TABLE 1. DISPLACEMENT OF [^{3}H]-ESTRADIOL FROM RECEPTOR BY UNLABELED COMPETITORS

Competitor	% Displacement + 1x competitor	+ 10X competitor
β-Estradiol	57	95
Estrone	38	87
Diethylstilbestrol	59	90
Dihydrotestosterone	19	53
Progesterone	14	49
Corticosterone	0	0

Partially purified cytosolic receptor was incubated with an equal volume of [^{3}H]-β-estradiol in the presence of a 1-fold or 10-fold excess of competitor (2 hr, 4 C) and assayed for displacement of bound [^{3}H]-β-estradiol.

FURTHER EVIDENCE FOR THE PRESENCE OF A SODIUM-CHLORIDE COTRANSPORT SYSTEM IN THE RECTAL GLAND OF SQUALUS ACANTHIAS

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INTRODUCTION--Since the first description of the chloride dependence and furosemide sensitivity of sodium uptake into plasma membranes isolated from the shark rectal gland (Eveloff et al., Pflueger's Arch. 378:87-92, 1978), two essentially different explanations for the results have been put forward. One explanation is that the rectal gland contains a sodium-chloride cotransport system, that translocates sodium and chloride simultaneously in analogy to other sodium-cotransport systems such as the sodium-glucose cotransport system. The other explanation assumes a combined action of a sodium/proton exchanger and of a chloride/hydroxyl exchanger. In order to investigate further which model can be applied to the rectal gland, the effects of amiloride, an inhibitor of the sodium/proton exchanger, and of DIDS, an inhibitor of the chloride/hydroxyl exchanger (Warnock and Eveloff, Amer. J. Physiol. 242(6):F561-F574, 1982) on the sodium uptake by plasma membranes isolated from rectal glands were investigated. The results obtained further substantiate the hypothesis that sodium chloride transport in the basal-lateral membrane of the rectal gland occurs via a NaCl cotransport system.

METHODS--Plasma membranes were isolated by differential centrifugation in a sucrose medium from rectal glands of Squalus acanthias as described previously (Eveloff et al., Pflueger's Arch. 378:87-92, 1978). For optimum results, freshly excised glands had to be used as starting material and the membrane vesicles had to be investigated either immediately after preparation or within 24 hours when frozen in dry ice. Sodium uptake was determined by a rapid filtration technique at 15°C. The composition of the intravesicular medium and of the incubation medium is detailed in the legends to Table 1. The experiments were performed in July, 1982.

Table 1.--Effect of Amiloride and DIDS on Sodium Uptake by Plasma Membrane Vesicles Isolated from Shark Rectal Glands

Incubation Conditions	Incubation Time	% Control	n
Amiloride	15 sec	90.2 ± 15	4
[8.3×10^{-4} M]	1 min	95.8 ± 14.6	4
	120 min	107 ± 4.7	4
DIDS	15 sec	100.5 ± 22	4
[2.5×10^{-5} M]	1 min	93.3 ± 12.4	5
	120 min	108.6 ± 15.8	4
Bumetanide	15 sec	54.1 ± 5.1	4
[4.3×10^{-4} M]	1 min	35.5 ± 0.7	4
	120 min	156 ± 13.6	4

The vesicles contained 200 mM mannitol, 20 mM Tris Hepes, pH 7.6, and 1.2 mM Mg $(NO_3)_2$. Uptake was determined in a medium containing 164 mM KCl, 0.42 mM $NaNO_3$, 20 mM Tris Hepes, pH 7.6, 1.2 mM Mg $(NO_3)_2$ and the compound indicated in the table. The sodium uptake of the control membranes amounted to 0.54 nmoles/mg protein after 15s, 1.17 nmoles/mg protein after 1 min and 0.63 nmoles/mg protein after 120 min of incubation. Mean values and standard deviation derived from four experiments are given.

RESULTS--In the upper part of Table 1 results of experiments are compiled in which the potential role of a sodium/proton exchanger in sodium uptake was investigated. At low sodium concentrations amiloride (in millimolar amounts) is known to inhibit the sodium/proton exchanger in brush border membranes isolated from mammalian kidney. As evident from Table 1, 8.3×10^{-4} molar amiloride does not affect sodium uptake into the vesicles significantly. The sodium concentration in these experiments was 0.42 mM, sufficiently low to detect any effect of amiloride on a sodium/proton exchanger.

A second series of experiments dealt with the possible role of a chloride/hydroxyl exchanger in the rectal gland. DIDS, an inhibitor of this transport system in mammalian renal and intestinal brush border membranes was added to the incubation medium and the sodium uptake in the presence of a potassium chloride gradient was investigated. DIDS at a concentration of 2.5×10^{-5}M had no effect on the sodium uptake.

Table 1 also indicates, for comparison, the effect of 4.3×10^{-4}M bumetanide, a known inhibitor of sodium chloride transport in the rectal gland (Kinne and Kinne-Saffran, Mar. Ecol. Prog. Ser. 1:123-132, 1979) on the sodium uptake into the same vesicle preparation. Bumetanide clearly inhibits sodium uptake, the maximum inhibition observed after 1 min of incubation is 64.5%.

DISCUSSION--All of the results reported above support the notion that sodium and chloride transport across the plasma membrane of the rectal gland are not the consequence of a separate movement of sodium through a sodium/proton exchanger and of chloride through a chloride/hydroxyl exchanger but rather occur via a sodium-chloride cotransport system. The strongest evidence for this conclusion from the current study is the lack of effect of amiloride on sodium uptake by the vesicles if one assumes that the sodium/proton exchanger in the dogfish and in mammalian tissue have a similar sensitivity to amiloride. The experiments with DIDS are restricted to a relatively low dosage of DIDS; because only the sodium salt was available, at higher concentrations an interference with the sodium concentration used in the transport assay would have been encountered. A further complication might be the high chloride concentration, a condition where the Cl/OH exchanger might not be rate limiting for sodium uptake. The results compare, however, favorably with studies of (Solomon et al., MDIBL Bull. 18:13-16, 1978) in the intact

gland where SITS concentrations below one millimolar applied over extended periods of time did not interfere with the secretion of the perfused rectal gland.

Thus, in conclusion, this study as well as other experiments published in this bulletin (Hannafin J. et al., this bulletin) strongly suggest that the rectal gland contains a sodium-chloride (potassium) cotransport system that is involved in the active secretion of chloride by the gland. This work was supported by NIH Grants AM27441, AM29927 and the American Heart Association, Maine Affiliate #E-4.

ION FLUXES AND N-METHYLFUROSEMIDE BINDING IN SQUALUS ACANTHIAS RECTAL GLAND PLASMA MEMBRANE VESICLES

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INTRODUCTION--Recently a potassium dependence of Na, Cl cotransport has been described in avian erythrocyte (Palfrey et al., Am.J. Physiol. 238(7):C129-148, 1980), Ehrlich ascites tumor cells (Geck et al., Biochim. Biophys. Acta 600:432-447 1980), thick ascending limb of Henle's loop (Greger and Schlatter, Pfluegers Arch. 392:92-94, 1982) and in Madin-Darby canine kidney cell line (McRoberts et al., J. Biol. Chem. 257:2260-2266, 1982). Since the rectal gland is also supposed to contain a loop diuretic sensitive Na, Cl cotransport system it was of interest to examine the role of potassium in the rectal gland system. To this end three parameters for the presence of a Na, K, Cl cotransport system were investigated in rectal gland plasma membrane vesicles: potassium dependence of sodium flux, sodium dependence of potassium (rubidium) flux, and sodium and potassium dependence of the binding of a loop diuretic.

MATERIALS AND METHODS--Dogfish caught by hook and line in Frenchman Bay were maintained in live cars and used within two days to one week of capture. Isolation of plasma membrane vesicles was carried out at 0-4°C as described by(E.veloff et al., Pfluegers Arch. 378:87-92, 1978) with modifications. Protein was determined by the method of (Lowry et al., J. Biol. Chem. 193:265-275, 1951) using bovine serum albumin as standard. Na-K-ATPase activity was determined by the method of (Kinne et al., Pfluegers Arch. 329:191-206, 1971). ^{22}Na uptake and ^{86}Rb uptake were followed by a rapid filtration technique as described previously (Pfluegers Arch. 378:87-92, 1978). The binding of tritiated N-methylfurosemide was measured by the following technique. The reaction was initiated by the addition of 20 µl membranes to 65 µl incubation medium. After a 20 min, a 20 µl sample was removed and pipetted directly onto a mixed cellulose ester filter (Millipore HAWP, pore size 0.45 u) in 15-20 discrete drops kept under suction. The filter was then washed with 3.5 ml ice cold stop solution; washing took less than 2 seconds. Experiments were performed (in duplicate for transport and in triplicate for binding) at 15°C. Composition of the incubation media is given in the figure and table legends. For evaluation of the amount of radioactivity taken up by or bound to the vesicles the amount of radioactivity present on the filters was corrected for the amount of radioactivity present on the filter in the absence of membrane.

RESULTS--As shown in Table 1 KCl-gradient dependent ^{22}Na uptake decreased 53% when the K-concentration was decreased from 164 to 5 mM by choline replacement. ^{22}Na-uptake in the presence of a RbCl-gradient was slightly higher than the KCl-gradient dependent uptake, indicating that Rb is an appropriate replacement for K. In addition, 0.8×10^{-3} M N-methylfurosemide inhibited KCl-gradient dependent ^{22}Na-uptake after 15 seconds by 35% (data not shown).

Rubidium tracer exchange in the vesicles was found to be stimulated by the presence of chloride and sodium but inhibited by bumetanide. The latter inhibition was observed only in the presence of sodium and chloride (see Table 2).

The binding of [^{3}H] N-methylfurosemide to the membranes could be resolved into at least two components. One component demonstrates saturation kinetics (see Figure 1), and is thought to represent high affinity binding while the