preparation was chosen to study the transport of glucose and p-aminohippurate (Eveloff et al., this bulletin) because of the ease of preparation and high enrichment of alkaline phosphatase.

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CALCIUM EFFECT ON SUGAR TRANSPORT IN TEASED RENAL TUBULES OF THE WINTER FLOUNDER *Pseudopleuronectes americanus*

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Previous studies have demonstrated a multiplicity of sugar transport systems in the renal tubule cells of several species. These transport systems are characterized not only by differing stereospecificities but also by differing cellular locations. This situation complicates any analysis of transport phenomena since the experimentally determined tissue sugar accumulation values may represent an integration of transport via two or more pathways. The use of teased flounder tubules circumvents to some degree these problems of localization since some evidence has been presented suggesting that sugar transport in this preparation reflects events taking place primarily at the antiluminal face (Kleinzeller and McAvoy, J. Gen. Physiol., 62:169-184, 1973). The experiments presented herein give further support to the validity of this view.

Trump (Federation Proceedings, 30:22-41, 1971) observed that incubation of teased flounder tubules in a calcium-free saline had two notable effects: (1) a marked cellular swelling and, (2) a weakened integrity of the "tight" intercellular junction (as evidenced by the inability of these tubules to concentrate chlorphenol red within their luminal space). If this ionic manipulation of the medium does indeed lead to markedly increased permeability of the intercellular junction, then sugars (or other compounds of low molecular weight) should gain access to that luminal space from which they are normally excluded in the teased tubule preparation. Accordingly, incubation of tubules in a Ca-free medium should give these tubules transport properties demonstrable in a system where the luminal face of the cell is freely accessible to the studied solute, e.g., clearance studies (Pritchard and Kleinzeller, Am. J. Physiol., 231:603-607, 1976). In particular, the brush-border localized, active transport system for methyl-α-D-glucoside (α-me-Glc), known to be affected by a Na-dependent, highly phlorizin-sensitive mechanism, should become apparent. The cellular uptake of D-galactose (Gal) should also be increased by the absence of Ca.

The present experiments involved incubation (or preincubation) of teased tubules in normal (1.4 mM Ca) or Ca-free saline and measurement of the tissue accumulation of three model sugars: α-meGlc, Gal, and 2-deoxy-D-galactose (2-dGal). Agents known to inhibit active sugar transport, i.e., phlorizin and ouabain, were also tested for their effects. Table 1 shows the effect of Ca-free medium on the accumulation of these sugars. α-MeGlc and Gal, which have been shown to be reabsorbed from the lumen in clearance studies (Pritchard and Kleinzeller, Am. J. Physiol., 231: 603-607, 1976), show greater accumulation in tubules incubated in Ca-free saline. The tissue to medium ratio (T/M) of α-meGlc rose well over 1.0, indicating active transport under these conditions. 2-dGal, however, is not reabsorbed from the lumen (Pritchard and Kleinzeller, Bull. MDIBL, in press, 1976) and was not found to accumulate to any greater degree in tubules incubated in Ca-free medium.

Phlorizin reduced the space of all three sugars to values close to that of the extracellular space. A further effect of Ca-free medium was to increase the water and Na content and to decrease

TABLE 1

Effect of Calcium-Free Medium on Total Sugar in Teased Tubules from Flounder Kidney

Sugar	Total Sugar Uptake (μmoles/g wet weight)	
	Control	Ca-free
Methyl-α-D-glucoside	0.26 ± 0.01	0.59 ± 0.05
Galactose	0.84 ± 0.03	1.16 ± 0.03
2-deoxy-galactose	1.52 ± 0.05	1.40 ± 0.08

Groups of tissue (4-5) were incubated aerobically (air) at 15°C for 60 minutes in normal (1.4 mM Ca) and Ca-free salines containing 0.5 mM of the respective sugars.

the K content of the cells. None of the above treatments significantly affected the T/M for the extracellular marker, i.e., polyethylene glycol (PEG).

The active transport of most sugars at the brush border is dependent upon the maintenance of a Na gradient; dissipation of this gradient inhibits this active cellular accumulation of the sugar. Accordingly, teased tubules were incubated in Ca-free medium containing 0.5 mM ouabain. Figure 1 shows that ouabain eliminates the active α-meGlc accumulation previously demonstrated in tubules incubated in Ca-free saline. Ouabain also caused a reduction of the α-meGlc accumulation in tubules incubated in normal saline, thus indicating that a portion, but not all, of the control sugar uptake values was Na dependent. It is concluded that incubation of tubules in

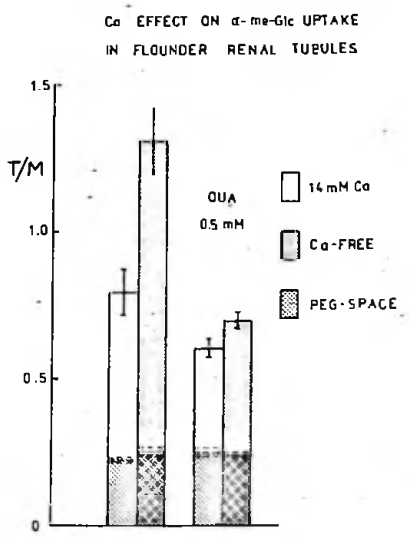

Figure 1. Effects of Ca^{++} on the uptake of methyl-α-D-glucoside by teased tubules of the winter flounder. For conditions of incubation see legend to Table 1. Ordinate: Tissue:Peritubular Medium for the sugar and PEG ± S.E. (N=5).

Ca-free saline increases the population of tubules which have both faces exposed to the medium and so causes a greater tissue accumulation of any sugar which is reabsorbed by an active process at the luminal brush border. Further work will show whether Ca-free incubation exerts similar effects on other sugars, specifically D-glucose, 2-deoxy-D-glucose, and mannose. If so, this technique, in concert with clearance studies and in vitro incubation of teased tubules in normal Ca-containing saline, will provide a means for determining the loci of the various sugar transport systems of the flounder kidney.

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ORGANIZATION OF THE HAMSTER RENAL PELVIS

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The mammalian renal pelvis is an epithelial-lined hollow invagination of the kidney which opens at the point where the ureter meets the renal parenchyma. Both the shape and size of the pelvis vary among mammals. As the urine is discharged from the papilla tip in rodents it is forced retrograde into the pelvis by the muscular contractions of the upper ureter. The urine is then in contact with the papillary epithelium which has previously been shown to be highly permeable to urea and water (K. H. Gertz, B. Schmidt-Nielsen and D. Pagel, Fed. Proc., 25:327, 1966). The possible role that the renal pelvis may have in the concentration of the final urine has been studied by Schutz and Schnermann (Pflügers Arch., 334:154-166, 1972).

Mammals placed on reduced dietary protein excrete a decreased fraction of filtered urea compared to those on a normal protein diet (B. Schmidt-Nielsen, Physiol. Rev., 38:139-168, 1958). Sheep and camel exhibit this urea conservation mechanism most profoundly while rat and man appear to be moderate urea conservers. It appears that mammalian species with a more extensive pelvis conserve urea most efficiently. To test the possibility that the upper extensions of the pelvis may be the site of the urea conservation mechanism we have begun by defining the anatomy and ultrastructure of the "high protein" hamster pelvis which will later be compared to the morphology of the hamster pelvis during urea conservation.

The cortex facing the urinary space in the lower half of the pelvis is covered by the expanded portion of the ureter, the pelvis wall (Figure 1). The transitional epithelium lining the lower pelvic wall is morphologically similar to the epithelium lining the ureter and bladder and has been shown to be impermeable to urea (R.M. Hicks, J. Cell Biol., 28:21-31, 1966). Eight scallop-shaped segments of the kidney parenchyma, the peripelvic columns (PPC) which are exclusively inner strips of the outer medulla, project into the pelvic space (Figure 2).

Adjacent PPC are separated by an interlobar artery and vein. At their upper extent (away from the hilus) each PPC fuses with the neighboring PPC to form a blind tunnel (the fornix) over the interlobars. The PPC are covered by a single layer of low cuboidal epithelium. The fornix region exposes the outer strips of the outer medulla and also the cortex to the pelvic urinary space. Both of these tissue zones are covered by a flattened cuboidal to squamous epithelium which is significantly thinner than the epithelium covering the PPC (inner stripe of outer medulla). All PPC fuse in the center of the pelvis from which extends the papilla (inner medulla). Cuboidal