It would appear from the above data that oviposition requires growth of the reproductive tract in order to accommodate the passage of the skate egg case. It is likely that this requisite growth occurs during follicle development and thus might be under hormonal stimulation. The possible hormonal regulation of the events associated with spawning is under investigation. This work was supported by NSF PCM 78-08201 to I.P.C.

28 FINE STRUCTURAL CHANGES ASSOCIATED WITH CELL VOLUME REGULATION IN SLICES OF DOGFISH (SQUALUS ACANTHIUS) RECTAL GLAND

S.K. Masur, J. Goldstein and A. Kleinzeller, Departments of Physiology and Biophysics, Mount Sinai School of Medicine and Department of Biological Sciences, Columbia University, New York, N.Y., Brown University, Providence, R.I. and Department of Physiology, University of Pennsylvania, Philadelphia, Pa.

Evidence for cell swelling induced by high external K^+ medium in incubated shark rectal gland slices has been derived from electrolyte and water content analysis (Kleinzeller et al., Bulletin, MDIBL 20:75, 1980). In other tissues, reversal of such swelling is accompanied by the appearance of cytoplasmic vesicles and vacuoles (Van Rossum and Russo, J. Membrane Biol. 59:191, 1981). We are reporting preliminary observations of similar changes in fine structure of rectal gland slices when swelling is reversed. We have begun morphological and cytochemical studies of these slices in normal medium, in swelling conditions and during swelling reversal.

Slices of rectal gland prepared as described by Goldstein et al. (Bulletin, MDIBL 19:3, 1979) were incubated for 1 to 2 hrs in either A) control medium (6 mM K^+) or B) high K^+ medium (280 mM K^+ replacing equivalently the Na^+ in the standard medium) or C) 1 hr in high K^+ followed by 5, 15 or 60 min in control medium.

For electron microscopy (EM), one or two slices were removed from an experimental sample and fixed in a modified Karnovsky's fixative (buffered, double aldehyde) (Doyle, Bulletin, MDIBL 17:34, 1977) for 1 1/2-3hrs, briefly rinsed in 0.2 M cacodylate, pH 7.4 and postfixed in "aged" 0.05% ruthenium red (RR) in 1% OsO_4 in 0.1M cacodylate for 1 - 3 hrs. Handley and Chan (Histochemistry 71:249, 1981) reported that ageing this mixture for 3 hrs prior to use allows the OsO_4 to oxidize the RR. RR is a morphologically detectable marker for regions which are surface connected at the time of fixation (Chambers, J. Cell Biol 57:874, 1973). Oxidized RR according to Handley and Chan penetrates tight junctions and therefore the aged RR could provide a marker for extracellulary connected compartments on luminal as well as basal-lateral borders. We found no evidence of RR penetration between the cells of the rectal gland slice, but the tissue was well preserved by this mixture. Following fixation the tissue was dehydrated in a graded series of alcohols, propylene oxide, embedded in Epon and 1-2 μm sections were cut on glass knives, stained with toluidine blue and examined by light microscopy (Figures 1,2,3). Thin sections were stained with uranyl and lead and examined in a Philips 201 EM (Figures 4 and 5).

Observations of Incubated rectal gland slices

A. Control medium, 1 - 2 hrs

Parenchymal cells of the incubated rectal gland slices maintain their polarity and their junctional complexes (Eveloff et al, J. Cell Biol. 83:16, 1979) for prolonged periods (Doyle, personal communication) (Fig. 1). Blunt microvilli project into the lumen which is bounded by the junctional complexes between adjacent cells. Immediately below the membrane, the apical region is filled with microfilaments and some microtubules; within this cytoskeletal material are membrane-bound vesicles. Going in a basal direction, we find bounded irregular tubules some of which have ribosomes and are therefore connected with the endoplasmic reticulum. Mitochondria are also found in this region along with the Golgi apparatus. The nucleus sits in the center of the cell. The basal-lateral membranes are plicated with little extracellular space between them and occasional coated vesicles near them. At the basal end of the cell, narrow bands of cytoplasm contain mitochondria and some rough ER vesicles.

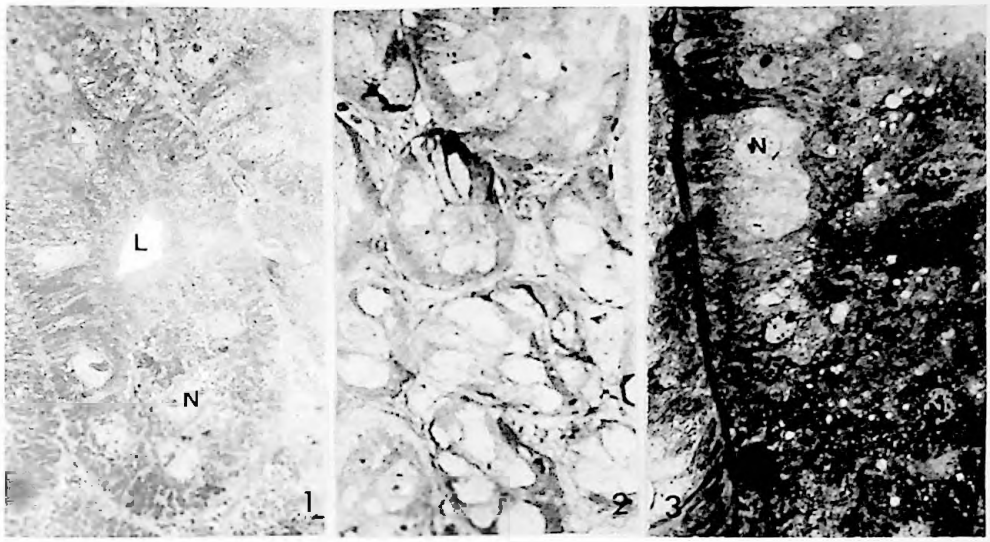

Figure 1.--Light micrographs of portions of several tubules from a rectal gland slice after 120 min in control medium. The lumen is seen at a central triansular space (L), the nuclei are at N and the "striped" cytoplasmic stain probably represents mitochondrial groups oriented within the basal cytoplasm X 1320.

Figure 2.--Light micrograph of tubules from a rectal gland slice exposed for 120 min to high K^+ medium. Most cells are swollen, lacking discernible nuclei. Denser outlining may represent cytoplasm of cells (connective tissue?) which can regulate their volume under extreme conditions. A central lumen is not seen. X 1320

Figure 3.--Light micrograph of tubules after exposure to conditions followed by 60 min of normal saline. The nuclei (N) are distinguishable from the cytoplasm, the denser staining regions absent in Fig. 2 (mitochondria?) are discernible. Vacuoles are obvious in regions apical to the nucleus and adjacent to where a lumen would be. X 1300

B. High K^+ medium, 1 - 2 hrs

The most obvious effect of the high K^+ medium is the swelling of the tubular cells which obliterates the lumen (Figs. 2 and 4). There is also a loss of cytoplasmic and nuclear electron-density and an enlargement of the osmotic space in the mitochondria, (Fig. 4).

C. High K^+ medium (1 hr) followed by return to control medium for up to 1 hr

The reversal of swelling under these conditions is detectable in a restoration of the control water and electrolyte content after 1 hr in control medium (Forrest and Kleinzeller, personal communication). Using EM we find the reappearance of the lumen within 5-15 min of return from high K^+ to control medium (arrow Fig. 5). The apical region of these cells is thrown up into irregular digit-like projections with microfilamentous cores. The apical region has an increased electron density of a stipled sort and vesicles and vacuoles are found in it. The observed vesiculation (Fig. 3) may be a contributing element to the net extrusion of H_2O and electrolytes found on transfer of the tissue from high K^+ to control medium.

The connective tissue cells do not obviously undergo the swelling changes (Fig. 4) and may contribute to the restrictive element postulated as an explanation for much less cell swelling than that predicted by the K^+ induced depolarization (Kleinzeller, ibid). We thank Hal Church and William Doyle for their kind and generous help. Supported in part by NIH-AM 25110 (SKM) and NIH-AM 12619 (A.K.) and a grant from the Whitehall foundation (A.K.) S.K. Masur has a Research Career Development Award from the NIH.

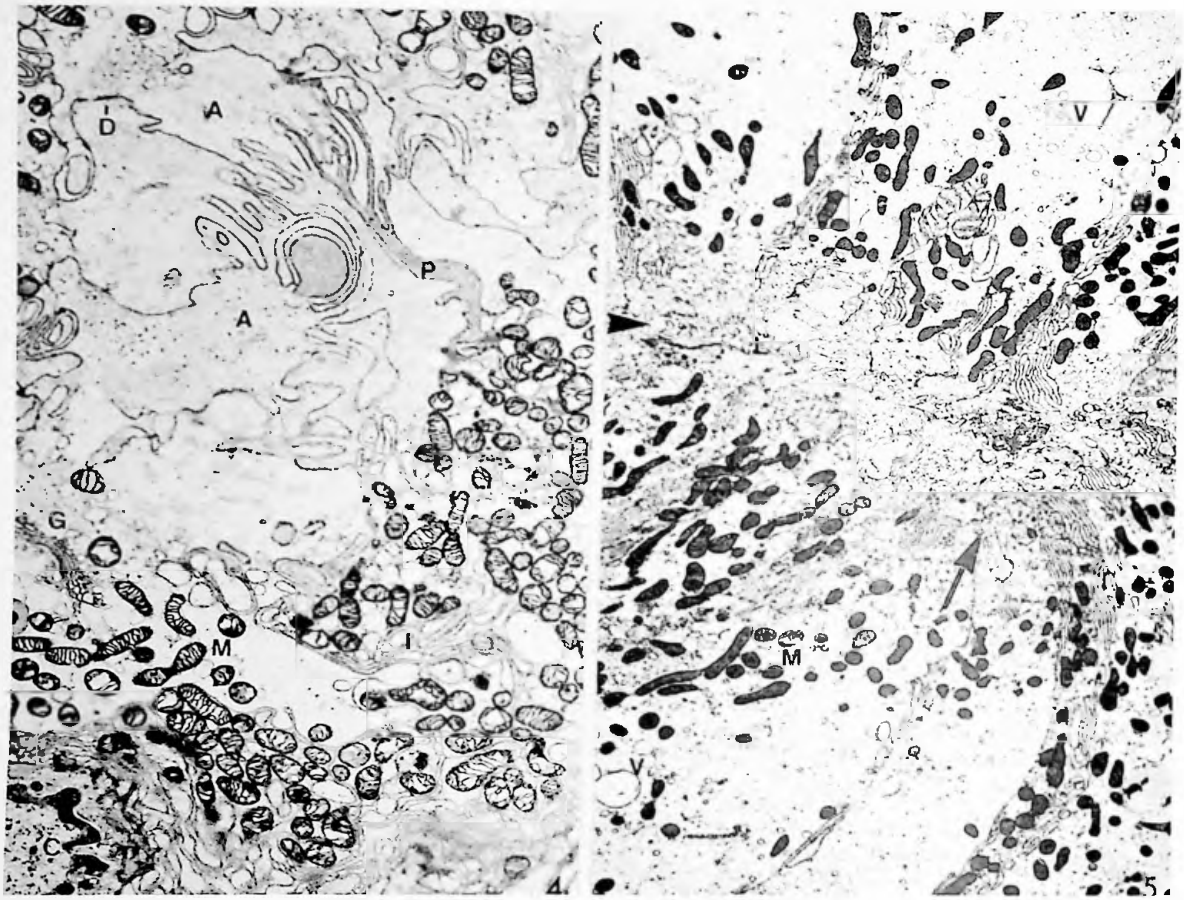

Figure 4.--Electron micrograph of apical regions of several cells after 60 min of incubation in high external K^+ mediu. The lumen and microvilli are absent as are vesicles which normally sit below the microvilli. The apical (A) cytoplasm has a stipled appearance suggestive of unoriented microfilamentous material. The membrane-bounded profiles (P) containing dense cytoplasm may be extensions of connective tissue cells (C) found at the base of tubules. Stacks of Golgi lammellae are seen at G. Basal-lateral membranes form interdigitating oval contours (I) with no apparent extracellular space (compare with accordian-like stacks in Fig. 5). The mitochondria (M) have enlarged "empty" compartments which appear to represent the cristae. Thickenings suggestive of desmosomes appear at D. X 7000.

Figure 5.--Electron micrograph of apical portions of cells surrounding the tubule lumen in a rectal gland slice which has been incubated for 10 min in control medium following 60 min in high K^+. The lumen is hook-shaped extending diagonally across the middle of the photograph and is indicated by L and arrows at either end. Elongated fingerlike cytoplasmic projections contain microfilaments as does the apical cytoplasm (A) (compare with Fig. 4). Small vesicles are also seen in this region and larger vesicles and vacuoles (V) predominate toward the central cytoplasmic regions. The mitochondria (M) appear normally electron-dense with narrow cristae and the contribution of two adjacent cell membranes is distinguishable within the basal-lateral folds. X 7000.

29 EFFECTS OF CRUDE OIL, DISPERSANT AND AN OIL-DISPERSANT EMULSION ON HERRING GULLS

D.S. Miller, D. Brier-Russell, F.A. Leighton, D. Phalen, D.A. Jeffrey, G. Lambert, D.B. Peakall, Mount Desert Island Biological Laboratory, Salsbury Cove, Me, Cornell University, Ithaca, N.Y., and Canadian Wildlife Service, Ottawa, Ontario, Canada