Ann. Zool Fennici 16: 217—221. 1979

Oxygen dissociation curves and oxygen capacities of blood of a freshwater fish, Salmo gairdneri

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Nikinmaa, M. & Soivio, A. 1979: Oxygen dissociation curves and oxygen capacities of blood of a freshwater fish, Salmo gairdneri. — Ann. Zool. Fennici 16: 217—221.

The O_2 dissociation curves of the blood of freshwater rainbow trout were determined by the mixing method at three different CO_2 tensions (0.19, 0.37 and 1.47 kPa). The 0.19 kPa CO_2 tension was close to the arterial and 0.37 kPa close to the venous CO_2 tension of the blood, and so the dissociation curves obtained at these two CO_2 tensions closely describe the oxygen dissociation characteristics of freshwater rainbow trout blood *in vivo*. The P_{50} values, determined at four CO_2 tensions (0.04, 0.19, 0.37 and 1.47 kPa), with average pH values of 7.85, 7.64, 7.53 and 7.40, were 1.76, 2.85, 4.41 and 7.41 kPa, respectively. The O_2 capacities, haematocrit values and P_{50} values were determined from blood samples taken by cardiac puncture and from cannulae implanted in the dorsal and ventral aortae. The sampling method used did not affect the values for O_2 capacity or P_{50}. However, the correlation between the haematocrit values and the O_2 capacities was markedly greater for the cannula samples than for the cardiac puncture samples. The regression equations indicated that in the samples taken by cardiac puncture the red blood cells had swelled.

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1. Introduction

The O_2 dissociation curves and P_{50} values of rainbow trout blood have been determined by the mixing method in several studies (cf. Hughes et al. 1975, Cameron 1971, Eddy 1971 and Weber et al. 1976). Many of these studies have used air and pure nitrogen as equilibrating gases (Hughes et al. 1975). In consequence, the equilibrating conditions are far removed from the physiological pH and P_{CO_2} of either arterial or venous blood, and the dissociation curves obtained cannot describe the physiological O_2 dissociation characteristics of rainbow trout blood. Eddy (1971) determined O_2 dissociation curves for rainbow trout at several CO_2 tensions covering the physiological P_{CO_2} range. But since the P_{CO_2} of neither was measured, information about the O_2 dissociation characteristics of arterial and venous blood is deficient. Sampling methods and the handling of the blood samples are known to cause changes in some blood parameters of fish, e.g. pH (Garey 1972), the haematocrit value (Soivio et al. 1973, 1974a, 1974b) and the concentration of plasma ions (Oikari and Soivio 1975).

The aim of this study was to construct O_2 dissociation curves at arterial and venous P_{CO_2} tensions and to ascertain whether the sampling methods used have any influence on the parameters describing the oxygen-carrying properties of the blood.

2. Material and methods

The experiments were carried out at Laukaa Fish Culture Research Station in June and July 1977. The fish used were 4-year-old rainbow trout (*Salmo gairdneri*). They were acclimated for 2 weeks to a temperature of 12 ± 0.4°C in 4 m² fibreglass tanks with a water content of 2 m³. The water supplied to the tanks (at 50 l/min) came from a nearby lake. During the experiments the O_2 saturation of the water was 80—90 %, the pH 6.3—6.5, the $P_{CO_2} < 100$ Pa and the specific conductivity 40 μ S/cm at 20°C. During the acclimation period the fish were fed on EWOS pelleted salmon food four times a day. Feeding was stopped a week before the experiments.

The fish were 43 in number (46.0±0.7 cm, 1200± 61 g, $\bar{x} \pm$ SE), 29 males and 14 females. In 19 of the fish (13 ♂♂ and 6 ♀♀) the dorsal aorta was cannulated according to the method of Soivio et al. (1972, 1975) and the ventral aorta was cannulated via the bulbus arteriosus. Before the experiments these fish were allowed to recover from the operation for 4 days, during the last two days of which they were placed in separate restrainers (Soivio et al. 1975). The blood samples were drawn into l-ml heparinized syringes. Blood samples from other fish were obtained by cardiac puncture in l-ml heparinized syringes. The O_2 capacities, haematocrit values and P_{50} values were determined for all the fish, but dissociation curves were constructed only for the cannulated fish. The O_2 capacity was measured according to Tucker (1967). This method requires blood to be equilibrated with air and after equilibration a sample of known volume is placed in a chamber containing degassed potassium ferricyanide, which liberates O_2 into physical solution. The O_2 capacity of the sample can be calculated from the change in the P_{O_2} of the solution, measured polarimetrically. The dissociation curves were determined by the mixing method (Haab et al. 1960, Edwards and Martin 1966) using seven double points for one curve. Each set of points took about 1 h to determine. All the P_{O_2} measurements were done with the Radiometer BMS3Mk2, PHM 71Mk2 system. The blood was oxygenated with air and deoxygenated with nitrogen in a rotating-type tonometer in celluloid chambers of 10 ml volume. The volume of the sample was 1 ml. The time taken for 99 % oxygenation or deoxygenation at 10°C was 15 min and for 95 % oxygenation or deoxygenation 5 min. The O_2 capacities for blood samples equilibrated with air and nitrogen having CO_2 tensions of 0.19, 0.37 and 1.47 kPa were also measured.

The dissociation curves were determined for blood samples equilibrated at the above CO_2 tensions. From data obtained previously 0.19 kPa was calculated to be the mean value of P_{CO_2} for arterial blood and 0.37 kPa the corresponding value for venous blood in the same experimental conditions. The gas mixtures (air + CO_2, N_2 + CO_2) were obtained using a modification of the Radiometer GMA1 gas-mixing apparatus. The P_{CO_2} of the blood was determined with the Radiometer CO_2 electrode and pH meter. For calibration, two known mixtures of N_2 and CO_2 were used. All determinations were performed at 12°C. The blood samples for haematocrit values were centrifuged within 3 min of sampling in Clay-Adams 1025 capillaries.

3. Results

The dissociation curves of rainbow trout blood in Fig. 1 are drawn from combined data of 14 fish for 0.19 kPa CO_2 tension, and 7 fish for 0.37 and 1.47 kPa CO_2 tension. Table 1 gives the means of P_{50} values, their pH values, and Root effects at different P_{CO_2} values. All three sets of values changed very significantly ($P < 0.001$) as the P_{CO_2} of the equilibrating gases was changed, except that no Root effect was observed when the P_{CO_2} of the equilibrating gases was changed from 0.04 to 0.19 kPa. The numerical value for the Bohr effect can be calculated from the following equation

Bohr constant = d log P_{50} value/ dpH

The value of the Bohr constant in the pH range 7.64—7.40 is —1.7 and in the pH range 7.85 —7.64 it is —1.0. Results for the pH of oxygenated and deoxygenated blood at 0.19 kPa CO_2 tension show a significant ($P < 0.01$) Haldane effect: deoxygenated blood has a higher pH (7.651 $n = 24$, SE = 0.008) than oxygenated blood (7.615, $n = 26$, SE = 0.007).

The total oxygen concentrations and P_{50} values of freshwater rainbow trout blood obtained by cardiac puncture or from the dorsal or ventral aortic cannulae are given in Table 2. There are no statistically significant differences

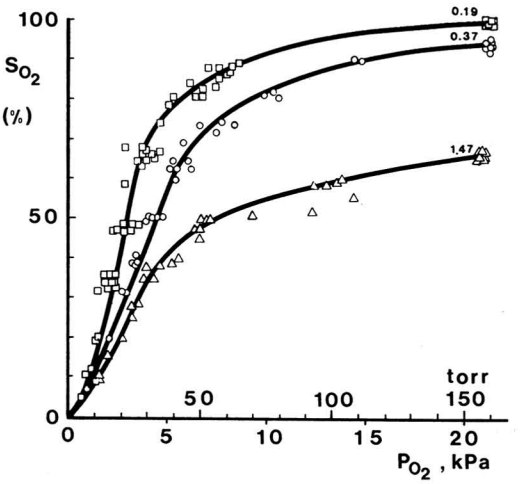

Fig. 1. The oxygen dissociation curves at 0.19, 0.37 and 1.47 kPa CO_2 tensions. The curves are based upon values from 14 fishes for CO_2 tension of 0.19 kPa and from 7 fish for the other two CO_2 tensions. All values were determined at 12°C.

Table 1. The P_{50} values, their pH values andsthe percentage Root effects at CO_2 tensions of 0.04, 0.19, 0.37 and 1.47 kPa. The means, standard errors of the means, and numbers of determinations are given. The determinations were made at 12°C.

P_{CO_2} (kPa)	P_{50} value (kPa)	pH	Root effect (%)
0.04	1.76±0.111 (8)	7.852±0.024 (11)	0 (8)
0.19	2.85±0.085 (22)	7.638±0.008 (13)	1±0.4 (14)
0.37	4.41±0.096 (8)	7.533±0.009 (7)	5.3±1.0 (7)
1.47	7.41±0.520 (5)	7.399±0.018 (7)	35.1±1.9 (6)

Table 2. The O_2 capacities and P_{50} values for blood samples taken with different sampling techniques. The means, standard errors of the means and numbers of determinations are given. The determinations were made at 12°C.

Sampling technique	O_2 capacity mmol O_2/l blood	P_{50} value (kPa) (pH = 7.63)
Cardiac puncture	4.241±0.118 (24)	2.48±0.131 (13)
Ventral cannulae	4.582±0.300 (8)	2.88±0.131 (10)
Dorsal cannulae	4.650±0.288 (8)	2.83±0.107 (12)

between the results obtained by the different sampling methods, although both P_{50} values and O_2 capacities seem slightly smaller for cardiac puncture samples than for the other two samples (Table 2). The correlation between O_2 capacities and haematocrit values, shown in Fig. 2, was greater at the 99.9 % confidence level for cannula samples than for cardiac puncture samples. For the former the correlation coefficient was 0.943 and for the latter 0.655. The respective regression equations were

total O_2 concentration mM = 0.140 Hct (%) + 0.91 (cannula samples)

» mM = 0.083 Hct (%) +2,27 (cardiac puncture samples)

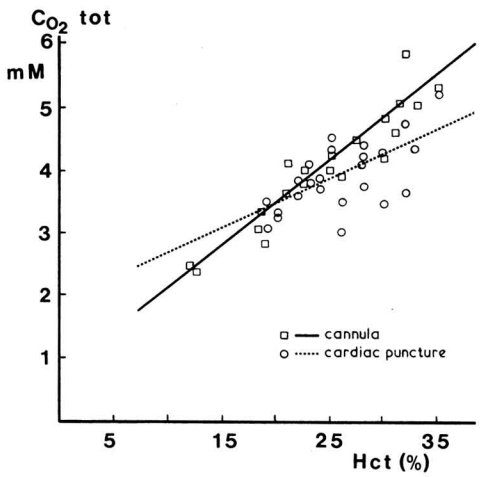

Fig. 2. The regression lines between O_2 capacity and haematocrit values for blood samples drawn via cannulae (n = 25) or taken by cardiac puncture (n = 24). All measurements were made at 12°C.

The standard deviation of regression coefficients was 0.0224 for cannula samples (n = 25) and 0.0448 for cardiac puncture samples (n = 24). The regression coefficients were significantly (P > 95 %) different when compared by the t test.

4. Discussion

In the mixing method, total deoxygenation of the blood is not necessary if the O_2 content of blood equilibrated with N_2 is measured (Torrance & Lenfant 1970). If any oxygen is left in the blood its amount has to be measured and corrections made in the mixing ratio, as otherwise the P_{50} values obtained will be too high. If the oxygenated blood is not fully saturated, the P_{50} values obtained will be too small. This source of error was avoided by equilibrating the blood with air until there was no further change in the O_2 content. If no corrections were made for the Root effect the P_{50} values obtained would again be too small. Therefore in this study we measured both the O_2 capacity of blood samples equilibrated with air and the O_2 capacity of samples equilibrated with gases having the P_{CO_2} in question, and then made corrections in the mixing ratio for the decrease in the O_2 capacity, i.e. the Root effect. When the above factors had been taken into account, the mixing method gave reproducible and accurate determinations for the whole blood oxygen dissociation curves.

The dissociation curves, as indicated by their P_{50} values, fit within the range of previous results obtained at different carbon dioxide tensions (cf. Irving et al. 1941, Eddy 1971, Cameron 1971, Hughes et al. 1975, Weber et al. 1976) and are essentially the same as those of Eddy (1971) and Cameron (1971) in air/nitrogen equilibration. However, these equilibrating conditions are far from the *in vivo* CO_2 tensions or pH values of either arterial or venous blood. It is difficult to correct the values obtained to physiological pH and P_{CO_2}, since the pH value of the blood fluctuates greatly when very low CO_2 tensions are used in the equilibrating gases, by up to 0.5 pH units, according to Eddy (1971). Also, since the Bohr effect is dependent on pH (Gillen & Riggs 1971, 1973, 1977, Iuchi 1973, Noble et al. 1975), the values of the Bohr constant at different pH values must be known before any corrections can be made to P_{50} values.

In addition to this, the Bohr effect may depend on the age of fish (Iuchi 1973) and on the O_2 level that the fish has experienced (Wood & Johansen 1973). Thus, at any given pH the value of the Bohr constant may vary, just as the P_{50} value varies, according to the environmental conditions (Johansen & Weber 1975). These factors make it almost impossible to correct the dissociation curves obtained in non-physiological equilibrating conditions to physiological values. Therefore, to obtain O_2 dissociation curves which closely resemble the physiological curves for arterial and venous blood, their respective CO_2 tensions should be used in the equilibrating gases.

The sampling method did not affect the values obtained for O_2 capacity or P_{50} of rainbow trout blood. It did, however, lead to a change in the correlation found between the haematocrit value and the O_2 capacity. The regression equation for cannulated fish in this study is about the same as that reported by Holeton & Randall (1967b): C_{O_2} (vol %) = 0.311 Hct + 0.7 (the value in this study was: C_{O_2} = 0.308 Hct + 1.2 expressed as volume % with dissolved O_2 subtracted).

The regression equations between O_2 capacity and haematocrit value suggest swelling of the red blood cells, which has been reported for the blood of rainbow trout by several authors (Irving et al. 1941, Holeton & Randall 1967, Soivio et al. 1973, 1974a, 1974b, 1977). The O_2 capacity changes only because of a change in temperature or a change in the haemoglobin concentration of the blood. There was no change in the average haemoglobin concentration in these experiments, so at a constant temperature changes in the O_2 capacity — haematocrit value regression coefficients could only be caused by a change in the volume of the red blood cells. As the regression coefficient was much smaller for the cardiac puncture samples than for the cannula samples, the red blood cells must have swelled during sampling by cardiac puncture. For this reason most of the haematocrit values given in the literature are probably higher than the physiological ones, since cardiac puncture has been used almost exclusively for blood sampling.

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Received 13. VI. 1979
Printed 13. XII. 1979