injections raised plasma chloride at the conclusion of the experiment from 143.3 ± 4.3 (n=8) to 147.4 ± 6.4 (n=5). Plasma clearance of ^{36}Cl in 6 eels pretreated in this fashion with hypertonic saline and exposed to freshwater was 3.20 ± 1.63 ml/100 g/hr. In contrast, injection of the same quantity of hypertonic saline at the conclusion of freshwater exposure, rather than at its onset, had only a slight effect in opposing freshwater turnoff (plasma ^{36}Cl clearance = 1.34 ± 0.76).

Because prolactin has been implicated in the freshwater adaptation of teleosts, ovine prolactin, 500 µg/100 g of body weight, was injected intraperitoneally in 5 seawater-adapted eels and the efflux of ^{36}Cl measured 2-3 hours later. Prolactin had no effect on chloride clearance, which averaged 4.65 ± 1.98 ml/100 g/hr after injection.

The effect of external calcium on the freshwater turn-off phenomenon was investigated by exposing 4 seawater eels for 2 hours to freshwater in which calcium chloride had been dissolved to seawater concentration (10 mM). This did not modify the ability of freshwater to turn off chloride clearance (0.49 ± 0.47 ml/100 g/hr).

Neurogenic transmitters might conceivably be implicated in the turn-off phenomenon, since parasympathetic and α-adrenergic agonists reduce active chloride transport across the operular membrane of *Fundulus heteroclitus*. Homatropine, 0.2 ml of 10^{-2} M per 100 g, a dose calculated to produce a concentration of 10^{-4} M per liter of extracellular fluid, was injected intraperitoneally in 4 eels at the beginning of exposure to freshwater, but turn-off was not interfered with; subsequent ^{36}Cl clearance averaging 0.62 ± 0.64 ml/100 g/hr. Likewise, phentolamine in the same dose did not affect freshwater turn-off in 4 eels; chloride clearance averaged 0.90 ± 0.68 mg/100 g/hr.

Exposure to freshwater for 2 hours induces a dramatic fall in chloride excretion by eels adapted to seawater, which can be prevented by injections of hypertonic NaCl given before, but not after the exposure. Calcium added to freshwater does not alter its effect, and injections of prolactin do not mimic freshwater turn-off. Although the time course of the phenomenon suggests neural or hormonal control, it was not modified by parasympathetic or α-adrenergic blockade.

ACTIN-LIKE MICROFILAMENTS ASSOCIATED WITH CELL SHAPE CHANGES IN *Ilyanassa* EGGS

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Polar lobe formation in fertilized eggs of the marine mudsnail, *Ilyanassa obsoleta*, involves a dramatic change in cell shape which mimics cytokinesis in animal cells not only in the overall cell shapes produced, but also in its sensitivity to specific drugs and arrangement of microfilament bundles Conrad, G. W. and D. C. Williams, Develop. Biol. 36:363-378, 1974).

We have reported previously (Bulletin MDIBL, 17:4-5, 1977) that heavy meromyosin (HMM), a molecule resulting from the tryptic digestion of myosin, binds to the microfilaments of the polar lobe constriction in an arrowhead pattern typical of HMM-actin complexes from muscle and non-muscle cells. Further study of HMM-treated and HMM-free control eggs has revealed that microfilaments are present throughout the cortex of eggs with a third polar lobe and in at least part of the cortex of round eggs. We also have found that glycerinated eggs treated with HMM and ATP or with HMM and sodium pyrophosphate have microfilaments which do not display an arrowhead pattern, responses to ATP and to pyrophosphate typical of F-actin. HMM preparations used in these experiments were demonstrated by SDS-polyacrylamide gel electrophores (SDS-PAGE) on slab gels to be free of contaminant actin.

As another assay of the actin-like nature of *Ilyanassa* microfilaments, eggs with polar lobe constrictions were glycerinated, incubated in buffer solutions containing 5 or 50 mM $MgCl_2$, and then examined by electron microscopy. The microfilaments of the lobe constriction from eggs treated with 50 mM $MgCl_2$ were arranged in bundles which formed an almost continuous band around the egg, in contrast

to the more dispersed and less organized microfilaments observed in the same region from eggs treated with 5 mM $MgCl_2$. These results suggest that *Ilyanassa* microfilaments are stabilized in the presence of 50 mM $MgCl_2$, another feature characteristic of actin-containing microfilaments.

Recent work (Pollard, T.C. et al., Cell Motility, Vol. B, p. 689, Cold Spring Harbor Laboratory) has demonstrated that tropomyosin can bind to muscle and *Acanthamoeba* F-actin and protect the filaments from fragmentation by osmium tetroxide. Similar studies were performed on *Ilyanassa* microfilaments incubated *in situ* after glycerination. Tropomyosin was extracted from chicken skeletal muscle with 1 mM dithiothreitol and isolated by ammonium sulfate and isoelectric precipitations. Glycerinated *Ilyanassa* eggs were incubated in buffer solutions containing tropomyosin and prepared for electron microscopy. Microfilaments from the lob constriction of tropomyosin-treated eggs often appeared long and straight, whereas microfilaments in control eggs most often assumed a network-like pattern. These results suggest that the actin-like protein in *Ilyanassa* microfilaments is capable of binding muscle tropomyosin, thereby acquiring resistance to OsO_4-induced fragmentation.

Ilyanassa eggs contain a prominent protein which, on SDS-PAGE slab gels, co-migrates with actin prepared from chick skeletal muscle. To determine whether the amount of this protein changes dramatically during polar lobe formation, we collected known numbers of eggs at each of several specific stages before and during the formation of the third polar lobe and concomitant first cleavage. Soluble proteins were extracted from yolk-containing, as well as from yolk-free fractions of detergent-lysed eggs and separated by SDS-PAGE on slab gels. The gels were stained with Coomassie blue and then scanned directly with a Joyce-Loebl double-beam recording microdensitometer (Model MK III C). The results indicated that the amount of soluble actin-like protein per egg rises by about 23% (above that in round eggs) as lobe formation begins, but then falls to about 30% below the level of round eggs when cleavage furrows and polar lobe constrictions display maximal numbers of microfilaments ultrastructurally.

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EFFECTS OF CALCIUM IONOPHORE A23187, CARBAMYLCHOLINE, AND RMI 12330A ON CHLORIDE SECRETION IN THE ISOLATED PERFUSED RECTAL GLAND OF *Squalus acanthias*

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Recent studies have described a cyclic AMP mediated active secretion of chloride in the rectal gland of the dogfish (Stoff et al., J. Expertl. Zool. 199:443-448, 1977; Silva et al., Amer. J. Physiol. 233(4):F298-F306, 1977). In many tissues in which activation of physiologic processes is mediated by cyclic AMP, calcium has been demonstrated to be a second intracellular messenger regulating the physiologic response. The divalent cation ionophore A23187 enhances calcium uptake or exchange in many epithelia and has been used extensively to study the effects of calcium on hormone mediated events. In mammalian intestinal tissue, A23187 has been demonstrated to stimulate chloride secretion in both rabbit ileal mucosa and the rabbit colon, without change in the intracellular level of cyclic AMP in these tissues (Bolton and Field, J. Membrane Biol. 35:159-173, 1977; Frizell, J. Membrane Biol. 35:175-187, 1977). In the present studies we investigated the effects of the calcium ionophore A23187 and carbamylcholine on chloride secretion in the isolated perfused rectal gland. The intestinal secretagogue carbamylcholine (carbachol) was chosen because stimulation of intestinal secretion by carbachol is independent of intracellular cyclic AMP and dependent on extracellular calcium (Bolton and Field, J. Membrane Biol. 35:159-173, 1977) and is thought to be mediated by increased cell permeability to calcium (Jaffer and Mitchell, Biochem. J. 160:163-169, 1976). In addition, we determined the effects of RMI 12330A, an organic cycloalkyl compound which inhibits both adenylate cyclase and cyclic AMP and ionophore A23187