

activating muscle contraction (accumulation by sarcoplasmic reticulum, activation of myofibrillar ATPase, and binding to troponin-tropomyosin) and generates muscle tension which is at least 90% of that generated by  $\text{Ca}^{+2}$ .  $\text{Sr}^{+2}$  also can substitute for  $\text{Ca}^{+2}$  in causing *Ilyanassa* eggs to form a lobe-like protuberance upon microiontophoretic injection. The timing and morphology of the response to injected  $\text{Sr}^{+2}$  are the same as to injected  $\text{Ca}^{+2}$ .

When *Ilyanassa* eggs are transferred from normal sea water, rinsed in  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  free artificial sea water (CMF-sea water) and then dispensed to solutions of normal sea water, CMF-sea water, or CMF-sea water + 10 mM EDTA, a majority of the eggs still form and resorb third polar lobes and undergo first cytokinesis in synchrony with control eggs left unrinsed in normal sea water. In CMF-sea water, approximately 70% of the rinsed eggs underwent normal development, whereas in CMF-sea water + 10 mM EDTA, approximately 65% developed normally (results of eight experiments). To determine whether the eggs in these CMF solutions were forming polar lobes and cleaving in the complete absence of both  $\text{Ca}^{+2}$  and  $\text{Mg}^{+2}$ , we analyzed the solutions by atomic absorption spectroscopy before and after they were used on the eggs. CMF-sea water + EDTA contained 3  $\mu\text{M}$   $\text{Ca}^{+2}$ ; after incubation with the eggs, such solutions contained 4-5  $\mu\text{M}$   $\text{Ca}^{+2}$ . CMF-sea water + EDTA contained 1  $\mu\text{M}$   $\text{Mg}^{+2}$ ; after incubation with the eggs, such solutions contained 3-4  $\mu\text{M}$   $\text{Mg}^{+2}$ . If  $\text{Ca}^{+2}$  (and  $\text{Mg}^{+2}$ ) are required for normal polar lobe formation and cytokinesis, the results obtained here suggest that they are derived from intracellular sources or are required in only very low exogenous concentrations.

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#### TENSIONS EXERTED BY CLEAVAGE FURROWS OF *Echinorachnius parma* EGGS TREATED WITH CYTOCHALASIN B

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Tensions exerted by the cleavage furrow of sand dollar *Echinorachnius parma* eggs can be measured with calibrated glass needles (Rappaport, Science 156:1241, 1967). The force exerted by the normal furrow of the first cleavage is about  $1.5 \times 10^{-3}$  dyne. Because the furrow contracts isometrically, the force measured by this method represents the maximum amount available in the division mechanism rather than the amount that is actually required for division. In this investigation we attempted to ascertain the least force that could accomplish division by measuring forces exerted by furrows that had been debilitated by chemical treatment. After each measurement the needles were removed from the cell to determine whether or not it could complete division.

The antibiotic Cytochalasin B reverses already functioning cleavage furrows at the same time that it causes the disappearance of the microfilamentous subsurface ring which is located at the base of animal cleavage furrows (Schroeder, Biol. Bull. 137:413, 1969). Concentrations of about 0.10  $\mu\text{g}/\text{ml}$  slow division without blocking it and alter the response of the surface to manipulation. The surface became more sticky and fragile. At these concentrations the surface of all Cytochalasin B-treated eggs was altered, but eggs from different females could differ in their sensitivity. The glass bottom of the operation chamber was covered with a thin film of Lubriseal, which discouraged cell adhesion and subsequent rupture. There was also some tendency for cytoplasm to leak from the egg at the point of entrance of the needle, but leakage did not usually interfere with cleavage, and it eventually stopped. Old eggs appeared more susceptible to treatment than freshly ovulated eggs.

Cytochalasin B concentrations of 0.08  $\mu\text{g/ml}$  were effective in reducing the contractile tension without making eggs unmanageable. The average tension exerted in that concentration was  $0.7 \times 10^{-3}$  dyne, but within that group of measurements furrows exerting as little as  $0.2 \times 10^{-3}$  dyne completed cleavage when the needles were removed. Concentrations of Cytochalasin B that reduced furrow tension also appeared to increase the time necessary for cytokinesis, but a careful analysis of the effect was not carried out. Although cleavage took place in concentrations of Cytochalasin B slightly greater than 0.08  $\mu\text{g/ml}$ , the stickiness of the egg surface prevented satisfactory measurement of the tensions exerted.

These results suggest that the force necessary to divide sand dollar eggs may be less than  $0.2 \times 10^{-3}$  dyne. However, it is possible that the treatment altered the resistance to deformation of the non-furrow surface, which might have affected the force necessary for division. This investigation was supported by Grant #PCM 74-18380 A02 from the National Science Foundation.

#### SPAWNING SEASON OF THE GREEN URCHIN, *Strongylocentrotus drobachiensis*

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E. B. Harvey reported in *The American Arbacia and Other Sea Urchins* (p. 59, Princeton University Press, 1956) that *S. drobachiensis* at Mt. Desert Island Biological Laboratory is ripe in March and April, and is spent by May 15th, although some males have ripe sperm beyond that date.

Animals were tested for ripeness from May 5th to July 5th, and functional gametes were obtained until the end of June. When the animals are at the peak of ripeness (March-April), gametes may be obtained by injection of 1 ml or less of 0.5 M KCl. To obtain the gametes at the end of the spawning season, we removed the jaw mechanism, the viscera and coelomic fluid, leaving only the gonads. The urchin was then inverted over a beaker of sea water and 0.5 M KCl (usually about 5-10 ml) was poured into the coelomic cavity.

The proportion of ripe animals and amount of eggs and sperm obtained from each ripe animal declined as the season progressed. However, as late as June 27th, a female produced about 35 ml of unpacked eggs, which fertilized well and subsequently divided normally. Urchins which were held in sea water tanks and fed generously with *Ascophyllum* performed better than animals which were collected and used directly from Frenchman's Bay in late June, even though there was no lack of seaweed in the natural habitat.

R. Hinegardner (Czihal, *The Sea Urchin Embryo*, pp. 11-12, Springer-Verlag, 1975) found that gamete production in captive *Lytechinus pictus* seems to depend on availability of food, and that unripe urchins ripen and produce gametes if well fed.

Satisfactory cleavage and subsequent development took place between 9°-12.5° C, with time of 1st cleavage 120-130 minutes after fertilization. Times of one hour from 1st to 2nd cleavage, and one hour from 2nd to 3rd cleavage were average. By July 5th, animals failed to release significant quantities of gametes after stimulation.

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