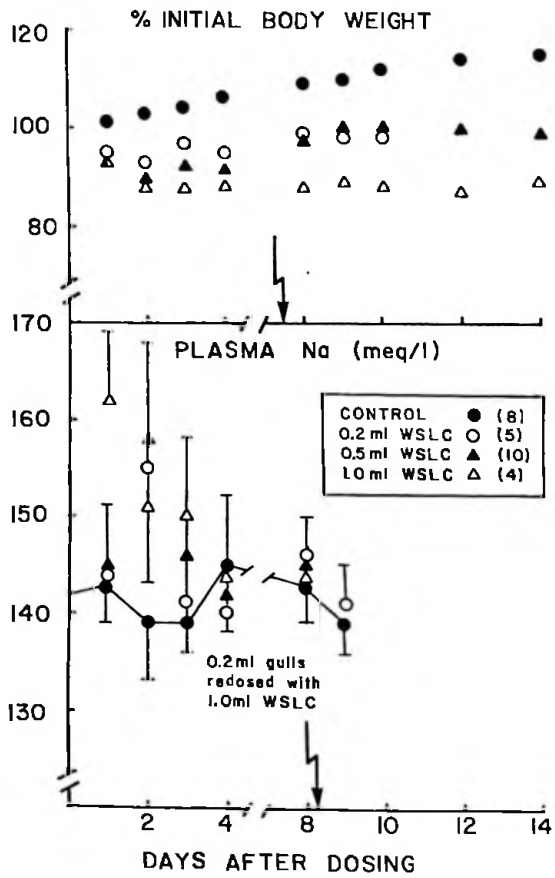

Figure 3. Effect of a single oral dose of WSLC on body weight and plasma Na concentrations in immature herring gulls maintained on sea water. Data expressed as mean ± SE with the number of birds in parentheses. Experimental body weights were significantly lower than controls ($P < 0.05$) from day 1 on. Plasma Na levels for birds dosed with 1.0 WSLC were significantly higher than controls on day 1.

INHIBITION OF CHLORIDE TRANSPORT IN FLOUNDER INTESTINE BY FUROSEMIDE AND CYCLIC AMP

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We previously showed that net Na and Cl fluxed across short-circuited flounder intestine are coupled, net Cl absorption ceasing in the absence of Na and vice versa (Field, Karnaky, Smith, Bolton and Kinter, J. Membrane Biol., in press; Field et al. MDIBL Bull. 1975). Furthermore, net Cl absorption is inhibited by ouabain suggesting that Na,K-activated ATPase provides the driving force for this transport. Similar observations have been made with rabbit ileum (Nellans et al. Am. J. Physiol. 226:1131, 1974) and gallbladder (Frizzell et al. J. Gen. Physiol. 65:79, 1975). In those two tissues, the coupling of net transepithelial fluxes of Na and Cl has been shown to originate with a 1:1 coupled influx across the brush border (luminal) membrane. Elsewhere in this bulletin Frizzell, Smith and Field show that Cl influx across the brush border of flounder intestine is also coupled to that of Na and that the coupled flux can be inhibited by increasing intracellular 3',5'-AMP (cAMP) or by adding furosemide to the mucosal medium. To establish the role of this brush border mechanism in transepithelial ion transport, changes in Cl and Na influxes must be related to changes in their transepithelial fluxes. The present study examines the effec of furosemide on transepithelial Cl fluxes and extends our prior observations on the effects of cAMP.

Furosemide: Cl fluxes were measured before and after adding furosemide (1 μmol/ml) to the mucosal medium. As shown in Table 1, J^{Cl}_{net} and I_{sc} were considerably smaller following furosemide. These changes can be attributed to the drug since, under control conditions, Cl fluxes remain constant for at least 3 h

TABLE 1

Effect of furosemide on Cl fluxes across flounder intestine

	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}	I_{sc}	G
Control period	5.8 ± .44	1.8 ± .30	4.0 ± .34	-2.4 ± .36	23.6 ± 1.0
+ furosemide, 1 mM	3.2 ± .23*	1.7 ± .27	1.5 ± .33*	-0.6 ± .13*	22.5 ± 1.1*

Results are means ± 1 SEM for 7 experiments on tissues from 4 flounder. Units for Cl fluxes and short-circuit current (I_{sc}) are μEq/h cm^2 and for conductance (G), mmhos/cm^2. Control fluxes were measured over 30 min beginning about 70 min after mounting tissues. Furosemide was then added to the mucosal medium and fluxes were again measured from 30 to 70 min thereafter. All tissues were stripped of muscle before mounting. The Ringer solution employed was the "high-HCO_3" - Ringer previously described (Field et al., MDIBL bulletin, 1975) with one modification: Cl concentration was reduced to 75 mM with equimolar amounts of SO_4 and mannitol. Bathing media were bubbled with 1 % CO_2 in O_2 and maintained at 15°.

*$p < .02$ compared to control period.

(Field et al., MDIBL Bull. 1976). It is of interest that some net Cl transport persisted despite 10^{-3} M furosemide. We do not know whether a small portion of transepithelial Cl transport is completely insensitive to furosemide or whether 10^{-3} is submaximal. The concentration of furosemide producing a half-maximal reduction in I_{sc} is between 10^{-6} and 10^{-5} M (see Frizzell et al. in this bulletin). It is also of interest that furosemide, unlike cAMP and ouabain (see below), does not increase the unidirectional serosa-to-mucosa Cl flux (J^{Cl}_{sm}). Thus inhibition of net Cl absorption is not inevitably associated with an increase in J^{Cl}_{sm}.

cAMP: We had previously shown (Field et al., MDIBL Bull. 1975, 1976) that cAMP reduces net Na and Cl absorption. We also found, however, that cAMP doubles J^{Cl}_{sm} (without affecting J^{Na}_{sm}), which raised the possibility that the nucleotide stimulates active Cl secretion in flounder intestine, as it does in mammalian intestine, and that the reduction in net Cl flux reflects a balance between continuing active absorption and the stimulated secretion. This seemed unlikely to us because the cAMP-induced increase in J^{Cl}_{sm} occurred also in the presence of ouabain, which inhibits Cl absorption in flounder intestine and which is known to inhibit Cl secretion in several other epithelia. In order to ascertain that the cAMP-induced increase in J^{Cl}_{sm} is not due to stimulation of a ouabain-insensitive active secretion, we measured Na and Cl fluxes across intestine pretreated with both ouabain and cAMP. As shown in Table 2, the net fluxes did not differ significantly from zero. Since ouabain inhibits net Cl absorption, any stimulation by cAMP of a ouabain-insensitive secretion should have resulted in net secretion of Cl. Since this clearly was not the case, the cAMP-induced increase in J^{Cl}_{sm} must reflect an increase in passive Cl permeability. The present results also confirm our prior observation (Field et al. MDIBL Bull. 1976) that ouabain itself markedly increases passive Cl permeability. The reason for this is not known, but the change is clearly specific for Cl (or anion), since J^{Na}_{sm} does not increase. This work was supported by NIH grant AM-21345.

TABLE 2

Na and Cl fluxes in the presence of ouabain, cyclic AMP and theophylline

	J_{ms}	J_{sm}	J_{net}
Na	9.6 ± .40	9.1 ± .46	0.5 ± .54
Cl	10.5 ± .63	10.3 ± .54	0.2 ± .57

Results are means ± 1 SEM for 18 experiments, 8 with tissues bathed in "low HCO_3" - Ringer and 10 with "high-HCO_3" - Ringer (see Field et al. MDIBL Bull. 1975 for composition of solutions). Results for the two groups did not differ significantly and therefore were pooled. OUabain, 0.5 µmol/ml, was added to the serosal medium 30-40 min after tissues were mounted in vitro. Thirty minutes later, theophylline, 5 µmol/ml, and cAMP, 2.5 µmol/ml, were also added to the serosal medium. Flux measurements were begun 1 h thereafter.

THE RENAL CLEARANCE OF D-MANNOSE IN THE WINTER FLOUNDER

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The clearance of D-mannose in the winter flounder, *Pseudopleuronectes americanus*, was investigated employing the technique described by Pritchard and Kleinzeller (Am. J. Physiol. 231:603-607, 1976 (Table 1). D-Mannose was readily reabsorbed (86%). No correlation was found between the percent of sugar reabsorbed and fluid reabsorption, glomerular filtration rate, or plasma concentration. Calculations were made from four to eight sequential clearance periods, representing the time taken to collect 98 µl of urine. The level of mannose in the plasma ranged from 82.84 to 41.55 nmol/ml. With plasma concentrations in excess of 90 nmol/ml, the percent of sugar reabsorbed was frequently observed to be depressed (60%).

TABLE 1

1. Clearance Data:

$C_{mannose}$ (ml/hr)	0.02 ± 0.003 (7)
C_{PEG} (ml/hr)	0.15 ± 0.02 (7)
$C_{mannose}/C_{PEG}$	0.15 ± 0.01 (7)

2. Tissue Values:

Inhibitor	t/p total mannose	t/p free mannose	t/p total mannose / t/p PEG	t/p free mannose / t/p PEG
None	1.01 ± .06 (9)	0.37 ± .01 (9)	5.77 ± .43 (9)	2.30 ± .10 (9)
Glucose	0.66 ± .04 (3)	0.35 ± .02 (3)	3.75 ± .40 (3)	1.93 ± .08 (3)
Phlorizin	1.28 ± .05 (1)	0.38 ± .04 (1)	4.85 ± .25 (1)	1.41 ± .25 (1)

Values shown are means ± SE for (n) number of fish.

The tissue to plasma ratio for free mannose was found to be larger than the corresponding ratio for PEG (Table 1). Since previous studies showed that D-mannose is not accumulated against a concentration gradient by flounder kidney tubules, it follows that D-mannose is actively taken up at the luminal face of the cell, and exits across the peritubular membrane via (carrier mediated) facilitated diffusion.

Glucose in doses of 2.5 mmol/Kg had only a transient effect, i.e., over four or less clearance periods on mannose reabsorption (Figure 1). Phlorizin in doses of 2.5 µmol/Kg showed no effect on the percent of