# VOLUME REGULATION OF MUSCLE FIBRES IN THE KILLIFISH, *Fundulus heteroclitus*

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When animal cells are exposed to acute changes in the osmolality of their bathing medium they act as osmometers according to Boyle-van't Hoff's law: the osmotically active water content (V) times the osmotic pressure ($\pi$) remains constant, V = k or $\pi_1/\pi_2 = V_2/V_1$.

Many cells studied in vitro have been shown to regulate their volume following the initial swelling in a hypoosmotic medium (F. M. Kregenow, J. Gen. Physiol., 58:372-395, 1971; P. M. Cała, J. Gen. Physiol., in press; M. A. Lang and H. Gainer, J. Gen. Physiol., 53:323-341, 1969). However, following shrinking in a hyperosmotic medium volume regulation is only rarely observed.

The present study deals with regulation of cell water and ion content in vivo in the muscle fibres of the killifish following transfer from fresh water to sea water and vice versa.

The fish were maintained in the laboratory for at least a week prior to the experiments in Living Streams (Frigid Units, Inc., Toledo, Ohio) in either sea water (SW) or pond water (FW) at 10°C. When transferred, they were placed in aerated plastic containers in an incubator at 10°C. $^{14}$C polyethylene glycol was used as a marker for extracellular fluid (B. Schmidt-Nielsen, J. L. Renfro and D. Benos, Bull. MDIBL, 12:99-104, 1972). It was injected in the caudal tail vein six hours prior to sacrifice. The fish were killed about 2, 4, 6 and 8 hours following transfer to the other salinity. Blood samples were taken from the heart with a heparinized glass cannula. Pieces of muscle were treated as described previously for determination of tissue water content and tissue ion concentration (B. Schmidt-Nielsen, J. L. Renfro and D. Benos, Bull. MDIBL, 12:99-104, 1972; B. Schmidt-Nielsen, Am. J. Physiol., 230:514-521, 1976). Using the plasma concentration as representing extracellular fluid, intracellular water content and amounts of ions were calculated as previously described (B. Schmidt-Nielsen, Am. J. Physiol., 230:514-521, 1976).

Fish acclimated to SW had higher plasma osmolality and sodium concentration than fish acclimated to FW. Cell water content was lower in SW vs FW acclimated fish (Table 1). During acclimation from SW → FW (Figure 1) cell water content did not increase with decreasing plasma osmolality, showing that volume regulation was taking place. $\pi_1/\pi_2$ eight hours after transfer to FW was 1.48 while $V_2/V_1$ was only 1.1. The final regulation of cell volume was not due to changes in the intracellular amounts of sodium and potassium but could be accounted for by a decrease in nonelectrolyte solutes from 390 ± 36 µmoles/gram dry cell solids (g.d.c.s.) (n=6) to 172 ± 25 µmoles/g.d.c.s. (n=3). During the period of acclimation intracellular sodium increased temporarily.

During acclimation from FW → SW cell water content decreased continuously (Figure 2). A temporary increase in intracellular sodium prevented the cells from shrinking proportionally to the decrease in osmolality. After 8 hours the average ratio $\pi_1/\pi_2$ equaled the average ratio $V_2/V_1$, both being 0.80. No change in intracellular amount of electrolytes or nonelectrolytes was observed. In fish acclimated for a week or more in SW the intracellular water content was significantly higher than that observed in fish 8 hours following transfer to SW (Table 1 and Figure 2).

In conclusion the results show that volume regulation in a hypoosmotic medium is evident within the first 2 hours of transfer and that it is primarily due to a loss of nonelectrolyte solutes from the cells. Volume regulation in a hyperosmotic medium is not apparent 8 hours following transfer. However, following a week of acclimation in SW the cell volume increases.

### TABLE 1

*FUNDULUS HETEROCLITUS* ACCLIMATED

| | SW | FW | P value |
|---|---|---|---|
| | Plasma Concentration mM | | |
| | n=6 | n=6 | |
| Osm | 409 ± 16 | 346 ± 4 | <.01 |
| Na | 194 ± 9 | 157 ± 4 | <.01 |
| K | 5.00 ± 0.23 | 4.54 ± 0.58 | ns |
| Cl | 156 ± 17 | 143 ± 13 | ns |
| | Intracellular Amount µmoles/g.d.c.s. | | |
| | n=13 | n=14 | |
| Na | 54.5 ± 4.3 | 53.3 ± 3.5 | ns |
| K | 476 ± 5 | 480 ± 7 | ns |
| | $gH_2O/g.d.c.s.$ | | |
| | n=14 | n=14 | |
| $H_2O$ | 3.36 ± .04 | 3.78 ± .07 | <.001 |

Mean ± S.E.

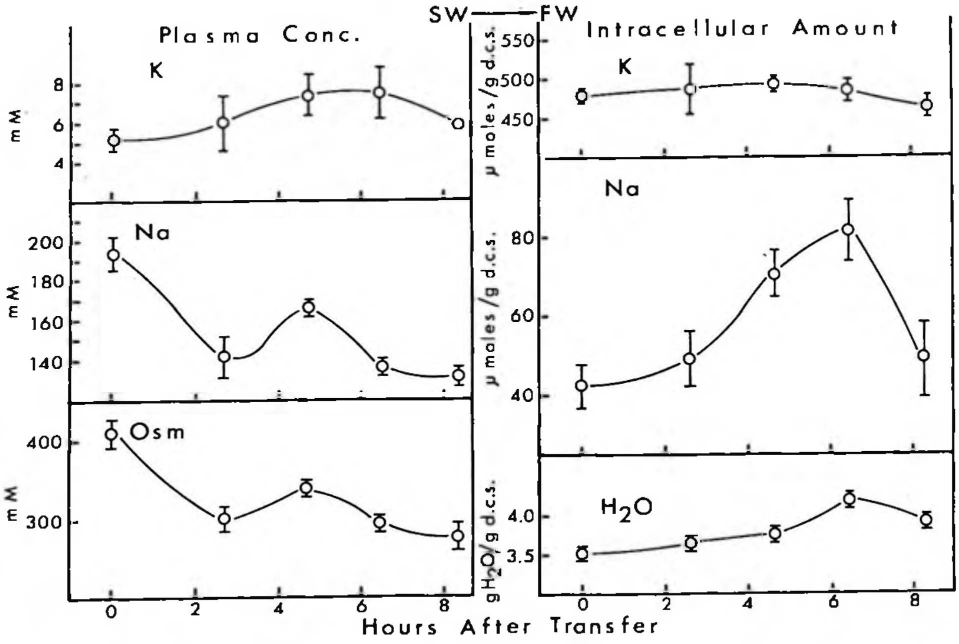

Figure 1. Plasma concentrations and intracellular amounts in muscle fibres of killifish following transfer from SW to FW. Each data point represents five fish, except the last which represents three fish. Two or three tissue smaples were taken from each fish. The values for plasma are expressed in mM. Values on the left represent Mean ± S.E. (n=6). The values for amounts are expressed in µmoles per gram dry cell solids (g.d.c.s.) (the product of the intracellular concentrations times the water content of the cells). Mean ± S.E.

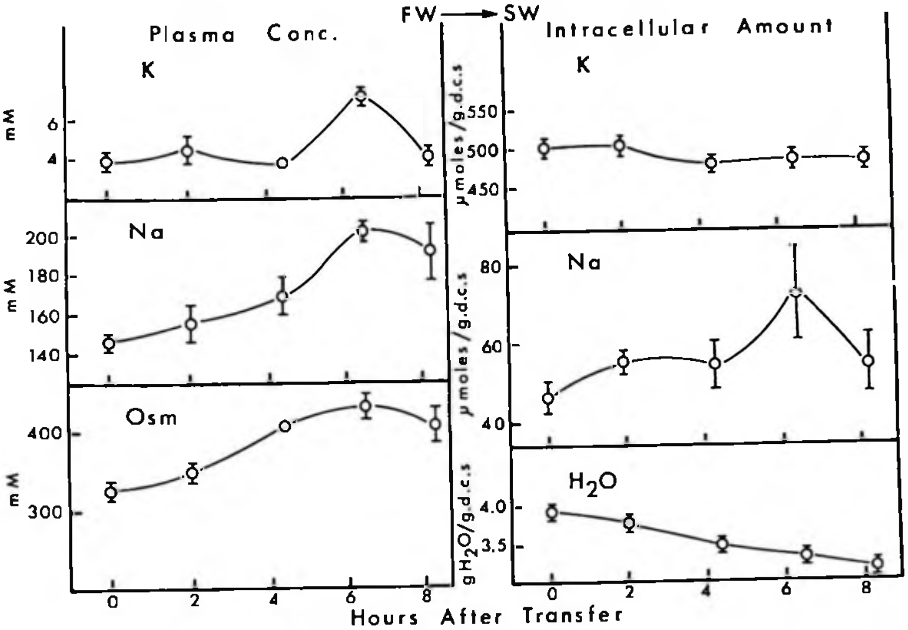

Figure 2. Plasma concentrations and intracellular amounts in muscle fibres of killifish following transfer from FW to SW. Each data point represents two fish. Three tissue samples were taken from each fish. Plasma concentrations Mean ± S.E. (n=2). Intracellular amounts Mean ± S.E. (n=6).

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## COMPARTMENTALIZATION OF FLUID IN THE MAMMALIAN RENAL PAPILLA: INTERSTITIAL FLUID MOVEMENT IN CHANNELS AROUND THE COLLECTING DUCT

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IN a previous study (B. Schmidt-Nielsen, Y. Patel, P. Patel and S. Zell, Bull. MDIBL, 13: 108-113, 1973) it was found that the interstitial fluid removed from the mammalian renal papilla by a centrifugation method (B. Schmidt-Nielsen, Am. J. Physiol., 230:514-521, 1976) is hypoosmotic to the cells of the papilla by 200 to 300 mOs in the undisturbed antidiuretic cat. (In osmotic diuresis the interstitial fluid has a higher osmolality than the cells.) This finding does not agree with the usual assumption that the urine in the collecting duct during antidiuresis is concentrated by diffusion of water into a hyperosmotic interstitium.

To compare the osmolality of interstitial fluid with that of collecting duct fluid micropunctures were performed on the renal papilla of the golden hamster. The following protocol was used: first, a sample of collecting duct fluid was taken, then interstitial fluid (0.2 nl), then