

result is attributed to the effects of elevated plasma electrolyte concentration.

In the specimens treated with thiocyanate in normal sea water there were no consistent effects on the tubular network. Concentrations higher than 10 mM did not permit adequate survival times for morphological changes in the t.r. to develop. In 10 mM thiocyanate after 48 hours there was some indication of reduced branching of the t.r. but only occasional cells showed aggregations of tubules.

Preliminary experiments on exposure to 10 and 20 mM potassium iodide in normal sea water showed substantial damage to membranes of the tubular reticulum.

That the tubular reticulum of the chloride cells is intimately involved in the regulation of transport processes in the gills receives some further support from these observations. In a stenohaline species *Pholis* exposure to half-strength sea water results in an apparently adaptational change in configuration of the tubular reticulum, while exposure to 1-1/2 times sea water causes severe disorganization and aggregation of the reticulum in basal regions of the cells. (Supported by a grant USPHS 5 SO1-RR05367-12).

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#### CHLORIDE TRANSPORT AND ITS INHIBITION IN GILLS OF SEA WATER TELEOSTS

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Chloride is transported outward against an electrochemical gradient by the gills of salt water teleosts. An active process involving a carrier is suggested by the fact that the efflux of chloride in sea water is sharply reduced in *Anguilla anguilla* by small quantities of NaSCN injected intraperitoneally while sodium efflux is unaffected. (Epstein, F.H., Maetz, J. and deRenzi, G.; Am. J. Physiol. 224:1295, 1973). The present study was undertaken to extend these observations to different species and to try to elucidate the mechanism of thiocyanate inhibition of chloride transport by the gill.

Chloride efflux was determined by injecting  $^{36}\text{Cl}$  intraperitoneally allowing one hour of equilibration, measuring the appearance of isotope in a known volume of aerated sea water at constant temperature of  $16^{\circ}\text{C}$  in which the fish lay immersed. Sampling was carried out every 10-15 minutes for one to two hours and at the end of the experiment blood was obtained so that the specific activity of  $^{36}\text{Cl}$  could be determined in plasma. Two microcuries of  $^{36}\text{Cl}$  per 100 grams body weight were injected in eels and flounders using an external bath of 1000 ml. To killifish weighing five to 10 grams, 0.5 to 1.0 microcuries of  $^{36}\text{Cl}$  were given and the bath volume was 150 ml.

Intraperitoneal injection of NaSCN (0.14 to 0.2mM per 100 gm body weight) reduced chloride efflux by an average of 50 percent in sea water specimens of *Anguilla rostrata* (n = 9) and *Pseudopleuronectes americanus* (n = 5). In *Fundulus heteroclitus* adapted to sea water however no inhibitory effect of thiocyanate could be detected (n = 6). All three species had evidence of a large exchange diffusion component to the chloride efflux in sea water with prompt falls of 75-80

percent in plasma clearance of  $^{36}\text{Cl}$  when fish were abruptly transferred to fresh water.

Perchlorate and iodide (0.14 to 0.2mM per 100 gm body weight) were also tested for an inhibitory effect on chloride efflux because of their position in the lyotropic series and their biological action to inhibit halide transport by thyroid cells. Neither sodium perchlorate (n = 5) sodium iodide (n = 3) altered chloride efflux by sea water adapted *Anguilla rostrata*.

The ratio of the concentration of chloride in sea water to that in eel plasma in the steady state is approximately 3.9 to 1 (504 mEq/L in sea water; 130 mEq/L in plasma). Thiocyanate might itself be bound and transported by the same carrier as that responsible for establishing the chloride gradient and if so the concentration of thiocyanate in the steady state should also be higher in sea water than in plasma. This was tested by injecting tracer amounts of  $^{35}\text{SCN}$  i.p. into four eels or placing it in their sea water bath and sampling bath and plasma daily until a steady state was achieved (this occurred in 48 hours). The ratio of  $^{35}\text{SCN}$  in sea water to that in plasma at four days averaged 3.0 (range 2.5 to 3.3), suggesting that SCN, like Cl, is actively transported outward in sea water presumably by the gill.

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#### THE EXCHANGE OF SEVERAL POLAR COMPOUNDS BETWEEN BLOOD, CENTRAL NERVOUS TISSUE, AND CEREBROSPINAL FLUID IN *Squalus acanthias*

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Examinations of the patterns of non-electrolyte permeability across various epithelial membrane systems such as the frog choroid plexus (J. Membrane Biol. 2: 127-149, 1970) and the rabbit gall bladder (Proc. Roy. Soc. B. 172: 227-271, 1969) have been reported. In general the rates of molecular transport increase with increasing lipid:water partition coefficients; however the smallest, most lipid-insoluble molecules (e.g., urea) cross these membranes at rates which are unusually rapid for their respective lipid solubilities. The purpose of this study was to investigate the exchange of three small organic non-electrolytes plus water between blood, central nervous tissue, and cerebrospinal fluid and to gain some information on the permeability properties of two "tight" membrane systems in the dogfish—the choroid plexus epithelium and the central nervous system (CNS) capillaries—similar to that presented for other multicellular membrane systems.

The radioactively labeled materials used in this study were  $^3\text{H}$ -water,  $^{14}\text{C}$ -urea,  $^3\text{H}$ -ethylene glycol, and  $^{14}\text{C}$ -thiourea. By a combination of intravenous and intramuscular injections constant or nearly constant plasma levels of the radioactive materials were achieved in free-swimming dogfish. Plasma samples were taken three or four times during each experiment to monitor the constancy of blood levels. Experiments in which the plasma concentrations varied by more than  $\pm 20\%$  from the final sample were discarded (about 50 percent of the experiments failed this test). Animals were killed from 10 minutes to 20 hours after the initial intravenous and/or intra-muscular injection(s) and samples of telencephalon (olfactory lobe), spinal cord, and CSF were rapidly obtained. Dupli-