We have first determined that it is justified to employ $^{86}Rb^+$ fluxes as a measure of E^+ fluxes: The 5 min influx of the label was taken to approximate the zero-time flux. Mean values, ± S.E.M. (5 measurements): $^{42}K^+$ influx: 2.97 ± 0.13 μmol/g tissue wet wt. (W.W.): $^{86}Rb^+$: 2.52 ± 0.07 μmol/g W.W. Within the limits of experimental error the fluxes of $^{42}K^+$ and $^{86}Rb^+$ are thus commensurate.

We measured the efflux of $^{86}Rb^+$ from slices preloaded with the label by 30 min aerobic incubation (standard saline with 1 mM $^{86}Rb^+$, 0.1 μCi/ml). The wash-out of the label from the blotted slices was followed a) in standard media; b) urea-free media. The efflux curves were resolved into two cellular components: Isotonic medium (890 mosM): $P = 0.33.e^{-7.2\,t} + 0.67.e^{-0.20\,t}$; urea-free (hypotonic) medium, 550 mosM: $P = 0.32.e^{-7.5\,t} + 0.68.e^{0.123\,t}$. The significance of the difference between the rate constants in the control and hypotonic saline for the fast efflux component is further documented in Figure 1; $p < 0.001$ (two experiments) for the slower rate constants. Hypotonicity thus decreases the efflux of both components.

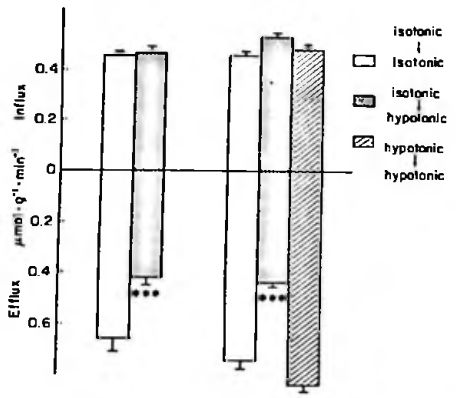

Figure 1.--Effect of saline tonicity on $^{86}Rb^+$ fluxes in slices of the dogfish rectal gland. Left pannel: Mean values, ± S.E., for 5 fish. Right pannel: 1 fish; mean values, ± S.E., of 5 measurements.

The zero-time (5 min) unidirectional fluxes of $^{86}Rb^+$ were then measured: The influx corresponds essentially to the operation of the sodium pump; the efflux is a measure of the K^+ "leak". It was assessed that under the given experimental conditions the contribution of the label in the extracellular tissue space introduced not more than a 5% error. Figure 1 (left pannel) shows that the change from isotonic to hypotonic media did not affect the $^{86}Rb^+$ influx, demonstrating that hypotonicity does not affect the Na^+-pump. On the other hand, hypotonicity significantly ($p < 0.001$) decreased the efflux of $^{86}Rb^+$ from the tissue, showing a reduction of the permeability of the K^+ channel.

The above data provide an explanation for the KCl accumulation in tissue incubated in hypotonic media: A decrease in K^+ efflux, at a constant influx, does indeed produce an increase in cell K^+.

An explanation was sought for the observation that KCl uptake by the tissue stops with the urea efflux, when also the electrochemical gradient of K^+ has reached that in the control. Therefore, zero-time unidirectional fluxes of $^{86}Rb^+$ were also measured after the tissue reached a new steady state in the hypotonic medium. Figure 1, right panel, shows that the influx was not affected during the whole procedure. On the other hand, the efflux, first reduced on transfer of the tissue from isotonic to hypotonic medium, returns at steady state to the value seen in the controls.

The data clearly demonstrate that medium hypotonicity produces a temporal decrease in the $^{86}Rb^+$ efflux, which disappears once the tissue has reached a new steady state of H_2O and electrolytes. Obviously, the cell volume control mechanism is not triggered by the actual cell volume, but by the changes in the osmotic or electrochemical gradients across the cell membrane. This study was supported in part by NIH Grant AM 12619, and by the Whitehall Foundation.

SUGAR TRANSPORT BY THE INTESTINAL MUCOSA OF THE WINTER FLOUNDER (Pseudopleuronectes americanus)

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Studies on the intestinal transport of sugars by the intestine of the flounder have been continued.

1.--GLUCOSE TRANSPORT--Previous reports have suggested that the conventional glucose-sodium cotransport is operative in the intestinal epithelial cells of the flounder (Pseudopleuronectes americanus) in spite of the fact that no net glucose transport or glucose stimulation of the short-circuit current (I_{sc}) had been found. Based on a comparison

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 $\mu A\ cm^{-2}$ in Cl^--free saline; 46.5 ± 12.6 $\mu A\ cm^{-2}$ 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 $\mu A\ cm^{-2}$) approximately 20 times greater than that of methyl-α-glucopyranoside (2.4 ± 0.9 $\mu A\ cm^{-2}$) 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 ^3H-phlorizin, was determined to be 7×10^{15} sites per gram dry weight of intestinal mucosa.

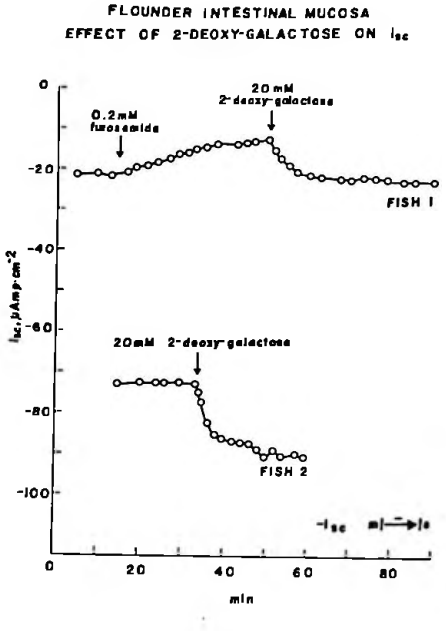

Figure 1.--Effect of serosal application of 2-deoxygalactose (20 mM mannitol added to mucosal solution to maintain osmotic balance). Representative traces.

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 $\mu A\ cm^{-2}$) 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