1972 #39

A NOTE ON THE ESTABLISHMENT OF CLEAVAGE FURROWS BY MITOTIC SPINDLES

R. Rappaport, Department of Biological Sciences, Union College, Schenectady, New York

This investigation was designed to determine whether the mitotic spindle of the sand dollar *Echinarachnius parma* egg can establish a cleavage furrow mechanism at the egg surface. Previous investigations showed that in cleaving echinoderm eggs only a pair of asters is required and that absence of one or both asters prevents furrowing. On the other hand in animal tissue cells the asters are much reduced and the volume of the mitotic apparatus is proportionately greater. No geometrical analysis of the role of different components of the mitotic apparatus of tissue cells in furrow establishment has been accomplished. Since spindles and asters appear to be composed of similar structures having similar chemical constitution, it appeared possible that both might be able to establish furrows given the proper geometric circumstances.

By micromanipulation techniques involving perforation, cutting, and artificial constriction, portions of the egg surface were moved close to or in contact with the equatorial spindle surface about 20 minutes before the position of the spindle is determined. The geometrical relations were such that the linear elements of the asters could not have been affecting the experimental surface region. In all cases furrows formed in surfaces adjacent to the asters and their time of appearance usually anticipated those located in the surface at the normal distance from the mitotic apparatus.

The spindle of cleaving eggs appears to be capable of furrow establishment even though its geometrical relation to the surface may prevent its participation in the normal process. In tissue cells on the other hand where the spindle is relatively large and close to the surface and the asters are small, the spindle may be primarily responsible for furrow establishment.

This investigation was supported by National Science Foundation Grant GB14743X3.

1972 #40

WATER AND IONIC MOVEMENTS ACROSS THE ISOLATED URINARY BLADDER OF THE WINTER FLOUNDER *Pseudopleuronectes americanus*

J. Larry Renfro, The Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

The urinary bladder of the teleost fish *Lophius americanus* was shown by Murdaugh, et al. (J. Clin. Invest. 42:959, 1963), to have the ability to alter the composition of the urine in as much as inulin and bicarbonate ions appeared to move through the bladder wall. Lahlou (Comp. Biochem. Physiol. 20:925-938, 1967) reported that the ureteral urine of the flounder *Platichthys flesus* was distinctly different from urine retained for a time inside the bladder. He stated that the bladder completed the work of the kidney by continuing to reabsorb Na^+ and Cl^- as a hyperosmotic solution resulting in dilution of the bladder urine. Lahlou and Fossat (C.R. Acad. Sc. Paris 273:2108-2110, 1971) found that an *in vitro* preparation of the freshwater trout bladder *Salmo irideus* reabsorbed a hyperosmotic sodium chloride solution, producing dilution of the mucosal fluid. Johnson,

et al. (Gen. Comp. Endocrin. 19:115-128, 1972), described sodium and water movement in a flounder urinary bladder *in vitro* *Platichthys stellatus* . They showed that more water was reabsorbed by the bladder from seawater-acclimated fish than from freshwater-acclimated fish, and presumed that Na^+ was transported actively.

The urinary bladder of teleosts is of mesodermal origin and its handling of salts and water may reflect similar mechanisms operating in some segments of the kidney tubules. The present study was an attempt to characterize the mechanisms operative in the teleost urinary bladder.

Winter flounder *Pseudopleuronectes americanus* weighing about 200 grams were maintained at the laboratory in flowing seawater (934 mOsm/Kg-water; 434 mEq Na/L; 8.8 mEq K/L; 51.5 mM Mg/L; 9.8 mM Ca/L; and 486.1 mEq Cl/L).

The unanesthetized animals were decapitated and the urinary bladder dissected free of mesentery. The ureteral end of the bladder was ligated. A flared PE 200 (Intramedic, Clay-Adams) tube was inserted through an incision in the bladder wall near the opening of the uropore. This tube was fastened into the bladder by two silk ligatures. The flared PE tube was then connected to a four-inch long glass tube which was held by a rubber stopper. This assemblage was then suspended in 100 ml of Forster's saline plus 5.5 mM/L glucose. Possible leakage was checked by distention of the bladder with Forster's saline plus Evans blue dye. The bladder was then rinsed two to four times with the ordinary Forster's solution and finally filled with the same solution. The serosal medium was continually aerated with filtered washed air. Temperature was maintained constant for this preparation at an arbitrary value of 10.4 C in a controlled temperature water bath (Fisher Isotemp).

The contents of each bladder were sampled after varying time periods (usually one to two hours) in the incubating bath. Bladders were usually discarded after eight hours however some were maintained overnight (18 to 24 hours) with only slight loss of transport ability.

In some cases after the rates of movement of water and ions across the bladder wall had been established with Forster's solution inside and outside, Forster's plus 10^{-4}M ouabain (Schwarz and Mann) was substituted for the normal medium both inside and outside the bladder.

Bladders were also filled with Forster's saline with and without 10^{-4}M ouabain and suspended in air inside moistened Erlenmeyer flasks. These flasks were submerged in the water bath at 10.4 C. The fluid which accumulated on the outside of the bladder was collected periodically (usually every 15 minutes) in capillary pipettes.

The electrical potential difference across the bladder wall was determined with double junction, silver-silver chloride electrodes (Orion Corp.) connected by 3 M KCl agar bridges to the luminal and the serosal fluids. The potential measurements could be continuously recorded with a digital millivolt meter equipped with a printer interface (Orion, Models 801 and 851).

With identical solutions inside and outside the bladder, there was a net movement of water, Na^+ and Cl^- from the mucosal to serosal side (Table 1). A small net movement of K^+ occurred in the opposite direction (Table 1). Na^+ and Cl^- were moved in approximately equal amounts and in direct proportion to the amount of water transported (Figure 1). The apparent concentration of Na^+ and Cl^- in the transported fluid averaged much more than the concentrations of these substances inside the bladder (Table 1).

Serial samples of the mucosal fluid of a single bladder over a 21-hour period showed that the quantity of K^+ inside progressively increased and the quantity of Na^+ and water progressively decreased (Figure 2). Na^+ content decreased more rapidly than water content.

TABLE 1

Net flux of water and ions across the bladder wall of the winter flounder with and without 10^{-4}M ouabain. The apparent concentrations of Na^+ and Cl^- in the transported fluid are also shown. The direction of flux is indicated as mucosal to serosal (M to S) or vice versa.

	Net Flux (μl or μEq per gm hr^{-1})		Concentration transported (mEq/1)	
	Control	Ouabain	Control	Ouabain
Water (M to S)	849.4 ± 90.5* (42)	189.8 ± 115.9 (6)	----------	----------
Sodium (M to S)	231.5 ± 27.6 (39)	1.9 ± 22.2 (6)	260.2 ± 20.7 (38)	498.3 ± 196.3 (3)
Chloride (M to S)	235.4 ± 33.7 (40)	79.7 ± 20.5 (6)	256.0 ± 32.1 (39)	819.6 ± 370.9 (5)
Potassium (5 to M)	6.12 ± 0.82 (40)	2.77 ± 1.01 (6)	----------	----------

* Mean ± standard error (n)

TABLE 2

The osmolality of the reabsorbed fluid from bladders suspended in air and filled with Forster's saline with and without 10^{-4}M ouabain is shown. The percentage decrease in osmolality of the mucosal fluid of bladders suspended in Forster's saline is also shown.

	mOsmoles/Kg-water		% decrease in mucosal fluid osmolality per hour
	Normal	Ouabain	
Reabsorbed fluid	335.7 + 12.4* (12)	279.7 + 9.7 (3)	1.95 + 0.23 (27)
Mucosal fluid	298.5 + 7.3 (12)	278.0 + 9.5 (3)	

* Mean + standard error (n)

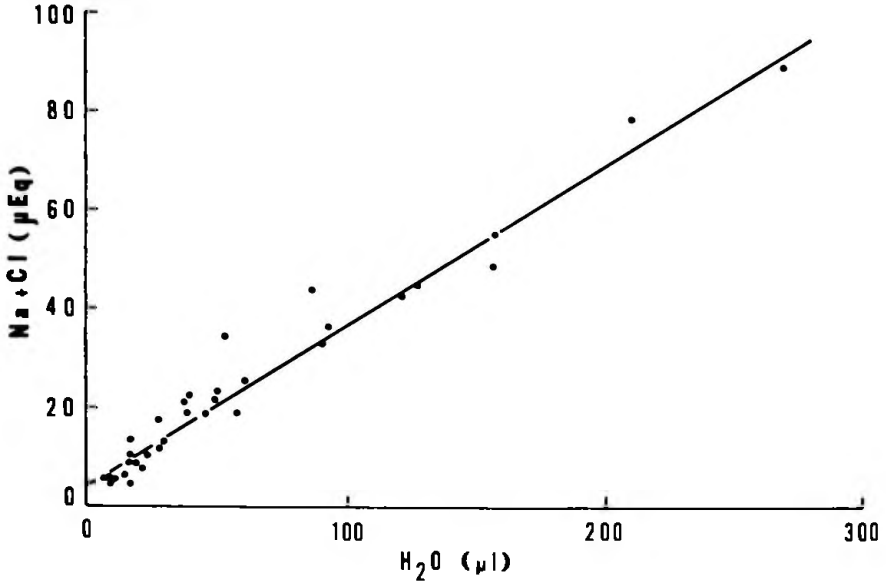

Figure 1. The relationship of the quantity of $Na^+ + Cl^-$ to the quantity of water moving out of the urinary bladder of the winter flounder in isomotic conditions. The diagonal line is the regression line.

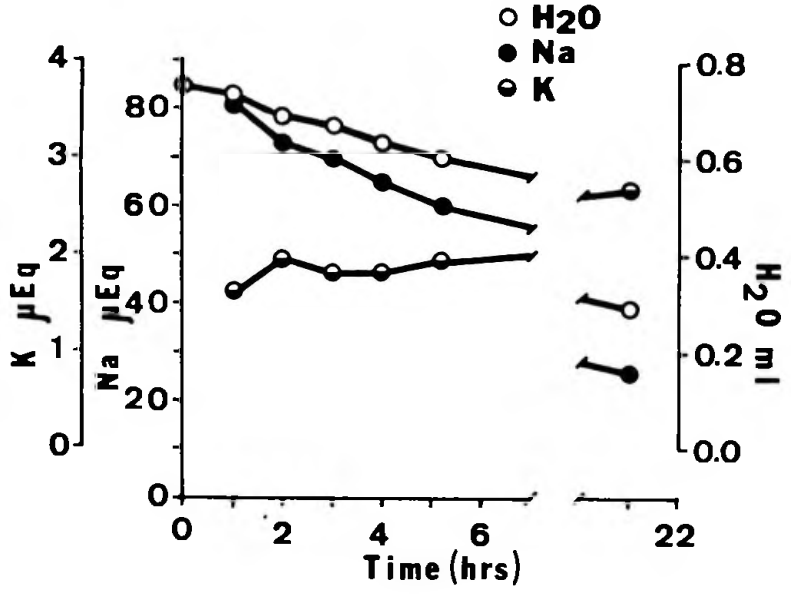

Figure 2. The changes in the absolute amounts of Na^+, K^+, and water in the mucosal fluid under isosmotic conditions are shown in relation to time in hours. Note that Na content declined more rapidly than the water content.

This apparent hyperosmotic transport was verified by direct observation of the osmotic pressure of the fluid transported by bladders suspended in air (Table 2). The osmotic pressure of this reabsorbed fluid averaged about 35 mOsmoles higher than the mucosal fluid. Routine measurements of the osmolality of the mucosal fluid of bladders suspended in Forster's saline consistently showed osmotic dilution of this fluid (Table 2).

The electrical potential difference measured across the urinary bladder wall with Forster's solution on both sides of the epithelium averaged 23.4 $\pm$ 4.8 (S.E.) (n=9) mV inside negative (range 10.4 - 53.1 mV). The time course for the development of the equilibrium potential and the maximum potential developed by each bladder were variable. The expected transepithelial electrical potential difference for passive distribution of ions under the conditions stated was of course zero. The same basic pattern was shown by all bladders with respect to the development of the equilibrium potential difference. Immediately after introduction of the mucosal fluid the P.D. was a few millivolts positive inside. After a variable time period the mucosal side became more negative with respect to the serosal side. Contractions of the bladder caused the mucosal side to become electrically more positive. Addition of 0.1 gm NaCN/1 to the external bath completely abolished the P.D.

The cardiac glycoside ouabain at a concentration of 10^{-4}M, greatly reduced the net flux of water and ions across the bladder wall (Table 1). Na^+ flux appeared to be more affected than Cl flux. K^+ secretion was also reduced. The presence of 10^{-4}M ouabain in the mucosal fluid of bladders suspended in air prevented hyperosmotic transport (Table 2). A very small amount of fluid was collected from the serosal side of these bladders in the presence of ouabain and found to be isosmotic to the mucosal fluid however this serosal fluid may have appeared because of hydrostatic pressure exerted by the fluid inside the suspended bladders.

The apparent concentrations of Na^+ and Cl^- in the transported fluid of the bladders treated with ouabain were extremely high (Table 1). However because of the greatly reduced flux in the presence of ouabain the error involved in determining these concentrations may be quite large and these values may be erroneous.

In the present study no chemical gradient existed to promote the movement of Na^+, Cl^-, K^+ or water. Ouabain sensitivity of transport indicated dependence on ATPase. Measurements of electrical potential difference across the bladder wall with the same solution inside and outside showed that a substantial electrical potential, negative inside, was maintained. Sodium is thus moving against an electrochemical potential and may be presumed to be actively transported. K^+ and Cl^- were not moving against the electrochemical gradient.

1972 #41

EFFECTS OF MERCURIALS ON SODIUM METABOLISM IN *Fundulus heteroclitus*

J. Larry Renfro, Dale Benos, and Bodil Schmidt-Nielsen. Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

Ample evidence exists to indicate that mercurials can alter the osmoregulatory ability of an organism. The primary evidence for this derives from the effect of mercurial diuretics on kidney