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COMPARATIVE RHEOLOGY OF HUMAN AND TROUT RED BLOOD CELLS

BY GERARD B. NASH AND STUART EGGINTON

Department of Haematology and Department of Physiology, The Medical School, University of Birmingham, Birmingham B15 2TT

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Summary

We have studied the comparative rheology of individual red blood cells from humans and rainbow trout (*Oncorhynchus mykiss*) at their natural body temperatures. Trout red blood cells were large ellipsoids (about 16 μm×11.5 μm×2.5 μm) with a mean volume of 250 fl, a surface area of approximately 350 μm^2 and an elongated nucleus of about 9 μm×5 μm. Although much larger than human red cells (diameter 8 μm, V=92 fl, A= 136 μm^2), both theoretical calculation and experimental aspiration into micropipettes indicated that the limiting size of a cylindrical vessel that both types of cell could enter was approximately 3 μm. Nevertheless, individual trout red cells had much longer transit times through 5 μm filter pores and were much slower to enter 3–4 μm diameter micropipettes. Interestingly, the relative deformability of the trout cells depended on the pore size and applied pressure, with entry times for trout and human cells converging as pipette diameter increased. The relatively poor overall cellular deformability of the trout cells reflected their membrane rigidity (shear elastic modulus 4–5 times higher than that of human membrane), as well as their large size and the presence of a prominent nucleus. Capillary diameters in trout muscle are similar to those in the human microcirculation (about 3 μm), while systemic driving pressures are much lower. Therefore, either red cell deformability is a less critical circulatory parameter than has previously been thought, or the apparently disadvantageous blood rheology of trout is adequate because of the lower demand for tissue perfusion.

Introduction

It is generally agreed that the ability of red cells to alter their shape reversibly, under the influence of flow forces, is necessary to facilitate their circulation (Chien, 1987). This deformability enables red cells to pass through capillaries with diameters smaller than the cellular dimensions and also reduces the bulk viscosity of the blood (Nash and Dormandy, 1989). Red cell deformability is thought to influence not only microcirculatory perfusion but also cell lifespan, because non-deformable cells may be trapped and destroyed in the spleen (Groom, 1980). However, the range within which this deformability must be maintained, in order to avoid circulatory impairment, is uncertain

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(Nash, 1991). The mechanical characteristics of red cells can vary widely between species (e.g. Chien *et al.* 1971, Waugh and Evans, 1976*a*), probably reflecting different functional requirements and/or geometry of the microvascular network. Analysis of comparative rheology of red cells may indicate which characteristics limit, or are adapted for, the differing demands of oxygen and nutrient delivery.

The mechanical properties of human red cells have been widely studied, in part at least because abnormalities may contribute to clinical pathology. These studies have shown that red cell deformability depends on cell geometry (volume, surface area and shape), cytoplasmic state (haemoglobin solution viscosity, presence of solid bodies) and membrane viscoelasticity. Each of these factors has been characterised in health and in a variety of disease states (see Chien *et al.* 1987, for a recent survey). Far less information is available for fish red cells. While bulk viscosity has been measured (e.g. Wells and Weber, 1991), studies of cellular rheology have concentrated on measurement of the filterability of blood or red cell suspensions (Hughes and Albers, 1988; Hughes *et al.* 1986; Chiocchia and Motais, 1989).

Comparative studies of fish and human red cells have been rare, although it has been shown that red cells from the toadfish have a more rigid membrane than human cells (Waugh and Evans, 1976*a*). A number of rheological studies have, however, compared red cells from human and mammalian species with nucleated avian or amphibian cells (Usami *et al.* 1970; Chien *et al.* 1971; Gaehtgens *et al.* 1981*a*,*b*). These studies generally showed that the larger, ellipsoidal, nucleated red cells were less deformable and had greater resistance to flow in bulk suspensions than the biconcave anucleate human cells. However, it was not clear which structural aspects of the different cells types were responsible for the differences in rheological behaviour. Here we report parallel studies of human and trout red cells, using techniques which test individual cells and their membrane properties and which we have previously applied to human blood.

Materials and methods

Blood sampling and preparation

Human blood was drawn from healthy volunteers by venepuncture, anticoagulated with heparin ($5 i.u.ml^{-1}$) and held at room temperature until required. The basic suspension medium for these cells was phosphate-buffered saline (PBS, Dulbeco A, Oxoid Ltd, London, UK, with $1 mgml^{-1}$ glucose added; pH7.4, $295 mosmolkg^{-1}$).

Blood was drawn from rainbow trout (*Oncorhynchus mykiss*) of about 650g body mass, maintained for 4–6 weeks in dechlorinated tapwater at 11–13°C, *via* chronically indwelling arterial catheters which had previously been implanted under 1:10000 MS222 anaesthesia. All fish were allowed to recover for 48–72h following surgery before sampling. These procedures allowed unobtrusive sampling of conscious fish and avoided sampling stress which leads to adrenergic stimulation of red cells. The blood was anticoagulated with heparin ($10 i.u.ml^{-1}$) and held on ice until required. The osmolarity of trout plasma was measured on one occasion and found to be $292 mosmolkg^{-1}$. The basic suspension medium for the red cells was a modified teleost Ringer's solution (TRS: NaCl $113 mmol l^{-1}$, KCl $4 mmol l^{-1}$, $(NH4)_2SO_4$ $0.1 mmol l^{-1}$, $NaHCO_3$ $13 mmol l^{-1}$,

$MgSO_4$ 1.2mmol l^{-1}, KH_2PO_4 0.4mmol l^{-1}, Na_2HPO_4 1 mmol l^{-1}, $CaCl_2$ 1.3mmol l^{-1}, sodium pyruvate 1.3mmol l^{-1}, glucose 5.6mmol l^{-1}, pH7.6 at 12˚C, 295mosmol kg^{-1}) (adapted from Payan and Matty, 1975; Perry *et al.* 1984).

Samples of human and trout cells prepared for micropipette and pore transit measurements were equilibrated in measurement media (see below) at 37˚C or 11˚C, respectively, for 1h before analysis. All procedures were completed within 4h of blood withdrawal.

Cell geometry

Cell morphology and dimensions were evaluated by video-microscopy using whole blood that had been diluted tenfold with 2% glutaraldehyde in PBS, or red cells that had been diluted for rheological analysis and mixed 1:1 with this fixative. Cell dimensions were measured using a BBC microcomputer fitted with a Genlock video-mixing board and a Basic, mouse-based program that determined distances between markers placed on the video screen. At a final magnification of 6000× on the video monitor, pixel resolution was 0.13 μm (i.e. better than optical resolution), and the coefficient of variation for repeated measurements of cell diameter was typically 2%.

Mean red cell volume (MCV) was measured for whole blood by determining haematocrit without correction for trapped plasma (microhaematocrit tube, centrifuged at 13000 ***g*** for 3min) and cell count (Coulter counter model ZF, Coulter Electronics Ltd, Luton, Bedfordshire). Previous studies have indicated that trapped plasma represents about 3% of the centrifuged cell column for human red cells and 6–7% for nucleated, ellipsoidal avian cells (Usami *et al.* 1970; Gaehtgens *et al.* 1981*a*). No data for trout red cells are available to our knowledge. Thus, there is probably an uncertainty of about 3–4% in comparative volume estimates for the human and trout cells in this study. Volume distributions (but not absolute cell volume) were measured with a pulse height and width analyser (PWHA; Bioengineering Unit, Strathclyde University, Glasgow) linked to the Coulter counter. This device analyses the height and width of each Coulter counter voltage pulse generated as a cell passes through the sensing orifice. The pulse height is proportional to the cell volume multiplied by a shape factor. Unless stated otherwise, the human or trout cells were fixed with glutaraldehyde (see above) before final dilution and measurement. This avoided the possibility of acute volume changes in the fish cells when subjected to ambient temperature in the Coulter counter, and also removed the possibility of the cells deforming and altering their shape factor as they flowed through the sensing orifice. Frequency distributions of pulse height were recorded, allowing changes in the cell volume distribution with time, temperature or preparative procedure to be followed.

The values for the length (L), width (W) and MCV of the trout cells were used to calculate the mean cell thickness (t) for each sample, by assuming the cells had ellipsoidal geometry. Overall mean values for L, W and t were then used to calculated a minimum value (area of two flat elliptical surfaces) and a maximum value (area of two elliptical surfaces separated by a band of thickness t) for the cell surface area. Values for the diameter of human red cells and surface area (directly measured by micropipette) were taken from Nash and Wyard (1980). Values for MCV and surface area were used to

calculate the minimum cylindrical diameter (MCD), i.e. the smallest-diameter cylindrical aperture into which the cells could fit (Canham and Burton, 1968).

Pore transit measurements

The time required for passage of individual cells through 5 μm pores was measured using a cell transit analyser (CTA; ABX International, Levallois, France) (Zhu *et al.* 1989). In this device, a dilute cell suspension is driven through a filter with 30 pores by hydrostatic pressure. The temperature of the filter assembly is controlled by a water jacket. Electrodes either side of the filter supply an a.c. current at 100kHz, and the conductance is recorded as a function of time by microcomputer. The widths of the resistive pulses detected as individual cells pass through the pores represent the transit times of these cells. The transit times are collected into a frequency distribution, and percentiles and measures of central tendency are automatically determined.

Human blood was diluted 1:1000 in PBS, and 2000 transit times were measured. The influence of white cells on these measurements is negligible (Zhu *et al.* 1989). However, since measurements on fish red cells have not been reported, and white cell counts were much higher (1 per 33 red cells; see Results) than those found for humans (typically 1 per 700 red cells), trout blood was first subjected to removal of the buffy coat. The blood was loaded into microhaematocrit tubes and centrifuged at 13 000 ***g*** for 3min. The tubes were cut with a diamond knife to remove the buffy coat and the red cells were washed into TRS. The cells were counted by Coulter counter and diluted to $10^6\,ml^{-1}$ in TRS. Pulses from 1000 cells were recorded. The hydrostatic driving pressure was 400Pa for human and 1000 Pa for trout cells. The higher pressure was used for the trout cells to keep the transit times in the mid-region of the instrument range and to maintain a similar sampling time.

Micropipette analysis

The microscope/video and micropipette system was similar to that previously described (Nash and Wyard, 1981; Nash and Meiselman, 1985). Blood was diluted 1:1000 in PBS (human cells) or TRS (fish cells), each containing 10% autologous plasma, equilibrated for 1h at the measurement temperature, and then placed in a chamber made of two glass coverslips separated by a U-shaped gasket. This chamber was placed in an aluminium block which had channels cut into it. Water was circulated through these channels at either 37˚C or 11˚C. Measurements with a thermocouple showed that the sample temperatures in the chambers actually averaged 36 and 12˚C for human and trout cells, respectively. The aluminium block was placed on the stage of a microscope and viewed using a water immersion 40× lens, which was also cooled or heated.

A micropipette was connected to a hydrostatic pressure system, with resolution of 0.5Pa and manipulated to enter the open side of the chamber. The tip of the pipette and the surrounding cells were viewed by black and white camera and video monitor. The video signal was first passed through a BBC microcomputer as described above.

Membrane rigidity

A membrane tongue was aspirated from the flattened side of red cells into a pipette

with internal diameter of approximately 1.5 μm (Fig. 1A). For trout cells, the membrane was tested in the peripheral region, not covering the nucleus. The length of the tongue (l) was measured *via* the microcomputer, at 3–5 increasing pressures (P), and the shear elastic modulus calculated from dl/dP as previously described (Nash and Wyard, 1981). This parameter represents the resistance of the membrane to shear deformation at constant area. Ten cells were measured in each sample.

Pipette entry time

Pipettes with internal diameter in the range 3.1–4.3 μm were used to aspirate red cells completely at a constant pressure (Fig. 1B,C). The chamber and hydrostatic pressure system each contained a Ag/AgCl electrode. A constant current of 0.1–0.2 μA was applied

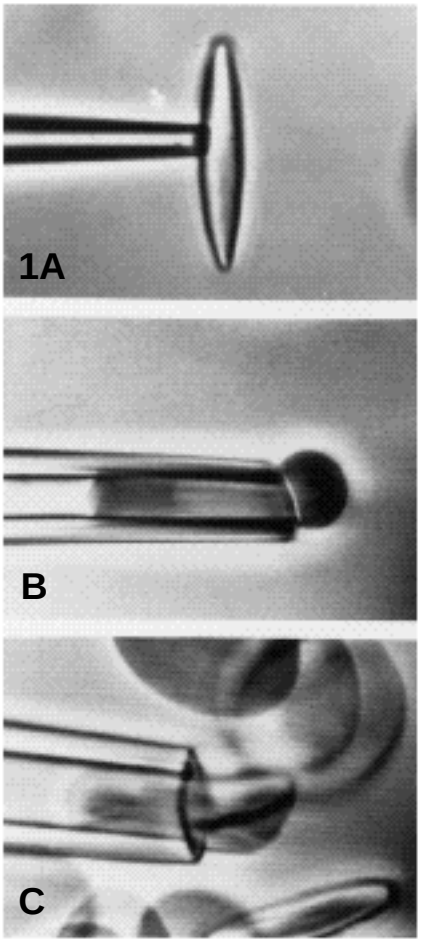

Fig. 1. Video photomicrographs of (A) a trout red cell with a portion of the cell membrane being aspirated into a micropipette with an internal diameter of 1.2 μm; (B) a trout red cell in the process of being fully aspirated into a micropipette with an internal diameter of 3.1 μm; (C) a trout red cell in the process of being fully aspirated into a micropipette with an internal diameter of 4.3 μm.

between the electrodes, depending on the pipette size, so that the voltage drop across the pipette was 0.2V. As each cell entered the pipette, the resistance, and hence the voltage, across the pipette increased. This voltage pulse was amplified and supplied to the analog/digital converter of a BBC microcomputer. The digitised pulses were analysed to measure the time between the voltage rising above a pre-set threshold and it falling to 90% of its peak height. This time represents the period between the cell first reaching the pipette aperture and completely entering. This equivalence was verified by comparison to video recordings played back a single frame at a time. The system has a time resolution of 0.01 s.

Results

The morphologies of human and trout red cells are compared in Fig. 2, and values for geometric parameters are shown in Table 1. Trout cells were essentially elliptical in all planar projections, although there was a tendency in some at least to bulge in the region of their clearly visible nucleus, when viewed side-on. This ellipsoidal nucleus had average dimensions of $8.7\pm0.7\times4.8\pm0.5\,\mu m$ (mean ± S.D. for 32 nuclei measured in two samples of trout blood). The trout cells had a larger volume, calculated surface area and major dimensions than the discoidal, human red cells. Given the volume and surface area of a cell, one can calculate the diameter of the smallest cylinder into which it can fit (Canham and Burton, 1968). The ability to adapt to a narrow capillary depends on the cell having a greater surface area than the minimum required to enclose its volume. Both the human and trout red cells had such an excess surface area and are predicted to be able to enter vessels with diameters of about 3 μm (Table 1).

The trout red cells maintained smooth elliptical morphology if kept as whole blood on ice or if diluted in TRS at 11˚C, but at room temperature their outline tended to become frilled and some haemolysis was apparent after a few hours. Sudden warming of unfixed trout red cells to room temperature for Coulter analysis caused cell swelling (average increase in volume 19% in six experiments). However, when diluted in TRS and held at 11–13˚C, trout cell volume remained nearly constant, with an average decrease of 6% over 2–4h (eight experiments). In all experiments, trout red blood cells were held for 1 h

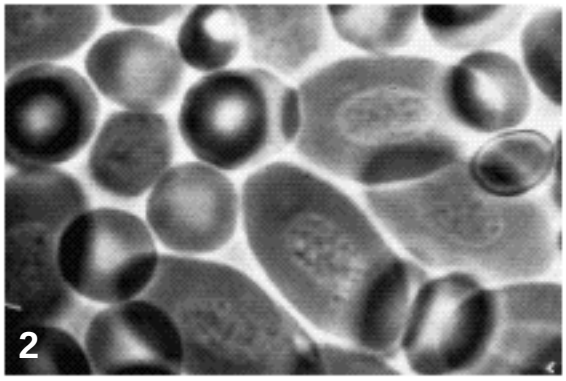

Fig. 2. Video photomicrograph of a mixture of glutaraldehyde-fixed human and trout red cells.

Table 1. *Geometry of trout and human red cells*

	Trout	Human
Dimensions	$L = 16.2 \pm 0.3$ $W = 11.5 \pm 0.7$ $t = 2.5 \pm 0.3$ ($N = 4$)	$D = 8.1 \pm 0.4$‡ ($N = 6$)
Mean cell volume (μm^3)	251 ± 19 ($N = 8$)	92.3 ± 4.6 ($N = 5$)
Surface area (μm^2)*	Min = 300 Max = 400	136±8‡ ($N = 5$)
Cylindrical diameter (μm)†	Min = 2.5 Max = 3.3	Min = 2.85

Data are mean ± S.D. of mean values from (N) samples.

L, cell length; W, cell width, t, cell thickness (calculated from data for cell volume, L and W, assuming ellipsoidal cell shape); 16 trout cells were measured in each sample. D, cell diameter.

*Estimated as the area of two flat elliptical surfaces (Min), or as the area of two elliptical surfaces separated by a band of thickness t (Max).

†Calculated from values for mean cell volume and surface area.

‡Data from Nash and Wyard (1980).

at the measurement temperature before rheological analysis. Human red cells showed no change in morphology or volume for up to 24h in PBS at 37˚C.

The volume distribution of trout red cells, but not human cells, was distinctly bimodal (Fig. 3). On average, 18.5% of the trout cells existed in a second peak with a modal volume 3.1 times that of the main peak (11 experiments). Microscopically, no morphologically distinct population of larger cells was evident. The second peak was present for fixed or unfixed cells, with or without removal of the buffy coat from the blood and at all storage times and temperatures. Removal of the buffy coat reduced the white blood cell count by 90% (from 59 to 6 per 2000 red cells counted microscopically in four samples), so that the population of larger cells could not represent white cells. The ratio of fish:human mean cell volume was 2.3, as judged by the Coulter counter/PWHA system and by MCV for two pairs of samples. This agreement between methods implies that the shape factors must have been similar for the fixed human and trout cells flowing through the Coulter aperture.

Portions of cell membrane were aspirated into micropipettes to characterise the resistance to shear deformation at constant area. Typical data for the variation in the length of aspirated membrane tongues as a function of pressure are shown in Fig. 4. The slopes of the lines fitted by linear regression are inversely proportional to the shear elastic modulus for the respective samples (Nash and Wyard, 1981). The membrane shear elastic modulus for trout red cells at 12˚C was $33.3 \times 10^{-6} \pm 5.9 \times 10^{-6}\,N\,m^{-1}$ and for human cells at 37˚C was $7.1 \times 10^{-6} \pm 0.2 \times 10^{-6}\,N\,m^{-1}$ (mean ± S.D. of three sample means in each case). The ranges of values for the 30 cells tested were 22.4–53.0 and 5.2–9.1 respectively. The trout membrane was therefore 4–5 times more rigid than that of human cells. Much higher pressures (150–3000Pa) were required to aspirate the fish cell

membrane compared to the human membrane (20–60Pa). If pressure was increased further for the human red cells, they buckled and a large portion of their membrane entered the pipettes. The lack of buckling of the fish cells at the higher pressures indicates that their membrane is more resistant to bending as well as to shear deformation (Evans, 1983). The relative resistance to bending is not accurately quantifiable, however, because of the different shapes of the fish and human cells.

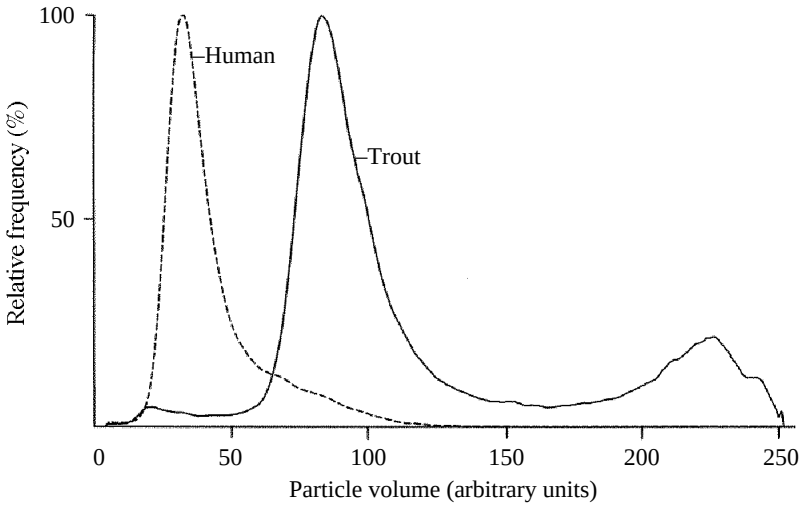

Fig. 3. Volume distributions of trout and human red blood cells obtained using a Coulter counter and pulse width and height analyser; the same instrument current and amplification settings were used for the two samples.

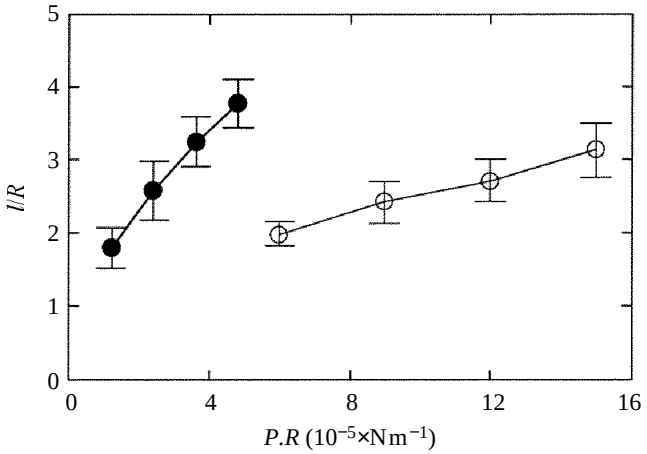

Fig. 4. Variation in the length (*l*) of membrane tongues aspirated into a micropipette as a function of the aspiration pressure (*P*). *R*, internal radius of the micropipette (0.6 μm). Data are mean ± S.D. of data from 10 red cells from a single sample of human (●) or trout (○) blood.

Individual red cells were aspirated into micropipettes with internal diameters in the range 3.1–4.3 μm. Resistance to entry (R) was defined as $dP.t_e$, where t_e is entry time, and the ratio of resistances for trout/human cells (R_t/R_h) was used for comparisons, because human and fish red cells could not always be tested at the same pressure. Fig. 5 shows variation in R_t/R_h with pipette diameter. Near the limiting diameter of 3 μm, trout cells had about a thousand times greater resistance to entry than human cells. This ratio dropped rapidly, to a value of approximately 10, as pipette diameter increased to 4.3 μm.

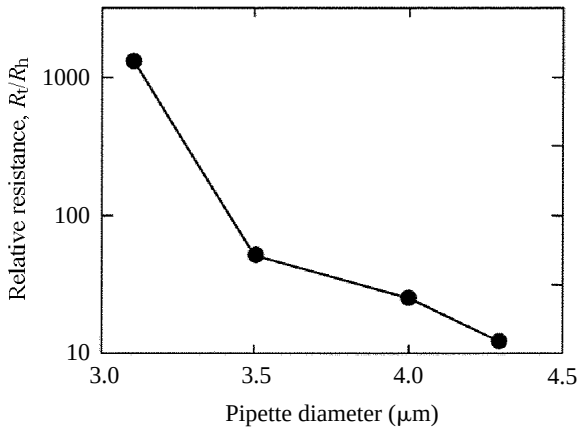

Fig. 5. Ratio of the resistances to micropipette entry of trout:human red cells (R_t/R_h) plotted as a function of micropipette internal diameter. Each point represents the median entry time multiplied by the aspiration pressure for trout cells divided by the same parameter for human cells measured with the same micropipette. For each pipette diameter, two trout samples (average 60 cells each) and two human samples (average 50 cells each) were tested.

Table 2. *Transit times for trout and human red cells flowing through 5μm diameter pores*

Transit time (ms)	Trout†	Human‡	Trout/human
Percentile			
25th	9.0±1.9	0.96±0.02	9.4
50th	11.6±2.1	1.15±0.06	10.1
75th	14.0±2.6	1.35±0.08	10.3
90th	17.4±4.1	1.66±0.10	10.5
90th/50th	1.48±0.1	1.40±0.06*	–
Mean (ms)	12.9±2.5	1.37±0.13	9.4
Coefficient of variation (%)	71±10	58±17*	–

Data are mean ± S.D. of values from six samples.
†Pressure 1.0kPa.
‡Pressure 0.4kPa.
All differences between trout and human cells were significant ($P<0.01$, by Student's t-test) except those marked with an asterisk.

These data indicate that the relative deformability of trout and human red cells depends on the diameter of the vessel through which they must pass. In absolute terms, entry times for human cells were of the order of 0.1–0.5s at pressures of 20–30Pa for the different pipettes and were 0.2–10s at pressures of 100–1000Pa for the trout cells.

Transit times through 5 μm pores (rather than entry times) were analysed automatically for individual cells using a cell transit analyser. Results are shown in Table 2. The median transit time was ten times higher for trout cells than for human cells, at a driving pressure which was 2.5 times higher (i.e. relative resistance=25). For higher transit time percentiles, there was an upward trend in the ratio of fish:human transit times. Also, the coefficient of variation of transit times was slightly, but not significantly, greater for the trout cells. The frequency distributions of entry times did not show obvious bi-modality or strong positive skewness for either trout or human cells (data not shown). However, the ratio of the 90th/50th percentile was slightly higher for the trout cells. Thus, the fish cells had a broader distribution of entry times, but evidence of a slower flowing subpopulation of cells was not marked. The relative resistance value of 25 for trout cells is higher than the value obtained using the 4.3 μm pipette and a much lower driving pressure (Fig. 5), so that relative deformability depends not only on pore diameter, but also on driving pressure.

Discussion

The red blood cells of humans and trout differ greatly in their mechanical attributes. The trout cells are larger, have a stiffer membrane (more resistant to shear and bending) and contain a large nucleus that is absent from the human cells. Regardless of their large size, the trout cells do have sufficient membrane surface area to enable them to adapt their shape to traverse capillaries. Thus, calculations and direct observation show that trout red cells can enter cylindrical apertures down to 3 μm in diameter. However, near this limiting diameter, their resistance to pore entry is about a thousand times higher than that of human red cells.

If cellular deformability is characterised by the ability to enter pores, then the relative deformability of trout:human red cells (R_f/R_h) depends on the pore diameter and driving pressure. As diameter increases, so the deformabilities converged (Fig. 5). However, for a 4 μm pipette and a driving pressure of about 10Pa, R_f/R_h was 10, while for 5 μm cell transit analyser pores and a driving pressure of about 1000Pa, R_f/R_h was 25. The greater relative resistance for the larger pores is not to be expected. The transit times through the larger pores were of the order of milliseconds, which is much shorter than the natural deformation time for human red cells (0.1s) and the pipette entry times. Deformation of the viscoelastic red cells in milliseconds will give a very high dynamic rigidity (defined as the elastic modulus multiplied by the ratio of the natural deformation time:imposed deformation time) (Evans *et al.* 1984). The results suggest that the dynamic rigidity of the trout cells increases more than that of the human cells when they are driven to deform so rapidly. This is equivalent to stating that the trout cells have a greater intrinsic, and/or membrane, viscosity than the human red cells.

The relatively poor overall deformability of the trout red cells could arise from their increased membrane rigidity, larger size and the presence of a large nucleus. Artificial rigidification of human red cell membrane has previously been shown to increase

resistance to pipette entry roughly in proportion to the change in shear elastic modulus (Nash and Meiselman, 1985). That the nucleus was itself relatively rigid was demonstrated if a membrane tongue was aspirated into a 1.5 μm pipette in its region. Much greater pressure was required to obtain visible deformation compared to regions away from the nucleus (data not shown). In addition, when aspirating a complete trout cell into 3–4 μm pipettes, it was often apparent that the cell entered more rapidly initially and slowed down when the nucleus reached the pipette entrance. Overall, it seems likely that the large size and nucleus of the trout cells had most effect at the lower pipette diameters, where relative resistance was very high, and that membrane rigidity would have a more uniform influence on deformability. However, it is difficult to be certain about this in the absence of cells with comparable geometry to that of trout cells, but rigidity close to that of human cells.

There have been few previous studies of the rheology of trout red cells. Hughes and co-workers have carried out a number of studies of the filterability and volume of fish red cells (Hughes and Albers, 1988; Hughes *et al.* 1986). They found that filtration rate and cell volume were increased when blood was sampled from trout during cannulation, compared to the subsequent recovery phase (Hughes *et al.* 1986). In addition, physiological saline could be used to give stable cell volumes and filterability over a period of hours. Suspensions of carp red cells in saline, however, had much poorer filterability than whole blood (Hughes and Albers, 1988). The filterability of the carp cells was sensitive to *in vitro* asphyxia, while the addition of adrenaline nullified this effect of asphyxia. Chiocchia and Motais (1989) found that, for trout red cells, adrenergic-stimulated swelling caused the filtration rate to be increased.

Human and fish red cells were not compared in the above studies. Measurements on osmotically swollen human red cells indicate that a small degree of swelling (up to about 10%) has little effect on filterability, but that further swelling impairs filterability (Stone *et al.* 1990). The degrees of cell swelling observed in the fish studies noted above were at least 10%. However, the decrease in cell surface area/volume ratio was presumably not great enough to impair pore transit. Reduction of internal viscosity might have reduced the filtration times. In the present study, cell volume was carefully monitored and found to be nearly constant during the measurement procedures.

The diameters of trout capillaries average 3 μm in slow muscle (Egginton and Rankin, 1991), and thus they are of a similar size to human capillaries (Bagge and Brânemark, 1977). Trout have a much lower circulatory driving pressure than humans, and generally have a lower haematocrit but similar bulk blood viscosity compared to values for humans (Wells and Weber, 1991). Cellular deformability is likely to have its greatest influence on resistance to flow when vessel diameters approach the cell dimensions (e.g. Gaehtgens *et al.* 1981*b*) and at the entry to capillaries. The major resistance vessels in the circulation are arterioles of greater diameter, so that impaired deformability would not necessarily have a significant influence on cardiovascular parameters such as cardiac output or overall circulatory resistance. However, microcirculatory efficiency and adequate tissue perfusion would be expected to be influenced by cellular deformability. Nevertheless, it seems that the greatly decreased cellular deformability of trout red cells is not a hindrance to microcirculatory efficiency.

This raises the question of whether red cell deformability is a much less critical circulatory parameter than has previously been thought (Nash, 1991). It may be that there is a considerable reserve capacity in the deformability of the human red cell, which might not be present in the trout red cell. Many clinical studies have suggested that impaired red cell deformability in humans has pathological consequences. However, firm association of loss of deformability with pathology is established in no more than a handful of conditions. In sickle cell disease, circulatory obstruction probably only occurs in the presence of polymerisation, when red cell deformability decreases by about a thousandfold (Nash *et al.* 1986). In hereditary spherocytosis, loss of deformability probably underlies premature destruction of red cells (Waugh and Agre, 1988). In contrast, other conditions with quite severe abnormality (e.g. Melanesian ovalocytosis; Saul *et al.* 1984) appear benign.

The current study suggests that a wide range of red cell deformability may be acceptable in circulatory terms. Other comparative studies of a number of animal species support this contention, because of the greatly varying rheological characteristics detected (Usami *et al.* 1970; Chien *et al.* 1971; Waugh and Evans, 1976*a*; Gaehtgens *et al.* 1981*a*,*b*). However, caution may be needed in unequivocally drawing this conclusion since, for example, trout have a much lower demand for tissue perfusion, reflecting a lower rate of oxygen uptake. In addition, Gaehtgens *et al.* (1981*b*) point out that a high cellular rheological component to flow resistance might be compensated by low capillary haematocrit (i.e. increased Fahreus effect) or a greater number of capillaries per unit tissue volume in species with nucleated red cells. Indeed, fish slow muscle has a capillary density exceeded only by that of the mammalian myocardium (Egginton and Johnson, 1983).

Trout present several other rheological and circulatory problems worthy of further investigation. The temperature of the human blood is closely controlled, but the trout circulation has to be able to cope with rapid changes in temperature (e.g. when traversing the boundary between different thermal masses in stratified lakes), as well as gradual seasonal changes. The effects of these temperature fluctuations on blood rheology and circulation in the trout are unknown. A decrease in temperature would at least be expected to increase blood viscosity (Barbee, 1973), while for the human red cell, it is known that the membrane becomes markedly more viscous and slightly more rigid with reduction in temperature (Waugh and Evans, 1976*b*; Hochmuth *et al.* 1979).

Similar behaviour need not necessarily be expected from trout cells. The viscoelasticity of the human red cell is believed to be imparted by the uniform protein structure (or skeleton) which underlies the membrane. This may not be the case for fish cells and some elliptical, nucleated red cells have additional resistance to deformation imparted by a structurally distinct marginal band (Waugh and Erwin, 1989). Also, it is known that some fish red cells undergo a ‘homeoviscous’ structural adaptation in their membrane composition, which may increase the membrane ‘fluidity’ at low temperatures (Nikinmaa, 1990). Whether this compensatory change in structure affects the macroscopic, as well as the molecular, rheology of the membrane is uncertain. Finally, trout red cells can also undergo rapid volume shifts in response to changes in the partial pressure of oxygen and other forms of stress, following adrenergic stimulation

(Nikinmaa, 1990). The circulatory and rheological effects of such factors are of interest, not only because of their effect on fish physiology. Comparative studies may shed further light on the critical parameters controlling normal human circulation and hence contributing to pathological conditions such as hypothermia.

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