small apical vesicles.

In all of our preparations there are numerous membrane bound profiles in the lumens of the secretory tubules. These may be artefacts of the fixation procedure or errors in the fusion of apical vesicles with plasma membrane. They are present in zero time control preparations and after ovabainperfusion as well as in the stimulated and control perfused glands. We do not suggest that a large portion of the secretory product is discharged by pinching off of apical membrane. If, however this process occurs normally, some residue of membrane as well as the secreted mucin should be detectable in rectal gland fluid.

The occurrance of mucin synthesis in these cells, and in the *Squalus* chloride cell invites consideration of its possible relation to salt secretion. These cells are quite unlike the ordinary mucous cells which occur in gills or the very large mucous cells scattered in the excretory duct of the rectal gland. The presence of a polyanionic substance in the Golgi of pancreatic acinar cells has been suggested by Palade (Science 189, 347, 1975) as a condensing mechanism, trapping cationic proteins and eliminating their osmotic effect, thereby allowing water to return to the cytoplasm. Polyanions have also been reported within the tubular reticulum of teleost chloride cells (Philpott and Copeland, J. Cell Biol. 18, 389, 1963).

Although a very large amount of polyanion would be required to trap the amounts of sodium present in rectal gland fluid, one wonders why this substance is synthesized in these situtions and whether it may play an auxiliary role in the production of hyperosmotic saline secretions. Further work on the detection and characterization of a mucin in rectal gland fluid is warranted. The evidence for reabsorption of tubular secretion by the excretory duct also indicates the need for micro puncture analyses. Supported in part by a grant from the National Science Foundation BMS 74-03529.

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The Effect of Phlorizin and its Derivatives on the sugar uptake by teased renal tubules of the winter founder (*Pseudopleuronectes americanus*).

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Phlorizin and its aglycone derivative phloretin have been used for some time in investigating the characteristics of carrier-mediated sugar transport systems. Accordingly, the effects of phlorizin, phloretin, and 2-deoxy-glucosyl phloretin on the sugar transport systems of teased flounder tubules were investigated. The uptake of sugars in this preparation predominately reflects transport at the basolateral face. Past research (Kleinzeller & Mullin, *Bull. MDIBL 15,* in press) has indicated the existence of three separate sugar transport systems: one shared by glucose, 2-deoxy-glucose and mannose (Glc-2dGlc); a second shared by galactose and 2-deoxy-galactose (Gal-2dGal); and a third system for the transport of φ-methyl-glucoside and glucose. Each system has been shown to be passively-equilibrating and to possess a characteristic structural specificity.

Teased tubules were incubated with 0.5 mM labelled sugar and 0.05 mM, 0.10mM, and 0.50mM concentrations of the three inhibitors. Phloretin, phlorizin, and 2-deoxy-glucosyl phloretin produced significant inhibition of the uptake of glucose, 2-deoxy-glucose, galactose, and 2-deoxy-galactose. In all cases, it was a decrease in the phosphorylated sugar which primarily accounted for the inhibition of sugar uptake; even at high concentrations of the inhibitors the apparent concentrations of cellular free sugars did not vary significantly from control values (Figure 1). Only in the case of 2-deoxy-galactose did the inhibitors cause any appreciable decrease in free sugar.

In the Gal-2dGal system, 0.05mM phloretin was more inhibitory than phlorizin or 2-deoxy-glucosyl phloretin. At high concentrations all three inhibitors had approximately the same effect on the transport of both galactose and 2-deoxy-galactose. The same relative potency of the inhibitors was seen in the Glc-2dGlc system. In both systems 0.05mM phlorizin significantly inhibited glucose and galactose uptake while it required 0.10 mM phlorizin to appreciably inhibit the uptake of the 2-deoxy derivatives. None of the inhibitors produced significant changes in the extracellular (PEG) space.

To assess the affinity of the inhibitors for the sugar transport sites, the tissue was pre-incubated with and without 0.5mM phlorizin for 30 minutes, washed in physiological saline for 2 minutes, and then incubated with or without 0.5mM phlorizin and with 0.5 mM labelled 2-deoxy-glucose. Tubules incubated with phlorizin showed the characteristic decreased sugar accumulation. Conversely, tubules which were only pre-incubated with phlorizin and then washed showed no inhibition of sugar uptake as compared with the control values (See Table II).

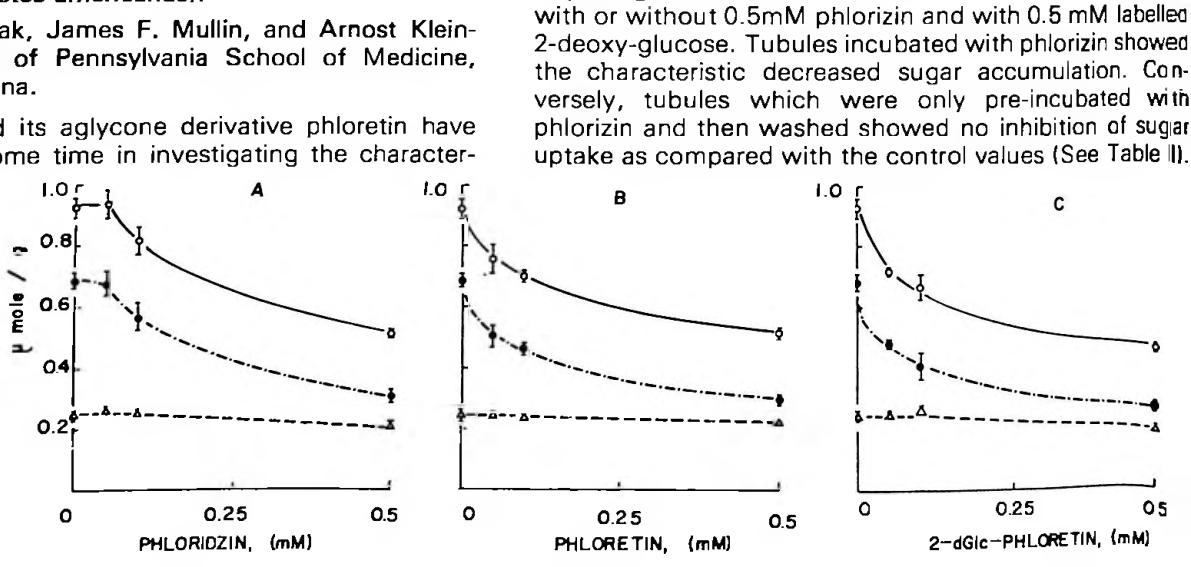

Figure 1

Table 1

Effect of Pre-Incubation and Incubation with Phlorizin on the Uptake of 2-deoxy-Glucose (2-dGlc) by Teased Tubules of Flounder Kidney.

ach figure represents the mean of five values, ±S.E.

re-Incubation medium	Incubation medium	2-dGlc accumulated (μmoles/gram wet weight) Total	Free	Phosphorylated
aline	Saline + 0.5 mM 2-dGlc	1.203 ±0.044	0.329 ±0.015	0.874 ±0.048
aline	0.5 mM Phlorizin + 0.5 mM 2-dGlc	0.556 ±0.015	0.260 ±0.014	0.297 ±0.045
.5 mM Phlorizin	Saline + 0.5 mM 2-dGlc	1.209 ±0.086	0.277 ±0.045	0.932 ±0.086

hese findings indicate a low binding affinity of the hibitor for the sugar carrier systems.

Two observations arise from these experiments. rst, all of the inhibitors affected sugar accumulation by ecreasing the amount of phosphorylated sugar rather an the amount of free sugar accumulated. Due to their ze and their common phenol moiety, there should be ppreciable entry of the inhibitors into the cell. Phosorylation of sugars has generally been considered an tracellular event. The present data, however, are not asily explained by this assumption; the possibility that hosphorylation may be intrinsically related with the tual translocation step must be considered.

Another set of observations concerns the relative finities of the phloretin-base inhibitors for the sugar ransport systems. There are three points to be seen in e present data. First, the binding of the inhibitors to the ugar transport systems is a low affinity type binding. econd, the inhibitors bind non-specifically to the various ugar transport systems. Thus, phlorizin and 2-deoxyucosyl-phloretin inhibit uptake not only in the Glc-2-dGlc pathway but also in the Gal-2dGal pathway. hloretin which completely lacks a sugar moiety also hibits transport in both systems. It would be reasonable o presume that it is the phenol moiety of these inhibitors hich engages in a non-specific binding to the sugar ransport systems. Finally, at low concentrations (10^{-4}M), hloretin is a more potent inhibitor than either of its lycoside derivatives.

These three observations correlate with previous ndings on the general effects of phlorizin and phloretin n sugar transport in a number of physiological systems. hlorizin has been found to be a much more potent hibitor (than phloretin) of actively transporting, Naependent sugar reabsorption whereas phloretin is enerally a greater or an equally potent inhibitor of Nadependent, passively equilibratsystems (e.g., edrytocytes). Thus Pritchard et al. (*Bull. MDIBL 14*: 89, 974) in clearance studies (which reflect transport events the luminal face) on the winter flounder, found that hlorizin was a high affinity inhibitor of the Na-dependent, active transport of φ-methyl glucoside. Similar very w concentrations of the inhibitor (25 μM) had no effect n the passively equilibrating transport of 2-deoxylucose in the same clearance studies.

The data from these experiments indicated a low-affinity, non-specific inhibition of sugar transport by phloretin and its glycoside derivates at the anti-luminal face of renal tubules. Such findings are consistent with previous findings concerning the effects of these inhibitors on passively equilibriating sugar transport systems. Similar effects of phloretin have been observed concerning the entry of sugars at the basolateral face of intestinal mucosa. (Kimmich, G. and Randles, J., *J. Membr. Biol. 23:* 57, 1975; Bihler I., in *Intestinal Permeation,* Proc. of Workshop Conference, Schloss-Reisensburg, 1975, Excerpta Medica, in press). The primary action of these inhibitors on the phosphorylated form of the sugars raises the possibility that phosphorylation may be an intrinsic part of the translocation mechanism.

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Adaptation to Freshwater by *Anguilla rostrata*

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During adaptation to seawater over a period of several days, sodium efflux across the gill of *Anguilla rostrata* slowly rises in parallel with a rise in Na-K-ATPase activity in gill homogenates (*Am. J. Physiol., 224,* 709-713, 1973). Adaptation to freshwater, on the other hand, has not been well studied, and we therefore undertook to correlate sodium efflux with gill content of Na-K-ATPase at intervals after specimens of *Anguilla rostrata* already adapted to seawater were transferred to freshwater.

Na-K-ATPase activaity in whole homogenates of gill filaments and sodium efflux (using ^{22}Na) were measured as previously described. Sodium efflux experiments with eels adapted to either sea water or freshwater were carried out in a seawater bath since even fully acclimatized seawater eels have negligible sodium efflux when immersed in freshwater.

Tracer quantities of ^{22}Na were injected intraperitoneally and allowed to equilibrate while the fish remained immersed in 1000 ml of aerated seawater at 16°C. The appearance of ^{22}Na in the external medium was then monitored over the next 30-60 minutes, and a sample of plasma was taken for measurement of stable and isotopic Na at the conclusion of the experiment.

The activity of Na-K-ATPase in gill homogenates of seven eels adapted to seawater for at least three weeks was 19.0 ± 1.4 μMPi/hr/mg protein (mean ± s.e.) and sodium efflux across the gills of intact fish averaged 857 ± 112 μEq/hr/100 gm. In eels acclimatized for at least one month in a freshwater pond the corresponding values were: Na-K-ATPase, 5.4 ± 0.6 μMPi/hr/mg protein and Na efflux, less than 50 μEq/hr/100 gm.