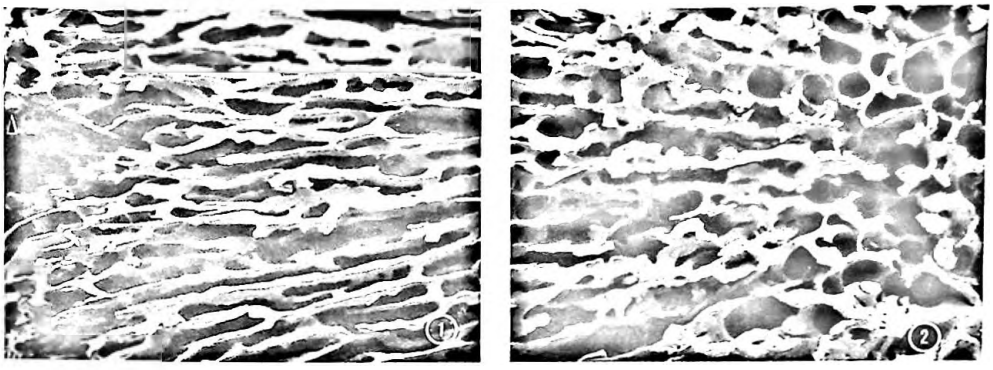

Figure 1. Scanning electron micrograph of outer tubular sinus vessels (75X). Flow is from left (capsular tissue) to right. Note small arteriole at left (arrow).

Figure 2. Scanning micrograph of inner tubular sinus vessels (75X). Plane of section is the same as Figure 1. Rectal gland lumen is out of view to the right and blood flows left to right.

The sinus vessels around the tubules appear to provide a pathway for unidirectional blood flow that is concurrent with tubular secretion as has been described previously (Bulger, Anat. Rec. 147:95, 1963; Doyle, Bull. MDIBL 17:34, 1977). However, the close association between the capsular arterial and venous vessels does suggest countercurrent exchange is possible between these vessels. The connections of the venous capsular vessels with veins leaving at the level of the mesenteric artery and the vessels entering the post-valvular intestine suggest that the direction of flow in the capsular area might be regulated by the relative resistances between the mesenteric and intestinal veins. Thus venous flow could be counter- or concurrent with arterial capsular flow. Although these studies could not conclusively demonstrate arterio-venous anastomoses between capsular vessels, this cannot be discounted and could perhaps contribute to A-V exchange. Clearly additional studies on flow patterns within rectal gland vasculature are warranted. This work was supported in part by Research Project #4901-01 and 02, Veterans Administration Medical Center, Bronx, New York and by NSF #PCM 76-16840.

INHIBITION OF CHLORIDE TRANSPORT BY ACETYLCHOLINE IN THE ISOLATED OPERCULAR EPITHELIA OF *Fundulus heteroclitus*. PRESENCE OF A MUSCARINIC RECEPTOR

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The isolated opercular epithelium of *Fundulus heteroclitus* contains great numbers of chloride cells and transports chloride ions from blood to seawater in in vitro conditions. In order to understand the mechanisms of control of this secretion, cholinergic agonists and antagonists have been tested on this preparation. An advantage of this model of study of fish osmoregulatory phenomena is that interference by vascular responses is excluded.

Opercular epithelia were dissected out and mounted in lucite chambers on sylgard covered disks as previously described (Degnan et al. J. Physiol. 277:155, 1977). After equilibration of the spontaneous potential difference the preparations were short circuited and drugs added to the serosal side in volumes of 25 µl or 250 µl in order to achieve the final concentrations referred to below. The volume of Ringers solution in the chambers was 2.5 ml. Radioisotopic fluxes were measured with $^{36}Cl^-$ which was added to the serosal or mucosal side in amounts ranging from 2 to 5 µC. Samples of 250 µl from the nonradioactive side and 25 µl from the radioactive side were taken at intervals of 30 minutes,

after a period of equilibration of the radioisotope with the membrane of 45 minutes. Two 30-minute control periods and two subsequent periods of similar duration were used to detect the effect of acetylcholine. The transient time during which the drug was producing its effect was not utilized in the calculations. The following drugs were utilized: acetylcholine chloride, acetylcholine bromide, acetyl-beta-methycholine chloride (metacholine), succinylcholine chloride, carbachol, muscarine chloride, dl-homatropine, d-tubocurarine chloride, eserine sulfate, Nicotine (1-methyl-2(3-pyridyl-pyrrolidine). All these compounds were dissolved in stock solutions in Ringers at concentrations of 10^{-2} or 10^{-4} M.

The addition of acetylcholine chloride at a final concentration of 10^{-5} M to the serosal side of the preparation produced a reduction of 70% in the short circuit current generated by the isolated operculi. A proportional reduction in potential difference was found without significant changes in the electrical resistance of the preparation. The action of acetylcholine was faster and reached stabilization quicker when it was added to the serosal (blood) side of the operculi than when placed in the mucosal (seawater) side. Therefore, additions of acetylcholine and other drugs were performed on the serosal side. The minimal concentration that produced a definite drop in current was 10^{-8} M with maximal effect at 10^{-3} M. The experiment shown in Figure 1 demonstrates the effect of acetylcholine on the current, and the dose response curve of Figure 2 constructed from a series of experiments demonstrates the behavior of this inhibition as a typical pharmacological receptor for acetylcholine in the opercular epithelium. It can be observed in Table 1 that the inhibition of the net flux of chloride occurs at the expense of a reduction in the efflux, that is in the chloride movement from blood to seawater side, without appreciable effects in the passive chloride flux. The net reduction in chloride transport was proportional to the reduction of the short circuit current.

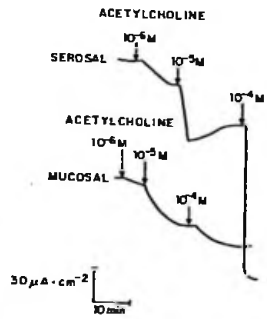

Figure 1. Inhibitory effect of acetylcholine chloride on the short circuit current of isolated opercular epithelium of *Fundulus heteroclitus*.

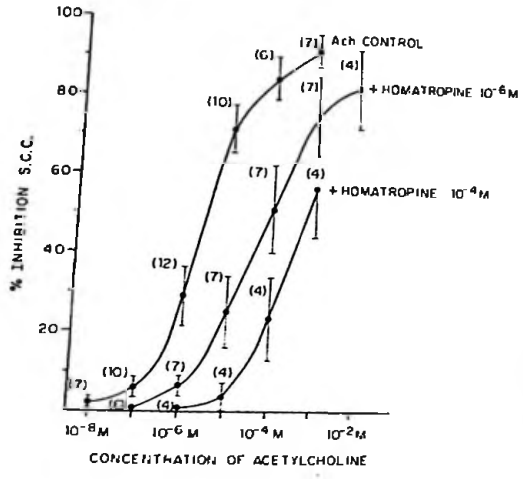

Figure 2. Dose response curves for acetylcholine inhibition of the chloride current of isolated opercular epithelia of *Fundulus heteroclitus*. Note the displacement to the right of the dose response curves at two different concentrations of homatropine. Similar dis placements were observed with atropine. Muscarine reproduced the inhibitory effect of acetylcholine.

TABLE 1

Effect on acetylcholine chloride (10^{-3} M)* on the chloride fluxes across the isolated opercular epithelium of *Fundulus heteroclitus*

	J_{out}	J_{in}	J_{net}		Inhibition SCC	Inhibition J_{net}
	$\mu M.cm^{-2}.hr^{-1}$	$\mu M.cm^{-2}.hr^{-1}$	$\mu M.cm^{-2}.hr^{-1}$	$\mu M.cm^{-2}$	%	%
Control (5)	6.78 ± 0.61**	1.071 ± 0.26	5.71	153.0	....	....
ACH (6)	3.04 ± 0.48	1.058 ± 0.26	1.98	53.0	77.9	65.3

*Though acetylcholine chloride produces inhibition at lower concentrations (see dose response curves) the maximal dose was used to demonstrate the effects on the uni-
**directional fluxes of chloride.
Mean ± SEM.

Once it was established that the effect of acetylcholine was on the chloride efflux, the type of receptor was characterized by drug interaction studies following the changes in the short-circuit current.

Homatropine at a concentration of 10^{-6} or 10^{-4} M blocks the effects of low concentration of acetylcholine. The dose response curve to acetylcholine shows a definite shift to the right as shown in Figure 2 indicating the effective action of homatropine, a competitive inhibitor of acetylcholine effects on the inhibition of the chloride current.

Atropine at a concentration of 10^{-5} M produced similar competitive inhibition of the acetylcholine response, in 3 experiments. The addition of muscarine chloride at concentrations ranging from 5×10^{-8} to 5×10^{-3} M produced inhibition of the chloride short circuit current, similar to the effects of acetylcholine. Muscarine, an agonist of the action of acetylcholine produces its effects by activating the same membrane receptor responsible for the inhibition produced by acetylcholine. The acetylcholine agonists carbachol, at a concentration of 10^{-8} to 10^{-3} M in 6 experiments, and methacholine at a concentration 10^{-6} to 10^{-5} M in 3 experiments produced inhibition of the short circuit current similar to acetylcholine and their effects were blocked by atropine.

The above described results indicate the presence of a so-called muscarinic receptor in the membranes of the chloride cells of the opercular epithelium.

A search for a nicotinic receptor produced negative results. Doses of nicotine from 2×10^{-8} to 2×10^{-3} M failed to produce any effect on the short circuit current in 3 experiments. After a test for any action of nicotine, the current could be inhibited by carbachol. Succinylcholine at a concentration of 10^{-7} to 10^{-5} M in 2 experiments and the neuromuscular blocking agents tubocurarine at a concentration of 4×10^{-5} M had no effects on the short circuit currents of the isolated operculi. Furthermore tubocurarine did not block the inhibition produced by acetylcholine, in 5 experiments.

Eserine sulfate, the inhibitor of acetylcholine esterase, was used at a concentration 4×10^{-3} M in 6 experiments. No potentiating effects of eserine were found on the acetylcholine response of the short circuit current.

It has been shown that the chloride cells of the fish gill which occupy 70% of the surface of the opercular epithelium are responsible for the transport of chloride ions from blood to seawater in *Fundulus heteroclitus*. We have to assume that the cell membranes of the chloride cell represent the site where the here described acetylcholine receptor is located. Most probably the membranes of the chloride cell facing the serosal or blood side are sensitive to acetylcholine. Acetylcholine produced vasoconstriction in the isolated perfused gill preparation (Renkin and Maetz, J. Endocrinol. 51:621-635,

1971), however it has not been successfully tested in intact fishes to ascertain its functions on ion translocation in the gills because of technical difficulties. The findings reported here of a muscarinic receptor indicate that this transmitter has a definite function in cell and internal medium osmoregulation in the teleost fish. Support for this work by the following funds is gratefully acknowledged: Student fellowship for George M. Rowing of the Science Research Council of Great Britain and NIH Research grants GM 25002 and EY 07009.

SATURATION OF THE OCULAR TRANSPORT MECHANISM FOR D-GLUCOSE IN THE SPINY DOGFISH, *Squalus acanthias*

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Work on the transport properties of the ocular barriers of the dogfish was continued. As regards D-glucose transport from the blood to the ocular humor, if one postulates a "carrier-mediated" facilitated diffusion transport mechanism, certain classical criteria can be tested. A number of these criteria, namely that D-glucose transport is downhill and faster than it should be on the basis of molecular size and lipid solubility, and that glucose transport is stereospecific with D-glucose being greatly preferred to its L-isomer, have been previously reported by us in this bulletin (Bull. MDIBL 17:106-110, 1977). This summer we addressed ourselves to the remaining question of whether this transport system is saturable.

Essentially our method consisted of elevating the blood D-glucose level, via intravenous infusion of unlabeled D-glucose solutions, for a period of 30 minutes and subsequently determining the cold glucose level at 10-minute intervals from plasma samples obtained; with the circulating plasma at a relatively stable, elevated glucose level, we then performed our determination of ocular D-glucose transport rate utilizing ^{3}H D-glucose and methods previously described (Bull. MDIBL 17:106-110, 1977).

Dogfish weighing from 1.7 to 4.9 kg were restrained on a wooden rack, head submerged nearly flat in tank of freshly flowing native seawater. The dorsal aorta was cannulated and fitted with a 2-way stopcock allowing free access to the dogfish plasma (the fish was heparinized to avoid clotting). The cold D-glucose solutions were infused using a constant delivery volume peristaltic pump which was calibrated, and run at pumping rates of .005 to .020 ml/min. We took care to infuse no more than 3 ml of solution/kg of fish during the whole course of the experiment in an effort to not excessively raise the plasma volume. When the total volume of blood samples withdrawn are considered, the infusion elevated the plasma volume only approximately 2-3%. At 10-minute intervals, 100 µl samples of whole blood were withdrawn and the concentration of D-glucose determined by the enzymatic colorimetric procedure using glucose oxidase and peroxidase supplied in kit form by Sigma Chemical Co. When the plasma glucose was sufficiently elevated, the pumping rate was lowered to a maintenance level and a final blood sample taken from which we could estimate the cold glucose concentration before injection of the labeled D-glucose. We also determined the cold D-glucose concentration at the end of the ocular rate transport determination and thus were able to estimate a mean D-glucose concentration, $\bar{c}$, during a particular experimental period for the determination of the ocular rate transport constants, Ki and Ko.

After the D-glucose infusion period, a bolus injection of (^{3}H) D-glucose was introduced into the central plasma compartment of the test animal at time 0. At intervals during the experimental period beginning with time 0, small samples of blood were taken and the concentration of radioactively labeled test substance determined. The times used were roughly: 2, 4, 6, 9, 12, 15, 20, 25, 30 minutes for a 30-minute experiment. From these data a mathematical description of concentration as a function of time was constructed having the form:

$$C_p = A + Be^{-b_2 t} + Ce^{-b_1 t}$$