separates the canalicular lumen from the lateral intercellular space. Paracellular pathways may be important in the formation of bile in this species.

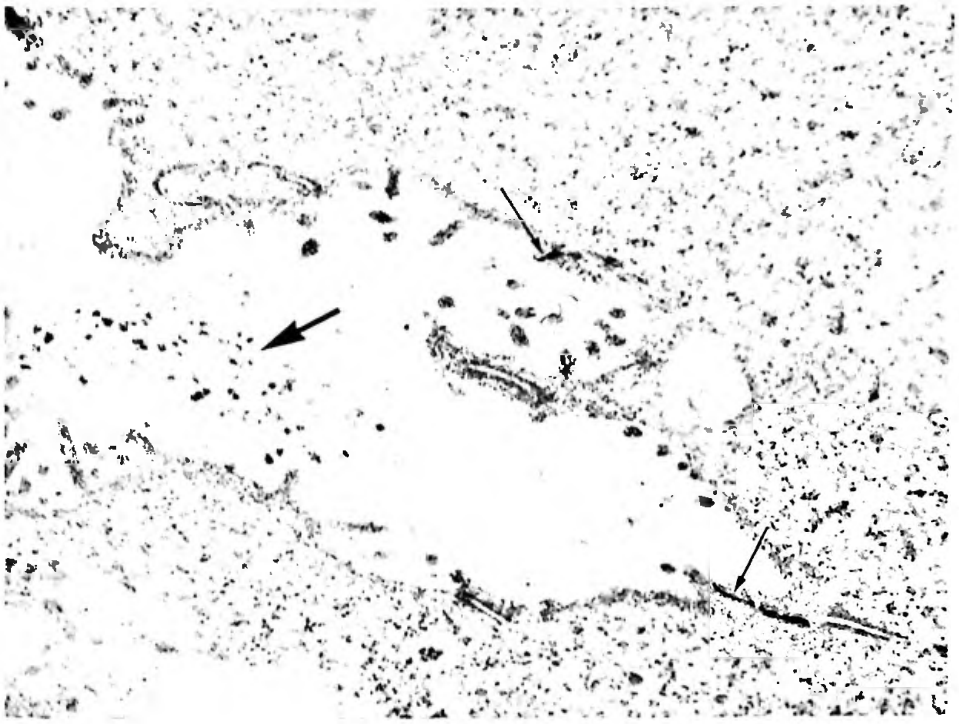

Figure 1. Representative Electromicrograph (X 25,000) of a skate liver canaliculus cut obliquely. Arrows show electron dense material (lanthanum) in both the junctional complexes (small arrows) and the canalicular lumen (large arrow). Stained with uranyl acetate and lead citrate.

MECHANISM OF CHLORIDE TRANSPORT IN THE RECTAL GLAND OF *Squalus acanthias*: IONIC SELECTIVITY

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The rectal gland of the spiny dogfish, *Squalus acanthias*, secretes chloride against an electrochemical gradient. It has been postulated that the active movement of chloride into the rectal gland cell is linked with the passive movement of sodium down its electrochemical gradient across the basolateral membrane. The gradient for sodium is maintained by the continued activity of Na-K-ATPase. Experimental observations using an isolated perfused rectal gland preparation have confirmed that the transepithelial movement of chloride in the rectal gland depends on the presence of sodium outside the cell (Bull. MDIBL 16:93-96, 1976) suggesting that the entry of chloride into the cell is coupled to that of sodium. We have also shown that ouabain 10^{-4} M blocks the transepithelial movement of chloride, providing evidence that Na-K-ATPase is energizing the process (Bull. MDIBL 15:69-71, 1975). If sodium and chloride enter the cell via a tightly linked carrier mechanism, the operation of this carrier would require chloride, and the secretion of both sodium and chloride will depend on the selectivity of the carrier mechanism for either ion. The experiments reported here were designed to explore these questions using the isolated perfused rectal gland. In addition, we sought to define further the participation of Na-K-ATPase in the process of chloride transport by inhibiting its activity while avoiding the use of ouabain.

Dogfish of either sex weighing 2 to 7 Kgs were taken by hook and line from Frenchman Bay and kept in marine livecars until they were killed, usually within three days of capture. The dogfish were killed by segmental transection of the cord and the rectal gland removed 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°-17°C by running sea water. The rectal glands were perfused by gravity at a pressure of 4 mm of Hg and a flow of 3 to 7 ml/min. The perfusion medium contained, unless otherwise specified, in mM: Na^+ 280; K^+ 5; Cl^- 290; 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 and 1% CO_2. 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 ten minute periods. Changes in the composition of the perfusate were made at the end of two or more collection periods. Experimental periods were usually of 30 minutes duration divided into 10 minute intervals. Determination of chloride was done by amperometric titration in a Buchler-Cotlove chloridometer. Sodium and potassium were measured in an IL 143 flame photometer. Results are expressed as mean ± SE (n).

Evidence for the participation of Na-K-ATPase in the transport of chloride in the rectal gland rests on the inhibition by ouabain of that portion of active chloride transport stimulated by theophylline and cyclic AMP. Another way of inhibiting Na-K-ATPase activity is to remove potassium from the medium perfusing the gland. Figure 1 shows the result of this maneuver. In the presence of 5 mM potassium the secretion of

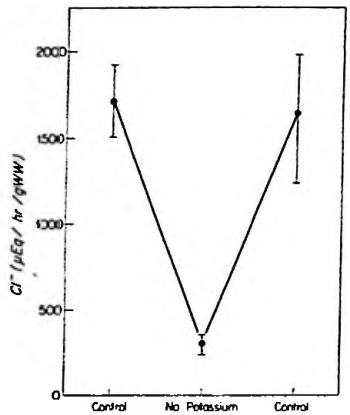

Figure 1. Effect of removing potassium from the medium perfusing six rectal glands. The secretion of chloride is reversibly inhibited by this maneuver. Dots represent means, bars represent ± S.E.M.

chloride averages 1714 ± 213 µEq/hr/g WW in 10 glands. When at the end of thirty minutes, potassium is left out of the perfusate replacing it with equimolar amounts of sodium, chloride secretion falls to 301 ± 5.7 in the final 10 minutes of a 30 minute experimental period, or 17.5% of control, a figure similar to that found after ouabain inhibition. In contrast to the experiments in which ouabain is used, chloride secretion returns to the initial level 1648 ± 371 when potassium concentration in the perfusate is restored to 5 mM. Since perfusion without potassium may deplete the rectal gland cells of potassium the concentration of potassium was measured in the venous effluent. Potassium was found in ever decreasing concentrations and after thirty minutes essentially no potassium could be measured in the venous effluent. Continuing perfusion without potassium for longer than thirty minutes decreases chloride secretion slightly more to 13.5% of control after 50 minutes of perfusion.

The selectivity of the hypothetical sodium and chloride facilitated diffusion for sodium was then tested by replacing all the sodium in the perfusion medium with lithium. We had previously shown that replacing perfusate sodium with either choline or Tris stopped chloride secretion virtually completely (Bull. MDIBL 16:93-96, 1977). Figure 2 shows the rate of Cl^- secretion in four glands during control periods (1417 ± 87 µEq/hr/g WW). After replacement of sodium by equimolar concentrations of lithium it fell to 51 ± 18, a value similar to that found when sodium was replaced by either choline or Tris. Following restoration of normal sodium concentration chloride secretion returns to control levels (1458 ± 280).

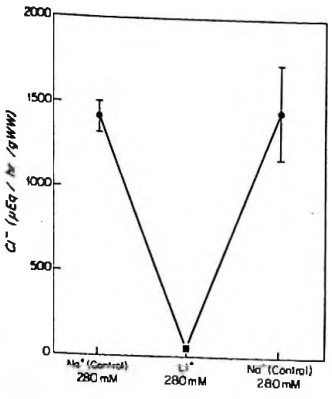

Figure 2. Effect of substituting lithium for sodium in the solution perfusing six rectal glands on the rate of chloride secretion in vitro. The secretory rate of chloride is reversibly inhibited by removing sodium from the perfusate. Lithium cannot replace sodium and inhibits in a reversible way chloride secretion. Dots represent means. Bars represent ± S.E.M.

The selectivity of the carrier mechanism for anions was then tested. Figure 3 shows that bromide can partially replace chloride in the process of rectal gland secretion. Following replacement of all the chloride in the perfusate with bromide the rate of halide secretion (bromide is measured as chloride in the chloridometer) falls by approximately 35%, from 1723 ± 221 to 1126 ± 180 in three glands. Following

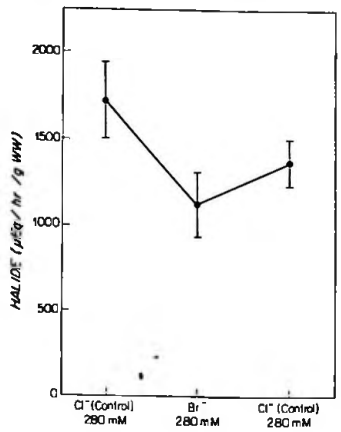

Figure 3. Effect of replacing chloride by bromide in the medium perfusing six rectal glands on the rate of halide secretion by the gland. Bromide can partially substitute for chloride. Dots represent means. Bars represent ± S.E.M.

restoration of normal chloride concentration, in the absence of bromide, chloride secretion rate rises to 1372 ± 132, a value not different from that of the initial control period.

Another anion examined was nitrate. Figure 4 shows that sodium secretion by the rectal glands falls dramatically when all the chloride in the perfusion medium is replaced by nitrate, from 1758 ± 157 in controls to 283 ± 61 in the absence of chloride. The secretion of chloride fell from 1782 ± 260 to 52 ± 18 μEq/hr/g. The potential difference across the gland was not eliminated by nitrate substitution but remained - 4.0 ± 0.4 mV duct lumen negative. The effect of removing chloride from the perfusate is readily reversible. Figure 5 shows that the rate of secretion of sodium, chloride and water is proportional to the concentration of chloride in the perfusate. As chloride in the perfusate decreases through 280, 140, 70, 0 mM so do fluid, sodium and chloride secretion in a fashion directly related to the concentration of chloride ($r = .88$, $p < .01$ for volume; $r = .87$, $p < .01$ for Cl; $r = .89$, $p < .01$ for Na^+). The fact that sodium secretion does not decrease as much as that of chloride, suggests that the gland transports nitrate to some extent.

The selectivity of the carrier mechanism for chloride was further tested by replacing chloride by another anion, acetate. Figure 6 shows the rate of fluid, chloride and sodium secretion by five rectal glands in which chloride was progressively replaced by acetate. As in the experiments in which chloride was substituted

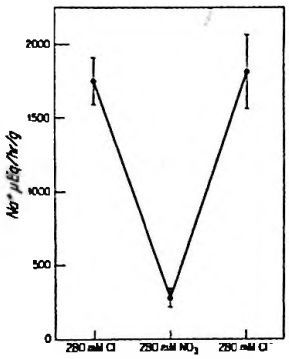

Figure 4. Effect of substituting nitrate for chloride in the perfusate of six rectal glands on the rate of secretion of sodium. Nitrate cannot replace chloride and inhibits the rate of secretion of sodium in a reversible manner. Dots represent means. Bars represent ± S.E.M.

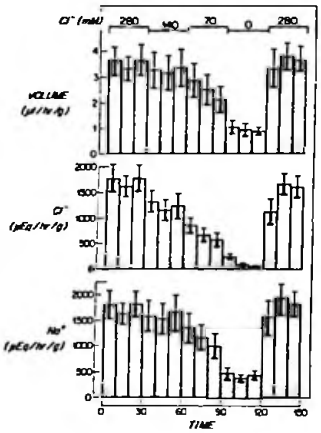

Figure 5. Substitution of nitrate for chloride. Effect of decreasing progressively the concentration of chloride in the perfusate from 280 to 140, 70, 0 mM and returning it to 280 mM on the rate of secretion of volume in the upper panel, chloride in the middle panel and sodium in the lower panel in six rectal glands. There is a linear relation between the rate of secretion of the three parameters shown and the concentration of chloride in the perfusate. It can be observed also that a small amount of nitrate is transported by the gland as evidenced by a larger decrease in the chloride secretory rate than that of sodium. Bars represent means ± S.E.M.

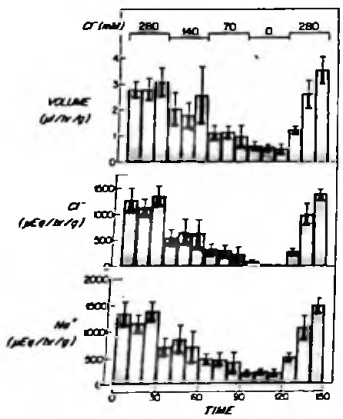

Figure 6. Substitution of acetate for chloride. Effect of decreasing progressively the concentration of chloride in the perfusate from 280 to 140, 70, 0 mM and returning it to 280 mM on: the rate of secretion of volume in the upper panel, chloride in the middle panel and sodium in the lower panel in four rectal glands. As with the nitrate substitution experiments there is a linear relation between the rate of secretion of all parameters and the concentration of chloride in the perfusate. As was the case with nitrate a small amount of acetate is transported across the gland. Bars represent means ± S.E.M.

by nitrate, when chloride concentration in the perfusate decreased through 280, 140, 70, and 0 mM the rate of rectal gland secretion falls progressively in a linear relation indicating that the secretory function of the rectal gland is dependent on chloride in the perfusate ($r = .86$, $p < .01$ for Cl; $r = .87$, $p < .01$ for Na). As was the case with nitrate, a small amount of acetate is transported across the gland epithelia as witnessed by the smaller decrease in sodium secretion compared with that of chloride.

These studies complement our previous experiments on the mechanism of chloride transport by the perfused rectal gland of the spiny dogfish. Evidence for the dependence of active secretion on Na-K-ATPase is strengthened by the fact that removal of potassium from the perfusion medium stops ion movement across the rectal gland. Secretion is dependent not only on the presence of sodium but also on that of chloride in the perfusate. Lithium cannot replace sodium and reversibly inhibits chloride secretion. Anions such as nitrate and acetate cannot replace chloride or do so to an extremely limited extent. They inhibit sodium secretion in a linear fashion and show clear dependence of sodium transport on chloride. Bromide can partially replace chloride in this system. Chloride secretion by the shark rectal gland is consistent with the presence of a linked sodium and chloride carrier that transports chloride into the cell against an electrochemical gradient. Na-K-ATPase is essential to maintain a gradient powering the downhill movement of sodium into the cell. The carrier that moves both sodium and chloride appears to be very selective for these ions.

THE OXYGEN COST OF CHLORIDE TRANSPORT IN THE RECTAL GLAND OF THE SPINY DOGFISH *Squalus acanthias*

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The isolated perfused rectal gland of the spiny dogfish, *Squalus acanthias*, offers many advantages for the study of the relationship between oxygen consumption and electrolyte transport. The artery and vein are cannulated and readily accessible for oxygen measurement. The rate of rectal gland secretion can be varied at will over a range that is tenfold that of basal secretion by stimulation with cAMP and theophylline. It remains viable for long periods of time and is perfused with an artificial medium lacking erythrocytes. We have previously shown that chloride secretion by the rectal gland is linearly related to oxygen consumption (Rosa et al. Bull. MDIBL 16:87, 1976). This relation was further studied in the present series of experiments.

Spiny dogfish weighing 2 to 7 Kg were taken by hook and line from Frenchman Bay, Maine and kept in marine live cars until killed, usually within three days. The dogfish were killed by segmental transections of the cord and the rectal glands removed by an abdominal incision. The rectal gland artery, vein and duct were catheterized with PE90 tubing. The glands were then placed in a plexiglass and aluminum chamber kept at 15° to 17° with running sea water. The glands were perfused by gravity at a pressure of 4 mm Hg with an artificial perfusion medium containing in mM: Na 280; K 5; Cl 290; 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 1% CO_2. Collections of arterial perfusate were obtained through a self-sealing rubber connector close to the arterial cannula. Rectal gland vein collections were taken anaerobically from the venous catheter. Rectal gland fluid was collected in 1.5 ml conical centrifuge tubes. Perfusate flow through the gland was measured directly by collecting all venous effluent in a graduated cylinder. Oxygen tension determinations were done using a polarographic oxygen electrode equipped with a constant temperature cell maintained at the same temperature as the perfused gland. Chloride in perfusate and rectal gland fluid was measured by amperometric titration with a Buchler-Cotlove chloridometer. Sodium and potassium measurements were done in an IL 143 flame photometer. Results are expressed as mean ± SE.

Figure 1 shows the relation between oxygen consumption and chloride secretion in 278 observations in 91 perfused rectal glands during resting unstimulated periods, represented by the + sign, and after varying degrees of stimulation with theophylline and dibutyryl cyclic AMP, shown as the open symbols. There is no correlation between the rate of chloride secretion and oxygen consumption in the resting state as illustrated by a correlation coefficient of 0.008. The line of best fit is a horizontal line exactly parallel to the