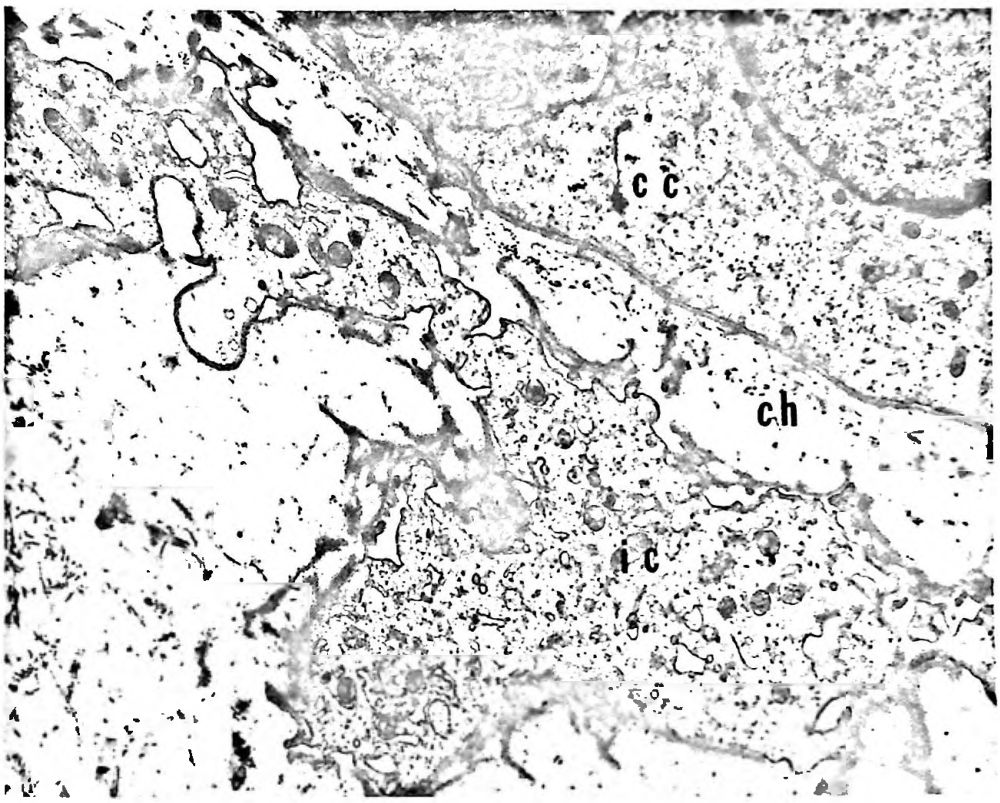

Figure 2. Electronmicrograph showing the channel next to the collecting duct. It was in these channels that the Alcian Blue was seen following injection of the dye within 50 μ of the papilla tip. The channel (ch) is bordered by the basal lamina of the collecting duct cells (cc) and by the interstitial cell (ic).

SODIUM DEPENDENT CHLORIDE SECRETION IN THE RECTAL GLAND OF THE SPINY DOGFISH, *Squalus acanthias*

Patricio Silva, Katherine Spokes, Jonathan Epstein, and Franklin H. Epstein, Department of Medicine and Thorndike Laboratory Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts

The secretion of chloride by the rectal gland of the spiny dogfish, *Squalus acanthias*, appears to be dependent on the continued activity of Na-K-ATPase. Experiments using isolated perfused rectal glands have shown that ouabain 10^{-4} blocks completely and irreversibly the chloride secretion by the gland. Since chloride is the ion actively transported across the rectal gland epithelium, it has been postulated that the active movement of chloride into the cell is linked with the passive movement of sodium down its electrochemical gradient across the basolateral cell membrane. The gradient for sodium flow depends on the maintenance of a low intracellular concentration of sodium by Na-K-ATPase. The movement of chloride into the cell would thus be dependent on the sodium concentration outside the cell. The experiments reported here were designed to test this hypothesis.

Dogfish of either sex weighing 2 to 5 kg were taken by hook and line from Frenchman's Bay and kept in marine live cars until sacrifice, usually within 3 days of capture. After segmental transsection of the cord, the rectal gland was resected via an abdominal incision. The rectal gland artery, vein and duct were cannulated with PE90 tubing. The glands were then placed in a plexiglass and aluminum chamber kept at 15° to 17°C by running sea water. The rectal glands were perfused by gravity at a pressure of 4 mm Hg and a flow between 3.5 and 7 ml/min. The perfusion medium contained in mM: K 5; Cl 280; HCO_3 8; phosphate 1; Ca 2.5; Mg 3; sulfate, 0.5; urea 350; glucose 5; pH 7.6 when gassed with 99% O_2 5% CO_2. In the experiments reported here the amount of sodium in the perfusate was varied from 0 to 280 mM substituting either choline or Tris to avoid osmotic changes. In all experiments the glands were stimulated continuously with 0.25 mM theophylline and 0.05 mM dibutyryl cyclic AMP. Rectal gland fluid was collected in 1.5 ml conical centrifuge tubes during 15 min periods. Changes in perfusate composition were made at the end of one or more collection periods.

Chloride determinations were made using a Buchler-Cotlove chloridometer. Sodium and potassium determinations were done in an IL 343 Flame photometer.

Figure 1 shows the result of substituting choline for sodium in the perfusion medium. Secretion of chloride in rectal glands averages 859.6 ± 276.5 µEq/hr/g wet weight when perfused with a normal (280 mM) sodium concentration. In the absence of sodium, chloride secretion drops to 58.8 ± 29.3 µEq/hr/g wet weight, representing essentially complete inhibition. Chloride secretion returns to the previous level (885.2 ± 386.8 µEq/hr/g wet weight) when sodium concentration in the

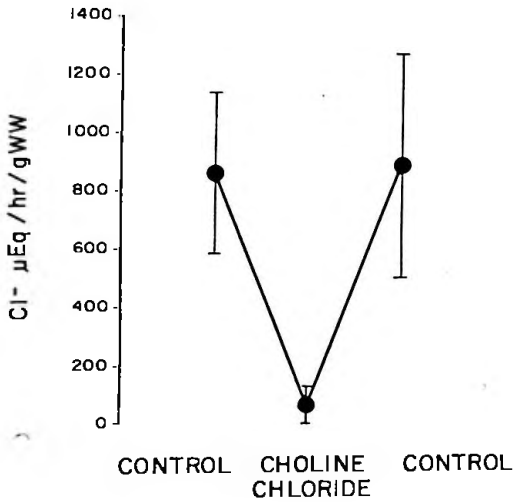

Figure 1. Effect of substituting choline for sodium in the solution perfusing five rectal glands on chloride secretion rate in vitro. The secretion rate of chloride is reversibly inhibited by removing sodium from the perfusate. Dots represent means, bars represent ± S.E.M.

perfusate is restored to 280 mM. Dependence of chloride secretion on the sodium concentration in the perfusate is further demonstrated by the experiment shown in Figure 2. The chloride secretion in five perfused rectal glands is directly proportional to the concentration of sodium in the perfusate. As sodium concentration varies through 0, 70, 140, 280 mM, chloride secretion increases from 0 to 735.2 ± 154.8.

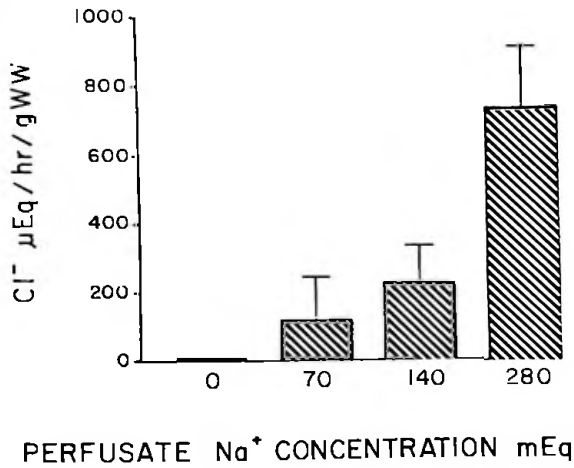

Figure 2. The effect of increasing progressively the concentration of sodium in the perfusate from 0 to 70, 140, 280 mM on chloride secretion rate in five rectal glands in vitro. There is a linear relation between the rate of chloride secretion and the concentration of sodium in the perfusate.

The possible effect of nonspecific cation substitution was then tested by substituting Tris for sodium. When Tris replaced sodium in the medium perfusing the stimulated rectal gland, chloride secretion decreased from 1667.5 ± 276.5 to 133.7 ± 35.6 µEq/hr/g wet weight. Restoring sodium concentration to 280 mM increased chloride secretion to 1403.9 ± 123.2 µEq/hr/g wet weight just as in the experiments with choline chloride.

The sodium-dependent chloride secretion exhibited by the stimulated rectal gland is reminiscent of the sodium-dependent electrolyte transport reported for mammalian intestine, pancreas, salivary gland, cornea, gastric mucosa, and frog skin. Similarly, other transport systems responsible for the reabsorption of glucose and amino acids are known to require sodium. The sodium-dependent chloride secretion observed in the rectal gland is one of the features that characterize a general hypothesis for chloride transport that has been previously proposed. The movement of chloride into the cell against an electrical gradient is coupled to the passive toward movement of sodium down its electrochemical gradient. Sodium and chloride move across the basolateral cell membrane via coupled facilitated diffusion. Maintenance of the gradient facilitating the movement of sodium into the cell is due to the continued activity of Na-K-ATPase located in the basolateral membrane of the cell, hence the inhibition of chloride secretion by ouabain. Movement of chloride across the luminal membrane of the cell is believed to be passive along an electrical gradient.

INTRACELLULAR CYCLIC AMP LEVELS AND ACTIVE CHLORIDE TRANSPORT IN THE RECTAL GLAND OF *Squalus acanthias*: THE EFFECT OF VASOACTIVE INTESTINAL PEPTIDE

Jeffrey S. Stoff, Maria T. Baranano, Patricio Silva, Robert M. Rosa, and Franklin H. Epstein,
Department of Medicine and Thorndike Laboratory of Harvard Medical School at Beth Israel Hospital, Boston, Massachusetts and Mount Desert Island Biological Laboratory, Salsbury Cove, Maine

The rectal gland of *Squalus acanthias* is a compound tubular gland which actively transports chloride. Previous work from our laboratory utilizing an in vitro perfusion technique has established