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.

When eels fully adapted to seawater are transferred directly to fresh water, serum sodium falls within 24 hours from the seawater level (156 ± 2.5 μEq/L, n = 6) to a lower value (146 ± 0.97, n = 4) characteristic of eels fully-acclimatized to freshwater (148.9 ± 3.1., n = 5), and does not change significantly thereafter. The changes responsible for freshwater adaptation therefore appear to be thrown into play rapidly. Exposure of seawater adapted eels to freshwater resulted in a rapid and profound reduction of sodium efflux without a corresponding reduction in the activity of Na-K-ATPase in gill homogenates. The latter remained elevated at levels characteristic of seawater fish for as long as 9 days after transfer to freshwater. A striking decrease in sodium efflux to 115 ⊞ 77 μEq/hr/100 gm (n = 4) was evident after only two hours of immersion in fresh water, though one hour of exposure to freshwater did not greatly affect sodium efflux (629 ± 77 μEq/hr/100 gm, n = 4). After one day of immersion in fresh water, sodium efflux in each of four eels was uniformly less than 50 μEq/hr/100 gm.

It seemed possible that the turn-off of sodium efflux might be mediated by prolactin, necessary in other euryhaline specied to maintain life in freshwater. Additional experiments were therefore carried out with 2-bromo-alpha-ergocryptine (CB154, Sandoz), an ergot derivative that has been shown to inhibit the release of prolactin in mammals. It is not known, however, whether the drug has a similar action in teleosts. Eight mg per 100 gm of body weight were injected intraperitoneally daily for one or two days before transfer to freshwater. Though this dose approached the toxic level it was ineffective in preventing the reduction in sodium efflux across the gill of *Anguilla rostrata* induced by a two-hour or one-day exposure to freshwater.

An important mechanism responsible for adaptation to freshwater, therefore, appears to be the capacity to turn off promptly the efflux of sodium across the gill. The change is stimulated by brief exposure to a hypotonic environment. It is not associated with a reduction in the activity of gill Na-K-ATPase. Since all measurements of efflux were carried out in a seawater bath, exchange diffusion of sodium, known to represent 80-90% of total sodium efflux under these circumstances, must have been affected. Although the time course of the response is consistent with hormonal mediation, it was not blocked by bromoergocryptine and further experiments will be necessary to clarify the possible role of prolactin. Dissociation of ion movements from gill Na-K-ATPase activity is a feature of early freshwater adaptation in *Anguilla rostrata*.

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Measurements of CSF-Brain-Blood Transport Rates in *Squalus acanthias*

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Perfusions and injections of tracer molecules an ions into the cerbrospinal fluid (CSF) compartment various mammalian species have been performed to stud their respective clearance rates from the CSF (e.g. *Am. Physiol. 203*: 775-781, 1962). Other investigators ha employed one or the other of these approaches alon with brain tissue sampling to determine the size of th brain extracellular space (e.g. *Life Science 1*: 43-4 1962). More recently the CSF perfusion technique ha been used to examine the rate of potassium exchang between extracellular fluid (ECF) and intracellular flu (ICF) (*Brain Res. 38*: 49-69, 1972) and of solute and wate flow between ECF and brain capillary blood (Patlak an Fenstermacher, *Am. J. Physiol*, in press). In the presen report the same two transport steps, ECF-ICF exchang and ECF-blood transfer, were studied in *Squalu acanthias* employing a method of intraventricular injectio for introducing the test substances into the CSF. Fo so-called extracellular markers plus two ions — Na and C — were used. In addition to the two exchange rate which were previously mentioned, the CSF disapperanc time, the ependymal permeability constant, and the bra ECF diffusion coefficient for all of these solutes wer measured from the data.

Dogfish, weighing from 2 to 4 kg, were placed in a large circular holding tank prior to beginning each experiment. While gently holding the fish at the side of the tank, a 20 gauge needle, which was attached to a 1.0 m syringe by about 6 inches of polyethylene tubing, was advanced through the skull and the underlying cerebellum until the tip reached the cerebellar ventricle. A sma volumer (0.2-0.3 ml) of fluid was withdrawn into the syringe, the syringe disconnected from the tubing, and the fluid tested for the presence (extradural fluid) o absence (CSF) of protein. If the aspirated fluid was CSF a second syringe which contained 0.3 ml of dogfish saline and two radioactively tagged materials was attached to the tubing and the solution injected into the cerebell ventricle. Mixing of the injected fluid throughout the ventricular system was accomplished by withdrawing and reinjecting the ventricular fluid (CSF plus injectate) three or four times. After the mixing was completed, the needle was removed and the fish were allowed to swim freely. At various times (1.0, 2, 3, 4, 8, and 20 hr) after making the injection, the fish were taken from the water, a sample o CSF obtained, and the brain removed. Subsequently the medulla was cut, starting at the ventricular or ependyma surface, into a series of 0.25 or 0.5 mm thick slices b means of a freezing microtome. The slices were weighed and analyzed for radioactivity by liquid scintillation spectroscopy. The following radioactivity labeled mater ials were used: ^{14}C-ethylenediaminetretracetic acid ^{3}H)sucrose, ^{3}H-mannitol, ^{22}Na, and ^{36}Cl.

The data which were obtained for a particular compound or ion were compared to computer-generated data for a CSF-brain-blood transport model system such as that employed by Pape and Katzman (*Brain Res. 38* 49-69, 1972). The parameters which were varied are listed as column headings in Table 1; the results of the comparative analysis of the dogfish medullary slice dat