

implication of this is that, either there is a pronounced species diversity in the nature of the natural secretagogue responsible for controlling rectal gland function, or that the response to VIP seen in *Squalus* is, in some way, non-specific or at least non-physiological. In support of the latter suggestion it should be pointed out that fairly high concentrations of VIP ( $> 10^{-8} \text{ mol l}^{-1}$ ) were required to produce a response in *Squalus*. The evidence suggests, therefore, that VIP is not the natural hormonal mediator of the secretory response in the rectal gland in vivo.

In contrast to this, the partially purified peptide extract obtained from the intestine of the elasmobranch *Scyliorhinus* and here described as Fraction 13, clearly contains a component (or components) that shows potent stimulatory activity in the rectal glands of all three widely differing species of elasmobranch studied to date. This component is therefore likely to play a major role in the control of secretion by the gland in vivo and possibly represents the native hormone responsible for determining secretion rate by the rectal gland. Although the precise constituents of Fraction 13 are, as yet, unknown preliminary evidence based on methanol solubility and retention time on HPLC gradients indicate properties distinct from those characteristic of VIP. Whilst its identity must await further purification and sequencing, we would like to propose the name "rectin" for this putative peptide hormone. This work was supported by grants from the U.K. Science and Engineering Research Council (GR/B/67063, GR/C/41777 and GR/B/68787).

#### BASOLATERAL CHLORIDE CONDUCTANCE IN FLOUNDER URINARY BLADDER

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The isolated urinary bladder of the winter flounder actively absorbs sodium and chloride and actively secretes potassium (Dawson and Andrew, Bull. MDIBL 19:46, 1981, and 20:89, 1982). The basolateral sodium transport step probably involves an electrogenic, Na/K ATPase (Dawson and Andrew, Bull. MDIBL 20:89, 1982), but the mechanism of chloride exit from the cell is unknown. The object of the present experiments was to provide a simple test for a basolateral chloride conductance in flounder urinary bladder.

Portions of flounder bladder were mounted as flat sheets in Ussing chambers as previously described (Dawson and Andrew, Bull. MDIBL 19:46, 1981), and were treated with ouabain (0.1 mM, serosal) to abolish the active transport of sodium, chloride and potassium. The ouabain-treated bladders were bathed on both sides by Ringer's solutions which contained (in mM) Na:147.5, K:2.5, Ca:1.5, Mg:1.0, Cl:5.0, gluconate:142.5, HEPES:15.0. Both sides were stirred with air and the pH was approximately 7.5 at room temperature. The serosal solutions also contained Verapamil ( $10^{-5} \text{ M}$ ) to inhibit smooth muscle contractions.

In these experiments we exploited the fact that the polyene antibiotic, amphotericin-B, when added to one side of a biological or an artificial membrane, forms pores which although they are moderately cation selective also possess a significant chloride conductance. Thus we added amphotericin-B ( $10 \mu\text{M}$ ) to the mucosal bathing solution to increase the chloride conductance of the apical membranes and then measured short circuit currents generated by imposing a chloride concentration gradient across the tissue. Rendering the bathing solutions initially low in chloride enabled us to change the chloride concentration by simply adding a small volume of concentrated NaCl to either bathing solution.

Figure 1 shows the results of an experiment in which the amphotericin-B was first added to the mucosal bath in the absence of any transmural chloride gradient. The short circuit current before addition of the polyene was near zero since active transport had been inhibited by ouabain. The addition of amphotericin-B increased the trans-epithelial conductance more than six-fold (from  $1.5$  to  $10 \text{ mS/cm}^2$ ), but had only a slight effect on the current. This result is expected in the absence of driving forces for transepithelial current flow. The addition of NaCl

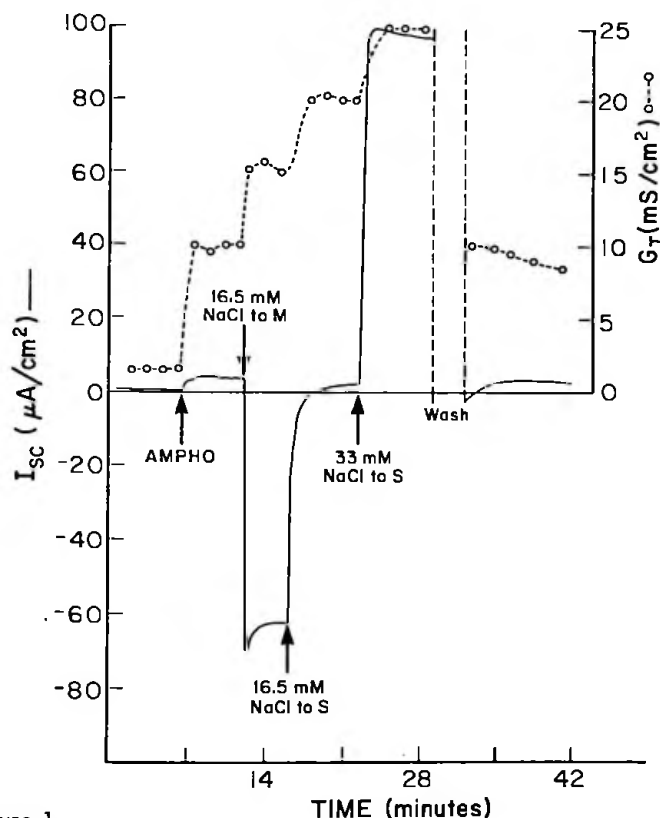


Figure 1. chloride emf. Addition of 16.5 mM NaCl to the serosal bath abolished the chloride gradient and reduced the current to zero although the conductance was further increased. Finally, increasing the chloride concentration in the serosal bath to 54.5 mM created a S to M chloride gradient and resulted in a current positive in the M to S direction as expected for S to M conductive chloride flow. Replacing both bathing solutions with chloride-containing Ringer's solution (without amphotericin-B) abolished the current, and reduced the tissue conductance.

These results are consistent with the notion that the basolateral membrane of the winter flounder contains a chloride conductance through which chloride ions move according to the direction of the prevalent electrochemical potential gradient. These experiments were carried out during the 1983 course in Epithelial Transport given at MDIBL. I thank Dr's. Mark Musch and John Stokes and all of the students who participated for their help. Supported by NIH AM29786.

#### POLAR LOBE FORMATION AND CYTOKINESIS IN FERTILIZED EGGS OF ILYANASSA OBSOLETA AND MYTILUS EDULIS

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Fertilized eggs of the marine mud snail, Ilyanassa obsoleta (Nassarius obsoletus), and of the blue, edible mussel, Mytilus edulis, form a constriction slightly below their equator that tightens and then relaxes several times before and during early cleavage. These shape changes represent an excellent model in studies of cytokinesis. Our purpose, in the projects summarized below, has been to determine what changes occur in intracellular ionic activities and in cytoskeletal proteins.

(16.5 mM) to the mucosal bath, however, resulted in the prompt development of a current, positive in the S to M direction, consistent with conductive chloride flow from M to S. This result has two important implications. First, the current induced by imposing a transmural gradient of NaCl can only be due to an overall conductance which is chloride selective, i.e., the current due to M to S sodium flow would have been oppositely directed. Second, the observation of anion selectivity rules out the possibility that these currents reflect the properties of the amphotericin channels in the apical membrane. It is well established that the so-called "one-sided" amphotericin channel is cation selective. Thus, the observed chloride selectivity must reflect the properties of the basolateral membrane of the epithelial cells.

Manipulating the transmural chloride gradient demonstrated that the direction of the current was determined by the orientation of the imposed