effects on cytokinesis were observed. The jelly, membranes and hyaline layer of fertilized E. parma eggs were removed by repeated washings with 1 M glycine beginning 1 min after fertilization. At various times thereafter, extensive portions of the surface were rapidly disrupted by contacting them with an expanding drop of mineral or silicone oil which was expelled from the nozzle of a micropipette. The immediate result was an explosive destruction of part of the surface, followed by massive outflow of endoplasm. Within seconds of the beginning of the outflow, new membrane rapidly appeared on the naked endoplasm. The new membrane connected with the persisting part of the original surface so that most of the egg contents were then covered by a combination of original and new surface. By mechanical pressure the mitotic apparatus and variable amounts of the surrounding endoplasm were coaxed into the region bounded by the new membrane, which subsequently protruded and often became completely separated from the remainder of the egg. As the volume of the protrusion increased, the thickness of the original surface increased as the area it covered decreased. Original and new surfaces became easily distinguishable because the original surface appeared to be covered with projecting, enmeshed linear structures and to be underlain by a cortical zone from which granular components were nearly absent. No granular free area or linear structures were associated with new surface.

In nucleated endoplasmic fragments, mitotic cycling continued and, at its maximum, the appearance of the mitotic apparatus was typically radiate. In the presence of a well-developed mitotic apparatus, the surface of the endoplasmic fragment developed typical furrows, the number and permanence of which were correlated with the number of asters and their size. Although failure of furrowing in endoplasmic fragments was not rare, the fact that failure also occurred in eggs in which the endoplasmic mass had previously rejoined the portion covered by the original surface suggests that the difficulties arose from disorganization rather than absence of an essential component. In cases where the endoplasm rejoined the portion covered by the original surface, the original and the new surface remained contiguous and discrete on the surface of the nearly spherical mass. These observations indicate that cleavage furrow establishment does not require a surface with a stable, long-standing organization. This investigation was supported by NSF Grant PCM7902624.

POLAR LOBE FORMATION AND CYTOKINESIS IN FERTILIZED EGGS OF ILYANASSA OBSOLETA AND MYTILUS EDULIS

Gary W. Conrad and David L. Sommerfeld, Division of Biology, Kansas State University, Manhattan, Kansas

Fertilized eggs of the marine mud snail, Ilyanassa obsoleta (Nassarius obsoletus), and of the blue, edible mussel, Mytilus edulis, form a constriction slightly below the equator that tightens and then relaxes several times before and during early cleavage. This shape change is an excellent mimic of cytokinesis. Our purpose has been to determine what accompanying changes occur in intracellular ionic activities and in the cytoskeletal proteins.

Electrodes. We attempted to construct Ca^{2+}-selective microelectrodes following the methods of Tsien and Rink (1981, J. Neurosci. Meth. 4:73) and of Lanter et al (1982. Anal. Chim. Acta 135:51). The calcium-selective neutral carrier, ETH 1001, was kindly donated by Professor W. Simon (Swiss Federal Institute of Technology, Zurich). Standard calibration solutions contained 0.125 M K^+ and free Ca^{2+} concentrations of 10^{-3}-10^{-8} M (Tsien and Rink, 1981). Silane treatments of microelectrodes with tri-n-butylchlorosilane in gaseous form or mixed with 1-chloronaphthalene were unsuccessful. However, when N-trimethylsilyldiethylamine was used (5%, v/v, in 1-chloronaphthalene), a few electrodes displayed excellent response to Ca^{2+}, even detecting a difference between pCa∞ and pCa 8, even after such electrodes were stored in air overnight. Two single barrel electrodes (tip diameter, 2 μm, O. D.) and two double barrel electrodes (overall tip diameter, 3 μm, O.D.; one barrel filled with 3 M KCl and used for determining membrane potential) calibrated satisfactorily, but insufficient numbers of Ilyanassa eggs were punctured to yield data on the intracellular pCa. Repeated attempts to produce more such single or double-barrel electrodes were unsuccessful.

Thiophosphorylation. Our previous experiments had suggested that polar lobe-like constrictions could be induced experimentally in Ilyanassa eggs when cAMP levels are raised by microiontophoretic injection of cAMP or by inhibiting

phosphodiesterases with methylated xanthines. We therefore sought to determine if changes in the phosphorylation state of proteins could be detected as Ilyanassa eggs underwent polar lobe formation and cytokinesis. Ilyanassa eggs, in spherical stages and in two stages of polar lobe constriction and cytokinesis, were lysed with solutions of Triton X-100, separated into yolky and non-yolky fractions, and incubated with (^{35}S)-adenosine 5'-(γ-thio)triphosphate (^{35}S-thioATP). Incubations were carried out for 30 min on ice or at 15°C in the presence of 5 μM ATP, 25 mM HEPES (pH 7.4), and 1.5 mM Mn Cl_2 or $MgCl_2$ according to Cassel and Glaser (1982. Proc. Nat. Acad. Sci. USA. 79:2231). Reactions were stopped by addition of SDS to 2.3% and heating to 60°C for 10 min. Such samples were subjected to electrophoresis in polyacrylamide gradient slab gels followed by autoradiography with X-ray film at - 80°C. Results indicated the thiophosphorylation of a high molecular weight area of protein near the top of the running gel in both yolky and non-yolky fractions of all samples. No qualitative differences were seen between samples from eggs in different degrees of polar lobe constriction.

Mytilus spawning. It is not convenient to get large numbers of Ilyanassa eggs to develop synchronously. Blue mussels, Mytilus edulis, however, release large numbers of gametes which are fertilized in open sea water and then undergo the same series of cell shape changes (polar lobe formation) that Ilyanassa eggs display. It has been difficult to study Mytilus embryos, however, because no reproducible method has been devised that elicits spawning (injection of 0.5 M KCl causes gamete shedding in echinoderms, but not in Mytilus). We therefore subjected freshly collected mussels to sea water containing a variety of hormones and neurotransmitters in an attempt to trigger spawning. Animals were placed in individual fingerbowls of sea water containing one of the following: 10^{-5}, 10^{-6} M 1-methyladenine: 10^{-5}, 10^{-6} M γ-amino-n-butric acid; 10^{-5}, 10^{-6} M acetylcholine chloride; 10^{-5}, 10^{-6} M progesterone in 1% or 0.1% DMSO; 10^{-6}, 10^{-7} M somatostatin; 10^{-5}, 10^{-6} M epinephrine; 1, 10 I. U./ml insulin; 10^{-5}, 10^{-6} M dexamethasone in 1% or 0.1% DMSO. Results: None of the treatments above elicited spawning in Mytilus. Supported by NIH Grant No. HDO7193.

ISOLATED RECTAL GLAND CELLS: OXYGEN CONSUMPTION STUDIES

Patricio Silva, Arthur Stevens, Patricio Silva, Jr., Katherine Spokes, and Franklin H. Epstein, Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory of Beth Israel Hospital, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts

Cells were separated from rectal glands by collagenase and hyaluronidase digestion of minced rectal glands using the technique previously described (MDIBL Bull. 20:38-39, 1980), with only minor modifications. The cells were kept on ice until used in a buffer of the following composition (in mM): Na 280; K 5; Cl 295; Mg 3; Ca 2.5; SO_4 0.5; phosphate 1; Urea 350; Hepes 40, pH 7.6. The same buffer was used for measuring oxygen consumption. When sodium was removed from the bath it was replaced by lithium. When chloride was removed it was replaced by nitrate. When both sodium and chloride were removed they were replaced by lithium nitrate. Cells that were stored in buffer containing sodium and chloride were washed twice in buffer of the same composition as that used for measuring oxygen consumption. Oxygen consumption was measured in a constant temperature (25°C) chamber containing 2 ml of buffer and 4 to 10 mg of cells using a Clark type polarographic oxygen electrode (YSI) connected to a recorder. The electrode was calibrated using the known solubility of oxygen at 25°C and the barometric pressure. The rate of oxygen consumption was calculated from the tangent of the recorded slope of the oxygen consumption, the volume of buffer in the measuring chamber, and the wet weight of a measured aliquot of the cells removed from the measuring chamber at the end of the experiment. Glucose 5×10^{-3} M, pyruvate 10^{-2} M, and acetate 2.5×10^{-3} M were used as metabolic substrates. Theophylline 2×10^{-3} M, dibutyryl cyclic AMP 10^{-3} M, ouabain 10^{-4} M, and bumetanide 10^{-4} M (final concentrations), were all added in a volume not exceeding 1% of the volume of the measuring chamber to avoid dilutional problems. Results are expressed as micromoles of O_2 consumed per hour per gram wet weight of cells. The results are summarized in Table 1.