

## BLOOD GAS RELATIONSHIPS IN THE RAINBOW TROUT *SALMO GAIIRDNERI*

By F. B. EDDY

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

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### INTRODUCTION

The equilibrium between mammalian haemoglobin and oxygen has been studied in detail. In the lower vertebrates, in particular the fishes, the oxygen equilibrium has not been studied as extensively. Krogh & Leitch (1919) found that fish blood exhibits the Bohr effect; Root (1931) showed that carbon dioxide caused the blood of certain marine fishes to become incompletely saturated with oxygen, even at high oxygen tensions (the Root effect).

Irving, Black & Safford (1941) showed that both effects are exhibited by the blood of salmonid fish. They constructed oxygen dissociation curves at various temperatures, but the partial pressures of carbon dioxide used were in general much higher than those normally encountered in the fish. The application of gas-exchange theory to the aquatic medium predicted that blood  $P_{\text{CO}_2}$  in fish would be lower than in higher vertebrates (Rahn, 1966; Hughes, 1964). Holeton & Randall (1967) found that at 15 °C the arterial blood of resting rainbow trout had a  $P_{\text{CO}_2}$  of 1-1.5 mmHg and venous blood had a  $P_{\text{CO}_2}$  of about 2.5 mmHg. In moderately exercised trout at 5 °C Stevens & Randall (1967) found that the venous  $P_{\text{CO}_2}$  rose to 8 mmHg. It therefore seems likely that changes of physiological significance in the oxygen dissociation curve of the trout occur at partial pressures of carbon dioxide between 1 and 8 mmHg.

Irving *et al.* (1941) found that in rainbow trout blood increasing temperature raised the partial pressure of oxygen necessary to half-saturate the blood ( $P_{50}$ ). However, prior to blood sampling the fish were not acclimated to the temperature at which the blood was equilibrated. Grigg (1969) showed that the blood oxygen affinity of bullheads was influenced by their thermal history. When equilibrated at the same temperature, blood from warm-acclimated bullheads showed an increased oxygen affinity compared with blood from cold-acclimated fish.

Rahn (1967) has indicated that temperature influences the blood pH of poikilotherms. Carp held at 30 °C had a blood pH of 7.6, while those held at 5 °C had a pH of nearly 8.0. This could be related to the effect of temperature on the ionization constant of water. These pH changes are quite large and their effect on blood oxygen affinity would be considerable; increasing pH increases the affinity of haemoglobin for oxygen (Barcroft, 1928).

The object of this work was to find out how the oxygen dissociation curve of the trout is influenced by the interaction of temperature,  $P_{\text{CO}_2}$ , and pH. A preliminary report of this work has been published (Eddy & Hughes, 1971).

## MATERIALS AND METHODS

Rainbow trout weighing between 300 and 600 g were obtained from a hatchery at Nailsworth, Gloucestershire. They were initially held in well-aerated water in 50 gal tanks at 15 °C; then some were transferred to similar tanks maintained at 6 °C and 20 °C. They were temperature-acclimated for at least 3 weeks before being removed from the tanks and anaesthetized with 50 mg/l MS 222 (Sandoz). Blood was removed from the dorsal aorta (Smith & Bell, 1964) into a heparanized syringe. Blood sampling normally took less than 5 min.

Oxygen dissociation curves were constructed by the mixing method described by Haab, Piiper & Rahn (1960). About 2 ml blood was transferred to each of two tonometers, similar to those described by Finley *et al.* (1960), but of about 10 ml capacity. The blood in one tonometer was equilibrated with nitrogen containing a known proportion of carbon dioxide and the other with air containing a similar proportion of carbon dioxide. The gases were first saturated with water vapour.

The gas mixtures were made up using gas mixing pumps (Wösthoff) and their content checked with a Lloyd Gallenkamp gas analyser.

The blood was equilibrated with the gas mixture for at least 30 min at the appropriate temperature. 0.5 ml samples were removed and analysed for oxygen by the method of Peters & van Slyke (1935); 50  $\mu$ l samples were taken to measure haematocrit (packed cell volume) using a microcapillary centrifuge (Hawksley) and pH using an Eschweiler micro pH electrode. Further samples of about 100  $\mu$ l were withdrawn from each tonometer and transferred in various proportions to a 250  $\mu$ l graduated Hamilton gas-tight syringe, whose dead space had been filled with mercury. Great care was taken to exclude air bubbles. The final quantity of blood in the syringe was normally 150  $\mu$ l; on shaking the syringe the mercury mixed the blood. The  $P_{O_2}$  of half the mixture was measured in an Eschweiler  $P_{O_2}$  electrode assembly; the remainder was transferred to the pH cuvette. Both cuvettes were maintained accurately at the appropriate temperature. The true oxygen saturation of the mixture could be calculated by making a correction for dissolved oxygen in the blood.

Normally between six and ten mixtures were used at each  $P_{CO_2}$ ; those representing approximately 50% saturation were determined in duplicate. Most curves were repeated 2–3 times using a different fish. The oxygen and pH electrodes were recalibrated at frequent intervals.

Several factors were found to give rise to discrepancies:

(i) When handled the blood showed a tendency to haemolyse, particularly at 20 °C; after  $P_{O_2}$  and pH measurements the blood samples were discarded. Black & Irving (1938) showed that rupture of the red cells caused large changes in the shape of the oxygen dissociation curve.

(ii) The most difficult part of the curve to determine was between 80 and 100% saturation. It is in this region that a small increase in saturation (and thus accuracy of mixing) caused a large change in  $P_{O_2}$ .

(iii) There was individual variation among fish, particularly in pH; under identical conditions blood from different fish varied by as much as 0.5 pH unit. This was most noticeable at  $P_{CO_2}$  of 1 mmHg and less.

RESULTS

The oxygen capacity of trout blood is reduced with increasing tensions of carbon dioxide (the Root effect). When the mixing method for oxygen dissociation curves is employed, it is necessary to know the blood oxygen capacity at each tension of carbon dioxide. The change in blood oxygen content with  $P_{CO_2}$  is shown in Table 1.

There is a large variation in individual oxygen capacities of rainbow trout. In individual experiments where the reduction in oxygen capacity at several different values of  $P_{CO_2}$  was determined, the reduction in percent oxygen saturation was similar to that shown in Table 1. Blood equilibrated with air (150 mmHg and about 0.3 mmHg  $P_{CO_2}$ ) was taken to be 100% saturated with oxygen; when equilibrated with higher tensions of carbon dioxide in air, the blood was less than 100% saturated, and the oxygen saturation of each mixture was reduced accordingly.

Table 1. *Oxygen capacity and percentage saturation of rainbow trout blood at various tensions of carbon dioxide and at different temperatures*

Temp. (°C)	$P_{CO_2}$ (mmHg)	$O_2$ capacity (vol. % $\pm 2$ S.E.)	No. of determinations	Saturation (%)
6	0.3	10.42 $\pm$ 0.44	14	100
	1.0	9.98 $\pm$ 1.64	5	96
	2.2	9.90 $\pm$ 1.72	6	95
	5.0	9.20 $\pm$ 0.66	5	88
	7.0	9.02 $\pm$ 1.42	4	86
15	0.3	8.93 $\pm$ 1.6	17	100
	1.0	8.74 $\pm$ 2.3	8	98
	2.0	8.35 $\pm$ 1.86	12	93
	3.6	8.01 $\pm$ 1.82	13	89
	7.5	7.49 $\pm$ 1.50	13	84
20	0.3	9.50 $\pm$ 1.06	7	100
	1-2.5	9.10 $\pm$ 3.04	4	95
	5-7	8.20 $\pm$ 1.68	7	87

*Oxygen dissociation curves*

Oxygen dissociation curves were constructed at 6, 15 and 20 °C, using carbon dioxide tensions of up to 7 mmHg. The results are shown in Table 2, and in Fig. 1A-C. The oxygen dissociation curves were drawn in the following way. All the data at any one  $P_{CO_2}$  and temperature were plotted with percent saturation as the ordinate and  $P_{O_2}$  the abscissa. A curve was then fitted to the points by eye.

The data in Tables 2 and 3 were used to construct Fig. 2A-C. These are similar to the  $O_2$ - $CO_2$  diagrams for respiratory gas-exchange analysis devised by Rahn & Fenn (1955). In Fig. 2,  $P_{O_2}$  is the abscissa and  $P_{CO_2}$  the ordinate. Percent oxygen saturation of the blood is indicated by a series of vertical isopleths and pH by horizontal isopleths.

*Effect of temperature on oxygen affinity*

In trout blood increasing temperature causes a decrease in oxygen affinity; that is,  $P_{50}$  is increased with temperature. Using the data on Tables 2 and 3, Fig. 3 was plotted; here the change in  $P_{50}$  with temperature is indicated, at carbon dioxide

Table 2. *Variation in  $P_{O_2}$  of trout blood with percentage saturation,  $P_{CO_2}$  and temperature*(Where there are four or more determinations for  $P_{O_2}$ , the mean, the standard error of the mean and the number of determinations are indicated.)

		Temperature: 6 °C								
0.3	Sat. (%)...	20	30	40	50	60	70	80	90	100
	$P_{O_2}$ (mmHg)...	2.3	6.8	6.0	—	12.0	10.5	24.0	46.0	150
		2.3	6.4	9.0	9.1	10.3	16.5	23.0	48.0	—
		—	—	—	±0.46	—	16.5	—	—	—
		—	—	—	5	—	—	—	—	—
1.0	Sat. (%)...	19	28.8	38.4	48	57.5	67	77	86.5	96
	$P_{O_2}$ (mmHg)...	3.0	—	10.5	—	—	—	—	—	150
		—	7.0	—	12.4	16.3	23.3	38.0	58.8	—
		—	±0.50	—	±0.89	±1.4	±1.8	±1.99	±6.7	—
		—	6	—	7	4	4	7	4	—
2.0	Sat. (%)...	18.5	28	37	46.5	56	65	74.5	84	93
	$P_{O_2}$ (mmHg)...	—	—	12.5	15.0	24.0	28.5	40.5	75.0	150
		—	8.5	13.5	13.0	—	33.0	39.0	—	—
		—	±0.35	15.0	—	—	—	45.0	—	—
		—	4	—	—	—	—	—	—	—
3.0	Sat. (%)...	18	27	36	45	54	63	72	81	90
	$P_{O_2}$ (mmHg)...	—	—	—	—	—	—	—	—	—
		—	10.4	—	19.1	25.5	33.0	60.0	69.0	150
		—	±0.62	—	±0.94	21.0	30.0	45.0	—	—
		—	4	—	4	22.5	31.5	—	—	—
6.0	Sat. (%)...	17	26	34	43	51.5	60	69	77.5	85
	$P_{O_2}$ (mmHg)...	9.0	10.5	10.5	—	19.5	—	—	88.5	150
		—	10.5	—	19.2	21.0	35.0	53.6	90.0	—
		—	12.0	—	±0.73	—	±1.26	±3.89	—	—
		—	—	—	5	—	5	4	—	—
		Temperature: 15 °C								
0.3	Sat. (%)...	20	30	40	50	60	70	80	90	100
	$P_{O_2}$ (mmHg)...	9.0	—	9.0	—	—	—	—	41.0	150
		5.0	8.5	10.0	13.2	17.1	21.5	29.9	38.0	—
		—	±0.57	9.0	±0.89	±0.82	±1.36	±2.45	43.0	—
		—	4	—	4	4	4	4	—	—
1.0	Sat. (%)...	19.0	28.8	38.4	48	57.5	67	73	86.5	96
	$P_{O_2}$ (mmHg)...	—	15.0	15.0	21.0	30.0	33.0	40.5	63.0	150
		—	13.5	13.0	15.0	28.5	31.5	—	67.0	—
		—	12.0	—	16.0	—	27.0	—	51.0	—
2.0	Sat. (%)...	18.5	28	37	46.5	56	65	74.5	84	93
	$P_{O_2}$ (mmHg)...	—	—	16.5	—	39.0	—	79.5	102.0	150
		—	14.7	—	22.9	36.0	47.8	55.5	84.0	—
		—	±0.52	—	±0.72	30.8	±1.98	—	—	—
		—	5	—	4	—	4	—	—	—
3.5-4.0	Sat. (%)...	18	27	36	45	54	63	72	81	90
	$P_{O_2}$ (mmHg)...	9.7	15.5	21.0	—	—	—	—	102.0	150
		—	±0.30	15.0	26.1	29.8	43.0	52.8	102.0	—
		—	5	17.3	±1.13	±2.22	±1.06	±3.72	105.0	—
		—	—	—	7	5	5	4	—	—

Table 2 (cont.)

$P_{CO_2}$ (mmHg)		Temperature: 20 C°									
6.5	Sat. (%)...	17	26	34	43	51.5	60	69	77.5	85	
	$P_{O_2}$ (mmHg)...	8.3	13.5	22.5	—	—	—	—	109.0	150	
		—	—	15.0	23.4	31.5	48.5	74.0	108.0	—	
		—	—	19.5	±2.79	±1.84	±1.0	±2.69	113.5	—	
		—	—	—	5	4	6	7	—	—	
0.3	Sat. (%)...	20	30	40	50	60	70	80	90	100	
	$P_{O_2}$ (mmHg)...	7.3	12.0	15.0	21.0	27.0	48.0	55.5	—	150	
		—	13.5	—	19.5	—	45.0	60.0	109.0	—	
		—	10.3	—	—	—	36.0	60.0	±2.8	—	
		—	—	—	—	—	—	4	—	—	
1.0	Sat. (%)...	19	29	38.5	48	57.5	67	77	86.5	96	
	$P_{O_2}$ (mmHg)...	19.5	—	—	24.0	18.0	42.0	—	93.0	150	
		18.0	19.0	—	31.5	40.5	52.0	62.8	91.0	—	
		16.5	±3.3	—	30.0	36.0	51.0	±3.9	—	—	
		—	4	—	—	—	4	—	—	—	
2.0-3.0	Sat. (%)...	18	27	36	45	54.5	64	73	89	91	
	$P_{O_2}$ (mmHg)...	12.0	—	—	—	—	—	—	—	150	
		10.5	15.9	21.0	27.9	34.7	41.0	60.1	91.2	—	
		—	±2.1	±3.9	±2.98	±2.1	±3.9	±4.5	±5.9	—	
		—	6	4	5	7	5	6	5	—	
6.5	Sat. (%)...	17	26	34	43	51.5	60	69	72.5	86	
	$P_{O_2}$ (mmHg)...	18.0	—	—	—	—	—	—	—	150	
		16.5	18.6	25.5	25.9	29.3	52.0	66.5	105.7	—	
		—	±1.8	±5.2	±2.1	±5.2	±3.2	±6.8	±4.9	—	
		—	5	4	4	4	6	5	5	—	

tensions between 0.3 and 6.0 mmHg. The slope of the line  $\Delta \log P_{50}/\Delta \text{pH}$  varied with  $P_{CO_2}$ ; at  $P_{CO_2}$  0.3 mmHg it was 0.0243 and at  $P_{CO_2}$  6 mmHg it was 0.0157.

In Fig. 4 the data in Tables 2 and 3 were used to plot the relationship between pH at  $P_{50}$  and temperature at various tensions of carbon dioxide.

The Bohr effect

The Bohr effect can be defined quantitatively by the expression  $\Delta \log P_{50}/\Delta \log \text{pH}$ : this is represented graphically in Fig. 5. The magnitude of the Bohr effect for each group of fish was:

Temperature (°C)	Bohr effect ( $\Delta \log P_{50}/\Delta \text{pH}$ )
6	-0.54
15	-0.57
20	-0.59

DISCUSSION

The oxygen dissociation curves for rainbow trout blood determined in this study are in reasonable agreement with those determined by other workers (Table 4).

The values agree with those found by Irving *et al.* (1941) over the same ranges of  $P_{CO_2}$  and temperature. There is agreement with the values of Eddy & Morgan (1969) at low  $P_{CO_2}$ , but at 10 mmHg  $P_{CO_2}$  the  $P_{50}$  found by them is lower than the extrapolated

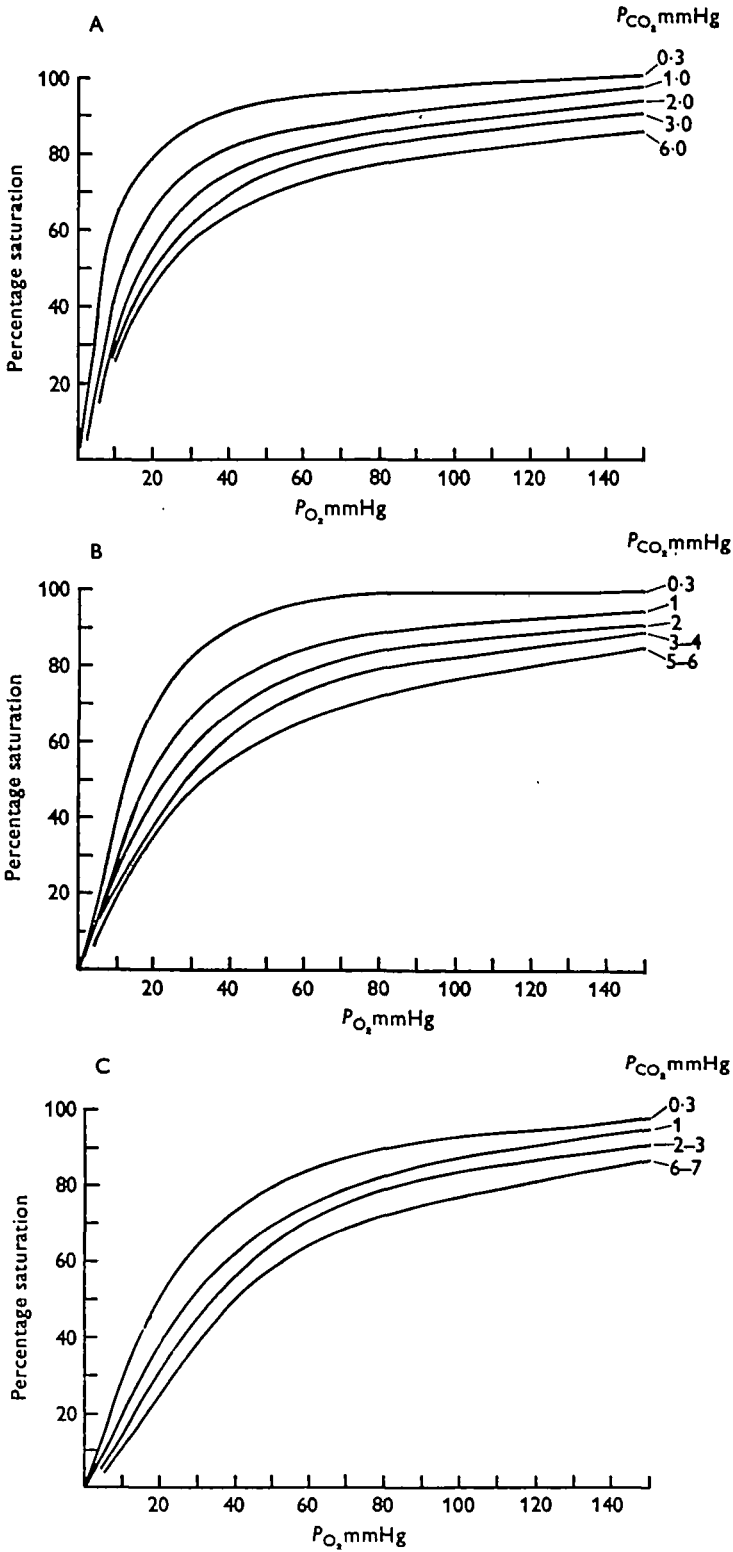


Fig. 1. Oxygen dissociation curves of rainbow trout blood at various temperatures and tensions of carbon dioxide. A, 6 °C; B, 15 °C; C, 20 °C.

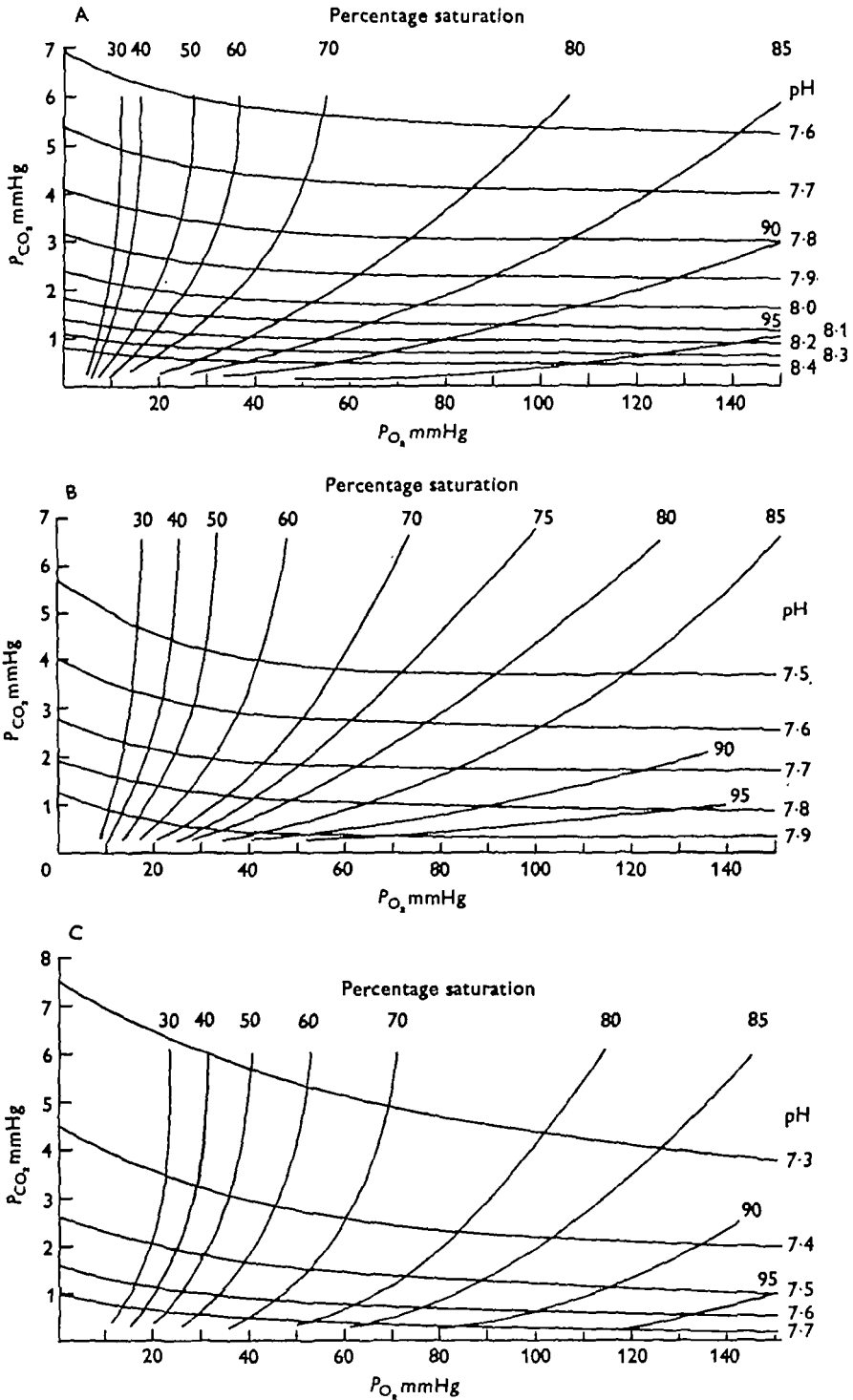


Fig. 2. The effect of pH on the oxygen dissociation curve of rainbow trout blood, at various temperatures and tensions of carbon dioxide.  $P_{O_2}$  is the abscissa and  $P_{CO_2}$  the ordinate. Percentage oxygen saturation of the blood is indicated by a series of vertical isopleths and pH by a series of horizontal isopleths. A, 6 °C; B, 15 °C; C, 20 °C. (This representation of the oxygen dissociation curve is based upon the  $O_2$ - $CO_2$  diagram described by Rahn & Fenn, 1955).







Table 3 (cont.)

$P_{CO_2}$ (mmHg)	Temperature: 20 °C									
	0	20	30	40	50	60	70	80	90	100
0.3	Sat. (%)...	—	—	—	7.40-7.95	—	—	—	—	—
	pH	—	—	7.70	—	—	7.86	7.32	7.85	7.61
	Mean Range	7.84 7.62-7.95	—	7.43-7.89	—	7.76 7.40-7.95	—	—	—	7.38-7.85
1.0	$H^+$ (nM/l)	—	—	20.0	—	—	13.8	49.7	14.1	24.3
	Mean Range	14.6 11.2-24.0	—	12.9-37.1	—	10.0-39.8	—	—	—	14.1-41.7
	Number	5	—	2	—	4	1	1	1	3
2.2-3.2	Sat. (%)...	0	18.6	27.9	37.2	46.5	55.8	74.8	83.7	93
	pH	7.71	7.81	7.65	7.60	7.68	7.52	7.56	7.42	7.51
	Mean Range	7.64-7.79	7.54-7.81	7.61-7.82	7.61-7.82	7.68 7.61-7.82	7.45-7.77	—	—	7.42-7.71
2.2-3.2	$H^+$ (nM/l)	19.3	15.5	22.3	25.1	21.0	21.0	27.5	38.0	31.0
	Mean Range	21.0-36.0	19.0-46.0	15.1-24.5	15.1-24.5	15.1-24.5	21.4-35.5	18.2-36.8	—	19.5-38.0
	Number	2	1	1	2	3	3	2	1	3
2.2-3.2	Sat. (%)...	0	18	27	36	45	48	63	72	81
	pH	7.53	7.45	7.46	7.44	7.45	7.40	7.46	7.38	7.48
	Mean Range	7.44-7.63	7.43-7.46	7.43-7.48	7.38-7.57	7.38-7.57	7.39-7.40	7.31-7.62	7.31-7.47	—
2.2-3.2	$H^+$ (nM/l)	29.5	34.5	34.7	35.9	35.2	40.1	34.9	41.9	33.1
	Mean Range	24.3-36.3	34.7-37.1	33.1-37.2	34.7-37.1	29.6-41.7	39.8-40.7	24.0-49.0	33.9-49.0	—
	Number	4	1	3	2	5	2	6	3	1
2.2-3.2	Sat. (%)...	90	—	—	—	—	—	—	—	—
	pH	7.35	—	—	—	—	—	—	—	—
	Mean Range	7.30-7.40	—	—	—	—	—	—	—	—
6.0-7.0	$H^+$ (nM/l)	44.2	—	—	—	—	—	—	—	—
	Mean Range	39.8-50.1	—	—	—	—	—	—	—	—
	Number	4	—	—	—	—	—	—	—	—
6.0-7.0	Sat. (%)...	0	17	25.2	33.6	42	50.4	67.3	75.6	84
	pH	7.33	7.32	7.32	7.26	7.26	7.35	7.26	7.22	7.23
	Mean Range	7.24-7.47	7.20-7.32	7.24-7.41	7.20-7.32	7.15-7.43	7.30-7.48	7.20-7.54	7.18-7.25	7.08-7.37
6.0-7.0	$H^+$ (nM/l)	45.3	47.9	48.1	55.5	55.3	44.5	54.7	59.7	58.6
	Mean Range	37.2-57.5	47.9-63.1	38.9-57.5	47.9-63.1	37.2-71.0	41.7-50.1	45.7-63.1	56.2-66.1	42.7-83.2
	Number	6	1	3	2	4	3	5	4	6

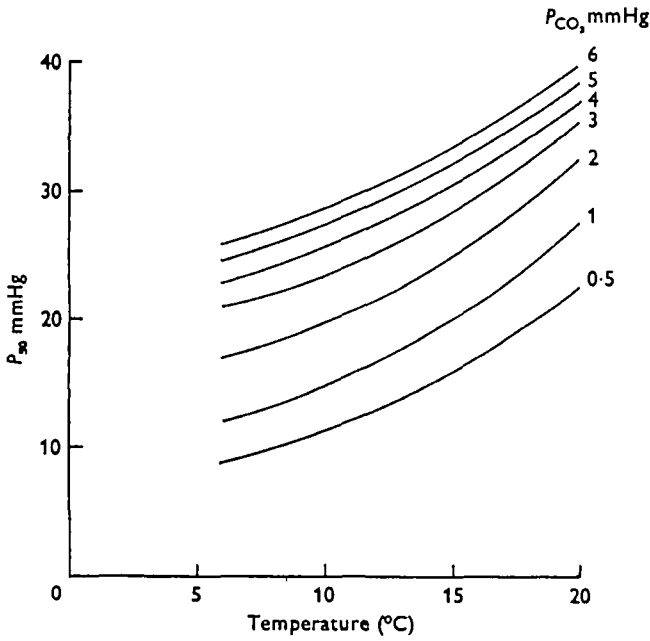


Fig. 3. The effect of temperature on the  $P_{50}$  value of rainbow trout blood at various tensions of carbon dioxide.

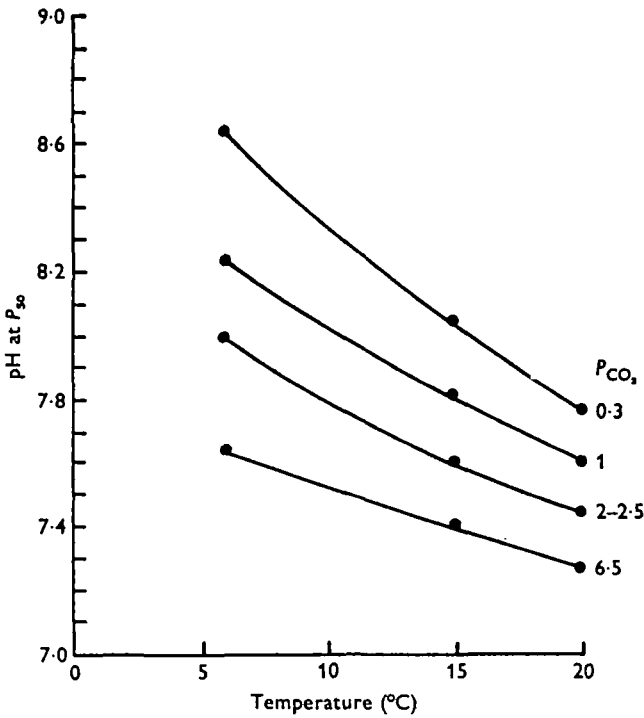


Fig. 4. The effect of temperature on the pH value at  $P_{50}$  of rainbow trout blood at various tensions of carbon dioxide.

values from the present study. The value for  $P_{50}$  (19 mmHg) at 7 mmHg  $P_{CO_2}$ , found by Garey & Rahn (1970) is lower than the value interpolated from the data in this study. The  $P_{50}$  value of 43 mmHg at 0.1 mmHg  $P_{CO_2}$ , obtained by Beaumont and Randall (unpublished) cited in Satchell (1971) is higher than that found here.

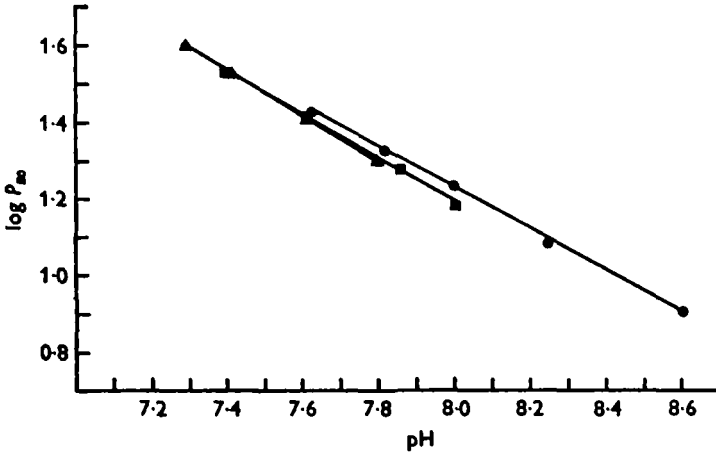


Fig. 5. The Bohr effect ( $\Delta \log P_{50} / \Delta \text{pH}$ ) in rainbow trout blood.  
●, 6 °C; ■, 15 °C; ▲, 20 °C.

Table 4.  $P_{50}$  of rainbow trout blood at different temperatures and at various tensions of carbon dioxide

$P_{CO_2}$	Temperature (°C)									
	6		10		15				20	
	a	e	a	b	a	c	d	e	a	e
< 1	—	8	—	—	—	15	—	14	—	20
1	14	13	15	—	20	—	43	20	27	27
6	—	26	—	19	—	—	—	34	—	40
10	32	—	33	—	42	27	—	—	45	—

(a) Irving *et al.* (1941). Fish weighed 680–1230 g.

(b) Garey & Rahn (1970): 10–14 °C,  $P_{CO_2}$  7 mmHg, fish weighed 500 g.

(c) Eddy & Morgan (1969): 13–19 °C, fish weighed 70–120 g.

(d) Beaumont & Randall (unpublished): cited in Satchell (1971).  $P_{CO_2}$  0.1 mmHg.

(e) This study: fish weighed 350–600 g.

#### *Effects of temperature, $P_{CO_2}$ and pH*

Temperature,  $P_{CO_2}$ , and hydrogen ions, whether raised independently or together, cause trout blood to have a lowered affinity for oxygen.

As temperature decreases a number of changes take place in the blood, all of which affect the shape of the oxygen dissociation curve.

(a) The solubility of carbon dioxide in blood and plasma increases.

(b) At lower temperatures the ionization of non-bicarbonate buffers (mainly proteins) decreases; thus carbon dioxide can become hydrated to form an increased amount of bicarbonate so maintaining ionic neutrality of the blood (Siggaard-Andersen, 1964).

(c) As temperature decreases, the ion product of water decreases; pure water at 25 °C is neutral when its pH is 7.0, and pOH is also 7.0. For neutrality to be maintained as temperature is lowered pH must increase (Rahn, 1967).

The available data suggest that trout blood behaves in a similar way to carp blood (Rahn, 1967), with changes in temperature. It appears that the trout maintains an  $[OH^-]/[H^+]$  ratio of between 16 and 20. This means that the ratio  $[HCO_3^-]/[H_2CO_3]$  is approximately 55 at 6 °C, 42 at 15 °C, and 32 at 20 °C. For  $[OH^-]/[H^+]$  to be maintained in the range of 16–20 1 mM  $H_2CO_3$  requires nearly twice as much  $HCO_3^-$  at 6 °C as at 20 °C.

These changes in the carbon dioxide equilibrium of blood have the following effects upon the oxygen affinity of blood.

(a) At lower temperatures blood pH is higher and blood oxygen affinity is increased.

(b) At lower temperatures there is more  $HCO_3^-$  available for buffering. Thus increases in  $P_{CO_2}$  produces less  $H^+$  than at higher temperatures. Therefore at lower temperatures similar increases in  $P_{CO_2}$  decrease blood oxygen affinity less than at higher temperatures.

The effects of temperature,  $P_{CO_2}$ , and pH on the oxygenation of trout blood can be expressed quantitatively in the following way.

Table 5. *The effect of temperature,  $P_{CO_2}$ , and pH upon the oxygenation of rainbow trout blood*

(At each temperature,  $P_{O_2}$  and  $P_{CO_2}$  are altered by known amounts and the resulting changes in percent saturation and hydrogen-ion concentration are followed. The hydrogen ion concentration is expressed in nanomoles per litre and as pH.)

Temperature (°C)	$P_{CO_2} = 1$ mmHg $P_{O_2} = 100$ mmHg		$P_{CO_2} = 6$ mmHg $P_{O_2} = 30$ mmHg		$\Delta$ (% sat.)	$\Delta$ [H <sup>+</sup> ]	$\frac{\Delta \% \text{ sat.}}{\Delta [H^+]}$
	Sat. (%)	[H <sup>+</sup> ] nM/l (pH)	Sat. (%)	[H <sup>+</sup> ] nM/l (pH)			
6	92	7.5 (8.13)	56	24 (7.60)	36	17.5	2.06
15	91	17 (7.77)	46	40 (7.40)	45	23	1.95
20	88	25 (7.60)	40	50 (7.30)	48	25	1.93

If blood at different temperatures and of known  $P_{O_2}$  and  $P_{CO_2}$  is subjected to a known increase in  $P_{CO_2}$  and reduction in  $P_{O_2}$ , then the changes in oxygen saturation can be determined. An example is given in Table 5. Here blood approximating to arterial ( $P_{O_2} = 100$  and  $P_{CO_2} = 1$  mmHg) is subjected to tensions of oxygen and carbon dioxide found in the tissues ( $P_{O_2} = 30$  and  $P_{CO_2} = 6$  mmHg) reported by Garey & Rahn (1970). At each temperature the change in oxygen saturation and in [H<sup>+</sup>] is shown. At 20 °C more oxygen is released than at 6 °C, but this is accompanied by a greater change in [H<sup>+</sup>]. In this particular case it appears that the ratio of oxygen released to change in [H<sup>+</sup>] remains about the same at each temperature.

*Comparison with in vivo results*

There is very little information available about how temperature changes affect blood-gas concentrations and pH in the living trout. Data from the work of Holeyton & Randall (1967) and Stevens & Randall (1967) are shown in Table 4. These authors derived the data for percentage saturation from the oxygen dissociation curves of Beaumont, Holeyton & Randall (unpublished, cited in Holeyton & Randall, 1967).

The results in Table 6 are in reasonable agreement with the results obtained in the present study (Fig. 1 A-C). The main differences are in the percentage saturation of arterial blood.

*The Bohr effect*

The Bohr effect is the change in  $P_{50}$  with unit change in pH, and can be defined quantitatively by the ratio  $\Delta \log P_{50}/\Delta \text{pH}$ ; its value depends upon the species, the

Table 6. *Mean blood gas values for rainbow trout blood at 5 °C and 15 °C*

Temp. (°C)	$P_{O_2}$		$P_{CO_2}$		Sat. (%)		pH	
	Art.	Ven.	Art.	Ven.	Art.	Ven.	Art.	Ven.
5	85	19	2.3	5.7	99.6	38	—	—
15	122	35	1-1.5	2-3	97	70	7.8	7.7

Table 7. *Values for the Bohr effect in various species*

(Unless otherwise stated, whole blood was used.)

Species	Temp. (°C)	$\frac{\Delta \log P_{50}}{\Delta \text{pH}}$	Remarks	Reference
Man	37	-0.467 to -0.558	$P_{CO_2}$ 12-55 mmHg, pH 7.3-7.7	Lenfant <i>et al.</i> 1969
Hedgehog	39	-0.45 to -0.49	Physiological pH and $P_{CO_2}$	Bartels <i>et al.</i> 1969
Turtle ( <i>Chelys fimbriata</i> )	26	-0.560	Physiological pH and $P_{CO_2}$	Lenfant <i>et al.</i> 1970
<i>Necturus</i> <i>Amphiuma</i> (Bullfrog)	20-22	-0.320 -0.131 -0.280	Physiological pH and $P_{CO_2}$	Lenfant & Johansen, 1967
<i>Protopterus aethiopicus</i>	20	-0.47	$P_{CO_2}$ 6-40 mmHg, pH 7.5-8.0	Lenfant & Johansen 1968
<i>Ictalurus nebulosus</i>	9, 24	-0.3079	$CO_2$ not increased above atmospheric pH 7.3-7.81	Grigg, 1969
<i>Trematomus borchgrevinkii</i> <i>T. bernacchi</i>	1.5	-0.70 -0.35	Antarctic fish with haemoglobin, $P_{CO_2}$ 0-1 mmHg, $O_2$ dis- sociation curves at pH 7.4, 7.5	Grigg, 1967
Rainbow trout	6 15 20	-0.54 -0.57 -0.59	$P_{CO_2}$ 1-7 mmHg, pH 7.2-8.4	This study
Mackerel	25	-1.2	pH 6.81-7.38, haemo- globin solutions used	Hall & McCutcheon, 1938

nature of the haemoglobin and the buffering of the blood. In Table 7 are values for the Bohr effect found in this study, together with values for other species.

The data in Table 7 show that where whole blood has been used to determine the Bohr effect, the values in fish are between  $-0.31$  and  $-0.70$ . For the trout and the lungfish *Protopterus* the values are similar to those for mammalian blood. The values for amphibians given in Table 7 are lower than those for many other vertebrates.

#### *Effect of acclimation temperature*

The results presented in this study are for fish acclimated to one particular temperature. It is not known if the same results would be obtained using blood from fish acclimated to one temperature and then tested at different temperatures. The fish used by Irving *et al.* (1941) were taken from streams of temperature between 6 and 12 °C; the blood was equilibrated in the range 0–38 °C and the  $P_{50}$  value was determined. The values found by them agree reasonably well with the results of the present study (Table 4), suggesting that acclimation temperature does not affect the dissociation curve in trout. Black, Kirkpatrick & Tucker (1966) found no difference in the oxygen dissociation curves of blood from brook trout acclimated to summer and winter temperatures. 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  $O_2$  affinity compared with blood from cold acclimated fish.

The  $O_2$  affinity of fish blood appears to be regulated by various factors, and it is not known whether any of these are altered by acclimation temperature. Forster & Steen (1969) found that eel haemoglobin contained two fractions, one showing a marked Bohr effect and the other showing no Bohr effect. Benesch & Benesch (1969) indicated that 2,3-diphosphoglycerate occurs in the mammalian erythrocyte in about equimolar concentrations to haemoglobin. This substance facilitates oxygen unloading from oxy-haemoglobin because it lowers oxygen affinity by preferentially binding to deoxygenated haemoglobin. Rapoport & Guest (1941) and Lenfant (unpublished, cited in Satchell, 1971) showed that fish erythrocytes contained high concentrations of soluble organic phosphates. It is not yet known if these substances are of any importance in altering the oxygen affinity of fish haemoglobins.

#### SUMMARY

1. Oxygen dissociation curves were determined using blood from rainbow trout, acclimated for at least 3 weeks to temperatures of 6, 15 and 20 °C. Carbon dioxide tensions in the range 0.3–7 mmHg produced both the Bohr and Root effects in the blood.

2. Increasing temperature,  $P_{CO_2}$  and hydrogen ions, whether raised individually or together, caused a decrease in blood  $O_2$  affinity (increased value for  $P_{50}$ ).

3. Blood at low temperatures had a higher pH than blood at high temperatures. This is related to the fact that the ionization constant of water is diminished with decreasing temperature. When blood was 50% saturated and  $P_{CO_2}$  was 1 mmHg, the pH value was 8.25 at 6 °C, 7.83 at 15 °C and 7.62 at 20 °C.

4. The factors influencing unloading of oxygen from the blood are discussed. To

release the same amount of oxygen from blood, a greater change in carbon dioxide tension is required at 6 °C than at higher temperatures.

5. The Bohr effect expressed quantitatively ( $\Delta \log P_{50}/\Delta \text{pH}$ ) was  $-0.54$  at 6 °C,  $-0.57$  at 15 °C and  $-0.59$  at 20 °C. These values are similar to those for the blood of many mammals, and are within the range reported for fish, where whole blood has been used.

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