J. Exp. Biol. (1961), 38, 93–107
With 1 text-figure
Printed in Great Britain

THE OXYGEN CAPACITY OF GOLDFISH (*CARASSIUS AURATUS* L.) BLOOD IN RELATION TO THERMAL ENVIRONMENT*

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(*Received* 26 *July* 1960)

INTRODUCTION

The exchange of respiratory gases between vertebrate animals and their environment always takes place through water and usually involves specialized structures such as lungs or gills. The transport of oxygen from the site at which it is acquired to the remaining parts of the body in which it is needed is the primary function of the blood. It is generally held that the presence of a respiratory pigment facilitates this function of the blood. The same pigment, haemoglobin, is found in the blood of all vertebrates, but, as Barcroft (1928) pointed out, no two haemoglobins appear to be the same.

When the conditions under which the respiratory exchange occurs are considered, it is evident that fish encounter greater variations in such important factors as hydrogen-ion concentration, oxygen concentration and temperature, than any air-breathing vertebrate. This is particularly true for freshwater species. Knowledge of the relationship between the biochemistry of haemoglobin and mammalian respiration raised questions regarding the adaptation of fish to the situations presented by their habits and habitats. Temperature, for instance, is known to have a marked effect upon the oxygen dissociation curve of mammalian blood (Krogh, 1941). By analogy, the oxygen transporting efficiency of fish blood would be expected to fall with decreasing temperature, yet many fish remain active over a fairly wide thermal range—particularly if the transition from one temperature to another is not too abrupt. Thus fish offer promising material with which to combine studies of ecology and physiology. A number of reviews deal with the literature relevant to the physiology of fish respiration; the more recent are by E. C. Black in Hoar, Black & Black (1951) and Fry in Brown (1957).

The general problem consists of relating the behaviour of the intact fish to the properties of its blood *in vitro*. The blood of fish exhibits a number of interspecific differences, including haemoglobin content (Hall & Gray, 1929), affinity for oxygen, and the extent to which the oxygen dissociation curve is displaced by carbon dioxide (Krogh & Leitch, 1919; Root, 1931; Willmer, 1934; Black, 1940).

* This study was carried out under tenure of scholarships from the National Research Council of Canada, and with assistance during one year from Department of Veterans Affairs, Canada.

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In addition to these biochemical differences, Hart (1943, 1944) showed that the amount of blood pumped by each beat of the heart varied with the species.

The term *Bohr effect* has been borrowed from mammalian physiology to describe the effect of carbon dioxide on the oxygen dissociation curve of the blood, although it is by no means certain that the mechanisms involved are the same. The greater sensitivity of fish blood in this respect prompted a number of studies of the effect of carbon dioxide on oxygen uptake by intact fish (Fry & Black, 1938; Irving, Black & Safford, 1939; Safford, 1940). These investigators sealed fish in bottles of water containing oxygen at atmospheric tension and varying amounts of carbon dioxide, left them to asphyxiate, and then measured residual oxygen and carbon dioxide tensions. Plotting these residual tensions of the two gases against one another, resulted in curves characteristic for a given species, and they appeared to bear the expected relationship to the sensitivity of the blood. The term *respiratory tolerance* has been used (Irving *et al.* 1939) to describe the ability of fish to remove dissolved oxygen from water in presence of carbon dioxide, but it could equally well apply to a tolerance of potassium cyanide. The specific term *carbon dioxide tolerance* is more descriptive of the phenomenon, and is used here.

The general relationship between these features of teleost respiration as revealed by previous investigations is shown in Table 1.

Table 1. *The general relationship between the habitats of fish and certain features of their respiratory physiology*

			Characteristics of the blood *in vitro*		
Ecological group	CO_2 tolerance	Stroke output of the heart	Hb content	Affinity for O_2	Sensitivity to CO_2
Cold-water species, e.g. trout	Low	Low	High	Low	High
Warm-water species, e.g. bullhead	High	High	Low	High	Low

Fry, Black & Black (1947) noted a seasonal change in the carbon dioxide tolerance curve of certain minnows, and reasoned that this change might be induced by differences in thermal environment. They tested this point by a series of asphyxiation experiments, using goldfish acclimatized to various temperatures over their thermal range, and found goldfish at higher temperatures to have greater carbon dioxide tolerance. Thus changes in thermal environment had induced, in the one species, differences previously considered to be interspecific.

The asphyxiation experiments do not indicate the manner in which thermal environment affects carbon dioxide tolerance, but Fry *et al.* (1947) suggested that their results might reflect changes in either the circulation of the blood or the chemistry of oxygen transportation.

It appeared that the blood of thermally acclimatized fish should be examined from at least three aspects: (1) oxygen capacity, (2) oxygen affinity, and (3) effects

of carbon dioxide upon the oxygen dissociation curve. This paper reports the results of measuring oxygen capacity of blood from goldfish acclimatized to temperatures near the extremes of their range.

MATERIAL AND METHODS

The oxygen capacity of blood is the amount of oxygen per unit volume with which it combines when fully saturated with that gas. Saturation is normally achieved with oxygen at its atmospheric pressure. Oxygen capacity is commonly expressed as volumes per cent (ml. O_2/100 ml. blood). The measurement may be made as O_2, Hb (haemoglobin) or Fe, and the following relationship between the three is generally accepted for mammalian blood:

Fe (mg. %)	Hb (g. %)	O_2 (vol. %)
1·00	0·298	0·40
3·36	1·000	1·34
2·50	0·744	1·00

The relationship shown above assumes that gasometric measurements are corrected for dissolved oxygen. For purposes of calculations in this paper, it is also assumed that this relationship is valid for fish blood, although direct clarification, which does not appear to have been made, is obviously desirable.

Experimental animals

Goldfish were chosen for these experiments because both their carbon dioxide and thermal tolerance, as well as their capacity for acclimatization, were known (Fry, Brett & Clawson, 1942; Fry, *et al.* 1947; Brett, 1946). They are also readily available and easily maintained.

A maximum of twelve to fifteen fish, averaging about 9 cm. in length, were kept in each of six aquaria measuring 12 × 12 × 20 in. and containing approximately 36 l. of water. The temperatures were controlled within ± 0·1° C. at 5, 10, 15, 20, 25 and 30° C.—or, as stated in the appropriate places, at temperatures near these levels. The constant temperature aquaria have been described by Brown (1951). Air bubbled vigorously through the water kept it thoroughly stirred and fully aerated.

The fish were fed a mixture of beef liver and baby food (Farex) prepared exactly as described by Farris (1950). Those at 5° C. were fed once per week; the remainder were fed every other day. The amount given was what they appeared to eat readily. Excess food and droppings were siphoned from the aquaria periodically.

Brett (1946) showed that acclimatization to thermal change is itself a function of temperature; i.e. acclimatization occurs more slowly at the cold end of the range than at the warm. His findings were borne in mind in acclimatizing fish used in these experiments. The fish were held at 10° C. for 2–3 weeks before transfer to the 5° C. aquarium, and no fish were used from that aquarium until they had been there a minimum of 3 weeks. The minimum acclimatization period for 15° C. and higher temperatures was 1 week.

Collection of blood samples

Blood from larger species of fish from local rivers and streams was used in working out analytical techniques. It was found that several analytical procedures applicable to mammalian blood were unsuitable for fish blood.

All fish were rendered unconscious before removal of blood. Larger fish were struck on the head, while for smaller ones one of two anaesthetics was used. Five per cent procaine hydrochloride was administered directly to the brain by hypodermic syringe after Kisch (1947). This rapidly produces unconsciousness without stopping the heart. Alternatively, 5 % urethane was administered by placing the fish in a bath until it lost consciousness. This method proved most satisfactory with goldfish. Blood was collected directly from the heart or ventral aorta. The pericardial cavity was exposed from the ventral side and the area dried with filter-paper. A paraffined needle attached to a small polyethylene tube was inserted, usually into the bulbus or ventral aorta, and the heart was allowed to pump out blood into a small polyethylene vessel under slight negative pressure applied by mouth. With very small fish the needle was inserted into the ventricle. Clotting was prevented by dusting the interior of the collecting apparatus with dry heparin. Goldfish at 5° C. often lacked sufficient cardiac vigour to pump blood into the tube. Where this occurred, the pericardial cavity was blotted dry, an incision was made in the heart, and the blood collected by suction as it leaked into the surrounding cavity, the area having been dusted with heparin.

Measurement of oxygen capacity

Attempts to measure the oxygen capacity of blood using the Van Slyke manometric apparatus and the method of Sendroy (1931) were disappointing because of lack of consistency in replicates. Following Prof. Roughton's suggestion, the carbon monoxide method was adopted, with minor modifications from Roughton & Root (1945). In what follows, the terms CO capacity and O_2 capacity are taken to be synonymous and used interchangeably.

Because of discrepancies between Van Slyke results and alkaline haematin analyses, to be described, it was thought advisable to check whether the van Slyke method could extract all the CO combined with the blood. This was not a question of time, since it had been ascertained that the 5 min. period was adequate for extraction to constant volume. It was a question of whether there was an unextractable fraction. The method was to reduce a sample of blood completely, then to add a known volume of CO, to measure the quantity of CO remaining after absorption by the blood, and finally to conduct a Van Slyke analysis. Results are shown in Fig. 1 A, and they indicate that the Van Slyke method as commonly used is entirely reliable. Hence it was taken as a standard to which other methods of analysis could be compared. The need for another method for measuring oxygen capacity arose from the fact that 0·1 ml. of blood was required for each measurement by the Van Slyke method, and this quantity was as much or more blood than could be obtained from each of the experimental fish. Preliminary experiments indicated

that oxygen capacity measurements were not comparable unless measurements of cellular content were also made concurrently. These considerations made it necessary to find a method whereby oxygen capacity could be determined using not more than 0·025 ml. of blood. Alternate methods that were tried included measurement of the iron content of the blood and several methods common to clinical haemoglobinometry.

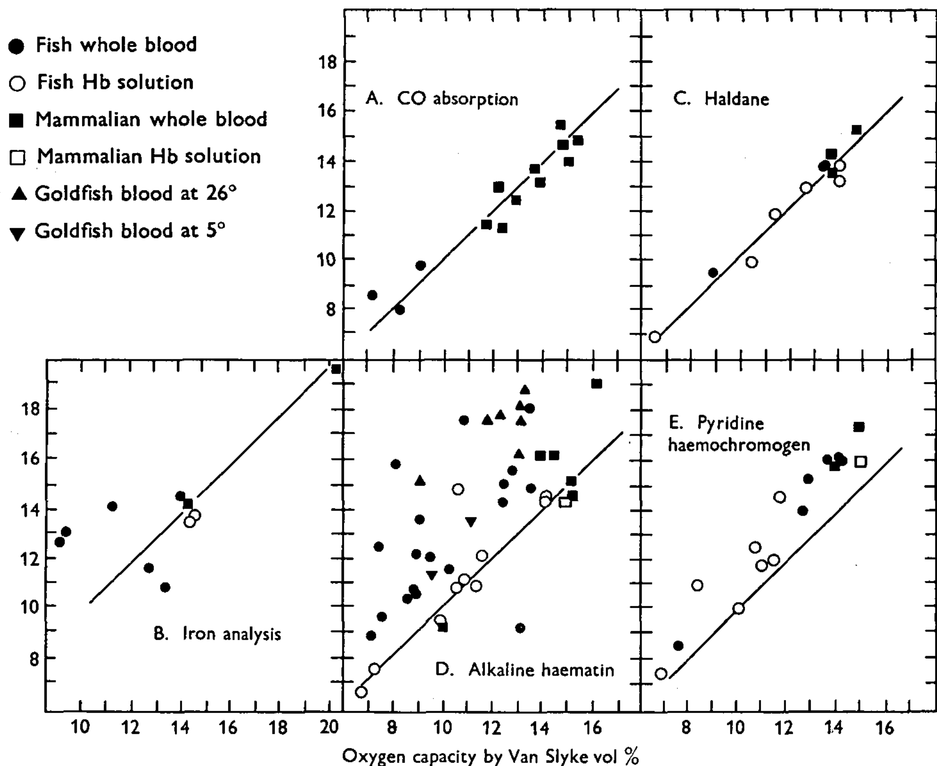

Fig. 1. Comparison of various methods of measuring O_2 capacity of blood. The standard method, gasometric determination of CO capacity with van Slyke apparatus, is checked against CO absorption in part A. In the remaining parts, various photometric methods of measuring Hb content are compared with the standard. Values are expressed as O_2 capacity in vol. %. Lines are drawn through points of equal capacity.

In dealing with mammalian blood, the measurement of iron content has frequently been taken as the standard to which other haemoglobinometric methods are compared, the oustanding instance being Harington (1952). Numerous methods have been prescribed for measuring iron in biological material and several were tried with goldfish blood. All of these methods begin with ashing the sample, either by incineration (dry ashing) or by means of powerful oxidizing agents such as HNO_3, H_2SO_4, $HClO_4$, H_2O_2, etc., used either singly or in various combinations (wet ashing). Ashing is followed by redissolving the iron, if necessary, and adjustment of pH and/or ionic state, depending upon the method by which the quantity of iron

is to be measured. Results presented in Fig. 1 B were obtained by wet ashing followed by photometric measurement using an absorptiometer and either α-α'-dipyridyl (Hill, 1931) or catechol-3 (the disodium salt of 1,2-dihydroxybenzene-3,5-disulphonate) (Yoe & Jones, 1944) as an indicator. The latter indicator was favoured because it is specific for ferric ion, hence its use precluded reduction, a step that frequently resulted in cloudy solution.

The quantity of Hb in blood is a diagnostic observation in human medicine, and it has long been considered important for the practitioner to be able to make reasonably accurate measurements of the pigment. Numerous methods and related apparatus have been developed in response to this need. Photometric methods involving five Hb derivatives were tried in the course of the present work: alkaline haematin, carboxy-haemoglobin (Haldane), cyanhaematin, oxyhaemoglobin, and pyridine haemochromogen. All but the latter derivative were extensively tested by the Medical Research Council (MRC) (Harington, 1952). Their recommendations as to preparing the derivative and selecting a suitable filter for light absorption measurements was followed closely. A brief description of these methods follows:

Alkaline haematin method

0·023 ml. of blood was added to 4·975 ml. of N/10 NaOH with rapid stirring and the solution heated for 4 min. in boiling water. After cooling, the solution was transferred to a 1 cm. optical cell and read in an absorptiometer using Ilford Spectrum filter 605. For most of the determinations, an artificial standard (Gibson & Harrison, 1945) was used. Five ml. of this standard were heated along with the blood samples, then cooled and read in the same cells. A natural haematin standard was prepared by dissolving 38·0 mg. of haematin hydrochloride (B.D.H.) containing 8·46 % iron in about 100 ml. of borate buffer (19·07 g. $Na_2B_4O_7 . 10H_2O$ and 100 ml. of N/1 NaOH per litre), leaving it in the refrigerator to 'age' for 2 days, and then making it up to 500 ml. with the same buffer. Results with either standard agreed within 1 %.

Cyanhaematin method

0·025 ml. of blood was added to 2·5 ml. of N/10 HCl, stirred into solution, and allowed to stand for 15 min. 2·5 ml. of N/10 NaOH containing 2 % NaCN was added, and the solution was thoroughly mixed. It was then transferred to 1 cm. optical cell and read in an absorptiometer using Ilford Spectrum filter 605. Standard cyanhaematin solution was prepared by dissolving 14·6 mg. of crystalline haematin containing 8·46 % iron in 500 ml. of N/10 NaOH containing 1 % NaCN.

Haldane (COHb) method

0·025 ml. of blood was added to dilute ammonia (0·4 ml. conc. NH_4OH per litre) in a standard Haldane tube and the volume was immediately made up to the graduation with the dilute ammonia. The tube was then stoppered with a finger tip and the blood was shaken into solution. A minute amount of capryl alcohol was used to prevent foaming while pure CO was bubbled through the solution for 2 min.

The sample solutions were compared with a standard Haldane solution sealed in a matching tube. A special metal adaptor enabled these comparisons to be made in the absorptiometer using Ilford Spectrum filter 605.

Oxyhaemoglobin method

In some instances the blood samples were treated as in the Haldane method except that neither capryl alcohol nor CO were added. No permanent standard is available, but the Haldane standard was used for comparison and its equivalence derived by a correction factor in accordance with the MRC method.

Alternatively, 0·025 ml. of blood was added to 4·975 ml. of the dilute ammonia as used in the Haldane method and stirred rapidly into solution. The solution was then transferred to a 1 cm. optical cell and read in an absorptiometer with Ilford Spectrum filters 604 or 605. The concentration of Hb was then computed from the optical density by employing the MRC factors 0·330 and 0·498 as the respective optical densities with the two filters of blood containing 14·8 g. % Hb and diluted 1:200.

Pyridine haemochromogen method

The following method was based upon that of Collier (1944). 0·025 ml. of blood was added to 4·975 ml. of N/10 NaOH and the solution was heated and cooled as in the alkaline haematin method. 2 ml. of pyridine were added to the solution and, after thorough mixing, it was left to stand for 30 min. It was then reduced by the addition of about 5 mg. of $Na_2S_2O_4$, transferred to a 1 cm. optical cell, and read in the absorptiometer with Ilford Spectrum filter 604. 0·25 ml. of standard haematin solution was treated in the same manner. The standard solution was prepared by dissolving 66 mg. of crystalline haematin hydrochloride containing 8·46 % iron in 100 ml. of N/10 NaOH. Since the standard was 1 mM with respect to iron, it was theoretically equivalent to an oxygen capacity of 22·4 vol. %, but the total quantity of liquid in the final dilutions of blood and standard samples differed, being 7·000 for blood and 7·225 for the standard. Consequently, the standard was taken to be equivalent to an oxygen capacity of $22{\cdot}4 \times 7{\cdot}000/7{\cdot}225 : 21{\cdot}70$ vol. %.

In using both the iron method and the clinical methods, advantage was taken of a service of the MRC whereby subscribers may obtain periodically samples of blood of which the Hb content has been measured by the best modern techniques. These samples, presumably human blood, make up some of the points in Fig. 1. All photometric measurements were made with a Hilger 'Spekker' Absorptiometer.

Measurement of cellular content

The erythrocytes were enumerated in the standard manner using a Thoma diluting pipette and a haemocytometer slide. At first Hayem's diluting fluid was used, but subsequently that of George (1952) was found to be more satisfactory. The concentration of stain was reduced by a factor of four in order to get better results with fish blood.

Microhaematocrit tubes were used to determine the percentage of erythrocytes in the blood. They were centrifuged at 3000 rev./min. for 30 min. Duplicates usually agreed within 1 %.

It should be emphasized that, in dealing with the experimental fish, all three measurements—oxygen capacity, haemocytometer and haematocrit—were made upon aliquots from the same sample of blood. Hb solutions were prepared from fish blood by laking erythrocytes that had been washed 3 times in about 10 vol. of cold 0·9 % NaCl with gentle centrifugation. Laking was brought about either by alternate freezing and thawing in 10 vol. of the saline solution or by adding 1 vol. of cells to 10 vol. of distilled water while stirring rapidly. The cellular debris was then removed by centrifugation. Solutions of mammalian Hb prepared in this manner are said to be about 95 % pure.

From haemocytometer counts of the number of red blood cells (RBC), the percentage of RBC in the blood (haematocrit), and the oxygen capacity of the whole blood, the following values were derived as shown:

(1) Mean corpuscular volume:

$$\text{MCV} = \frac{\%\ \text{RBC} \times 10}{\text{Millions of RBC/mm.}^3} = \mu^3.$$

(2) Oxygen capacity of 100 ml. of erythrocytes:

$$\text{RBC} = \frac{\text{Whole blood capacity} \times 100}{\%\ \text{RBC}} = \text{vol. }\%.$$

(3) Oxygen capacity of single cells:

$$\frac{\text{Whole blood capacity} \times 10}{\text{Millions of RBC/mm.}^3} = \mu^3.$$

RESULTS

The results of tests to find a micro-method for measuring oxygen capacity are shown in Fig. 1. Fig. 1 B shows that iron analyses agree with Van Slyke measurements upon mammalian blood and fish Hb solutions, but exhibit considerable scatter when whole fish blood is used. While the results are too few to be conclusive, they draw attention to the fact that difficulties attending the use of iron content as a basis for haemoglobinometry are not limited to analytical techniques, but extend to interpretation of results. The scatter may have been due to varying amounts of non-Hb iron. Part of this non-Hb iron is in the plasma, but, as Lemberg & Legge (1949) point out, a larger portion is contained within the erythrocytes. The observation of Catton (1951) that there is a greater amount of debris in films of fish blood prepared for histological examination than in corresponding preparations of mammalian blood, has been confirmed in the present work. There appeared to be more 'ghosts' and general debris in haemocytometer preparations from fish blood than from human or sheep blood, and this was so even where clarity of plasma in haematocrits showed that no haemolysis had occured. If the debris results from

erythrocytes breaking down in the blood stream, then presumably the Hb becomes denatured, and the scatter observed in results of iron analysis may reflect Catton's morphological observation. In any event, the measurement of iron content of fish blood was not considered to be a suitable alternative to the Van Slyke method of measuring oxygen capacity.

The use of the alkaline haematin method for determining Hb in mammalian blood is fairly well established from reports of the MRC and from the work of Gibson & Harrison (1945), who prepared an artificial standard that is commercially available. The results of the present work are shown in Fig. 1 D and they indicate that while the method is satisfactory for mammalian blood and fish Hb solutions, with fish blood it gives very erratic results that tend to be much higher than Van Slyke.

There have been conflicting reports of the suitability of the alkaline haematin method to other than mammalian blood. Bankowski (1942), for instance, concluded that it was not satisfactory for avian blood, which resembles fish blood in having nucleated erythrocytes. From the present results it is thought to be unsuitable for fish blood.

The cyanhaematin method proved unsuitable because of difficulty with the first step, the conversion of Hb in the blood to acid haematin. Fish blood mixes more readily with acid than with alkaline solution, but the preparations frequently become cloudy. This is scarcely surprising inasmuch as Hb denatured by acid does not form a true solution but a colloidal suspension (Lemberg & Legge, 1949). The acid haematin method has been widely used in clinical medicine, where it is associated with the name of Sahli (Wintrobe, 1942), and has frequently been applied to non-mammalian blood, e.g. Schlicher (1926), Dombrowski (1953) and Black (1955). Admirable ingenuity has been displayed by some workers attempting to avoid or overcome the difficulties of applying the acid haematin method (Dukes & Schwarte, 1930; Elvehjem, 1931), but it appears simpler and more advisable to abandon the method.

The most obvious derivative to use is oxyhaemoglobin, but its use in an absorptiometer suffers from lack of a permanent standard. The MRC have attempted to overcome this lack by publishing what amount to extinction coefficients for a number of filters used in conjunction with certain Hb derivatives in both visual and photoelectric photometers. While the use of extinction coefficients is well established in spectrophotometry where very narrow portions of the spectrum are used, it has not been widely practised in obsorptiometry where exact reproducibility of filter characteristics is regarded with some doubt. This method was tried both by using the MRC factors for Ilford filters 604 and 605 directly, with the solution in 1 cm. cuvettes, and also by using the Haldane tubes and correcting the value of the Haldane standard by means of the MRC factors. Both of these methods gave good results, nevertheless more confidence was felt in using a method for which a permanent standard was available.

Results of the Haldane method (Fig. 1 C) were found to agree consistently with the Van Slyke methods; indeed the two methods appear to be interchangeable.

The pyridine haemochromogen method is not as convenient as the others in the series, but was found to be quite simple in practice, and gave precise results as is shown in Fig. 1E. All results fell above the line of theoretical agreement with the Van Slyke method, but agreement is achieved by multiplying the pyridine haemochromogen values by 0·878. Theoretically the Haldane method should be better than the pyridine haemochromogen one for measuring oxygen capacity, since any so-called 'inactive' fractions of Hb present in blood would be converted to haemochromogen. It is of interest to note that the unidentified substances which interfered with the alkaline haematin method appeared to have no effect upon measurements of pyridine haemochromogen.

Table 2. *Measurements on blood of pike and roach*

These fish had not been acclimatized to any specific temperature, but had been taken from the river and kept in aquaria at temperatures ranging from 10 to 15° C. The column headings have the following meanings: size is recorded as length in cm., weight in g. RBC 10^6 is the haemocytometer count in millions of erythrocytes per mm.^{3} of blood. Crit is the haematocrit reading in % erythrocytes. MCV (μ^3) is mean corpuscular volume in cubic microns; its derivation is shown in the text. Oxygen capacities are recorded in % by volume for whole blood and erythrocytes (cells), and in cubic microns for single cells. Capacity of the blood was measured gasometrically as carbon monoxide capacity and each recorded value is a mean of two or more determinations.

					Oxygen capacities		
	Size (cm./g.)	RBC (10^6)	Crit (%)	MCV (μ^3)	Whole blood (%)	Cells (%)	Single cell (μ^3)
Pike	38/417	1·7	27·0	159	8·07	30·2	48
	36/384	1·8	27·3	152	8·91	32·6	50
	32/224	1·8	30·8	171	9·67	31·4	54
	38/374	1·7	28·8	156	8·99	33·5	53
	39/371	1·4	31·7	223	7·65	25·5	54
	35/411	1·7	30·3	180	9·55	30·1	57
	37/363	1·6	29·5	185	9·86	33·4	62
Mean	36/364	1·7	29·1	175	8·96	31·0	54
Roach	23/207	2·0	48·5	242	14·50	29·9	73
	25/274	2·1	56·5	269	14·90	26·4	71
	23/195	1·7	43·0	253	11·53	26·9	68
	26/241	1·9	36·0	189	12·45	34·6	65
Mean	24/229	1·9	46·0	238	13·35	29·5	69

Records of measurements on the larger species of fish are included in Table 2. They had not been acclimatized to any particular temperature, but are included to give an indication of the differences in oxygen capacity shown by different members of the same species, and to show that the variation is reduced, hence the results made more comparable, if the erythrocyte content of the blood is taken into account. Thus oxygen capacity measurements on pike whole blood have a standard deviation of $\pm 16\%$. This deviation is reduced to about half if cellular content is considered; the corresponding measurements of the oxygen capacity of the erythrocytes have a standard deviation of $\pm 9\%$.

Time did not permit measurements of the capacity of goldfish blood acclimatized to a complete series of temperatures. The observations were therefore limited to temperatures near each end of the range, in the hope of obtaining maximal effects.

Some difficulty was experienced in maintaining fish in a condition of good health at temperatures below 10° C. Some individuals in the cold, which appeared to be otherwise healthy, had blood that was abnormal in appearance. Only values obtained from blood of normal appearance from fish of normal appearance are quoted in Table 3.

Table 3. *Measurements on blood of thermally acclimatized goldfish*

The column headings have the same meanings as for Table 2. The capacity measurements were made upon whole blood by the Haldane (carboxyhaemoglobin) method using an absorptiometer and each value is a mean of two or more determinations.

	Size (cm./g.)	RBC (10^6)	Crit (%)	MVC (μ^3)	Oxygen capacities: Whole blood (%)	Oxygen capacities: Cells (%)	Oxygen capacities: Single cell (μ^3)
Goldfish acclimatized to 5° C.							
	8·0/ 8·3	2·1	39·3	184	11·20	28·6	52
	8·0/ 8·9	2·0	35·9	178	10·67	29·7	53
	8·8/12·1	1·7	32·5	188	8·92	27·5	52
	—	2·0	32·5	166	9·71	29·8	50
	—	2·2	31·0	144	10·60	34·2	48
Mean	8·2/9·1	2·0	34·2	172	10·22	30·0	51
Goldfish acclimatized to 6° C.							
	10·4/20·3	1·2	24·8	212	6·69	27·0	57
	9·0/ —	2·2	40·0	180	9·75	24·4	44
	11·0/26·5	2·7	45·5	171	15·54	34·1	58
	9·8/19·5	2·1	33·5	163	13·18	39·3	64
Mean	10·1/22·1	2·1	36·0	181	11·29	31·2	56
Goldfish acclimatized to 26° C.							
	10·8/25·0	2·1	41·0	193	13·20	32·2	62
	9·5/19·5	1·6	31·4	193	9·20	29·3	56
	9·5/17·5	1·6	36·7	233	13·11	35·7	84
	9·5/18·5	1·7	38·7	226	13·11	33·9	77
	8·7/15·5	1·7	41·0	240	12·31	30·0	72
	9·8/19·0	1·6	39·3	240	13·31	33·9	81
	9·3/17·1	1·7	34·1	205	11·78	34·5	71
Mean	9·6/19·0	1·7	37·5	219	12·29	32·9	72
Goldfish acclimatized to 30° C.							
	9·4/12·6	1·3	25·0	188	6·65	26·6	50
	9·6/15·5	1·8	33·0	185	9·36	28·3	52
	9·4/16·2	2·0	38·3	192	12·50	32·6	63
	9·8/14·2	1·8	29·5	165	9·96	33·8	56
	9·8/14·9	1·9	28·5	152	8·59	30·2	46
Mean	9·6/14·7	1·8	30·9	176	9·41	30·3	53

The peculiar features which appeared in blood of some of the fish at low temperatures included low counts and low haematocrit values. The blood also was sometimes 'fragile', that is, exhibited considerable spontaneous haemolysis, usually without clotting. Excessive amounts of cellular debris and white cells were observed in the haemocytometer preparations. The erythrocytes also appeared to have an unusual refractive index, for they were nearly invisible in the counting fluid.

The results of measuring the oxygen capacity of blood from thermally acclimatized goldfish are shown in Tables 3 and 4, the former showing the variation from fish to fish. Before discussing these results, consideration should be given to what might be expected by way of changes in oxygen capacity.

Changes in the Hb content of blood in response to the environment might come about either by a change in the number of erythrocytes or by a change in the Hb concentration of the individual cells. Lemberg & Legge (1949) point out that the mean corpuscular Hb concentration is remarkably constant even with different vertebrate species, and in spite of variations in size and number of erythrocytes. This observation suggests that the second possibility is unlikely to be the basis of the mechanism. Numerous observations have shown that an increase in altitude of residence brings about an increase in oxygen capacity of the blood of various mammals, and that this change is in the number of erythrocytes (Prosser, Bishop, Brown, John & Wulf, 1950). In the experiments upon mammals, the oxygen tension has been lowered: in the present work, oxygen tension remained constant at about 155 mm. Hg, while the environmental change was via temperature. When mammals move to higher altitudes, oxygen demand is constant in the face of diminishing tension, whereas with fish living at increasing temperatures the oxygen demand is increasing in face of constant tension. The result could conceivably be the same; a drop in tissue oxygen which presumably would stimulate an increase in the oxygen capacity of the blood. Whether or not the point of stimulation is reached depends for mammals upon the increase in altitude, and for fish it may depend upon the increase in temperature. With men the point is passed in something less than 15,000 ft. altitude (Reynafarje, Berlin & Lawrence, 1954); with fish we do not know whether they can reach a temperature which would have a corresponding stimulatory effect. It is possible that fish may be unable to acclimatize to a temperature at which oxygen at a tension of 150 mm. will not meet the demand. It might be predicted from these considerations that if a change in the oxygen capacity of fish blood did occur with increase in temperature, it would be in the direction of increased capacity.

When measurements upon thermally acclimatized goldfish are reduced to oxygen capacities per 100 ml. of cells, it is apparent that no marked change occurs in the cellular content of Hb over the range from 5° to 30° C. This might have been predicted from the observations of Lemberg & Legge (1949), although observations of the constancy in erythrocyte Hb concentration stem mainly from homoiotherms. Table 3 shows that there is a fairly wide spread in cell count and in whole blood capacity at either end of the thermal range, but it is also apparent that any differences that exist at the temperature extremes are small and that differences resulting from a change of a few degrees in environmental temperature could not be discerned in these results. Hence all of the results have been placed in two groups, 'Warm' and 'Cold', in Table 4.

Calculations based upon Table 4 show that there is a significantly higher red cell count in the cold group of goldfish. This is not reflected in the whole blood capacity, for while the number of erythrocytes has increased in the cold group, their average size is smaller, with the net result of no change in capacity. It is noteworthy that the changes in question are so small as to require statistical tests to establish their validity. It follows from what has been said that there must be some difference in the oxygen capacity, and hence Hb content, of the individual

Table 4. *Summary of measurements on blood of thermally acclimatized goldfish*

The data from Table 3 are grouped at 'cold' (5 and 6° C.) and 'warm' (26 and 30° C.) for comparative purposes. *T*-tests ($P = 0.05$) show the values marked with an asterisk to be significantly different.

Group	No. of fish	RBC (10^6)	Crit (%)	MCV (μ^3)	Oxygen capacities Whole blood (%)	Cells (%)	Single cell
Cold	9	2·0*	35·0	176*	10·70	30·5	53*
Warm	12	1·7*	34·7	201*	11·09	31·7	64*

erythrocytes. It might appear that this statement is at variance with the observation of Lemberg & Legge (1949) and the conclusion reached in considering the capacities per 100 ml. of cells in the current work, but such is not so. The absolute amount of Hb per cell (mean corpuscular Hb, usually expressed in microgrammes per cell) may vary—i.e. small cells contain less Hb—but the concentration of Hb within each cell (mean corpuscular Hb concentration, expressed as %) remains fairly constant.

It should be mentioned that measurements by the alkaline haematin method upon the blood of the same goldfish showed an increase in 'alkaline haematin' content with increase in environmental temperature. Difficulties with this method have already been discussed. It was concluded that, if a true increase has been shown, it is an increase in non-haemoglobin substance.

SUMMARY

1. The Roughton & Root (1945) modification of the Van Slyke method for measuring haemoglobin content of blood as carboxyhaemoglobin is satisfactory for fish blood, provided at least 0·1 ml. is available.

2. Measurements of iron content are of doubtful value in estimating the oxygen capacity of fish blood.

3. The following photometric methods of determining haemoglobin content were found to be unreliable with fish blood: acid haematin, alkaline haematin and cyanhaematin.

4. The following photometric procedures gave satisfactory measurements of the haemoglobin content of fish blood: Haldane (carboxyhaemoglobin), oxyhaemoglobin and pyridine haemochromogen.

5. Blood from goldfish acclimatized to temperatures near the extremes of their thermal range shows no difference from normal blood in oxygen capacity.

I wish to thank Prof. F. J. W. Roughton, F.R.S., for his inspiring interest during the course of this work and Prof. Sir James Gray, F.R.S., for generous accommodation in the Zoology Laboratory. My attention was drawn to this problem by Prof. F. R. Hayes, F.R.S.C., and I am further indebted to him for assistance in preparing this paper.

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