THE STRUCTURAL REQUIREMENTS FOR TWO PATHWAYS OF D-GLUCOSE TRANSPORT INTO RENAL TUBULAR CELLS OF THE FLOUNDER *Pseudopleuronectes americanus*

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Evidence has been presented previously (Kleinzeller and McAvoy, Bull. Mt. Desert Island Biological Lab. 12: 64, 1972; J. Gen. Physiol. 62: 169, 1973) indicating the presence of three carrier-mediated pathways by which sugars are transported across the antiluminal face of the flounder renal tubule, i.e., 1) the pathway for α-methyl-D-glucoside which is only partly inhibited by D-glucose; 2) a pathway shared by D-glucose and 2-deoxy-D-glucose; 3) a pathway shared by D-galactose and 2-deoxy-D-galactose. The specificity of the first two of these transport pathways was now investigated in more detail.

Teased renal tubules of the winter flounder *(Pseudopleuronectes americanus)* were used employing previously described experimental and analytical techniques. The data are expressed as the tissue/medium ratio (T/M) or as μ mole sugar/g wet tissue wt.

Increasing concentrations of D-glucose inhibited the tissue uptake of 0.5 mM α-methyl-D-glucoside-^{14}C. Even at 5 mM, i.e., at a molar ratio of 10 glucose to one substrate, the cellular space occupied by the glucoside was still one-third that of the control without inhibitor. Thus D-glucose is a poor inhibitor of the glucoside transport system. Other sugars (5 mM) were tested as possible competitive inhibitors: the cellular uptake of 0.5 mM α-methyl-D-glucoside was significantly inhibited by β-methyl-D-glucoside, and slightly by 1,5-anhydro-D-glucitol and 3-O-methyl-D-glucose. Thus these sugars, and D-glucose, share the carrier involved in the transport pathway for α-methyl-D-glucoside. Ineffective as inhibitors were: D-galactose, D-mannose, 2-deoxy-D-glucose, 2-deoxy-D-galactose, D-xylose, the corresponding α-methyl-D-glycosides, and D-sorbitol. These results suggest a rather narrow structural specificity of the transport pathway for α-methyl-D-glucoside. In order to share the carrier sugars require a hemiacetal group and hydroxyls on C_2, C_4, and C_6 in positions found in D-glucose. Hydroxyls on C_1 and possibly on C_3 do not appear to represent a structural requirement for transport by this pathway.

The specificity of the transport pathway shared by D-glucose and 2-deoxy-D-glucose was studied by investigating the transport of D-glucose-^{3}H and D-mannose-^{14}C.

Figure 1 shows that D-glucose (1 mM) readily entered the tissue and was present in both free and phosphorylated form. The steady-state level of free glucose was always markedly lower than that in the incubation medium. Phosphorylated glucose appeared in the tissue only when a considerable amount of free sugar was present in the cells (i.e., in excess of the inulin space, corresponding to a T/M of 0.35). This observation supports previous contention that the phosphorylation step is located after the transport of free sugar across the cell membrane.

The properties of the transport system for D-glucose were examined by studying the effect of various inhibitors on the total uptake of D-glucose (free plus phosphorylated) by the tissue; such procedure is justified on the assumption that the phosphorylation step follows the transport process.

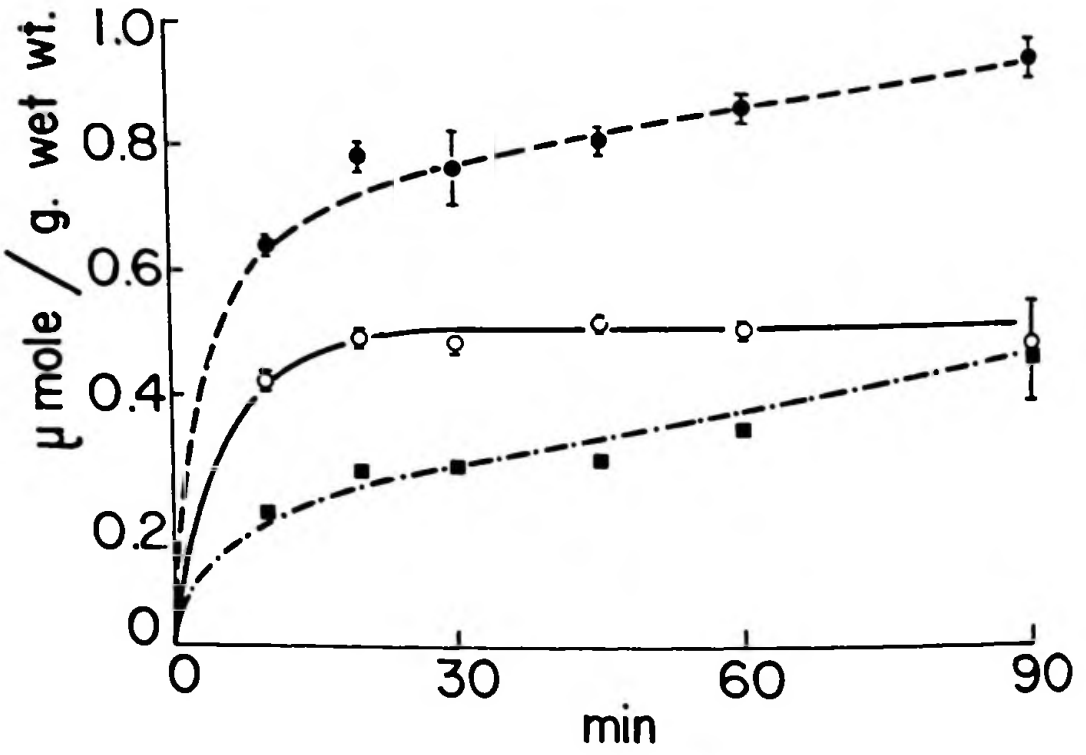

Figure 1. Uptake of D-glucose-^{3}H by flounder renal tubules. Groups of teased tubules (4 per experimental point) were incubated aerobically (air) in standard medium at 15°C containing 1 mM D-glucose-^{3}H. The values are the means, ± S.E. Free sugar, ○; total tissue sugar, ●; phosphorylated sugar (total minus free), ■.

The results given in Figure 2 are expressed as the T/M ratio (1 h aerobic incubation, 15°C), taking the T/M for the control (without inhibitor) as 100 percent. The uptake of D-glucose was greatly inhibited by 0.5 mM phlorizin or phloretin and was not affected by 0.5 mM ouabain. Of various sugars tested D-mannose and 2-deoxy-D-glucose competitively inhibited the uptake of D-glucose whereas α-methyl-D-glucoside, 1,5 anhydro-D-glucitol, and D-galactose were ineffective. The structural requirements for D-glucose transport are therefore identical with those observed for 2-deoxy-D-glucose including a mandatory C_1-OH. Moreover α-methyl-D-glucoside did not inhibit the uptake of D-glucose whereas this hexose did inhibit though weakly the transport of α-methyl-D-glucoside. Therefore it is conluded that D-glucose can be transported across the antiluminal face of the flounder renal tubule by both transport pathways.

The time-curve for the uptake of 1 mM D-mannose by the tissue was similar to that presented for D-glucose (Figure 2), both free and phosphorylated mannose being found in the tubular cells. The phlorizin and phloretin sensitive uptake of D-mannose was inhibited by 5 mM D-glucose and 2-deoxy-D-glucose whereas D-galactose, α-methyl-D-glucoside, α-methyl-2-deoxy-D-glucoside, and α-methyl-D-mannoside were ineffective as inhibitors. The specificity pattern of D-mannose transport is thus identical with that of D-glucose and 2-deoxy-D-glucose. It is suggested that the carrier shared by these three hexoses has the following minimal structural requirements (Figure 3): a hemiacetal group, C_1-OH, C_4-OH and possibly C_3-OH because competitive inhibition of 2-deoxy-D-glucose by

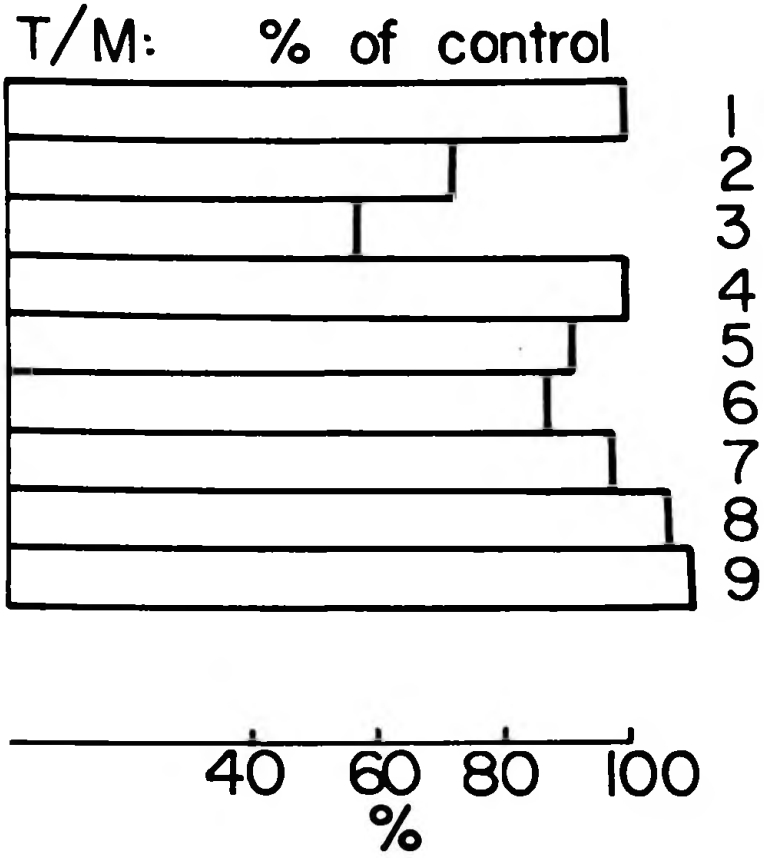

Figure 2. Uptake of D-glucose by flounder renal tubules: Effect of inhibitors.
Groups of teased tubules (at least 4 per experimental group) were incubated aerobically (air) for 1 h at 15° C in standard medium containing 1 mM D-glucose-^{3}H without (control) or with inhibitors. The results are expressed as the T/M of total sugar, in % of that in the control. 1: Control, 2: Phloridzin, 0.5 mM; 3: Phloretin, 0.5 mM; 4: Ouabain, 0.5 mM; 5: D-mannose, 5 mM; 6: 2-Deoxy-D- glucose, 5 mM; 7: D-Galactose, 5 mM; 8: α-Methyl-D-glucoside, 5 mM; 9 1,5-Anhydro-D-glucitol, 5 mM.

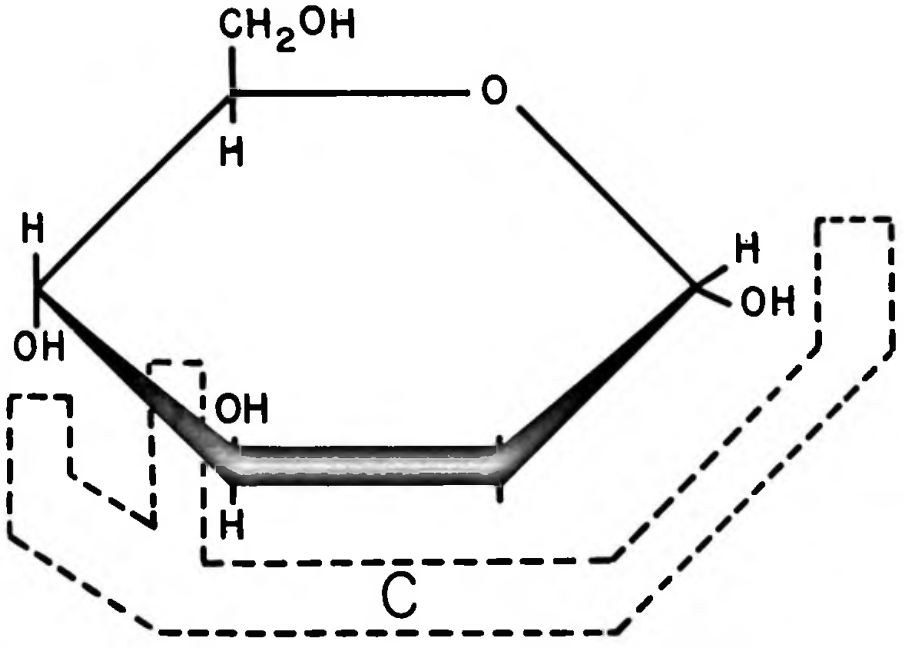

Figure 3. The structural requirement for the transport pathway shared by D-glucose, 2-deoxy-D-glucose and D-mannose.
C: Carrier

3-0-methyl-D-glucose, as previously reported, could not be confirmed in this study; C_2-OH is not required. This investigation was supported in part by USPHS Grant AM-12619.

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THE EFFECT OF PHLORETIN ON RENAL UREA AND SODIUM TRANSPORT IN *Squalus acanthias*

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The dogfish kidney is capable of reabsorbing urea against a large concentration gradient and can produce a urine with a urea concentration substantially lower than that of blood independent of the urinary sodium concentration. The purpose of the present study was to examine the effect of phloretin, a potent inhibitor of amide movement across several biological membranes, on the ability of the dogfish kidney to reabsorb urea.

Free-swimming, inulin-loaded fish were studied in tanks having a brisk flow of running sea water. Catheters were placed in the urinary sinus and dorsal aorta for collection of samples and injection of test substances. After a two-hour equilibration period and one 2-hour baseline period, the fish were injected with one to 1.2 ml of a test solution consisting of phloretin (20-23 mg/kg) dissolved in ethanol (0.14-0.16 ml/kg) and dogfish Ringer's (0.08-0.09 ml/kg), over a period of 10-15 minutes. Following the injection urine and blood were collected for two additional two-hour periods. A second group of fish was treated similarly but received only ethanol and Ringer's, without phloretin in order to ascertain the effect of ethanol alone on urea reabsorption.

On each plasma or urine sample, volume, urea, sodium, and inulin concentrations were determined. Analysis of data was done by comparing the mean of second and third periods with the first by the method of pair analysis.

Data for four control fish are shown in the left half of Table 1 for one period before and the average of two periods after the addition of ethanol. Ethanol did not lead to a significant alteration in urinary volume (V), GFR, fractional urea excretion or urinary excretion of urea or sodium. A small although significant decrease was seen in fractional excretion of sodium.

Results for the five fish receiving phloretin are shown in the right half of Table 1 for one period before and the mean of the two periods after the administration of phloretin. Urinary urea excretion increased markedly after phloretin administration. Fractional urea excretion also increased greatly while absolute urea reabsorption fell. Therefore phloretin significantly decreased urea reabsorption by the dogfish kidney. Urinary sodium excretion did not change significantly after phloretin but a decrease in absolute reabsorption and an increase in fractional excretion were observed. Urinary osmolality changed insignificantly from 705 ± 19 before phloretin to 723 ± 42 after phloretin. There was a small increase in urinary volume and a small decrease in GFR neither of which was significant. However fractional water excretion did increase significantly from 24 ± 2 to 37 ± 2 percent after phloretin ($p < 0.01$).

Thus phloretin causes a marked decrease in urea reabsorption in the dogfish kidney associated with a decrease in sodium reabsorption as well. Its action here is similar to that seen in the toad