TABLE 3

Tissue level of vasoactive intestinal peptide (pg/100 mg wet weight)

Brain	66 ± 7.02	Duodenum	142 ± 18.0 *
Gill	47 ± 2.52	Rectum	135 ± 28.2 *
Pancreas	55 ± 2.52	Kidney	50 ± 1.15
Liver	56 ± 2.83	Rectal gland	88 ± 0
Stomach	63 ± 6.19	Mean all tissue[a]	56.2 ± 2.98

Each value represents Mean ± SEM. N is 4 for all samples except rectal gland where n is 2.

*$p < .001$ when compared to Mean all tissues.

[a]Mean all tissue excluding duodenum and rectum.

VIP were generally higher than those found in mammalian tissue by this laboratory. Especially interesting was the high concentration of hormone localized to the gastrointestinal tract of the fish, in particular the duodenum and rectum.

These studies provide further evidence that vasoactive intestinal peptide is an important humoral regulator of chloride secretion in the rectal gland. It seems likely that during feeding the ingested load of hypertonic sea water stimulates the release of VIP from the intestinal epithelia into the blood. This response must be mediated by an increase in luminal hypertonicity rather than in extracellular space since plasma VIP levels declined following intravenous hypertonic volume expansion. The high circulating level of VIP produced after gastrointestinal hypertonic infusion activates chloride secretion by a cyclic AMP-dependent mechanism. This effect may be modulated in part by endogenous prostaglandins. The increase in rectal gland secretion of sodium chloride produced by the hormone would then restore salt homeostasis.

OPEN-CIRCUIT Na^+ AND Cl^- FLUXES ACROSS ISOLATED OPERCULAR EPITHELIA FROM SEAWATER-ADAPTED *Fundulus heteroclitus* AND THE INFLUENCE OF ADRENERGIC STIMULATORS

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The opercular epithelium of the killifish, *Fundulus heteroclitus*, contains an abundance of chloride cells (Burns and Copeland Biol. Bull. Mar. Biol. Lab. Woods Hole 99:381-385, 1950) identical in ultrastructure to the gill chloride cell (Karnaky, Jr. and Kinter, J. Exptl. Zool. 199:355-364, 1977). When isolated and mounted in a lucite chamber under short-circuited conditions, this epithelium actively secreted Cl^- at a rate equivalent to the short-circuit current (Isc) with no significant net flux of Na^+ (Karnaky, Jr. et al Science 195:203-203, 1977; Degnan et al. J. Physiol. 271:155-191, 1977). More real life conditions can be experimentally studied by monitoring the open-circuit isotope fluxes across these epithelia when bathed on the mucosal side with seawater (SW) and on the serosal side with Ringer.

Opercular epithelia from SW-adapted killifish were isolated, pinned out as a flat sheet on a Sylgard (Dow Corning) disk with a 0.24 cm^2 aperture, and mounted in lucite chambers. Two epithelia were obtained from each fish, mounted in matching chambers, bathed on the mucosa with artificial SW (gassed with air, pH 7.9) and on the serosa with Ringer (gassed with 95% O_2/5% CO_2, pH 7.15), and kept open-circuited. The Na^+ and Cl^- concentrations of the SW and Ringer were determined with a flame photometer and a chloridometer respectively. The SW and Ringer Na^+ concentrations were 480.6 and 151.0 m-equiv/l. respectively and the SW and Ringer Cl^- concentrations were 533.1 and 142.5 m-equiv/l respectively. Isotope fluxes were performed on paired epithelia from the same fish. The transepithelial potential differences (p.d.) ranged from 23 to 38 mV (seawater side negative) and the criterion for good pairing was that the p.d.'s for the two preparations agreed within 10% of each other. The predicted flux ratios were calculated using Ussing's equation (Ussing, Hand. der Experiment. Pharmakol. 13:1-195, 1960), the average p.d. for the paired preparations, and activity coefficients given by Robinson and Stokes (Electrolyte Solutions, Academic Press, N.Y., 1959). The adrenergic stimulators were tested by first obtaining suitable control fluxes, adding isoproterenol to the serosal solution and obtaining new steady-state fluxes, and then adding arterenol to the serosal solution and obtaining further steady-state fluxes. Ouabain was tested by first obtaining suitable control fluxes, adding ouabain to the serosal solution, and obtaining additional fluxes starting one hour after the addition of ouabain.

The mean Cl^- and Na^+ unidirectional fluxes and mean p.d.'s across the paired epithelia are presented in Table 1. The Cl^- effluxes (serosa to mucosa) ranged from 1.260-4.678 $\mu equiv \cdot cm^{-2} \cdot hr^{-1}$ while the Cl^- influxes (mucosa to serosa) ranged from 1.522-4.445 $\mu equiv \cdot cm^{-2} \cdot hr^{-1}$. Isoproterenol, a β-adrenergic stimulator, and arterenol, primarily an α-adrenergic stimulator, stimulate and inhibit respectively the Isc and Cl^- secretion across opercular epithelia from SW-adapted fish (Degnan and Zadunaisky, unpublished observations). With these open-circuit epithelia isoproterenol stimulated the Cl^- efflux and arterenol inhibited the Cl^- efflux. These changes in the Cl^- efflux occurred in the face of p.d. changes which would have the opposite effects if Cl^- behaved passively. The changes in the Cl^- influxes in response to these drugs, however, changed in accordance with the p.d. changes. These results suggest active Cl^- efflux and passive Cl^- influx. The calculated Nernst equilibrium potential for Cl^- (E_{Cl^-}) across these open-circuited epithelia was 32.5 mV (seawater side positive), indicating that this anion was not at equilibrium.

TABLE 1.

Cl^- and Na^+ open-circuited fluxes across isolated opercular epithelia of seawater-adapted *Fundulus heteroclitus*

$^{36}Cl^-$ fluxes	Efflux	Influx ($\mu equiv \cdot cm^{-2} \cdot hr^{-1}$)	Net Flux	Mean p.d. (Efflux) mV	Mean p.d. (Influx) mV	Significance (P)
Control (5)	2.350 ± 0.638	2.854 ± 0.595	-0.504 ± 0.609	26.0 ± 3.8	26.0 ± 3.7	>0.60
Isoproterenol (5) 10^{-5} M, serosa	3.071 ± 0.705	3.477 ± 0.800	-0.406 ± 1.017	30.3 ± 3.3	28.2 ± 4.5	>0.70
Arterenol (5) 10^{-5} M, serosa	0.933 ± 0.104	2.625 ± 0.485	-1.692 ± 0.390	15.3 ± 3.3	14.7 ± 4.4	>0.10
$^{22}Na^+$ fluxes						
Control (7)	3.325 ± 0.718	3.869 ± 0.717	-0.544 ± 0.952	31.5 ± 1.0	30.6 ± 1.6	>0.50
Ouabain (7) 10^{-6} M, serosa	1.838 ± 0.434	3.310 ± 0.601	-1.472 ± 0.693	13.1 ± 1.7	14.2 ± 2.0	>0.10

mean ± s.e.m.; Number of paired experiments in parentheses; p.d. orientation is seawater side negative. Significance levels given for the difference between the mean efflux and influx.

The Na^+ effluxes ranged from 1.199-5.757 $\mu equiv \cdot cm^{-2} \cdot hr^{-1}$ while the Na^+ influxes ranged from 1.442-6.859 $\mu equiv \cdot cm^{-2} \cdot hr^{-1}$. Ouabain produced a 44.7% decrease in the Na^+ efflux and a 14.4% decrease in the Na^+ influx. The decreased efflux could be accounted for by the decreased p.d. but the influx would be expected to increase in response to this same p.d. change. The E_{Na^+} for these epithelia was 28.4 mV (seawater side negative) which was close to the mean p.d. of 31.1 ± 0.9 mV (seawater side negative), suggesting that Na^+ was at equilibrium across these epithelia.

The predicted and observed flux ratios for the experiments listed in Table 1 are presented in Table 2. Although further studies are required to establish statistical significance, the present data suggested that Cl^- was not at equilibrium across these epithelia and that the Cl^- efflux was active and the Cl^- influx was passive. The data suggested that Na^+ was at equilibrium and that the unidirectional Na^- fluxes

TABLE 2

Cl^- and Na^+ flux ratios across isolated opercular epithelia of seawater-adapted F. *heteroclitus*

	Cl^- Flux Ratios	
	Predicted	Observed
Control (5)	10.11 ± 1.21	1.42 ± 0.34
Isoproterenol (5) 10^{-5} M, serosa	11.48 ± 1.27	1.36 ± 0.43
Arterenol (5) 10^{-5} M, serosa	6.59 ± 0.79	2.77 ± 0.29
	Na^+ Flux Ratios	
	Predicted	Observed
Control (7)	0.91 ± 0.03	1.52 ± 0.43
Ouabain (7) 10^{-6} M, serosa	1.78 ± 0.10	2.14 ± 0.32

Mean ± S.E.M.; Number of paired experiments in parentheses.

across these epithelia were passive processes. These results were in good agreement with those obtained with several intact SW-adapted teleosts (see data compiled by Maetz and Bornancin, Fortschr. Zool. 23: 322-362, 1975). Relatively large fluxes of both Na^+ and Cl^- across the gills in both directions were observed. Cl^- was secreted against an electrochemical gradient, suggesting active transport, and the measured gill potentials were similar to the calculated Na^+ equilibrium potential. The presence of α- and β-adrenergic receptors in the gill are known (Pic et al. Comparative Physiology, pp. 293-321, North Holland Press, 1973). Stimulation of the α-receptors inhibits branchial Cl^- secretion while stimulation of the β-receptors reportedly alters the branchial hydraulic conductivity. These results with opercular epithelia suggest that α-adrenergic inhibition of gill Cl^- secretion does not result from changes in the branchial hemodynamics. No observations that β-adrenergic stimulation increases gill Cl^- secretion have been reported. These studies were supported by NIH grants EY 01340 (JAZ) and EY 05059 (KJD).

EVALUATION OF THE RENAL HANDLING OF 2,4-D BY THE WINTER FLOUNDER

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Among the most widely used pesticides today are 2,4-D (2,4-dichlorophenoxyacetic acid) and related herbicides. These compounds are generally thought to be relatively non-toxic to mammals, at least in part due to their rapid renal excretion via the organic acid transport system (Toxicol. Appl. Pharmacol.