Table 3.--Intracellular Cl activities

	ψ_t	ψ_a	a_i^{Cl}	a_{eq}^{Cl}
Control	- 3.5	-59 ± 4	46 ± 4	13 ± 1
high serosal K (170 mM)	-11.0	-42 ± 3	84 ± 7	37 ± 2
Control	- 3.5	-63 ± 2	39 ± 2	11 ± 1

mean ± SEM of 4 to 7 impalements in a single tissue, ψ:mV, a_i^{Cl}: mM.

would be expected if the inward K gradient caused Cl to be transported into the cell. The observed change in Cl activity is much larger than expected for passive equilibration across the basolateral membrane.

In summary, the apical membrane K conductance appears to be linked to the rate of Cl absorption. This linkage results in an increase in K conductance when coupled uptake is impaired experimently by reduced concentration of the transported ions or by application of an inhibitor. In addition, a coupled K:Cl cotransport in the basolateral membrane may be involved in Cl absorption.

PRIMARY CULTURES OF TUBULAR CELLS OF THE RECTAL GLAND OF SQUALUS ACANTHIAS

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Primary cell cultures were initiated with cells and tubular fragments of the rectal gland. Under optimal conditions confluent monolayers of tissue, approximately 0.5 cm in diameter, were produced within 72 hours. Tissue outgrowths remained viable for 4-5 days as evidenced by trypan blue exclusion.

TISSUE PREPARATION

Rectal glands aseptically removed from male and female adult dogfish were first sliced into thin sections and then minced to obtain a suspension of tubule fragments and individual cells. This suspension was washed three times with shark Ringer's solution (NaCl, 280 mM; KCl, 6 mM; $CaCl_2$, 5 mM; $MgCl_2$, 3mM; Na_2SO_4, 0.5 mM; $NaHCO_3$, 8mM; Urea, 350 mM; glucose, 5mM) with 2x antibiotics (Penicillin, 200 units/ml; Streptomycin, 200 µg/ml; Fungizone, 0.5 µg/ml; M. A. Bioproducts). Preperfusion of the gland with chilled Ringer's did not notably improve growth, but removal of calcium from the Ringer's did decrease subsequent viability. Silva has also noted that removal of calcium from Ringer's reduces oxygen consumption of rectal gland cells (personal communication). Before transfer to tissue culture flasks, fragment suspensions were trypsinized (0.25% Worthington trypsin) for 20-30 minutes. Digests of 1) trypsin and collagenase; 2) collagenase and hyaluronidase; 3) collagenase, hyaluronidase and trypsin, and 4) trypsin alone were also examined. Digests treated with trypsin consistently provided greater cell growth.

CULTURE MEDIA

After removal of the digest solution, cells and tubular fragments were transferred to culture flasks. Several different standard media were tested: 1) Minimum Essential Media (M.A. Bioproducts), 2) Medium 199 (M.A. Bioproducts), and CL 2 (a mixture of Hams F 12 and Liebovitz L 15 as used by Handler et al. Proc. Nat. Acad. Sci. USA 76:4151, 1979), containing amphibian Ringer's salts and 8mM HCO_3. All media were modified further to contain the solute content of elasmobranch Ringer's by the addition of NaCl, urea and trimethylamine oxide. Glutamine (20 mM) was also added to all media to promote attachment and growth. Best results were obtained in CL 2 with NaCl 280 mM, 350 mM urea and 50-100 mM TMAO. The effect of serum addition was monitored with 1) no sera, 2) 10% fetal calf serum (Gibco 309), 3) 2-5% female shark plasma and 4) combinations of 2-5% female shark plasma and 2-5% fetal calf serum. Growth, as evidenced by the number of tissue patches produced per unit area after 72 hours, was always superior with a 10% fetal calf serum addition. Cultures could be maintained contamination free with the addition of 1x antibiotics. All flasks were fed every 24-48 hours.

GROWTH CONDITIONS

Flasks were maintained at either 25°C or 16°C. At the higher temperature both sealed flasks and those open to a 1% CO_2 atmosphere were examined; at 16° all flasks were sealed in room air. Although initial settling of fragments occurred under all conditions, extended growth was observed at the lower temperature. Since flasks were frequently fed, pH was maintained relatively constant at 7.4-7.5. However, it is expected that for longer term culture, 1% CO_2 or HEPES buffering would improve growth.

Attempts to culture cells on collagen coated surfaces were also made using both commercially prepared collagen (Sigma, acid soluble rat tail collagen), and rat tail extracts (Hall et al., Proc. Nat. Acad. Sci. 79:4672-5676, 1982) as prepared by Dr. John Valentich. No growth was observed on collagen, but in three separate batches, when fragments were placed on semi-solid gels, they appeared to reorient and form clusters with central lumina similar to those described in MDCK cells (Hall et al., op. cit., 1982).

GROWTH CHARACTERISTICS OF PRIMARY CULTURES

Within an hour of plating, cells appeared individually and in small clusters scattered over the surface. These cells were flattened epithelioid with winged pseudopods. Frequently, cells appeared to secrete droplets of a low density material. These droplets did coalesce but did not mix with the culture medium, indicating some lipid content.

Growth of epithelioid cells from larger tubule fragments occurred 24-48 hours after initial plating. Patches of outgrowth were characterized by the presence of large, highly vacuolated cells at the leading edge and uniform cells with large oval nuclei and dense cytoplasmic granular inclusions throughout the field (Figure 1). Fibroblastic cells in-

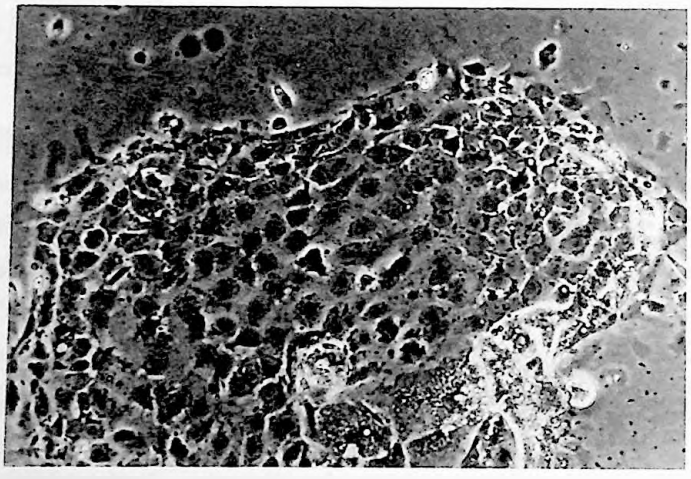

Figure 1.--Primary monolayer culture of shark rectal gland tubule cells (48 hours). Phase contrast, 125x.

frequently appeared 48-72 hours after plating, and followed the epithelioid patch growth. Primary cultures were occasionally contaminated by both bacteria and/or yeast but infection was minimal when prewashes with triple antibiotics were used.

Attempts to pass the cells using either trypsinization or cell scraping were not successful. There was no growth and very poor viability in transferred flasks.

In summary, primary monolayer cultures of tubular cells of the rectal gland were obtained and were viable for 4-5 days. These cells appear sufficient large and numerous for further characterization of transport properties using patch clamping and other electrophysiological techniques.

ATP INDIRECTLY STIMULATES CHLORIDE SECRETION BY HYDROLYSIS TO ADENOSINE IN THE RECTAL GLAND OF *SQUALUS ACANTHIAS*

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ATP has been shown to affect many tissues either directly through an ATP receptor (Burnstock, G.,