Table 1.--Effect of $BaCl_2$ on chloride secretion and electrical potential in the shark rectal gland

Perfusate $BaCl_2$ concentration M	Chloride secretion micro Eq/hr/g	PD* -mV
0	1567 ± 134 (10)	6.7 ± .9 (6)
10^{-4}	1297 ± 142 (6)	4.2 ± 1.0 (5)
5×10^{-4}	941 ± 77 (6)	2.7 ± 1.3 (4)
10^{-3}	906 ± 144 (6)	1.5 ± .4 (5)
2×10^{-3}	491 ± 74 (2)	NM**
5×10^{-3}	280 ± 25 (6)	1.0 ± .5 (4)
0	1487 ± 144 (10)	5.1 ± 1.1 (5)

Values are mean ± SEM; *Potential difference across the rectal gland is lumen negative
**NM: not measured.

The model for chloride secretion for the rectal gland predicts that a reduction in cellular potassium efflux produced by by barium should lower the potential difference across the luminal membrane, resulting in a reduction in the driving force for chloride across that membrane and a consequent reduction in the rate of chloride secretion and in the transglandular electrical potential. The positive correlation between the rate of chloride secretion and the transglandular electrical potential is consistent with this hypothesis. Although it is tempting to ascribe the effects of barium to a reduction in intracellular potential, this remains an unproven assumption since membrane potential was not measured. The possibility remains that barium has unappreciated effects on cellular metabolism that might be responsible for its effect on potassium efflux and ultimately, in the rectal gland, on chloride transport.

ION REQUIREMENTS FOR OXYGEN CONSUMPTION BY ISOLATED RECTAL GLAND CELLS

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Cells were isolated from rectal glands using the technique previously described (MDIBL Bull. 20:38-39, 1980), with only minor modifications. The cells were kept on ice until used in a buffer of the following composition (in mM): Na 280; K 5: Cl 295; Mg 3; Ca 2.5; SO_4 0.5 ; phosphate 1; Urea 350; Hepes 40, pH 7.6. The same buffer was used for measuring oxygen consumption. When sodium was removed from the bath it was replaced by lithium. When chloride was removed it was replaced by nitrate. When both sodium and chloride were removed they were replaced by lithium nitrate. Cells that were stored in buffer containing sodium and chloride were washed twice in buffer of the same composition as that used for measuring oxygen consumption. Oxygen consumption was measured in a constant temperature (25°C) chamber using a Clark type polarographic oxygen electrode (YSI) connected to a recorder. The electrode was calibrated using the known solubility of oxygen at 25°C and the barometric pressure. The rate of oxygen consumption was calculated from the tangent of the recorded slope of the oxygen consumption, the volume of buffer in the measuring chamber, and the wet weight of a measured aliquot of the cells removed from the measuring chamber at the end of the experiment. Glucose 5×10^{-3}M, pyruvate 10^{-2}M, and acetate 2.5×10^{-3}M were used as metabolic substrates. Theophylline 2×10^{-3}M, dibutyryl cyclic AMP 2×10^{-3}M, ouabain 10^{-4}M, and bumetanide 3×10^{-5}M (final concentrations), were all added in a volume not exceeding 1% of the volume of

the measuring chamber to avoid dilutional problems. Results are expressed as micromoles of 0_2 consumed per hour per gram wet weight of cells. The results are summarized in Table 1.

Table 1.--Sodium and chloride requirement for oxygen consumption in isolated rectal gland cells

	Basal	Theophylline 2 mM dibutyryl cyclic AMP 2 mM		
			Ouabain 10^{-4}M	Bumetanide 3×10^{-5}M
Control	38.6 ± 5.3 (7)	66.1 ± 13.0 (7)*	18.7 ± 5.0 (4)**	37.2 ± 7.8 (2)**
Lithium chloride (no sodium)	37.1 ± 8.8 (4)	36.5 ± 6.8 (4)**	31.8 ± 6.4 (4)	
Sodium nitrate (no chloride)	25.4 ± 8.9 (4)	28.6 ± 8.5 (4)**		28.8 ±10.3 (4)
Lithium nitrate (no sodium or chloride)	22.5 ± 7.8 (4)*	25.1 ± 7.4 (4)**		23.8 ± 7.1 (4)

Values are mean micromoles 0_2/hr/g wet wt. ± SEM.

*Significantly different from basal control.

**Significantly different from stimulated control.

In both the basal and stimulated states, oxygen consumption was higher than the values observed last year, owing to the higher temperature of incubation (25°C) as compared with 15°C in the experiments of 1980. Oxygen uptake was stimulated an average of 71% by cyclic AMP and theophylline. After stimulation, QO_2 was inhibited to or below the basal level by ouabain or bumetanide. Omission of Na^+, or Cl^-, or both, from the bathing medium prevented stimulation of oxygen uptake. Basal QO_2, on the other hand, was little affected by the presence or absence of sodium or chloride.

These experiments demonstrate the feasibility of using isolated rectal gland cells for the study of changes in metabolism related to transport. While our previous studies of intact stimulated rectal glands showed that active secretion and QO_2 could be inhibited by omitting sodium or chloride from the perfusion medium, it was possible that this might have resulted from the development of electrochemical gradients opposing secretion across the intact epithelial surface of rectal gland tubules. The present results, however, are consistent with the hypothesis of coupled transport of both sodium and chloride into individual rectal gland cells across their plasma membrane borders.

CREATINE PHOSPHOKINASE (CPK) IN ELASMOBRANCH TISSUES

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Phosphocreatine is thought to act as a reserve of high-energy phosphate in mammalian cardiac muscle, skeletal muscle and brain, preventing depletion of ATP and accumulation of ADP via the reversible reaction, PCr + ADP $\rightleftharpoons$ ATP + Cr, which is catalyzed by creatine phosphokinase. The possible role of this reaction in elasmobranchs was investigated by assaying the activity of CPK and the content of phosphocreatine and ATP in various tissues of Squalus acanthias and Raia erinacea.

The assay for CPK was based on the method of Rosalki (J. Lab. Clin. Med., 69:696, 1967) using reagents supplied by Sigma (no. 45 UV), in which ATP generated by the CPK reaction is utilized in a hexokinase/glucose 6-phosphate dehydrogenase system which ultimately yields an amount of NADPH proportional to the CPK activity.