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.

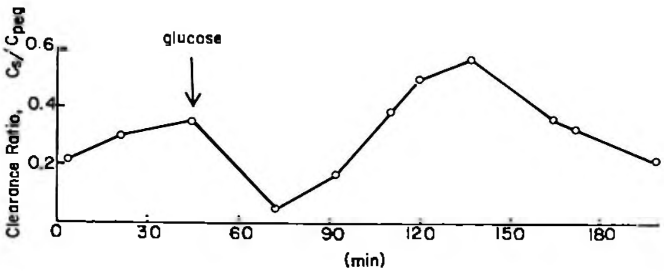

Figure 1. The effect of glucose (2.5 mmol/Kg) on D-mannose clearance. The ratio of mannose cleared to GFR(C_{PEG}) is plotted against the midpoint of each clearance period.

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.

Bidirectional fluxes of Na and Cl were determined using Na^{22} and Cl^{36}. Na-free media were prepared by replacement of Na with choline and Cl-free media were prepared by replacement of Cl with SO_4 with mannil employed to maintain isotonicity.

Results

The results of paired studies (a) under control conditions, (b) when the tissue was bathed by the Na-f media, and (c) when tissues were bathed by the Cl-free media are summarized in Table 1. The important poi

Table I

Condition	n	ψ_{ms} (mV)	R_t (ohm.cm^2)	I_{sc}	J^{Na}_{ms}	J^{Na}_{sm}	J^{Na}_{net} (μEq/cm^{2}hr)	J^{Cl}_{ms}	J^{Cl}_{sm}	J^{Cl}_{net}
Control	8	3.2	85	1.4	10.9	8.4	2.5	3.6	2.0	1.6
		±0.3	±4	±0.1	±0.9	±1.0	±0.7	±0.3	±0.2	±0.1
Control	5	3.3	104	1.3	-	-	-	4.6	2.6	2.0
		+0.4	+6	±0.2				±1.4	±1.3	±0.1
Na-Free	5	0.9*	695*	0.04*	-	-	-	4.0	2.6	1.4*
		±0.2	±43	±0.02				±0.5	±0.4	±0.2
Control	5	2.9	103	1.1	8.6	6.2	2.4	-	-	-
		±0.3	±10	±0.2	±0.8	±0.6	±1.0			
Cl-Free	9	2.3	184*	0.6	9.6	7.6	2.0	-	-	-
		±0.2	±15	±0.1	±1.8	±1.5	±1.0			

J_{ms} is the unidirectional flux from mucosa to serosa; J_{sm} is the flux from serosa to mucosa; $J_{net} = J_{ms} - J_{sm}$

n = number of tissues studied; all errors are S.E.M.; (*) significant difference from paired control, $p<0.05$.

to be noted are: (1) both Na and Cl are actively absorbed under short circuit conditions; (2) in the absence of Na, active Cl absorption is slightly reduced principally as a result of a decrease in the muco to-serosa unidirectional flux; (3) replacement of Na with choline essentially abolishes the ψ_{ms} and I_{sc} a brings about a marked increase in the R_t; (4) in the absence of Cl the rate of active Na absorption appea to be slightly reduced; and (5) replacement of Cl with SO_4 results in an 80% increase in the R_t.

Finally preliminary studies indicate that bidirectional and net fluxes of Na and Cl are not affected when HCO_3 is omitted from the bathing media; the ψ_{ms} and I_{sc} are not affected by cAMP (7.5 mM); and, Cl fluxes are not affected by 0.1 mM methazolamide.

Conclusions

The results of these studies are too preliminary to permit many firm conclusions. Nevertheless certa features of ion transport by gallbladder of *Squalus acanthias* have already emerged clearly.

First, this epithelium is distinctly different from gallbladders that have been described for a wide variety of species including the teleost fish, inasmuch as Na and Cl absorption are for the most part uncoupled. Active Na absorption is only minimally affected in a Cl-free medium and appears to be respons for the transepithelial electrical potential difference and short-circuit current. Active Cl absorption is mediated by one or more neutral processes but is only minimally affected by the removal of Na from the bathing media. A preliminary "model" consistent with our findings is illustrated in Figure 1 and is proposed simply to serve as a guide for more detailed studies in the future.

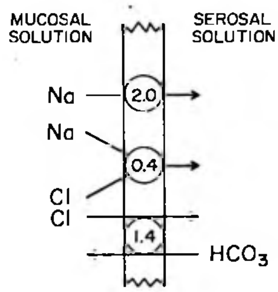

Figure 1

Second, the gallbladder of *Squalus acanthias* is a relatively leaky epithelium (like gallbladders from a variety of other species) and the paracellular conductance is predominantly due to Na. Thus replacement of Na in both bathing media with choline resulted in a 6-fold increase in tissue resistance whereas replacement of Cl in both bathing media with sulfate resulted in only a twofold increase in R_t. This notion is further supported by the fact that the serosa-to-mucosa fluxes of Na are 2-3 times greater than those of Cl. Thus it appears as if the paracellular shunt pathway of this epithelium sharply prefers Na over Cl, a feature that is relatively uncommon in leaky epithelia.

Finally, the results of our preliminary studies indicating that ion transport across this epithelium is not affected by amiloride, cAMP or a very potent carbonic anhydrase inhibitor strongly suggests that the mechanisms responsible for Na and Cl transport warrant further investigation.

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PARTIALLY INDUCED HEPATIC MICROSOMAL MIXED-FUNCTION OXIDASE SYSTEMS IN INDIVIDUAL WINTER FLOUNDER, *Pseudopleuronectes americanus*, FROM COASTAL MAINE

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The induction of hepatic microsomal benzo(a)pyrene hydroxylase (AHH) activity in fish has been suggested as a biochemical monitor for petroleum pollution in the marine environment (Payne, Science 191:945, 1976). However, as chemicals other than polycyclic aromatic hydrocarbons (PAH), including certain halogenated dioxin and biphenyl isomers, occur as aquatic pollutants and also dramatically induce AHH activities in various species of fish (Bend and James, Biochem. Biophys. Perspect. Marine Biol. 4: in press) and since hepatic AHH of some marine species, such as the Atlantic stingray (*Dasyatis sabina*), is not increased by repeated PAH administration (Bend, James, and Dansette, Ann. N. Y. Acad. Sci. 298:505, 1977), caution must be exercised in correlating elevated hepatic AHH activities with pollution by petroleum products.

In fish, as in mammals, dramatic increases in hepatic microsomal AHH activities appear to be related to the formation of cytochrome P-448, although not enough of this new form of the hemoprotein is present to cause