the inactive cells until there is a balance between the passive force of the inactive cells and the sum of the active and passive force of the active cells. This mechanism explains how increasing damage lowers the peak tension and result in a curved relation for the twitch tension versus the initial sarcomere length. It also explains the nonlinear relation between twitch tensi-n and sarcomere shortening if it is assumed that the red light beam through the center of the preparation is preferentially scattered by undamaged cells. The model described here ascribes the same nonlinear length tension curve for the series and parallel elastic element. The maximum twitch tension is remarkedly constant, 220 ± 40 mg/cm (6 preparations). In this respect, the model predicts significant curvature of the ascending limb with only a small decline in maximum twitch tension. The constancy of the twitch tension in various preparations indicates that there is little variation in the degree of activation from preparation to preparation.

The experiments reported above suggest that laser diffraction images can be used to study the contractile process of the single cell layered myoepithelium at the sarcomere level. The results are similar to those obtained in mammalian heart. Series elastic components seem to exist in varying degrees. Sarcomere length-tension curves were only measured in 3 successful experiments of all which yielded diffraction images of less than optimal quality. The experiments are, however, promising enough to spure continued investigation. An improved design for the laser diffraction system may well improve the quality of the diffraction patterns to a degree where it is possible to clamp the sarcomere length instead of the length of the preparation.

EFFECTS OF MICROIONTOPHORETIC INJECTION OF CALCIUM IONS AND CYCLIC AMP ON CELL SHAPE.

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Fertilized eggs of some mollusks and some annelids undergo a series of shape changes before and during early cleavage, a process known as polar lobe formation. These shape changes are seen in most dramatic form in the eggs of the common marine and snail, *Ilyanassa obsoleta*. A polar lobe results from formation of a constriction slightly below the equator of the fertilized egg. This constriction greatly resembles a cleavage furrow.

Previous work has shown that a ring of microfilaments is present in the cortical cytoplasm at the base of the polar lobe constriction, analogous to the microfilament bundle at the base of a cleavage furrow (Devel. Biol. 36:363-378 (1974)). The microfilament ring is present only when the polar lobe constriction is present and disappears when the constriction relaxes during lobe resorption. Integrity of the microfilaments can be disrupted by treatment with cytochalasin B, with simultaneous regression of the lobe constriction. We wish to determine what normally regulates the constriction of the microfilament ring associated with lobe formation. Previous experiments have shown that, if spherical *Ilyanassa* eggs are placed in high concentrations of exogenous calcium ions, cell shapes change dramatically within 15 min.in a process which appears to mimic normal polar lobe formation (Devel. Biol. 37:280-294 (1974)). Eggs placed in solutions of other ions, such as sodium, potassium, magnesium, or manganese, do not show such shape changes. On the other hand, the normal process of polar lobe formation, as well as of cytokinesis, continues without any inhibition when eggs are placed in calcium-free sea water. This suggests that if calcium ions <u>are</u> involved in regulating the constriction of microfilament rings, they derive from intracellular pools. We therefore wish to know if changes in intracellular concentrations or activities of ions like calcium play a role in normal polar lobe formation.

In the present experiments, we injected inorganic ions, cyclic nucleotides, and other substances into *Ilyanassa* eggs and noted the changes in cell shape. Experiments were done in the following manner. Fertilized eggs were removed from egg capsules and placed in a petri dish of sea water on the stage of a dissecting microscope fitted with photographic optics. Glass micropipettes were made (tip diameter, 3-5 μ) and filled with test solutions. The buffer in all test solutions was HEPES (10 mM, pH 7.5); test salts (all in chloride form) were used at a concentration of 0.5 M, whereas cyclic nucleotides were at 50 mM. The microiontophoresis apparatus used the glass micropipette filled with the test solution as one electrode (a KCl-treated silver wire was inserted into the back of the micropipette, in the test solution) and a KCl-treated silver wire as the other electrode. The latter wire electrode was placed directly in the sea water at the edge of the petri dish. The micropipette then was fastened to a micromanipulator and, under continuous observation with the dissecting microscope, eggs were pierced by the tip of the micropipette. Eggs could be impaled at any point on their surface.

At the site of insertion of the micropipette tip, one of three things occurred: no bleb formed around the tip, a small bleb formed which slowly enlarged, or a reasonably large bleb formed very quickly. Large diameter micropipette tips or dirty tips led to large blebs. Bleb formation occurred without any current being injected from the microiontophoresis apparatus, i.e., it resulted simply from insertion of a micropipette tip.

A standard interval of 15 sec was used for all microiontophoretic injections, during which time each egg was observed continuously. Eggs were photographed immediately before and immediately after microiontophoretic injection. Injection of calcium ions at any point on the egg surface causes the formation of a localized change in cell shape within 15 seconds after injection. A large lobe-like protuberance forms near the tip of the pipette, i.e., either around the point where the needle was inserted or (if the needle was pushed almost all the way through the egg) opposite the site of needle insertion. By varying the input current, we determined the minimum amount of calcium that had to be injected in order to get a shape change. This minimum current, 125 nanoamps, was approximately the same throughout early development of the fertilized egg, except that during the period of normal lobe resorption we could find no current of injected calcium which would lead to the formation of a protuberance. When the micropipette was filled instead with buffered solutions of NaCl, KCl, or $NgCl_2$ microiontophoretic injections at a wide range of input currents did not lead to changes in cell shape. Moreover, microiontophoretic injections of control solutions, such as HEPES buffer alone or sea water, did not result in the formation of protuberances.

Changes in calcium concentrations in cells may be related to changes in the concentrations of cyclic nucleotides. We therefore determined the effects of injecting derivatives of cyclic AMP and cyclic GMP on the shapes of *Ilyanassa* eggs. When the micropipette was filled with the HEPES buffer solution containing 3',5'-cyclic AMP, microiontophoretic injection at currents above 300 nanoamps caused eggs to form lobe-like protuberances very similar to those caused by the injection of calcium ions. Response to injection of this derivative was slightly slower than that to calcium injection. On the other hand, when the micropipette contained derivatives of other cyclic nucleotides, such as either 2',3'-, or 3',5'-cyclic GMP, microiontophoretic injection did not result in a change in cell shape. Injection of 2',3'-cyclic AMP occasionally gave a slight change in shape, but such changes were not observed as consistently as when eggs were injected with calcium or with 3',5'-cyclic AMP.

To determine the extent to which the cell shape changes described above were dependent upon exogenous calcium ions, injections of calcium ions or of 3',5'-cyclic AMP were performed when eggs were in petri dishes containing calcium-free sea water. Under such circumstances, the simple insertion

of the micropipette tip did not result in the formation of any blebs around the needle. Moreover, microiontophoretic injection of neither calcium ions nor of 3',5'-cyclic AMP resulted in any change in cell shape. However, when calcium was added to the petri dish, the ability of such cells to form blebs around the point of needle insertion returned, as did the ability to form lobe-like protuberances upon microiontophoretic injection of calcium ions or of 3',5'-cyclic AMP.

In summary, the data suggest there is a system that can be found anywhere beneath the surface of the *Ilyanassa* egg which, when supplied intracellularly with either calcium ions or with 3',5'-cyclic AMP, will generate a lobe-like protuberance of the cell surface so long as exogenous calcium ions are present. One possible explanation for the results may be that microiontophoretic injection of calcium ions or of 3',5'-cyclic AMP quickly initiates changes in the properties of the plasma membrane that lead to an increase in membrane permeability to exogenous calcium ions. The resultant formation of a lobe-like protuberance therefore, although morphologically very similar to normal lobe formation, appears to be an aberrant mimic of the process, for, as noted above, normal polar lobe formation does not appear to be dependent upon exogenous calcium ions.

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ION HOMEOSTASIS IN CEREBROSPINAL FLUID IN THE DOGFISH, *Squalus acanthias*, AND LITTLE SKATE, *Raja erinacea*.

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In higher vertebrates the concentrations of potassium, magnesium and calcium in cerebrospinal fluid (CSF) and in brain interstitial fluid (ISF) are maintained relatively constant, independent of changes in plasma ion concentration. Potassium homeostasis has also been demonstrated for elasmobranch CSF, but only in dogfish (Cserr and Rall, Comp. Biochem. Physiol. 21:431-434, 1967). In view of the paucity of information on CSF homeostasis in elasmobranchs and, further, in view of differences in the ultrastructure of the blood-brain barrier between elasmobranchs and higher vertebrates (Brightman et al. Progr. Neuropathol. 1:146-161, 1971) we have investigated CSF ion homeostasis in two elasmobranch species, dogfish and little skate. The comparison of CSF electrolytes between dogfish and little skate is also of interest since the ventricular cavities and choroid plexuses are large in dogfish and extremely reduced in the skate.

CSF was obtained from the cerebellar ventricle in dogfish (0.5 to 1.0 ml) and from the third ventricle in skates (5 to 15 microliters). To test for homeostasis, the plasma concentration of one of the three cations was elevated for four hours. Magnesium or calcium was given as an initial i.m. injection (4.8 ml/kg 333 mM $MgCl_2$ or 6.0 ml/kg 333 mM $CaCl_2$) followed by 1/4 of the original dose after two hours. For potassium, the initial injection (5 ml/kg 400 mM KCl) was followed by a continuous i.m. infusion (approx. 5 ml/kg·hr). Analyses of plasma before and at hourly intervals after injection confirmed that plasma ion concentration was elevated throughout the experimental period. Extradural fluid (EDF), which bathes the outer surface of the elasmobranch brain, was also sampled and analyzed for electrolytes. Concentrations were converted to mEq/kg H_2O using values for % water content of plasma, CSF and EDF of 94%, 97% and 96%, respectively (Cserr, Fenstermacher and Rall, in press).

Results are summarized in Table 1. CSF concentrations of magnesium and calcium in control dogfish and skates are in the same range as mammalian values (Katzman & Pappius, In: "Brain Electrolytes and Fluid Metabolism," 1973), and further, increasing plasma concentration by 2- to 4-fold resulted in only a small increase in CSF concentration, demonstrating homeostasis of CSF calcium and magnesium in both