SEARCH FOR CARBONIC ANHYDRASE IN FERTILIZED AND UNFERTILIZED SAND DOLLAR (ECHINARACHNIUS PARMA) EGGS

Andrew Fine
Department of Pharmacology and Therapeutics
University of Florida College of Medicine
Gainesville, FL 32610

The sand dollar egg experiences a rise in intracellular pH following fertilization. Another echinoderm, the sea urchin, Strongylocentrotus droebachiensis undergoes a similar change. In the sea urchin "increase in intracellular pH through activation of Na^+-H^+ antiporter during growth stimulation has been demonstrated" (S.S. Shen, in Mechanisms of Egg Activation, ed. R. Nucitelli et al., Plenum Press, New York, 1989, pp. 173-199). Since carbonic anhydrase (CA) is involved elsewhere in acid-base regulation, establishing its presence or absence in sand dollar eggs seemed fundamental. Both fertilized and unfertilized eggs were tested. NO CA was found in either case.

Mature sand dollars were stimulated to shed eggs by Dr. Raymond Rappaport. The sample was collected, centrifuged and diluted with an equal volume of distilled water. This, along with stirring caused the cells to lyse. When fertilized eggs were used, the sperm was added and left undisturbed for at least 15 min. before centrifuging and diluting. The solution was kept cool throughout the determination that followed. The method for carbonic anhydrase determination was that in which 100% CO_2 is bubbled through a reaction vessel submerged in a water bath (0° C) and the pH change of barbital buffer monitored colorimetrically by bromthymol blue. The method is described in J. Pharm. Exp. Ther. 130, 26-39, 1960; now we use different buffer and indicator. When no CA is present in the sample, the time elapsed before color change occurs (about 60 seconds) does not differ from the time elapsed when water is used in place of the sample. With CA present the time narrows, depending on the enzyme concentration.

Four overall egg dilution ratios were used ranging from 80:1 to 16:1. One ml of the original 1:1 dilution with water was further diluted with seven parts of reaction mixture giving an overall dilution of 1:16. Each determination, therefore, contained 0.5 g wet original sample. The dilution ratio was reduced in an attempt to maximize sensitivity of the procedure. Eggs were tested 30 min. after ovulation (in the unfertilized sample) or fertilization in separate trials 0.5 and 5 hours after fertilization. The data collected when overall dilution was reduced to 16:1 is given in Table 1. T^U is the time of the uncatalyzed reaction; T^S is the time found for the various samples. Clearly, there is no real difference in these times. The sensitivity of the test is such that it can detect enzyme in the tissue at concentration of about 3×10^{-8} M, or 10^{-3} the concentration in mammalian red cells. This absence of CA implies the existence of mechanisms for the pH change present after fertilization, which do not involve buffering by CO_2.

TABLE 1. CARBONIC ANHYDRASE ASSAY IN UNFERTILIZED AND FERTILIZED SAND DOLLAR EGGS

Sample	T^u (sec)	T^s (sec)
Unfertilized (4)	62	63
Fertilized (5) -30 min	63	65
Fertilized (4) -5 hours	66	64

Overall dilution of sample is 16:1. n = ( ).

This research was supported in part by NIH student training grant #2-T35-HL07489.