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VOLUME REGULATION IN RED BLOOD CELLS

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SUMMARY
Hagfish erythrocytes lack the anion exchanger (band 3 protein) in their membranes. This protein has been suggested to play a key role in the activation of a regulatory volume decrease in teleost and elasmobranch fishes. A total lack of the ability of cellular volume regulation in hagfish erythrocytes supports this notion. The lack of volume regulation is accompanied by an extreme stability of hagfish erythrocytes when exposed to anisotonic media.

19.1 INTRODUCTION
The extracellular fluid of hagfish is iso-osmotic or very slightly hyperosmotic to seawater. In fact, the composition of plasma is not very different from that of seawater. Only the concentrations of calcium, magnesium and sulphate are significantly lower in plasma than in seawater (Morris, 1965). This situation resembles that of many marine invertebrates, but is unique among vertebrates where osmotic equilibrium with full strength seawater is always partly obtained by high plasma concentrations of organic substances like urea or TMAO (trimethyl amine oxide). Hagfishes are osmoconformers, i.e. they do not defend their plasma osmotic concentration when transferred to either more dilute or more concentrated seawater. They are normally considered to be stenohaline, and indeed *Myxine* cannot survive acute transfer to less than 70–80% of full strength seawater. This is related to a very slow clearance of the initial osmotic water influx across the gills and body surface (McFarland and Munz, 1965), which is probably due to the limited filtration capacity of the agnathan kidney (Fels et al., this volume; Riegel, this volume). If, however, dilution or concentration is accomplished by small daily changes over several weeks, *Myxine* may be acclimated to salinities ranging from approximately 60% to 150% of full strength seawater (Cholette et al., 1970). During the process of acclimation, the parietal muscle cells regulate their volume by adjusting the intracellular concentration of free amino acids, most notably proline, alanine, leucine and threonine. The ability of the parietal muscle cells to regulate their volume is retained although it is very likely that *Myxine* has been living in an osmotically stable environment for millions of years. Unlike parietal muscle cells, the erythrocytes, however, do not seem to be able to regulate their volume.

19.2 PROPERTIES OF HAGFISH ERYTHROCYTES
The erythrocytes of hagfishes are approximately ellipsoidal in shape and are nucleated. They are larger than teleost red blood cells,
the two major axes being 25 and 20 μm, and having a volume of 1160 μm³. Even though the blood plasma in which they are suspended is practically pure seawater, the concentrations of the major inorganic ions Na⁺, Cl⁻ and K⁺ are not very different from those in teleost fishes (Fincham et al., 1990; Peters and Gros, this volume; Figures 19.2 and 19.4). Unlike in teleosts, however, the concentrations of free α-amino acids are very high (Fincham et al., 1990). In Eptatretus stoutii the most abundant amino acids are proline, alanine, leucine and threonine. The erythrocyte membrane of Eptatretus stoutii has been shown to be deficient of the anion exchanger (the band 3 protein), spectrin and several other cytoskeletal elements normally found in red blood cells (Ellory et al., 1987). In Myxine, Brill et al. (1992) found only little binding of the anion exchange inhibitor DIDS (4,4'-diisothiocyanostilbene-2,2'-disulphonate) to the membrane of the erythrocytes, which indicates that the anion exchanger is also absent in this species. The absence of the anion exchanger in the membrane of the red blood cells has consequences for CO₂ and O₂ transport (Fago and Weber, this volume) as well as for cellular volume regulation.

19.3 VOLUME REGULATION

When suspended in hypotonic media, teleost red blood cells (RBCs) initially behave as nearly perfect osmometers undergoing rapid, osmotic cell swelling according to the high membrane permeability to water. Subsequently the cells regulate their volume back towards the initial value by a regulatory volume decrease (RVD) and the final change in cell volume is consistently smaller than expected from van’t Hoff’s law. The mechanism involved in RVD in teleost and elasmobranch RBCs is the activation of coupled potassium-chloride effluxes and often also of a net efflux of β-amino acids, especially taurine. These fluxes are followed by osmotically obliged water (Hoffmann and Simonsen, 1989; Nikinmaa, 1992). Treating trout RBCs with DIDS inhibits RVD as well as all the ion pathways normally activated by hypotonic swelling, which suggests that the anion exchanger of the erythrocyte membrane plays an essential role in the activation of the response (Garcia-Romeu et al., 1991). It has been shown that expression of rainbow trout band 3 in Xenopus oocytes induces chloride and taurine transport, which strengthens the view that band 3 protein plays a key role in the activation of volume regulation (Fievet et al., 1995).

It has recently been demonstrated that, while erythrocytes from the lampreys Petromyzon marinus and Lampetra fluviatilis are capable of RVD when suspended in hypotonic medium, Myxine RBCs appear to be unable to regulate their volume in hypotonic medium (Nikinmaa et al., 1993). In the later investigation, however, the initial osmotic disturbance was obtained by a dilution of the suspension medium of only 20%. Compared to this, skate RBCs did not show any volume regulation when the medium was diluted by 30%, whereas a 50% dilution induced potassium transport activity as well as a volume regulatory response (Dickman and Goldstein, 1990). We therefore examined the osmotic behaviour of Myxine RBCs under a larger range of conditions.

When challenging red blood cells from Myxine in hypotonic media of differing degrees of hypotonicity it turned out that they were extremely stable, being able to swell to more than 3 times their original volume with very little or no apparent haemolysis. There were no signs of volume recovery when the medium was diluted by 25% (Figure 19.1) in agreement with earlier findings by Nikinmaa and co-workers (1993). Similarly, concentration of the medium by 25% did not elicit a regulatory volume increase. Addition of adrenaline in isotonic medium, which elicits a cell volume increase in trout red blood cells by activating the Na⁺/H⁺ exchanger (Borgese et al., 1987), was