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MECHANISM AND CONTROL OF HYPEROSMOTIC NaCl-RICH SECRETION BY THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

BY FRANKLIN H. EPSTEIN, JEFFREY S. STOFF AND PATRICIO SILVA

Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, U.S.A., Charles A. Dana Research Institute, Harvard-Thorndike Laboratory of Beth Israel Hospital and the Department of Medicine, Harvard Medical School and Beth Israel Hospital, Boston, Massachusetts, U.S.A.

SUMMARY

Secretion of chloride from blood to lumen is accomplished in the rectal gland of elasmobranchs by a process of secondary active transport involving the co-transport of Cl^- with Na^+ across the basolateral membranes of rectal gland cells. Energy is provided by ATP *via* membrane Na-K-ATPase, which establishes an electrochemical gradient favouring Na^+ influx into the cell. The involvement of K^+ in the co-transport mechanism, so as to provide a ratio of $1\,Na^+ : 1\,K^+ : 2\,Cl^-$ entering the cell, would increase the energetic efficiency of the process, and is consistent with the Cl/O_2 ratio of 27–30 observed in secreting rectal glands. Secretion is stimulated by cyclic AMP (cAMP) and by vasoactive intestinal peptide (VIP) and adenosine, which activate adenylate cyclase. Activation of the gland *in vivo* probably occurs *via* VIP-secreting nerves as well as circulating agents; it is inhibited by somatostatin. Cyclic AMP probably stimulates chloride secretion by at least three mechanisms: (1) increasing chloride conductance across the luminal cell membrane, (2) enhancing the co-transport pathway for transmembrane movements of Na^+, K^+ and Cl^- and (3) activating Na-K-ATPase.

INTRODUCTION

Although elasmobranch fishes, including sharks and rays, are said originally to have developed in fresh water, they have lived for millions of years in the salty ocean where they are subjected to osmotic stresses that menace the composition of their body fluids. The shark swims in a sea containing about 500 mequiv l^{-1} of sodium chloride, or 1000 mosmol l^{-1}. The serum sodium of the shark is approximately 260–290 mequiv l^{-1}, but sodium and its associated anions in the extracellular fluid of the shark amount to only half the external osmotic pressure of the ocean. In order to counterbalance the hypertonicity of the sea, which if unopposed would rapidly dehydrate the animal, elasmobranchs synthesize urea, which circulates in high concentration in the blood and also permeates all cells. The concentration of mineral salts plus urea

Key words: Rectal gland, chloride secretion, Na-K-ATPase.

precisely balances the osmotic concentration of sea water, preventing the depletion of body water. Nevertheless, sodium chloride tends to diffuse inward through the gills and is also absorbed when food is swallowed. Thus, the shark must combat a tendency to become hypernatremic. The mechanism that elasmobranchs have devised for getting rid of unwanted increments of salt is the rectal gland (Burger & Hess, 1960; Burger, 1962).

The rectal gland of the spiny dogfish shark, *Squalus acanthias*, is a relatively simple structure that looks like a human appendix. It is composed of tubules packed tightly together looking superficially in histologic section like mammalian kidney cortex, and well supplied with capillaries. Electron microscopy reveals a uniform population of cells with extensive basolateral infoldings (Fig. 1). These are rich in Na-K-ATPase, as demonstrated autoradiographically by the binding of radioactive ouabain in histological sections (Eveloff *et al.* 1979). The rectal gland has a single artery, vein and central duct. It is therefore a simple matter to isolate the gland, remove it from the shark and perfuse it in the laboratory at the temperature of sea water with a shark Ringer's solution resembling an ultrafiltrate of shark plasma.

RECTAL GLAND SECRETION *IN VIVO*

The properties of fluid secreted by the rectal gland of *Squalus acanthias*, are shown in Table 1. In comparison with shark plasma, the fluid is concentrated with respect to sodium chloride, but isosmotic by virtue of the fact that it contains almost no urea. The concentration of sodium and chloride therefore approximates that of sea water, or about 500 mequiv l^{-1}. The isolated perfused gland secretes a fluid that is entirely similar in composition to that produced *in vivo*. When perfused in the laboratory at a pressure comparable to the blood pressure of the shark (20–25 mmHg), the potential difference across the gland, serosal (blood) side to apical (duct lumen), is orientated with the duct negative. Since the concentration of chloride in the duct is almost twice that in the perfusate, chloride is transported against both an electrical and a chemical gradient, while sodium moves down an electrical gradient that roughly balances the opposing chemical gradient (Silva *et al.* 1977).

The intact, free-swimming shark secretes rectal gland fluid at a highly variable rate. Small intravenous infusions of hypertonic sodium chloride stimulate profuse

Table 1. *Characteristics of rectal gland secretion*

	Intact fish	Perfused gland
Volume	0·1–2·0 ml h^{-1} kg body wt^{-1} 0·2–4·0 ml h^{-1} g $gland^{-1}$	same
Na^+	500 mequiv l^{-1} (plasma = 260–280 mequiv l^{-1})	450–500 mequiv l^{-1}
Cl^-	500 mequiv l^{-1}	450–500 mequiv l^{-1}
Urea	15 mmol l^{-1} (plasma = 350 mmol l^{-1})	30 mmol l^{-1}
Mg^{2+}	0	0
Ca^{2+}	0	0
Duct potential	?	−5 to 15 mV

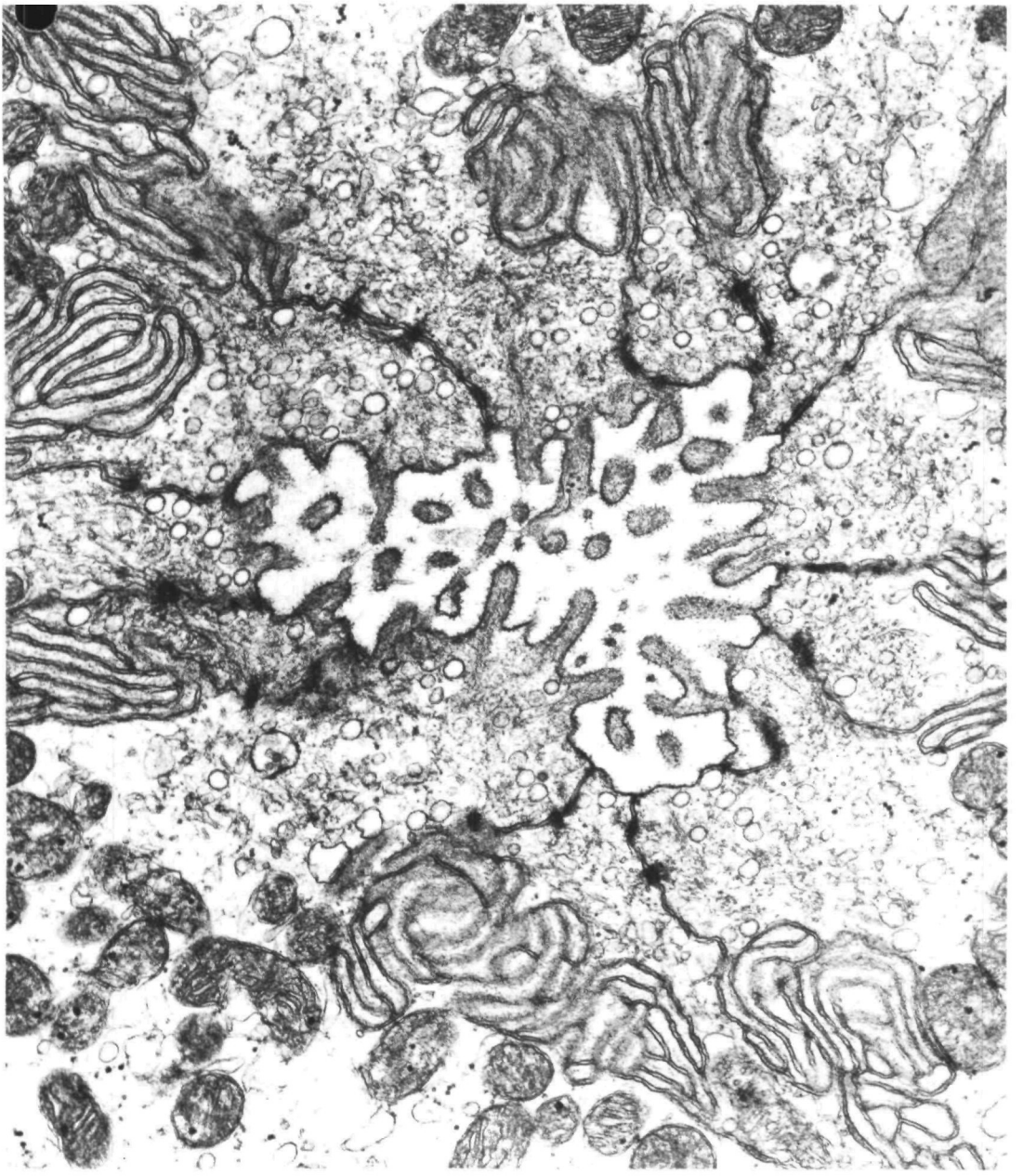

Fig. 1. Electron microphotograph of rectal gland. Note central duct lumen into which project apical microvilli, numerous mitochondria at lower left, and extensive whorled basolateral infoldings. Courtesy of Dr William P. Doyle.

stimulation. An equally effective stimulus to rectal gland secretion is the isotonic expansion of body fluids with shark Ringer's solution (Solomon *et al.* 1980). It seems likely that in live fish, swimming in the ocean in osmotic equilibrium with their surroundings, changes in the osmotic activity of body fluids are immediately translated into changes in body volume, so it is not surprising that isotonic expansion should be a potent stimulus to the rectal gland. Intravenous infusions stimulate rectal gland secretion promptly, within the first half-hour of collection. The volumes attained [2–4 ml(g gland wet weight)$^{-1}$ h^{-1}] are comparable to the rates of secretion obtained when the isolated gland is maximally stimulated *in vitro*. Since the mean arterial blood pressure is not altered by these intravenous infusions, it seems likely that the afferent stimulus for rectal gland secretion lies on the venous side of the circulation.

CHARACTERISTICS OF RECTAL GLAND SECRETION

Some key characteristics of rectal gland secretion are listed below, as elucidated by studies of isolated glands perfused with shark Ringer's solution containing 5 mM-glucose and aerated with 99 % O_2, 1 % CO_2 (pH 7·5–7·6) (Silva *et al.* 1977).

(1) Chloride is actively secreted against an electrical and chemical gradient, from blood to duct.

(2) Secretion is inhibited by ouabain and by omitting potassium from the perfusing solutions. It is therefore likely to depend on the activity of Na-K-ATPase, which lines the basolateral border of rectal gland cells.

(3) Secretion of chloride depends on the presence of sodium in the perfusate, and secretion of sodium depends on the presence of chloride in the perfusate.

(4) Secretion is inhibited by furosemide and furosemide analogues, agents which block coupled sodium-chloride transport in other tissues. Furthermore, the potency of 'loop diuretics' in inhibiting rectal gland secretion follows the same rank

Table 2. *Intracellular chloride concentration, chloride activity and electrical potential in basal and stimulated rectal glands*

	Intracellular Cl^- concentration (mequiv l^{-1})	Intracellular Cl^- activity (mequiv l^{-1})	Intracellular potential (mV)	Duct potential (mV)
Basal	78 ± 4 (1) 74 ± 5 (2)	57 ± 6 (3) 60 (3)	−78 ± 2 (3) −81 ± 4 (4) •−90 (6)	−6·8 ± 0·9 (5) −6·2 ± 1·2 (7)
Stimulated	44 ± 4 (1) 54 ± 6 (2)	52 ± 7 (3)	−73 ± 3 (3) •−60 (6)	−15·0 ± 1·6 (5) −16 ± 1·3 (7)

• Measurements performed on isolated rectal gland cells.
All other references are to studies of isolated perfused glands.
(1) Silva, Stoff & Epstein, 1979.
(2) Silva, Spokes & Epstein, 1979.
(3) Welsh *et al.* 1980.
(4) Duffey *et al.* 1978.
(5) Silva *et al.* 1977.
(6) Morad, Mitra & Cleeman, 1982.
(7) Silva *et al.* 1980.

order as their potency in blocking sodium-chloride co-transport in avian erythrocytes or in the thick ascending limb of canine kidneys (Palfrey *et al.* 1979).

(5) The intracellular concentration of chloride as deduced from chemical measurements, as well as the activity of chloride in cellular cytoplasm as determined directly by micropuncture with chloride electrodes, is 60–80 mequiv l^{-1} (Duffey *et al.* 1978; Welsh, Smith & Frizzell, 1980). This is two to four times higher than that predicted by the Nernst equation from the simultaneously measured electrical potential inside cells, which has been variously measured at −60 to −100 mV (Table 2). Chloride must therefore be transported uphill into the cell across the basolateral border.

These features of rectal gland secretion strongly suggest the hypothesis of a 'secondary active' co-transport of NaCl across basolateral membranes, in which chloride is transported into the cell against an electrochemical gradient, coupled to the downhill movement of Na^+. A downhill electrochemical gradient for Na^+ is maintained by activity of Na-K-ATPase (Fig. 2). More direct evidence for this hypothesis has been obtained from experiments on membrane vesicles.

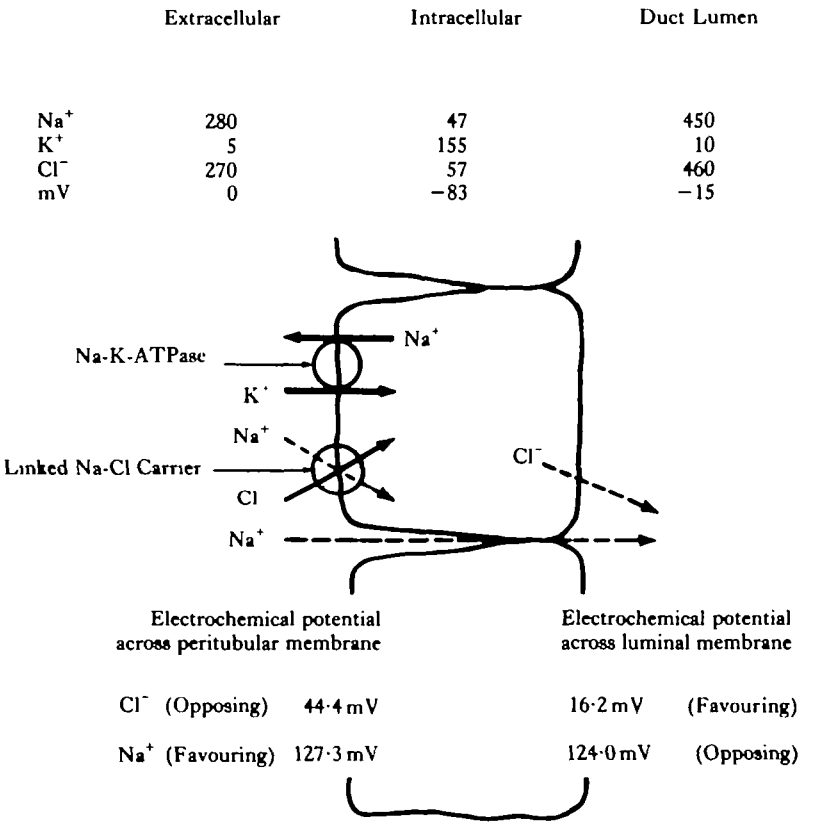

Fig. 2. Model for the transepithelial transport of chloride. Cl^- moves into the cell against an electrochemical gradient, *via* a coupled NaCl carrier. The energy for this entry step is provided by the electrochemical gradient for sodium directed into the cell, which is maintained by the activity of Na-K-ATPase. Cl^- leaves the cell across the luminal border down an electrochemical gradient that favours its efflux. Na^+ moves passively into the lumen through a paracellular pathway.

DIRECT EVIDENCE FOR SODIUM CHLORIDE CO-TRANSPORT IN MEMBRANE VESICLES FROM RECTAL GLAND

A membrane fraction rich in basolateral plasma membranes (as evidenced by the specific activity of Na-K-ATPase) was prepared from the rectal gland of the spiny dogfish and the uptake of ^{22}Na into the plasma membrane vesicles investigated by a rapid filtration technique (Eveloff *et al.* 1978). Sodium uptake was greatest in the presence of a potassium chloride gradient directed into the vesicles. It was strikingly reduced when chloride was replaced with nitrate and was even slower with gluconate (Fig. 3). If the membrane vesicles were pre-equilibrated with potassium chloride or potassium nitrate plus valinomycin, to minimize the influence of any electrical driving forces on sodium movement, the uptake of sodium was still greatest in the presence of chloride and remarkably decreased in the presence of nitrate. Furthermore, saturation of sodium uptake by increasing sodium chloride concentrations was observed, suggesting a saturable carrier. Furosemide at concentrations of 10^{-3} M and 10^{-4} M decreased sodium uptake into the vesicles in a dose-dependent manner only in the presence of chloride. Analogues of furosemide were effective in proportion to their comparative diuretic potency (Kinne *et al.* 1979). On the other hand, sodium uptake was not inhibited by amiloride, an inhibitor of sodium-proton exchange, or by 4-amino-4′-isothiocyanostilbene-2,2′-disulphonate (SITS), which blocks anion-exchange mechanisms in other systems (Kinne, Friedman & Kinne-Saffran, 1982), nor do these agents inhibit secretion by perfused rectal glands (unpublished data). Interestingly, the transport properties of membranes isolated from glands stimulated by cAMP and theophylline do not differ significantly from those isolated from glands in the basal state (Hannafin *et al.* 1981).

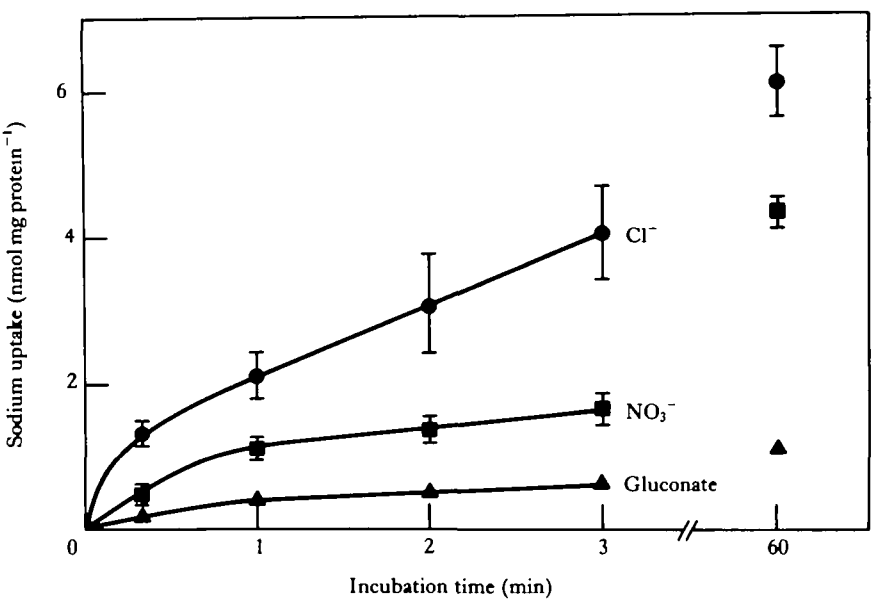

Fig. 3. Uptake of ^{22}Na into plasma membrane vesicles of shark rectal gland. Sodium uptake is accelerated in the presence of chloride but retarded by nitrate or gluconate.

These results provide direct evidence for a coupling of sodium and chloride flux across the plasma membrane of the rectal gland via a co-transport system sensitive to furosemide. They are strong support for the co-transport hypothesis of chloride secretion illustrated in Fig. 2.

HORMONAL CONTROL OF RECTAL GLAND SECRETION

The addition of theophylline or of dibutyryl cAMP to solutions perfusing the isolated rectal gland results in a burst of secretory activity (Stoff *et al.* 1977). When secretion is stimulated in this way the electrical potential difference between duct and perfusate becomes more negative (−5 to −15 mV), the flow of duct fluid is increased (from about 0·3 to 2–4 ml $h^{-1} g^{-1}$), and the concentration of sodium chloride in the secretion frequently increases slightly (from 450 to 500 mequiv l^{-1}). Thus, cAMP stimulates the movement of chloride against an even higher electrochemical gradient than existed previously, underlining the active nature of chloride transport in rectal gland secretion. A relatively constant rate of secretion *in vivo* (about 1200 μequiv $h^{-1} g^{-1}$) can be obtained by perfusing with 0·25 mM-theophylline and 0·05 mM of dibutyryl cAMP. The addition of cyclic GMP to the perfusate has no effect, either to stimulate secretion, or to block secretory stimuli.

An extensive search for the 'first messenger' of rectal gland secretion was carried out in perfused glands (Stoff *et al.* 1979). A number of hormones have been studied at pharmacological concentrations and found to have no significant effect on chloride secretory rate. Vasoactive intestinal peptide was the only hormone studied that stimulated chloride secretory rate.

The effect of VIP to increase chloride secretory rate appears to be mediated by cAMP. The hormone induces a significant rise in intracellular cAMP over the entire concentration range studied (10^{-6}–10^{-8} M). Theophylline, an inhibitor of phosphodiesterase, enhances the cAMP response to VIP at each concentration of the hormone tested. These findings indicate that the effect of VIP in this tissue occurs *via* activation of adenylate cyclase. The effect of theophylline on cAMP metabolism parallels its augmentation of VIP-stimulated volume and chloride secretory rate and supports the view that intracellular cAMP mediates active chloride transport in the rectal gland. Although secretin and glucagon share marked structural homologies with VIP, neither of these hormones produces a significant increase in secretion or in intracellular cAMP. These observations suggest the presence of a highly specific cell receptor for VIP at the contraluminal surface of rectal gland epithelial cells.

It is likely that since VIP functions as a neurotransmitter in other animals, at least one mode of stimulation of the rectal gland *in vivo* will be found to involve neurogenic stimulation *via* nerve terminals containing VIP. VIP has, in fact, recently been identified by immunohistochemical means in nerve fibres of isolated perfused glands (R. Lechan & J. Stoff, unpublished observations). Veratridine (or the cruder preparation, veratrine), which depolarizes nerves, stimulates secretion when added to the medium perfusing isolated glands. The stimulation by veratridine is prevented by tetrodotoxin, which blocks neural impulses (Erlij, Lodenquai & Rubio, 1981). Furthermore, veratridine stimulation requires the presence of nerves, since preparations of isolated rectal gland cells devoid of neural elements cannot be stimulated

crease their oxygen consumption by veratridine, whereas their respiration is increased by VIP or cAMP (Silva *et al.* 1982).

Adenosine, known to activate adenylate cyclase in other tissues, also stimulates gland secretion at a concentration of 10^{-5} M, presumably *via* activation of A_2 (excitatory) membrane receptors (Erlij, Silva & Reinoch, 1978; Forrest, Rieck & Murdaugh, 1980). At lower concentrations, adenosine might also activate inhibitory (A_1) receptors, thereby retarding secretion. Both the inhibitory and excitatory actions of adenosine are inhibited by theophylline (Daly, 1982).

Studies of electrolyte secretion by mammalian gastrointestinal tract and pancreas indicate that somatostatin may inhibit secretory transport (Carter, Bitar, Zfass & Makhlouf, 1978). Both somatostatin and VIP have been identified by immunohistochemical methods at similar sites in several species, including *Squalus acanthias*. The effect of somatostatin on VIP-induced secretion was therefore studied in the isolated perfused rectal gland. Somatostatin ($1{\cdot}4 \times 10^{-7}$ M) had no effect alone on the secretory rate for chloride, but completely inhibited chloride secretion induced by VIP. The effect of somatostatin was reversible; i.e., chloride secretion induced by VIP could be restored by removal of somatostatin from the perfusion medium (Stoff *et al.* 1979).

Somatostatin blocks the transient stimulation of isolated rectal glands perfused with veratrine (Fig. 4), consistent with the surmise that veratrine depolarizes VIP-releasing nerves in this preparation. Interestingly, somatostatin does not prevent stimulation of the gland by adenosine or by forskolin, a compound that directly activates adenylate cyclase. The fact that somatostatin markedly inhibits VIP but exerts little or no inhibition on adenosine and forskolin suggests a specific point of action on the VIP-receptor-adenylate cyclase complex (Epstein *et al.* 1982).

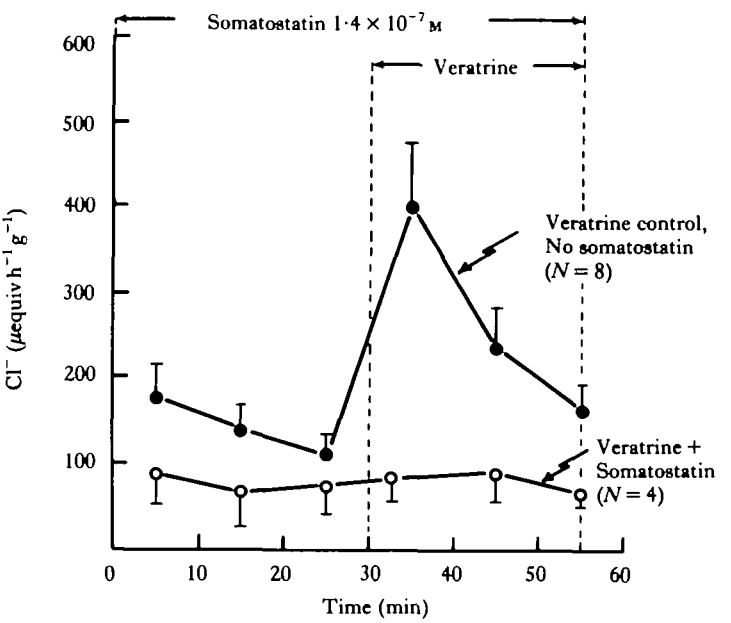

Fig. 4. Effect of somatostatin on rectal gland stimulation by veratrine. Values are mean ± S.E. Stimulation was carried out by infusing veratridine at a concentration of 2×10^{-5} M or an equivalent amount of veratrine (3 mg/100 ml).

The primacy of neural as opposed to hormonal regulation of rectal gland secretion in live sharks has been called into question by the experiments of Solomon *et al.* (1981) on explanted glands. In these experiments the rectal gland of a spiny dogfish was excised completely and attached *via* its artery to the circulation of another fish which was maintained alive at a normal arterial pressure and P_{O_2} in running sea water. Following infusion of 150 ml of isotonic shark Ringer's solution, secretion by the explanted gland increased dramatically, accompanied by an increase in blood flow to the gland, although there was no change in the pressure of perfusion. The increases in secretion and blood flow and the reduction in vascular resistance equalled those produced by similar infusions in rectal glands *in situ* with intact neural connections. The effect of volume expansion on secretion could be inhibited by infusing somatostatin into the circulation of the explanted gland, but not by theophylline, implying that VIP rather than adenosine is likely to have been the excitatory agent (Solomon *et al.* 1981). Despite these suggestive findings, we have thus far been unable to demonstrate a convincing and consistent correlation between arterial blood levels of VIP (as determined by a rather difficult radioimmunoassay) and the rate of rectal gland secretion in intact sharks.

MECHANISMS BY WHICH CYCLIC AMP ACTIVATES SECRETION BY THE RECTAL GLAND

Increase in apical chloride conductance

The activation of secretion by rectal gland cells involves changes in the permeability and enzymatic characteristics of both luminal and basolateral cell membranes. The chloride content of stimulated cells is consistently slightly lower than in the basal or resting state. Furthermore, measurements of intracellular potential indicate that after cAMP stimulation it becomes less negative (i.e., cAMP depolarizes the membrane) (Table 2). At the same time, duct potential becomes more negative, so that the voltage difference across the apical membrane falls. Thus, the electrochemical force driving chloride across the apical membrane into the duct lumen is diminished during excitation at the same time that the secretory flux of chloride is greatly increased. Chloride conductance across the luminal membrane of the cell must therefore be increased by stimulation. This appears to be analogous to the augmented chloride conductance produced by epinephrine across the apical membrane of corneal epithelium (Klyce & Wong, 1977; Nagel & Reinach, 1980) and induced by secretagogues in the luminal (apical) membrane of canine tracheal epithelium (Welsh, Smith & Frizzell, 1983). It should be noted that a change in luminal permeability as the result of activation of adenylate cyclase located in basolateral membranes is characteristic of many other hormone-induced alterations in epithelial transport, such as those of vasopressin and parathyroid hormone.

Increase in conductance of basolateral membranes

In addition to facilitating the exit of Cl^- from the cell across its luminal border, it seems likely that the permeability of basolateral membranes to the movements of other ions is also increased when rectal gland secretion is stimulated. It is not yet cl[illegible]

ether this is a primary or a secondary effect. When the active sodium-potassium pump (Na-K-ATPase) of perfused rectal glands is blocked by ouabain, the concentration of intracellular ions changes in the direction of equilibrium with extracellular fluid. These changes were examined when isolated perfused glands were in the basal state and also when they were stimulated to secrete with cAMP and theophylline, to see whether stimulation affected the passive movement of sodium, potassium and chloride across cell membranes (Silva, Spokes & Epstein, 1979). In basal glands ouabain induced an increase of 30 mequiv l^{-1} in intracellular Na^+ concentration and a decrease of 50 mequiv l^{-1} in intracellular K^+ after 30 min, while the intracellular concentration of Cl^- was unchanged. In stimulated glands, these movements were exaggerated. The increase in intracellular Na^+ averaged 112 mequiv l^{-1} and the decrease in intracellular K^+ averaged 96 mequiv l^{-1} ($P < 0{\cdot}01$), while mean intracellular Cl^- concentration rose by 80 mequiv l^{-1}. Furosemide, 10^{-4} M, partially reversed the accelerated changes in intracellular electrolytes that are seen after ouabain is added to stimulated glands (Fig. 5). Similar results have been obtained with slices of the rectal gland of the European dogfish, *Scyliorhinus canicola* (Shuttleworth & Thompson, 1980). These data are consistent with an action of cAMP

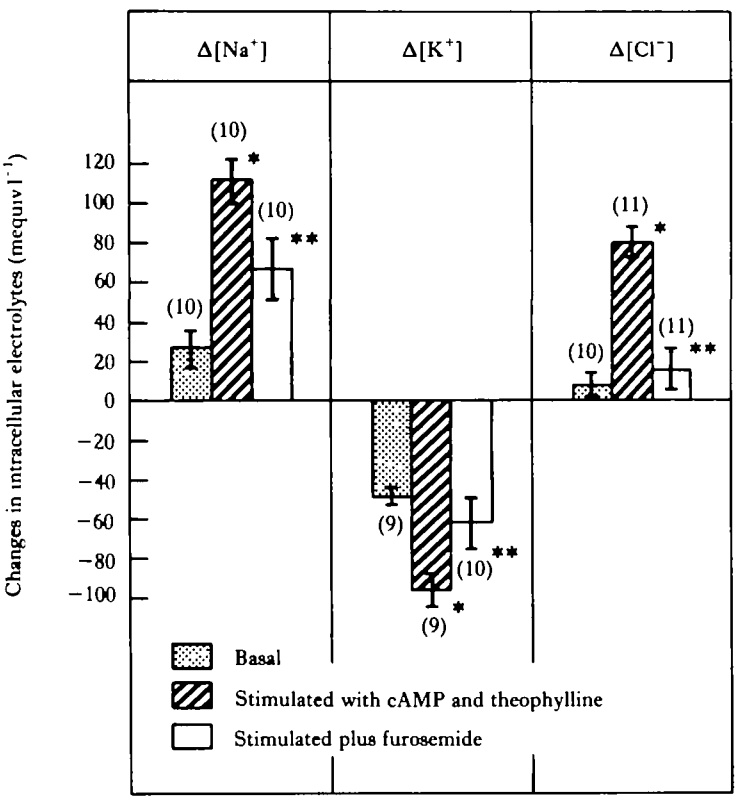

Fig. 5. Changes in intracellular electrolytes in perfused rectal glands after ouabain (10^{-4} M). Net movements of Na^+, K^+ and Cl^- are more marked in glands pre-stimulated with dibutyryl cAMP and theophylline than in basal glands. This increase is partially reversed by 10^{-4}M furosemide. * Significantly different from basal ($P < 0{\cdot}01$). ** Significantly different from stimulated glands without furosemide ($P < 0{\cdot}01$). Values shown are mean ± S.E.

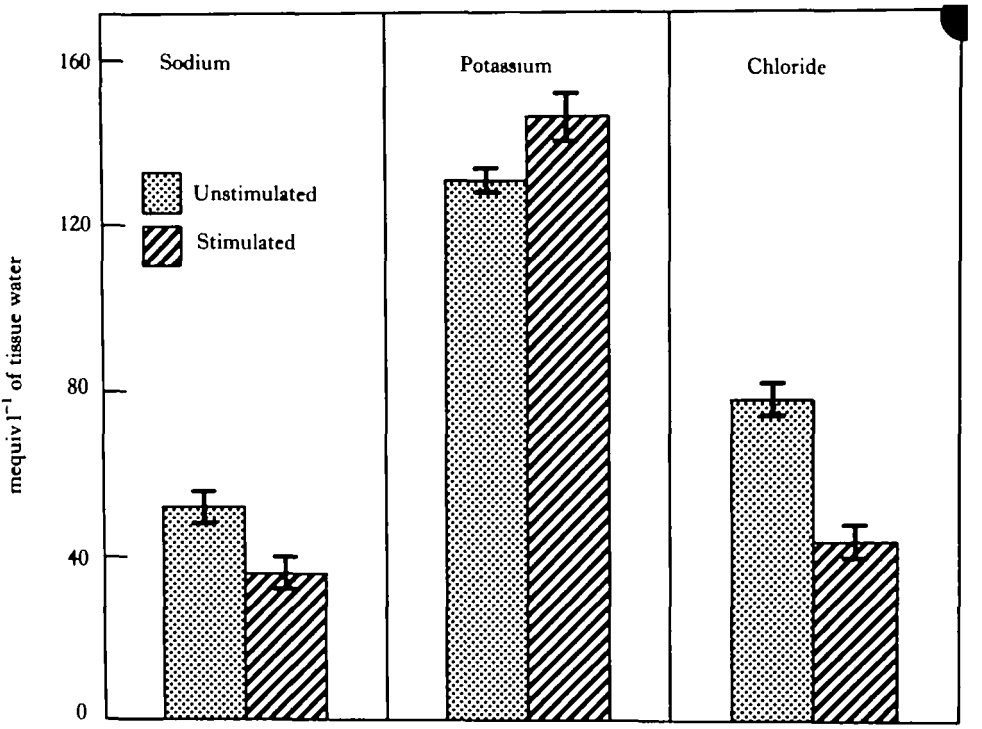

Fig. 6. Intracellular concentration of Na, K and Cl in unstimulated and stimulated perfused rectal glands. With stimulation, intracellular concentrations of sodium and chloride fall, while potassium tends to rise. Bars represent mean ± S.E. ($N = 6$).

upon a ouabain-insensitive transport of sodium, potassium and chloride in the rectal gland analogous to that described in avian erythrocytes (see Palfrey & Greengard, 1982). An increase in coupled NaCl entry across the basolateral cell border is intuitively necessary to permit the large transcellular secretory flux of chloride that is stimulated by cAMP, and an increase in basolateral potassium permeability has been postulated to accompany increased Na/K pump activity in Na-absorbing epithelia (Schultz, 1981). In a system analogous to the rectal gland, the tracheal epithelium of the dog, an increase in basolateral conductance with the onset of active Cl^- secretion has now been demonstrated by more rigorous means (Welsh *et al.* 1983).

Direct activation of Na-K-ATPase

Finally, it is probable that hormonal stimulation of the rectal gland activates Na-K-ATPase directly. The oxygen consumption of stimulated glands is more than four-fold higher than in the basal state. Ouabain-inhibitable oxygen consumption, presumably a reflection of Na-K-ATPase activity, is more than six-fold greater after stimulation (Silva, Stoff & Epstein, 1979). Fig. 7 shows the estimated intracellular concentrations of Na, K and Cl in unstimulated and stimulated perfused rectal glands. With stimulation, intracellular concentrations of sodium and potassium change reciprocally, resulting in a fall in intracellular sodium and in many experiments but not all, a rise in intracellular potassium. Thus, it is difficult to ascribe the activation of Na-K-ATPase to an increase in intracellular sodium content.

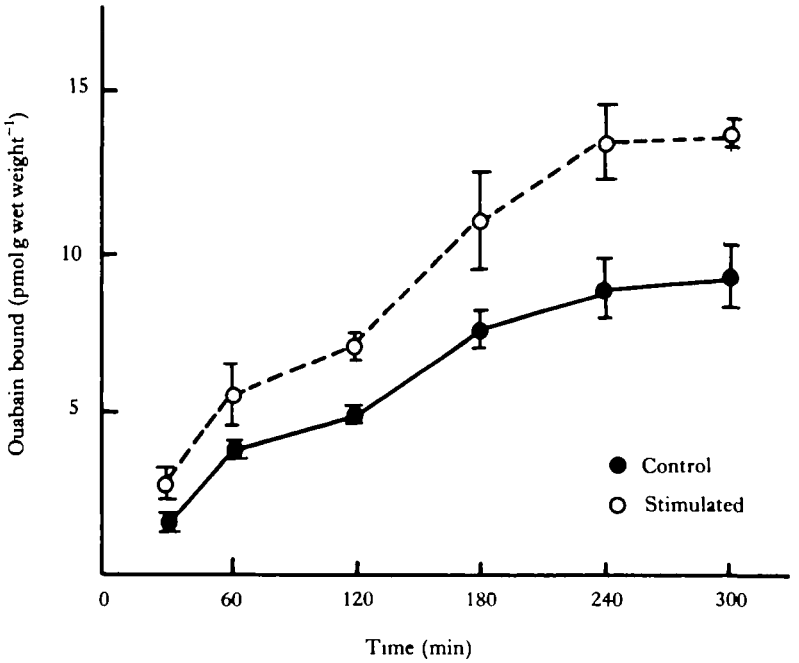

Fig. 7. Time course of ^{3}H-ouabain binding (10^{-9} M) to slices of rectal gland at 25 °C. Each point represents the mean of four, duplicate experiments. Steady state binding was reached after 4 h of incubation. Binding was increased by 50% in gland slices stimulated by theophylline 10^{-2} M and dibutyryl cAMP, 10^{-3} M. Values are mean ± S.E.

Ouabain binding to rectal gland slices is increased after cAMP stimulation (Shuttleworth & Thompson, 1978, 1980) but the Na-K-ATPase activity of cell homogenates is unchanged (Silva, Stoff & Epstein, 1979). In an attempt to examine the mechanism of enzyme activation, the binding of ouabain to rectal gland cells of *Squalus acanthias* has been measured before and after stimulation with cAMP and theophylline (J. Epstein *et al.* 1981). Stimulation significantly accelerates the rate of binding and increases the amount of ouabain bound at equilibrium in the presence of low concentrations of ouabain (10^{-9} M to 10^{-7} M) (Fig. 7). Scatchard plots suggest at least two classes of binding sites, one of high and one of low affinity. Stimulation with cAMP and theophylline appears to increase the ouabain affinity of the high-affinity site. Ouabain binding at this high-affinity site is increased by cAMP and theophylline even when rectal gland secretion is inhibited by furosemide or bumetanide, and when Li^+ is substituted for Na^+, or NO_3^- for Cl^-. The changes in ouabain binding induced by cAMP and theophylline in this species do not appear, therefore, to be secondary to secretory activity. In this respect they differ from the findings in *Scyliorhinus canicola*, where ouabain binding was thought to vary with Na-K-ATPase turnover (Shuttleworth, 1980). Furthermore (again unlike *Scyliorhinus*, Shuttleworth *et al.* 1982), ouabain binding is not increased in *Squalus* rectal gland cells when cell ATP hydrolysis is stimulated by the addition of a sodium ionophore that increases sodium entry into the cell (Silva *et al.* 1982). Thus, the increase in ouabain affinity produced by cAMP in this species seems not to be a simple consequence of increased Na-K-ATPase turnover nor of an increase in internal cell sodium concentration, but

presumably reflects a change produced by cAMP in the configuration, environment or location of existing enzyme so as to enhance its activity.

HIGH-ENERGY PHOSPHATE AND TRANSPORT IN RECTAL GLAND

The shark rectal gland contains substantial amounts of phosphocreatine, creatine phosphokinase, and adenine phosphonucleotides, and therefore provides a useful model in which to study the sequence of events involving high-energy phosphate turnover when secretory work is abruptly stimulated. Kinetic sequential studies of this sort can be done in the isolated perfused gland by nuclear magnetic resonance (n.m.r.), as described for other organs like heart, liver and kidney (Gadian & Radda, 1981; Ross, 1981). Fig. 8 illustrates the effect of stimulation with cAMP and theophylline on the ^{31}P n.m.r. spectrum of an isolated rectal gland perfused at 16 °C within the bore of a superconducting magnet (Oxford Instruments) in the laboratory of Dr George Radda. Stimulation produced little or no change in ATP, but a distinct fall in phosphocreatine and a reciprocal rise in the inorganic phosphate peak (F. H. Epstein, K. Thulborn & R. S Balaban, unpublished data). Thus, in the shark rectal gland as in skeletal muscle or mammalian heart, sudden substantial energy drains are met by changes in cellular levels of creatine phosphate in such a way as to tend to conserve the level of ATP within the cell.

STOICHIOMETRY OF CHLORIDE TRANSPORT IN RECTAL GLAND

The relationship of oxygen uptake to chloride transport in isolated perfused rectal glands has stimulated speculation that more than one Cl^- is coupled with the movement of one Na^+ into the cell across the basolateral membrane (Silva *et al.* 1980). The molar ratio of net Cl^- secretion to oxygen consumption in stimulated glands (Cl/O_2 ratio) consistently averages 27 to 30 (a ratio, it may be remarked, that is also characteristic of the mammalian kidney). If 3 mol of Na^+ are transported per mol of ATP hydrolysed by rectal gland membrane Na-K-ATPase, and if the P/O ratio of rectal gland mitochondria is 3:1, then the ratio of Na^+ transported to O_2 consumed should be 18:1, and if 1 mol of Cl^- were transferred for every mol of Na^+ transported, the ratio of Cl^- to O_2 would also be 18:1. The ratio of 30:1 observed in the experiments of Silva *et al.* (1980) clearly suggests the possibility that two or more Cl^- may be linked to the movement of one Na^+. Fig. 2 illustrates that from a thermodynamic standpoint, the electrochemical potential driving Na^+ into the cell is more than enough to account for the simultaneous entry of more than one Cl^-.

One mechanism by which more than one Cl^- could be transported into the cell with one Na^+ is provided in the recent demonstration (Hannafin, Kinne-Saffran, Friedman & Kinne, 1983) that K^+ is required for the co-transport of Na^+ and Cl^- by rectal gland membrane vesicles. Moreover, radioactive rubidium ($^{86}Rb^+$), a marker for potassium, is accumulated by such vesicles by a process that depends on the presence of Cl^- and is blocked by loop diuretics. This suggests that, as in avian erythrocytes (Palfrey & Greengard, 1982), Ehrlich ascites cells (Geck *et al.* 1980) and mammalian thick ascending limb (Greger & Schlatter, 1981), Cl^- co-transport in the rectal gland might involve the linkage of one Na^+, one K^+ and two Cl^-. Note that little additional

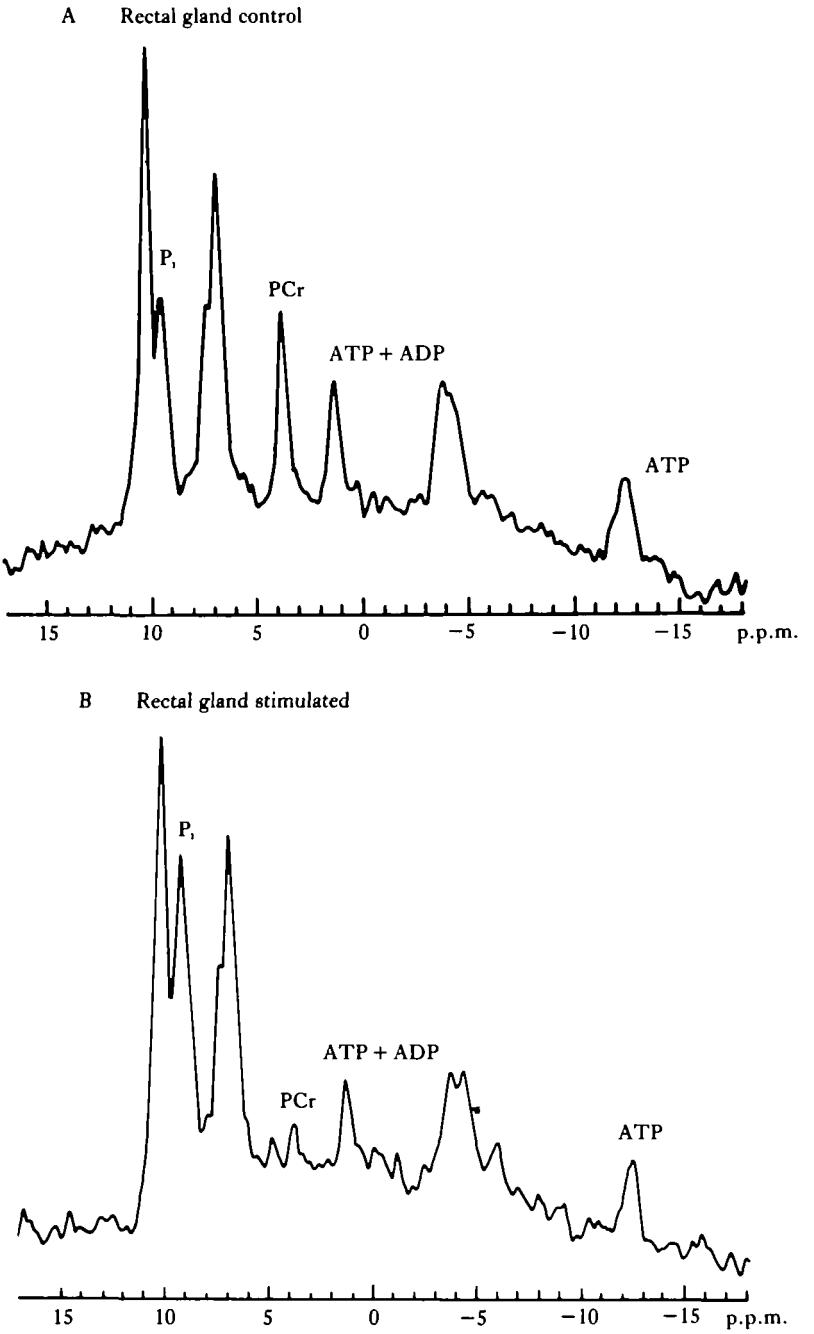

Fig. 8. ^{31}P n.m.r. spectrum obtained from perfused rectal gland in the basal state (8A) and after stimulation (8B). The unlabelled peak at 7 p.p.m. is also seen in spectra of renal medulla and represents glycerol phosphorylcholine. After stimulation there is no significant change in ATP peaks. The decrease in phosphocreatine (PCr) is approximately equal to the rise in inorganic phosphate (P_i). Sum of 300 scans at 2-s intervals, 20 μs pulse.

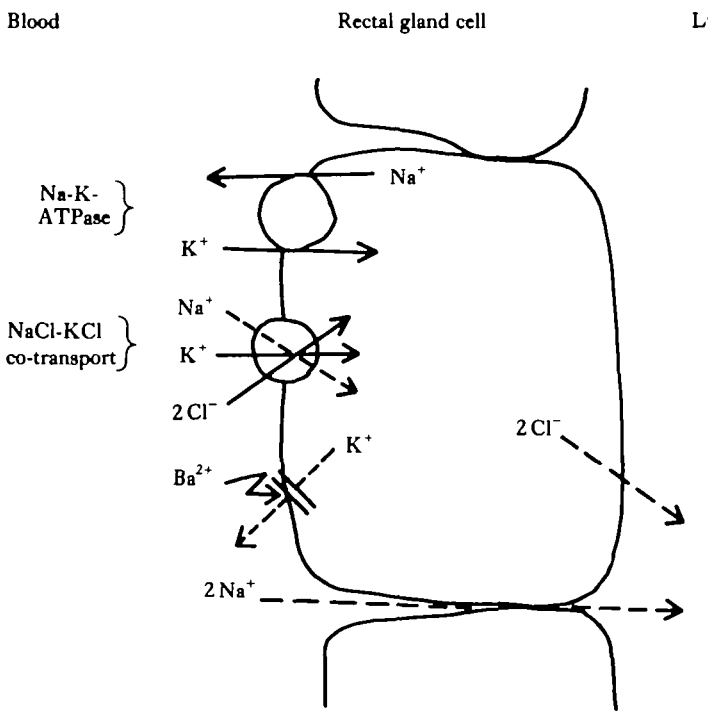

Fig. 9. Model for secondary active transport of Cl^- in rectal gland, involving co-transport of one Na^+, one K^+ and two Cl^-. Active transport of one Na^+ via Na-K-ATPase permits the transepithelial transport of two NaCl. The linked NaCl-KCl carrier is inhibited by furosemide. Ba^{2+} blocks a potassium channel permitting downhill efflux of K^+ across basolateral membranes. Solid lines indicate active transport; interrupted lines indicate downhill, passive transport.

thermodynamic work is required for the inward transport of K^+; since K^+ is in approximate electrochemical equilibrium across the basolateral membrane, the chemical gradient opposing influx is roughly balanced by the electrical gradient favouring entry. Potassium entering the cell in this fashion would diffuse back out across the basolateral membrane. Sodium would be pumped out by Na-K-ATPase into the intercellular space, and diffuse passively into the duct lumen across intercellular junctions. Cl^- would leave the cell chiefly across the luminal membrane, to be joined in the lumen by Na^+. The net result would be an amplification of the efficiency of the cell, permitting two NaCl to be secreted into the duct lumen at the cost of transporting only one Na^+ *via* Na-K-ATPase (Fig. 9).

The importance to this system of K^+ movement across the basolateral membrane is supported by the effects of Ba^{2+} on rectal gland secretion. A competitive inhibitor of K^+ conductive pathways, Ba^{2+} (2–5 mM) reversibly depressed rectal gland secretion while reducing transglandular electrical potential (Silva *et al.* 1981). Potassium recycling from cell to interstitial space was presumably inhibited by Ba^{2+}. The model depicted in Fig. 9 predicts that such a change would lower the potential difference across the luminal membrane and thereby reduce the driving force for chloride secretion.

The general features of this model for active chloride transport linked to Na^+ and K^+ appear to be applicable to a variety of transporting epithelia in addition to th

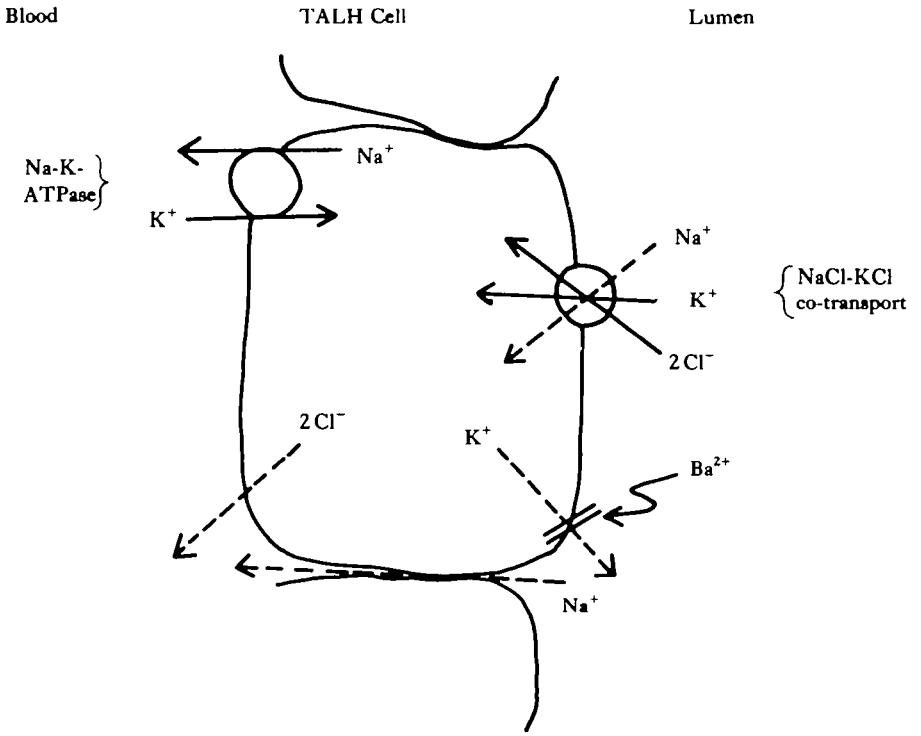

Fig. 10. Model for secondary active transport of Cl^- in thick ascending limb of Henle (TALH). See text. Solid lines indicate active transport; interrupted lines indicate downhill, passive transport.

rectal gland. These include canine tracheal epithelium (Welsh *et al.* 1983), teleost intestine (Field, Kimberg, Orellana & Frizzell, 1981) and the thick ascending limb of Henle's loop (Greger & Schlatter, 1981). A modification of the model is required for epithelia in which Cl^- is actively reabsorbed (as in intestinal mucosa and the thick ascending limb of Henle) rather than secreted (as in canine tracheal epithelium and rectal gland). In reabsorbing epithelia, the Na-K-Cl transporter must be on the apical surface of the cell (as evidenced by the site at which furosemide inhibits), as is the chief Ba^{2+}-sensitive channel for K^+ exit. These relationships are illustrated in Fig. 10. Further delineation of the precise molecular steps by which ion movements are activated in the rectal gland of the shark should provide important clues to the way in which transport is controlled by hormones in other tissues.

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REFERENCES

Burger, J. W. (1962). Further studies on the function of the rectal gland in the spiny dogfish. *Physiol. Zool.* **35**, 205–217.

Burger, J. W. & Hess, W. W. (1960). Function of the rectal gland in the spiny dogfish. *Science, N.Y.* **131**, 670–671.

CARTER, R. J., BITAR, K. N., ZFASS, A. M. & MAKHLOUF, G. M. (1978). Inhibition of VIP-stimulated intestinal secretion and cyclic AMP production by somatostatin in the rat. *Gastroenterology* **74**, 726–730.

DALY, J. W. (1982). Adenosine receptors: targets for future drugs. *J. medicinal Chem.* **25**, 197–207.

DUFFEY, M. E., SILVA, P., FRIZZELL, R. A., EPSTEIN, F. H. & SCHULTZ, S. G. (1978). Intracellular electrical potentials and chloride activities in the perfused rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **18**, 73–74.

EPSTEIN, F. H., STOFF, J. S., SILVA, P., SPOKES, K. & MYERS, M. (1982). Somatostatin inhibition of rectal gland secretion. *Bull. Mt. Desert Island Biol. Lab.* (in the press).

EPSTEIN, J., STEVENS, A., SPOKES, K., SILVA, P., JR. & EPSTEIN, F. H. (1981). Effect of stimulation of the rectal gland on ouabain binding: evidence for a direct action of cyclic AMP. *Bull. Mt. Desert Island Biol. Lab.* **21**, 103–108.

ERLIJ, D., LODENQUAI, S. & RUBIO, R. (1981). Nervous control of secretion of rectal gland of the dogfish: pharmacological evidence. *Bull. Mt. Desert Island Biol. Lab.* **21**, 74–76.

ERLIJ, D., SILVA, P. & REINACH, P. (1978). Effects of adenosine and other purine derivatives on the secretion of salt and water by the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **18**, 92–93.

EVELOFF, J., KARNAKY, K. J., JR., SILVA, P., EPSTEIN, F. H. & KINTER, W. B. (1979). Autoradiographic localization of (^{3}H)ouabain-sensitive Na,K-ATPase in dogfish *Squalus acanthias* rectal gland. *J. Cell Biol.* **83**, 16–32.

EVELOFF, J., KINNE, R., KINNE-SAFFRAN, E., MURER, H., SILVA, P. & EPSTEIN, F. H. (1978). Coupled sodium and chloride transport into plasma membranes prepared from dogfish rectal gland. *Pflügers Arch. ges. Physiol.* **378**, 87–92.

FIELD, M., KIMBERG, L. S., ORELLANA, S. A. & FRIZZELL, R. A. (1981). Potassium dependence of chloride transport in the intestine of the flounder, *Pseudopleuronectes americanus*. *Bull. Mt. Desert Island Biol. Lab.* **21**, 93–95.

FORREST, J. N., JR., RIECK, D. & MURDAUGH, A. (1980). Evidence for a ribose-specific adenosine receptor (Ra) mediating stimulation of chloride secretion in the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **20**, 152–155.

GADIAN, D. G. & RADDA, G. K. (1981). NMR studies of tissue metabolism. *Ann. Rev. Biochem.* **50**, 69–83.

GECK, P., PIETRZYK, C., BURCKHARDT, B. C., PFEIFFER, B. & HEINZ, E. (1980). Electrically silent cotransport of Na^+, K^+, and Cl^- in Ehrlich cells. *Biochim. biophys. Acta* **600**, 432–447.

GREGER, R. F. & SCHLATTER, E. (1981). Presence of luminal K^+, a prerequisite of active NaCl transport in the cortical thick ascending limb of Henle's loop of rabbit kidney. *Pflügers Arch. ges. Physiol.* **392**, 92–94.

HANNAFIN, J. A., FRIEDMAN, D., KINNE-SAFFRAN, E., EVELOFF, J. & KINNE, R. (1981). Sodium chloride cotransport by *Squalus acanthias* rectal gland: potential sites of regulation by cyclic AMP. *Bull. Mt. Desert Island Biol. Lab.* **21**, 83–86.

HANNAFIN, J. A., KINNE-SAFFRAN, E., FRIEDMAN, D. & KINNE, R. (1983). Presence of a sodium-potassium chloride cotransport system in the rectal gland of *Squalus acanthias*. *J. Membr. Biol.* (in the press).

KINNE, R., FRIEDMAN, D. & KINNE-SAFFRAN, E. (1982). Further evidence for the presence of a sodium-chloride cotransport system in the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **22** (in the press).

KINNE, R., KINNE-SAFFRAN, E., EPSTEIN, J., SILVA, P., EPSTEIN, F. H., MILLER, D. & EVELOFF, J. (1979). Effect of 'loop diuretics' on sodium transport by plasma membrane vesicles isolated from the rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **19**, 92–95.

KLYCE, S. D. & WONG, R. K. S. (1977). Site and mode of adrenalin action on chloride transport across the rabbit corneal epithelium. *J. Physiol., Lond.* **266**, 777–799.

MORAD, M., MITRA, R. & CLEEMAN, L. (1982). Measurements of membrane potential and membrane resistance in isolated cells from dogfish rectal glands. *Bull. Mt. Desert Island Biol. Lab.* **22** (in the press).

NAGEL, W. & REINACH, P. (1980). Mechanism of stimulation by epinephrine of active transepithelial Cl transport in isolated frog cornea. *J. Membr. Biol.* **56**, 73–79.

PALFREY, H. C. & GREENGARD, P. (1982). Hormone-sensitive erythrocyte cation transport pathways as analogs for epithelial transport systems. *Ann. N.Y. Acad. Sci.* **372**, 291–308.

PALFREY, H. C., SILVA, P., EPSTEIN, J. A., EVELOFF, J., KINNE, R. & EPSTEIN, F. H. (1979). Inhibition of cyclic AMP-stimulated chloride transport in the rectal gland of *Squalus acanthias* by a related series of 'loop' diuretics. *Bull. Mt. Desert Island Biol. Lab.* **19**, 58–60.

ROSS, B. D. (1981). Application of phosphorus nuclear magnetic resonance to problems of renal physiology and metabolism. *Proc. 8th Int. Congr. Nephrol., Athens*, pp. 841–849.

SCHULTZ, S. G. (1981). Homocellular regulatory mechanisms in sodium-transporting epithelia: avoidance of extinction by 'flush through'. *Am. J. Physiol.* **241**, F579–F590.

SHUTTLEWORTH, T. J. (1982). Rapid Communication. Amphotericin and the elasmobranch rectal gland: Implications for the relationship between oxygen consumption and ion transport. *J. exp. Zool.* **231**, 255–258.

SHUTTLEWORTH, T. J. & THOMPSON, J. L. (1978). Cyclic AMP and ouabain-binding sites in the rectal gland of the dogfish *Scyliorhinus canicola*. *J. exp. Zool.* **206**, 297–302.

SHUTTLEWORTH, T. J. & THOMPSON, J. L. (1980). The mechanism of cyclic AMP stimulation of secretion in the dogfish rectal gland. *J. comp Physiol.* **140**, 209–216.

SILVA, P., STOFF, J., FIELD, M., FINE, L., FORREST, J. N. & EPSTEIN, F. H. (1977). Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. *Am. J. Physiol.* **233**, F298–F306.

SILVA, P., SPOKES, K. & EPSTEIN, J. (1979). Action of cAMP and theophylline on ion movement during ouabain inhibition of Na-K-ATPase in perfused rectal gland of *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **19**, 70–72.

SILVA, P., STOFF, J. & EPSTEIN, F. H. (1979). Indirect evidence for enhancement of Na-K-ATPase activity with stimulation of rectal gland secretion. *Am. J. Physiol.* **237**, F468–F472.

SILVA, P., STOFF, J. S., SOLOMON, R. J., ROSA, R., STEVENS, A. & EPSTEIN, J. (1980). Oxygen cost of chloride transport in perfused rectal gland of *Squalus acanthias*. *J. Membr. Biol.* **53**, 215–221.

SILVA, P., EPSTEIN, J., MYERS, M., STEVENS, A. & EPSTEIN, F. H. (1981). Inhibition of chloride secretion by $BaCl_2$ in the rectal gland of the spiny dogfish, *Squalus acanthias*. *Bull. Mt. Desert Island Biol. Lab.* **21**, 12–13.

SILVA, P., STEVENS, A., SILVA, P., JR., SPOKES, K. & EPSTEIN, F. H. (1982). Isolated rectal gland cells: oxygen consumption studies. *Bull. Mt. Desert Island Biol. Lab.* **22** (in the press).

SOLOMON, R., ROSA, R. M., STOFF, J. S., SILVA, P. & EPSTEIN, F. H. (1980). Effect of infusions of saline in the dogfish *in vivo* on the rate of secretion by the rectal gland. *Bull. Mt. Desert Island Biol. Lab.* **20**, 138–141.

SOLOMON, R., TAYLOR, M., STOFF, J., SILVA, P. & EPSTEIN, F. H. (1981). Volume induced secretion by the rectal gland is mediated by a humoral agent(s). *Bull. Mt. Desert Island Biol. Lab.* **21**, 16–19.

STOFF, J. S., ROSA, R., HALLAC, R., SILVA, P. & EPSTEIN, F. H. (1979). Hormonal regulation of active transport in the dogfish rectal gland. *Am. J. Physiol.* **237**, F138–F144.

STOFF, J. S., SILVA, P., FIELD, M., FORREST, J., STEVENS, A. & EPSTEIN, F. H. (1977). Cyclic AMP regulation of active chloride transport in the rectal gland of marine elasmobranchs. *J. exp. Zool.* **199**, 443–448.

WELSH, M. J., SMITH, P. L. & FRIZZELL, R. A. (1980). Intracellular chloride activities in the isolated perfused shark rectal gland. *Bull. Mt. Desert Island Biol. Lab.* **20**, 101–103.

WELSH, M. J., SMITH, P. L. & FRIZZELL, R. A. (1983). Chloride secretion by canine tracheal epithelium. III. Membrane resistances and electromotive forces. *J. Membr. Biol.* (in the press).