other work (Czech, J. Biol. Chem., 248:3636-3641, 1973) that CyB acts on sugar transport at the membrane level and our studies show that CyB primarily affected the accumulation of sugar phosphate, it is reasonable to consider the possibility that sugar transport at the antiluminal face of renal cells involves a phosphorylating mechanism. The possibility that the inhibition of sugar uptake was related to an inhibition of cellular energy metabolism is unlikely in view of a lack of effect on tissue water and ionic distribution.

The sulfhydryl reagents PMB and PCMBS have been shown to be inhibitors of sugar transport in erythrocytes (Smith and Ellman, J. Memb. Biol., 12:177-188, 1973). In the teased tubule preparation both PMB and PCMBS inhibited the uptake of all sugars tested; PCMBS was consistently a more potent inhibitor than PMB. Like CyB, these inhibitors primarily affected the tissue levels of the respective sugar phosphates. A comparison of the inhibitory effects on the various model sugar transport systems showed that PMB and PCMBS were considerably less inhibitory on the Glc-2-dGlc system than on the Gal-2-dGal system; α-meGlc transport had an intermediate sensitivity to thg inhibitors. It would thus appear that the active center of the Glc-2-dGlc system has its reactive sulfhydryl groups in a less accessible position than either of the other sugar transport systems.

FDNB acted as a potent inhibitor of transport in both the Glc-2-dGlc and Gal-2-dGal systems; again it was the accumulation of sugar phosphate which was most affected. FDNB has been demonstrated to inhibit glucose transport in erythrocytes and, in addition, to enter the cells rapidly (Forsling, Remfry, and Widdas, J. Physiol., 194:535-543, 1968). Therefore it is possible to conceive two mechanisms of its inhibitory effect in renal cells (wherein a very high percentage of accumulated sugar is phosphorylated): first, the FDNB could bind to the cell membrane and so directly affect the translocation of the sugar; and second, the FDNB could act either as a respiratory inhibitor or uncoupler and so lead to ATP deletion which would subsequently inhibit the formation of sugar phosphate.

In summary, the various inhibitors used in these experiments affected sugar transport in flounder renal cells in a way consistent with the actions of phlorizin and phloretin: that is, by primarily affecting the accumulation of the phosphorylated form of the sugar.

This study was supported in part by USPHS Grant AM 12619. The assistance of Mr. Jonathan Goldstein is appreciated.

FAILURE OF HYPERTONIC SODIUM INJECTIONS OR EXTERNAL POTASSIUM TO INCREASE CHLORIDE EFFLUX ACROSS THE GILL IN *Anguilla rostrata*

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Active extrusion of chloride by the gill is the mechanism by which seawater teleosts excrete excess salt to maintain a constant internal environment. Chloride efflux in seawater-adapted fish has been reported to rise in response to two stimuli: (1) administration of hypertonic sodium chloride and (2) addition of potassium to a fresh water bathing medium.

The first question addressed in these experiments was whether chloride efflux across the gill responded immediately and automatically to induced hypernatremia. American eels (*Anguilla rostrata*) were adapted to seawater for six weeks. Serum Na averaged 160 mEq/L, serum Cl 140 mEq/L and gill Na-K-ATPase was at levels characteristic of salt-water-adapted fish (14-18 μMPi per mg protein per hr). Chloride efflux was measured over 30 minute periods by injecting 2-3 μCi of ^{36}Cl, allowing it to

equilibrate for 40 minutes, monitoring the appearance of $^{36}Cl^-$ in aerated seawater bath and measuring the specific activity of $^{36}Cl^-$ in plasma at the conclusion of the experiment. After a 30-minute control period, 1.0 M NaCl, $NaNO_3$ or Na acetate was injected intraperitoneally in an amount calculated to raise serum sodium 15 to 30 mEq/L. Chloride efflux was then measured again over the next 30 minutes.

Surprisingly, hypertonicity did not elicit any consistent change in chloride efflux, whether plasma chloride was elevated by the injection of sodium with chloride or slightly lowered by the administration of sodium with another anion. After hypertonic NaCl, plasma Na rose to 186 ± 8 mEq/L and plasma Cl to 166 ± 10 (mean ± s.d.). Chloride efflux before the injection was 1962 ± 655 uEq/hr and afterwards 2081 ± 746 (n=6). With sodium acetate (n=4) plasma Na rose to 192 ± 15 mEq/L and Cl to 131 ± 6; chloride efflux was 1186 ± 244 before and 1148 ± 215 after the salt was injected. After sodium nitrate (n=4) plasma sodium was 175 ± 6 and Cl 130 ± 3, while chloride efflux was essentially unchanged (1450 ± 709 before and 1266 ± 343 after). These data indicate that, at least in *Anguilla rostrata*, chloride efflux does not increase promptly or automatically in response to an elevation of the concentration of sodium or chloride in extracellular fluid.

In the next series of experiments we examined the effect of adding external potassium to fresh water on the efflux of ^{36}Cl and ^{22}Na in *Anguilla rostrata* adapted to seawater. The radioisotope was injected and allowed to equilibrate for 40 minutes while the fish was in seawater. The eel was then transferred to 1 liter of aerated fresh water at 16°C where two 30-minute clearance periods were obtained. Between the first and the second periods, 10 mM KCl was added to the freshwater bath. Sodium efflux was almost doubled by the addition of external K+, from 174 ± 104 to 320 ± 51 (n=6). Chloride efflux by contrast, was unchanged during the second 30-minute period (241 ± 210 in FW vs 210 ± 92 in FW-K, n=6). These results differ from those previously reported in *Anguilla anguilla* (Am. J. Physiol., 224: 1295-1299, 1972) in which addition of K+ to a freshwater bath increased ^{36}Cl efflux within less than ten minutes; it is not yet clear whether the reason for the difference lies in the time course of stimulation by external K+ or whether there is a species difference in this response.

SULFATE-STIMULATED ATPase IN KIDNEY HOMOGENATES OF MARINE VERTEBRATES

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Sulfate is excreted into the urine of all seawater fish in excess of the quantity filtered, presumably by a process of active transport and secretion by renal tubular cells. Since different anions have varying effects on hydrolysis of Mg-ATP by broken-cell homogenates of different tissues (Enzyme, 12:499-507, 1971) it seemed reasonable to ask whether sulfate might stimulate ATP breakdown by membrane fragments of cells that habitually secreted this divalent ion.

Mg-ATPase was determined in sucrose-EDTA-deoxycholate homogenates of kidney tissue as previously described (Am. J. Physiol., 218:607-611, 1970). The concentration of Na+ in the Mg-ATPase medium was 120 mM, present as sodium chloride in the "chloride medium" and as sodium sulfate in the "sulfate medium." The final concentration of Mg-ATP was 6 mM; pH was 7.8, and incubation was for 15 minutes at 37°C. Results are shown in Table 1.

Sulfate stimulated ATPase by about 20% in kidney homogenates of the long-horned sculpin, *M. octodecimspinosus*, a species with few and vestigial glomeruli. However, there was no evidence for sulfate-enhancement of ATP hydrolysis in kidneys of the aglomerular goosefish, *Lophius americanus*. In