Electron microscopic studies revealed that the glomerular endothelium functioned as a partial barrier to the entry of the injected macromolecules into the subendothelial space. This was indicated by the finding that the density of IDC particles was clearly lower in the mesangial matrix than in the capillary lumen at all times of study.

Phagocytosis of IDC by mesangial cells was observed by was modest when compared to occasional intra-capillary phagocytes loaded with IDC.

It is concluded that the hagfish mesangium exhibits different morphologic and functional features when compared to other vertebrates. Due to the barrier function of the glomerular endothelial layer, the delivery of IDC to the circumferential mesangium is limited. Therefore, the mesangium does not exhibit conspicuous activity as a clearing system for the glomerular filter, an important feature of higher vertebrate kidneys (Sterzel, R.B., Lovett, D.H., Stein, H.D. and Kashgarian, M., Klin. Wochenschr. 60:107-109, 1982). Supported by NIH, DFG and DAAD.

CHLORIDE ABSORPTION BY INTESTINE OF WINTER FLOUNDER: RELATION TO APICAL MEMBRANE ELECTRICAL P.D. AND CELLULAR Cl ACTIVITY

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Previous studies of electrolyte transport across isolated flounder intestine have demonstrated that inhibition of Cl absorption leads to a hyperpolarization of the apical membrane electrical potential difference, ψ_a (Halm et al., Bull, MDIBL 21:88, 1981; Halm et al., Bull. MDIBL 22:80, 1982). The response of ψ_a to transport inhibition suggests that apical membrane K conductance increases with reduced transport. The results presented here indicate that the size of the response is proportional to the degree of inhibition.

METHODS

Conventional microelectrodes were employed to measure ψ_a and intercellular Cl activities were calculated from the data obtained with Cl-selective microelectrodes. The criteria for successful impalement and methods for fabrication of ion-selective electrodes have been described by Duffey et al (J. Memb. Biol. 50:331, 1979).

RESULTS AND DISCUSSION

The presence of barium in the mucosal solution (2 mM) was shown to decrease the apparent K conductance of the apical membrane (Halm et al., Bull. MDIBL 21:88, 1981, Halm et al., Bull. MDIBL 22:80, 1982). Subsequent inhibition of Cl absorption increases apical membrane K conductance as indicated by a hyperpolarization of ψ_a and an increased sensitivity of ψ_a to changes in $[K]_m$. Table 1 shows that the cyclic-nucleotide cGMP, which inhibits apical

Table 1.--Hyperpolarization of ψ_a by Cyclic-nucleotides

	Control		Mucosal barium	
	$\Delta\psi_a$	n	$\Delta\psi_a$	n
cGMP	-14 ± 3*	5	-26 ± 3*	3
cAMP	-1 ± 4	4	0 ± 1	3

mean $\pm$ SEM for n tissues, $\Delta\psi$:mV. Asterisks indicate significant difference from zero.

membrane Cl uptake (Rao et al., Bull MDIBL 22:85, 1982), hyperpolarized ψ_a while cAMP did not alter ψ_a. This result supports the hypothesis that cGMP acts on the uptake of Cl across the apical membrane and that cAMP only alters the permselectivity of the paracellular pathway (Krasny et al., Bull MDIBL 22:82, 1982).

Figure 1 shows that ψ_a hyperpolarized progressively as either $[Cl]_m$ or $[Na]_m$ was reduced (in the presence of

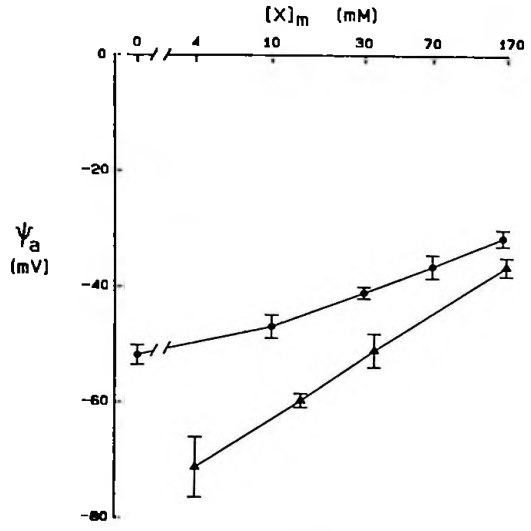

Figure 1.--Dependence of ψ_a on $[Cl]_m$ and $[Na]_m$. ψ_a was measured in the presence of mucosal solution Ba (2 mM) at several $[Cl]_m$ (▲, n=7) or $[Na]_m$ (●, n=5), mean ± SEM.

2 mM mucosal solution barium). This supports the concept that inhibition of coupled Na:Cl uptake leads to the hyperpolarization of ψ_a. The basolateral membrane has been shown to exhibit a Cl conductance (Halm et al., Bull. MDIBL 22:80, 1982). Measurement of intracellular Cl activity demonstrated that, in the presence of barium, Cl is at electrochemical equilibrium across the basolateral membrane. Since Cl would be expected to remain near electrochemical equilibrium as coupled uptake is inhibited, the intracellular Cl activity can be estimated from ψ_b. This analysis indicates that intracellular Cl is maintained relatively constant (13 to 23 mM) over a wide range of transport rates (20 to 80% of maximal).

Measurements of ion concentration in the intestinal fluid in vivo show that both the Na and Cl concentrations are lower than in plasma while the Mg and SO_4 concentrations are elevated above seawater values (Hickman, Can. J. Zool. 47:457, 1968). An artificial intestinal fluid (AIF) was designed to mimic the invivo concentration of ions (mM): 30 Na, 5K, 100 Mg, 10 Ca, 81 Cl, 100 SO_4, 26 N-methyl-D-glucamine, 5 EPPS and 5 mannitol. Table 2 shows the electrophysiological changes which occur when

Table 2.--Artificial Intestinal Fluid

	ψ_t	ψ_a	ψ_b	f_R	G_T
control	- 3.5 ± 0.5	-64 ± 8	-60 ± 9	0.20 ± 0.01	28 ± 5
AIF	-21.0 ± 2.1	-82 ± 3	-61 ± 1	0.27 ± 0.12	15 ± 2
Δ	-17.5 ± 2.6*	-18 ± 6*	- 1 ± 8	+0.07 ± 0.11	-13 ± 3*

mean ± SEM for 3 tissues, ψ:mV, G_T: mS/cm^2. Asterisks indicate significant difference from zero.

the apical membrane is bathed by AIF. The decrease in tissue conductance occurs primarily due to the reduction in $[Na]_m$, which is the most permeable ion in the paracellular pathway. Accordingly, the change in ψ_t can be attributed to a Na diffusion P.D. in the paracellular pathway. The change in ψ_a is nearly identical to this change in ψ_t, which could imply that the paracellular diffusion P.D. is responsible for the hyperpolarization of ψ_a. However, the fractional resistance of the apical membrane, f_R (0.27), indicates that ψ_a would change by only 5mV, and ψ_b by 13 mV, due to the paracellular diffusion P.D. The anticipated change in ψ_b possible could be offset by a fall in intracellular Cl activity (due to reduced $[Na]_m$ and $[Cl]_m$) which tends to hyperpolarize ψ_b and mask the depolarizing effect of the paracellular diffusion P.D. The addition of bumetanide in the presence of AIF decreased ψ_t by about 3mV suggesting a small contribution of active transport to the generation of ψ_t under these conditions.

The exit of Cl across the basolateral membrane has been postulated to occur partially through coupled KCl cotransport (Stewart et al., Bull. MDIBL 20:92, 1980), and evidence for such a mechanism in the Necturus gallbladder has been presented (Reuss, Nature 305:723, 1983). Table 3 shows the intracellular Cl activity measured with a Cl-sensitive microelectrode. Increasing $[K]_s$ from 5 to 170 mM resulted in a large increase of cell Cl activity, as

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.