The stickleback was examined in order to determine the characteristics of the apical tubular system in its chloride cells. This system, which we described for freshwater *Anguilla*, has been reported to be more prominent in three-spined stickleback *Gasterosteus* (Bierther, Ztschr. f. Zellforsch. 107, 421, 1970) after gradual adaptation to freshwater. The nine-spined stickleback, *Pungitius*, was found in moderate abundance in Northeast Creek in association with *Fundulus*. In the laboratory it withstands abrupt changes in salinity which may make it a preferred species especially for study of specific ion effects on configurations of the intracellular tubular membrane systems. The morphology is well suited to electron microscopic study.

The rock-eel, *Pholis gunnellus*, was collected from beneath rocks exposed at minus low tides at the laboratory point. It lives well in laboratory aquaria. Its gills have abundant chloride cells of the usual seawater configuration as found in *Anguilla*, *Fundulus*, and *Pungitius*. This normal configuration can be summarized as consisting of an evenly spaced distribution of the abundant mitochondria in intimate association with the highly branched tubular reticulum which is characteristically present in the cytoplasm of chloride cells. In *Anguilla*, Epstein has reported that 7mM NaSCN in seawater adapted eels causes a 65 percent fall in chloride efflux within a few minutes and a similar but delayed response to 10mM SCN in the external medium. Rock-eels in 10mM SCN in seawater became sluggish in 20-24 hours and died in 30-36 hours. The fine structure of their chloride cells taken at 2.5 to 18 hours of exposure to thiocyanate was essentially normal including normal Golgi areas in the 2.5 hour specimen. Specimens taken at 23 hours showed marked alterations in all chloride cells. Some, located beneath the superficial epithelium in the distended intra-epithelial lymph space, were maximally rearranged and probably moribund. These cells were rounded, with the mitochondria and matrix cytoplasm clumped centrally and surrounded by a broad peripheral zone entirely composed of parallel arrays of *unbranched* tubules in dense packing. Cross sections of such arrays showed up to 800 densely-packed tubules and sections of longitudinal arrays showed 20-30 tubules of uniform diameter. These tubules are about twice the diameter of those in normal cells. Other cells of the thiocyanate specimens, which were still in the superficial epithelium, showed transition stages in dissociation of tubules from mitochondria, loss of branching, and association in small parallel arrays. This response is unlike other varieties of degenerative changes seen in chloride cells and probably represents a response to specific ions. Although this morphological response is delayed several hours beyond the onset of the physiological effect, it would seem to implicate the tubular reticulum of the chloride cell as a site of action of thiocyanate.

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THIOCYANATE INHIBITION OF ADENOSINE TRIPHOSPHATASE IN GILLS; POSSIBLE RELATION TO CHLORIDE TRANSPORT.

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Thiocyanate specifically inhibits the efflux of chloride across the gill of European eels *(Anguilla anguilla)* adapted to seawater, blocking the net extrusion of NaCl and eventually producing

hypernatremia and death (Epstein, F.H., Maetz, J. and deRenzis, G., unpublished observations). Specific inhibition of chloride efflux without marked change in sodium efflux after injections of NaSCN was confirmed in two American eels fully adapted to seawater. We therefore wished to see if thiocyanate had any action on the adenosine triphosphatases of gill tissue, since SCN^- and other anions are known to block MgATPase in other tissues, according to the position of the anion in the lyotropic series (Katz, A.I. and Epstein. F.H., Enzyme 12:499, 1971).

Substitution of 100 mM SCN^- for 100 mM Cl^- in the assay for Na-K-ATPase (Jampol, L. and Epstein, F.H., Am. J. Physiol. 218:607, 1970) produced no change in the specific activity of Na-K-ATPase in whole gill homogenates of eight specimens of *Anguilla rostrata* adapted to seawater (average $\pm$ s.d. = 9.6 $\pm$ 2.6 μMPi/mg protein/hr in Cl^- medium vs. 10.0 $\pm$ 2.9 in SCN^- medium). In gill homogenates of 10 freshwater eels, however, a thiocyanate medium inhibited Na-K-ATPase slightly (mean $\pm$s.d. in Cl^- medium = 5.1 $\pm$ 1.0 vs. 3.8 $\pm$ 1.6 in SCN^- medium).

Thiocyanate caused inhibition of MgATPase by approximately 50 percent in gill homogenates of eels adapted to fresh and salt water. The thiocyanate-inhibitable ATPase (measured as the difference between MgATPase in Cl^- medium and SCN^- medium) was 5.0 $\pm$ 1.0 μMPi/mg protein/hr in seawater and 3.9 $\pm$ 1.3 in freshwater, a difference that is not significant ($p > 0.05$; $t = 1.87$).

If "thiocyanate-inhibitable ATPase" were part of the mechanism by which chloride is actively transported by gills in seawater, one might expect a considerable increase in the specific activity of this moiety in the gill, in the process of adaptation of freshwater eels to seawater. In the present experiments however the difference between freshwater and seawater eels was small and did not reach the level of significance. It is possible that larger differences would be observed if plasma membrane or microsomal fractions were assayed, rather than whole homogenates of gill filaments. Nevertheless the present data do not support the notion that chloride extrusion by the gill is related to that portion of MgATPase in gill homogenates that is inhibited by thiocyanate.

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MEASUREMENT OF THE BRAIN EXTRACELLULAR SPACE IN *Squalus acanthias*

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The size of the extracellular space (ECS) in various tissues is usually measured by determining the steady state tissue to plasma ratio of some relatively large, metabolicly inert foreign compound such as inulin, which has been infused or injected into the venous circulation. The use of this experimental procedure for measuring the brain ECS in a variety of vertebrate species has yielded peculiarly low values and has suggested that the exchange of these extracellular-type compounds across the capillaries of the brain is too slow, the so-called bloodbrain barrier effect, to allow an accurate determination of the brain ECS in that manner (Comp. Biochem. Physiol. 42A: 73-38, 1972). Never-theless reasonable estimates of the brain ECS have been made by introducing inulin and other extracellular markers into the cerebrospinal fluid (CSF) and measuring tissue to CSF ratios of the marker