

subjected to Student's t-test. The resistance at mucosal-positive voltages greater than the breakpoint (R_2) is significantly ($0.02 < P < 0.05$) decreased by the increase in P_{O_2} , as determined from the ratio of the resistance (1.38 ± 0.15) in the two conditions. Variability between tissues makes the comparison of resistances not significant when unpaired values are used. It would appear that any information regarding acid secretion is contained in the R_2 resistance region.

In frog skin, it has been suggested (Helman, O'Neil and Fisher, Am. J. Physiol. 229:947, 1975) that the breakpoint around +140 mV, which agrees with the calculated emf of a Na^+ electrogenic pump, is a measure of this pump potential. The dogfish gastric mucosa secretes H^+ from a serosal (and presumably cellular) pH of around 7.4 into a region with a calculated pH 0.5 or so. For this process, the pump emf should be about -380 mV, which is far beyond the range explored in these experiments. However, if the primary secretion is HCl at this pH, the required pump emf for Cl^- is +80 mV, which is in rough agreement with the values observed in those cases where a serosal-positive breakpoint can be found. Caution is indicated, however, by the experience with the frog gastric mucosa, in which a large number of breakpoints are found at voltages from +200 to -150 mV. It seems likely that more breakpoints will be found when the dogfish tissue is explored over a wider range.

It is recognized that the present experiments should be repeated under better conditions. The method of altering the secretory rate could be improved, higher currents are required, and the recording of voltage pulses used in this study ignores time-dependent factor which may be of importance. It is clear, however, that this tissue does show a multilinear I-V relationship, which must be explained as a part of any complete understanding of the electrophysiology of the tissue. Supported by NSF PCM 77-03336.

FURTHER STUDIES OF ADAPTATION TO FRESHWATER BY *Anguilla rostrata*

Franklin H. Epstein, Gregg Kormanik and Patricio Silva, Dept. of Medicine and Thorndike Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts

When American eels (*Anguilla rostrata*), fully adapted to seawater, are placed for brief periods of time in freshwater, the efflux of sodium and chloride is markedly depressed and remains low even after re-immersion in full-strength seawater. Outward transfer of salt is thus inhibited even though gill Na-K-ATPase remains at the high level characteristic of seawater adaptation (Bull. MDIBL, 15:31-32, 1975). Neither the afferent stimulus nor the effector mechanism responsible for this turn-off of gill secretion has been identified.

Anguilla rostrata weighing 40-100 g, trapped in fresh water, were adapted to seawater for at least 3 weeks before they were studied. The efflux of ^{36}Cl was measured by injecting 2 microcuries intraperitoneally in a volume of 0.2 ml, allowing equilibration for at least an hour, determining the quantity of isotope appearing in the aerated bathing medium (1 liter of seawater at 16°C) over the course of the subsequent hour, and dividing by the specific activity of ^{36}Cl in plasma.

The plasma clearance of ^{36}Cl in 8 eels fully adapted to seawater was 5.74 ± 3.30 ml/hr/100 g (mean \pm s.d.) and in 8 freshwater eels, 0.8 ± 0.3 . After one hour of exposure to freshwater, ^{36}Cl clearance remained high, averaging 3.70 ± 2.07 ($n=8$), but after 2 hours of freshwater exposure it fell to $0.61 \pm .59$ ($n=12$). Previous studies had demonstrated a similar sequence of events for sodium efflux (Bull. MDIBL, 15:31-32, 1975). It is natural to suppose that dilution of the blood in freshwater is the signal for the turn-off of sodium and chloride efflux, but plasma sodium concentration in 8 eels was not significantly lowered by 2 hours of freshwater exposure (157.3 ± 3.4 mEq/L before vs. 156.7 ± 3.3 after), while plasma chloride fell only slightly, from 146.8 ± 5.5 mEq/L to 142.1 ± 4.5 . Nevertheless, preliminary injection of hypertonic saline (0.3 ml of 2.5 M NaCl per 100 g) successfully prevented the turn-off of chloride efflux normally produced by 2 hours of freshwater exposure. Such

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 $\mu\text{g}/100$ 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

Barbara Schmidt, Molly May and Gary W. Conrad, Division of Biology, Kansas State University
Manhattan, Kansas

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 μM MgCl_2 were arranged in bundles which formed an almost continuous band around the egg, in contrast