

Figure 1. The effect of hypotonicity on tissue water and electrolytes in slices of the dogfish rectal gland. The tissue was incubated aerobically ( $O_2$ ) for 60 min in standard dogfish saline (853 mosM, control) and in a saline in which only the NaCl concentration was halved, all other constituents being the same as in the control (601 mosM). Steady state values, means  $\pm$  S.E. are given.

The rather small swelling effect of ouabain suggests the presence of an ouabain insensitive component of cell volume regulation in the dogfish rectal gland.

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#### RENAL CLEARANCE OF DMO AND METHYLAMINE BY THE WINTER FLOUNDER (*Pseudopleuronectes americanus*)

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Intracellular pH is most often measured from the distribution of an inert weak acid across the cell membrane (Physiol. Rev. 49:285, 1969). One critical postulate is that the marker be passively distributed between the intra- and extracellular fluid in accordance with the hydrogen ion gradient. Failure to monitor successfully changes in the intracellular pH of teased flounder tubules using 5,5-dimethyl-2,4-oxazolidinedione (DMO), made us suspect that the kidney may be able to handle actively this molecule. Therefore, clearance studies were carried out on DMO, as well as on another often used marker for measuring pH, i.e., methylamine, employing the technique described by Pritchard and Kleinzeller (Am. J. Physiol. 231:603, 1976).

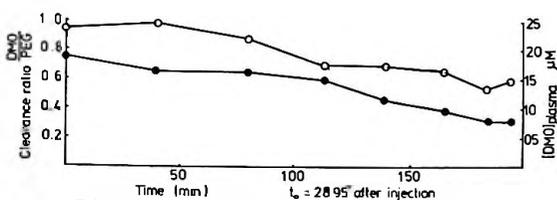


FIGURE 1 RENAL CLEARANCE OF DMO

● Clearance of DMO to PEG  
○ Plasma concentration of DMO

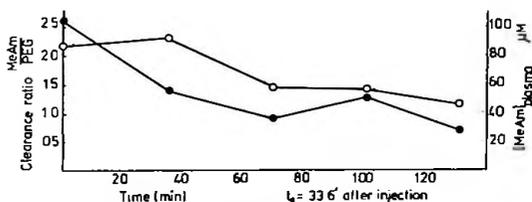


FIGURE 2 RENAL CLEARANCE OF METHYLAMINE

● Clearance of Methylamine to PEG  
○ Plasma concentration of Methylamine

As may be seen from Figure 1, the clearance ratio of DMO to PEG (polyethylene glycol) was less than one, and was observed to decline over time. The clearance ratio for two fish ranged from 1.0 to 0.32. The mean rate of urine flow was  $0.30 \pm 0.04$  ml/hr, based on a total of sixteen clearance periods each of which represents 96  $\mu$ l of urine. (All values are expressed as mean  $\pm$  the standard error.) The mean clearance of PEG was  $0.45 \pm 0.09$  ml/hr. Although there is notable scatter in these two values, neither parameter was observed to vary in any defined manner.

To determine the extent of DMO tissue accumulation, the kidneys of two fish were extracted, sectioned into five pieces of near equal weight, and treated appropriately. The tissue to plasma ratio of DMO over that for PEG thus obtained was  $2.40 \pm 0.15$ .

Further experiments will need to be carried out to show unequivocally active uptake of DMO by the flounder kidney. In any event, these preliminary results raise questions as to the advisability of using DMO to measure the intracellular pH of kidney cells.

As may be seen from Figure 2, the clearance ratio of methylamine to PEG also declined with time. However, in this case, the clearance ratio was considerably greater than one which indicates that methylamine is actively secreted. The clearance ratio from three fish ranged from thirty to seven.

The mean ratio of urine flow for these fish was  $0.23 \pm 0.05$  ml/hr, based on fourteen clearance periods. The mean clearance of PEG was  $0.28 \pm 0.07$  ml/hr. The tissue to plasma ratio of methylamine to that for PEG, determined for one fish, was  $16.49 \pm 0.51$  ( $n = 5$ ).

The data thus suggests that methylamine is secreted into the lumen by the transport system for weak (organic) cations (see, e.g., Am. J. Physiol. 225:1123, 1973).

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#### THE RENAL HANDLING OF D-FRUCTOSE BY THE WINTER FLOUNDER (*Pseudopleuronectes americanus*)

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The renal handling of D-fructose by the winter flounder was characterized employing the teased tubule preparation and clearance studies.

The *in vitro* studies, using teased renal tubules, were carried out as previously described (Kleinzeller et al., Am. J. Physiol. 232:F227, 1977). The tissue to medium ratio (T/M) for seven pieces of tissue, incubated for 60 minutes in saline having an initial concentration of 1 mM D-fructose, was  $0.61 \pm 0.02$ . (All values are expressed as mean  $\pm$  the standard error.) No significant difference in the T/M was observed over a concentration range of 0.05 to 5.0 mM; i.e., saturation of a carrier site could not be demonstrated. Glucose, 2-deoxyglucose and mannose, which have previously been shown to share the same carrier at the antiluminal face of the cell, did not inhibit the uptake of D-fructose when present at a fivefold molar excess. Also, 0.5 mM phlorizin, phloretin or ouabain did not significantly reduce the T/M value.

Clearance studies were carried out as previously described (Am. J. Physiol. 231:603, 1976). The clearance of D-fructose was not significantly different from the GFR (clearance of PEG) at a plasma fructose range of 50 to 100  $\mu$ M. The mean clearance ratio obtained from six fish was  $1.05 \pm 0.06$ , based on 29 clearance periods. No correlation was observed between either the GFR or the rate of urine flow and the clearance of D-fructose. An additional load of D-glucose, estimated to be 1 mM, had no effect on the clearance of D-fructose.

In brief, no active reabsorption of D-fructose by the winter flounder kidney was observed. Judging from the results obtained with teased tubules, fructose enters the cells at the antiluminal face by a pathway which is not carrier mediated. This investigation was supported in part by USPHS grant AM 12619 and the Whitehall Foundation. The assistance of Mr. Jonathan Goldstein was appreciated.

#### THE HANDLING OF D-MANNOSE AT THE BRUSH BORDER OF THE KIDNEY IN THE WINTER FLOUNDER (*Pseudopleuronectes americanus*)

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Studies on the handling of D-mannose by the winter flounder (*Pseudopleuronectes americanus*) were continued in view of persistent reports (Silverman, Biochim. Biophys. Acta 457:303, 1976) that in the brush border of the mammalian kidney a specific carrier is responsible for the phlorizin-insensitive reabsorption of this sugar.