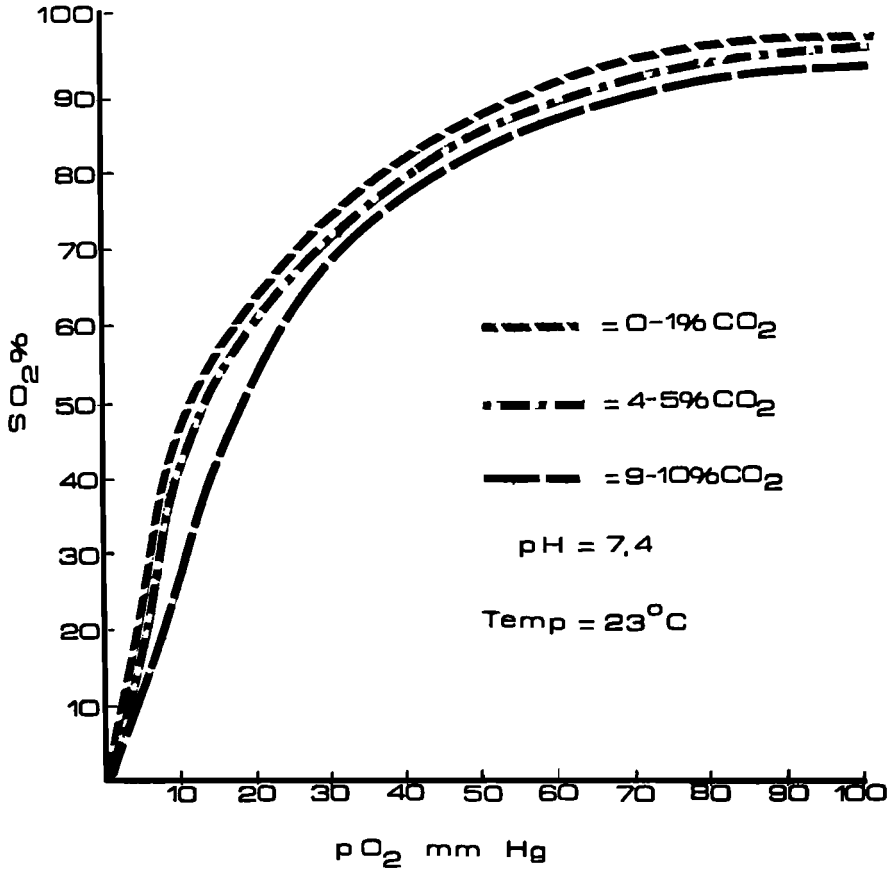


FIGURE 1
Mean oxygen dissociation curves of *L. umbratus* and *L. capensis* obtained with different concentrations of CO₂.

DISCUSSION

The concentration of methaemoglobin in the blood of mudfish is relatively high and values reported in this study correspond to those published by Cameron (1971). This high concentration of methaemoglobin could explain why the actually determined oxygen capacity of the blood does not correspond to the "active" haemoglobin content, but it is clear from Table 2 that this is not the only factor. It is also possible that methaemoglobin formation in the blood during equilibration with the gas mixture is rapid and that less "active" haemoglobin is therefore available. A third possibility is that 1 g of fish haemoglobin does not necessarily bind with 1.36 ml of oxygen as is the case with the human (Bartels & Harms 1959). Great care was taken in equilibrating the blood with the gas mixtures and it is clear that in the case of the fish studied the oxygen capacity of the blood cannot be merely calculated from the haemoglobin content.

The dissociation curves depicted in Figure 1 are sigmoid. The n value for the 0–1% CO₂ curve had a value of 1.2 and this implies that slight interaction occurs between the haem groups. Active fish inhabiting waters of high oxygen content tend to have sigmoid oxygen curves e.g., *Salmo gairdneri*, *Lates albertianus* and *Scomber scombrus* (Fish 1956 and Root 1931). The mudfish are definitely not very active and tend to inhabit water with low oxygen contents. The low content of oxygen in blood (Table 1) confirms this. According to Du Toit *et al* (1973) both have identical haemoglobins on electrophoresis and it is therefore not surprising that the two species studied have similar dissociation curves.

The Bohr factor and Root effect fall into the range published for other fish species (Satchell 1971). The Bohr effect is the result of conformation changes in the haemoglobin molecule due to an increase in hydrogen ion concentration which inhibits the binding to oxygen (Satchell 1971) and the Root effect is apparently a pH dependant negative haem-haem interaction (Manwell 1964).

According to Satchell (1971) the oxygen affinity of fish blood is related to the environmental oxygen content of the habitat. The P₅₀ of 11.84 mm Hg at a concentration of 0–1% CO₂ determined for mudfish blood is very low compared to a P₅₀ of 43 mm Hg for *Salmo gairdneri* (Satchell 1971). Together with the slightly sigmoid dissociation curves this implies that the mudfish inhabit water low in oxygen and this in turn corresponds to field observations (Schoonbee – personal communication). The magnitude of the Bohr effect also implies that these fish will not tend to inhabit waters high in carbon dioxide seeing that loading of haemoglobin with oxygen would then be difficult.

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