Hepatic microsomal B-GT activity was determined as previously reported (Roy Chowdhury, J. et al., Comp. Biochem. Physiol. 66B:523-528, 1980) and PNP-GT was assayed by a radioassay (Tukey, R., et al, Biochem. J. 171: 659-663, 1978). Specific B-GT activity in the fetal and adult liver microsomes were similar ($P > 0.2$). Specific PNP-GT activity was slightly greater in the fetal microsomes, but the difference was not statistically significant ($P > 0.2$) (Table 2).

Table II. UDP-glucuronyl transferase activities in adult and fetal hepatic microsomes

	Bilirubin-UDP-glucuronyl transferase (nmol/mg protein.20 min)	p-nitrophenol-UDP-glucuronyl transferase (nmol/mg protein.15 min)
Adult	2.5 ± 0.8	25.7 ± 2.2
Fetal	2.0 ± 0.4	34.4 ± 3.5

Data represent means of 6 assays ± SEM.

The results indicate that, unlike the findings in the Rhesus monkey and man, isomeric composition of bilirubin in dogfish fetal bile and meconium resemble those in the adult bile. This is consistent with the finding of adult levels of PNP-GT and B-GT activity in the fetal hepatic microsomes.

NEUTRAL NaCl ABSORPTION BY FLOUNDER URINARY BLADDER

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The urinary bladder of the winter flounder absorbs NaCl by a process independent of electrical activity across the epithelium (Renfro, Am. J. Physiol. 228:52-61, 1975; Renfro, J. Exp. Zool. 199:383-390, 1978). Although in some tissues there is a measurable short circuit current, this current is entirely attributable to K^+ secretion and can be inhibited by mucosal Ba^{++} without affecting NaCl absorption (Dawson and Andrew, Bull. MDIBL 20:89-92, 1980). Among the previously documented characteristics of NaCl absorption are the interdependence of each ion on the other, the similar net absorptive rates for Na and Cl under several conditions, and the dependence on a ouabain-sensitive basolateral membrane Na-K ATPase (Renfro et al., Am. J. Physiol. 231:1735-1743, 1976). The present experiments were designed to further characterize the nature of this absorption process and to examine whether the mucosal-to-cell transport step might be, a) a neutral transport process dependent on mucosal K^+, b) a parallel Na^+-H^+, Cl^--OH^- exchanger, or c) a simple NaCl entry process.

Urinary bladders were dissected from flounder which had been maintained in tanks of flowing seawater for 1-10 days. The bladder was opened longitudinally, mounted in concentric plastic rings, and placed in an Ussing chamber as previously described by Dawson and Andrew (Bull. MDIBL 19:46-49, 1979). The tissue was short circuited and transepithelial resistance was measured by periodically (~2 minutes) passing sufficient current to clamp the voltage to 10 mV for 0.5 sec. The mucosal and serosal bathing solutions contained (mM) NaCl, 140; KCl, 2.5; $CaCl_2$ 1.5; $MgCl_2$ 1.0; Na HEPES, 7.5; HEPES, 7.5; glucose, 5.0. Both solutions were continuously gassed with room air and pH = 7.5. All tissues were exposed to 10 μM verapamil during mounting and during the flux studies. Previous experiments have shown that this concentration of verapamil reduces the spontaneous contractions of the muscular layer thus preventing spontaneous fluctuations in electrical activity while having no effect on ion transport.

The protocols were designed to measure the effect of a certain maneuver on the active Na and or Cl (M→S) flux. The passive component was estimated by measuring the M→S flux after treatment with 0.5 mM papaverine to the mucosal solution and 0.1 mM ouabain to the serosal solution. Previous measurements of unidirectional fluxes of Na and Cl have indicated that these agents reduce net transport to 0. Similar results were obtained in several experiments where S→M fluxes were compared to M→S fluxes in the presence of papaverine and ouabain.

The unidirectional fluxes were calculated after measuring the appearance of the appropriate tracer in the opposite (usually serosal) side of the chamber. Equilibration periods of at least 30 minutes were allowed after adding isotope or making a perturbation. Flux periods were 20 minutes and usually two periods were averaged for each intervention. All experiments were conducted at room temperature which averaged 21°C. Time control experiments indicated that unidirectional flux was stable for over 6 hours.

In all experiments, I_{sc} ranged from 0 to 20 $\mu A/cm^2$ and averaged ~3 $\mu A/cm^2$. Transepithelial resistance (R_T) ranged from 1500 - 5000 Ω-cm^2. In agreement with previously reported data, the I_{sc} and R_T did not correlate with the magnitude of the net Na or Cl flux.

Table 1 demonstrates the effect of raising mucosal [Cl] from < 1 mM to 6 mM to 20 mM (gluconate substituted for Cl) on mucosa-to-serosa Na flux (J^{ms}_{Na}). There was a progressive increase which was inhibited by papaverine and ouabain. Similar values were obtained when mucosal [Na] was increased from 2 mM to 8 mM to 20 mM (choline substitution with Tris buffer). These results confirm the interdependence of Na and Cl for their net transport.

TABLE 1: Effect of Mucosal [Cl] on J^{ms}_{Na} (n=7)

Mucosal [Cl]	1 mM	6 mM	20 mM	Ouabain + Papaverine
J^{ms}_{Na} ($\mu Eq/cm^2 \cdot h$)	0.59 ±0.13	1.61 ±0.33	2.29 ±0.45	0.65 ±0.09

To examine whether Na and/or Cl transport required K^+, experiments were conducted where K^+ was omitted from the mucosal solution and J^{ms}_{Na} and J^{ms}_{Cl} were measured (in separate bladders). Sequential addition of K^+ to the mucosal solution resulted in the measured [K] values reported in Table 2. Increasing bulk mucosal [K] from below detectable values to 1.1 mM produced no change in J^{ms}_{Cl} or J^{ms}_{Na} yet these fluxes were significantly inhibited by papaverine and ouabain. These results make it extremely unlikely that Na^+ and Cl^- enter the cell coupled to K^+.

Another possible mechanism for neutral NaCl entry into the cell is by parallel Na^+-H^+ and Cl^--OH^- exchangers. In other systems these exchangers have been sensitive to amiloride in high concentrations (in the presence of low Na) and the disulfonic stilbene DIDS, respectively. However, when 1.0 mM amiloride was applied to the mucosal surface when mucosal [Na] was 15 mM, J^{ms}_{Na} was not affected. Likewise, DIDS (0.1 mM) had no effect on J^{ms}_{Cl}. These experiments suggest that parallel cotransport systems do not play a role in NaCl absorption.

TABLE 2: Effect of Mucosal [K] on J_{Na}^{ms} and J_{Cl}^{ms}

Mucosal [K]	<0.05 mM	0.1 mM	0.6 mM	1.1 mM	Ouabain + Papaverine
J_{Na}^{ms} (μEq/cm^2·h)	4.05	4.18	4.35	4.24	0.58
(n = 7)	±0.41	±0.36	±0.39	±0.32	±0.11
J_{Cl}^{ms} (μEq/cm^2·h)	3.61	3.80	3.77	3.85	1.71
(n = 6)	±0.47	±0.49	±0.51	±0.66	±0.18

Furosemide has been shown to inhibit NaCl entry in several types of epithelia and Renfro has previously reported an inhibitory effect on the flounder bladder using a concentration of 1 mM. The effect of this concentration of furosemide did not uniformly produce inhibition of J_{Na}^{ms} and J_{Cl}^{ms}. J_{Na}^{ms} was reduced only in bladders where control values were greater than 3 μEq/cm^{2}h. In bladders where inhibition occurred, there was only a partial inhibition of net flux. The effect on J_{Cl}^{ms} was similar but there tended to be some inhibition even at low (<3 μEq/cm^{2}h) rates. When the effect of furosemide was examined on J_{Na}^{ms} and J_{Cl}^{ms} measured simultaneously, J_{Cl}^{ms} was inhibited to a greater extent than was J_{Na}^{ms}. The data are displayed in Table 3. The reduction in J_{Na}^{ms} was 0.70 ± 0.30 while the reduction in J_{Cl}^{ms} was 1.33 ± 0.38. Thus the reduction in J_{Cl}^{ms} was almost twice the reduction in J_{Na}^{ms} ($p < 0.01$).

TABLE 3: Effect of 1 mM Furosemide on J_{Na}^{ms} and J_{Cl}^{ms} (n=12)

	Control	Furosemide	Ouabain + Papaverine
J_{Na}^{ms} (μEq/cm^2·h)	3.52	2.82[a]	0.63[c]
	±0.56	±0.30	±0.06
J_{Cl}^{ms} (μEq/cm^2·h)	4.49	3.16[b]	1.38[c]
	±0.70	±0.34	0.08

[a] $p < 0.05$ compared to control; [b] $p < 0.005$ compared to control; [c] $p < 0.001$ compared to furosemide.

These data, when considered together, provide evidence that Na and Cl absorption is a coupled process which occurs by an electroneutral mechanism. Evidently it does not occur via a parallel exchange system nor does it need mucosal K^+ to operate optimally. Although furosemide does not inhibit net Na and Cl absorption completely, it does reduce the absorptive flux when control values are high. The net rates of Na and Cl absorption are not different but furosemide reduces J_{Cl}^{ms} to a significantly greater degree than it does J_{Na}^{ms}. Although the changes in unidirectional flux were differentially reduced by furosemide, there was no evidence of any change in the electrical properties of the epithelium. Thus the most likely explanation for this differential effect is an effect on both the neutral NaCl transport system and a parallel effect on a Cl-Cl exchange system. This work was supported by a grant from the National Institutes of Health AM 25231. We appreciate the advice and assistance of David Andrew and David Dawson.

FURROWING ACTIVITY IN ENDOPLASMIC FRAGMENTS OF THE SAND DOLLAR EGG

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The physical mechanism which accomplishes cytokinesis is localized anew in each cell cycle in the equatorial surface as a consequence of interaction between the surface and the mitotic apparatus. In order to learn more of the organizational factors involved in the interaction, the original egg surface was drastically disrupted and the consequent