

## IONIC REQUIREMENTS FOR AMINO ACID TRANSPORT INTO EMBRYOS OF *Echinarachnius parma*

Richard B. Crawford, Trinity College, Hartford, Connecticut

One of the early events in the developing echinoderm is a modification of the ionic requirements for amino acid transport. Epel has described the activation of a  $\text{Na}^+$ -dependent amino acid transport system within a few minutes after fertilization of sea urchin eggs (Exptl. Cell Res., 72, 74, 1972). In order to pursue these studies in the sand dollar *Echinarachnius parma* the ionic requirements for transport of amino acids in these embryos was determined. These studies showed that for optimal transport at least  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$  are required.

Amino acid transport in fertilized sand dollar eggs was measured by following the accumulation of labelled amino acids in the trichloroacetic acid (TCA) soluble fraction. Fertilized eggs were obtained by adding five drops of a one percent suspension of sperm to 50 ml of a two percent suspension of eggs. The zygotes were washed two times with 100 ml sea water 15 minutes after fertilization. Development was allowed for three hours at  $16^\circ$  by which time the embryos were at the four to eight cell stage. Two ml aliquots were removed and put into a 12 ml centrifuge tube. The embryos were washed two times with 10 ml of the appropriate incubation medium. Final volume in each centrifuge tube was adjusted to 2 ml and then the labelled amino acid was added ( $0.4 \mu\text{Ci } ^3\text{H-valine}$  or  $0.2 \mu\text{Ci } ^{14}\text{C-glycine}$ ). Incubation was carried out at  $16^\circ$  for 15 minutes and then terminated by the addition of 8 ml of ice-cold medium. The embryos were sedimented in a hand centrifuge and then washed three times by resuspending them in 10 ml cold medium followed by centrifugation. The embryos

TABLE 1  
Transport of Glycine and Valine into *E. parma* Embryos

Incubation Medium <sup>a</sup>	cpm in Aliquot from TCA-Soluble Fraction	
	Glycine <sup>b</sup>	Valine <sup>c</sup>
sea water	34,546	5,227
$\text{Na}^+$	27	539
$\text{K}^+$	9	74
$\text{Mg}^{++}$	57	1,072
$\text{Na}^+$ , $\text{Mg}^{++}$	11,473	1,871
$\text{Na}^+$ , $\text{K}^+$	27	270
$\text{K}^+$ , $\text{Mg}^{++}$	72	816
$\text{Na}^+$ , $\text{K}^+$ , $\text{Mg}^{++}$	25,706	5,483

<sup>a</sup>Concentration of ions given in text.

<sup>b</sup>Uniformly labelled  $^{14}\text{C}$ -glycine.

<sup>c</sup>Randomly labelled  $^3\text{H}$ -valine.

were suspended in 5 ml of 10 percent TCA and allowed to stand with frequent shaking for one hour. After centrifuging 1 ml of the supernatant 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:  $\text{Na}^+$ , 0.5 M NaCl;  $\text{K}^+$ , 0.01 M KCl;  $\text{Mg}^{++}$ , 0.052 M  $\text{MgCl}_2$ . 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  $\mu$  membrane.

Results of studies on glycine and valine transport may be seen in Table 1. Of the ions tested,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$ , it is clear that no one of them alone will support transport. A combination of  $\text{Na}^+$  and  $\text{Mg}^{++}$  allows for about one third the rate seen in sea water.  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$  together allow a transport approximating that observed with sea water. The levels of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$  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  $\text{Na}^+$ -dependent, but also requires  $\text{K}^+$  and  $\text{Mg}^{++}$ . Since it has been demonstrated that in the case of some amino acids the  $\text{Na}^+$ -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  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Mg}^{++}$ . 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

W.L. Doyle, University of Chicago, Chicago, Illinois

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  $\text{K}_2\text{SO}_4$  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