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SHORT COMMUNICATION

USE OF FILTRATION METHODS IN EVALUATION OF THE CONDITION OF FISH RED BLOOD CELLS

BY G. M. HUGHES* AND C. ALBERS
Physiological Institute, University of Regensburg, FRG

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Although the vertebrate erythrocyte is a very specialized type of cell it has provided material for a whole variety of general studies which are important not only for an understanding of red cell function, but also of the nature, structure and properties of cell membranes. More recently, suspensions of these cells have been used to investigate ionic, and other, mechanisms which are important in the respiratory function of vertebrate blood (Nikinmaa, 1982; Cossins & Richardson, 1985). The nucleated nature of non-mammalian red cells endows them with longer life and metabolic properties more directly related to other cells (Schindler & de Vries, 1986). Regulation of the volume of these cells, including the role of catecholamines, has consequences in relation to the concentration of modifying chemicals within the cell which have important influences on the affinity, and other aspects, of haemoglobin gas transport properties (Borgese *et al.* 1987; Nikinmaa & Huestis, 1984; Weber, 1982).

Because of the complex nature of blood plasma, especially in relation to the varying concentrations of circulating catecholamines and other chemicals, many investigators have tended to replace the plasma with a suitable suspending medium. Assessment of the suitability of such media has utilized a variety of criteria including haematocrit and mean cell volume (MCV). Although these features are important, they have not included tests for the maintenance of normal mechanical properties of the red cells, except for the absence of haemolysis. As the remarkable deformability properties of vertebrate red cells are one of their most important adaptations to functioning at the microcirculatory level, it would seem appropriate that this property should be tested to establish the extent to which any red cell suspension may claim to be fully representative of whole blood and, hence, whether any studies of special properties of the red cells using such suspensions truly reflect their condition in the normal circulation. It was on this basis that a recent study of trout red blood cell suspensions was made (Hughes *et al.* 1986), confirming the suitability of Cortland saline (Wolf, 1963) as a suitable medium for this fish.

* Usual address: Research Unit for Comparative Animal Respiration, Bristol University, Bristol BS8 1UG, England.

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The aim of the present experiments was to investigate the effects of equilibration with different gas mixtures on the deformability of carp (*Cyprinus carpio*) erythrocytes. Although the results indicated the presence of such effects, the most significant finding was that in all cases blood equilibrated in tonometers differed from blood which had not been tonometered. Preliminary attempts to produce red cell suspensions with the same filtration properties as whole blood have not been successful.

The present study is mainly based upon experiments carried out at 20°C using blood samples taken from chronically cannulated carp using methods described by Hughes *et al.* (1983). The blood was taken into heparinized syringes and used in several types of experiments. These included studies of whole blood, comparing samples before and after tonometry (intermittent rotating IL type 237) with different gas mixtures which reproduced normoxic (P_{O_2} 55 mmHg, P_{CO_2} 3·5 mmHg; 1 mmHg = 133·3 Pa) and asphyxic (P_{O_2} 7 mmHg, P_{CO_2} 14 mmHg) conditions in carp dorsal aorta blood. Other samples were used in parallel studies with suspension of the red cells made in the following medium (mmol l^{-1}): KCl, 4·0; NaCl, 124·0; sodium L-lactate, 0·6; $CaCl_2$, 3·6; $NaHCO_3$, 12·6; KH_2PO_4, 1·0; $MgSO_4.7H_2O$, 1·0; D-glucose.H_2O, 3·5: pH 7·85; 20°C: osmolarity 268 mosmol l^{-1}. These suspensions were also used for pilot studies on the oxygen consumption of carp red cells.

Haematocrit (microcentrifuge) and red cell count (Coulter counter) were routinely determined in duplicate, and were adjusted to a standard haematocrit value of 20 %. After modifying the composition of the suspending medium to that given above, and always making it up freshly, suspensions were obtained in which haemolysis was absent.

To test the filtrability of whole blood and red cell suspensions the apparatus developed by Kikuchi *et al.* (1983), as modified by Hughes *et al.* (1987), was used to determine the timecourse of blood flow through Nuclepore filters containing pores of 8 or 5 μm. From the recordings a slope value was used to calculate the filtration time of the 0·3-ml sample. All samples used in the filtration tests were heparinized to the same extent (500 i.u. ml^{-1} blood or suspending medium). The filtration time of the suspending medium alone was determined before each measurement and a new filter used each time. Filtration times for carp plasma were also determined, as was the content of total protein using a refractometer. The latter measurements were confirmed by direct chemical analysis (Lowry *et al.* 1951).

Cannulation time was very brief with rapid recovery of all specimens when returned to the aquaria. Nevertheless, clear changes in haematocrit and MCV were observed. All experiments showed a significant decrease ($P < 0·05$) in both haematocrit and MCV during postoperative recovery. Some of this occurred within 90 min but it was most marked after 24 h. Pore passage time for single red blood cells (PPT) sometimes decreased during this period, as was to be expected from the corresponding reductions in haematocrit and MCV. In this respect results with carp blood were less consistent than in comparable studies using rainbow

Table 1. *Pore passage time (ms) for single red blood cells through 8 μm pores and total plasma protein (g dl^{-1}) of blood samples after tonometry with different gas mixtures (see text)*

Expt no.	Normoxia		Asphyxia		Asphyxia + adrenaline		Air		No tonometry (means)	
	PPT	TP	PPT	TP	PPT	TP	PPT	TP	PPT	TP
1	0·772	2·7	0·554	2·8	0·525	2·9	0·536	2·8	0·356	3·1
2	2·445	3·0	4·22	3·1	2·169	3·0	0·769	2·9	0·495	3·2
3	1·48	2·9	1·055	2·6	0·912	2·6	1·223	2·6	0·558	3·13
4	1·961	2·9	3·908	2·7	1·93	2·7	2·699	2·7	0·799	3·0
5	0·601	3·1	2·46	3·5	0·476	3·5	1·073	3·3	0·542	3·73
6	0·399	2·9	0·352	2·8	0·493	3·0	0·444	2·7	0·295	3·13
Means	1·28		2·09		1·08		1·12		0·58	

PPT, pore passage time; TP, total plasma protein.

Values for the same samples immediately after sampling, i.e. without tonometry, are given as means of the four samples taken from that fish.

Each of the six sets of determinations was made using four separate samples from the same carp.

Analysis of variance (two-way) for the PPT data showed significant differences between the five columns at the 5% level, with a least significant difference of ±0·266.

trout (Hughes *et al.* 1986) where PPT increased in spite of decreasing MCV and haematocrit. However, most of the carp showed some increase in PPT during the 24-h recovery period.

Comparisons were made between results obtained using blood samples immediately following their removal from the fish (= whole blood) and samples from the same blood after it had been kept in tonometers for varying periods. No changes were observed if more than 1 ml was placed in the tonometer. However, tonometry of a smaller volume for longer than 20 min was associated with an increase in PPT and also significant reductions (10%) in total plasma proteins (Table 1). This reduction in plasma protein was observed even after 10 min of tonometry but did not seem to occur when more than 1 ml of blood was placed in the tonometer. Further experiments involving tonometry under asphyxic conditions, either with or without the addition of adrenaline (10^{-6} mol l^{-1}), gave comparable results, i.e. reduction in plasma proteins and increase in PPT with longer tonometry. An indication was also obtained (paired *t*-test: $0{\cdot}10 > P > 0{\cdot}05$) that adrenaline reduces the pore passage time when compared with blood tonometered under asphyxic conditions alone (Table 1). No significant effect of adrenaline was found when the blood was tonometered with air.

Although fresh suspensions had been prepared and gave repeatable results when used in oxygen consumption measurements, it was found that these suspensions filtered very badly in spite of many attempts to modify the suspension procedure in ways comparable to those used for rainbow trout (Hughes *et al.* 1986). These included adequate heparinization and repeated passage through a

syringe needle (18 gauge) to reduce red cell aggregates. Pore passage times of all suspensions were very long so that even 8 μm filters became blocked quite quickly. Estimates of the filtration rate could only be obtained at the very beginning of the recordings.

The results obtained here confirm other research showing that the filtration technique can give useful information regarding the condition of both whole blood and red cell suspensions. The intention at the beginning of the study was to investigate filtration properties of carp red cell suspensions equilibrated with different gas mixtures and compare them with whole blood. However, at an early stage it became apparent that the filtration times of the suspensions used were so long that such a study was not possible. Furthermore, parallel experiments using whole blood revealed properties of great interest and are reported here.

Changes in deformability following anaesthesia have been observed in rainbow trout where PPT decreased in spite of red cell swelling (Hughes *et al.* 1986). In carp this effect was observed in some specimens, but in others PPT increased with the increase in MCV. This difference may reflect variability in hypoxic levels during surgery and also that carp are not affected as much as trout when subjected to the same degree of hypoxia. A very striking effect, however, was the decrease in filtration time of blood tonometered with adrenaline and under asphyxic conditions relative to blood with asphyxia alone. Furthermore, such an effect was not observed with normoxic blood. These effects are related to comparable studies (Fuchs & Albers, 1988) showing an effect of adrenaline on MCV which occurred under conditions of hypercapnic hypoxia but not normoxia.

In all cases tonometered blood showed an increase in pore passage time relative to blood which had not been tonometered and its filtration time determined immediately after sampling from the fish. It is this effect which is mainly responsible for the significant difference which analysis of variance showed between columns in Table 1. Tonometered blood also showed a significant reduction in total protein compared with identical samples which had not been tonometered.

It is concluded: (i) that red cells in blood tonometered under the particular conditions of these experiments show different mechanical properties to those of whole blood; (ii) that adrenaline decreases the filtration rate of whole blood but only when equilibrated with low-oxygen and high-CO_2 mixtures; and (iii) that red cell suspensions which otherwise appeared to maintain respiratory properties similar to those of whole blood evidently have changed mechanical properties as they do not pass through filters containing pores of 8 μm whereas whole blood samples can do so with little difficulty.

Prolonged tonometry is commonly used in some methods for determining the oxygen dissociation characteristics of whole blood and/or acid–base properties, and such determinations might easily be influenced by modifications to the condition of the red blood cells. As yet no studies have been made comparing these characteristics following tonometry under different conditions. It has been pointed out (Albers, 1972), that the volume ratio of gas to blood must be adequate

and depends on the type of tonometer. It should be emphasized that no comparisons have been made between the relative merits of the many types of tonometer used in different laboratories.

The use of red blood cell suspensions is often encouraged to reduce some of the variability conferred by blood plasma. As a result of this study it seems possible that adverse changes in the condition of the red cells may occur which must be weighed against these advantages. It is suggested that routine monitoring of filtration characteristics might be incorporated in future studies using red cell suspensions to provide an index of the mechanical condition of the material. More extended studies on suspensions of carp red cells are planned to assess the factors which tend to reduce their filtrability.

References

ALBERS, C. (1972). Analyse von Gasen in Flussigkeiten. In *Klinisch-Physiologische Untersuchungsmethoden* (ed. W. Koenig). Stuttgart: Thieme-Verlag.

BORGESE, F., GARCIA-ROMEU, F. & MOTAIS, R. (1987). Control of cell volume and ion transport by β-adrenergic catecholamines in erythrocytes of rainbow trout, *Salmo gairdneri*. *J. Physiol., Lond.* **382**, 123–144.

COSSINS, A. R. & RICHARDSON, P. A. (1985). Adrenaline-induced Na^+/H^+ exchange in trout erythrocytes and its effects upon oxygen-carrying capacity. *J. exp. Biol.* **118**, 229–246.

FUCHS, D. A. & ALBERS, C. (1988). Effect of adrenaline and blood gas conditions on red cell volume and intraerythrocytic electrolytes in the carp, *Cyprinus carpio*. *J. exp. Biol.* **137**, 457–476.

HUGHES, G. M., ALBERS, C., MUSTER, D. & GOETZ, K.-H. (1983). Respiration of the carp, *Cyprinus carpio* L. at 10 and 20°C and the effects of hypoxia. *J. Fish Biol.* **22**, 613–628.

HUGHES, G. M., KIKUCHI, Y. & BARRINGTON, J. (1986). Physiological salines and the mechanical properties of trout red blood cells. *J. Fish Biol.* **29**, 393–402.

HUGHES, G. M., OXENHAM, R. K. C. & SCHROTER, R. C. (1987). Photoelectric recording of the time course of blood filtration – an index of red cell deformability. *J. Physiol., Lond.* **388**, 6P.

KIKUCHI, Y., ARAI, T. & KOYAMA, T. (1983). Improved filtration method for red cell deformability measurement. *Med. Biol. Eng. Comput.* **21**, 270–276.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. & RANDALL, R. J. (1951). Protein measurement with the Folin–phenol reagent. *J. biol. Chem.* **193**, 265–275.

NIKINMAA, M. (1982). Effects of adrenaline on red cell volume and concentration gradient of protons across the red cell membrane in the rainbow trout, *Salmo gairdneri*. *Molec. Physiol.* **2**, 287–297.

NIKINMAA, M. & HUESTIS, W. H. (1984). Adrenergic swelling of nucleated erythrocytes: cellular mechanisms in a bird, domestic goose, and two teleosts, striped bass and rainbow trout. *J. exp. Biol.* **113**, 215–224.

SCHINDLER, J. F. & DE VRIES, U. (1986). Scanning cytophotometry of carp, *Cyprinus carpio* L., erythrocyte populations: the influence of short-term hypoxic environment and the recovery period following severe bleeding. *J. Fish Biol.* **28**, 741–752.

WEBER, R. E. (1982). Intraspecific adaptation of haemoglobin function in fish to oxygen availability. In *Exogenous and Endogenous Influences on Metabolic and Neural Control* (ed. A. D. F. Addink & N. Spronk), pp. 87–101. Oxford, New York: Pergamon Press.

WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progve Fish Cult.* **25**, 135–140.