J. Exp. Biol. (1973), **58**, 281–293
With 5 text-figures
Printed in Great Britain

OXYGEN DISSOCIATION CURVES OF THE BLOOD OF THE TENCH, *TINCA TINCA*

BY F. B. EDDY*

Research Unit for Comparative Animal Respiration, University of Bristol, Bristol BS8 1UG

(*Received* 10 *July* 1972)

INTRODUCTION

The oxygen dissociation curves of mammalian blood have been studied in greater detail than those of the lower vertebrates. In fish the oxygen dissociation curves have been examined most closely in the salmonids (Irving, Black & Safford, 1941; Cameron, 1971; Eddy, 1971) and in the lungfish (Johansen, 1970). The oxygen-binding characteristics of blood from cyprinid fish have received little attention; Black & Irving (1937) determined the oxygen dissociation curve of carp blood and found that there was a high affinity for oxygen and that the Bohr and Root effects were present.

Aspects of respiration in the tench have been studied. The mechanisms and control of respiration have been examined by Hughes & Shelton (1958, 1962), Randall & Shelton (1963) and Schumann & Piiper (1966). In the interpretation of results from these experiments a knowledge of the oxygen dissociation curve is useful, and it was the object of the present work to provide some information about the interaction between oxygen, pH, P_{CO_2} and temperature in tench blood.

MATERIALS AND METHODS

Experimental animals. Tench weighing between 100 and 400 g were obtained from a fish farm at Great Stambridge, Essex. They were initially held in 50 gal glass aquaria maintained at approximately 13 °C; after a few days some fish were transferred to similar aquaria held at 5 °C and 20 °C where they were temperature-acclimated for at least 3 weeks. The water temperature was maintained to within ± 1 °C. They were fed twice weekly on maggots and chopped sheep heart.

Anaesthesia and blood sampling. Tench were anaesthetized with approximately 130 mg/l MS 222 (Sandoz) and then placed on an operating table. Blood was removed from the dorsal aorta by a method essentially that of Smith & Bell (1964).

Blood analysis. The haematocrit of 50 μl samples was measured using a micro-capillary centrifuge (Hawksley). The pH of similar-sized samples was measured in an Eschweiler micro pH assembly which was capable of an accuracy of 0·01 pH unit. The pH electrode was calibrated with phosphate buffers (Pye Ingold and EIL) whose temperature coefficient was known (Mattock, 1962).

The oxygen content of 10 μl samples of blood was measured by the method of

* Present address: Department of Biological Sciences, University of Lancaster, Bailrigg, Lancaster.

Tucker (1967). The stated accuracy of the method is 0·03 ml oxygen/100 ml blood, expressed as the mean difference from variations obtained by the van Slyke method (Tucker, 1967). In the present study all determinations were made in duplicate or until values differing by less than 0·5 vol % had been obtained. The assemblies for measuring pH and oxygen were maintained at the same temperature as the tonometer water bath.

The oxygen capacity refers to the maximum amount of oxygen with which the blood haemoglobin can combine; its value is obtained by subtracting the oxygen dissolved in the blood from the total blood oxygen. Calculations involving dissolved oxygen are based on the data given by Sendroy, Dillon & van Slyke (1934). The percentage saturation of the blood can be defined as

$$\frac{(\text{oxygen capacity of blood} - \text{dissolved oxygen}) \times 100}{\text{oxygen capacity of blood}}.$$

Production of gas mixtures. It seemed probable that the P_{50} value for tench blood would be similar to that reported for carp blood, 1–5 mmHg (Black & Irving, 1937; Garey, 1970). Thus it was necessary to produce accurately oxygen tensions in the range 0–20 mmHg. The following system was used. Two gas mixtures were made up: (*a*) a known tension of CO_2 in nitrogen, and (*b*) the same as (*a*) but containing a known tension of O_2 as well, e.g. 3 mmHg P_{CO_2}, 30 mmHg P_{O_2} and the balance N_2. The composition of each gas mixture was determined with a Lloyd–Gallenkamp gas analyser. The two gas mixtures were then mixed together at known and controlled flow rates using a water manometer (0–50 ml/min) and a Flowstat (G. A. Platon) 0–500 cc/min. Suitable adjustment of the flow rates produced oxygen tensions in the range 0–40 mmHg. The apparatus was calibrated by setting the flowmeter rates and allowing the emerging gas to flow over an Eschweiler micro P_{O_2} electrode connected to an Eschweiler amplifier. The P_{O_2} of these gas mixtures was measured and the gas flows through the meters was noted; repeated calibrations showed that the P_{O_2} at any particular setting differed by less than 0·5 mmHg on each occasion. The P_{CO_2} of the mixture remained unaltered since it was the same (within 0·5 mmHg) in each inflowing gas.

Construction of oxygen dissociation curves. About 0·5 ml freshly drawn blood was placed in a 15 ml tonometer similar to that described by Finley *et al.* (1960) and a humidified gas mixture was passed through it. The gas emerging from the tonometer was passed through a strong sodium hydroxide solution and then through a second tonometer containing a further 0·5 ml of blood; this was for the determination of the dissociation curve in the absence of CO_2 and for the determination of oxygen capacity. The removal of the CO_2 caused a change in the P_{O_2} for which a correction was made. At each new oxygen tension used at least 10 min was allowed for equilibrium to be reached and then duplicate blood samples were removed for the analysis of oxygen and pH.

Blood was used on the same day as it was removed from the fish; during each experiment haematocrit was determined at intervals and if there was evidence of haemolysis the blood and those results were discarded. The P_{O_2} and pH cuvettes together with the water bath containing the tonometers and gas humidifiers were maintained at the stated temperature ±0·5 °C.

Table 1. *Haematological values for the tench*

	Hb (g/100 ml)	Ht %	RBC ($\times 10^6$/ mm^3)	MCV (μm^3)	MCH (pg)	MCHC (g Hb/ 100 ml)	Oxygen capacity (vol. %)	Weight (g)
Mean	6·78	24·1	1·05	244·9	74·7	33·1	7·67	276·9
Number	21	26	13	13	14	14	33	16
Standard error	0·54	1·62	0·08	16·3	5·33	2·92	0·48	19·44
Range	2·7–11·1	8–36	0·51–1·47	95–352	49–112	20–60	2·8–10·9	150–421

Hb = Haemoglobin, Ht = haematocrit (packed cell volume), RBC = Red blood cells, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.

Haematology. Red cell counts were carried out in quadruplicate using Hendrick's fluid (1952). Haemoglobin was estimated by the cyanohaemoglobin method (Wintrobe, 1956) using standards supplied by British Drug Houses.

RESULTS

Haematology. Haematological values for the tench are shown in Table 1. There was considerable variation between individuals. The mean value for oxygen capacity was 7·67 vol %, for haematocrit 24·1 and for haemoglobin 6·78 g/100 ml. Oxygen capacity and haematocrit (Ht) were related by the equation.

$$\text{Oxygen capacity} = 0{\cdot}24 \pm 0{\cdot}049\,(\text{Ht}) + 1{\cdot}69 \pm 1{\cdot}25 \quad (N = 26)$$

and oxygen capacity and haemoglobin were related by the equation

$$\text{Oxygen capacity} = 0{\cdot}51 \pm 0{\cdot}15\,\text{Hb} + 2{\cdot}98 \pm 1{\cdot}07 \quad (N = 21).$$

The standard deviation and the number of paired determinations are indicated. In the tench these values appeared to be independent of temperature; the data in Table 1 are composed of data from all three acclimation temperatures investigated.

Oxygen capacity of tench blood. In the salmonids and in many other fish carbon dioxide prevents the haemoglobin from becoming completely saturated with oxygen even at high oxygen tensions, the Root effect. This effect was observed in tench blood, Fig. 1. Its magnitude showed no temperature dependence, therefore the results from all temperatures were pooled.

Oxygen dissociation curves. Oxygen dissociation curves of tench blood were constructed at 5, 13 and 20 °C and they are shown in Fig. 2(*a–c*), where percentage saturation of the blood is plotted against the oxygen partial pressure. In some cases all the experimental data is plotted; where at least four curves for a given P_{CO_2} and temperature were determined the standard error of the percentage saturation is indicated.

Increasing temperature lowered the oxygen affinity of the blood; thus when P_{CO_2} was 0 mmHg the P_{50} value was 0·5 mmHg at 5 °C, 2 mmHg at 13 °C and 3 mmHg 20 °C. At all temperatures increasing P_{CO_2} had the effect of decreasing the P_{50} value; here the decreased oxygen affinity of the haemoglobin is associated with increased hydrogen ion concentration (the Bohr effect).

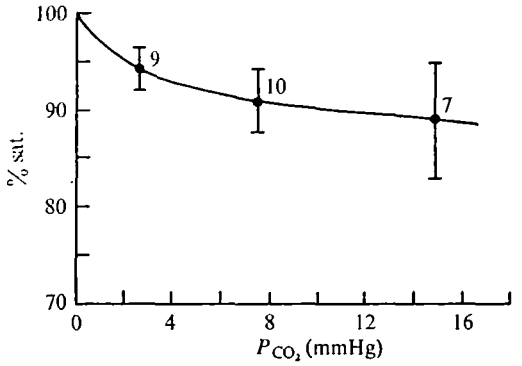


Fig. 1. The effect of carbon dioxide on the oxygen capacity of tench blood (the Root effect). The number of determinations are indicated and the vertical bars indicate ± 2 standard errors.

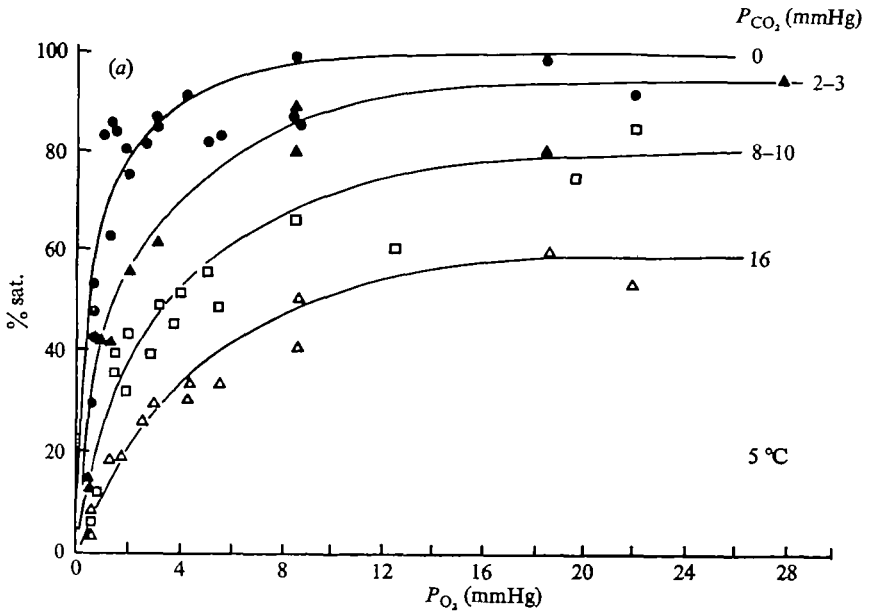
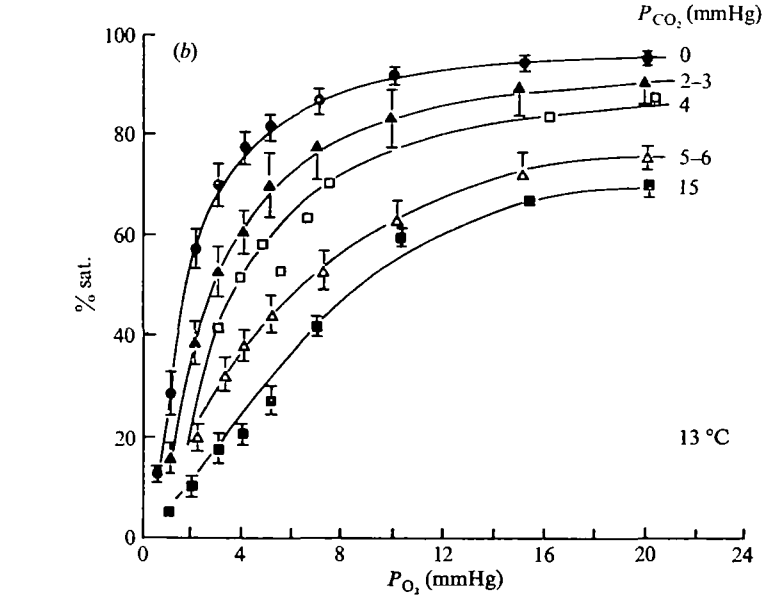


Fig. 2a. For legend see facing page.

The effect of increasing hydrogen ion concentrations on the oxygen affinity of the blood is indicated in Fig. 3 (*a–c*). Here at various P_{CO_2} values the percentage saturation of the blood is plotted against the blood pH. It is evident, particularly low CO_2 tensions, that there is a scatter of blood pH values. This may reflect the buffering properties of the blood at pH values higher than about pH 7·8. The data in Fig. 3 (*a–c*) show that there is a tendency for oxygenated blood to have a lower pH value than de-oxygenated blood. Carbon dioxide dissociation curves of tench blood (Eddy unpublished) indicated that de-oxygenated blood contained 1–2 vol % more CO_2 than oxygenated blood. This indicates that as in mammalian blood oxyhaemoglobin can dissociate more hydrogen ions and is therefore a stronger acid than reduced haemoglobin (the Haldane effect).



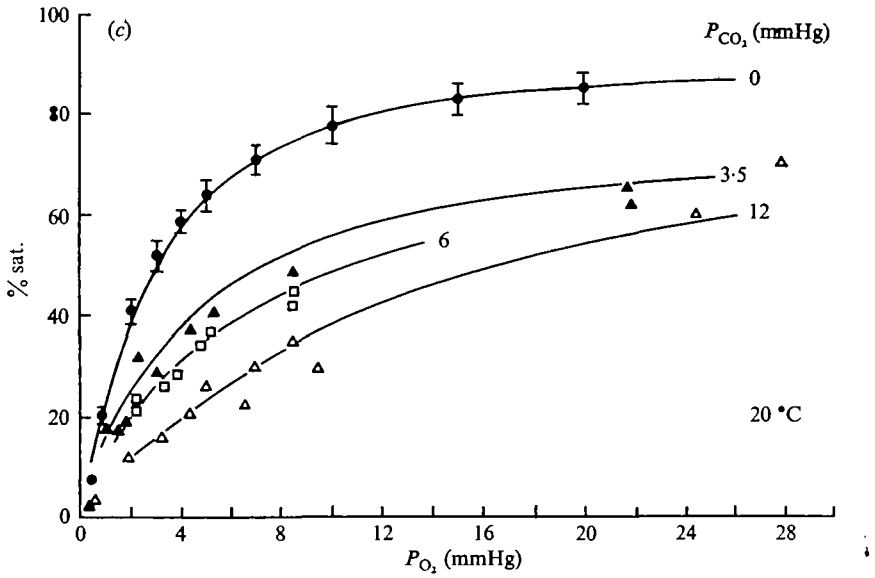


Fig. 2. Oxygen dissociation curves of tench blood at various temperatures and carbon dioxide tensions. The vertical bars indicate the standard error of the mean. (*a*) 5 °C, (*b*) 13 °C, (*c*) 20 °C.

In studies on the oxygen equilibria of mammalian blood it has often been useful to express the dissociation curve at constant pH; this makes it convenient to compare curves obtained under different conditions, for example, at different CO_2 tensions. In most mammalian studies the extreme high and low pH values obtained are usually in the range 7·0–7·5; however, in tench blood there is a much greater spread of pH

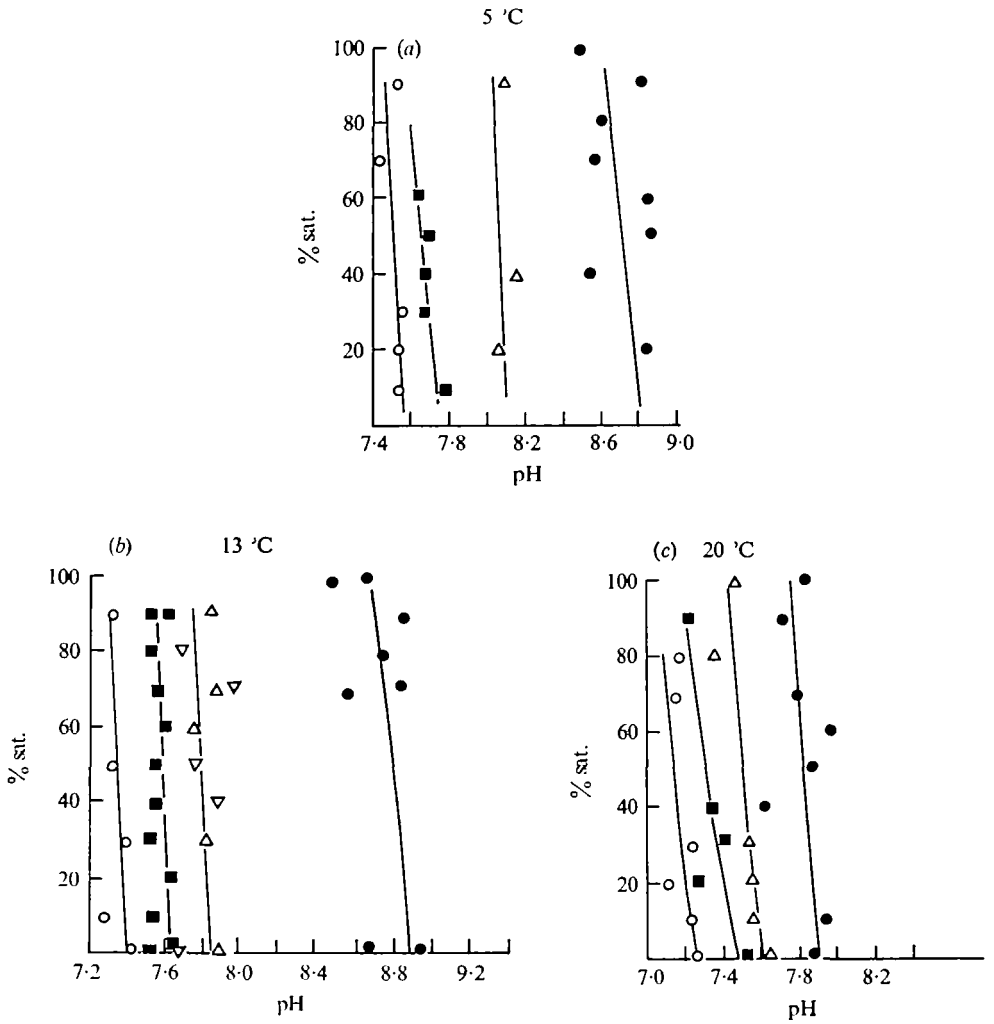


Fig. 3. The effect of increasing carbon dioxide tensions on the pH and oxygen saturation of tench blood at various temperatures. P_{CO_2} values in mmHg: (*a*) ●, 0·0; △, 2·5; ■, 7–10; ○, 16; (*b*) ●, 0·0; △, 3–6; ■, 7–11; ○, 14–16; (*c*) ●, 0·0; △, 3–3·5; ■, 5·5–6·6; ○, 12.

values ranging from pH 7·1 at 20 °C to pH 9·2 obtained at 5 °C, which creates difficulties in the construction of dissociation curves at constant pH; it is not possible to select a pH value which would be common to all curves at each temperature.

The information in Figs. 2 and 3 were combined to make a quantitative determination of the Bohr effect which can be defined as the change in the P_{50} value with unit change in pH ($\Delta \log P_{50}/\Delta$ pH). This relationship is presented graphically in Fig. 4; the values obtained for the Bohr effect are −0·75 at 5 °C, −0·64 at 13 °C and −0·69 at 20 °C. The differences between these values are likely to be insignificant because of the variation in pH between individual fish.

The data in Figs. 2 and 3 were combined to show the effect of temperature on oxygen affinity of the blood at various carbon dioxide tensions (Fig. 5). It appears

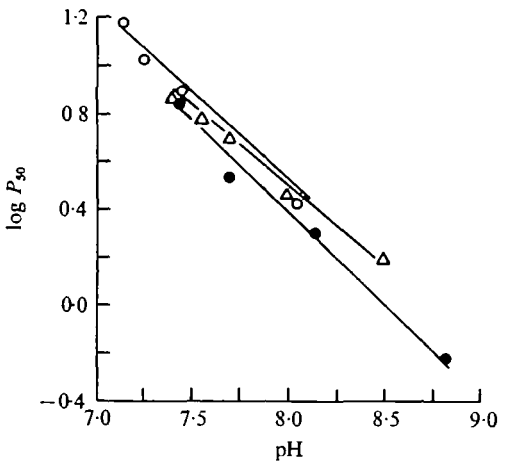


Fig. 4. The Bohr effect (Δ log P_{50}/Δ pH) in tench blood. ●, 5 °C; △, 13 °C; ○, 20 °C.

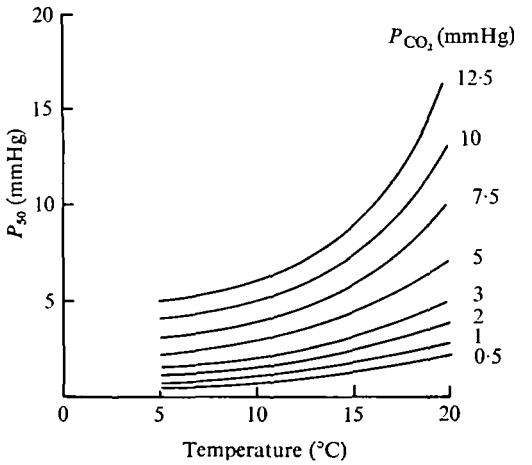


Fig. 5. The effect of temperature on the P_{50} value of tench blood at various carbon dioxide tensions.

that high carbon dioxide tensions together with high temperatures have the greatest effect in decreasing the oxygen affinity of tench blood.

DISCUSSION

Validity of methods. The likely sources of error in the measurement of pH at different temperatures have been discussed by Mattock (1959, 1962). The effects of temperature on pH measurement can be minimized if the pH values of the buffer and of the test solutions are similar and if both solutions are maintained at the same temperature. The difference in liquid-junction potential between buffer solution and the sample solution will be incorporated into all observations. Whole blood may give different liquid-junction potentials from plasma because of the presence of

suspended particles; this factor may produce differences between one blood sample and another of up to 0·01 pH unit.

Albers & Pleschka (1967) have indicated a number of difficulties in the measurement of pH in dogfish blood. The ionic strength of dogfish blood is high (about 0·3) while the pH scale of the National Bureau of Standards is based on solutions with an ionic strength of 0·1. Hence it is almost impossible by electrometric measurements to arrive at the true pH value of solutions of high ionic strength. The ionic strength of carp plasma is 0·15, similar to the value for human plasma of 0·167 (Albers, 1970); it is likely that the ionic strength of tench plasma is similar to that of carp plasma and therefore the measurement of pH in tench blood should present the same problems as the measurement of pH in human blood at different temperatures.

The effects of temperature, pH and P_{CO_2} on the oxygen affinity of the blood

The effects of temperature on the oxygen affinity of tench blood are in general of the same nature as the effects observed in trout blood (Eddy, 1971). Decreasing temperature caused an increase in pH and in oxygen affinity of the blood; at low temperatures the solubility of carbon dioxide increases, more plasma bicarbonate is formed and there is a decrease in the ionization of the blood proteins (Siggaard-Andersen, 1964). The temperature-dependent changes involving hydrogen ions can be discussed in terms of the ideas advanced by Rahn (1967) and by Howell *et al.* (1970). As temperature decreases the ion product of water decreases; pure water at 25 °C is neutral when the pH is 7·0 and *p*OH is also 7·0. For neutrality to be maintained with decreasing temperature the pH must increase. Howell *et al.* acclimated turtles and frogs to various temperatures and found that at the lower temperatures the pH value of arterial blood was elevated. They suggested that it was the ratio of hydroxyl to hydrogen ions ($[OH^-]/[H^+]$) which was maintained approximately constant with temperature changes.

At 15 °C the pH value of rainbow trout arterial blood is about 7·8, yielding an $[OH^-]/[H^+]$ ratio of 20; at 13 °C the pH of tench arterial blood is about 8·1 giving an $[OH^-]/[H^+]$ ratio of about 45. It is not known whether these ratios are maintained over a wide temperature range but this might be expected if the body fluids of trout and tench behave in the same way as those of the poikilotherms described by Howell *et al.* (1970). It is interesting to note that at a similar temperature the pH value of tench blood is higher than that for trout blood; the reasons for this are unknown but Rahn (1967) suggested that each species maintains its own particular $[OH^-]/[H^+]$ ratio.

The Bohr effect. The change in the oxygen affinity of the haemoglobin with pH can be described by the Bohr effect (Fig. 4). In the tench the differences between the values found at each temperature are unlikely to be significant because of the variation of pH values for each fish. The values for the Bohr effect in tench blood are a little higher than those for trout blood ($-0{\cdot}56$; Eddy, 1971) and fall in the upper part of the range of values for the blood of many vertebrate species (Eddy, 1971; Jones, 1972).

Tench haemoglobin. The relationship between oxygen partial pressure (p) and percentage oxygen saturation (y) can be expressed by Hill's equation:

$$y = Kp^n/(1+Kp^n).$$

If $\log (y/1-y)$ is plotted against $\log p$ for values of y between 20 and 80 then straight lines of slope n result. Although this is an empirical relationship between P_{O_2} and percentage oxygen saturation it is often useful for analytical reasons when studying oxygen equilibria of haemoglobins. If $n = 1$, then there is no co-operativity and the oxygen-binding sites of the haemoglobin molecule remain functionally independent of each other, the resulting dissociation curve being hyperbolic. Higher values of n indicate co-operativity between the oxygen-binding sites and the dissociation curves become increasingly sigmoid. The values of n for most mammalian haemoglobins are generally between 2·8 and 3·0; in fish the values are generally between 1·0 and 2·0 (Riggs, 1970). In tench blood n had values in the range of 0·84–1·75, which suggests little co-operativity between the haemoglobin units. It should be remembered that n is usually estimated for haemoglobin solutions, which represent a somewhat less complicated system than whole blood.

It has been shown that fish have multiple haemoglobins which can be separated by electrophoresis and chromatography. Rainbow trout haemoglobin has four components (Binotti *et al.* 1971) and tench haemoglobin at least two (Callegarini & Cucchi, 1968). The two major haemoglobin fractions found by Binotti *et al.* (1971) had different Bohr effects, but Noble, Lawrence & Gibson (1970) could find no indication of haemoglobin fractions with differing Bohr effects in their studies on carp haemoglobins. The respiratory significance of multiple haemoglobin in tench blood remains obscure.

Effect of acclimation temperature. The results presented in this study are for fish acclimated to one particular temperature. It is not known whether the same results would be obtained using blood from fish acclimated to one temperature and tested at different temperatures.

Black, Kirkpatrick & Tucker (1966) found no difference in the oxygen dissociation curves of brook trout acclimated to summer and winter temperatures and tested at the same temperature. However, Grigg (1969) showed that the oxygen dissociation curve of brown bullheads was influenced by acclimation temperature. When equilibrated at the same temperature blood from warm-acclimated bullheads showed an increased oxygen affinity compared to blood from cold-acclimated fish. Cameron (1971) constructed oxygen dissociation curves at 10, 15 and 20 °C for rainbow trout acclimated to 7–9 °C; he also constructed dissociation curves at 15 °C for a group of fish acclimated at 18 °C for 3 weeks and could detect no difference between the two groups.

The oxygen affinity of fish blood appears to be regulated by several factors but it is not known whether any of these are affected by acclimation temperature. Wilkins & Iles (1966) have reported that electrophoretic patterns of herring red cell haemolysates change during the life-cycle. It is not known whether similar changes occur during temperature acclimation in the tench.

Benesch & Benesch (1969) indicated that 2,3-diphosphoglycerate is present in the mammalian erythrocyte in about equimolar concentrations to haemoglobin. This substance facilitates oxygen unloading from oxyhaemoglobin because it lowers oxygen affinity by preferentially binding to de-oxygenated haemoglobin.

Rapoport & Guest (1941) and Lenfant (unpublished, cited in Satchell, 1971) showed that fish erythrocytes contained high concentrations of soluble organic

Table 2. *Haematological data for the tench and for other teleost fish*

	RBC	Ht	Hb	Author
Tench	1·05 ± 0·08	24·1 ± 1·62	6·78 ± 0·54	This study
Carp	1·4–1·8	26–31	6·0–8·0	Houston & DeWilde (1968)
Trout	1·29	31·6	7·42	Houston *et al.* (1968)
Mackerel	3·0	37·1	10·9	Root (1931)

RBC = red blood cells × 10^6/mm^3, Ht = haematocrit, Hb = haemoglobin g/100 ml.

phosphates. Working with haemoglobin solutions from the freshwater teleost *Chiclasoma cyanoguttatum* Gillen & Riggs (1971) found that ATP (adenosine tri-phosphate) was the major organic compound of the red cells. This substance appeared to modify the oxygen equilibria in the same way that 2,3-diphosphoglycerate does in mammalian blood.

Haematology. The haematology of the tench is similar to that of the carp reported by Houston & DeWilde (1968). The values for cyprinid fish appear to be lower than the values for more active fish such as the trout and the mackerel (Table 2). The oxygen capacity of tench blood (about 8 vol %) is lower than that for active fish, for example, trout 9–10 vol % (Eddy & Morgan, 1969) and mackerel 15 vol % (Root, 1931). This supports the idea put forward by Root that active fish have blood with a high oxygen capacity while inactive fish have blood with low oxygen affinity.

The role of the blood in respiration. Compared to the blood of many fish species tench blood has a high affinity for oxygen. At 13 °C and at low carbon dioxide tensions when the P_{O_2} is about 10 mmHg the haemoglobin is more than 85% saturated; at higher carbon dioxide tensions 85% saturation is achieved when the P_{O_2} is 30–40 mmHg.

Using the data of Garey & Rahn (1970) and of Garey (1970) for gas tensions in carp blood and the oxygen dissociation curves of tench blood at 13 °C (Fig. 2*b*) the arterial (efferent) blood P_{CO_2} can be estimated to be about 2–3 mmHg; the haemoglobin will be over 85% saturated when the arterial P_{O_2} exceeds 10 mmHg, and under these conditions the pH of the arterial blood will be about 7·8–7·9. In venous (afferent) blood the P_{CO_2} can be estimated to be 4–5 mmHg and the haemoglobin will be about 40% saturated at a P_{O_2} of about 3 mmHg; here the pH value can be estimated to be 7·7–7·8.

There are no data available for *in vivo* gas tensions in carp blood or tench blood over a range of temperatures. However, Howell *et al.* (1970) using frogs measured blood pH and P_{CO_2} finding that increasing temperature caused a rise in arterial P_{CO_2} and a decrease in arterial pH both *in vivo* and *in vitro*. Thus in poikilotherms changes in body temperature are likely to have considerable effects on the oxygen-carrying capability of the blood. At low temperatures the blood pH will be high, the P_{CO_2} low and the haemoglobin will have a high affinity for oxygen; it will be oxygen-saturated at relatively low oxygen tensions and its oxygen loading and unloading tensions will be relatively low. Thus the P_{O_2} values of circulating blood are likely to be low and the oxygen tension gradient between blood and tissues will be relatively small; this might suggest that the tissues are able to function at lower oxygen tensions when temperature is decreased.

When the body temperature is increased the reverse argument applies; that is, the

conditions prevailing in the blood favour relatively high oxygen loading and unloading tensions and the P_{O_2} values of circulating blood are relatively high.

It would be interesting to know whether the oxygen-transporting capacity of the blood remains the same over a wide range of temperatures or whether the efficiency is diminished at low temperatures, because a tissue P_{O_2} low enough to dissociate sufficient oxygen from the haemoglobin is physiologically impracticable; or whether the efficiency of the system is impaired at high temperatures because to release sufficient oxygen the hydrogen-ion concentration changes involved are too large and physiologically unacceptable.

The ideas presented so far are an oversimplification because they take no account of the temperature dependence of the circulatory system. The data presented by Brett (1971) indicate that the standard oxygen uptake of salmon increases with increasing temperature and that the heart rate and cardiac output are also increased. Thus oxygen transport by the blood at different temperatures depends upon the ability of the haemoglobin to remain an efficient oxygen carrier and also upon the temperature-dependent characteristics of the circulatory system.

SUMMARY

1. Oxygen dissociation curves of tench (*Tinca tinca*) blood were constructed for fish which had been acclimated to 5, 13 or 20 °C for at least 3 weeks.

2. Compared to the blood of an active fish such as the rainbow trout tench blood has a high affinity for oxygen; at 13 °C and a P_{CO_2} of 2–3 mmHg the blood was half saturated with oxygen at a P_{O_2} of 4 mmHg.

3. Increasing temperature, increasing P_{CO_2} and increasing hydrogen ion concentration decreased the oxygen affinity of the blood.

4. At low temperatures the blood had an elevated pH value compared to blood at high temperatures. This is discussed in terms of the temperature dependence of ionization constants, in particular that of water.

5. The Bohr effect and the factors influencing the loading and unloading tensions of oxygen in tench blood are discussed. The role of the blood in respiration and some properties of fish haemoglobins are also discussed.

My thanks go to Dr G. Shelton for his helpful remarks about the manuscript. This work was financed by Natural Environment Council funds made available to Professor G. M. Hughes.

REFERENCES

ALBERS, C. (1970). Acid-base balance. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 173–208. London and New York: Academic Press.

ALBERS, C. & PLESCHKA, K. (1967). Effect of temperature on CO_2 transport in elasmobranch blood. *Respir. Physiol.* **2**, 261–73.

BENESCH, R. & BENESCH, R. E. (1969). Intracellular organic phosphates as regulators of oxygen release by haemoglobin. *Nature, Lond.* **221**, 618–22.

BINOTTI, I., GIOVENCO, S., GIARDINA, B., ANTONINI, E., BRUNORI, M. & WYMAN, J. (1971). Studies on the functional properties of fish haemoglobins. II. The oxygen equilibrium of isolated haemoglobin from trout blood. *Archs Biochem. Biophys.* **142**, 274–80.

BLACK, E. C. & IRVING, L. (1937). The effect of carbon dioxide upon the oxygen capacity of the carp (*Cyprinus carpio*). *Trans. R. Soc. Can.*, Sect. V, 29–32.

BLACK, E. C., KIRKPATRICK, D. & TUCKER, H. H. (1966). Oxygen dissociation curves of the blood of brook trout (*Salvelinus fontinalis*) acclimated to summer and winter temperatures. *J. Fish. Res. Bd Can.* **23**, 1–13.

BLACK, E. C., MANNING, G. T. & HAYASHI, K. (1966). Changes in levels of haemoglobin, oxygen, carbon dioxide, pyruvate and lactate in venous blood of rainbow trout (*Salmo gairdneri*) during and following severe muscular activity. *J. Fish. Res. Bd Can.* **23**, 783–95.

BRETT, J. R. (1971). Energetic responses of salmon to temperature. A study of some thermal relations in physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerca*). *Am. Zool.* **160**, 264–66.

CALLEGARINI, C. & CUCCHI, C. (1968). Intraspecific polymorphism of haemoglobin in *Tinca tinca*. *Biochem. Biophys. Acta* **160**, 264–66.

CAMERON, J. N. (1971). Oxygen dissociation characteristics of the blood of the rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **38**A, 699–704.

EDDY, F. B. (1971). Blood gas relationships in the rainbow trout *Salmo gairdneri*. *J. exp. Biol.* **55**, 695–711.

EDDY, F. B. (1973). The role of the blood in respiratory gas exchange in the tench *Tinca tinca*. (In preparation.)

EDDY, F. B. & MORGAN, R. I. G. (1969). Some effects of carbon dioxide on the blood of the rainbow trout *Salmo gairdneri* Richardson. *J. Fish. Biol.* **1**, 361–72.

FINLEY, T. N., LENFANT, C. N., PIIPER, J. & RAHN, H. (1960). Venous admixture in pulmonary circulation in dogs. *J. appl. Physiol.* **15**, 418–24.

GAREY, W. F. (1970). Cardiac output of the carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.* **33**, 181–9.

GAREY, W. F. & RAHN, H. (1970). Gas tensions in the tissues of trout and carp exposed to diurnal changes in oxygen tension in the water. *J. exp. Biol.* **52**, 575–82.

GILLEN, R. C. & RIGGS, A. (1971). The haemoglobins of a freshwater teleost *Chiclasoma cyanoguttatum* (Baird & Girard). 1. The effects of phosphorylated organic compounds upon the oxygen equilibria. *Comp. Biochem. Physiol.* **34**, 585–95.

GRIGG, G. C. (1969). Temperature changes in the oxygen equilibrium curve of the blood of the brown bullhead, *Ictalurus nebulosus*. *Comp. Biochem. Physiol.* **28**, 1203–23.

HENDRICKS, J. L. (1952). Erythrocyte counts and haemoglobin determinations in two species of suckers, genus *Catostomus* from Colorado. *Copeia* **4**, 255–6.

HOUSTON, A. H. & DEWILDE, M. A. (1968). Thermoacclimatory variations in the haematology of the common carp, *Cyprinus carpio*. *J. exp. Biol.* **49**, 71–81.

HOUSTON, A. H., REAVES, R. S., MADDEN, J. A. & DEWILDE, M. A. (1968). Environmental temperature and the body fluid system of the freshwater teleost. I. Ionic regulation in the thermally acclimated rainbow trout *Salmo gairdneri*. *Comp. Biochem. Physiol.* **25**, 63–81.

HOWELL, J. B., BAUMGARDNER, F. W., BONDI, K. & RAHN, H. (1970). Acid-base balance in cold blooded vertebrates as a function of body temperature. *Am. J. Physiol.* **218**, 600–5.

HUGHES, G. M. & SHELTON, G. A. (1958). The mechanism of gill ventilation in three freshwater teleosts. *J. exp. Biol.* **35**, 807–23.

HUGHES, G. M. & SHELTON, G. (1962). Respiratory mechaniams and their control in fish. In *Advances in Comparative Physiology and Biochemistry*, vol. 1 (ed. O. Lowenstein), pp. 275–364. London: Academic Press.

IRVING, L., BLACK, E. C. & SAFFORD, V. (1941). The influence of temperature upon the combination of oxygen with the blood of the trout. *Biol. Bull. mar. biol. Lab., Woods Hole* **80**, 1–17.

JOHANSEN, K. (1970). Air breathing in fishes. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 361–411. New York and London: Academic Press.

JONES, D. J. (1972). *Comparative Physiology of Respiration*. London: Edward Arnold.

MATTOCK, G. (1959). Electrochemical aspects of blood pH measurements. In *A Synopsis on pH and Blood Gas Measurement* (ed. R. F. Woolmer), pp. 19–33. London: J. & H. Churchill.

MATTOCK, G. (1962). Laboratory pH measurements. In *Advances in Analytical Chemistry and Instrumentation*, vol. 2 (ed. C. N. Reilly), 35–121. New York: John Wiley.

NOBLE, R. W., LAWRENCE, J. P. & GIBSON, Q. H. (1970). The effects of pH on the reactions of oxygen and carbon monoxide with the haemoglobin of the carp *Cyprinus carpio*. *J. biol. Chem.* **245**, 6628–33.

RAHN, H. (1967). Gas transport from the external environment to the cell. In *Development of the Lung* (ed. A. V. S. de Reuck and R. Porter), pp. 3–23. *Ciba Fdn Symp.* London: J. and A. Churchill.

RANDALL, D. J. (1966). The nervous control of cardiac activity in the tench (*Tinca tinca*) and in the goldfish (*Carassius auratus*). *Physiol. Zool.* **39**, 185–92.

RANDALL, D. J. & SHELTON, G. (1963). The effects of changes in environmental gas concentrations on the breathing and heart rate of a teleost fish. *Comp. Biochem. Physiol.* **9**, 229–39.

RAPOPORT, S. & GUEST, G. M. (1941). Distribution of acid soluble phosphorus in the blood cells of various vertebrates. *J. biol. Chem.* **138**, 269–82.

RIGGS, A. (1970). Properties of fish haemoglobins. In *Fish Physiology*, vol. 4 (ed. W. S. Hoar and D. J. Randall), pp. 209–52. New York and London: Academic Press.

ROOT, R. W. (1931). The respiratory function of the blood in marine fishes. *Biol. Bull. mar. biol. Lab., Woods Hole* **61**, 427–56.

SATCHELL, G. S. (1971). *Circulation in Fishes*. Cambridge University Press.

SCHUMANN, D. & PIIPER, J. (1966). Der Sauerestoffbedarf der Atmung bei Fischen nach Messungen an dernarkotisierten Schleie (Tinca tinca). *Pflügers Arch ges. Physiol.* **288**, 15–26.

SENDROY, J., DILLON, D. T. & VAN SLYKE, D. D. (1934). Studies of gas and electrolyte equilibria in blood. XIX. The solubility and physical state of uncombined oxygen in the blood. *J. biol. Chem.* **105**, 597–632.

SIGGAARD-ANDERSEN, O. (1964). *The Acid-base Status of the Blood*. Copenhagen: Munksgaard.

SMITH, L. S. & BELL, G. R. (1964). A technique for prolonged blood sampling in free swimming salmon. *J. Fish. Res. Bd Can.* **21**, 329–37.

TUCKER, V. A. (1967). Method for O_2 content and dissociation curves on microlitre blood samples. *J. appl. Physiol.* **23**, 410–14.

WILKINS, N. & ILES, T. (1966). Haemoglobin polymorphism and its ontogeny in the herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). *Comp. Biochem. Physiol.* **17**, 1141–58.

WINTROBE, M. M. (1956). *Clinical Hematology*, 4th ed. Lea & Febiger, Philadelphia.