were supended in 5 ml of 10 percent TCA and allowed to stand with frequent shaking for one hour. After centrifuging 1 ml of the supernatent was added to 15 ml of "Aquasol" for counting in the liquid scintillation spectrometer.

When their presence is indicated concentrations of the ions in the various media were as follows: Na^+, 0.5 M NaCl; K^+, 0.01 M KCl; Mg^{++}, 0.052 M $MgCl_2$. In any medium in which NaCl was missing choline chloride was present at a concentration of 0.49 M. Sea water was filtered through a 0.45 μ membrane.

Results of studies on glycine and valine transport may be seen in Table 1. Of the ions tested, Na^+, K^+, and Mg^{++}, it is clear that no one of them alone will support transport. A combination of Na^+ and Mg^{++} allows for about one third the rate seen in sea water. Na^+, K^+, and Mg^{++} together allow a transport approximating that observed with sea water. The levels of Na^+, K^+, and Mg^{++} used are similar to the concentrations of these ions found in the sea water.

The transport system for glycine and valine into three-hour embryos of the sand dollar is not only Na^+- dependent, but also requires K^+ and Mg^{++}. Since it has been demonstrated that in the case of some amino acids the Na^+- dependency for their transport into echinoderm eggs requires fertilization it may be important to examine the emerging pattern for other ionic dependencies. Also when considering mechanisms to explain amino acid transport in echinoderm embryos it seems more than merely coincidental that membrane-associated ATPase often requires Na^+, K^+, and Mg^{++}. Thus it will be of interest to explore the levels of ATPase and the pattern of ionic dependence of the enzyme in the developing sand dollar.

1973 #12

EFFECTS OF THIOCYANATE AND OSMOTIC STRESS ON SURVIVAL OF THE ROCK EEL *Pholis* AND ON THE ULTRASTRUCTURE OF THE CHLORIDE CELLS

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Chloride cells in fish gills are characterized by an abundance of mitochondria evenly dispersed in relation to an elaborate, regularly branched, tubular reticulum (t.r.) which communicates with the plasma membrane. Thus the lumen of the t.r. constitutes an internalized compartment whose composition may be altered to some degree by changes in plasma electrolyte composition. In euryhaline forms subjected to adaptation from salt to freshwater there are changes in conformation of the t.r. correlated with the time required for adaptation (usually several days) (Doyle and Epstein, Cytobiologie 6, 58-73, 1972). Additional evidence from other investigations implicates the membranes of the t.r. as the site of ion transport. Substances such as SCN or K_2 SO_4 which result in prompt flux changes across the gill have been found to produce striking changes in the configuration of the t.r. (Doyle unpublished). In an attempt to alter the ionic environment of the membranes of the t.r. to a greater extent than occurs under the influence of the adaptive mechanisms present in euryhaline fish we have subjected a stenohaline species *Pholis gunnellus* to similar stresses.

Rock eels collected at low tides at the laboratory point were maintained throughout the summer in sea water aquaria furnished with rocks and opaque shelter tubes. They were fed in early evening

on pieces of *Mytilus* and appeared to thrive. Experimental animals were transferred instantaneously to the various concentrations of sea water and thiocyanate to determine survival times and cytological effects.

TABLE 1

Survival time (hours) in stated concentrations of seawater and sodium thiocyanate

Concentration of Seawater	Concentration of NaSCN (mM)			
	0	10	20	40
1/3	60			
1/2	72-168	42 ± 6	30	
1	>8 weeks	41 ± 7	35 ± 5	18
1 1/2	60 ± 12	29 ± 1		
2	22			

The survival times are given in Table 1. It should be noted that rock eels did not feed in the experimental solutions and that it was usual for them to leave their shelters and to swim erratically a few hours before they became moribund. By courtesy of John Forrest, serum sodium concentration was found to be 205 m Eq./L for *Pholis* in normal sea water. Thus half-strength sea water was assumed to be hypertonic to normal serum and one-third sea water to be hypotonic. With the addition of 10 and 20 mM thiocyanate the survival time in half-strength sea water was about the same as that in normal sea water. In 1-1/2 times sea water the toxicity of thiocyanate was enhanced. In the guppy *Lebistes* we have observed that 20 mM thiocyanate is nontoxic in fresh water but toxic to guppies adapted to half-strength sea water.

For electron microscopy gills were fixed at appropriate intervals in all test conditions given in Table 1. In addition because cytological changes in the t.r. take 24 to 48 hours to develop some fish were treated for a few hours with thiocyanate and transferred to normal sea water to see whether an initial damage to transport processes would persist and result in a subsequent morphological change. The cytological effects which can be summarized here exclude gross damage and post mortem changes in the cells. In half-strength sea water specimens surviving for seven days showed the development of a distinct apical region in which the tubules were of a uniform diameter consistently smaller than that of the t.r. throughout the rest of the cell. Since an apical tubular system was observed in freshwater eels it seems probable that with more gradual changes in salinity *Pholis* might fully adapt to half-strength sea water with these apical tubules being developed. In 1-1/2 times sea water for 52 and 67 hours there were consistent significant alterations in the majority of chloride cells in all specimens. Consistent with some cell shrinkage the apical pit is enlarged and there is increased cytoplasmic density. The lumen of the t.r. is distended irregularly in comparison with normal animals. At the basal regions of the cells (especially near capillaries) these are distinct areas devoid of mitochondria occupied by clumped rather sparsely branched tubules. This

result is attributed to the effects of elevated plasma electrolyte concentration.

In the specimens treated with thiocyanate in normal sea water there were no consistent effects on the tubular network. Concentrations higher than 10 mM did not permit adequate survival times for morphological changes in the t.r. to develop. In 10 mM thiocyanate after 48 hours there was some indication of reduced branching of the t.r. but only occasional cells showed aggregations of tubules.

Preliminary experiments on exposure to 10 and 20 mM potassium iodide in normal sea water showed substantial damage to membranes of the tubular reticulum.

That the tubular reticulum of the chloride cells is intimately involved in the regulation of transport processes in the gills receives some further support from these observations. In a stenohaline species *Pholis* exposure to half-strength sea water results in an apparently adaptational change in configuration of the tubular reticulum, while exposure to 1-1/2 times sea water causes severe disorganization and aggregation of the reticulum in basal regions of the cells. (Supported by a grant USPHS 5 SO1-RR05367-12).

1973 #13

CHLORIDE TRANSPORT AND ITS INHIBITION IN GILLS OF SEA WATER TELEOSTS

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Chloride is transported outward against an electrochemical gradient by the gills of salt water teleosts. An active process involving a carrier is suggested by the fact that the efflux of chloride in sea water is sharply reduced in *Anguilla anguilla* by small quantities of NaSCN injected intraperitoneally while sodium efflux is unaffected. (Epstein, F.H., Maetz, J. and deRenzis, G.; Am. J. Physiol. 224:1295, 1973). The present study was undertaken to extend these observations to different species and to try to elucidate the mechanism of thiocyanate inhibition of chloride transport by the gill.

Chloride efflux was determined by injecting ^{36}Cl intraperitoneally allowing one hour of equilibration, measuring the appearance of isotope in a known volume of aerated sea water at constant temperature of 16°C in which the fish lay immersed. Sampling was carried out every 10-15 minutes for one to two hours and at the end of the experiment blood was obtained so that the specific activity of ^{36}Cl could be determined in plasma. Two microcuries of ^{36}Cl per 100 grams body weight were injected in eels and flounders using an external bath of 1000 ml. To killifish weighing five to 10 grams, 0.5 to 1.0 microcuries of ^{36}Cl were given and the bath volume was 150 ml.

Intraperitoneal injection of NaSCN (0.14 to 0.2mM per 100 gm body weight) reduced chloride efflux by an average of 50 percent in sea water specimens of *Anguilla rostrata* (n = 9) and *Pseudopleuronectes americanus* (n = 5). In *Fundulus heteroclitus* adapted to sea water however no inhibitory effect of thiocyanate could be detected (n = 6). All three species had evidence of a large exchange diffusion component to the chloride efflux in sea water with prompt falls of 75-80