Effects of Temperature and Transfer from Seawater to Freshwater on Blood Microrheology in Pacific Salmon

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Summary Blood of Pacific salmon was studied with particular interest in red blood cell (RBC) deformability in relation to migration. Blood samples were taken via cardiac puncture or chronic cannula placed in the dorsal aorta and heparinized. As an index of RBC deformability the mean passage time of single RBCs through micropores of 8 μm in diameter and 10 μm in length was determined under a pressure difference of 10 cmH₂O. Despite about 100 mOsmol/l difference in plasma osmolality, there was no marked difference in RBC passage time between fish in seawater and those well acclimatized to freshwater. However, it seemed probable that a transient decrease in RBC passage time, i.e., an increase in RBC deformability, occurred immediately following transfer from seawater to freshwater. Plasma osmolality decreased to about 300 mOsmol/l within 1 hr after the transfer and showed no fluctuations thereafter. The temperature dependence of RBC deformability was much smaller in comparison with those previously observed in yellowtail and carp; salmon RBCs were still highly deformable even at 5°C, a possible temperature of cold river water.

Key words: red blood cell deformability, temperature, osmolality.

In lower vertebrates physicochemical properties of blood are readily modified by environmental conditions. Changes in physical properties of red blood cells (RBCs) can have direct effects on microcirculation. Under normal conditions, because of their remarkable deformability, RBCs can be easily squeezed through fine capillary vessels or narrow openings whose diameters are less than that of an
If RBCs lose this deformability under different environmental conditions, the survival of the animals will be severely restricted. However, little consideration has been given to this subject by comparative physiologists.

RBC deformability can be tested in vitro by squeezing RBCs through micro pores with a diameter smaller than that of an RBC, i.e., under similar conditions to in vivo flow situations. Recent studies using an improved filtration method (Kikuchi et al., 1980, 1983) have shown that RBC deformability in yellowtail and carp varies considerably with temperature, suggesting a relation with their normal ambient temperature (Hughes et al., 1982; Kikuchi et al., 1982). It was further notable that a marked increase in RBC deformability was induced in rainbow trout exposed to aquatic hypoxia (Hughes and Kikuchi, 1984). Similar mechanisms appeared to work in exercised rainbow trout (Kikuchi et al., 1985). Some evidence has been obtained of such positive changes not only in fish but in mammals; rats kept at high altitudes showed an increased RBC deformability in comparison with those kept at low altitudes (A. Sakai et al., in preparation). These findings suggest that RBC deformability and its regulation are significantly involved in the acclimatization of animals to different environments and in acute responses to external stresses. In order to extend such knowledge of microcirculation, the present study examined RBC deformability in Pacific salmon in relation to their migration from sea to rivers, first effects of temperature and then effects of transfer from seawater to freshwater.

Pacific salmon (Oncorhynchus keta, weighing 3–4 kg) captured in sea near estuaries of rivers of southern Hokkaido (Mukawa and Shiraoi, water temperature about 15°C) were kept in laboratory seawater and freshwater circulation (10 in seawater and 6 in freshwater) at the same temperature of 15°C in an aquarium (Sun-Piazza Aquarium, Sapporo). After taking a minimum of 3 days for acclimatizing the fish to the laboratory circulation, the cannulation of the dorsal aorta was tried first for the fish in seawater. Techniques modified from those described in Soivio et al. (1975) were used under anesthesia with MS222 (1 g/10 l). However, good preparations were obtained only in three specimens because of difficulty in accurately puncturing the vessel. Cardiac puncture was made immediately when preparations appeared to be unsuccessful. Blood samples obtained via cardiac puncture were used mainly for measurement of RBC deformability at different temperatures. The cannulation of the dorsal aorta was also tried for the fish in freshwater after more than two weeks of acclimatization, and was successful in two specimens. Blood samples were obtained via cardiac puncture from the others and compared with those from the fish in seawater with effects of temperature on RBC deformability. The cannulated fish in seawater were transferred to freshwater 24 hr after the operation so that they could recover well from the anesthesia and surgical invasions. To our regret, one of the three was lost before the transfer because it jumped out of the tank at night. Blood sampling was made immediately before and one and four hours after the transfer. In the second...
specimen one sampling was added at 30 min after the transfer. Two ml of blood was taken each time into 500 units of heparin. The same ratio of heparin to blood was used for the blood sampling via cardiac puncture.

As an index of RBC deformability the average time required for single RBCs to pass through pores of 8 µm diameter and 10 µm length in a Nuclepore filter was determined under a pressure difference of 10 cm H2O. This method for measuring RBC deformability was given in KIKUCHI et al. (1980) and in more detail in KIKUCHI et al. (1983). A 0.35 ml blood sample was used for each determination of the RBC passage time. The temperature of the blood samples was varied by equilibrating them in a water bath for 20 min. The filtration apparatus was also thermostated with water from the water bath. Three water baths at three different temperatures were used at the same time so that the time loss for varying the water temperature was minimized. A small portion of each blood sample was used for measurement of hematocrit (microhematocrit, 11,000 G for 5 min, in duplicate), RBC count (Neubauer counter), and plasma osmolality (freezing point depression method, Knauer Halbmikro-osmometer, FRG).

Changes in RBC passage time with temperature obtained for the fish in seawater are shown in Fig. 1A and those for the fish in freshwater in Fig. 1B. Plasma osmolality and mean corpuscular volume are also given in the figures. For the sake of comparison, mean ± S.D. of the RBC passage times in Fig. 1A are shown in Fig. 1B by the region with hatching. As is clear from Fig. 1B, there were no marked differences in RBC passage time at any temperatures measured between the fish in seawater and the fish in freshwater in spite of about 100 mOsmol/l difference in plasma osmolality. The relative changes in RBC passage time with temperature were small; even at 5°C the RBC passage times were not considerably greater than the values at 15°C. Above 20°C the blood flow rate through the filter often decreased with increasing temperature. Those changes, however, appeared to be dependent on the amount of heparin and hence due to microclot formations. Therefore, the increases in calculated RBC passage time are shown by broken lines in the figures and the values were excluded from the calculation of means and S.D.

Although there appeared to be no marked differences in RBC deformability in the fish well acclimatized to seawater and/or freshwater, some transient changes were observed in RBC passage time following transfer from seawater to freshwater. The changes are shown in Fig. 2 together with changes in plasma osmolality. The regions with hatching in the figure show mean ± S.D. of values obtained from the cannulated fish in seawater before the transfer (including values at 6 hr after the operation) and those obtained from the cannulated fish in freshwater, respectively. RBC passage time became shorter immediately following the transfer, indicating an increase in RBC deformability, and then gradually increased toward the values for the fish acclimatized to freshwater. Plasma osmolality decreased rapidly to about 300 mOsmol/l within 1 hr after the transfer and showed no fluctuations thereafter.
Fig. 1. Changes with temperature in RBC passage time through 8 μm pores in a Nuclepore filter. A: RBCs in blood samples taken from fish kept in seawater. B: RBCs in blood samples taken from fish well acclimatized to freshwater. The blood samples were taken via cardiac puncture in both cases. The region with hatching in B shows mean±S.D. of the values given in A. See text for the broken lines.
Salmon migrating from sea to rivers are subjected to rapid changes in water salinity and water temperature. Although a number of studies have been made on the osmoregulation in diadromous fish (see Foskett et al., 1983), few investigators have paid attention to the microcirculation which is essential for osmoregulatory organs to function. For example, effects of temperature on RBC deformability will become important since salmon will not be able to migrate upstream even if the osmoregulation is complete unless their RBC can maintain high deformability at temperatures as low as 5°C. The present results of the RBC passage time-temperature relationship are thus consistent with the activities of salmon in cold rivers. Conversely, the greater temperature dependency of RBC deformability observed in yellowtail and carp (Hughes et al., 1982; Kikuchi et al., 1982) accords with the fact that they are more active at relatively high temperatures.
The changes in plasma osmolality after the transfer from seawater to freshwater were very rapid. The osmoregulation appeared to be “switched” from that of the seawater teleost to that of the freshwater teleost. It is known that chloride cells transport chloride ions in the reverse direction as soon as they accept hormones (Foskett et al., 1983). It will be noteworthy that such rapid changes in plasma osmolality or “switching” of the osmoregulation appear to provide more difficult conditions for the cells to acclimatize themselves to the new osmolality of their environments. The present observation of plasma osmolality will be valuable since such phenomena will have been detected only by using chronically cannulated fish.

A marked increase in RBC deformability immediately following the transfer might be related to some hormones which may increase in the circulation in response to the water osmolality change. Changes in plasma membrane physical properties are known to occur in cells which accepted hormones (Lewis, 1983). Such changes might be suggested to also occur in red blood cells from the present observation of RBC deformability. Whatever the mechanism is, this increase in RBC deformability will be favorable for the microcirculation and hence hormonal control of osmoregulatory organs.

We wish to express our thanks to Sun-Piazza Aquarium and its staff for their kind assistance and support throughout the present study. We are also much indebted to the Royal Society who provided travel funds for GMH.

REFERENCES


Japanese Journal of Physiology