TABLE 1

Electrical properties and chloride fluxes of opercular epithelia of *Fundulus heteroclitus* during treatment with prolactin

	Cl efflux $\mu Eq.h^{-1}.cm^{-2}$	Cl influx $\mu Eq.h^{-1}.cm^{-2}$	Pd mV	R $\Omega.cm^2$
Seawater control	6.4 ± 0.98* (n = 5)	1.1 ± 0.21 (n = 6)	10.7 ± 1.57 (n = 11)	87 ± 10 (n = 11)
Seawater + prolactin	1.2 ± 0.30 (n = 5)	1.7 ± 0.31 (n = 6)	2.0 ± 0.30 (n = 11)	135 ± 14 (n = 5)
Fresh water adapted	0.95 ± 0.28 (n = 6)	-	0.9 ± 0.3 (n = 5)	296 ± 81 (n = 4)

*Mean ± S.E.M. between brackets: Number of experiments.

the expense of the unidirectional flux in the direction serosa to mucosa. The value of this flux under prolactin treatment is comparable to the one obtained in fresh water adapted fish.

Changes in resistance of the operculum produced by prolactin could be related to the tightness of the junctions between the different cells constituting the epithelium (P. Claude, J. Memb. Biol. 39: 2-9, 1978). Leaky junctions have been observed by Sardet (personal communication) and Bentley, Sardet and Mayer Gostan (personal communication) in neighboring sister cells of the "chloride cells" that have many interdigitating arms on their apical surface. Prolactin could then act on these junctions. Prolactin appears to be the main hormone responsible for the crucial decrease in ion movements which accompany the freshwater adaptation. This work was supported by NIH Research Grants EY 01340 and GM 25002.

SUGAR TRANSPORT ACROSS THE INTESTINE OF WINTER FLOUNDER

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Previous studies have shown that no net absorption of D-glucose takes place in the intestine of winter flounder (*Pseudopleuronectes americanus*), although L- and D-tyrosine and L-tryptophan are actively absorbed by this tissue (Rout, W.R., Lin, D.S.T. and Huang, K.C., Proc. Soc. Exptl. Biol. Med. 118:935, 1965). The absence of a nearly ubiquitous transport process from the intestine of the winter flounder aroused our curiosity, and led us to study this phenomenon further. Renal clearance studies of D-galactose in winter flounder show that this sugar is poorly absorbed from the renal tubules, whilst the high clearance ratio of 2-deoxy-D-galactose, indicates that this sugar is actively secreted into the tubule lumen (Pritchard, J.B., Booz, G. and Kleinzeller, A., Amer. J. Physiol. 234(5):F424, 1978). Since there is usually close similarity between the sugar transport processes in the kidney and intestine, it was considered worthwhile to investigate the transport of 2-deoxy-D-galactose and of galactose by the intestine of winter flounder in order to characterize the secretory process and the absent absorptive process.

Winter flounders weighing 250-350 g were obtained by trawling off Mt. Desert Island during July and August and were held in flowing aerated seawater. After the intestine was removed from a freshly killed fish, the serosa and external muscle layers were removed by a simple stripping procedure. The

TABLE 1

Fluxes of D-galactose across the intestinal epithelium of the winter flounder. Transepithelial fluxes of D-galactose (1 mM) and the accumulation ratio of total and free sugar in tissue water. Mean values ± S.E. are given; the number of measurements, n, is shown in parentheses.

	J_{ms}	J_{sm}	J_{net}	Sugar cellular accumulation nmole sugar/g tissue water	
	nmoles.cm^{-2}h^{-1}			total	free
Control	51 ± 5 (10)	38 ± 6 (10)	13 ± 8 (10)	1.13 ± 0.14 (10)	0.67 ± 0.04 (5)
Mucosal phlorizin (0.1 mM)	60 ± 6 (9)	83 ± 7 (9)	-23 ± 5 (9)	0.70 ± 0.07 (9)	0.50 ± 0.05 (3)

TABLE 2

Transepithelial fluxes of 2-deoxy-D-galactose in the flounder intestine. The transepithelial fluxes of 2-deoxy-D-galactose (1 mM) and the accumulation ratio of the total and free sugar in tissue water are shown. Mean values ± S.E. are given, the amount of measurements in parentheses.

	J_{ms}	J_{sm}	J_{net}	Sugar cellular accumulation nmole sugar/g tissue water	
	nmoles.cm^{-2}h^{-1}			total	free
Control	54 ± 6 (11)	178 ± 5 (11)	-124 ± 8 (11)	2.4 ± 0.26 (11)	0.76 ± 0.06 (6)
Ouabain (0.1 mM) in serosal solution	56 ± 6 (6)	65 ± 6 (6)	- 9 ± 8 (6)	1.65 ± 0.04 (6)	0.74 ± 0.03 (5)
Choline Ringer (both sides)	56 ± 16 (3)	76 ± 11 (3)	- 20 ± 9 (3)	1.65 ± 0.14 (3)	-

TABLE 3

Effects of modifiers on 2-deoxy-D-galactose transport across the winter flounder intestine. Concentration of 2-deoxy-D-galactose 0.1 mM. For all other details, see legend to Table 2.

	J_{ms}	J_{sm}	J_{net}	Sugar cellular accumulation nmole sugar/g tissue water	
	nmoles.cm^{-2}h^{-1}			total	free
Control	3.0 ± 0.2 (5)	12.0 ± 0.6 (5)	-9.0 ± 0.7 (5)	2.3 ± 0.2 (33)	0.81 ± 0.04 (33)
Phloretin, 0.1 mM (both sides)	7.2 ± 0.7 (5)	7.4 ± 0.4 (5)	-0.2 ± 1.0 (5)	1.3 ± 0.1 (5)	0.56 ± 0.04 (5)
Phloretin, 0.1 mM (serosal side)	3.4 ± 0.4 (3)	3.7 ± 0.2 (3)	-0.3 ± 0.3 (3)	2.3 ± 0.3 (3)	-
Phloretin, 0.1 mM (mucosal side)	3.4 ± 0.2 (3)	11.4 ± 0.8 (3)	-8.0 ± 1.0 (3)	2.5 ± 0.2	-
Galactose, 10 mM (both sides)	5.7 ± 0.5 (5)	5.6 ± 0.5 (5)	0.1 ± 0.5 (5)	1.1 ± 0.04 (4)	0.84 ± 0.05 (4)

intestine was then mounted as a flat sheet on a lucite flux chamber, which contained six identical ports for measuring transepithelial fluxes simultaneously. The area of each tissue exposed to bathing solution was 0.2 cm^2 (serosal surface). The solutions bathing the mucosal and serosal side were maintained at ambient temperature (18 ± 2°C) and were aerated and stirred by a continuous stream of 95% air + 5% CO_2. Flounder Ringer contains $NaHCO_3$ 15 mM, $KHCO_3$ 5 mM, NaCl 150 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1 mM, NaH_2PO_4 0.3 mM, Na_2HPO_4 1.65 mM, pH of aerated solution 7.3. Na-free Ringer contained choline HCO_3 (Sigma Chemicals Ltd.) 20 mM, choline Cl 150 mM, KH_2PO_4 0.3 mM, K_2HPO_4 1.65 mM, KCl 1.5 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1.0 mM. Usually, the media also contained 1 mM of the respective sugars.

Radiochemicals: ^{3}H and ^{14}C-labeled D-galactose, ^{14}C labeled α-methyl-D-glucoside and ^{14}C labeled β-methyl-D-galactoside, ^{3}H and ^{14}C labeled polyethylene glycol were all obtained from New England Nuclear Corp. ^{3}H-2-Deoxy-D-galactose was prepared and purified as described previously (Kleinzeller, A.,

TABLE 4

Transport of α-methyl-D-glucoside across the winter flounder intestine. Transepithelial and the net fluxes of α-methyl-D-glucose (1 mM and 0.1 mM) are given. The reported accumulation ratio represents the value derived for the free sugar. Means ± S.E. are presented.

	J_{ms}	J_{sm}	J_{net}	Sugar cellular accumulation
	nmoles.cm^{-2}h^{-1}			nmole/g tissue water
Control, 1 mM	44.8 ± 2.9 (6)	25.8 ± 3.0 (6)	19 ± 4.2 (6)	1.1 ± 0.06 (6)
Control, 0.1 mM	6.6 ± 0.9 (3)	2.9 ± 0.3 (3)	3.7 ± 1.1 (3)	0.73 ± 0.04 (3)
Phlorizin, 0.1 mM (mucosal side) Substrate: 0.1 mM	5.6 ± 0.5 (3)	5.3 ± 0.8 (3)	-	0.53 ± 0.02 (3)
Ouabain, 0.1 mM Substrate: 0.1 mM	4.8 ± 0.3 (3)	4.6 ± 0.4 (3)	-	0.52 ± 0.02 (3)
Galactose, 10 mM (both sides) Substrate: 0.1 mM	5.7 ± 0.3 (5)	5.6 ± 0.5 (5)	-	0.84 ± 0.05 (5)

Tam, I., Kanter, R.K. and McAvoy, E.M. (Biochim. Biophys. Acta 373:397, 1974); the ^{14}C-labeled sugar was a generous gift from Prof. D. Keppler, University of Freiburg, Germany.

Flux measurements: simultaneous bidirectional transepithelial sugar fluxes across sheets of flounder intestine were measured in a flux chamber as previously described (Naftalin, R.J. and Holman, G.D. (Biochim. Biophys. Acta 373:453, 1974). Usually 1.25 μCi ml^{-1} ^{3}H-label and 62.5 nCi ml^{-1} of ^{14}C-labeled compound were added to the mucosal and serosal bathing solutions respectively; the positions of these compounds were reversed occasionally to check for artifacts.

No difference between the fluxes of ^{14}C and ^{3}H-labeled materials was observed. At hourly intervals for two to three hours, 0.5 ml of bathing solution was removed for counting in a triton-toluene based scintillant. After incubation, the tissue was washed free of surface label for 2 min in ice cold Ringer, then the tissue was weighed, and extracted for 1 to 2 hr in distilled water and the whole extract + tissue residue counted. The tissue extracellular space was estimated using polyethylene glycol and the intracellular water was obtained by subtracting the extracellular space from the total tissue water. Intracellular water = 0.69 ± 0.04 ml water/g wet weight of tissue (n=33).

From the above data, J_{ms} (the mucosal-serosal flux), J_{sm} (the serosal-mucosal flux), as well as their difference, i.e., the net flux J_{net} were calculated. The unidirectional entry and exit fluxes of the sugars across both faces of the epithelium (Naftalin, R. and Curran, P.F. J. Memb. Biol. 16:257, 1974) were also determined. Details of these measurements are not presented here.

The total concentration of sugar, i.e., free + phosphorylated sugar =

$$\frac{\text{total amount of labeled sugar in tissue}}{\text{wet weight of tissue} \times 0.7} \text{ . mM } l^{-1} \text{ tissue water.}$$

The concentration of free sugar was estimated by precipitating the sugar phosphates from tissue extracts with $Ba(OH)_2$ and $ZnSO_4$ as described previously (Kleinzeller, A. and McAvoy, E.M. Biochim. Biophys. Acta 455:126, 1976). The tissue extracts were analyzed by paper chromatography, also as described in that paper. The major phosphorylated products are galactose-1-P, or 2-deoxy-D-galactose-1-P.

When only one form of label was available, as with α-methyl-D-glucoside and β-methyl-D-galactoside, estimates of bidirectional fluxes and accumulation ratio were measured in opposite directions in adjacent tissues. Such measurements involve no significant increase in error. The specific activity ratio was estimated from the ratio of tissue concentration of sugar coming from mucosal and serosal solutions.

Results

Bidirectional transepithelial fluxes of D-galactose across sheets of winter flounder intestine

In control experiments with 1 mM D-galactose on both sides of the tissue there is a small but nonsignificant mean net galactose absorptive flux. The tissue:medium ratio of total sugar (free + phosphorylated) sugar does not exceed unity and the free galactose concentration within the tissue is 0.6 mM. The absence of net galactose transport in flounder intestine confirms the result of Rout et al (1965).

Effects of phlorizin on D-galactose transport

Phlorizin was added to the mucosal bathing solution to see if there was any Na^+-dependent D-galactose absorption.

It can be seen in Table 1 that with 0.1 mM phlorizin present in the mucosal bathing solution there is a significant net secretion of D-galactose. This secretion arises from an apparent stimulation of s-m flux ($p < 0.01$). No significant change in the free galactose concentration within the tissue is observed with phlorizin in the mucosal solution.

The apparent stimulation of galactose secretion by phlorizin is unexpected as phlorizin abolishes net galactose absorption across rabbit ileum by inhibiting m-s flux (Estep, J.A. and Goldner, A.M. Biochim. Biophys. Acta 367:371). In winter flounder renal tubules, phlorizin enhances the already high clearance ratio of 2-deoxy-D-galactose (Pritchard et al., 1978).

These results show that winter flounder intestine is similar to flounder renal tubules inasmuch as both possess two transport systems for D-galactose: a net absorptive system, which can be inhibited by phlorizin and a net secretory system stimulated by addition of phlorizin to the mucosal bathing solution.

The presence of two transport systems working in opposite directions explains why net absorption of galactose is negligible in the winter flounder intestine.

Transport of 2-deoxy-D-galactose

2-Deoxy-D-galactose is actively secreted across flounder intestine (see Table 2). This finding is similar to the observed secretion in winter flounder renal tubules (Pritchard et al., 1978). Net secretion, J_{ms}, and J_{sm} are all saturable functions of the 2-deoxy-galactose concentration in the external bathing solution (results not shown). The K_m for all three fluxes are in the range 12-15 mM, the Vm's are 0.75, 1.4 and 2.2 μmole $cm^{-2}h^{-1}$ respectively.

Increasing the external sugar concentration above 1 mM reduces the total sugar accumulation ratio within the tissue. This effect is entirely due to saturation of the sugar phosphorylation reaction. There is no significant change in the accumulation ratio of free sugar of 2-deoxy-galactose within the tissue in the concentration range 0.1 mM-25 mM. The main flux asymmetry is across the mucosal border; although 2-deoxy-galactose movement across the baso-lateral border is rapid, the opposing fluxes balance each other more closely. Since the sugar is not accumulated within the tissue, it therefore seems, that the active secretory event is across the mucosal border.

Effects of modifiers on 2-deoxy-galactose secretion

a) Replacement of Ringer Na with choline. When Ringer Na is entirely replaced in both mucosal and serosal bathing solutions, net 2-deoxy-galactose secretion is abolished: however, if Na replacement is only unilateral, secretion is unaffected. This effect is similar to the result of Rout et al. (1965) who found that unilateral replacement was ineffective in inhibiting tyrosine absorption by flounder intestine.

b) Effect of 0.1 ml ouabain in serosal solution on secretion of 2-deoxy-galactose. When ouabain (0.1 mM) is added to the serosal solution net secretion of 2-deoxy-galactose is abolished. This effect

is due to the significant reduction in s-m flux, no significant effect of ouabain is seen on m-s flux, or on the accumulation of free sugar.

These results indicate that secretion of 2-deoxy-galactose in the intestine is related to the tissue Na-pump activity.

c) Effect of phloretin. Phloretin is known to inhibit sugar fluxes across the serosal border of intestine (Bihler, I. and Cybulsky, R. Biochim. Biophys. Acta 298:429, 1973). Phloretin (0.1 mM), when added to both mucosal and serosal sides of the tissue, or alternatively to the serosal side alone, abolishes net secretion of 2-deoxy-galactose; addition of 0.1 mM phloretin to the mucosal solution has no effect on secretion (Table 3). This result poses an interesting problem; active secretion occurs at the mucosal border and 2-deoxy-galactose movements across the serosal border are passive, yet phloretin inhibits active secretion by acting exclusively at the serosal border, as it does not cross the tissue.

d) Effect of galactose on 2-deoxy-galactose secretion. D-Galactose when present at 10 mM in both mucosal and serosal solutions completely abolishes net secretion of 0.1 mM 2-deoxy-galactose. This effect is seen mainly as reduction in s-m flux; D-galactose also reduces the total amount of 2-deoxy-galactose present in the tissue, but is without significant effect on the free 2-deoxy-galactose concentration within the tissue (Table 3).

The result in this section indicates that 2-deoxy-galactose and galactose show a common secretory pathway across winter flounder intestine.

Transport of α-methyl-D-glucoside

α-Methyl-D-glucoside is actively absorbed by winter flounder intestine (Table 4), although it is not accumulated to a higher concentration within the tissue than in the external bathing solution. The specific activity ratio, R, of this sugar is significantly above unity indicating that the unidirectional uptake of this sugar across the mucosal border is twice as fast as across the baso-lateral border. It can be seen in Table 4 that ouabain (0.1 mM), when present in the serosal solution, or phlorizin when present in the mucosal solution, both abolish net absorption of α-methyl-D-glucoside by reducing m-s flux. D-galactose, when present in both mucosal and serosal bathing solutions, also abolishes net absorption of α-methyl-glucoside. The results in this section confirm that the winter flounder intestine does possess an Na-dependent absorptive pathway for certain sugars and that D-galactose uses this pathway.

Discussion

The results show that in the winter flounder intestine there are two distinct active transport pathways for sugars having different specificities for sugars and inhibitors. Both systems are sensitive to ouabain and therefore dependent on the activity of the Na-pump. There is a phlorizin-sensitive absorptive pathway which transports α-methyl-glucoside, β-methyl-D-galactoside, and D-galactose which appear identical to the well characterized Na-dependent brush-border sugar transport system. Additionally, there is a second secretory pathway which is inhibited by phloretin acting at the baso-lateral border, which is stimulated by phlorizin in the mucosal solution and which transports 2-deoxy-galactose, galactose and presumably D-glucose (Rout et al., 1965). Since D-galactose can be transported by both active transport pathways, little or no net transepithelial flux of this sugar is observed. This dual transport system for sugars in the intestine also operate in the renal tubule of the flounder (Pritchard et al., 1978).

Our working hypothesis of the mechanism of active secretion, is that the secreted sugar is concentrated in an intracellular compartment near the apical pole of the epithelial cells by a process which depends on the function of the Na-pump (perhaps fluid withdrawal from the cell due to osmotic gradients created by the Na-pump activity). The sugar then moves passively out of the cell across the mucosal border. Phloretin acts by slowing entry across the serosal border, thereby preventing the sugar

from accumulating within the putative intracellular compartment. Phlorizin, acting at the mucosal border inhibits the uptake of sugars across the mucosal entry pathway, but does not affect 2-deoxy-galactose, or galactose movement via the alternative pathway, hence does not affect active secretion, indeed it may enhance it. This investigation was supported by a travel grant from the Royal Society (London) and a Dahlgren Fellowship from MDIBL to R.J. Naftalin, as well as by a grant from the Whitehall Foundation to A. Kleinzeller.

MICROVASCULAR ORGANIZATION OF THE GILL OF *S. acanthias*

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Physiological studies of pressure-flow relationships in the gill of *S. acanthias* demonstrate a marked increase in resistance to flow within the gill vasculature in response to various stimuli such as hypoxia and hypercapnia (Kent and Peirce, Comp. Biochem. Physiol. 60(C):37-44, 1978). Recent studies using thermal washout techniques suggest that the vasomotion may include A-V shunting (Eid et al., Bull. MDIBL 17:91-94, 1977). Anatomic evidence for such shunts is lacking (Kempton, Biol. Bull. 136: 226-240, 1969); indeed the literature on the microvasculature of the gill of dogfish is quite sparse. Recently techniques utilizing vascular casting and SEM studies have proved fruitful in detailing the gill vasculature in teleosts (Olson et al., Fed. Proc. 37:387, 1978). In the present studies these techniques have been applied to the dogfish.

Four dogfish ranging in weight from 4 to 6 kg were anesthetized with sodium pentobarbital (20 mg/kg), heparinized via the caudal artery (1000 units/kg) and placed in a seawater trough ventral side up. Fresh seawater from tubes in the spiracles ran over the gills. The conus arteriosus was cannulated with a short, large bore cannula (PE270) through which dogfish Ringer's (Robin) was pumped at a pressure similar to ventral aortic pressure (40-50 mmHg). The atrium was opened and Ringer's was allowed to run through the vasculature of the fish for at least 10 minutes or until red cells were completely washed out. A plexiglass resin and catalyst (Mercox) was pumped in following the Ringer's at the same pressure for 10 minutes. Excess Mercox escaped from the open atrium when the circulatory system was completely filled. The fish were transferred to a 50°C water bath for an hour and the gill basket was removed and put in a 20 % NaOH solution where tissue was digested. The Mercox cast of the gills was then rinsed with distilled water and air dried.

Specimens were examined microscopically with a Wilde M5 microscope at the South Bend extension of Indiana University Medical School and with a Cambridge Stereoscan 600 scanning electron microscope at the Notre Dame University Biology Department.

The distribution of the afferent branchial artery in the first hemibranch is seen in Figure 1. In Figure 1 primary filamental afferent arteries (16 shown) leave the afferent branchial artery and distribute to the mid-portion of either one or two filaments. Here the vessel bifurcates into a recurrent branch running to the basal end of the filament and a concurrent branch which supplies the filament to the outer tip. Along the entire length of the filament both the recurrent and concurrent filamental afferent arteries give rise to lateral and medial afferent sinuses. These join with those of adjacent filaments along the outer margin of the hemibranch as seen in Figure 1. This figure also shows that the distal end of the concurrent filamental afferent artery divides and becomes indistinguishable from the sinuses at the outer margin. Figure 2 shows a detail of the lateral view of the medial afferent sinus as it comes off the filamental afferent artery. Part of the lateral sinus has been removed from this section of the artery. The afferent lamellar arterioles can be seen arising from the