

taken from each organ. The cpm/gram of tissue was calculated for both isotopes for each sample and total organ flow determined. The percent of cardiac output found in each organ was calculated as follows:
$$\frac{\text{average activity/gm} \times \text{organ weight}}{\text{total dose activity}}$$

There was no significant difference in the distribution pattern between the isotopic tags given in the control period. The acidemia which lowered pH from $7.36 \pm 0.109(\text{SD})$ to $7.151 \pm 0.328(\text{SD})$ produced a variety of blood flow shifts in various organs of individual fish. The only consistent pattern within the group was an increase in blood flow to the kidneys after the acid load in five out of six fish. Excluding the one fish in which flow fell from 3.4 to 1.9 percent of cardiac output, the average of the remaining five fish rose from 5.17 percent ± 2.76 SEM to 6.53 percent ± 2.55 SEM after the acid injection. By the time of the terminal sample the pH of the fish had returned to 7.39 ± 0.23 (SD).

The lack of consistent changes in blood flow distribution to all parts of the body excluding the kidney indicates the degree of acidemia produced in this study is not a factor in controlling the amount of blood flow to most of the vascular beds of the dogfish. This may be due in part to the fact that acidosis was already present in the control periods (pH = 7.36 ± 0.109); moreover the time course of a change in blood flow due to changing pH may not coincide with our period of observation. It has been found that a fall in arterial pH comparable to the one used in this study is not reflected in the urinary pH (Sharks, Skates, and Rays, 1967, p.254) and that the gills play an important role in non-respiratory acid excretion in dogfish (#28, MDIBL, 1968). However the trend for the kidneys to receive a larger portion of the cardiac output with a fall in pH is of interest and should be examined in a larger group of fish exposed to a variety of conditions in which pH is lowered.

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SUGAR TRANSPORT ACROSS THE PERITUBULAR FACE OF RENAL CELLS OF THE FLOUNDER *Pseudopleuronectes americanus*

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Previous studies on the transport of sugars in teased tubules of flounder kidney (Bull MDIBL 10:34, 1970) have been extended. An improved analytical technique (to be published) allowed determination in the same tissue extract of both free and total sugars; the difference between these values represents phosphorylated sugar. As opposed to the previously employed $\text{ZnSO}_4 + \text{Ba}(\text{OH})_2$ deproteinizing procedure, the new technique prevents breakdown of sugar phosphate to free sugars.

The uptake of α -methyl-D-glucoside- ^{14}C (α -MGLU), 2-deoxy-D-glucose- ^{14}C (2-DGLU), D-galactose- ^{14}C (GAL) and 2-deoxy-D-galactose- ^3H (2-DGAL) were tested. α -MGLU entered the cells as free sugar only, whereas the other sugars were present in the tissue both in free and phosphorylated form. The cellular uptake of all sugars was inhibited practically completely by 0.5 mM phlorhizin, 0.3 mM phloretin and also by 1 mM N-ethylmaleimide, but not by 0.5 mM ouabain or

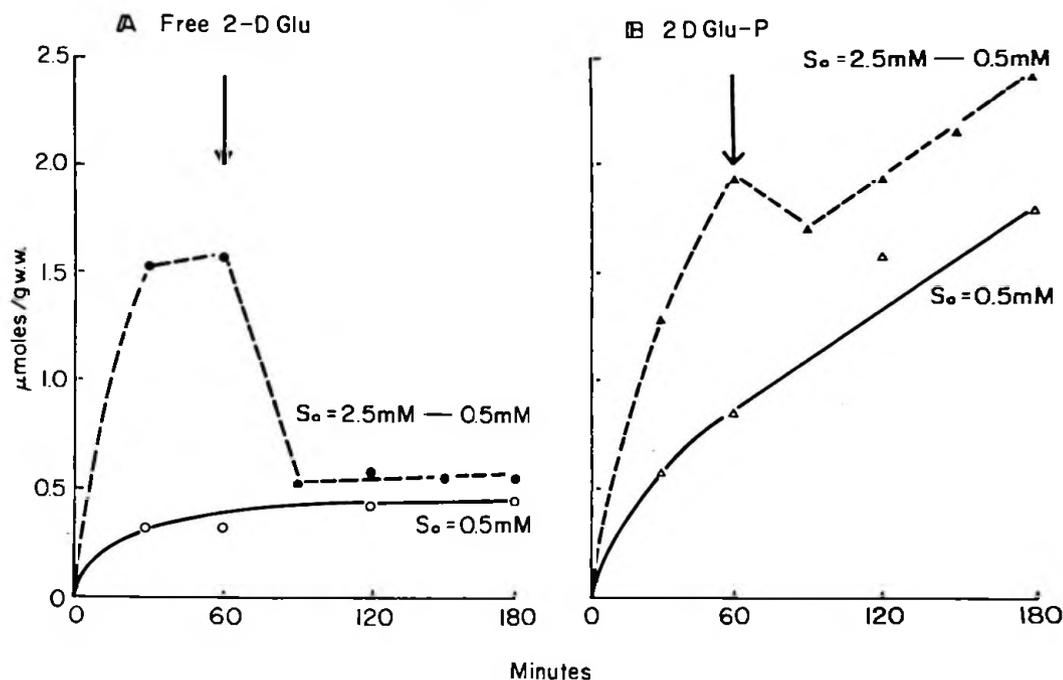
absence of external Na. No mutual interference between the uptake of chlorphenol red (0.025 mM, final conc.) and these sugars was found.

Competitive studies provided evidence for three pathways of sugar transport localized at the peritubular face of renal cells:

1. The α -MGLU pathway: Under all experimental conditions the steady-state tissue/medium ratio (T/M) was below that of the water space and was decreased to levels approaching the inulin space (0.377 ± 0.011 [S.E.] kg/kg tissue wet wt.) by phlorhizin. This observation may be taken as an indication that in teased tubules α -MGLU enters the cells only at the peritubular face since studies *in vivo* have shown that α -MGLU available at the luminal face of the renal cells is actively taken up by a concentrating process (see Bull. MDIBL 12: 64, 1972). A saturable entry process was indicated by a decrease of the T/M with increasing medium concentrations (S_0) from 0.05 to 5 mM. The steady-state level of α -MGLU ($S_0 = 0.5$ mM) was only slightly depressed by 5 mM D-glucose (GLU) and was not inhibited by GAL. Thus the pathway of α -MGLU does not appear to share its carrier with GLU (see also below).

Accumulation Of 2-Deoxy-D-Glucose In Flounder Kidney Tubules

Reversibility



LEGEND

Teased tubules were first loaded with 2-DGLU- 14 C at $S_0 = 0.5$ mM (O, Δ) and 2.5 mM (O, Δ). At the time indicated by arrows the tissue was transferred from medium with 2.5 mM sugar to fresh medium at $S_0 = 0.5$ mM, and the changes in tissue 2-DGLU levels were followed. A. Free tissue 2-DGLU; B. 2-DGLU-phosphate (i.e. total minus free tissue sugar).

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 α -MGLU, GAL or 2-DGAL, indicating a carrier shared by D-glucose and 2-deoxyglucose; a free hydroxyl on C₁ and hydroxyls on C₃ and C₄ 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 α -MGLU.

The uptake of 2-DGAL was inhibited by GAL but was not affected by GLUcose, α -MGLU or 2-DGLU. The carrier shared by GAL and 2-DGAL thus requires the hydroxyls on C₃ and C₄ 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 α -methyl-D-glucoside (α -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 ¹⁴C or ³H was presented intraperitoneally (250 mg/kg) 24 hours prior to the experiment. At time zero 50 μ mole/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 α -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 ZnSO₄ + 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).