doorbell buzzer mounted on the manipulator. By controlling the amplitude and flexibility of the needle, cytoplasmic flow along the needle could be induced. Astral fibers extending to the underside of the equatorial surface remained intact and division was normal.

These experiments demonstrate a hitherto unsuspected durability of the stimulus mechanism and of the linear elements of the mitotic apparatus. Furrow establishment apparently can occur under circumstances that would discourage maintenance of sharply defined diffusion gradients.

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SOME CHARACTERISTICS OF ION TRANSPORT PROCESSES IN THE URINARY BLADDER OF THE WINTER FLOUNDER *Pseudopleuronectes americanus*

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The *in vitro* preparation of the distal expansion of the fused archinephric ducts (urinary bladder) of the winter flounder *Pseudopleuronectes americanus* has been shown to transport Na, Cl, and water from the mucosal to the serosal fluid (Renfro, J.L., Bull. MDIBL 12, 81, 1972). These processes were found to be ouabain sensitive at a concentration of 10^{-4}M. The present study is a continuation of our effort to characterize the role of the urinary bladder in teleost salt and water balance.

The maintenance of the live animals, removal and maintenance of the urinary bladder, and the sac-type preparation used in a portion of these studies has been described previously (Renfro, *op. cit.*). An additional method involving continuous perfusion of the isolated bladder was employed to maintain a constant transepithelial electrical potential difference (P.D.). PE 200 tubing was inserted into each end of the tube-like bladder and fastened with silk suture. This preparation was then suspended in a 10 ml polystyrene tube and sealed at the bottom end. Perfusion fluid was delivered by gravity feed at a regulated flow rate. The incubation medium was Forster's saline (J. Cell Comp. Physiol., 51, 259-1958) modified by omission of the biphosphate-bicarbonate buffer system and substitution of 3 mM Imidazole (pH = 7.8). The external bath volume was exactly 4 ml of aerated solution maintained at a constant temperature of 10°C. Unidirectional fluxes were performed sequentially rather than simultaneously with ^{22}Na and ^{36}Cl. The external bath was renewed frequently to avoid back diffusion of isotope.

P.D. was determined with double junction Ag-$AgCl_2$ reference electrodes (Orion Corp.) as previously described (Renfro, *op. cit.*). Voltage clamping electrodes were Ag-$AgCl_2$ wires connected to the mucosal and serosal fluids by PE 60 tubing filled with three percent agar-3M KCl. The voltage supply was a Bioelectrics NF-1 amplifier.

Unidirectional fluxes across perfused bladders in open-circuited conditions with identical solutions on the two sides showed that movement of Na^+ and Cl^- from the bath (serosa) to the lumen (mucosa) of the bladder was about one-sixth of the reverse flux (Table 1).

The range of potentials among bladders was -0.9 to + 22.6 mV with reference to the mucosal side; however the P.D. was constant for each individual bladder.

Unidirection fluxes of Na^+ and Cl^- in short-circuited conditions with the same solution inside

TABLE 1

Unidirectional fluxes of Na^+ and Cl^- in the isolated, perfused urinary bladder of *Pseudopleuronectes americanus*.

	Na^+ ($\mu Eq/cm^2 \cdot hr^{-1}$)			Cl^- ($\mu Eq/cm^2 \cdot hr^{-1}$)		
P.D., mV	J_{SM}	J_{MS}	J_{net}	J_{SM}	J_{MS}	J_{net}
+4.4	0.812	5.320	4.505	1.153	6.156	4.993
±1.7 (15)	±0.168 (14)	±0.871 (14)	±0.764 (14)	±0.311 (6)	±1.119 (6)	±1.037 (6)

All values are mean ± standard error (n). The bladders were open-circuited with identical solutions on the two sides. The sign of P.D. is given with respect to the mucosal side. J_{SM} = serosal to mucosal flux; J_{MS} = mucosal to serosal flux.

TABLE 2

Estimation of the epithelial active transport potentials (E_{ion}) for Na^+ and Cl^-.
(Ussing and Zerahn, Acta Physiol. Scand., 23, 111, 1951)

Na ($\mu Eq/cm^2 \cdot hr^{-1}$)			Cl ($\mu Eq/cm^2 \cdot hr^{-1}$)		
J_{SM}	J_{MS}	E_{Na} mV	J_{SM}	J_{MS}	E_{Cl} mV
0.586	3.099	-40.7	1.637	5.406	+29.2
1.397	8.343	-43.6	2.459	9.899	+33.9
0.061	0.905	-65.8	0.967	6.595	+46.9
0.609	3.095	-39.7	0.571	2.500	+36.0
			1.390	11.604	+51.8
			1.051	7.010	+46.3
			0.543	8.008	+65.7
Mean ± S.E.M. (n)	47.5 ± 6.2 (4)			44.3 ± 4.7 (7)	

The unidirectional fluxes were obtained on short-circuited bladders with identical solutions on the two sides. Na^+ and Cl^- fluxes were measured on separate bladders. The sign of E_{ion} refers to the mucosal side. J_{SM} = serosal to mucosal flux; J_{MS} = mucosal to serosal flux.

TABLE 3

Effect of voltage clamping on unidirectional fluxes of Na^+ and Cl^- through the isolated, perfused urinary bladder of *Pseudopleuronectes americanus*

Imposed P.D. mV	Ion	$J_{SM}/J_{SM(OC)}$	$J_{MS}/J_{MS(OC)}$
0.0	Na	1.086 ± 0.222 (5)	0.949 ± 0.190 (5)
	Cl	0.995 ± 0.085 (7)	1.011 ± 0.128 (7)
+50.0	Na	1.069 ± 0.195 (4)	0.933 ± 0.158 (6)
	Cl	1.101 ± 0.055 (7)	0.772 ± 0.056 (7)
-50.0	Na	1.140 ± 0.129 (4)	0.822 ± 0.093 (6)
	Cl	0.986 ± 0.124 (6)	0.821 ± 0.122 (7)

All values are mean ± standard error (n). The P.D. (transepithelial potential difference) was clamped at the indicated voltage for 15 minute periods during which the fluxes were measured. The fluxes are expressed as a ratio with the open-circuited (OC) flux determined before and after each clampling period. J_{SM} = flux from serosa to mucosa. J_{MS} = flux from mucosa to serosa.

TABLE 4

Net flux across the isolated bladder wall of water, monovalent, and divalent ions with a 57 mM Mg concentration inside and Forster's saline outside. Data obtained on sac-type preparations.

		J_{net} µl or uM/$cm^2 \cdot hr^{-1}$	%Δ C_i/hr
water	(M to S)	9.107 ± 1.925 (7)	----
Na	(M to S)	1.413 ± 0.332 (7)	-10.40 ± 3.03
Cl	(M to S)	1.342 ± 0.254 (7)	+ 0.43 ± 0.76
K	(S to M)	0.034 ± 0.014 (7)	+13.91 ± 4.10
Mg	(M to S)	0.115 ± 0.019 (5)	+ 6.89 ± 1.44
Ca	(M to S)	0.002 ± 0.002 (5)	+ 6.89 ± 2.44

All values are mean ± standard error (n). Fluxes except for potassium were mucosal (M) to serosal (S). The percentage change in mucosal fluid concentration (%Δ C_i/hr) is indicated as increased (+) or decreased (-).

and outside enabled calculations of the epithelial E.M.F. (E_{ion}) (Ussing, H.H., and K. Zerahn, Acta Physiol. Scand., 23, 111, 1951) for these two ions from the relation: $E_{ion} = \frac{RT}{zF} \ln \frac{J_{MS}}{J_{SM}}$. R is the universal gas constant; T is absolute temperature (283 K.); z is the valance of the ion; F is Faraday's number; J_{MS} and J_{SM} are the fluxes from lumen to bath and bath to lumen, respectively. It is seen in Table 2 that if Na^+ were transported alone one could predict a P.D. of about 40 mV mucosal side negative. If Cl^- were transported alone a P.D. of about 40 mV mucosal side positive is predicted. The average observed P.D. was about 4 mV mucosal side positive. Voltage clamping at either 0, +50 or -50 mV had no effect on unidirectional fluxes of either Na^+ or Cl^- (Table 3). These data may indicate that Na^+ and Cl^- pass through the epithelial membranes predominately as a neutral complex. Diamond (J. Physiol., London, 161, 474, 1962) explained a similar phenomenon in the gall bladder of a teleost by assuming that Na^+ and Cl^- pumps were coupled.

Water reabsorption from the bladder was shown to be closely correlated with the active NaCl reabsorption (Renfro, *op. cit.*). One probable function of this reabsorption may be seen in Table 4. When the Mg concentration of the mucosal fluid was raised to 57 mM (normal marine teleost urine contains 50-200 mM Mg) the effect of salt and water reabsorption was to increase the concentration of divalent ions in the mucosal fluid. Since divalent ions are the primary excretory products of marine teleostean kidneys the ability of the bladder to increase their concentration in the urine would be a significant asset to the water economy of the fish.

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AVIAN SALT GLAND: INTRA- AND EXTRACELLULAR ION CONCENTRATIONS IN SECRETING AND IN INACTIVE GLANDS

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The salt gland of the herring gull *Larus argentatus* secretes a fluid with Na and Cl concentrations from 600 to 800 mM and osmolalities from 1200 to 1600 mOs. Apparently the secreted fluid is produced by tubules which open into a central canal (Fänge, Schmidt-Nielsen and Osaki, Biol. Bull., 115, 162-170, 1958). The cells of the secretory tubules of ducklings raised on sea water (Ernst and Ellis, Cell Biol., 40, 305-321, 1969) are highly specialized with deeply-folded lateral and basal surfaces forming complex extracellular spaces. Active sodium transport involving Na-K-ATPase is indicated by the finding that the duct of the secreting gland is electrically positive to the blood and that ouabain injected retrograde into the duct inhibits secretion (Thesleff and Schmidt-Nielsen, Am. J. Physiol., 202, 597-600, 1962). Furthermore Ernst et al. (Biochim. Biophys. Acta, 135, 682-692, 1967) found a direct relationship between the previous osmotic stress of ducklings and the Na-K-ATPase activity in the salt gland. The findings that ouabain inhibits from the luminal side have been interpreted to mean that the transport of sodium takes place across the luminal surface of the cells.