It is concluded that fluid does leave the dogfish gut vascular compartment more promptly and freely when vascular volume is elevated. The loss of fluid appears to become significant when the resulting intra-vascular pressure approaches 6-7 mm Hg. This is a high venous and possibly capillary pressure for a dogfish, but well within the range observed in our saline-loading experiments. This finding may contribute to our failure to observe a sustained elevated vascular pressure after vascular volume loading.

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1974 #28

RENAL CLEARANCE OF SUGARS BY THE WINTER FLOUNDER, *Pseudopleuronectes americanus*

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In recent years Kleinzeller et al. (Bull. MDIBL 13:67-69, 1973 and J. Gen. Physiol. 62:169-184, 1973) have shown that several distinct sugar transport systems of differing specificity are located at the peritubular (antiluminal) membrane of renal tubular cells in the winter flounder, *Pseudopleuronectes americanus*. Supporting data have been developed using the in vitro teased tubule preparation where the sugars are presented exclusively to the peritubular face of the cells. In the studies reported below, sugars are presented in vivo, and thus reach the luminal face of the tubular cell by filtration and the antiluminal face via the peritubular capillaries. Sugars characteristic of each of the three carrier systems described by

Kleinzeller et al. at the peritubular membrane were examined in vivo, 1) α-methyl-D-glucoside (α-m-G), 2) 2-deoxy-D-glucose (2-d-G), and 3) D-galactose (D-Gal). Combining the results of these studies with the data derived in vitro we are now able to assess and characterize luminal transport.

The clearance studies were carried out according to procedures originally described by Maack and Kinter (Amer. J. Physiol. 216:1034-1043, 1969) as modified by Williams et al. (Bull. MDIBL 13:128-131, 1973). The method is described in detail elsewhere in this volume (Pritchard, Cauthen et al. Bull. MDIBL 14, 1974). Plasma concentrations of ^{14}C-sugars were 0.05-0.1 mM. Polyethylene glycol-^{3}H (PEG) was used as the glomerular marker (0.5 mg/ml plasma). Inhibitors were injected intravenously via the caudal vein. The fish were sacrificed by decapitation at the conclusion of the final clearance period and samples of kidney and plasma were taken for analysis of total and free sugar according to Kleinzeller and McAvoy (J. Gen. Physiol. 62:169-184, 1973). Radioactivity was determined by liquid scintillation spectrometry.

Clearance data and tissue to plasma ratios (T/P) for each sugar are shown in Table 1 and the effects of inhibitors are shown in Figure 1. α-methyl-D-glucose was effectively reabsorbed and its T/P exceeded unity. Since the peritubular carrier system is not capable of accumulation against a concentration gradient (Kleinzeller and McAvoy, J. Gen. Physiol. 62:169-184, 1973), reabsorption may be pictured as active luminal transport into the cell and passive exit via facilitated diffusion at the peritubular membrane. Both glucose (Figure 1) and phlorizin (not shown) produce rapid inhibition of reabsorption. The kinetics of this response showed an abrupt halt in luminal transport followed by a period of net secretion as free sugar from the elevated intracellular compartment moved into the urine down its concentration gradient (terminal T/P α-m-G = 1.94 vs U/P α-m-G = 1.74 after glucose in Fish 4).

TABLE 1

Sugar	Cl_{sugar}	Cl_{PEG}	$\frac{Cl_{sugar}}{Cl_{PEG}}$	T/P Free Sugar	$\frac{T/P\ Free\ Sugar}{T/P\ PEG}$
α-m-G	0.07 (7)# ± 0.03	0.30 (7) ± 0.07	0.21 (7) ± 0.05	1.81 (5) ± 0.25	2.51 (5) ± 0.39
2-d-G	0.45 (6)# ± 0.12	0.49 (6) ± 0.14	0.93 (6) ± 0.04	1.24 (4) ± 0.31	1.24 (4) ± 0.10
D-Gal	0.05 (4)# ± 0.02	0.19 (4) ± 0.06	0.27 (4) ± 0.02	0.55 (2) ± 0.13	0.84 (2) ± 0.17

$Cl_{\alpha\text{-m-G}}$ vs Cl_{PEG} $P<0.02$; $Cl_{2\text{-d-G}}$ vs Cl_{PEG} no sign. diff.; $Cl_{D\text{-Gal}}$ vs Cl_{PEG} $P<0.1$

Table 1: Summary of the clearance values (Cl) and terminal renal tissue to plasma ratios (T/P) for α-methyl-D-glucoside (α-m-G), 2-deoxy-D-glucose (2-d-G), D-galactose (D-Gal), and the glomerular marker polyethylene glycol (PEG) after *in vivo* presentation in the winter flounder. Data expressed as the mean ± the standard error of the mean for (N) animals. On the average 5 sequential clearance measurements and 4 separate tissue content analyses were made in each animal.

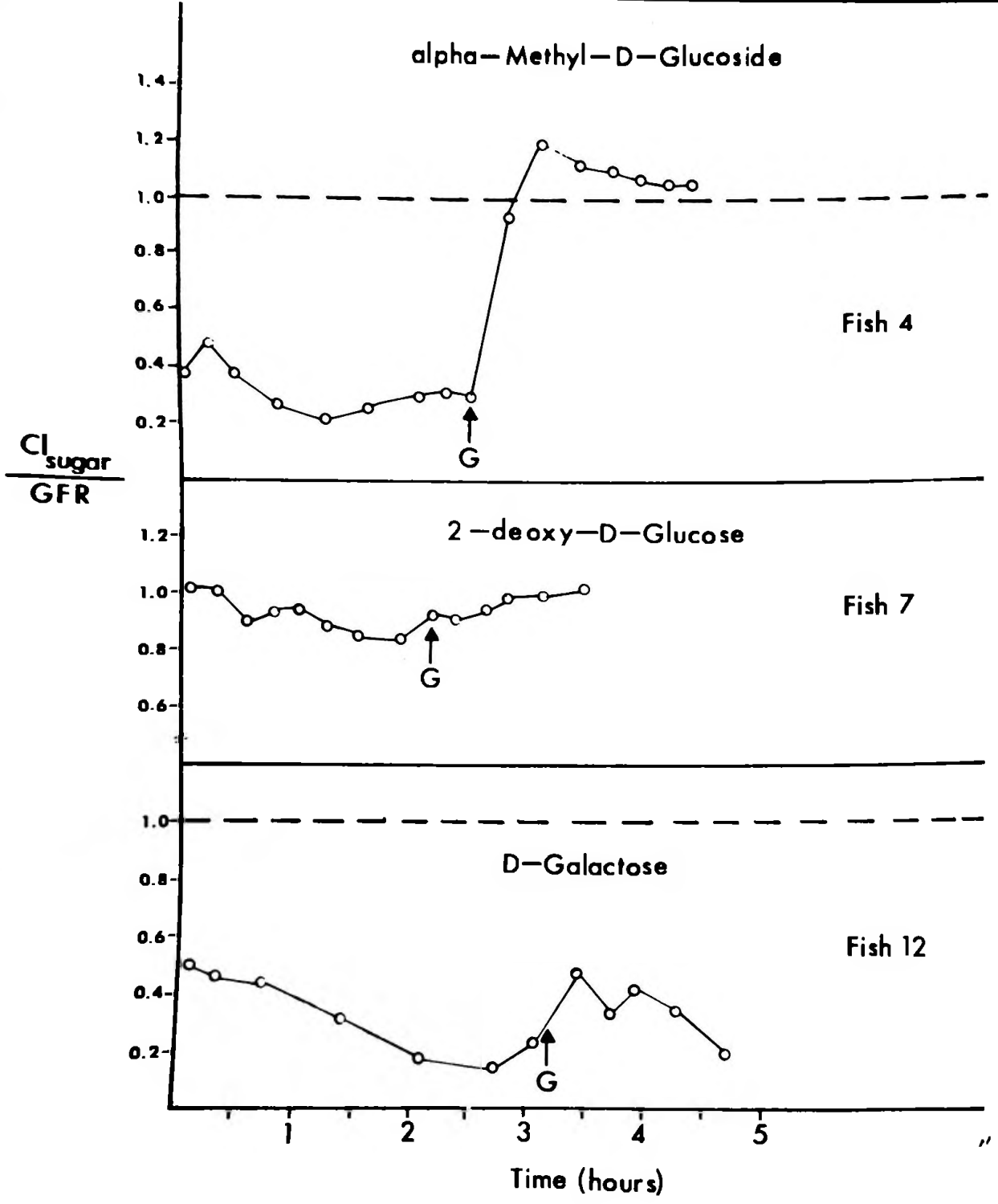

Figure 1: Effect of glucose on the clearance of three sugars in the winter flounder. Clearances (Cl_{sugar}) are expressed as a fraction of the simultaneously measured glomerular filtration rate (GFR) and plotted against the midpoint of the collection period. The time of intravenous glucose injection is shown by the arrows. Plasma ^{14}C-sugar concentrations were 0.05-0.1 mM and plasma glucose was approximately 10 mM after injection. A clearance ratio of less than 1 denotes net reabsorption of filtered sugar, greater than 1 indicates net secretion by the tubules.

T/P α-m-G after glucose or phlorizin were consistently lower than control values (1.81±0.25 for control vs 1.40±0.31 plus inhibitors) but these differences were not statistically significant.

Clearance of 2-d-G was not significantly different from the glomerular filtration rate (GFR). Nevertheless, T/P for total 2-d-G ranged from three to eight and T/P for free 2-d-G always approached or exceed unity. Neither glucose (Figure 1) nor phlorizin altered the clearance of 2-d-G. Thus there is no evidence for luminal transport. Furthermore since there is no net transport from the lumen, the source of cellular 2-d-G must have been entry from the plasma via the peritubular transport system previously characterized (Kleinzeller et al., Bull. MDIBL 13:67-69, 1973).

The third sugar, D-Gal, was reabsorbed by a mechanism which generated T/P of 1-2 for total sugar and of much less than unity for the free sugar. In addition both glucose (Figure 1) and phlorizin produced a slight transient but statistically significant ($p < 0.01$) increase in the clearance of D-Gal. The significance of this effect is unclear at present but certainly neither inhibitor is as effective in altering D-Gal reabsorption as in halting α-m-G reabsorption.

These data also provide evidence for the tightness of epithelial membranes in the flounder tubule. For example the tubule does not show net accumulation of α-m-G when it is presented in vitro (i.e. to the peritubular face of the cell); yet when α-m-G reaches the luminal membrane by filtration in vivo, net accumulation occurs. Thus the tubules behave as if they do not permit sugars to cross the epithelium, i.e. the "tight" junctions between cells are not leaky to these sugars. Therefore we may now conclude that accumulation of 2-d-G in vitro must represent peritubular uptake, not leakage

between cells to the lumen and accumulation by luminal transport. Additional support for this conclusion is provided by 1) the lack of net reabsorption in vivo and 2) the inability of glucose and phlorizin to alter luminal uptake while effectively inhibiting in vitro uptake (Kleinzeller et al. Bull. MDIBL 13:67-69, 1973).

Two conclusions may now be drawn. First, each sugar is handled differently at both faces of the tubular cell. Second, the handling of either α-m-G or 2-d-G by the luminal membrane is markedly different from the handling of the same sugar at the opposite pole of the cell (i.e. the peritubular membrane).

1974 #29

RENAL HANDLING OF ^{14}C-DDA IN VIVO BY THE WINTER FLOUNDER *Pseudopleuronectes americanus*

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Recent in vitro studies in two species of fish and on mammal have provided strong evidence for active transport of the polar DDT metabolite, DDA [2,2-bis-(p-chlorophenyl) acetic acid], by the kidney (Pritchard, et al., Bull. MDIBL 14 (in press); Pritchard, Pollution and Physiology of Marine Organisms, F.J. Vernberg, Ed., Academic Press (in press); and unpublished observations). Such in vitro techniques focus primarily on transport in a secretory direction at the peritubular membrane of the renal cell. They must be coupled with in vivo clearance studies to assess the contribution of secretory transport to net excretion, thus to evaluate the role of organic