STUDIES OF OSMOREGULATION IN TELEOSTS AND ELASMOBRANCHS: WATER AND SOLUTE PERMEABILITIES OF APICAL MEMBRANES ISOLATED FROM THE GILLS OF FLOUNDER (*PLEURONECTES AMERICANUS*) AND SHARK (*SQUALUS ACANTHIAS*)

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Teleosts and elasmobranchs deal with the severe osmotic challenge of living in sea water (1000 mOsm/kg) in very different ways. Teleosts excrete excess ingested salt through chloride cells in the gills and absorb water along the integument of the gut, while elasmobranchs oppose water loss by maintaining high concentrations of urea in the blood. The gills with highly folded absorptive surfaces have maximized surface area to volume in order to extract oxygen which is present at variable and at greater depths, low partial pressures. In utilizing such structures however, there is the potential for massive, osmotically driven water loss across these surfaces. We hypothesized that these two branches of marine life have evolved epithelial gill surfaces which present a barrier to the external environment and limits the losses of vital water and solutes to the ocean.

We investigated the passive permeabilities of flounder and shark gill apical plasma membranes to water, urea, ammonia and protons. To make these measurements we prepared plasma membrane vesicles in the presence of 2 mM carboxyfluorescein (CF - an intravesicular volume and pH indicator) using the method of Booth and Kenny (Biochem. J. 142: 575-581, 1974). Membrane permeabilities were measured by stopped-flow fluorometry using well established techniques (Rivers R. *et al.*, Am. J. Physiol. 274:F453-F462, 1998). Enzyme activities demonstrated enrichment of the apical membrane marker ADPase in flounder and shark of ~6.5-fold compared to homogenate, while the basolateral marker Na,K-ATPase was not enriched. Biotinylation of membrane proteins demonstrated a 5.7-fold enrichment of flounder gill membrane, thus validating the marker enzyme data. (see accompanying paper). Membrane permeabilities to water, urea, NH_3 and protons at 15 °C revealed values (mean ± SD, n = 4) for flounder and shark, respectively, of $6.58 \pm 1.8 \times 10^{-4}$ and $7.36 \pm 1.3 \times 10^{-4}$ cm/s (water), $5.88 \pm 1.06 \times 10^{-7}$ and $4.26 \pm 2.92 \times 10^{-7}$ cm/s (urea), $19.3 \pm 5.6 \times 10^{-3}$ and $14.5 \pm 1.9 \times 10^{-3}$ cm/s (NH_3) and $1.68 \pm 0.7 \times 10^{-1}$ and $3.31 \pm 1.2 \times 10^{-1}$ cm/s (protons). Thus apical gill membranes from both species have extremely low water and solute permeabilities and were, except for protons not very different from each other. Activation energy for water permeation was 12 kcal/mol for flounder and 9.2 kcal/mol for shark apical membranes suggesting the absence of aquaporins. Addition of 1 mM $HgCl_2$ to membrane vesicles for 1 hour at 4 °C resulted in markedly reduced water permeability of flounder membrane vesicles and a small reduction in shark membrane vesicles. Dose response curves to $HgCl_2$ revealed that shark membranes were sensitive to Hg^{2+} at higher concentrations. Both membranes also exhibited a reduction in NH_3 permeability upon treatment with Hg^{2+}. Given the high activation energy for water transport and the similar effect on NH_3 permeation we conclude that Hg^{2+} probably interacts with membrane phospholipids or

extracellular matrix to alter permeability, rather than with membrane proteins. A quantitative difference exists between the response of flounder and shark to this heavy metal with the flounder apical membrane exhibiting greater sensitivity.

To determine whether the vesicular permeabilities we had measured are reflected *in vivo*, we initiated preliminary studies aimed at measuring the permeabilities of intact gill epithelia. We utilized the concentration-dependent fluorescent dye, calcein AM as a marker of cellular volume. Excised gill arches from flounder were maintained in sea water and loaded for approximately 1 hour with 10 μM calcein AM. Confocal microscopy was used to image the gills (Fig. 1). Preliminary data indicate that we could successfully load living gill epithelia with the dye and image individual cells. Live cell microscopy under perfusion with solutions of different osmolality will allow time-dependent changes in cell volume to be monitored and image analysis performed, with subsequent derivation of osmotic permeability coefficients. These experiments should allow independent confirmation of the measurements performed on isolated plasma membranes and set the stage for future studies of osmoregulation in these divergent species.

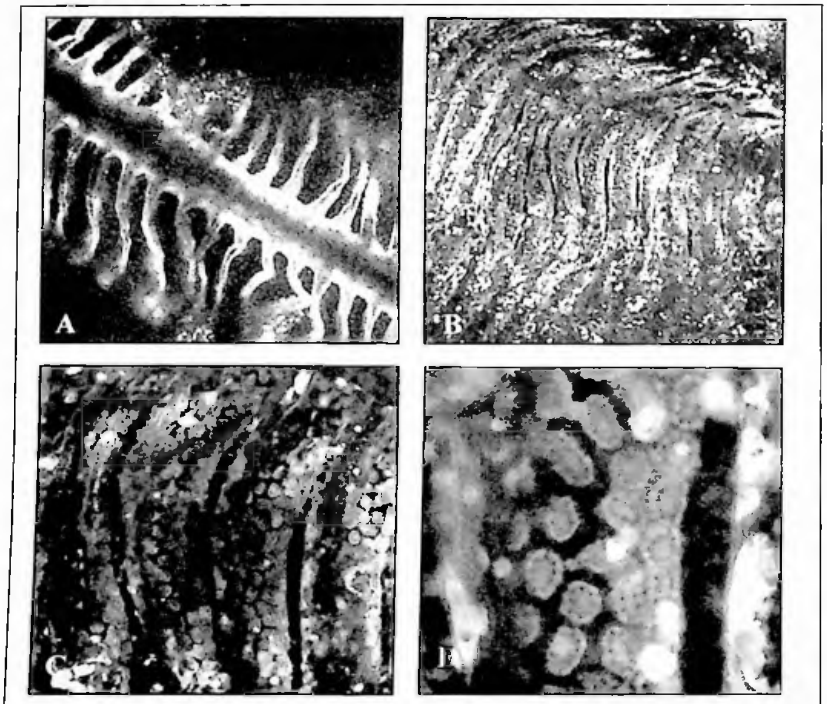

Fig. 1. Flounder gill incubated for 1 hour with 10 μM calcein AM and then imaged at different magnifications with fluorescent confocal microscopy. A. Segment of intact gill arch (cartilage) with attached epithelium-covered gill filaments, B-D. Scalpel-scraped filaments. Progressively higher magnification views (10x, 20x, 100x) of calcein-labeled epithelial cells on individual filaments.

Acknowledgments: This work was supported by grant NIEHS (see Bulletin report by us) to JPS, RK and EKS. We also thank the members of the Dr. Fricker group for advice in the use of the confocal microscope.