Table I

Sulfate- and Chloride-Stimulated ATPase in Kidney Homogenates

	Chloride medium Mg-ATPase µMPi/mg protein/min	Sulfate medium Mg-ATPase µMPi/mg protein/min	p
Sculpin n = 5	16.5 ± 2.3	19.7 ± 2.4	<0.01
FW eel n = 8	15.1 ± 3.2	14.8 ± 3.6	n.s.
SW eel n = 9	15.4 ± 2.0	17.3 ± 3.8	n.s.
Goosefish n = 4	18.1 ± 5.5	20.9 ± 8.8	n.s.
Flounder n = 1	17.2	16.6	
Skate n = 1	23.2	25.0	
Dogfish n = 1	22.5	23.2	

kidneys of the euryhaline American eel, *Anguilla rostrata*, sulfate failed to stimulate ATPase more than chloride, whether the eels had been adapted to seawater (thus secreting large quantities of sulfate) or fresh water (no sulfate secretion by the kidneys). Neither mitochondria nor microsomes prepared from whole kidney homogenates of saltwater-adapted eels demonstrated sulfate ATPase activity. Single experiments in the winter flounder, *Pseudopleuronectes americanus*, the little skate, *Raia erinacea*, and the spiny dogfish, *Squalus acanthias*, also did not show specific stimulation of ATPase in kidney homogenates by sulfate.

These experiments do not support the hypothesis that an ATPase stimulated specifically by the sulfate ion is involved in the active transport of sulfate across renal tubular epithelium. Since sulfate can be substituted completely for chloride without altering Mg-ATPase activity in most kidney homogenates, the data also argue against the concept of a chloride-ATPase concerned with the transport of Cl^- in the kidneys of marine vertebrates.

TRANSPORT OF PAH AND GLUCOSE INTO PLASMA MEMBRANE VESICLES PREPARED FROM FLOUNDER KIDNEY TUBULES

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In the teleost proximal tubule, two active transport steps for organic acid secretion have been postulated, one at the contraluminal or basal-lateral border of the cell and a second at the luminal or brush border membrane (Kinter, Fortschritte der Zoologie, 23:223-231, 1976). The use of isolated plasma membranes now provides a tool for further elucidating these individual transport steps (Berner and Kinne, Pflugers Arch., 361:269-277, 1976). Using membrane vesicles containing predominantly brush border membranes prepared from flounder kidney (Kinne et al., this bulletin) evidence for carrier-mediated transport of both p-amino-hippurate (PAH) and D-glucose was obtained.

A renal homogenate was prepared from the kidneys of winter flounder (*Pseudopleuronectes americanus*) by the method of Kinne et al., (this bulletin). A brush border membrane fraction was isolated by a modification of the method of Booth and Kenny (Biochem. J., 142:575-581, 1974) using a high concentration of calcium. The membranes were suspended in 100 mM mannitol, 2 mM $CaCl_2$, 25 mM Tris-HEPES buffer, pH 8.2, to obtain a protein concentration of around 10 mg/ml. The uptake studies were initiated by adding 20 µl of the membranes, kept at 0°C, to 150 µl of the incubation medium, kept at 10°C, which generally contained 100 mM NaCl, 100 mM Mannitol, 2 mM $CaCl_2$, 18 mM Tris-HEPES buffer, pH 8.2, and 10.8 µM tritiated PAH or 0.11 mM tritiated D-glucose. The incubation period was stopped at different time periods (0.3, 1,2,3,5,20 or 60 min) by diluting a sample of 20 µl with 1 ml ice-cold incubation medium which did not contain PAH or D-glucose. The membrane vesicles were collected by rapid filtration on Millipore filters (pore size 0.45 µ) and washed once with 3 ml incubation medium. Brush border membrane preparations were regularly analyzed for alkaline phosphatase, a marker for the brush border membrane, and for Na^+-K^+-ATPase, a marker for basal-lateral contamination; the enzyme activities were comparable to those obtained by Kinne et al. (this bulletin).

Since sodium-dependent D-glucose transport is generally found in the brush border membrane of renal tubules, this transport process was used as a criterion of functional integrity for brush border vesicles. Uptake of D-glucose by vesicles initially containing no sodium is shown in Figure 1. In the

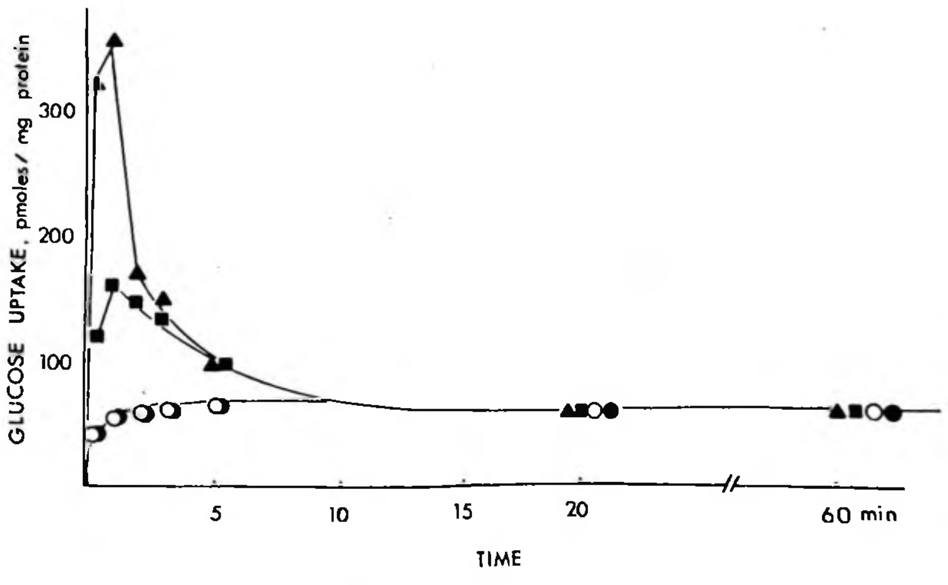

Figure 1. D-glucose uptake into brush border vesicles from flounder kidney tubules. Vesicles were prepared in 100 mM mannitol, 25 mM Tris-HEPES buffer, pH 8.2, 2 mM $CaCl_2$, and incubated at 10°C for indicated time periods in media containing 0.11 mM ^{3}H-D-glucose, 2 mM $CaCl_2$, 18 mM Tris-HEPES buffer, pH 8.2, and generally 100 mM mannitol. In addition the incubation medium contained one of the following: 100 mM NaCl (triangle), 30 mM NaCl plus 140 mM mannitol (squares), 100 mM KCl (open circles), or 0.1 mM phloridzin plus 100 mM NaCl (solid circles). The data points are the means of 3 experiments.

presence of a sodium gradient across the membrane, i.e., between the external incubation medium and the intravesicular space, glucose uptake was transiently stimulated so that the concentration inside the vesicle exceeded the concentration in the surrounding medium. Lowering the sodium gradient to 30 mM NaCl lowered the height of the overshoot and replacing NaCl in the external medium with 100 mM KCl eliminated the overshoot. Phloridzin, a specific inhibitor of carrier-mediated glucose transport, completely inhibited the glucose overshoot but did not affect the equilibrium concentration. These results indicate that, as in brush border vesicles of rat and rabbit proximal tubules (Aronson and Sacktor, J. Biol. Chem., 250:6032-6039, 1975 and Kinne et al., J. Memb. Biol., 21:373-395, 1975), glucose uptake in brush border vesicles of flounder tubules is driven by the electrochemical potential difference for sodium ions across the membrane.

In contrast, PAH uptake did not show the overshoot phenomenon and required longer than glucose to attain equilibrium (Fig. 2). Competitive inhibitors of PAH secretion in intact flounder tubules were tested to determine whether PAH uptake into brush border vesicles could also be inhibited. Chlorphenol red (Fig. 2) and 2,4-dinitrophenol were the strongest inhibitors of PAH uptake, i.e., more than 50% of

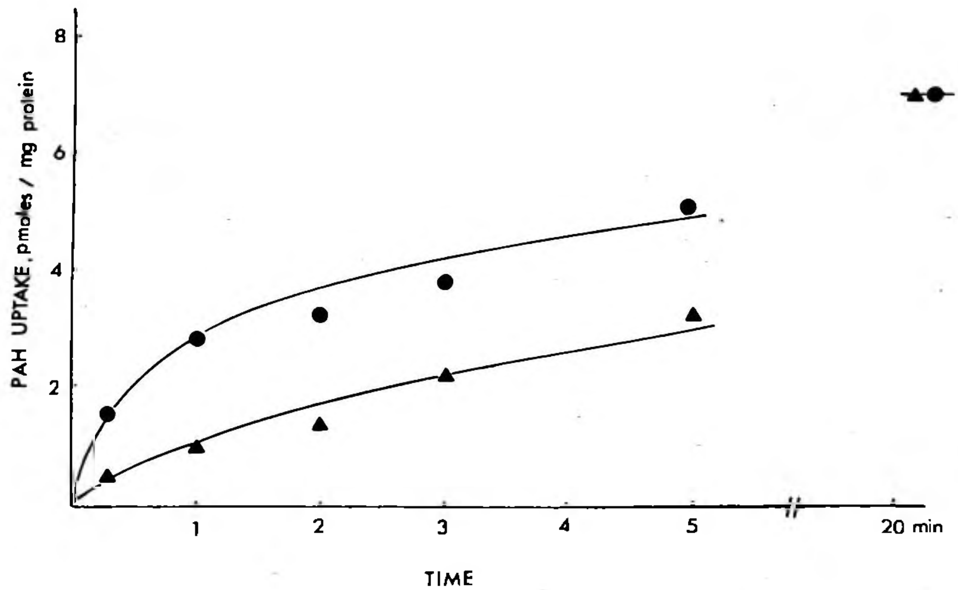

Figure 2. PAH uptake into brush border vesicles from flounder kidney tubules. Vesicles were prepared as in Figure 1. The incubation medium contained 100 mM mannitol, 100 mM NaCl, 2 mM $CaCl_2$, 18 mM Tris-HEPES buffer, pH 8.2, and 10.8 μM ^{3}H-PAH in the absence (circles) or presence (triangles) of 5 mM chlorphenol red. The symbols represent the means of 4 experiments.

uptake was inhibited after 1 min of incubation. At the same concentration, 5 mM, phenol red inhibited PAH uptake to a lesser degree and probenecid had no demonstrable effect. In order to determine whether the PAH uptake really reflected transport into vesicles, uptake was carried out under conditions where the osmolarity of the external medium was modified by the addition of sucrose. The uptake of PAH decreased in a linear fashion as the osmolarity of the medium was increased, indicating transport into an osmotically reactive space, i.e., the vesicle. The contribution of PAH binding to the vesicular membranes was estimated by extrapolation of the uptake data to infinite osmolarity and was found to be negligible. Using equilibrium uptake values for D-glucose and PAH (60 and 7 pmoles/mg protein, respectively) and assuming that intravesicular and extravesicular concentrations are equal (0.11 mM and 10.8 μM, respectively) it is calculated that 1 mg of membrane protein represented an intravesicular space of 0.5 μl for both solutes. Thus, PAH and D-glucose are transported into the same vesicular space.

The results described above provide evidence for carrier-mediated transport of PAH as well as D-glucose into plasma membrane vesicles prepared from flounder kidney tubules. Since this membrane preparation contains predominantly brush border membranes, it is tempting to conclude that both transport systems are located in the brush border membrane. This conclusion is supported by combined studies on sugar transport in the intact flounder kidney and teased kidney tubules (Pritchard and Kleinzeller, Am. J. Physiol., 231:603-607, 1976) in which energy-dependent phloridzin-sensitive transport of D-glucose-like sugars was demonstrated at the luminal side of the tbular cells. Definitive cellular localization of the PAH transport system (s) in flounder tubules, however, requires improved separation of luminal from contraluminal plasma membranes.

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DISTRIBUTION OF ENZYMES IN MEMBRANES OF RAT KIDNEY CORTEX, WITH PARTICULAR REFERENCE TO CARBONIC ANHYDRASE

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Carbonic anhydrase is present in the microsomal membranes of the rat and human kidney cortex as well as in the soluble fraction (Maren and Ellison, Mol. Pharm., 3:503, 1967; McKinley and Whitney, BBA, 445:781, 1976). These preparations represent plasma membranes, endoplasmic reticulum and ribosomes. Data are lacking, however, on the specific localization of the enzyme in the renal cortical cell. The present experiments were undertaken to determine whether carbonic anhydrase is present in the total plasma membrane and in a brush border membrane preparation from rat kidney cortex.

Male Sprague-Dawley rats weighing 300-600 gm were killed by decapitation, the kidneys were removed and the cortex excised after perfusion through the renal artery with cold sucrose-Tris buffer (0.25 M sucrose - 0.01 M Tris OH, pH 7.6 with HCl) to remove contaminating red blood cells. Homogenized cortical tissue plasma membranes were isolated by the method of Fitzpatrick et al. (J. Biol. Chem., 244: 3561, 1969) and brush border membranes were prepared using a modification of the method of Booth and Kenney (Biochem. J., 142:575, 1974). In this modification, 10 mM $CaCl_2$ was added to the renal cortical homogenate and aggregated subcellular organelles and basal-lateral membranes were removed by low-speed centrifugation. The supernatant fraction was centrifuged at 15,000 x g to yield a pellet rich in brush border membranes. Prior to measuring the enzyme activities, all preparations were freeze dried and stored at -20°C. Marker enzymes for cell membranes were assayed as follows: alkaline phosphatase, a plasma membrane enzyme (Monod et al., Nature New Biol., 240:126, 1972), ($Na^+ + K^+$)-ATPase a basal-lateral membrane marker (Kinne et al., Pflugers Arch., 329:191, 1971), HCO_3^--ATPase, a brush border membrane enzyme (Kinne-Saffron and Kinne, Proc. Soc. Exptl. Biol. Med., 146:751, 1974), Ca^{++}-ATPase, a plasma membrane enzyme (Parkinson and Radde, Biochem. Biophys. Acta, 242:238, 1976) and succinic dehydrogenase, a mitochondrial enzyme (Gibbs and Reimer, Proc. Soc. Exptl. Biol. Med., 119:470, 1965). Carbonic anhydrase was measured by the method of Maren (J. Pharm. Expt. Therap., 130:26, 1960). Protein determinations were carried out according to Lowry et al. (J. Biol. Chem., 193:265, 1951). The techniques