2. The 2-DGLU and GLU pathway: Using the improved analytical procedure it was confirmed that 2-DGLU is actively accumulated in renal cells by a transport process localized at the peritubular cellular face; a considerable accumulation of 2-DGLU-phosphate also takes place. Kinetic studies revealed a fast efflux of the free sugar from the cells whereas 2-DGLU phosphate only slightly and temporarily decreased during the washout of free sugar (Figure 1 A and B). This result indicated a rather slow hydrolysis of 2-DGLU-P to free sugar, suggesting that phosphorylation takes place subsequent to the transport of the free sugar into the cells. The cellular accumulation of 2-DGLU was markedly inhibited by GLU but not by *a*-MGLU, GAL or 2-DGAL, indicating a carrier shared by D-glucose and 2-deoxyglucose; a free hydroxyl on C_1 and hydroxyls on C_3 and C_4 in the transposition appear to be structural requirements of the sugar molecule for this carrier.

3. The GAL and 2-DGAL pathway: A considerable amount of GAL taken up by the cells is found in phosphorylated form; kinetics indicate that GAL is transported across the cell membrane as free sugar. The entry of GAL was completely inhibited by 2-DGAL but was not affected by D-glucose or *a*-MGLU.

The uptake of 2-DGAL was inhibited by GAL but was not affected by GLUcose, *a*-MGLU or 2-DGLU. The carrier shared by GAL and 2-DGAL thus requires the hydroxyls on C_3 and C_4 to be in the cis-position.

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THE RENAL HANDLING OF SOME SUGARS BY THE FLOUNDER

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The renal handling of *a*-methyl-D-glucoside (*a*-MGLU) and 2-deoxy-D-glucoside (2-DGLU) by winter flounder *(Pseudopleuronectes americanus)* was examined *in vivo* using the clearance techniques of Maack, *et al.* (Bull. MDIBL 9: 29-30, 1969). Inulin labeled with ^{14}C or ^{3}H was presented intraperitoneally (250 mg/kg) 24 hours prior to the experiment. At time zero 50 μmole/kg labeled sugar was given via the caudal vein. Urine was collected continuously in samples of 0.3-0.7 ml. Blood samples of 0.3 were taken from the caudal vein at time zero and the conclusion of each clearance period. Clearances were calculated correcting for the dead space in the catheter. Tissue inulin and sugar were determined immediately after the final clearance period.

Marked tubular reabsorption of *a*-MGLU was seen in all clearance periods of both flounder (Table 1). The clearance of this sugar was only one tenth that of inulin. Furthermore the terminal tissue to plasma (T/P) ratio for the sugar was about 2.7 while that for inulin was only 1.1, indicating cellular accumulation during reabsorption. No significant difference was seen between total sugar and free sugar (i.e., after $ZnSO_4$ + $Ba(OH)_2$ precipitation); thus there was no apparent phosphorylation in the tissue. The observed T/P values of inulin (mean 1.16) are in agreement with those observed by B. Schmidt-Nielsen and L. Renfro (personal communication).

TABLE 1

Mean Clearance and terminal tissue to plasma (T/P) ratios ± standard error of the mean for *in vivo* presentation of inulin, *a*-methyl-D-glucoside, and 2-deoxy-D-glucose in the winter flounder. N equals 4 for all data except the clearances of the sugars where N equals 3.

	CLEARANCE		TERMINAL T/P RATIO		
Flounder #	Inulin	Sugar	Inulin	Total Sugar	Free Sugar
		a-Methyl-D-Glucoside			
3	0.402 ± 0.030	0.030 ± 0.001	1.136 ± 0.009	2.658 ± 0.278	2.736 ± 0.298
4	0.152 ± 0.12	0.016 ± 0.001	0.933 ± 0.027	1.511 ± 0.101	1.161 ± 0.024
		2-Deoxy-D-Glucose			
5	0.877 ± 0.070	0.833 ± 0.129	1.628 ± 0.131	8.957 ± 0.173	2.121 ± 0.204
6	0.750 ± 0.048	0.765 ± 0.092	0.959 ± 0.042	6.619 ± 0.192	1.186 ± 0.116

In vitro experiments with teased kidney tubules showed that *a*-MGLU rather sluggishly entered the tissue. Under all experimental conditions that steady-state tissue/medium ratio (T/M) for *a*-MGLU was lower than 1.0 and at an external sugar concentration of 0.5 to 1.0 mM the T/M was around 0.6. Table 2 shows that the T/M was significantly reduced by 0.5 mM phlorhizin ($P << 0.01$) to levels below the T/M for mannitol and approached the T/M for inulin. If the assumption is correct that in teased flounder tubules inulin does not enter the tubular lumen (i.e., that tight junctions between the tubular cells do not allow the penetration of inulin) the above result indicates that also *a*-MGLU does not freely pass through the tight junctions; consequently the phlorhizin-sensitive entry of *a*-MGLU into renal cells takes place at the peritubular face of the cells. Data indicate that this transport process for *a*-MGLU is saturable and is independent of Na (Bull. MDIBL 12: 30, 1972), suggesting facilitated diffusion.

TABLE 2

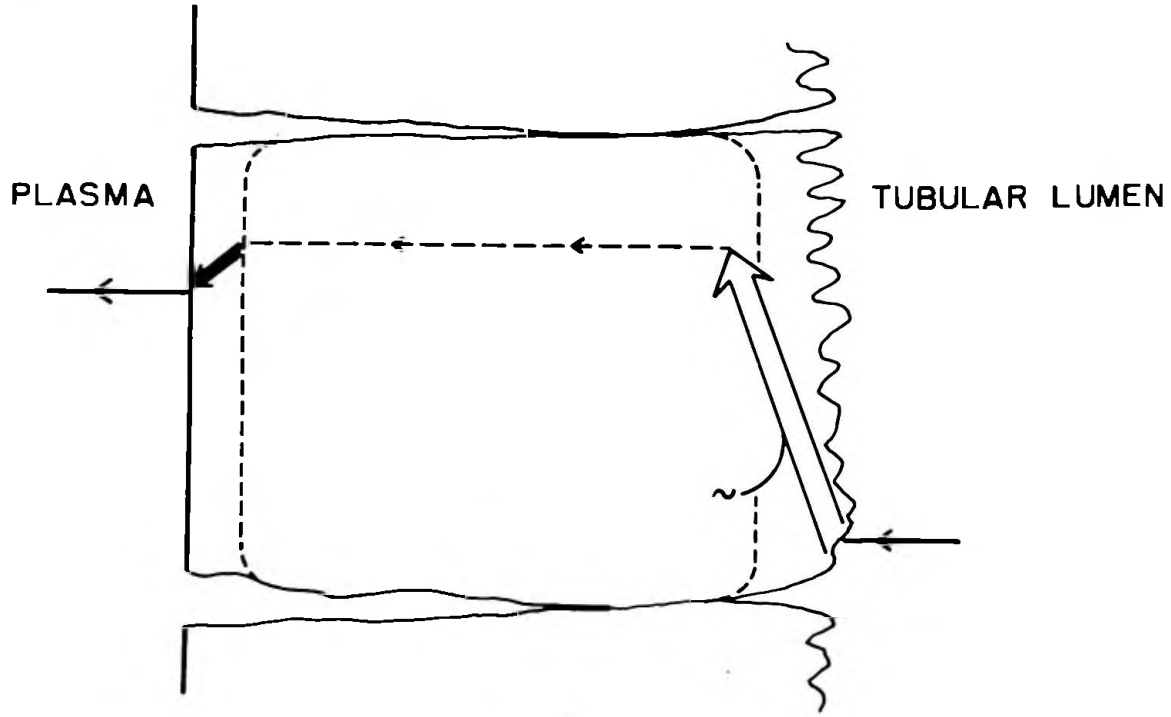
STEADY-STATE LEVELS OF SOME SACCHARIDES IN TEASED TUBULES OF FLOUNDER KIDNEY

Tubules were incubated 1 h in salines containing 0.5 mM *a*-MGLU-^{14}C and 0.1 percent (w/v) inulin-^{3}H or 1 mM mannitol-^{3}H, without (control) or with 0.5 mM phlorhizin Values of mean T/P ± S.E. (n not less than 6) are given.

Saccharide	T/P	
	Control	0.5 mM phlorhizin
a-MGLU	0.513 ± 0.020	0.398 ± 0.021
Inulin	0.322 ± 0.015	0.318 ± 0.029
Mannitol	0.476 ± 0.015	0.449 ± 0.028

2-deoxy-glucose had clearance ratios similar to inulin and was therefore subject to no net tubular transport (Table 1). Nevertheless terminal tissue to plasma ratios for the free sugar were significantly greater than inulin, indicating cellular accumulation. Marked phosphorylation was shown by the large difference between total and free sugar. Under *in vitro* conditions a carrier-mediated active transport of the sugar into the cells takes place (Bull. MDIBL 12: 30, 1972).

These data argue that the two sugars are handled quite differently by the renal tubule. *a*-MGLU is reabsorbed against urine to tissue concentration gradient; thus must be actively transported at the luminal face of the cell. At the peritubular cellular face a carrier-mediated equilibration between sugar and plasma levels of the sugar take place as schematically shown in Figure 1. The pathway of *a*-MGLU reabsorption in the kidney tubules thus resembles that for D-galactose (Bull. MDIBL 10: 34, 1970).



LEGEND TO FIGURE

Figure 1 Scheme of *a*-Methyl-D-glucoside reabsorption across the tubular cell of the flounder kidney. Solid lines: levels of *a*-MGLU in the tubular urine and plasma, respectively. Broken line: diffusion within cell. White arrow: active transport (~ indicates energy source). Black arrow: facilitated diffusion.

2-Deoxy-glucose, on the other hand, does not show net reabsorption; yet tissue to plasma ratios for the free sugar are greater than one, suggesting cellular accumulation. The source of this sugar must have been the plasma, indicating active uptake at the antiluminal face of the cell. This interpretation is compatible with the *in vitro* demonstrated capability of the cells to accumulate actively 2-DGLU by a process localized at the peritubular face.

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