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DO FISH ACCLIMATED TO LOW TEMPERATURE IMPROVE MICROCIRCULATORY PERFUSION BY ADAPTING RED CELL RHEOLOGY?

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Summary

We have measured the rheological properties of individual red blood cells from fishes inhabiting different thermal environments and have also investigated the effects on red cells of acute *in vitro* temperature changes. The membrane shear elastic modulus (rigidity) increased markedly with decreasing temperature, and the dependence was similar if temperature was varied acutely *in vitro* or if cells were measured at normal body temperatures. Red cells from trout and *Notothenia coriiceps* had almost equal membrane rigidity at comparable temperatures and showed similar temperature-sensitivity in acute experiments. Entry times of trout cells into narrow (approximately 4 μm diameter) micropipettes also increased during *in vitro* reduction of temperature, and this could be explained largely by the temperature-dependence expected of aqueous solutions. Perhaps surprisingly, entry times did not vary when trout living at different temperatures were tested at these temperatures. Transit times of individual cells through somewhat larger pores (5 μm) in oligopore filters again increased with decreasing temperature *in vitro* (partly due to increasing fluid viscosity), but such transit times did show a similar temperature-dependence for cells from trout living at different temperatures. Thus, the temperature-dependence of cellular flow resistance appears to arise from variations in membrane rigidity and in the viscosity of fluid components, along with unquantified variations in components such as microtubular structures (which we found did not influence membrane rigidity but did affect pore entry time) and the cell nucleus. Thermal acclimation did not involve adaptation to compensate for increased membrane rigidity or a large pore transit time, with, at most, minor compensation in entry times into smaller pores. We conclude that impaired cellular rheology is not a major factor influencing circulation in fish at low temperature.

Key words: erythrocytes, rheology, trout, fish, *Oncorhynchus mykiss*, *Notothenia coriiceps*, *Tilapia mossambicus*.

Introduction

Ectotherms may adopt various methods to overcome the effects of changes in environmental temperature on physiological processes. Some display behavioural thermoregulation, i.e. they migrate with the temperature gradient in order to keep their body temperature constant. Others become torpid in the cold, while many temperate-zone species, e.g. salmonids, undertake physiological adaptation during seasonal changes of temperature. In addition, they may also have to cope with acute temperature changes, e.g. during diurnal cycles or vertical migrations in the water column. Because of the efficient branchial heat exchange, changes in ambient temperature are rapidly transferred to the blood. The effects of such temperature variation on the rheological properties of the red blood cells and the implications for the microcirculation are considered here.

Resistance to blood circulation and tissue perfusion depend on the rheological properties of the red cells as well as the vascular architecture. The rheological properties of human red cells have been widely studied (see reviews by Chien, 1987; Nash and Dormandy, 1989). The rheological component of resistance to flow in large vessels is represented by the blood viscosity, which is dependent on the concentration of red cells (haematocrit), on plasma viscosity and, at low shear rates, on red cell aggregation mediated by plasma proteins. Red cell deformation at high shear rate allows reduction in blood viscosity but the mechanical properties of the individual cells are thought to be more important in microvessels, particularly the capillaries, with a diameter that is smaller than the dimensions of the red cells.

The mechanical factors which affect flow in microvessels are the red cell geometry (surface-to-volume ratio, size and shape), internal viscosity and membrane viscoelasticity. To

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allow adaptation of its shape, it is essential that the red cell has an excess of surface area over the minimum required to enclose its volume (i.e. as a sphere) because the membrane itself is highly resistant to expansion (Evans *et al.* 1976). The excess surface ultimately defines the smallest capillary which the cell can enter. Changes in cell volume not only affect the surface area:volume ratio, but can also influence haemoglobin concentration and hence cytoplasmic viscosity. The red cell membrane has characteristic viscous and elastic components of resistance to deformation, mainly determined by the protein skeleton that underlies the lipid bilayer of its cell membrane (see review by Mohandas and Chasis, 1993). In the circulation, the cell deforms by a combination of membrane bending and shearing, and the elastic resistance to shear deformation (shear elastic modulus) is much greater than the elastic resistance to bending (Evans, 1983). All of these cellular rheological factors are affected by changes in temperature. With decreasing temperature, internal viscosity will increase in parallel with water viscosity, the membrane area of human red cells contracts slightly, the shear elastic modulus (rigidity) increases slightly, and the membrane viscosity increases more markedly (Waugh and Evans, 1976; Hochmuth *et al.* 1980).

Although similar mechanisms are likely to operate in fish, relatively few rheological studies have been made. These have concentrated on the filterability of cell suspensions (e.g. Hughes and Albers, 1988; Hughes *et al.* 1986) and blood viscosity (Fletcher and Haedrich, 1987; Wells and Weber, 1991). With regard to the effects of temperature, it has been demonstrated that for trout, ray and carp, resistance to flow through filters decreases as temperature increases (Kikuchi *et al.* 1982; Hughes and Kikuchi, 1984), and it has been suggested that some Antarctic fish compensate for increased blood viscosity at their low ambient temperature by having an unusually low concentration of red blood cells (Wells *et al.* 1990). In a previous comparative study of red cell rheology (Nash and Egginton, 1993), we found that trout red cells were more resistant to flow through small pores than were human red cells at their respective body temperature and that trout cells had a relatively rigid membrane. Nevertheless, it is interesting that, even with the relatively great resistance to flow of the trout cells and the slightly lower systemic blood pressure in fish compared with that of humans, the limiting size of capillaries is about 3 μm for both trout and human red cells.

The present study attempts to answer the following questions: (i) what are the effects of acute temperature change on the rheology of fish red cells; (ii) what are the rheological properties of fish red cells after acclimation to different temperatures?

Many cellular processes in temperate-zone fishes show acclimation during the seasonal cycle, but it is not known whether rheological adaptation is necessary to avoid microcirculatory problems related to exposure to cold. Changes in rheological properties might otherwise reduce blood flow or even cause blockage of small capillaries, which would diminish gas exchange and cause tissue hypoxia. There is reason to believe that adaptation is possible because membrane fluidity at the molecular level is compensated to avoid reduction at low temperature (Cossins and Bowler, 1987). In this study, therefore, we investigated whether the rheological properties of red blood cells of trout varied during seasonal changes of environmental temperatures to a greater or lesser degree than during acute *in vitro* temperature variation. For comparison, we also measured the specific membrane rigidity (shear elastic modulus) of two species which live at stable but widely disparate temperatures (*Notothenia coriiceps* and *Tilapia mossambicus*) and tested the response of the membrane of one of these (*Notothenia coriiceps*) to acute temperature variation. Such comparisons should show whether any variation in the rigidity of the trout red cell membrane during acclimation was part of a trend found for other species of fish living at different temperatures and whether acute variation in rigidity *in vitro* was comparable for different species.

Materials and methods

Blood sampling and preparation

Rainbow trout, *Oncorhynchus mykiss* (Walbaum), of approximately 650 g body mass were obtained from a commercial trout farm and kept in laboratory tanks, supplied with aerated dechlorinated tapwater with a flow of 5 cm s^{-1}. *Tilapia* (=*Oreochromis) mossambicus* were obtained from a commercial supplier. *Notothenia coriiceps* Richardson came from Signy Island (60˚43′ S; 45˚36′ W) and were kindly supplied by the British Antarctic Survey. Water in the tanks for *T. mossambicus* and *N. coriiceps* was kept static. Oxygen saturation of the water was over 95 %. Fish were allowed to acclimate to laboratory conditions for a minimum of 1 week before use. The water temperature was maintained at 4 ˚C in winter, 11 ˚C in spring and 18 ˚C in summer for trout, 25 ˚C for *T. mossambicus* and 0 ˚C for *N. coriiceps*. Trout and *T. mossambicus* were fed daily with commercial pellets and *N. coriiceps* with squid.

Dorsal aortic cannulations of trout and *N. coriiceps* were carried out under MS-222 anaesthesia (1:10 000; see Egginton 1994, for details). Trout were enclosed in individual restrainers and allowed to recover from surgery for 2 days before withdrawal of blood, which was drawn *via* the cannula into heparinized syringes (5 i.u. ml^{-1}). *T. mossambicus* were lightly anaesthetised with MS-222 and blood was drawn *via* caudal vein puncture into heparinized syringes.

Blood haematocrit was determined by centrifugation (3 min, 12 000 ***g***) and haemoglobin concentration by the cyanmethaemoglobin method. Red cell count was measured by a Coulter counter model ZF (Coulter Electronics UK, Ltd, Luton, UK). Mean cell haemoglobin concentration (MCHC) was calculated by dividing the haemoglobin concentration by the haematocrit, and mean cell volume (MCV) by dividing the haematocrit by the red cell count. A portion of blood was centrifuged and the plasma was harvested for measurement of osmolarity (Advanced Micro-osmometer, model 3MO, Advanced Instruments, Inc., Massachusetts, USA).

For the rheological measurements, whole blood was suspended in aerated physiological buffer (112.8 mmol l^{-1} NaCl, 4.2 mmol l^{-1} KCl, 0.1 mmol l^{-1} $(NH_4)_2SO_4$, 13.1 mmol l^{-1} $NaHCO_3$, 1.2 mmol l^{-1} $MgSO_4$, 0.4 mmol l^{-1} KH_2PO_4, 1.0 mmol l^{-1} NaH_2PO_4, 1.3 mmol l^{-1} $CaCl_2$ and 1.3 mmol l^{-1} sodium pyruvate), and the suspension was equilibrated at the measurement temperature for 1 h before analysis. pH and osmolarity of the buffer were adjusted to be equal to the pH and osmolarity of fish plasma at appropriate temperatures. Osmolarity of *N. coriiceps* plasma was not measurable by freezing-point depression because of the presence of glycoprotein antifreeze. Although the exact composition of nototheniid plasma is not known, for these fish, additional NaCl was added to the buffer (final concentration 150 mmol l^{-1}) in line with buffers for other marine teleosts. Because different buffers may influence cell volume and alter flow of trout cells through narrow pores (Hughes *et al.* 1986), we routinely monitored red cell volume during the experimental protocols. Distributions of cell volumes were measured by the Coulter counter and associated pulse width and height analyser (PWHA; Bioengineering Unit, Strathclyde University, Glasgow) after fixing the cells in 1 % glutaraldehyde in buffer. Absolute volumes were not determined, but shifts in cell volume were quantified as percentage changes in the median pulse height (Nash and Egginton, 1993). For *N. coriiceps* and *T. mossambicus*, only membrane rigidity was determined (see below), and this property should not be influenced by minor volume shifts. For all fish, preservation of smooth, elliptical, red cell morphology was ensured microscopically.

Rheological measurements

Rheological measurements were similar to those previously described for trout red cells (Nash and Egginton, 1993).

Micropipette analysis

Whole blood was diluted 1:1000 in buffer containing 5 % autologous plasma and placed in a chamber made of glass coverslips and a U-shaped gasket. The chamber was placed on the stage of a microscope and viewed using a water immersion 40× objective lens. The temperatures of the chamber and lens were controlled separately by water jackets and a circulator. A micropipette connected to a hydrostatic pressure system was moved into the chamber using a micromanipulator, and its tip was viewed by videomicroscopy (final magnification ×5000 on the video monitor).

The membrane shear elastic modulus (rigidity or resistance to shear deformation at constant area) was measured using a micropipette with an internal diameter of 1.6 μm. A membrane tongue was aspirated from the flattened side of the red cell into the pipette. The membrane was aspirated from the peripheral region of the cell, between the nucleus and the cell edge. The length (L) of the tongue was measured *via* a linked computer and video mixer at several increasing pressures (P), and the shear elastic modulus was calculated from the slope of the linear regression of L *versus* P (Nash and Wyard, 1981). Ten cells were measured in each sample.

The time taken for cells to enter a micropipette was measured using a pipette with an internal diameter of 4.2 μm and a hydrostatic driving pressure of 200 Pa. Cell entry was video-recorded and the time taken measured by retrospective analysis. Typically 50 cells were measured. The same pipette was used for all experiments.

Pore transit time

Transit times for individual cells flowing through 5 μm pores were measured using a cell transit time analyser (CTA; ABX International, Levallois, France) (Zhu *et al.* 1989) with custom-made software (Fisher *et al.* 1992). Dilute blood suspension (10^6 cells ml^{-1} buffer) flowed through an oligopore filter under 1000 Pa hydrostatic pressure. The same filter, which contained thirty 5 μm pores, was used throughout the experiments. Between measurements, the filter was cleaned by ultrasonication. Electrodes on either side of the filter generated an a.c. current at 100 kHz so that each cell generated a voltage pulse as it passed through a pore. The pulses were amplified, digitised and their widths determined by microcomputer and used as a measure of the cell transit time. 400 cells were measured in each sample. This filtration method differs from others (see Stone *et al.* 1990, for a review) by measuring transit times for individual cells, rather than a bulk average, and allows a frequency distribution of transit times to be derived for the population of cells tested. Because the white cell count for fish is relatively high compared with that of humans, we tested whether white cells affected transit times by carrying out measurements with or without buffy coat removal. We found that the influence was negligible and diluted whole blood was used for all subsequent measurements.

Study design

Two types of investigation were carried out. (1) Measurement of the effects of acute, *in vitro* temperature change. Red blood cells (RBCs) from fish living at various temperatures were exposed to temperatures in the range 0–25 °C. Diluted samples were first cooled to 0 °C and equilibrated for 1 h. After measurement at this temperature, samples were warmed slowly stepwise and remeasured. When each desired temperature was reached, 15 min of equilibration was allowed before measurements. (2) Measurements on fish living at various temperatures. RBCs from trout living at 4, 11 or 18 °C during the appropriate season were compared by measuring pipette entry time, pore transit time and membrane rigidity at the same temperatures. RBCs from *N. coriiceps* living at 0 °C and *T. mossambicus* living at 25 °C were measured at the appropriate temperature.

The possible rheological effect of the marginal band or microtubular structures found in most nucleated red cells was investigated by incubating trout red cells for 4 h with 100 μmol l^{-1} colchicine. This agent dissociates such structures and 100 μmol l^{-1} is the maximum concentration that has been used in previous rheological studies (Frank, 1990; Betticher *et*

Table 1. *Haematological data for fish living at various temperatures*

	Haematocrit (%)	[Haemoglobin] ($g\,l^{-1}$)	MCHC ($g\,l^{-1}$)	$10^{-9}\times$ red cell count ($cells\,ml^{-1}$)	Mean cell volume (fl)	Plasma osmolarity ($mosmol\,l^{-1}$)
Trout at 4 °C	18.5±1.5	49.2±4.9	295±24	0.71±0.07	279±7	304±4
Trout at 11 °C	20.3±0.9	53.4±5.4	268±20	0.77±0.05	269±15	295±3
Trout at 18 °C	24.2±3.2	70.6±8.2	297±16	1.07±0.13	241±19	273±4
Notothenia coriiceps at 0 °C	23.9±3.6	63.5±10.0	252±17	0.83±0.17	312±20	–
Tilapia mossambicus at 25 °C	22.2±2.5	59.0±6.6	268±16	1.12±0.10	201±23	313±14

Data are mean ± S.E.M. for 4–11 fish at each temperature.

MCHC, mean cell haemoglobin concentration.

Plasma viscosity was not measurable by freezing point depression for *Notothenia coriiceps* because of the presence of the glycoprotein antifreeze.

Factorial analysis of variance (ANOVA) showed that red cell count ($P<0.05$) and plasma osmolarity ($P<0.01$) varied significantly with temperature for trout (*Oncorhynchus mykiss*).

al. 1993). Here, the membrane shear elastic modulus (for trout living at 4 °C) and micropipette entry time (for trout at 11 °C) were compared with and without colchicine treatment.

Results

Values for haematological indices and plasma osmolarity for trout, *N. coriiceps* and *T. mossambicus* living at different temperatures are summarised in Table 1. While haematocrit, red cell count and haemoglobin concentration each tended to increase for trout living at higher temperature, the MCHC did not vary and MCV tended to decrease. Plasma osmolarity also decreased significantly with increasing temperature for trout. In agreement with our previous studies (Nash and Egginton, 1993), when the temperature of trout red cells was varied acutely *in vitro*, cell volume assessed by Coulter counter did not vary provided that a period of equilibration was allowed (data not shown).

Values for the membrane shear elastic modulus (rigidity) of fish red cells are shown in Figs 1 and 2. Considering first the effect of acute *in vitro* temperature change on red cells of trout (living at 11 °C) and *N. coriiceps* (living at 0 °C), rigidity increased with decreasing temperature, particularly for temperatures below 4 °C (Fig. 1). It was notable that the rigidities for trout and *N. coriiceps* had a similar temperature-dependence and were also very close in absolute terms when measured at comparable temperatures. When trout were tested after long-term acclimation to different temperatures, membrane rigidity again increased with decreasing temperature, and the temperature-dependence closely followed the trend for the acute *in vitro* experiments (Fig. 2). *N. coriiceps* living at 0 °C and *T. mossambicus* living at 25 °C had levels of rigidity that appeared to follow the trend for variation in rigidity of trout cells acclimated to different temperature. Thus, overall, membrane rigidity showed marked temperature-dependence, but there was no evidence that adaptation of this rheological parameter occurred during acclimation of trout.

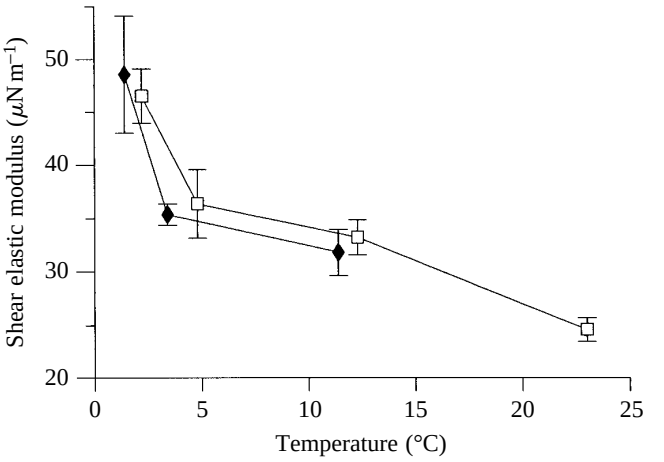

Fig. 1. Effect of acute *in vitro* variation in temperature on the rigidity of fish red cell membrane. Data are mean ± S.E.M. for three experiments on trout (□) or *Notothenia coriiceps* (◆). For trout, there was a significant variation in elastic modulus with temperature, as judged from linear regression of individual experimental means against measurement temperature ($P<0.01$). For *N. coriiceps*, linear regression did not show a significant correlation of elastic modulus with temperature, but in individual experiments, the elastic modulus at 1 °C was always significantly greater than the modulus measured at higher temperature ($P<0.05$; Student's *t*-test).

Trout and *N. coriiceps* had membranes with similar rigidity when measured at the same temperature and also showed a similar sensitivity to temperature change *in vitro*.

Cellular deformability was assessed for trout red cells by measuring resistance to flow into micropipettes with a diameter of approximately 4 μm (close to the limit for entry) and through pores of 5 μm diameter. During acute *in vitro* temperature manipulations, entry time into micropipettes increased with decreasing temperature, with the effect being more marked for trout living at 18 °C than for those living at 11 °C (Fig. 3). If the entry time was normalised by dividing it

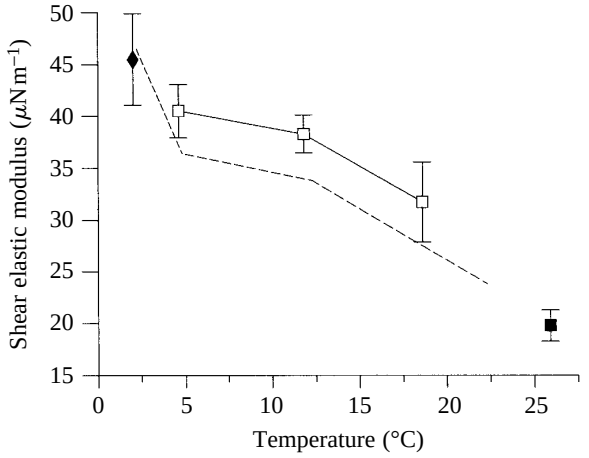

Fig. 2. Rigidity of red cell membrane for fish living at different temperatures. Data are mean ± S.E.M. for five specimens of trout (□), *N. coriiceps* (◆) or *Tilapia mossambicus* (■). For trout, there was a significant variation in elastic modulus with temperature, as judged from linear regression of mean values for individual specimens against measurement temperature (P<0.05). The dashed line represents data for variation in rigidity of trout red cell membrane with acute *in vitro* temperature change (shown in Fig. 1).

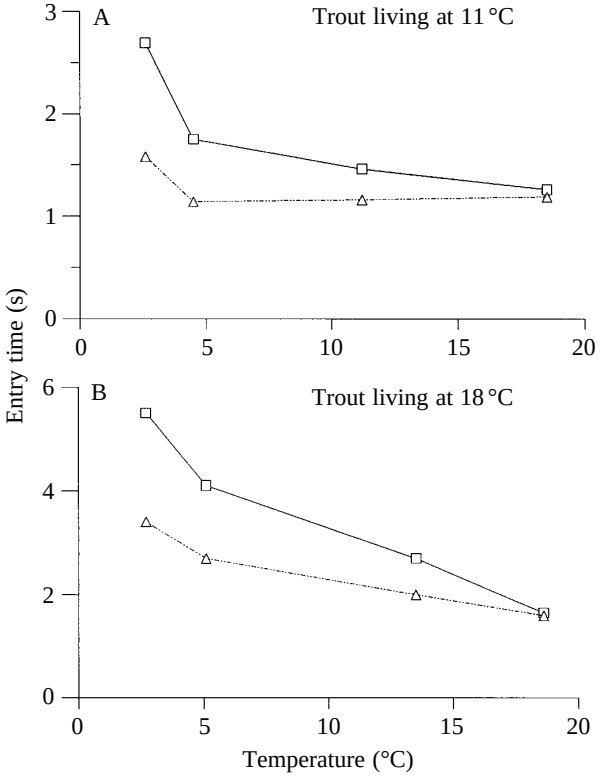

Fig. 3. Effect of acute *in vitro* variation in temperature on the entry time of trout red cells into a 4 μm pipette. (A) Trout living at 11 ˚C; (B) trout living at 18 ˚C. Data are averages from two experiments in each case. Absolute entry times (□) and normalised entry times (△) (entry time divided by the viscosity of water in mPa s; units=mPa^{-1}) are shown separately. Linear regression indicated that significant variation with temperature only occurred for absolute entry time for trout living at 18 ˚C (P<0.05).

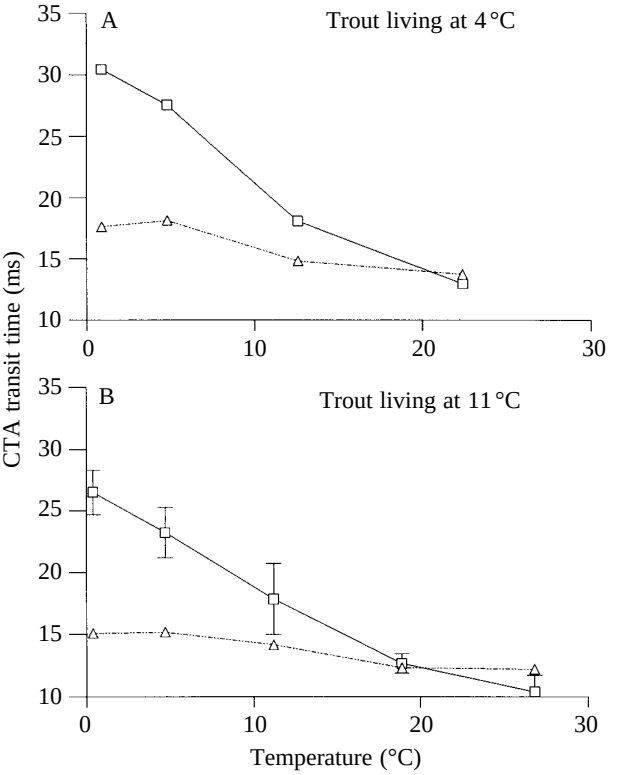

Fig. 4. Effect of acute *in vitro* variation in temperature on the 5 μm pore transit times (CTA) of trout red cells. (A) Trout living at 4 ˚C; (B) trout living at 11 ˚C. Data are averages from two or mean ± S.E.M. from three experiments respectively. Absolute transit times (□) and normalised transit times (△) (transit time divided by the viscosity of water in mPa s; units=mPa^{-1}) are shown separately. Linear regression indicated that significant variation with temperature occurred for absolute entry time (P<0.01) and for normalised entry time (P<0.05) for trout living at either temperature.

by the viscosity of water at the same temperature, then the effect of temperature was reduced and was not statistically significant. This procedure is equivalent to subtracting the expected effect of temperature on fluid viscosity, so that any residual temperature-dependence might be attributed to another (e.g. solid) structural component of resistance. In experiments of similar design, pore transit time for red cells from trout living at 4 ˚C or 11 ˚C showed a similar temperature-dependence (Fig. 4). Normalisation for fluid viscosity yielded a reduced, but significant, residual temperature-dependence of transit time.

Red cells from trout acclimated to different temperatures were also subjected to micropipette analysis at their ambient temperature. There was no evidence of variation in pore entry time with temperature (Fig. 5). However, it should be remembered that the variation in entry time over the same temperature range was not significant in the experiments where temperature was acutely varied *in vitro* (Fig. 3A; trout living at 11 ˚C). In contrast, pore transit times for trout acclimated to different temperatures did show a strong temperature-dependence (Fig. 6). The data followed closely the trend

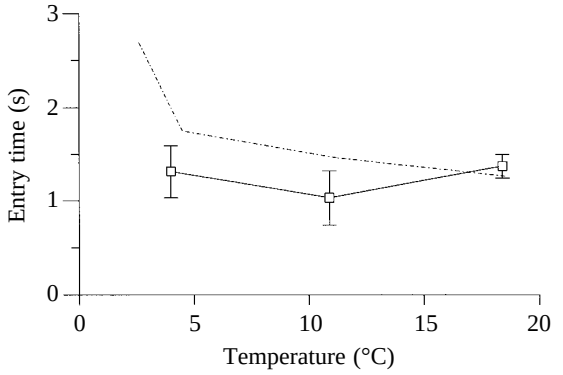

Fig. 5. Entry time of red cells into a 4 μm diameter pipette for trout living at different temperatures. Data are mean ± S.E.M. for four or six specimens of trout. There was no significant variation in entry time with temperature. The dashed line represents the entry times for red cells from trout acclimated to 11 ˚C, subjected to acute temperature variation *in vitro* (data from Fig. 3A).

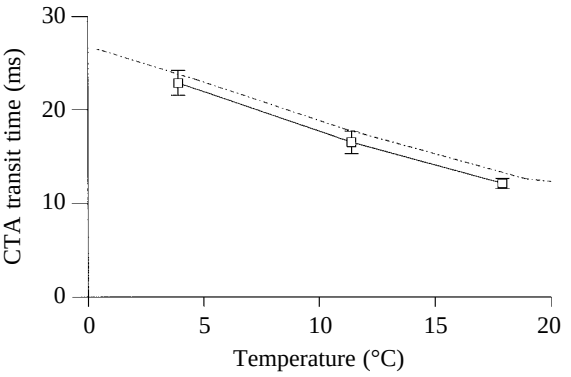

Fig. 6. Transit times of red cells through 5 μm pores (CTA) for trout living at different temperatures. Data are mean ± S.E.M. for four or five specimens of trout. Linear regression indicated that significant variation in transit time occurred with temperature (P<0.01). The dashed line represents the transit times for red cells from trout acclimated to 11 ˚C, subjected to acute temperature variation *in vitro* (data from Fig. 4B).

previously noted for acute temperature variation and thus again showed no evidence of adaptation.

In addition to membrane rigidity and fluid (e.g. cytoplasmic) viscosity, another component possibly influencing flow resistance is the marginal band found in many nucleated red cells (Cohen, 1978). When trout red cells were treated with colchicine to dissociate microtubular structures, the membrane shear elastic modulus did not change: for untreated red cells, the modulus was 38±10 μN m^{-1} *versus* 41±8 μN m^{-1} for colchicine-treated cells (mean ± S.D. of means from three comparative experiments). Thus, as might be expected, the membrane rigidity was independent of these structures. However, cell entry time into a micropipette was reduced by about 25 % after colchicine treatment: entry time 2.3±0.7 s for control *versus* 1.7±0.7 s after treatment (mean ± S.D. of means from three comparative experiments; entry time was significantly reduced by treatment in each experiment, P<0.01 by unpaired t-test).

Discussion

Not only do different species of fish live at a wide range of temperatures, but many are also able to acclimate to seasonal fluctuations in ambient temperature. In fish living at low temperature, it might be expected that adaptation of the flow properties of the blood would be needed to compensate for the inevitable increase in resistance to flow, although modification of the cardiovascular system might achieve the same result. Rheological compensation could occur both at the bulk flow level (e.g. reduction of haematocrit or plasma protein concentration to reduce blood viscosity) and/or at the cellular level (e.g. reduction of the flow resistance of individual red cells). Here, we have studied the rheological properties of individual fish red cells to investigate their dependence on temperature and to determine whether there is evidence of adaptation to life in the cold.

Rigidity and resistance to flow into and through narrow pores increased when red cells were cooled *in vitro*. Similar changes were noted when red cells from trout living at different temperatures were measured at comparable temperatures. Thus, judging from the temperature-dependence of membrane rigidity or pore transit time, we found no evidence of rheological adaptation associated with acclimation of trout to lower temperature. In contrast, micropipette entry times were essentially constant for red cells from trout living at different temperatures. However, even in the experiments where temperature was varied acutely *in vitro*, variation in entry time was barely statistically significant (particularly over the range of living temperatures). The 4 μm aperture of the micropipette is close to the limiting size for entry defined by cell geometry, and cells must elongate (shear) as well as bend (or fold) during entry. Passage through the large pores can be achieved simply by folding and so may not be so sensitive to changes in cell structure. However, given the increased membrane shear rigidity and lack of reduction in cell volume or MCHC with acclimation to lowered temperature, no adaptation in pipette entry time would be expected. There remains the possibility of some other structural adaptation (perhaps in the nuclear structure) that would influence flow into smaller apertures. It should also be borne in mind that the pipette entry measurement showed much wider variation between fish than the cell transit analysis, and the latter measurement has greater methodological precision. We conclude that the evidence does not suggest a significant rheological adaptation to the cold for individual red cells.

In terms of identifying the structural factors that control resistance to flow through narrow vessels, our studies of temperature-dependence imply that increased fluid viscosity is a major component, possibly identifiable with the cytoplasmic viscosity and/or the membrane viscosity. The remaining temperature-dependence after correction for fluid viscosity could arise from the strong temperature-dependence of the

membrane rigidity. Human erythrocyte membrane rigidity is only weakly dependent on temperature, but membrane viscosity is more strongly so (Hochmuth *et al.* 1980). If trout red cell membrane viscosity (not investigated here) also has a strong temperature-dependence, it could contribute to the 'dynamic rigidity' (Evans *et al.* 1984) experienced during rapid entry into small pores. Other structural components that could play a role are the putative marginal band found in many nucleated red cells (Cohen, 1978) and the nucleus itself. We tried, but could not identify, a distinct marginal band in trout red cells by electron microscopy (not shown), but did find that resistance to pore entry was reduced by treatment of cells with colchine. This supports the contention that another structural component (independent of the membrane rigidity, which was not affected by colchicine) affected cell deformability. The nucleus appears rigid when aspirating trout cells into small micropipettes (Nash and Egginton, 1993), but we have not identified a means to evaluate its resistance separately or with respect to temperature-dependence.

There have been relatively few studies of the rheology of fish red cells and particularly of the effects of temperature. The temperature-dependence of the viscosity of the blood of Antarctic fish has been investigated (see Macdonald and Wells, 1991, for a review), but we are not aware of comparable data for trout. Red-blooded Antarctic fish tend to have a blood viscosity that is more strongly temperature-dependent than that of the haemoglobin-free icefishes (Wells *et al.* 1990), perhaps suggesting that variation in red cell rheology with temperature can influence bulk flow resistance. At the individual cellular level, our own previous study (Nash and Egginton, 1993) detailed the rheology of trout cells at a single temperture (11 °C) and demonstrated the high rigidity and flow resistance of these cells compared with those human red cells. Others have described the resistance of bulk suspensions of fish red cells (most often from trout) to filtration through multipore filters (Hughes and Albers, 1988; Hughes *et al.* 1986; Chiocchia and Motais, 1989). Studies of the *in vitro* temperature-dependence of these measurements indicated that, for a range of species, passage times through 8 μm pores decreased with increasing temperature (Hughes and Kikuchi, 1984; Kikuchi *et al.* 1982). This broadly agrees with our current results, although no correction was made for the temperature-dependence of fluid viscosity, which in such large pores would include the viscosity of the suspending medium. By analogy to human cells at least, cytoplasmic viscosity might be expected to be the major cellular determinant of flow resistance in larger pores, with relative insensitivity to membrane and cell geometry compared with smaller pores (Reinhart and Chien, 1985; Stuart *et al.* 1985). With regard to membrane rheology, the present study is the first to describe the effect of temperature on the elastic modulus of fish red cells and the failure to adapt this property during acclimation. Membrane lipid fluidity (reflecting resistance to molecular motion within the lipid bilayer) decreases with decreasing temperature, and fish are believed to regulate the membrane lipid constituents to reduce this effect (Cossins and Bowler, 1987; Macdonald and Wells, 1991, for reviews). This does not necessarily signify that the macroscopic membrane viscosity or elasticity (reflecting resistance to membrane motion) must be compensated, as these properties depend largely on the membrane protein skeleton.

The physiological relevance of the present study remains open to conjecture. If rheological adaptation is unnecessary for trout acclimating to seasonally lower temperature, is another adaptation required to compensate for increased flow resistance? The most obvious would be the alteration of vascular architecture to lower peripheral resistance (e.g. by vasodilation, opening of anastomoses or shunts or growth of new vessels) or an increase in perfusion pressure. We know of no data describing cyclical variation in these parameters. It is possible that reduced oxygen demand, due to reduced levels of activity and hence $\dot{V}_{O_2}$, would obviate the need to maintain perfusion at the same levels as at warmer temperatures. It is interesting to note that, in terms of membrane rigidity at least, the rheological properties of the different species (*N. mossambicus*, trout and *T. coriiceps*) appeared to have a similar temperature-dependence. This again seems to imply that variation of this property is not a specific feature of adaptation to life in different thermal environments, but could represent a common temperature-dependent change in membrane structure. Overall, therefore, our results suggest either that red cell deformability is not the critical factor limiting microvascular perfusion, as has been generally assumed, or that decreased red cell flux in capillaries at low temperature is accommodated by other cardiovascular adaptations.

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