of methylglucoside fluxes in the flounder and rabbit, Naftalin and Kleinzeller (Am. J. Physiol. 240:G392-G400, 1981) suggested that the number of such transport sites was decreased in the flounder relative to mammalian systems. A further examination of this proposal was undertaken.

The oxidation of ^{14}C-U-glucose to $^{14}CO_2$ by intestinal mucosal strips was found, in part, to be inhibited by a) 0.5 mM ouabain, b) 0.5 mM phlorizin and c) absence of sodium (choline-saline). Cellular glucose uptake is also known to be inhibited by phlorizin and ouabain. Previously we reported (Bull. MDIBL 21:62, 1981) a phlorizin and ouabain inhibitable stimulation of the I_{sc} following addition of glucose analogues to the mucosal surface of intestinal epithelia bathed in chloride-free saline. These results are consistent with a mucosal glucose uptake system demonstrating the same ionic requirements and inhibitor sensitivities as the sodium-glucose co-transport described in other organisms.

Mucosal addition of L-leucine to short-circuited flounder intestine induces an increase in a serosally directed current which is evident in the presence of the tissue chloride current (mean increase of 13.3 ± 3.2 μA cm^{-2} in Cl^--free saline; 46.5 ± 12.6 μA cm^{-2} with Cl^-). A kinetic analysis of the effect of increasing concentrations of the amino acid on the short-circuit current reveals a V_{max} value (50.0 ± 6.6 μA cm^{-2}) approximately 20 times greater than that of methyl-α-glucopyranoside (2.4 ± 0.9 μA cm^{-2}) suggesting that the sugar transport sites were fewer in number than those for amino acids. This is not unexpected in view of the proteinaceous nature of the organism's diet. The number of glucose transport sites present, as estimated by the specific binding of ^{3}H-phlorizin, was determined to be 7×10^{15} sites per gram dry weight of intestinal mucosa.

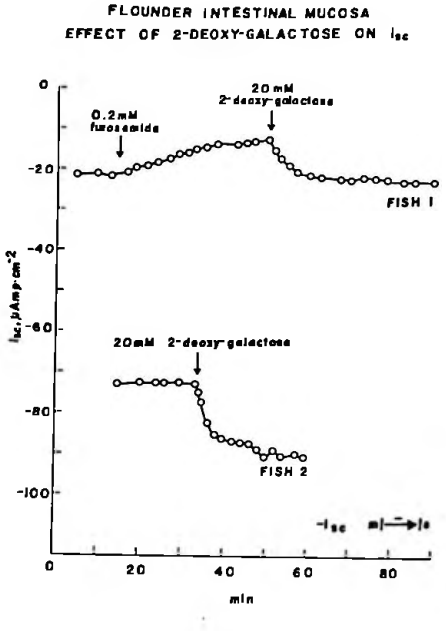

Figure 1.--Effect of serosal application of 2-deoxygalactose (20 mM mannitol added to mucosal solution to maintain osmotic balance). Representative traces.

2.--2-DEOXY-GALACTOSE TRANSPORT--In addition to its absorptive capacities, flounder intestine is known to secrete the sugars galactose and 2-deoxy-galactose. This secretion process is inhibited by serosal application of ouabain or phloretin. Addition of 2-deoxy-galactose to the serosal surface of short-circuited intestinal mucosa bathed in Cl^- containing saline results in an increase in a mucosally directed current (20 mM induced ΔI_{sc} - 8.2 ± 0.7 μA cm^{-2}) Figure 1. This response did not appear to be saturable with increased concentrations of the sugar (1 to 40 mM). Preliminary experiments indicate that removal of chloride abolishes the stimulatory effect of the sugar on the I_{sc}. Efforts to correlate the increased I_{sc} with ionic fluxes across the tissue have failed so far to deminstrate any significant effect of the sugar on the unidirectional movements of the tracers ^{22}Na, ^{86}Rb or ^{36}Cl. This study was supported in part by NIH Grant AM 12619 and by the Whitehall Foundation.

INTERSTITIAL CELLS IN THE INNER MEDULLA OF THE HAMSTER KIDNEY: ARE THEY BEGINNING LYMPHATICS?

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In the inner medulla of the kidney, fluid reabsorbed from the collecting ducts must be removed to maintain a constant water content of the tissue. It is currently believed that this reabsorbate is quantitatively removed via the

capillaries and vasa recta (Jamison, The Kidney, Eds. Brenner and Rector, pp391-441, 1976). It is further believed that no interstitial fluid is transported from the inner medulla to the renal lymphatics (Kriz, Am. J. Physiol., 241: R3-R16),). Movement of interstitial fluid and production of lymph in any organ stops if the organ is kept immobile. Conversely, if an organ such as the renal papilla, is in continuous motion, we can expect interstitial fluid to be propelled along. Our earlier studies (Schmidt-Nielsen et al., Kidney Internatl. 18:419-431, 1980; Reinking and Schmidt-Nielsen, Kidney Internatl. 20:55-60, 1981) have shown that the mammalian renal papilla is continuously milked by the peristaltic contractions in the renal pelvic wall. We have also shown that tubular fluid, as well as vasa recta blood, moves readily toward the outer medulla in both descending and ascending limbs of the loops of Henle and vasa recta (Schmidt-Nielsen and Graves, Kidney, Internatl. 22:413-625, 1982). Consequently, we can also expect interstitial fluid to be propelled in the same director, by the renal pelvic contractions.

I have earlier described our observations of movements of dyes through the renal medulla (Schmidt-Nielsen, Fed. Proc. 38:2493-2503, 1977). A number of new observations now implicate the interstitial cells (C) in conducting this interstitial fluid toward the outer medulla.

To trace interstitial fluid movements, we have used positively and negatively charged markers of various molecular sizes (Table 1). The protocol was as follows: In the anesthetized hamster, the right kidney was exposed

TABLE 1 MARKERS USED

	FW	Charge
Alcian blue	1,299	+
Ruthenium red	786	+
Lissamine green	577	-
Evans blue	961	-
Ferritin (AF)	=400,000	-

and the fat cleared from the renal pelvis. Then, a small opening was made in the pelvic wall to expose the terminal 50 μm of the papilla tip. Through this opening, a micropipette, filled with a dye or mixture of dye and electron-dense marker, was introduced and the pelvic epithelium punctured. The marker was injected into the interstitial space with great care. Using 200X magnification we could distinguish whether the marker entered the interstium, collecting ducts, tubules, or capillaries. In some instances, a marker was injected through the renal pelvic wall into the papillary tip. This latter procedure had the advantage that the pelvic peristalsis was maintained intact but, the disadvantage that the injection was blind and that some of the marker might enter tubules or capillaries.

When dyes such as lissamine green or Evens blue (which in contrast to Alcian blue do not bind to tissue components) were injected into the papillary interstitium, the dye could be seen in vivo to distribute itself diffusely throughout the interstitium and move toward the outer medulla. Approximately 5 to 10 seconds after the end of the injection, the dye was no longer visible in the tissue, indicating that it had been removed. As for lissamine green the dye could presumably have been removed via the capillaries since the molecular size is small enough for the dye to traverse the capillary walls. For Evans blue, which birds to proteins, it is also possibly, but a little less likely that the dye should be quantitatively removed via the capillaries.

In light micrographs (LM) of sections from papillas (fixed in situ through the pelvic wall, Schmidt-Nielsen and Graves, Kidney Internatl. 613-625, 1982) immediately after the injection, all markers (that can be detected by LM) were seen in the interstitium several hundred micrometers above the site of injection. However, in the case of Alcian blue and Ruthenium red, the markers were also seen within the capillaries. This finding was verified on electron micrographs. Consequently, we had no evidence that the markers had been transported in interstitial spaces rather than within capillaries. To avoid this ambiguity we used markers with larger molecular sizes such as ferritin. When ferritin was injected, it was found to be concentrated within special extracellular

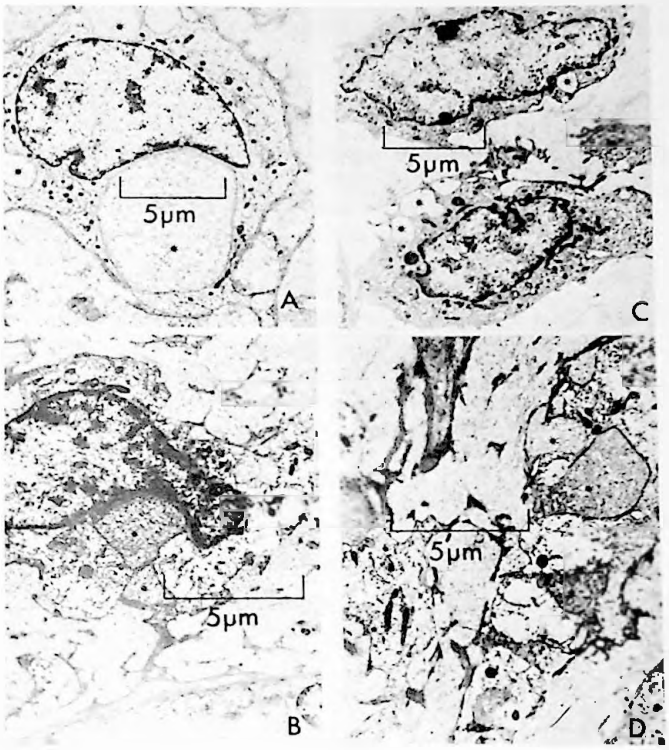

Figure 1.--The IC spaces in a renal papilla that was snared (fluid movement into and out of papilla stopped) when the pelvic wall was in the relaxed state. A and B show cross sections of interstitial cells. Some of the IC spaces are marked with asterisks. C and D show IC spaces revealed by longitudinal sections of the papilla. Note that the spaces are often open to the interstitium. Note also that the interstitial cells have an incomplete basal lamina.

areas almost totally enclosed within interstitial cells (IC). These areas had been noticed in earlier experiments (Schmidt-Nielsen and Graves, Kidney Internatl. 22:618-625, 1982). When the renal pelvic wall was relaxed, these extracellular spaces (IC spaces), were particularly prominent (Figure 1). The IC resemble cells of lymphatic vessels in that they have an incomplete basal lamina. Serial 1 μm sections for light microscopy through several hundred μm of the renal papilla showed that the IC were usually about 14 μm longitudinally. Furthermore, the cells have extensive projections, reaching in all directions through the interstitium. The extracellular spaces surrounded by the cells appear open to the interstitium, forming a network through the cell which continue from cell to cell.

The finding that ferritin was concentrated specifically in these IC spaces (Figure 2) suggested that they conduct papillary interstitial fluid toward the outer medulla. When ferritin was injected through the intact renal pelvic wall for 30 sec and the renal papilla snared (i.e. all fluid movement to and from the renal papilla stopped instantaneously) 15 sec after the end of the injection, the ferritin was again found in the IC spaces, but in a much lower concentration. It was found in sections 200, 300, and 400 μm from the papilla tip (Figure 3).

In an attempt to visualize the pathway the fluid carrying the tracer must take, as it travels through the inner medulla, we have made longitudinal and cross sections from the same areas throughout the inner medulla. Below 750 μm from the tip, the cells appear rather randomly scattered throughout the interstitium. The cell bodies are about as tall as they are wide (10-16 μm without the extensions). Above 750 μm the cells are only about 2-4 μm tall and are arranged between the capillaries or vasa recta as rungs in a ladder. In cross sections, however, they are as wide in all directions as the cells nearer the tip. In all areas the cells have long and elaborate extensions and appear to communicate extensively with one another. In the sections nearer the border between inner and outer medulla, they are particularly prominently arranged within the vascular bundles. It is interesting to note that it was in these areas Alcian blue was seen in our earlier study (Schmidt-Nielsen, Fed. Proc. 36:2496-2503, 1977).

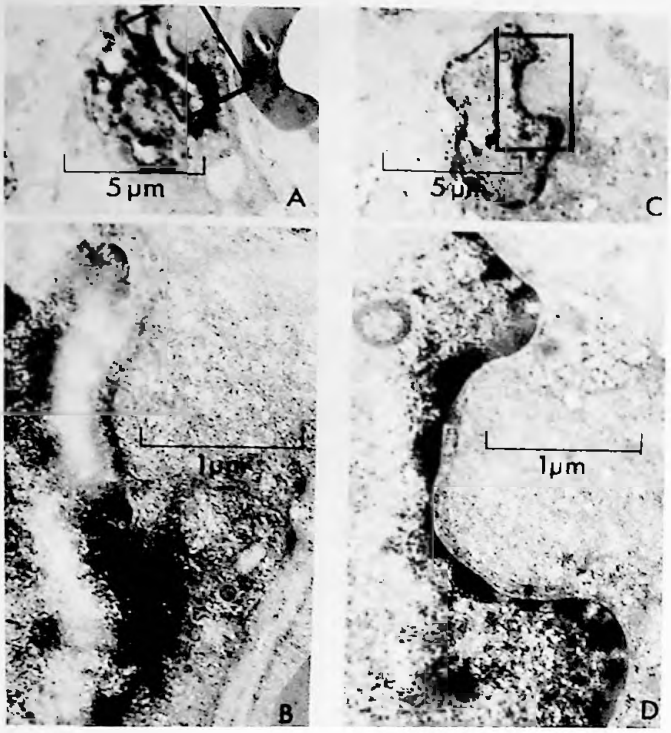

Figure 2.--The IC spaces in a renal papilla, with an opened pelvis, that was microinjected with ~50 nl of a 10% ferritin solution for about 1 min prior to the snaring of the papilla. A and B show the same cell at two different magnifications. These cross sections were taken 250 µm from the tip of the papilla. The small black ferritin granules are seen to be concentrated in the IC spaces. C and D also show one cell at low and at high magnification, respectively. These cross sections are 300 µm from the tip of the papilla. Again the IC spaces are filled with ferritin granules.

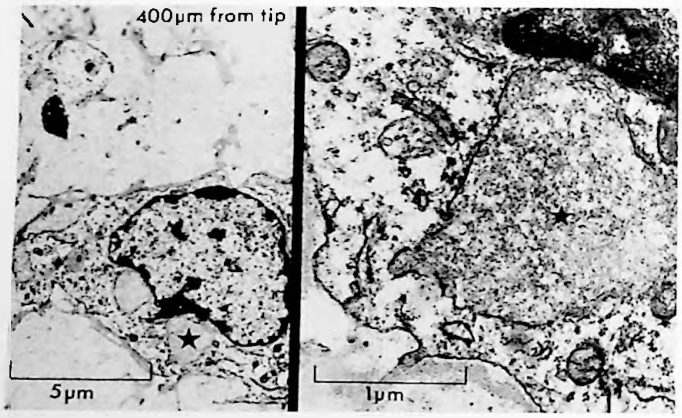

Figure 3.--The IC spaces in a renal papilla injected through the intact renal pelvic wall with approximately 50 nl of a 10% ferritin solution. The injection lasted 30 sec, the papilla was snared and fixed 15 sec after the end of the injection. The figure to the right is a higher magnification of the IC space shown in the figure to the left. The ferritin granules were sparse in this section as well as in the sections below. This may indicate that the ferritin has been rather effectively removed in this papilla. The removal has probably been facilitated by the peristaltic contractions of the intact renal pelvic wall.

The IC of the inner medulla have not previously been implicated in fluid transport. They are known to be the site of prostaglandin synthesis. Thus, in both rats and rabbits about half of the medullary synthesis of PGE_2 takes place in the IC. Churchill et al (Fed. Proc. 41: pX, 1982) reported that in antidiuretic rats the IC have almost 70% higher exectrolyte concentrations than the surrounding interstitium and collecting duct cells. At this time it is not known if this high electrolyte concentration is involved in moving fluid into the IC spaces. An important question to be answered is whether the IC spaces connect with the lymphatics at the border between the outer medulla and cortex. Our current experiments are addressing this point.

CORNEAL WOUND HEALING IN SCULPIN AND SHARK

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Corneal epithelial wound healing in mammalian species has been quantified and occurs by epithelial cell