J. exp. Biol. 111, 253–257 (1984)
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EFFECTS OF *IN VIVO* AND *IN VITRO* CHANGES IN P_{O_2} ON THE DEFORMABILITY OF RED BLOOD CELLS OF RAINBOW TROUT (*SALMO GAIRDNERI* R.)

BY G. M. HUGHES AND Y. KIKUCHI*

Research Unit for Comparative Animal Respiration, Bristol University, Woodland Road, Bristol BS8 1UG, England

Accepted 3 February 1984

Changes in haematocrit value are known to occur during hypoxia of rainbow trout and this has sometimes been interpreted as a result of an increase in red cell volume which is observed *in vitro* following equilibration with gas mixtures of low P_{O_2} (Black & Irving, 1938; Soivio, Westman & Nyholm, 1974). The possibility that there is also an increase in red cell number is still under discussion (Thomas & Hughes, 1982). The rise in blood haemoglobin content associated with such an increase would have physiological advantages for oxygen transport. However, an increase in haematocrit may increase resistance to blood flow and could impair the supply of oxygen at the tissue level. It is of interest, therefore, to know what are the effects of hypoxia both environmentally induced and under *in vitro* conditions on the flow properties of fish blood, for such effects have received little attention. This paper summarizes measurements of red cell deformability following changes from normoxic to hypoxic conditions, both *in vivo* and *in vitro*. A technique of filtration through a Nuclepore membrane has been used to provide an index of red cell deformability. During studies on yellowtail blood (Hughes, Kikuchi & Watari, 1982) variability in red cell deformability appeared to depend upon conditions of sampling and differences were observed in the filtration time of blood samples from normoxic and asphyxic fish. This effect has now been studied under more controlled conditions in rainbow trout.

Fish kept at $15 \pm 1\,^{\circ}C$ were cannulated through the dorsal aorta. Samples were taken immediately, while the fish was still under MS222 anaesthesia ($0{\cdot}1\,g\,l^{-1}$), or some days later after complete recovery from surgery. Blood samples were taken from the same specimens kept in well aerated water or following the bubbling of nitrogen through water in the holding tanks so that P_{O_2} fell to 40–60 mmHg. The particular level to which the hypoxia was extended varied according to the response of individual fish. In some individuals a lowering of P_{O_2} below 60 mmHg initiates quite strong movements and such effects were avoided during these experiments. The slightest indication of any increase in locomotory activity was taken as a warning when to reduce the N_2 bubbling and to maintain that particular P_{O_2}. In this way the results are considered to show the effects of lowering of blood P_{O_2} without complications due to effects induced by increased activity. The reduction from normoxia to this hypoxic condition occurred over a period of 30 min. No blood samples were taken from a fish

* Usual address: Division of Physiology, Institute of Applied Electricity, Hokkaido University, Sapporo, Japan.

Key words: Trout, erythrocyte, hypoxia.

until it had been held at the hypoxic level for at least 30 min. In no cases were fish maintained at the lower P_{O_2} for longer than 2 h. Blood samples obtained under normoxic and hypoxic conditions were equilibrated *in vitro* with N_2/air or O_2/air mixtures respectively. Syringes containing the blood and gas mixture were shaken as in a tonometer. In this way a comparison was made of the pore passage time of blood at similar P_{O_2} values, in one case from fish equilibrated at low P_{O_2} and the sample equilibrated *in vitro* at high P_{O_2} and *vice versa*.

The filtration technique is essentially that summarized earlier (Hughes *et al.* 1982) and in more detail by Kikuchi, Arai & Koyama (1983). The Nuclepore filters contain pores of 8 μm diameter, the blood flow was due to a pressure difference of 10 cm H_2O. Timing was by an electronic clock and in all cases measurements were preceded by control measurements using saline which was left in the pores of the filter so as to reduce variability due to enclosed air bubbles. From the data obtained for blood passage time (BPT), calculations were made of the pore passage time for individual red blood cells (RBC−PPT) using equations already given (Hughes *et al.* 1982). Duplicate haematocrit measurements and red cell counts were carried out from which mean corpuscular volumes were calculated. Mean values were derived and paired *t*-tests carried out for the blood obtained from the same fish, or for the same samples when they were equilibrated under normoxic or hypoxic conditions *in vitro*.

Blood samples from cannulated fish under normoxic and hypoxic conditions

From previous experiments it was known that the results obtained from individual fish varied. In particular there was a range in normoxic haematocrit values which was reflected in the passage times (Fig. 1). Although the mean values summarized in Table 1 are of interest, the wide standard errors tend to obscure changes which were

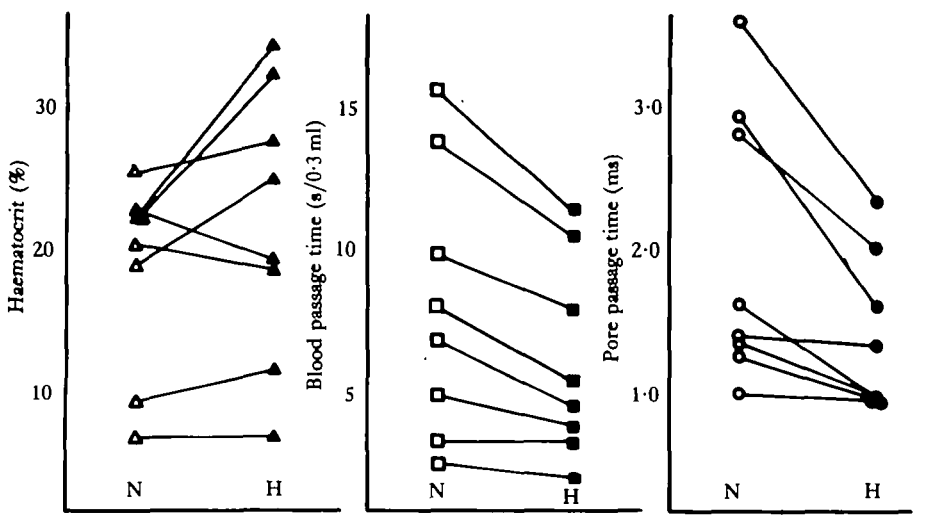

Fig. 1. Relationships between measurements of haematocrit value (%), blood passage time (s/0·3 ml) and the pore passage time (ms) for single red cells in blood samples taken from trout under normoxic (N, open symbols) and hypoxic (H, closed symbols) conditions. Measurements obtained for individual fish are joined.

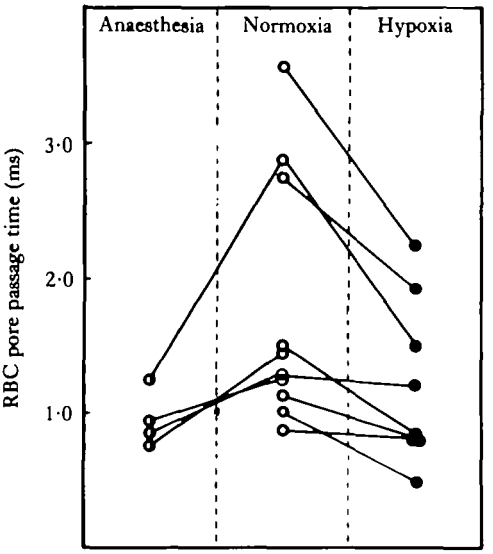

Fig. 2. Comparison between the RBC pore passage time of blood samples obtained from trout under three conditions: anaesthesia ( ), normoxia (○), hypoxia (●). Lines join measurements obtained from the same individuals.

very clear when results using blood from individual fish were analysed separately. This is apparent when data for individual specimens is plotted as in Fig. 1. For nearly all individuals the following changes were observed:

1. There was a decrease in passage time for 0·3 ml of blood (BPT) through the 8 μm pores.

2. The haematocrit value (HCt) of samples from fish equilibrated in hypoxic water was higher than that of blood samples from the same specimens under normoxic conditions.

A decrease in the passage time calculated for individual red blood cells

Table 1. *Summary of results of measurements using blood samples from* Salmo gairdneri *kept under normoxic and hypoxic conditions*

	Normoxia (Pa_{O_2} 80–110 mmHg)					Hypoxia (Pa_{O_2} approx 20 mmHg)		
	Mean	S.E.	*N*	Paired '*t*' test	(*N*)	Mean	S.E.	*N*
Blood passage time/0·3 ml (s)	5·94	0·83	16	5·58*	(11)	5·38	0·68	19
Haematocrit value (%)	16·96	1·79	16	2·353***	(11)	28·93	2·87	19
Mean cell volume (μm^3)	230·7	5·29	16	3·497**	(12)	274·8	8·62	19
RBC pore passage time (ms)	1·50	0·184	16	4·614*	(12)	0·973	0·093	19

Student's paired '*t*' test was only possible where samples were taken from the same fish under both conditions. Significance probabilities: * 0·001, ** 0·01, *** 0·05.

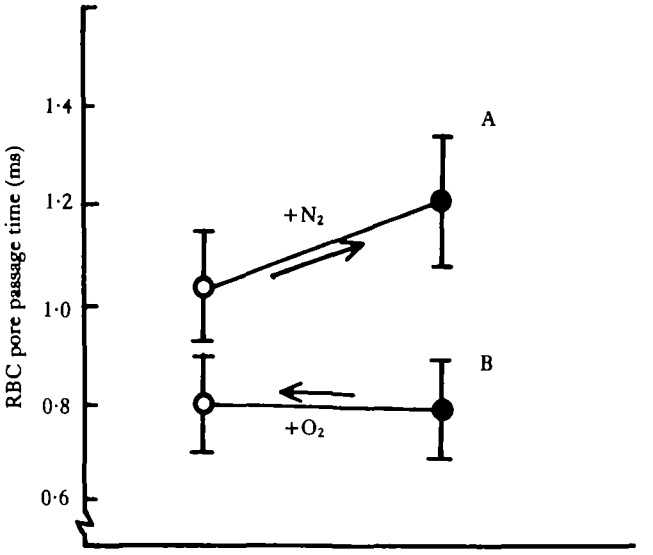

Fig. 3. Mean values (±S.D.) for RBC pore passage time obtained from blood samples taken from: (A) normoxic fish and equilibrated with low oxygen gas mixtures ($P_{O_2} < 5$ mmHg), (B) blood samples taken from fish in hypoxic water and equilibrated with high oxygen mixtures ($P_{O_2} > 160$ mmHg). Arrows show the direction of the *in vitro* change in P_{O_2}.

(RBC−PPT) was observed, and this was in spite of an increase in mean red cell volumes. As is apparent from the analyses summarized in Table 1 these differences in passage time between samples taken from the same individuals were significant at the 0·1 % level when compared using Student's paired *t*-test.

Similar changes to those observed in trout equilibrated with normoxic water and hypoxic water were found when comparisons were made between blood samples taken post-operatively under anaesthesia, when the arterial P_{O_2} was low (10–25 mmHg), and following recovery. These results are set out in Fig. 2 and again paired *t*-tests showed these differences to be statistically significant.

Measurements on blood samples in vitro

Blood from normoxic trout equilibrated with high nitrogen-containing mixtures seemed to show an increase in RBC pore passage time (Fig. 3A) but statistical analysis showed that this was not significant at the 5 % level. Blood taken from hypoxic fish when equilibrated at high P_{O_2} values showed no change in mean RBC pore passage time (Fig. 3B).

The most striking result of these experiments is the finding of an increase in deformability of red cells in blood from hypoxic fish. Furthermore, this occurred in spite of an increase in cell volume associated with a rise in haematocrit. That no significant increase in deformability was observed *in vitro*, when blood from normoxic fish was subjected to comparable lowering of P_{O_2}, suggests that changes other than corpuscular volume alone are involved *in vivo*.

From results obtained by previous investigators with rainbow trout under hypoxia there are indications that a rise in haematocrit may be due to at least two other causes: (a) increase in red cell number, by the liberation of fresh red cells from organs such

the spleen and (b) a reduction in total plasma volume. Exactly what has happened in the present experiments is not certain, but there is little doubt that haematocrit increased and was accompanied by an increase in mean red cell volume. Both of these features are normally associated with an increase in the time for a given volume of blood to pass through a Nuclepore filter containing 8 μm pores. The average dimensions of trout red blood cells are $9{\cdot}0 \times 11{\cdot}0\,\mu$m and it is clear, therefore, that the red cells must undergo deformation. The results from blood samples taken from individual trout during hypoxia clearly involve several other factors which could change haematocrit, whereas for *in vitro* samples an increase in red cell number is not possible nor can there be any reduction in plasma volume. Thus any change in haematocrit must be associated with a change in corpuscular volume. The absence of any statistically significant changes in RBC−PPT of *in vitro* samples, therefore, tends to emphasize the possibility that liberation of RBCs and haemoconcentration are involved in the changes which occur in the whole animal.

Additional factors which could be involved in the whole fish are changes in the composition of the RBC population, plasma proteins and inorganic constituents of the blood, or in the concentration of circulating catecholamines. Fish red cells are, in fact, known to be more sensitive to catecholamines than are those of mammals (DeVries & Ellory, 1982). The fact that changes in red cell deformability were observed in samples obtained from fish that had fully recovered from hypoxia, but without marked change in blood pH, indicates that at least some of these factors are not involved.

It would appear that during hypoxia trout blood undergoes several adaptive modifications. An increase in cell size must bring them closer to the water/blood barrier and hence reduce the plasma component of the resistance to oxygen uptake. Any increase in haematocrit resulting from a release of RBCs would raise the O_2 carrying capacity. It is now apparent that these changes are accompanied by an increase in RBC deformability which effectively reduces the resistance to blood flow through the gills and other parts of the microcirculation. In view of the known differences in response of the trout ventilatory and cardiovascular systems to different regimes of hypoxia, a more detailed study of rheological aspects of their adaptation would elucidate further details of the modifications indicated by the present study.

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