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

sugar reabsorbed. Before the injection of phlorizin, plasma concentration shows no correlation with the percent of sugar reabsorbed (r^2 = .05). After injection, the correlation is good (r^2 = .95). It thus seems likely that phlorizin acted solely to inhibit antiluminal transport. Neither glucose or phlorizin had any effect on the tissue accumulation of either free or phosphorylated sugar.

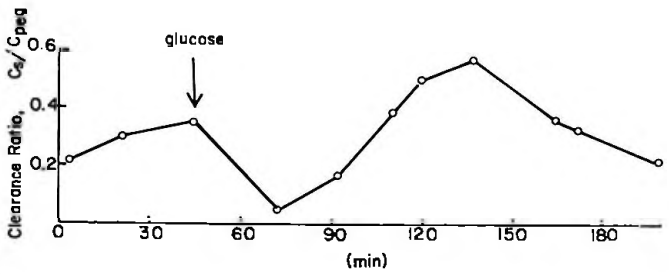

Figure 1. The effect of glucose (2.5 mmol/Kg) on D-mannose clearance. The ratio of mannose cleared to GFR(C_{PEG}) is plotted against the midpoint of each clearance period.

Silverman et al. (Am. J. Physiol. 218:743-750, 1970), have provided evidence in the dog for distinct mannose and glucose sites on the luminal membrane. The glucose carrier is phlorizin sensitive, whereas the mannose site is not. The preliminary results of our study would tend to support the possibility of a similar model for the winter flounder. This work was supported by USPHS grant ST 326M 0729-03.

ION TRANSPORT BY GALLBLADDER OF *Squalus acanthias*

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Gallbladders from a variety of species including the teleost fish (Diamond, J. M. J. Physiol. 161:474, 1962), rabbit and guinea pig (Diamond, J. M. J. Gen. Physiol. 48:1, 1964) actively absorb both sodium (Na) and chloride (Cl) in an electrically neutral manner; that is, NaCl (or more generally Na-anion) absorption does not generate a significant transepithelial electrical potential difference. Several years ago Frizzell et al. (J. Gen. Physiol. 65:769, 1974) demonstrated that neutral NaCl absorption by rabbit gallbladder is the result of a neutral NaCl entry mechanism at the mucosal membrane similar to one that had been demonstrated earlier in rabbit ileum (Nellans et al. Am. J. Physiol. 225:467, 1973). Neutral NaCl transport mechanisms have now been demonstrated in gastrointestinal epithelia from a wide variety of animals including mammals, amphibia, fish, molluscs and arthropods.

The objectives of this research program were to initiate studies of ion transport by the gallbladder of *Squalus acanthias* and, in collaboration with Dr. William Armstrong, attempt to relate transepithelial ion transport to intracellular ionic activities determined using ion-selective microelectrodes.

Methods

Squalus acanthias were killed by severing the spine at several locations. The abdomen was opened and the gallbladder was detached from the liver by blunt dissection; care was taken to prevent rupture of the gallbladder and an outpouring of bile. The sac was then opened, rinsed free of bile and the muscle layers were stripped off using fine optical forceps. Segments of tissue were mounted as flat sheets in a modified Ussing apparatus (Schultz, S. G. and Zalusky J. Gen. Physiol. 47:567, 1964) and bathed on both surfaces with shark Ringer (Rosa et al. MDIBL Bull. 16:87, 1976) maintained at 15 C. All experiments were carried out under short circuit conditions and the short-circuit current (I_{sc}), transepithelial electrical potential difference (ψ_{ms}) and tissue resistance (R_t) were recorded at 10 min intervals.