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

Franklin H. Epstein, Kate Spokes and Patricio Silva, Department of Medicine and Thorndike Laboratory, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts

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

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

Jull Eveloff, Rolf Kinne, and William B. Kinter, Mount Desert Island Biological Laboratory, Salsbury Cove, Maine; and Max Planck Institute for Biophysics, Frankfurt, West Germany

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