When eels fully adapted to seawater are transferred directly to fresh water, serum sodium falls within 24 hours from the seawater level (156 ± 2.5 μEq/L, n = 6) to a lower value (146 ± 0.97, n = 4) characteristic of eels fully-acclimatized to freshwater (148.9 ± 3.1., n = 5), and does not change significantly thereafter. The changes responsible for freshwater adaptation therefore appear to be thrown into play rapidly. Exposure of seawater adapted eels to freshwater resulted in a rapid and profound reduction of sodium efflux without a corresponding reduction in the activity of Na-K-ATPase in gill homogenates. The latter remained elevated at levels characteristic of seawater fish for as long as 9 days after transfer to freshwater. A striking decrease in sodium efflux to 115 ⊞ 77 μEq/hr/100 gm (n = 4) was evident after only two hours of immersion in fresh water, though one hour of exposure to freshwater did not greatly affect sodium efflux (629 ± 77 μEq/hr/100 gm, n = 4). After one day of immersion in fresh water, sodium efflux in each of four eels was uniformly less than 50 μEq/hr/100 gm.

It seemed possible that the turn-off of sodium efflux might be mediated by prolactin, necessary in other euryhaline specied to maintain life in freshwater. Additional experiments were therefore carried out with 2-bromo-alpha-ergocryptine (CB154, Sandoz), an ergot derivative that has been shown to inhibit the release of prolactin in mammals. It is not known, however, whether the drug has a similar action in teleosts. Eight mg per 100 gm of body weight were injected intraperitoneally daily for one or two days before transfer to freshwater. Though this dose approached the toxic level it was ineffective in preventing the reduction in sodium efflux across the gill of *Anguilla rostrata* induced by a two-hour or one-day exposure to freshwater.

An important mechanism responsible for adaptation to freshwater, therefore, appears to be the capacity to turn off promptly the efflux of sodium across the gill. The change is stimulated by brief exposure to a hypotonic environment. It is not associated with a reduction in the activity of gill Na-K-ATPase. Since all measurements of efflux were carried out in a seawater bath, exchange diffusion of sodium, known to represent 80-90% of total sodium efflux under these circumstances, must have been affected. Although the time course of the response is consistent with hormonal mediation, it was not blocked by bromoergocryptine and further experiments will be necessary to clarify the possible role of prolactin. Dissociation of ion movements from gill Na-K-ATPase activity is a feature of early freshwater adaptation in *Anguilla rostrata*.

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Measurements of CSF-Brain-Blood Transport Rates in *Squalus acanthias*

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Perfusions and injections of tracer molecules an ions into the cerbrospinal fluid (CSF) compartment various mammalian species have been performed to stud their respective clearance rates from the CSF (e.g. *Am. Physiol. 203*: 775-781, 1962). Other investigators ha employed one or the other of these approaches alon with brain tissue sampling to determine the size of th brain extracellular space (e.g. *Life Science 1*: 43-4 1962). More recently the CSF perfusion technique ha been used to examine the rate of potassium exchang between extracellular fluid (ECF) and intracellular flu (ICF) (*Brain Res. 38*: 49-69, 1972) and of solute and wate flow between ECF and brain capillary blood (Patlak an Fenstermacher, *Am. J. Physiol*, in press). In the presen report the same two transport steps, ECF-ICF exchang and ECF-blood transfer, were studied in *Squalu acanthias* employing a method of intraventricular injectio for introducing the test substances into the CSF. Fo so-called extracellular markers plus two ions — Na and C — were used. In addition to the two exchange rate which were previously mentioned, the CSF disapperanc time, the ependymal permeability constant, and the bra ECF diffusion coefficient for all of these solutes wer measured from the data.

Dogfish, weighing from 2 to 4 kg, were placed in a large circular holding tank prior to beginning each experiment. While gently holding the fish at the side of the tank, a 20 gauge needle, which was attached to a 1.0 m syringe by about 6 inches of polyethylene tubing, was advanced through the skull and the underlying cerebellum until the tip reached the cerebellar ventricle. A sma volumer (0.2-0.3 ml) of fluid was withdrawn into the syringe, the syringe disconnected from the tubing, and the fluid tested for the presence (extradural fluid) o absence (CSF) of protein. If the aspirated fluid was CSF a second syringe which contained 0.3 ml of dogfish saline and two radioactively tagged materials was attached to the tubing and the solution injected into the cerebell ventricle. Mixing of the injected fluid throughout the ventricular system was accomplished by withdrawing and reinjecting the ventricular fluid (CSF plus injectate) three or four times. After the mixing was completed, the needle was removed and the fish were allowed to swim freely. At various times (1.0, 2, 3, 4, 8, and 20 hr) after making the injection, the fish were taken from the water, a sample o CSF obtained, and the brain removed. Subsequently the medulla was cut, starting at the ventricular or ependyma surface, into a series of 0.25 or 0.5 mm thick slices b means of a freezing microtome. The slices were weighed and analyzed for radioactivity by liquid scintillation spectroscopy. The following radioactivity labeled mater ials were used: ^{14}C-ethylenediaminetretracetic acid ^{3}H)sucrose, ^{3}H-mannitol, ^{22}Na, and ^{36}Cl.

The data which were obtained for a particular compound or ion were compared to computer-generated data for a CSF-brain-blood transport model system such as that employed by Pape and Katzman (*Brain Res. 38* 49-69, 1972). The parameters which were varied are listed as column headings in Table 1; the results of the comparative analysis of the dogfish medullary slice dat

Table 1

CSF-Brain-Blood Transfer Constants Determined for *Squalus acanthias* by Intraventricular Injection and Serial Sampling of Medullary Tissue

Material	$t_{1/2}$ of CSF Disappearance (hr)	Ependymal Permeability Coefficient (cm/sec)	Tissue Diffusion Constant ($\times 10^6$ cm^2/sec)	Cellular Exchange $t_{1/2}$ (hr)	Capillary Exchange $t_{1/2}$ (hr)
Inulin	50	1×10^{-4}	0.8	∞	10
EG	28	5×10^{-4}	1.1	150	4.0
DTA	19	5×10^{-4}	1.2	500	4.5
ucrose	16	1×10^{-3}	1.2	300	8.0
Mannitol	7	2×10^{-3}	1.6	250	4.5
^{22}Na	5	3×10^{-3}	2.8	0.5	2.0
^{36}Cl	6	1×10^{-3}	4.6	0.75	1.5

e listed in Table 1 (the exchange half-times or $t_{1/2}$'s hich are used in Table 1 were obtained by dividing the antity, 1n 2, by the appropriate rate constants).

The rate of disappearance from the CSF correlated ell with the molecular weight of the material. The gest compound, inulin, had the longer $t_{1/2}$ (slowest rate disapperance), whereas the two ions had the shortest $_2$'s. This observation differs from that of Prockop *et al.* *Pharmacol. 135*: 266-270, 1962) who found that the earance rates of four water soluble compounds of ergent sizes (dextram to mannitol) from the CSF mpartment of the rabbit were virtually identical. Our ding suggests that molecular exchange between CSF d blood and/or brain plays a more important role in *qualus* than in mammals where much of the solute earance from the CSF occurs by entrainment in the sorbed CSF, i.e. by bulk flow.

The ependymal permeability and tissue diffusion nstants of these markers showed the same general ern. The smaller solutes yielded higher coefficients, as d be expected for diffusional flow across a mem- e (the ependyma) or through an aqueous medium e ECF). The tissue diffusion coefficients listed for each stance in Table 1 equaled about 30% of their spective diffusion constants in water at 15°C. Such an parent reduction in diffusability of solutes and water ough tissue has been previously reported by Suenson *al.* for cat heart muscle (*Am. J. Physiol. 227*: 16-1123, 1974) and attributed to diffusion of these terials into dead-end pores within the tissue.

All of the extracellular compounds had very large ular exchange $t_{1/2}$'s, which indicated the relative im- meability of brain cells to this group of molecules. In ntrast, Na and Cl entered some or all brain cells quite pidly as shown by their short disapperance half-times.

The differences in the rates of exchange between tracellular compounds and the two ions were not so rked for the brain capillaries as for the brain cells. The od-brain ECF transfer $t_{1/2}$'s ranged from 10 hr (inulin) 1.5 hr (Cl). With the exception of sucrose, the capillary nsfer constants for all of these solutes correlated quite ll with their respective diffusion coefficients in water, ggesting that the movements of such materials across e brain capillary complex occurred by diffusion through ueous channels.

In summary, the rates of disappearance from the CSF, transport across the ependyma, diffusion in the brain ECF, and exchange across the brain capillary complex for a series of "extracellular" molecules and ions were consistent with their relative molecular weights and water diffusion coefficients; however the movement of Na and Cl into the cells of the medulla appears to occur at rates which are much (30 to 100 fold) faster than those of the "extracellular" compounds. Such a difference suggests that some or all of the transport of these two ions across the cellular membranes of neurons and/or glia in the brain of *Squalus* occurs by special (other than simple diffusion) processes.

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Chloride Transport Across the Isolated Intestinal Mucosa of *Pseudopleuronectes americanus*: Relation to Sodium Transport and Effect of Cyclic AMP

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Mammalian small intestine can absorb and also secrete Cl against an electrochemical gradient. Cyclic 3', 5'-AMP (cAMP) appear to inhibit the former process and to stimulate the latter (Nellans et al., *Am. J. Physiol.* 228, 1808, 1975). It is possible that active secretion arises from cells in the crypts of lieberkuhn. Teleost intestine is devoid of crypts and has not thus far been found to secrete water and electrocytes.

In some marine teleosts including the eel (Ando et al., *Comp. Biochem. Physiol.* 51A, 27, 1975) and the flounder (Huang and Chen, *Am. J. Physiol.* 220, 1734, 1971), the serosal side of the intestine is electronegative relative to the lumen and, under short-circuit conditions, Cl is absorbed at a faster rate than Na. It has recently been postulated that these phenomena are due to a Na-independent electrogenic Cl pump (Smith et al., *Pfluger's Archiv*, in press). In mammalian small intestine, in contrast, the serosa is usually electropositive relative to the lumen and Na and Cl absorptive processes appear to be tightly coupled (Schultz et al. *Ann. Rev. Physiol.* 36, 51, 1974).

We have further examined these differences between mammalian and teleost intestine, using the isolated intestinal mucosa of the winter flounder, *Pseudopleuro-*