

hypernatremia and death (Epstein, F.H., Maetz, J. and deRenzi, G., unpublished observations). Specific inhibition of chloride efflux without marked change in sodium efflux after injections of NaSCN was confirmed in two American eels fully adapted to seawater. We therefore wished to see if thiocyanate had any action on the adenosine triphosphatases of gill tissue, since  $\text{SCN}^-$  and other anions are known to block MgATPase in other tissues, according to the position of the anion in the lyotropic series (Katz, A.I. and Epstein, F.H., *Enzyme* 12:499, 1971).

Substitution of 100 mM  $\text{SCN}^-$  for 100 mM  $\text{Cl}^-$  in the assay for Na-K-ATPase (Jampol, L. and Epstein, F.H., *Am. J. Physiol.* 218:607, 1970) produced no change in the specific activity of Na-K-ATPase in whole gill homogenates of eight specimens of *Anguilla rostrata* adapted to seawater (average  $\pm$  s.d. =  $9.6 \pm 2.6$   $\mu\text{MPi/mg protein/hr}$  in  $\text{Cl}^-$  medium vs.  $10.0 \pm 2.9$  in  $\text{SCN}^-$  medium). In gill homogenates of 10 freshwater eels, however, a thiocyanate medium inhibited Na-K-ATPase slightly (mean  $\pm$  s.d. in  $\text{Cl}^-$  medium =  $5.1 \pm 1.0$  vs.  $3.8 \pm 1.6$  in  $\text{SCN}^-$  medium).

Thiocyanate caused inhibition of MgATPase by approximately 50 percent in gill homogenates of eels adapted to fresh and salt water. The thiocyanate-inhibitable ATPase (measured as the difference between MgATPase in  $\text{Cl}^-$  medium and  $\text{SCN}^-$  medium) was  $5.0 \pm 1.0$   $\mu\text{MPi/mg protein/hr}$  in seawater and  $3.9 \pm 1.3$  in freshwater, a difference that is not significant ( $p > 0.05$ ;  $t = 1.87$ ).

If "thiocyanate-inhibitable ATPase" were part of the mechanism by which chloride is actively transported by gills in seawater, one might expect a considerable increase in the specific activity of this moiety in the gill, in the process of adaptation of freshwater eels to seawater. In the present experiments however the difference between freshwater and seawater eels was small and did not reach the level of significance. It is possible that larger differences would be observed if plasma membrane or microsomal fractions were assayed, rather than whole homogenates of gill filaments. Nevertheless the present data do not support the notion that chloride extrusion by the gill is related to that portion of MgATPase in gill homogenates that is inhibited by thiocyanate.

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#### MEASUREMENT OF THE BRAIN EXTRACELLULAR SPACE IN *Squalus acanthias*

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The size of the extracellular space (ECS) in various tissues is usually measured by determining the steady state tissue to plasma ratio of some relatively large, metabolically inert foreign compound such as inulin, which has been infused or injected into the venous circulation. The use of this experimental procedure for measuring the brain ECS in a variety of vertebrate species has yielded peculiarly low values and has suggested that the exchange of these extracellular-type compounds across the capillaries of the brain is too slow, the so-called bloodbrain barrier effect, to allow an accurate determination of the brain ECS in that manner (*Comp. Biochem. Physiol.* 42A: 73-38, 1972). Nevertheless reasonable estimates of the brain ECS have been made by introducing inulin and other extracellular markers into the cerebrospinal fluid (CSF) and measuring tissue to CSF ratios of the marker

at various times after injection, infusion, or perfusion. For mammals the values for the brain ECS obtained using this technique range from 15-20 percent (Am. J. Physiol. 219: 1528-1533, 1970); determination of the brain ECS of elasmobranchs by injection or infusion of the marker into the CSF have not been reported. In this study an attempt was made to measure the size of the brain extracellular space in *Squalus acanthias* from the CSF to brain ratios of three intraventricularly injected extracellular markers.

Dogfish, weighing from 2 to 3 kg, were taken directly from a live car and securely lashed into a trough-shaped restraining board with their dorsal surface up. A 20-gauge needle, attached to a one ml glass syringe, was advanced percutaneously through the chondrocranium until the tip reached the cerebellar ventricle and a small volume of fluid could be aspirated into the syringe. While holding the needle firmly in position, the syringe was disconnected and a drop of the withdrawn fluid tested for the presence (extradural fluid) or absence (CSF) of protein. If the aspirated fluid was CSF, a second syringe containing 0.3 ml of artificial dogfish CSF was attached to the needle and the solution injected into the cerebellar ventricle. Mixing of the injected fluid throughout the ventricular space was accomplished by withdrawing and reinjecting the ventricular fluid (CSF plus injectate) four or five times. After the mixing was completed, the needle and syringe were removed and the fish returned to a large, seawater-filled swimming pool. During the entire procedure, the fish were out of the water for one to two minutes. The injection solution contained both a  $^3\text{H}$ - and a  $^{14}\text{C}$ -labelled extracellular marker. The marker combinations used were:  $^3\text{H}$ -mannitol and  $^{14}\text{C}$ -inulin;  $^3\text{H}$ -mannitol and  $^{14}\text{C}$ -sucrose; and  $^3\text{H}$ -sucrose and  $^{14}\text{C}$ -inulin. At either four or 24 hours after the injection the fish were caught, a sample of CSF obtained, and the brain removed. The medulla was dissected out of the brain and frozen onto a microtome stage. A series of 0.5 mm thick slices of medullary tissue, running from the ventricular surface inward, were obtained and analyzed for radioactivity by liquid scintillation spectroscopy. The distribution space for each molecule was calculated by dividing the concentration of labelled material (cpm/mg) in the first or ventricular piece by the concentration (cpm/ $\mu\text{l}$ ) in the terminal CSF sample and multiplying the quotient by 100 percent.

The spaces (percent  $\pm$  S.D. plus the number of animals) obtained at four hours were: mannitol =  $16.2 \pm 0.3$ , N = 3 and sucrose =  $15.8 \pm 1.1$ , N = 3. At 24 hours the values were: mannitol =  $28.6 \pm 3.1$ , N = 4; sucrose =  $20.0 \pm 1.9$ , N = 7; and inulin =  $14.2 \pm 1.9$ , N = 11.

Computer modeling of the kinetics of CSF brain exchange following an intraventricular injection of an extracellular material indicated that the concentration of the compound in the extracellular fluid of the first medullary slice would be virtually in equilibrium with the CSF within four hours for compounds with diffusion coefficients at  $15^\circ\text{C} \geq 1.5 \times 10^{-6} \text{ cm}^2/\text{sec}$  (mannitol and sucrose), providing that brain capillary exchange is slow (half-times for blood to brain extracellular fluid equilibration  $\geq$  six hours). For compounds with lower diffusion coefficients such as inulin, equilibration between the first slice and the CSF would be obtained after slightly longer periods of time. On the basis of the four-hour sucrose and inulin data, the 24-hour inulin data, and the computer modeling, a reasonable estimate of the ECS in the medulla of *S. acanthias* is 14 to 16 percent. This value corresponds quite closely to those found for the brain extracellular space in a variety of other vertebrate species (Am. J. Physiol. 219: 1528-1533, 1970). The high values for the mannitol space at 24 hours are similar to those found for the cat cerebral cortex under normothermic conditions (Exp. Neurol. 27: 101-114, 1970) and suggest that significant cellular uptake of this "extracellular" marker occurs within the brain tissue of both species.