

**EFFECT OF ETHACRYNIC ACID AND OUABAIN ON THE EFFLUX OF SODIUM AND CHLORIDE ACROSS THE GILL OF *Anguilla rostrata*.**

John N. Forrest, Jr., and Franklin H. Epstein, Yale School of Medicine, New Haven, Connecticut and Harvard Medical School, Boston, Massachusetts

Both ethacrynic acid and ouabain have been shown to block active sodium transport in several systems. It was therefore of interest to study their effect upon the efflux of sodium and chloride across the intact gill of seawater teleosts since net extrusion of sodium chloride by the gill is an important component of biological adaptation to a seawater environment.

Specimens of *Anguilla rostrata* weighing 60-150 grams were adapted to seawater for at least three weeks before use.  $\text{Na}^{22}$  or  $\text{Cl}^{36}$  were injected intraperitoneally or intramuscularly as the NaCl salt, and 40-60 minutes allowed for equilibration in an external bath containing 1000 ml. of seawater. Samples of the bath were then taken at intervals of 10-15 minutes and counted for radioactivity. At the conclusion of the experiment plasma was obtained, and appropriate back corrections made to calculate the efflux of electrolytes throughout the course of the experiment. Results are expressed in ml of plasma cleared of Na or Cl per 100 grams body weight per hour. In some experiments the efflux of  $\text{Na}^{22}$  and  $\text{Cl}^{36}$  were determined simultaneously, making use of their differential counting characteristics.

In six experiments ethacrynic acid in a dose of 1.3 to 2.3 mg/100 grams body wt i.m. had no significant effect on sodium efflux (mean plasma clearance  $\pm$  s.d. =  $4.6 \pm 1.6$  ml/100gm/hr before and  $4.3 \pm 1.6$  after ethacrynic acid). The clearance of chloride was similarly unchanged in four experiments ( $5.8 \pm 1.7$  before and  $6.6 \pm .3$  after ethacrynic acid).

The effect of ouabain injected into the eel intramuscularly or intraperitoneally was variable and difficult to separate from its systemic effect on the animal. A dose of 700  $\mu\text{g}/\text{Kg}$  body weight produced hyperkalemia and killed the eel within 4 hours. Four hundred  $\mu\text{g}/\text{Kg}$  was fatal within 12 hours, and 200  $\mu\text{g}/\text{Kg}$  was usually fatal in 24 hours. Sodium efflux was not consistently inhibited by either 200  $\mu\text{g}$  or 400  $\mu\text{g}/\text{Kg}$  within the first hour after systemic injection. During the second hour after injection of 400  $\mu\text{g}/\text{Kg}$  of ouabain, sodium efflux was inhibited by 60-70% in 2 of 6 animals, but unchanged in the remaining four, in which Cl flux also remained essentially unaffected.

Measurement of Na-K-ATPase specific activity in gill homogenates one and two hours after injection of these doses of ouabain showed variable inhibition, from none to 50 percent, using the activity in gills of control saltwater eels as a standard.

Despite the potent effects of ethacrynic acid on salt transport in other systems it does not appear to block sodium or chloride efflux across the gill in eels adapted to seawater, and the effect of large doses to elevate the serum sodium (Bulletin Mount Desert Island 10:18, 1971) may therefore be due to its action on gut or kidney. Ouabain has a variable effect on sodium chloride efflux, related perhaps to the difficulty in achieving full inhibition of Na-K-ATPase in gills without causing death of the animal when the drug is injected systemically.