STIMULATION OF PROTEIN SYNTHESIS IN UNFERTILIZED SEA URCHIN EGGS BY PRIOR METABOLIC INHIBITION

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It is widely held that the mature unfertilized egg is incapable of protein synthesis (Hultin, 1952; Gross et al., 1964; Stafford et al., 1964; Nakano and Monroy, 1958). It has been shown, however, that eggs of the surf clam, Spisula solidissima, are active in protein synthesis (Reeder and Bell, 1967). The present study presents evidence that mature unfertilized eggs of two species of sea urchins synthesize protein at characteristic rates and that fertilization results in a fourfold increase in one, Arbacia punctulata, and a thirtyfold increase in the other, Strongylocentrotus purpuratus. Mature unfertilized eggs can be released from controls which regulate their normal level of protein synthesis. If egg metabolism is temporarily suppressed with either puromycin or nitrogen (anaerobiosis), after recovery from the inhibitor protein synthesis is increased by a factor of one and one half to two.

MATERIALS AND METHODS

Eggs of Strongylocentrotus purpuratus and Arbacia punctulata were obtained and handled by standard methods. Millipore filtered sea water containing penicillin (50 ug/ml) and streptomycin (30 ug/ml) was used in all experiments. C^{14}-labeled reconstituted protein hydrolysate -- a mixture of L amino acids with specific activity about 150 mc/mmole (Schwarz Bio-Research, Orangeburg, N.Y.) -- was used as a protein precursor. To measure incorporation after exposure of eggs to labeled amino acids, a sample of eggs was rinsed once with cold sea water, once with homogenization medium (Nemer and Spirin, 1965) and dissolved in 8 M urea. Portions of this homogenate were assayed for trichloracetic acid precipitable incorporation and total incorporation (the sum of acid precipitable and acid soluble incorporation). Another portion was used for estimation of proteins by the method of Lowry et al. (1951).

In preliminary experiments eggs were pulse labeled with varying concentrations of C^{14} amino acids. We found that acid precipitable incorporation is a constant fraction of total incorporation under these conditions. We concluded that the ratio (acid precipitable incorporation)/(total incorporation) is a valid expression of the rate of protein synthesis under conditions of varying permeability to amino acids. In later experiments we used this method of expressing the rate of protein synthesis to compensate for increases in total incorporation.

Bacterial contamination was monitored by plating samples of the egg suspension on agar made up in 80% sea water and 20% Waymouth medium. Contamination was negligible (less than 10 viable bacteria/ml at the end of an experiment) in all cases. Eggs were examined in all experiments for the presence of oocytes. If they were present in significant numbers, eggs were rejected. Most preparations used contained one to two oocytes per 500 eggs.

To show that all eggs were incorporating isotope into protein, whole unfertilized eggs were labeled with C^{14} amino acids, fixed and extracted with 5% TCA, mounted on slides and coated with Kodak NTB-3 emulsion. Controls were labeled in the presence of puromycin. When the developed slides were examined it was clear that all unfertilized eggs of both species incorporated significant amounts of isotope into protein, thus confirming the results of Brachet et al. (1963). Puromycin reduced labeling to near background levels.

RESULTS AND DISCUSSION

To compare the rates of incorporation in fertilized and unfertilized eggs independent of permeability to amino acids, unfertilized eggs were exposed to C^{14} amino acids for several hours and then washed free of exogenous amino acids. An aliquot of the washed eggs was fertilized. Samples were taken at intervals to determine incorporation of labeled precursor. The details of procedure and results of a typical experiment are shown in Figure 1. The average incorporation rate during the first hour after fertilization was 4.3 times the rate of incorporation rate in unfertilized eggs of Arbacia at 21-22°C. Unfertilized Strongylocentrotus eggs at 20°C incorporate at a rate 30 times lower than zygotes, and maintain a constant rate of incorporation for at least 24 hours. The rate of incorporation in unfertilized Arbacia eggs dropped abruptly by 30-60% after about ten hours.

This method of comparing rates of synthesis in fertilized and unfer-

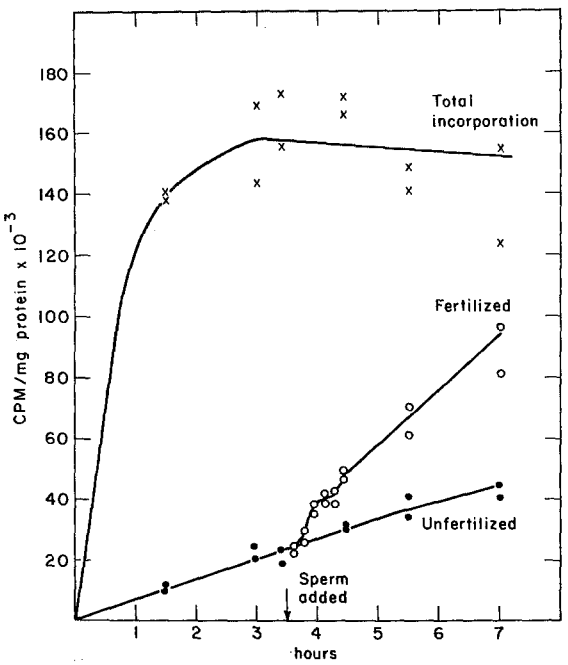

FIGURE 1. One ml of unfertilized Arbacia eggs in 10 ml sea water was incubated with 5 μc/ml C^{14} amino acids for three hours, washed four times with sea water, and divided into two aliquots, each of which was diluted to 50 ml. Three and one half hours after the initial introduction of isotopes, one aliquot of the washed eggs was fertilized. TCA precipitable and total incorporation were measured at intervals during the procedure. The slopes of the curves of TCA precipitable incorporation measure the rate of protein synthesis. In this experiment all samples were counted in a liquid scintillation counter using a dioxane-base scintillation fluid.

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tilized eggs is valid only as long as free amino acid pools remain undiluted by mobilization of stored acids or de novo synthesis of amino acids. The results of Kavanau (1954) suggest that dilution occurs only after the two cell stage, so that the relative rate of synthesis in the first hour after fertilization is unaffected by this complication.

Puromycin at 235 ug/ml reduced amino acid incorporation in eggs of both Arbacia and Strongylocentrotus to 10% of control levels within one hour and to 5% of control after two hours. When Arbacia eggs were treated with puromycin at this concentration for four hours at 21°C. and then placed in puromycin-free sea water, they soon resumed a normal rate of protein synthesis. When treatment was extended to seven or more hours, a normal rate of synthesis was never resumed. After five hours of treat-

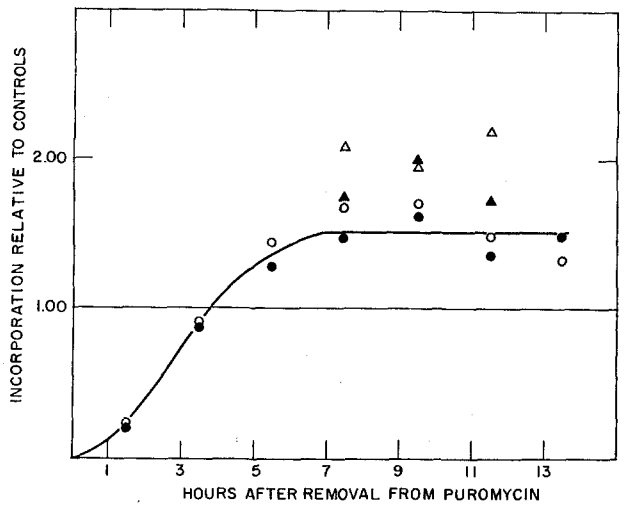

FIGURE 2. Unfertilized Strongylocentrotus eggs were pulse labeled (2 hours, 1 μc/ml) with C^{14} amino acids at various times after five hours (○) or seven hours (△) of treatment with puromycin at 235 ug/ml. Results are expressed as both TCA precipitable CPM/mg protein relative to controls and as (TCA precipitable incorporation)/(total incorporation) relative to controls (filled symbols). The effect of a five hour treatment on permeability is seen to be small in this instance; the seven hour treatment may have produced a slightly enhanced permeability increase.

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ment, the return to puromycin-free sea water was marked by rates of protein synthesis up to two times control levels. Similar results were obtained with Strongylocentrotus although the range of effective treatment times was slightly wider (Figure 2). Puromycin treatment often provoked a permeability increase (an increase relative to controls in the value of total incorporation). This increase apparently was not directly connected with the increase in rate of protein synthesis, since its occurence and magnitude were variable. It was corrected for as described above and in the figure legends.

The possibility was considered that puromycin might cause leakage of amino acids from the eggs. However, when eggs were exposed to labeled amino acids, washed, and then exposed to puromycin, no change was observed in the value of total incorporation during at least six hours of puromycin treatment.

Exposure of eggs to anoxia inhibited amino acid incorporation more than 90% after one hour. Release of inhibition after several hours resulted in increased rates of protein synthesis. Again the magnitude of

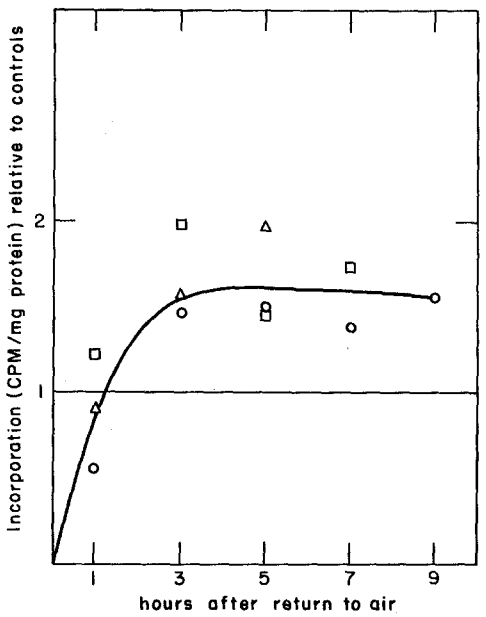

FIGURE 3. Unfertilized eggs of Arbacia were pulse labeled (1 hour, 1 μc/ml) with C^{14} amino acids following exposure to anaerobic conditions (N_2) for 3 (O), five (□) or six (△) hours. The first pulse was begun immediately after the return to air.

the increase was about 1.5 to 2 fold over controls (Figure 3). The effect of anoxia on permeability to amino acids was variable. In the experiments reported here, no increase was observed.

Eggs were capable of normal development after at least seven hours of anoxia if fertilized at least two hours after termination of the treatment. Puromycin stimulated eggs never developed normally, however.

It is clear from these results that fertilization of sea urchin eggs results in an increase in the rate of protein synthesis rather than in its initiation. In both species studied temporary supression of the eggs' metabolism seems responsible for releasing eggs from at least one control normally imposed on synthetic activity in the mature unfertilized egg.

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