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

Franklin H. Epstein, Rafael Rubio and David Erlij, Department of Medicine, Harvard Medical School and Beth Israel Hospital; Department of Physiology, University of Virginia; Department of Physiology, Downstate Medical Center of the State University of New York

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.

Approximately 30-50 mg of tissue was homogenized with a Tekmar high-torque homogenizer in 2 ml of 1.15% KCl solution containing 1.2 mM dithiotheritol, and 0.01 M Hepes-NaOH, pH 7.4 at 4°C. The protein content of the homogenate was measured with Bio Rad reagent. The content of ATP and phosphocreatine was determined enzymatically in tissues from pithed animals freeze-clamped in vivo with tongs cooled in liquid nitrogen (Methods of Enzymatic Analysis, 2nd Edition, edited by H.U. Bergmeyer, Academic Press, 1974, vol. 4, pp 1777-1781). The results are shown in Table 1.

TABLE I

ATP, phosphocreatine, and creatine phosphokinase in elasmobranch tissues

Tissue	CPK activity units	ATP μmoles/g	PCr μmoles/g
Squalus acanthias (spiny dogfish)			
Rectal gland	661 ± 37 (6)	1.46±.06 (3)	.570±.14 (3)
Skeletal muscle, white	833 ± 95 (5)		
Skeletal muscle, red	640 ± 69 (3)		
Heart	24 ± 5 (6)	3.123±.126 (4)	.716±.039 (4)
Brain	484 ± 96 (4)		
Kidney	166 (1)		
Pancreas	7 (1)		
Colon mucosa	198 (2)		
Raia erinacea (little skate)			
Heart	535 ± 100 (3)	2.142±.255 (2)	.593±.086 (2)

Values are mean ± s.e. (n). CPK activity is given in nanomoles creatine phosphorylated per minute at 25°C.

The high CPK activity found in shark rectal gland where energy demands for transport work can be switched on abruptly, is consistent with the presence of phosphocreatine in this tissue and with the fall in phosphocreatine that has been shown to occur when salt secretion is stimulated.

A surprising result was the extremely low level of CPK activity in the heart of the dogfish shark, Squalus acanthias, in the face of substantial amounts of both phosphocreatine and ATP. The low enzyme activity did not seem to be the result of an enzyme inhibitor in myocardial tissue, since homogenates of Squalus die not inhibit CPK activity in skeletal muscle or in homogenates of the heart of Raia erinacea. This unexpected finding may afford an opportunity to investigate the relationship of creating phosphokinase and phosphocreatine to myocardial function.