Table I

Hg in Kidney of Fundulus Calculated from ^{209}Hg and from ^{14}C Following Injection of Labelled Methylmercury

	p.p.m.				Ratio kidney/gill			
	Hg only		Hg + Se		Hg only		Hg + Se	
	^{203}Hg	^{14}C	^{203}Hg	^{14}C	^{203}Hg	^{14}C	^{203}Hg	^{14}C
5 hrs	0.63 ±0.07	0.47 ±0.08	0.20 ±0.08	0.39 ±0.18	1.78 ±0.20	1.42 ±0.09	0.86 ±0.23	0.87 ±0.16
25 hrs	0.91 ±0.22	0.87 ±0.21	0.67 ±0.22	0.58 ±0.28	1.59 ±0.12	1.60 ±0.15	0.80 ±0.15	0.75 ±0.11
73 hrs	1.28 ±0.22	1.62 ±0.45	0.67 ±0.24	0.73 ±0.10	1.80 ±0.22	1.76 ±0.55	0.91 ±0.11	0.99 ±0.08

Mean ± S.D.

The fish in the columns labelled Hg + Se were pretreated with Na_2SeO_3 ½ hour before the injection of methylmercury.

e'enium does not change methylmercury retention in *undulus heteroclitus*; 3) pretreatment does change stribution in the tissues; and 4) pretreatment does not crease breakage of the chemical bond in methyl- ercury.

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1 • 1975

itimulation of Rectal Gland Secretion by Cyclic AMP.

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The isolated rectal gland of *Squalus acanthias*, when erfused *in vitro*, secretes a fluid high in sodium and nloride. The rate of secretion is lower than that ommonly observed in live dogfish, however, and eclines with time. Because it seemed possible that rectal and secretion is regulated by circulating humoral factors esent in the intact fish, we tested the hypothesis that, e some other secretory organs, active transport in the ctal gland is stimulated by cyclic adenosine mono- osphate.

Dogfish of either sex weighing 2 to 5 kg were taken hook and line from Frenchman's Bay and used for all periments. They were ketp in marine live-cars and crificed within 3 days of capture. Rectal glands taken om freshly killed animals were placed in a plexiglass and uminum chamber cooled with running sea water emperature 16 ± 1°C). The artery and duct were nnulated with PE 90 polyethylene catheters. The rectal and was perfused by gravity at a flow between 4 and 9 l/min with a medium at pH 7.6 containing (mM/L) :280; K:6; Mg:3; Ca:2,5; Cl:290; Phosphate:1; $SO_4$0.5; O:8; Urea:350; Glucose:5. The perfusion medium was ssed with a mixture of 99% O_2 and 1% CO_2. Duct fluid s collected at timed intervals (usually 10 min) for up to hours. Potential differences were measured using KCl ar bridges and a Hewlett Packard 410-C electronic voltmeter equipped with two calomel electrodes. All pharmacological agents used were previously dissolved in perfusion medium and added to the perfusion reservoir. Na and K were measured with an IL 343 flame photometer and Cl by amperometric titration. Cyclic AMP was assayed by a protein binding assay at two dilutions of rectal gland fluid stored at -20°C.

The addition of a single dose of 1 mM theophylline to the perfusion medium was followed by a significant change in the magnitude of the PD (from 6.8 ± 2.1 to 19.7 ± 7.6 mV, p 0.05, duct negative), a rapid gland fluid from 202 to 2982 l/hr/gWW (p 0.001) and a resultant rise in the rate of excretion of Cl from 104 to 1525, Na from 105 to 1604 and K from 6.2 to 46.1 Eq/hr/gWW. The concentration of Na and Cl exceeded 500 meq/L in duct fluid and, if below that level during control studies, rose appreciably after theophylline stimulation. The concentration of cyclic AMP in rectal gland fluid ($5x10^{-9}$ - $5x10^{-8}$M) did not change, though the total amount excreted increased owing to the large increment in the rate of fluid secretion. After secretion is stimulated by 0.25 mM theophylline, a slow and continuous decline in function supervenes which can be reversed by the subsequent addition of dibutyryl cyclic AMP (0.1 mM). The response to theophylline was dose dependent and increased in magnitude without a plateau as the concentration of this drug was increased in the perfusion medium from 0.01 mM to 5 mM. The rate of fluid secretion was increased 23 times by the highest dose (5 mM) of theophylline tested. Dibutyryl cyclic AMP (0.05 mM) produced the same effects as theophylline when added alone, although in the absence of ptheophylline the effects were less marked and of shorter duration. Perfusion of the rectal gland in the presence of 0.25 mM theophylline and 0.05 mM dibutyryl cyclic AMP resulted in stable stimulation of the rectal gland for up to 140 minutes.

Since cyclic AMP is the common intracellular effector of a number of different hormones, a search for the most likely candidates was conducted in an effort to determine their possible role in the regulation of the activity of the rectal gland. Vasopressin (Pitressin R), and

Table I

The effect of ouabain, furosemide and thiocyanate on the rate of secretion of sodium, potassium, chloride and water in the isolated perfused rectal gland of squalus acanthias

	OUABAIN 10^{-4}M		FUROSEMIDE $3x10^{-6}$M			THIOCYANATE 10^{-3}M		
	CONTROL	EXP	CONTROL	EXP	RECOVERY	CONTROL	EXP	RECOVERY
SECRETION RATE µl/hr/gWW	1099 ±272 (5)	328** ±258 (5)	1780 ±183 (4)	826** ±47.2 (4)	1629 ±264 (4)	1244 ±510 (3)	534* ±188 (3)	581 ±180 (3)
Na^+ SECRETION RATE µeq/hr/gWW	437 ±109 (5)	118** ±81 (5)	749 ±86 (4)	331** ±24 (4)	658 ±113 (4)	602 ±256 (3)	262* ±103 (3)	278 ±63 (3)
K^+ SECRETION RATE µeq/hr/gWW	9.0 ±0.9 (5)	2.6** ±1.6 (5)	13.3 ±1.9 (4)	6.0** ±0.8 (4)	11.3 ±2.3 (4)	13.0 ±6.9 (3)	4.9* ±1.5 (3)	4.4 ±1.6 (3)
Cl^- SECRETION RATE µeq/hr/gWW	444 ±117 (3)	57** ±34 (3)	883 ±84 (4)	379** ±31 (4)	780 ±128 (4)	635 ±300 (3)	257* ±99 (3)	277 ±75 (3)

Values are expressed as the mean ± standard deviation and the number of observations are in parenthesis. Perfusion fluid $[Na^+]$ = 268 meq/L, $[K^+]$ = 4.69 meq/L, $[Cl^-]$ = 253 meq/L by measurement. Significance between figures was calculated using the student "t" test.
* $p < 0.05$
**$p < 0.001$

three substituted oxytocins normally found in the posterior lobe of the pituitary of *Squalus acanthias* (aspartocin, valitocin and vasotocin) were found to have no appreciable effect at a concentration of 1 g/ml (approx 1 M/L). No response was seen after either epinephrine ($5x10^{-5}$M) or secretin (75 m /ml). A wider search for another hormone(s) seems warranted.

The mechanism by which the rectal gland secretes a fluid rich in NaCl was then explored. When not stimulated by theophylline or cAMP, the basal secretion of the perfused rectal gland is insensitive to inhibitors of both NaK-ATPase and carbonic anhydrase, as well as to furosemide (*Bull. MDIBL, 14*: 122, 197 and *13*: 113, 1973). The situation is different in the stimulated gland perfused with a solution containingi 0.25 mM theophylline and 0.05 mM dibutyryl cyclic AMP. Addition of ouabain to a final concentration of 10^{-4}M produced a consistent reduction of 80 - 90% in the rate of secretion of Na, Cl and K, a drop in PD and a marked reduction in rectal gland fluid volume of 87.0 ± 6.2% (Table I). This concentration of ouabain causes complete inhibition of NaK-ATPase when added to homogenates of rectal gland *in vitro* and measurements of rectal gland NaK-ATPase done after perfusion with 10^{-4}M ouabain show complete inhibition of the enzyme.

Rectal gland secretion was also inhibited by furosemide, $3x10^{-4}$M. The inhibition produced by furosemide was reversible, for when furosemide was removed from the perfusate, secretion rates rose to control levels (Table I).

Thiocyanate inhibits chloride transport in many tissues, including the teleost gill and the unstimulated rectal gland. Following the addition of 10 mM SCN- to the perfusion medium, the rate of fluid excretion and of electrolyte secretion was decreased (Table I). Unlike the inhibition produced by furosemide, that caused by thiocyanate was not reversed by perfusing the gland with a solution free of inhibitor.

Although the rectal gland is rich in carbonic anhydrase its function is unclear. Ethoxolamide, a powerful inhibitor of this enzyme, was added at a concentration of 10^{-4}M to stimulated perfused rectal glands in three difference experiments. No change in the rate of fluid or electrolyte excretion was seen.

These experiments indicate that the rectal gland of *Squalus acanthias* is a salt secreting organ regulated by the adenyl cyclase - cyclic AMP system. When secretion is stimulated by theophylline or dibutyryl cAMP, the concentration of Cl in duct fluid rises and the duct fluid becomes more electronegative, fulfilling criteria for active transport of chloride. NEvertheless, secretion is blocked by ouabain, implying a key role for NaK-ATPase in the transport process. Furosemide and theiocyanate also inhibit rectal gland secretion, though carbonic anhydrase inhibitors do not. The perfused rectal gland thus appears to offer an attractive opportunity for study of the molecular mechanisms of active chloride secretion.

Key facts that must be taken into account in any general hypothesis of chloride transport in the rectal gland include the following: 1. Chloride moves across the gland against its electrochemical gradient. 2. Blockade of NaK-ATPase inhibits Cl transport. 3. The intracellular concentration of Cl probably exceeds that predicted from the Nernst equation by a wide margin (*Bull. MDIBL 14*: 116, 1974). These facts are consistent with active transport of Cl into the cell together with Na in a way linked to the movement of Na down its own electrochemical gradient across the basal cell membrane. The movement of Cl into the cell and its net secretion depend upon the maintenance of a low intracellular concentration of Na by the action of the NaK-ATPase pump. Chloride would then be extruded from the cell across the luminal cell membrane by electrical forces. A chloride carrier (ass uggested by Dr. Michael Field) analogous to those esponsible for the linked transport of glucose or amino acids into cells together with Na, is hypothesized to be present in the basal plasma membranes of rectal gland cells.

12 • 1975

Single Nephron Handling of Electrolytes (Na, K, Mg, Ca, Cl, P, S) in the Little Skate, *Raja erinacea*

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The kidneys of elasmobranchs regulate the excretion of electrolytes in the urine by tubular reabsorption and secretion. The present study was undertaken to obtain information on the handling of electrolytes by the individual segments of the nephron in the kidney of the little skate, *Raja erinacea*.

Experiments were performed on 16 female skates, weighing from 600 to 3,100 g. Each fish was anesthetized by i.v. injection of 1.5 mgs/kg sodium pento-barbital (Diabutal) and 1 mg/kg curare in the lateral tail veins. The fish was placed either on the ventral or dorsal side depending on which side of the kidney was used for micropuncture. The spiracles were perfused with undiluted seawater at 1 - 1.5 ml/min at 10°C (Bull. MDIBL 11: 91, 1971).

Tubular fluid was taken by micro glass capillaries (O.D. 8 - 15) from the proximal tubular segment I (PTS I) from the dorsal surface of the skate kidney, and from the proximal tubular segment II (PTS II) from the ventral surface. (PTS I probably corresponds to segment III, and PTS II to segment IV described by Deetjen and Antkowiak, Bull. MDIBL 10: 5, 1970). For further details see also Lacy, Schmidt-Nielsen, Galaske and Stolte, this volume. To collect fluid from the end of the collecting duct (ECD) small catheters of PVC-tubing (O.D. 150) were inserted.

The collected fluid, as well as plasma and seawater, was analyzed for osmolality by the freezing point depression method of Ramsay-Brown and for electrolytes by electron probe microanalysis (Lechene, Proc. Fifth National Conference on Electron Probe Analysis, New York, 32A-32C, 1970; Lechene, in : *Microprobe Analysis as Applied to Cells and Tissues*, ed. by Theodore Hall, Patrick Echlin and Rudolf Kaufmann, pp. 351-368, Academic Press, London, 1974).

Figure I summarizes the tubular fluid to plasma ratio (TF/P) for osmolality and electrolyte concentrations. The osmolality in the individual segments was not significantly different from that of plasma. Sodium concentration was already below that of plasma in the PTS I and declined further in the PTS II (TF/P o.86 + 0.05, n = 16 and 0.52 + 0.04, n = 22). Chloride showed a similar transtubular concentration ratio. In the final urine (FU), chloride concentration was lower than that of Na. There was no change in the TF/P ratio for potassium in the PTS I whereas it increased significantly in the PTS II and increased further until the end of the collecting duct (ECD). The major finding is the high transtubular concentration difference for magnesium and phosphate in the PTS II with a ratio of tubular fluid to plasma with 47.29 +

TABLE I

	HCT %	Osm mOsm	Na mM	K mM	Mg mM	Ca mM	Cl mM	P mM	S mM
SW	-	858 ± 24 (9)	425 ± 7 (7)	9.06 ±0.23 (7)	46.9 ± 0.6 (6)	10.3 ± 0.4 (6)	503 ± 6 (6)	1.55 ±0.21 (9)	25.7 ± 1.7 (9)
PLASMA	19.6 ± 1.1 (16)	968 ± 16 (16)	267 ± 6 (12)	5.40 ±0.51 (12)	1.19 ±0.19 (9)	1.74 ±0.24 (9)	301 ± 13 (9)	1.86 ±0.33 (9)	5.36 ±0.45 (9)

Osmolalities and electrolyte concentrations in seawater in comparison to plasma in the little skate, *Raja erinacea*, and the mean hematocrit (HCT).