

when incubated in Forster's balanced saline medium at 14°C. Townsley and Scott (J. Fish. Res. Bd. Canada 20:243, 1963) reported that systaltic action of muscular cells, lining the tubular epithelium, was present for up to one month when the tubular masses were incubated in Medium 199 at 5°C; however, they did not test the viability of the tubular cells. In the present study, a modification of the culture conditions employed by Townsley and Scott was used and the viability of the tubular cells was tested by the capacity of the tubules to concentrate chlorophenol red in the luminal fluid, a process which requires expenditure of cell energy.

Flounder kidneys were removed from decapitated animals. The caudal kidney was teased in ice-cold Forster's saline medium and about 10 explants, 2 to 3 mg in weight each, were transferred to small plastic culture flasks containing 2 ml of the culture medium. The latter consisted of Medium 199 Hanks base with L-glutamine and with or without 10% fish serum added. NaHCO₃ was added to give concentrations up to 10 mM and pH's from 7.2 to 7.8. Penicillin, 100 U/ml and in some cases, streptomycin, 10 µg/ml were also added. The culture flasks were kept at 4°C in a horizontal position so that only a thin film of medium covered the tubular masses. The medium was changed every three days at which time one explant of each culture flask was dissected further and transferred to Forster's saline medium containing 2.5×10^{-5} M chlorophenol red. Under the microscope and at room temperature dye uptake was followed for one hour and compared to the dye uptake of freshly dissected tubular masses of a control kidney.

Maintenance of near normal rates of dye uptake for three weeks required the following conditions: (1) incubation temperature, 4°C, (2) pH of medium above 7.6, (3) 10% fish serum added to incubation medium, (4) penicillin was necessary to suppress contamination but streptomycin had deleterious effect upon the culture. Flounder tubular cells remained viable for up to 10 days when incubated in Forster's medium alone at 4°C. After the third week dye uptake by the tubular cells diminished but did not disappear for up to one month of culture. Mitotic figures were not detected in the preparations. Systaltic muscular action was observed occasionally.

This preparation should be useful in studies of intracellular phenomena during protein uptake as well as in *in vitro* studies of induction phenomena of organic acid transport.

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1970 #26

WATER ABSORPTION BY ISOLATED EEL INTESTINE DURING SEA WATER ADAPTATION

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Sea water adaptation by the eel is associated with an increase in sodium, chloride and water absorption by the intestine (Bull. MDIBL 9:23-26, 1969) which appears to be controlled by cortisol (Gen. and Comp. Endocrinol. 11:373-80, 1968) via the pituitary. An increase in water absorption by *in vitro* eel intestine can also be elicited in fresh water adapted eels by daily injection of cortisol for 2 weeks prior to the study. The increase in water absorption during sea water adaptation appears to be sensitive to ouabain (Bull. MDIBL 9:23-26, 1969). However ouabain does not completely inhibit water absorption in either fresh or sea water adapted eels. The purpose of the present investigation was to determine the effect of ouabain and diamox on water absorption *in vitro* by intestines from freshwater adapted, 3 days in sea water and sea water

adapted eels and to determine the time course for the effects of cortisol injection on water absorption by eel intestine.

The eels, *Anguilla rostrata*, used for the present study were caught in estuaries in northern Maine and held in the laboratory for at least 2 weeks in either fresh water or sea water before any experimental manipulation. The fish were not fed. Immediately following removal of the intestine from the fish a polyethylene cannula was tied in one end and the intestine was placed in isotonic saline at 0°C. When all of the intestines had been prepared, they were preincubated at room temperature for 1/2 hour in saline. If metabolic poisons were to be used, they were placed in this saline. During the preincubation period the intestines were frequently flushed with saline. Water absorption was measured in vitro in non-everted sacs of the entire intestine by the method previously described (Bull. MDIBL 9:23-26, 1969). When metabolic inhibitors were used both the serosal and mucosal surfaces of the intestine were exposed to these agents.

The absorption of water by intestines from both fresh water and sea water adapted eels was inhibited by 10^{-4} M ouabain but was not affected by 10^{-4} M diamox (Table 1). In contrast to previous observations (Bull. MDIBL 9:23-26, 1969) nearly all of the water absorption was ouabain

Table 1

EFFECT OF OUABAIN, DIAMOX AND 2,4-DINITROPHENOL ON WATER ABSORPTION BY NON-EVERTED EEL INTESTINAL SACS

	Water absorption (μ l/gm.hr.)				
	Temp. C	Control	Ouabain 10^{-4} M	Diamox 10^{-4} M	DNP 10^{-4} M
Sea water adapted > 2 weeks	16	202 \pm 20 (7)	83 \pm 19 (7)		
	24	366 \pm 68 (9)		366 \pm 96 (7)	190 \pm 45 (6)
Freshwater adapted > 2 weeks	24	85 \pm 14 (15)	5 \pm 3 (12)	95 \pm 21 (6)	
Then 3 days in sea water	24	311 \pm 17 (10)	70 \pm 13 (10)		

Values are mean \pm standard error. The number of determinations in each sample is in parentheses.

sensitive in fresh water adapted eels, but not all of the increase in water absorption seen during sea water adaptation was ouabain sensitive. The discrepancy in ouabain sensitivity of water absorption may be explained by differences in the history of the eels used in the two studies. In 1969 eels were obtained from fresh water ponds while the eels used in the present report were obtained from estuaries and were probably in various stages of sea water adaptation. Even after 2 weeks in fresh water these eels could tolerate direct transfer to 100% sea water while the eels used in 1969 could not. Although in vitro water absorption by fresh water eel intestines used in the present study was not significantly different from that observed in 1969, the change in water absorption during sea water adaptation was different. Water absorption increased rapidly during sea water adaptation but did not "over shoot" as was previously observed (Bull. MDIBL 9:23-26,

1969). Instead, water absorption increased to approximately sea water adapted levels after 3 days in sea water (Table 1). This increase in water absorption was not accompanied by an increase in Na-K-ATPase level of gut mucosa which was not significantly different in eels from fresh water ($0.69 \pm 0.07 \mu\text{Moles } P_i/\text{mg protein} \cdot \text{hr}$, $n = 5$ at 15°C) and after 3 days in sea water (0.72 ± 0.12 , $n = 5$) but was significantly higher in eels adapted to sea water for 2 weeks (1.44 ± 0.07 , $n = 5$, $p < 0.001$). Water absorption by intestines from sea water adapted eels was inhibited 51% by 10^{-4}M 2,4-dinitrophenol (DNP) (Table 1). When fresh water adapted eels were injected with cortisol ($400 \mu\text{gm}/100 \text{gm} \cdot \text{day}$ of cortisol hemisuccinate) for 3 days, gut water absorption was not significantly different ($p < 0.001$) from that in sea water adapted eels (Table 2).

Table 2
EFFECT OF HYDROCORTISONE INJECTION ON WATER ABSORPTION
BY INTESTINAL SACS FROM FRESHWATER EELS

Days of hydrocortisone injection	0	1-1/2	3	10
Water absorption ($\mu\text{l}/\text{gm} \cdot \text{hr}$.)	85 ± 14 (15)	250 ± 58 (10)	357 ± 85 (7)	363 ± 62 (8)

Although most of the increase in water absorption following sea water adaptation was ouabain sensitive, a large component of water absorption was not ouabain or diamox sensitive. An increase equal to the increase in water absorption during sea water adaptation can be produced in 3 days in fresh water adapted eels by hydrocortisone injection. Although Na-K-ATPase may be important in water absorption, gut water absorption can increase markedly without increase in Na-K-ATPase and in sea water adapted eels only 50% of water absorption was inhibited by 2,4-dinitrophenol which would eliminate ATP as a substrate for Na-K-ATPase. Water absorption by eel intestine appears to be linked to more than one salt absorptive mechanism but further studies which directly measure salt transport are required in order to define these processes more accurately.

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1970 #27

RATES OF SODIUM, CHLORIDE, AND BICARBONATE ACCESSION TO CEREBROSPINAL FLUID (CSF) IN Squalus acanthias

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This study is part of a general program concerned with the relations among ion movements, fluid production, and physiological role of CSF. In the accompanying paper (Report #28) the movement of CO_2 between plasma and CSF has been studied and its physiological role suggested. In this paper the rates of radioactive sodium and chloride loss from plasma and entry into CSF were studied, followed by an analysis of the effect of carbonic anhydrase inhibition on these processes. The data are the first to show the quantitative relations for CSF entry among the three major