1972 #6

## EFFECTS OF CYTOCHALASIN-B AND COLCHICINE ON POLAR LOBE FORMATION IN EGGS OF THE MARINE MUDSNAIL, *Ilyanassa obsoleta*

Gary W. Conrad, Division of Biology, Kansas State University, Manhattan, Kansas

The constrictions of the plasma membrane involved in polar lobe formation in mollusk embryos resemble cleavage furrows except that polar lobe constrictions do not cleave the cell completely, they are not associated with a spindle apparatus visible at the level of the light microscope, and they are relaxed quickly after polar body formation and early cleavage. Factors regulating polar lobe constrictions are unknown. Cytochalasin-B and colchicine, drugs which affect the function of microfilaments and microtubules respectively were used to determine the extent to which the polar lobe constrictions behaved similarly to first cleavage furrows. Fertilized eggs were treated for varying periods of time with sea water containing one or both of these drugs at times prior to second or third polar lobe formation. The eggs were then allowed to progress through early cleavage. Experiments using cytochalasin-B at concentrations of 0.1 - 10 μg/ml showed that (1) the formation of polar lobes could be completely prevented by cytochalasin-B. (2) the second and third polar lobes, once formed, were rapidly resorbed when exposed to this drug, (3) the formation of the second polar lobe could be prevented at concentrations which still allowed formation of the third polar lobe, (4) the formation of the third polar lobe could be prevented at concentrations which still allowed first cleavage, and (5) the formation of a third polar lobe did not depend on the prior formation of a second polar lobe. Experiments using colchicine at concentrations of 0.1 - 1000 μg/ml showed that polar lobes could form in all concentrations but that concentrations which inhibited first cleavage also prevented the rapid resorption of polar lobes. In the presence of colchicine stabilized lobes were resorbed much more rapidly if cytochalasin-B was also present. These data suggest that the formation of polar lobe constrictions and their stability once formed involve only cytoplasmic filaments, whereas their resorption may depend in some way on microtubule function.

This work was supported by a grant from Sigma Xi and a Dahlgren fellowship.

1972 #7

## STUDIES ON THE MECHANISM OF POLAR LOBE FORMATION IN EGGS OF THE MARINE MUDSNAIL, *Ilyanassa obsoleta*

Gary W. Conrad, Division of Biology, Kansas State University, Manhattan, Kansas

Snail eggs undergo a series of shape changes preceding first cleavage. These deformations of the fertilized egg result in the formation of a protuberant bulge of cytoplasm, the polar lobe. This lobe contains morphogenetic substances of an unknown nature, probably sequestered somewhere near the plasma membrane, which are necessary for the normal differentiation of the shell, operculum, foot, statocysts, heart, intestine, and eyes in the older embryo. The purpose of the present study was to elucidate the mechanism of polar lobe formation in the fertilized egg.

The hypothesis tested proposed the existence of a band of material encircling the spherical egg which polymerizes into filaments and, like actin and myosin filaments in muscle, can contract and relax. Contraction of such a band could deform a spherical egg into a polar lobe shape. Similar models have been proposed to account for cytokinesis. If such a model is valid it should be possible to demonstrate (1) a band of cytoplasmic microfilaments, (2) essential roles for calcium and magnesium ions and ATP, and (3) inhibition of polar lobe formation by inhibiting divalent cation-activated ATPases but not by inhibiting monovalent cation-activated ATPases.

Methods: *Ilyanassa obsoleta* was collected on the northeast side of Thompson Island and kept in running sea water aquaria at MDIBL. Egg capsules were collected from the side of the aquarium each day and opened with iris scissors. The eggs, which developed synchronously within a single capsule (30-300 eggs/capsule), were suspended in sea water and kept in plastic petri dishes on a running sea water table in the laboratory. Under these conditions the eggs develop normally at temperatures below 25° C. Observations of living eggs were made with an inverted phase microscope over a period of two to three hours. For electron microscopy eggs were fixed at room temperature for 45 minutes in solutions of 2 percent glutaraldehyde, pH 7.1, followed by 15 minutes at 2-4° C. Glutaraldehyde solution (1080 mOsm) contained 0.081 M sodium phosphate, 0.185 M NaCl, 0.145M KCl, and $10^{-5}$ M $MgCl_2$. Eggs were then rinsed in cold buffer solution (1398 mOsm) containing 0.15M sodium phosphate, 0.342 M NaCl, 0.268 M KCl, and $10^{-5}$ M $MgCl_2$. Next they were treated with cold solutions of 2 percent $0_s0_4$ in the same buffer, rinsed in 0.1 M maleate buffer (pH 5.4), stained for 45 minutes in 0.5 percent aqueous uranyl acetate in maleate buffer, dehydrated in acetone, and flat embedded in Spurr's ERL resin formulation B (hard). These embedded eggs were sent back to Kansas State University where my collaborator, Dr. Dan Williams, sectioned them in specific orientations with diamond knives on a Reichert ultramicrotome III. The sections were stained with lead citrate or 1 percent aqueous uranyl acetate followed by lead citrate and examined at 100 KV in an RCA EMU-4 or at 60 KV in a Phillips 201 electron microscope in the Division of Biology at Kansas State University.

Our results suggested the following: (1) Cytoplasmic microfilaments were present in the form of a ring at the base of the polar lobe constriction. (2) Polar lobes continued to be formed and resorbed on a normal time schedule even when the eggs were exposed to calcium –, magnesium–free sea water $\pm$ 10 mM EDTA. (3) Polar lobe formation and cleavage occurred normally in the presence of 2,4-dinitrophenol ($\leq 1 \times 10^{-5}$M) and more slowly at higher concentrations. Neither process was completely prevented even by a concentration of $5 \times 10^{-4}$M. (4) Ouabain ($\leq 1.25 \times 10^{-3}$M) had no effect on polar lobe formation or cleavage, whereas both mersalyl acid ($\geq 5 \times 10^{-4}$M) and ethacrynic acid ($\geq 5 \times 10^{-3}$M) inhibited polar lobe formation and cleavage.

Insufficient data are available to determine whether polar lobes form by a mechanism strictly analogous to that of muscle contraction. Whereas a band of microfilaments is present at the base of the polar lobe constriction, the degree to which these filaments are actin-like, i.e., will bind heavy meromyosin, is not known. Moreover the meaning of the inhibitor experiments above remains uncertain until levels of ATP and mono-and divalent-cation activated ATPases are measured directly and correlated with the degree of inhibition of polar lobe formation.

The electron microscopy was done in collaboration with Dr. D.C. Williams. We thank Drs. L.E. Roth for use of an electron microscope and ancillary facilities and J.P. Hayslett for a gift of ethacrynic acid. This study was supported by a grant from Sigma Xi and grant HD3462 from NIH to Dr. L.E. Roth.

1972 #8

## EFFECTS OF DDT ON *Fundulus Heteroclitus:* SURVIVAL, UPTAKE, AND DISTRIBUTION

R.B. Crawford, J.B. Anderson, and A.M. Guarino, Trinity College, Hartford, Connecticut and Laboratory of Toxicology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

The teleost *Fundulus heteroclitus* was selected as an appropriate model system for the study of effects of pesticides on reproduction and embryogenesis. Therefore studies were conducted on the effects of a pesticide, DDT (1,1,1 trichloro-2,2 bis (p-chlorophenyl) ethane), on the adult organism and the distribution of DDT in the surviving animals. It is of particular interest to determine an effective route for preloading gametes with the pesticide.

Fish were kept in 1000 ml Erlenmeyer flasks containing 750 ml sea water maintained at 13°C. Under standard conditions each flask held four fish. All sea water was filtered through membrane filters (0.45 $\mu$ pore size) to eliminate the influence of other marine organisms and particles on DDT uptake by *Fundulus.* Each flask was aerated from a central pump *via* an aquarium stone. The water, whether it contained DDT or not, was changed daily. DDT solutions were prepared by adding 2.0 ml of ethanolic solutions to 1000 ml filtered sea water immediately prior to use. Control fish were kept in 0.2 percent ethanol-sea water.

Lethal dosage of DDT for *Fundulus* was determined under a variety of exposure conditions. Keeping the fish in DDT continuously gave results as seen in Table 1. The survivorship after 24 hours of treatment was over 90 percent for fish in 0.1 ppm DDT. Further reduction in concentration was not lethal within 24 hours.

TABLE 1

Percentage Deaths in Fundulus Maintained in DDT-Containing Media

| DDT | Hours in DDT solution | | | | | |
|---|---|---|---|---|---|---|
| ppm | 6 | 10 | 19 | 22 | 24 | |
| 1.0 | 0 | 0 | 33 | 67 | 100 | (3)[a] |
| 0.5 | 0 | 0 | 0 | 55 | 82 | (11) |
| 0.1 | 0 | 0 | 0 | 0 | 9 | (56) |
| 0.07 | 0 | 0 | 0 | 0 | 0 | (8) |
| 0.05 | 0 | 0 | 0 | 0 | 0 | (24) |
| 0.01 | 0 | 0 | 0 | 0 | 0 | (8) |
| control | 0 | 0 | 0 | 0 | 0 | (16) |

[a]Figure in parentheses refers to number of fish studied.