Further experiments were performed to characterize the mechanisms involved in active secretion of electrolytes. Since the rectal gland is rich in carbonic anhydrase (Physiol. Rev. 47: 595, 1967) the role of this enzyme in active NaCl transport was studied by comparing the gland flow rate and sodium excretion before and during perfusion of 14 glands with ethoxzolamide in a concentration of 0.1 mM. The total CO_2 content of the perfusate under control conditions was 6.6 ± 0.6 mM/L as compared to 6.8 ± 0.6 (P=NS) in the experimental period. Inhibition of carbonic anhydrase reduced neither flow rate nor the rate of sodium secretion. Glandular flow rate was 5.5 ± 0.9 µl/min/g control and 7.2 ± 1.1 experimental (P=NS) while the control level of sodium secretion was 2.7 ± 0.5 µEq/min/g compared to 3.3 ± 0.6 experimental (P=NS).

In an additional group of experiments the effect of thiocyanate, 10 mM on electrolyte secretion was examined since a recent report (Amer. J. Physiol. 224: 129, 1973) demonstrated that this compound inhibited active chloride transport in salt water teleost and that efflux of chloride and sodium were closely linked. Following the addition of NaSCN to the perfusate fluid in four glands, flow rate fell 36 percent from 3.9 ± 1.2 µl/min/g to 2.5 ± 0.7 ($P < 0.05$), and sodium secretion was reduced 64 percent from a control value of 1.9 ± 0.6 µEq/min/g to 0.7 ± 0.2 ($P < 0.05$).

These data indicate that the rate of formation and composition of rectal gland fluid during *in vitro* perfusion is comparable with values obtained *in vivo*. A sodium concentration gradient of 1.5 or greater serves as a useful index of active secretion since lower gradients are characterized by passive diffusion. Moreover while there was no evidence that carbonic anhydrase influenced the rate of electrolyte secretion, inhibition by thiocyanate supported the previous hypothesis that chloride is actively transported by the rectal gland.

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THIOCYANATE INHIBITABLE ATPase IN THE GILL OF *Anguilla rostrata*

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The presence of a Mg-ATPase which is inhibited by thiocyanate (SCN) but not ouabain and requires neither Na or K has been described in a number of tissues in which active transport of anions may occur. (Kasbekar, D.K., and Durbin R.P. An adenosine triphosphatase from frog gastric mucosa. Biochim. Biophys. Acta 165:472, 1965.) The gills of teleosts are known to be a major regulator of ionic balance, particularly in sea water where the external environment contains four times the concentration of Na and Cl as the extracellular fluid. Chloride efflux is greatly increased in sea water and net transport of chloride occurs against both a concentration and an electrical gradient. Thiocyanate inhibits chloride efflux, suggesting that an active, carrier-mediated process is involved. It was therefore of interest to study the *in vitro* effects of SCN on ATPases.

Eels adapted to fresh water or sea water for at least three weeks were used. Gill filaments, removed immediately from eels were placed in an ice cold 20/1 solution (v/w) of 5mM EDTA, 0.25M sucrose, 10mM imidazole, 0.1 percent desoxycholate at pH 6.8. Homogenization was carried out in a glass homogenizer immersed in ice using a Teflon pestle at 1725 rpm. The homogenate, after

filtering through two layers of nylon mesh, was used in the subsequent assay (whole homogenate) or centrifuged in the Sorvall and/or Spinco preparative centrifuges to obtain cell fractions. The whole homogenate or cell fraction was incubated at 37°C in a metabolic shaker in each of three media (all of pH 7.8 and containing 6mM Mg-ATP as 10mM Imidazole): (a) 100mM NaCl and 20mM KCL; (b) 120mM NaCl or (c) 120mM NaSCN. The reaction was stopped after 15 minutes by the addition of 35 percent TCA and immersion in ice. Na-K-ATPase was determined by the difference in activity between medium (a) and (b); SCN inhibitable ATPase was determined by the difference in activity between medium (b) and (c).

The inorganic phosphate released was measured by the method of Fiske-Subbarow and proteins by the method of Lowry. Succinate dehydrogenase was determined by the method of Green and Kohout using reduced cytochrome c as an end product.

Thiocyanate inhibitable ATPase was found in both the whole homogenate and cell fractions of fresh water and sea water adapted eels. Its pattern of distribution among cell fractions paralleled that that of succinic dehydrogenase rather than Na+K ATPase. Thiocyanate inhibitable ATPase was significantly higher in the whole homogenate of SW eels but not in the microsome fraction while Na+K ATPase was significantly higher in both whole homogenate and microsomes of SW eels.

These findings suggest that SCN inhibitable ATPase is located predominately in the mitochondrial fractions and not in the microsomes where fragments of plasma membranes, the site of active transport are found. The higher SCN inhibitable ATPase in whole homogenate of SW eels is consistent with the increase in mitochondria observed in adaptation from FW to SW. The finding of detectable levels of SCN inhibitable ATPase in the microsome fractions devoid of succinate dehydrogenase activity suggests that a small percentage of the total SCN inhibitable ATPase may be located in either the endoplamic reticulum or the plasma membranes (both sedimenting with the microsome fraction) although differences in the sensitivities of the SDH and ATPase assay does not rule out the possibility of mitochondrial contamination of the microsome fraction. In either case, the failure of microsomal SCN inhibitable ATPase to increase in adaptation from FW to SW, when chloride efflux markedly increases, does not support a role of this ATPase in the active transport of chloride.

A Mg-ATPase inhibited by thiocyanate is described in broken-cell homogenates of gill tissue. It is predominantly associated with mitochondria though activity can also be detected in microsomes free of the mitochondrial enzyme succinic dehydrogenase. SCN-inhibitable ATPase in gill microsomes does not rise in an adaptive fashion when chloride transport increases as a result of transfer

from FW to SW. This finding, therefore, cannot be used to establish a connection between SCN inhibition of ATPase and active chloride transport by the gill.

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AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-OUABAIN BINDING BY Na-K-ATPase IN PERFUSED GILLS OF *Fundulus heterclitus*

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Since the activity of sodium-potassium-activated adenosine triphosphatase (Na-K-ATPase) is especially high in tissues specialized for high level electrolyte transport and its activity appears to be correlated with the osmotic work load of the gland or tissue it has frequently been suggested that this enzyme plays an important role in transepithelial electrolyte transport. Clearly one of the first steps in evaluating this role is localization of the enzyme in the epithelial layers and cells responsible for transport. Our approach to the localization of Na-K-ATPase has taken advantage of the specific binding and inhibitory action of the cardiac glycoside, ouabain. We have examined a most important effector organ of teleost osmoregulation, the branchial epithelium, which in euryhaline fish adapted to varying concentrations of sea water exhibits clearly different rates of sodium transport and activities of Na-K-ATPase (Maetz, J., Phil. Trans. B, 262, 209, 1971).

Specimens of *Fundulus heteroclitus* were adapted for two to five weeks to varying concentrations (2X, 1X, and 0.1X) of artificial sea water (SW) maintained at 18-22°C. For experimentation, specimens were pithed, and mounted in a tray and the perfusion fluid, with or without ^{3}H-ouabain, was introduced at a rate of about 0.1 ml/minute via a cannula in the bulbous arteriosus. The liver was cut to provide for drainage. The gill preparation was maintained near 20 °C and kept moist by irrigation with saline solution, usually 280 mM NaCl. The perfusion fluid consisted of a modified Forster's fish medium (Science, 108, 65, 1948) with 135 mM NaCl, 7.5 mM $NaHCO_3$ and one to two percent albumin. Blanching of the gill filaments and tracer dye, Lissamine green, served as