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-*

Table 1

Ion Fluxes Across the Isolated Intestinal Mucosa of the Flounder

Composition of medium in mmol/l ($Na/Cl/HCO_3$)	N	Na fluxes (μEq/h cm^2)			Cl fluxes (μEq/h cm^2)			SCC	G
		m→s	s→m	net	m→s	s→m	net	(μEq/h cm^2)	(mmhos/cm^2)
168/150/20	13	13.6 ±2.7	11.6 ±2.9	2.0 ±1.0	8.4 ±1.8	3.0 ±1.2	5.5 ±1.4	-3.2 ±0.8	26.9 ±5.6
157/157/5	7	12.1 ±2.1	10.7 ±1.9	1.4 ±1.6	5.0 ±1.7*	3.0 ±0.8	2.0 ±1.7*	-1.2 ±0.4*	21.4 ±3.1
168/0/20	5	14.8 ±0.7	15.0 ±1.9	-0.2 ±1.2	–	–	–	0.0 ±0.1*	21.5 ±1.5
0/150/20	11	–	–	–	6.3 ±2.4†	6.1 ±2.9*	0.2 ±2.2*	-0.2 ±0.1*	10.3 ±2.8*

All solutions were gassed with 99% O_2, 1% CO_2. Temperature was maintained at 15-16° C. Values shown are means ±1 S.D. for N animals. Superscripts * and † indicate differences from corresponding results in first row — $p<.005$ and $p<.05$, respectively. In addition to concentrations of ions shown above, solutions also contained in mmol/l: k, 5; Ca, 1; Mg, 1; SO_4, 2; and H_2PO_4-HPO_4, 3. Na was replaced by choline and Cl by SO_4 (75 mM) and mannitol (75 mM). D-Glucose (5 mmol/l) was added to the serosal bathing medium and mannitol (5 mmol/l) to the luminal medium.

nectes americanus. Flounder (275-500 g) were taken from sea water and killed by a blow to the head. Intestine from about 2.5 cm below the stomach to about 5 cm above the anus was removed, stripped of muscle and mounted in Ussing chambers (1.12 cm ^{2}of mucosa exposed) as previously described for rabbit ileum (Field et al., *Am. J. Physiol.* 220, 1388, 1971). In preliminary experiments we determined that the intestine was uniform throughout in its electrical properties and permeabilities to Na and Cl. Usually 4 chambers were mounted with intestine from one fish. The compositions of bathing solutions employed are specified in table 1 and its legend. Electric potential difference (PD), short-circuit current (SCC), electrical resistance, and unidirectional mucosa (m)-to-serosa(s) and s-to-m fluxes of ^{22}Na and ^{36}Cl were determined as previously described (Field et al., *Am. J. Physiol.* 22 1388, 1971).

Flux measurements in the absence of added cA are given in table 1 and measurements in the presence cAMP are given in table 2. These data can be summarize as follows:

(1) Under control conditions (25 mM HCO containing NaCl Ringer), the PD varied between -3 a -5mV (luminal reference). This PD was reduced to zero 0.1 mM ouabain added on the serosal side, 50% inhibi occurring in 7 min (data not shown).

(2) There were net m-to-s fluxes of Na and Cl acro the short-citcuited mucosa, the ratio of net Cl to net N fluxes being 2.7.

(3) The difference between net Cl and Na fluxes wa

Table 2

Effect of cAMP on Ion Fluxes Across Isolated Intestinal Mucosa of the Flounder

	Na fluxes (μEq/h cm^2)			Cl fluxes (μEq/h cm^2)			SCC	G
	m→s	s→m	net	m→s	s→m	net	(μEq/h cm^2)	(mmhos/cm^2)
Control	15.5 ±2.1	13.3 ±2.6	2.2 ±0.9	9.1 ±1.9	3.0 ±1.4	6.1 ±1.3	-3.1 ±0.6	30.3 ±6.8
(6)								
+cAMP, theo	14.3 ±1.8	12.8 ±2.2	1.5 ±1.6	10.7 ±2.7	6.7 ±1.4*	4.1 ±2.4	-0.9 ±0.9*	33.8 ±5.3
Control	13.8 ±0.7	12.1 ±1.4	1.7 ±1.1	9.1 ±0.8	3.5 ±1.5	5.6 ±1.9	-3.1 ±1.1	24.5 ±0.2
(3)								
+db-cAMP	14.0 ±1.6	11.8 ±2.7	2.2 ±1.3	10.8 ±2.4	6.2 ±3.7	4.6 ±1.3	-2.2 ±1.1	28.0 ±5.9
pooled control	14.9 ±1.9	12.9 ±2.2	2.0 ±1.0	9.1 ±1.6	3.2 ±1.3	5.9 ±1.4	-3.1 ±0.7	28.4 ±6.1
(9)								
pooled cAMP	14.2 ±1.6	12.5 ±2.2	1.7 ±1.5	10.8 ±2.5	6.5 ±2.2*	4.2 ±2.0	-1.3 ±1.2*	31.8 ±5.9

Values are means ±1 S.D. for (N) paired experiments. Bathing solutions as in row 1, table 1. cAMP (2 μmol/ml) and theophylline (5 μmol/ml) or dibutyryl cAMP (0.25 μmol/ml) added to serosal bathing solution. Flux measurement begun 30 minutes thereafter. * different from control, $p<.005$.

out equal to the SCC, suggesting the absence of HCO_3 nsport.

(4) The s-to-m unidirectional Cl flux was only about e-fourth of the s-to-m Na flux, indicating a greater rmeability to cations than to anions. The permselecti-y of the "tight" junction for cations may in fact be derestimated by the ratio of s-to-m unidirectional fluxes ce a portion of the Cl flux may not be due to extra-llular diffusion. Replacement of Na with a less rmeant cation (choline) increased Cl permeability.

(5) Reduction of the HCO_3 concentration of the dium to 5 mM reduced net Cl absorption from 5.5 to and SCC from -3.2 to -1.2 μEq/h cm^2. This is in eping with the observation of Oide (*Comp. Biochem. siol.* 46A, 639, 1973) that fluid absorption by eel estine is greatly enhanced by increasing luminal pH m 7.2 to 9.0.

(6) Replacement of all Na in the medium with ine reduced net Cl flux and SCC to zero. Replace-nt of all Cl with SO_4 and Mannitol reduced net Na flux SCC to zero. Thus Na and Cl transport processes are sely coupled. Huang and Chen (*Am. J. Physiol.* 220, 34, 1971) previously reported the apparent absence of ch coupling in flounder intestine. Their choline-Ringer ntained 25 mM Na, however, and their SO_4-Ringer ntained 5 mM Cl. Furthermore, they added glucose to r SO_4-Ringer to maintin constant osmolality and thus y may have specifically stimulated co-transport of cose and Na.

(7) Addition of cAMP and theophylline (table 2) ought the SCC closer to zero ($p<.001$) and reduced ntly the net absorptive fluxes of Na and Cl, although ther change was significant statistically. The ratio of t Cl to net Na fluxes remained at 2.7. Dibutyryl cAMP d a similar effect although the change in SCC was less ng.

(8) Addition of cAMP and theophylline or of dibutyryl AMP doubled the s-to-m Cl flux but had no effect on Na xes. Thus cAMP appears to selectively increase the ssive Cl conductance of the tissue.

The apparent absence of cAMP-mediated Cl cretion in flounder intestine (Cholera toxin also failed to cit secretion - see Rout et al. *Fed. Proc.* 32, 424Abs, 73) provides circumstantial evidence for the crypt cell gin of secretion in mammalian intestine. The absence either crypts or immature cells in flounder intestine was nfirmed histologically (We are indebted to William B. nter and his associates for these examinations).

The apparent coupling of "active" Na and Cl fluxes ust be reconciled with the large difference in their gnitudes. It is possible that NaCl is pumped by the thelial cell into the lateral space and that the nsmural PD is largely a diffusion potential across the ht junction, which appears to be highly cation-selective e the explanation of Machen and Diamond, *J. Membr. l.* 1, 194, 1969) for the small negative PD across gall adder epithelium). This view implies that under ort-circuit conditions most of the Na entering the lateral ace returns to the luminal solution via the "tight" nction whereas most of the Cl enters the serosal solu-n. For this to be the case, about two-thirds of the resistance to transmural Na diffusion must reside in the lateral space beyond the junctional complex.

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Selective Adaptation of Secretory Function in the Flounder Nephron

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An important function of the kidney of a marine teleost appears to be the secretion of magnesium and sulphate enabling the fish to maintain plasma concentrations of these ions at least fifty times lower than those of the surrounding seawater. (*Can. J. Zool. 46*: 439, 1968). Urine to plasma concentration ratios for magnesium of 100-300 are achieved almost entirely by tubular secretion, glomerular filtration accounting for less than 3% of the magnesium excreted. Other routes of excretion, such as the gills, do not participate in the homeostasis of these ions and, although some processing of ingested seawater does occur in the bowel (*Can. J. Zool. 46*: 457, 1968), the large amounts of both magnesium and sulphate that are added to the body fluids daily require a powerful control system.

A second, extensively studied, tubular transport system governs the excretion of a wide variety of organic anions such as diodrast, p-ammohippurate (PAH) and dyes of the phenol red class (*Am. J. Physiol., 211*: 1152, 1966). Despite the widespread interest in this secretory system, its physiological significance remains obscure with no information available as to the normal metabolites handled by this system or to its importance in overall homeostasis.

The present studies examined the response of the remaining nephrons of the flounder kidney to the removal of approximately half of the total renal mass in order to establish the relative importance of the two secretory systems in overall regulation of body fluid composition.

The teased flounder tubule preparation described by Forster (*Science, 108*: 65, 1948) was used to examine the ability of the tubules to concentrate substanced within their lumina. The in-vitro properties of this system have been shown to correlate closely with the in-vivo behaviour of the kidney (*Bull. MDIBL, 9*: 29, 1969).

Winter flounder (*pseudopleuronectes americanus*) 150-400 g in weight were anesthetized with intravenous sodium pentobarbital (0.2 g/kg body weight) and the single fused kidney lying in apposition to the spinal column exposed via a small incision. The renal tissue was cauterized along its whole length using a red hot wire or a heated tungsten filament. Approximately 50% of renal tissue was destroyed by this means without obvious damage to the large vessels running through the center of the kidney. Control flounder were sham-operated using the identical procedure apart from the removal of renal tissue, and the fish returned to tanks of running seawater. On the sixth postoperative day the fish were removed from the tanks, a venous blood sample drawn, and all viable renal tissue removed and weighed.