pH and Chloride. Work with chloride-sensitive single-barrel microelectrodes, and with pH-sensitive double-barrel microelectrodes during previous summers at MDIBL has generated the following results based on calculations done at the Laboratory during 1983: 1) Unfertilized Ilyanassa eggs have a membrane potential, ψ_m, of -13.7 ± 6.4 mV (n=34), an intracellular chloride activity, $(Cl)_c$, of 29.9 ± 2.6 mM (n=11), and a calculated intracellular chloride activity at equilibrium, $(Cl)^e_c$, of 155.7 mM. These data suggest that chloride is transported out of unfertilized eggs. 2) Fertilized eggs have a ψ_m of -78.2 ± 1.5 mV (n=12), a $(Cl)_c$ of 18.6 ± 2.8 mM (n=33), and a $(Cl)^e_c$ of 12.1 mM. These data suggest that fertilized eggs, in contrast to unfertilized ones, transport chloride into the cell. 3) Intracellular pH, pH_c, is 7.25 ± 0.28 (n=58). 4) Recordings indicated that ψ_m, $(Cl)_c$, and pH_c did not change significantly as fertilized Ilyanassa eggs underwent polar lobe formation and first cleavage.

Ca-selective microelectrodes. Using the methods of Tsien and Rink (1981. J. Neurosci. Meth. 4:73) and of Lanter et al (1982. Anal. Chim. Acta 135:51), single barrel Ca^{2+}-selective microelectrodes were prepared weekly in Kansas, packaged in electrolyte-filled tubes, and shipped to MDIBL. Such electrodes were calibrated with standard solutions containing 0.125 M K^+ and free Ca^{2+} concentrations of 10^{-3} - 10^{-8} M (Tsien and Rink, 1981). The calibrations of these electrodes were stable for 2 - 3 days. Because the electrode tip diameters were 5 - 7 μm, great difficulty was encountered when impaling cells. The only technique which allowed the ψ_m to be maintained somewhat (as measured with a 3M KCl - filled microelectrode inserted first into the cell) was to vibrate the tip of the calcium electrode with a tuning fork applied to the micromanipulator. Using such techniques, two Ilyanassa fertilized eggs were impaled successfully with the pair of electrodes (for ψ_m and Ca^{2+}) and gave calculated concentrations of intracellular Ca^{2+} of 6 - 11 μM (ψ_m = -28 to =36 mV) and 7 - 16 μM (ψ_m = -12 to -16 mV).

Spawning of Mytilus edulis. The method of Dan and Wada (1955, Biol. Bull. 109:40) was used for electrical stimulation of Mytilus edulis to induce spawning. An A.C. transformer was used, together with electrodes made of a Ag/AgCl wire and a cotton wad wick, to stimulate the mussels with a range of voltages (5 - 25 v) for 15 - 60 sec. Stimulated animals were then incubated in a noncirculating bowl of sea water at sea water temperature. These experiments were repeated steadily throughout June and July, but no shedding of eggs or sperm was observed from any animal. A reliable technique for experimentally inducing the shedding of gametes from Mytilus edulis has not been found. Supported by NIH HDO7193.

THE EFFECTS OF SEA WATER ADAPTATION ON CARASSIUS auratus (GOLDFISH)

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It has been suggested that sodium loading, induced by administration of salt and mineralocorticoid hormone or impairment of renal sodium excretion, in several mammalian species is associated with a depression of vascular and cardiac $Na^+ - K^+$ ATPase activity attributable to a circulating ouabain-like inhibitor ("endoxin"). In a previous study (J. Lowenstein, J. Zadunaisky, A. Evans, Bull Mt. Desert Island Biol. Lab. 22, 103-105, 1982), we reported that adaptation of goldfish (Carassius auratus) to 1/3 sea water resulted in 33% reduction in ^{86}Rb uptake in cardiac slices. In the present study, the effects of sea water adaptation on sodium pump activity in the aorta of Carassius auratus were examined.

Carassius auratus weighing 16 to 60 grams were adapted to 1/3 sea water ($[Na^+]$ = 169.5 mM, $[K^+]$=3.6 mM) for 10 days, or maintained in fresh water ($[Na^+]$ = 6.0 mM, $[K^+]$ = 0.1 mM). After pithing, the suprabulbar aorta was removed, the adventitia removed by blunt dissection and the tissue divided into 2 or 4 segments. Tissues were incubated in Ringer's solution (NaCl 135 mM, KCl 2.5 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.0 mM, Na HCO_3 16 mM,

glucose 5.5 mM) to which carrier free ^{86}Rubidium chloride (4 nM) was added. Incubations were carried out at room temperature; the incubation bath was bubbled with 95% oxygen·5% CO_2. After 15 minutes, the tissue was removed, blotted and digested overnight with .1 ml concentrated nitric acid. After addition of 1.0 ml distilled water .5 ml aliquots were taken for liquid scintillation counting and protein determination (micro Lowry) using standards prepared by nitric acid digestion of human serum albumin. Blood samples collected directly from the aorta were centrifuged in capillary tubes for measurement of hematocrit and plasma sodium and potassium.

Results

Sea water adaptation was associated with a significant increase in plasma sodium concentration; plasma potassium concentration and hematocrit did not differ in fresh water and sea water adapted goldfish (Table 1).

Table 1.--Effects of 1/3 Sea-Water Adaptation on Carassius auratus

	Fresh Water (n=11)	Sea Water adapted (n=17)
Plasma Na^+ (mM)	141.7 ± 3.5	183.1 ± 4.2
Plasma K^+ (mM)	4.03 ± .34	5.80 ± .69
Hematocrit (%)	34.0 ± 1.99	30.3 ± 1.24
Ouabain sensitive ^{86}RB uptake (pmole ^{86}Rb/mg tissue protein/15 min)	12.84 ± 0.38	10.32 ± 0.37
Ouabain insensitive ^{86}Rb uptake (pmole ^{86}Rb/mg tissue protein/15 min)	21.12 ± .29	26.13 ± 0.16

Ouabain-sensitive ^{86}Rb intake (the difference between intake in the presence and absence of 10^{-4}M ouabain)averaged 12.85 pmole/mg tissue protein/15 min in fresh water and 10.32 pmole/mg/15 min in sea water adapted Carassius. The difference was not statistically significant. Ouabain-insensitive ^{86}Rb uptake was not significantly different in fresh water and sea water adapted fish (Table 1).

Discussion

From the observed ouabain-sensitive ^{86}Rb uptake, averaging 12.85 pmole/mg tissue protein/15 minutes, assuming ^{86}Rb to be transported in a manner identical to K^+, utilizing the "specific activity" of the incubation bath (.004 μmole ^{86}Rb/2.5 μmold K^+) it can be estimated that the uptake of potassium into the aortic segments averaged 150 mmole K^+/gram tissue/min. This value agrees well with the ouabain sensitive uptake of ^{42}K into rabbit carotid artery, averaging 160 mmole/gram/min reported by Heidlage and Jones (Fed. Proc. 37:917, 1978).

The data suggest that sodium pump activity of aortic tissue is not significantly altered by adaptation of Carassius auratus to 1/3 sea water for 10 days despite significant increase in extracellular sodium, as evidenced by a significant increase in plasma sodium concentration and unchanged hematocrit in sea water adapted fish. This finding differs from our previous observation of depressed ^{86}Rb uptake in cardiac slices from sea water adapted Carassius. Additional studies will be required to confirm whether cardiac tissue and vascular smooth muscle respond differently to sea water adaptation, i.e., salt loading.

The finding that plasma sodium concentration was significantly increased in sea water adapted fish rases the possibility that increased intracellular sodium concentration in the aortic tissue of these fish stimulated $Na^+ - K^+$ ATPase activity and served to offset the effects of a circulating inhibitor of the pump.