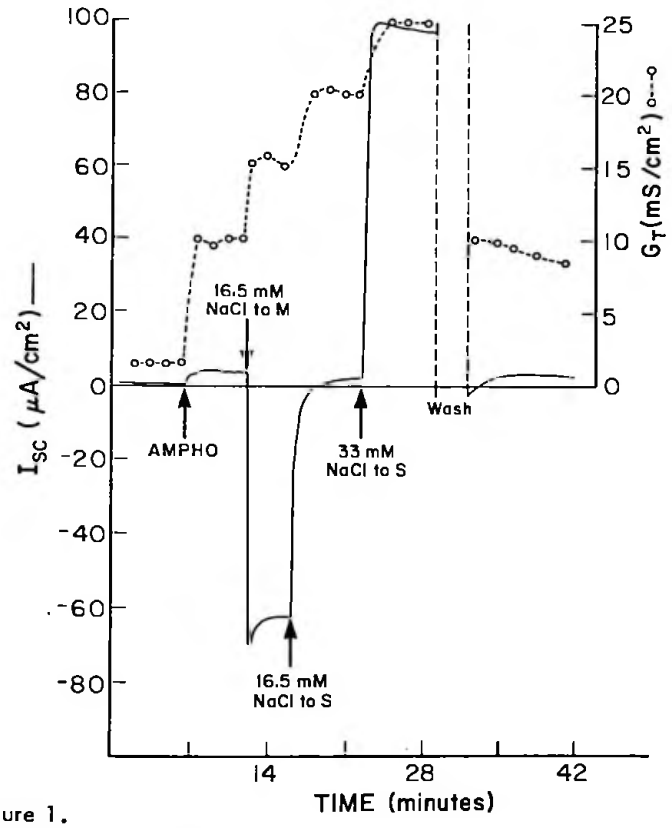

Figure 1.

(16.5 mM) to the mucosal bath, however, resulted in the prompt development of a current, positive in the S to M direction, consistent with conductive chloride flow from M to S. This result has two important implications. First, the current induced by imposing a transmural gradient of NaCl can only be due to an overall conductance which is chloride selective, i.e., the current due to M to S sodium flow would have been oppositely directed. Second, the observation of anion selectivity rules out the possibility that these currents reflect the properties of the amphotericin channels in the apical membrane. It is well established that the so-called "one-sided" amphotericin channel is cation selective. Thus, the observed chloride selectivity must reflect the properties of the basolateral membrane of the epithelial cells.

Manipulating the transmural chloride gradient demonstrated that the direction of the current was determined by the orientation of the imposed chloride emf. Addition of 16.5 mM NaCl to the serosal bath abolished the chloride gradient and reduced the current to zero although the conductance was further increased. Finally, increasing the chloride concentration in the serosal bath to 54.5 mM created a S to M chloride gradient and resulted in a current positive in the M to S direction as expected for S to M conductive chloride flow. Replacing both bathing solutions with chloride-containing Ringer's solution (without amphotericin-B) abolished the current, and reduced the tissue conductance.

These results are consistent with the notion that the basolateral membrane of the winter flounder contains a chloride conductance through which chloride ions move according to the direction of the prevalent electrochemical potential gradient. These experiments were carried out during the 1983 course in Epithelial Transport given at MDIBL. I thank Dr's. Mark Musch and John Stokes and all of the students who participated for their help. Supported by NIH AM29786.

POLAR LOBE FORMATION AND CYTOKINESIS IN FERTILIZED EGGS OF ILYANASSA OBSOLETA AND MYTILUS EDULIS

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Fertilized eggs of the marine mud snail, Ilyanassa obsoleta (Nassarius obsoletus), and of the blue, edible mussel, Mytilus edulis, form a constriction slightly below their equator that tightens and then relaxes several times before and during early cleavage. These shape changes represent an excellent model in studies of cytokinesis. Our purpose, in the projects summarized below, has been to determine what changes occur in intracellular ionic activities and in cytoskeletal proteins.

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,