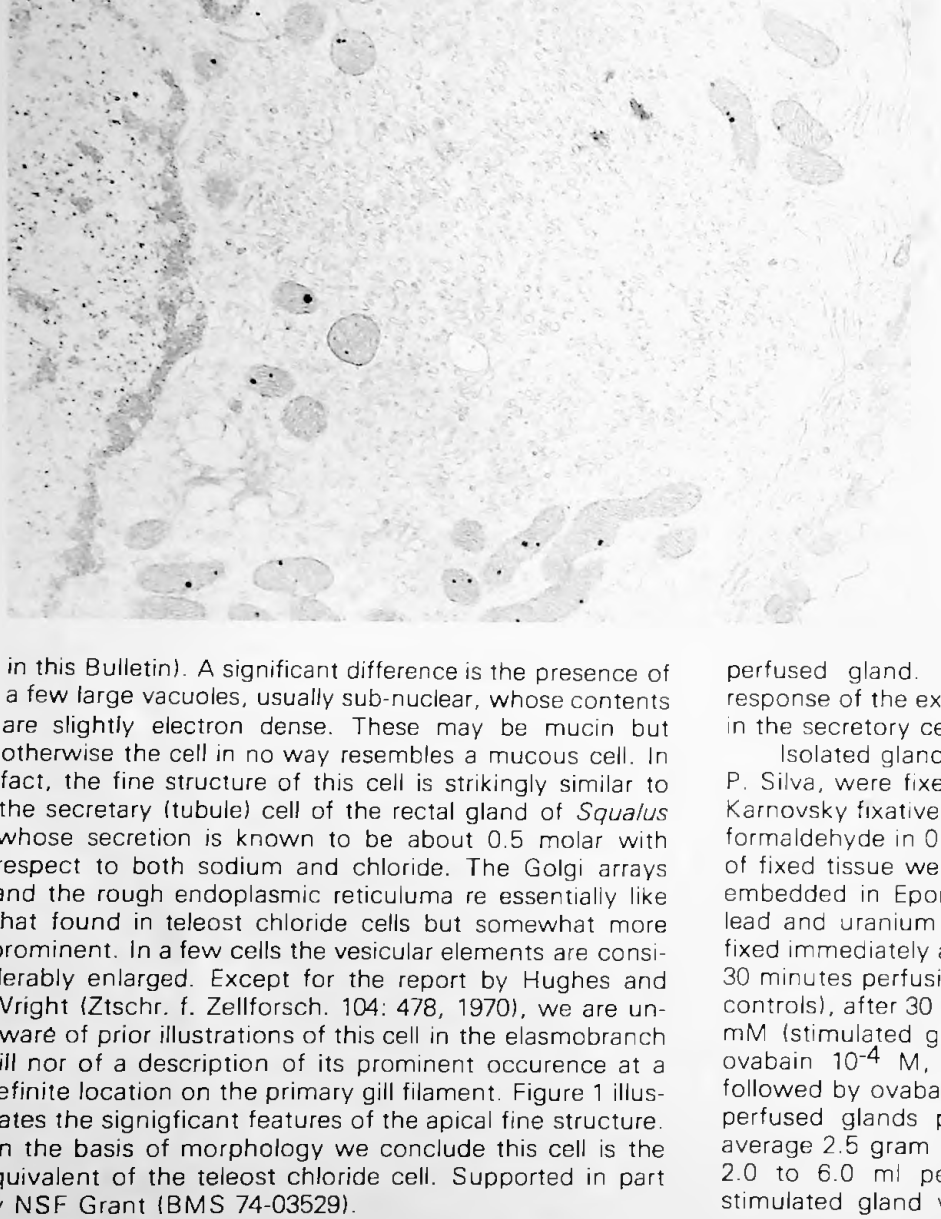

Figure 1 A portion of the supranucle cytoplasm of the "chloride cell" in *Sq lus* (12,300 X). Nucleus at the left and adjacent respiratory epithelial cell at right with dense mucous vesicles bene the surface ridges. Between the two ce a prominent intercellular space and in digitating cell processes. The small v icles and elongate tubules in the center the micrograph are closely packed and present the branching tubular reticu of the teleost chloride cell.

in this Bulletin). A significant difference is the presence of a few large vacuoles, usually sub-nuclear, whose contents are slightly electron dense. These may be mucin but otherwise the cell in no way resembles a mucous cell. In fact, the fine structure of this cell is strikingly similar to the secretary (tubule) cell of the rectal gland of *Squalus* whose secretion is known to be about 0.5 molar with respect to both sodium and chloride. The Golgi arrays and the rough endoplasmic reticuluma re essentially like that found in teleost chloride cells but somewhat more prominent. In a few cells the vesicular elements are considerably enlarged. Except for the report by Hughes and Wright (Ztschr. f. Zellforsch. 104: 478, 1970), we are unaware of prior illustrations of this cell in the elasmobranch gill nor of a description of its prominent occurence at a definite location on the primary gill filament. Figure 1 illustrates the signigficant features of the apical fine structure. On the basis of morphology we conclude this cell is the equivalent of the teleost chloride cell. Supported in part by NSF Grant (BMS 74-03529).

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Fine structure of the Stimulated Gland of *Squalus*

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Initiated by the discovery this summer of the mechanism for stimulation of secretion in the rectal gland by Silva and coworkers (#40 this Bulletin) the histology and fine structure of the gland has been reexamined. The findings of Hoskins (J. Morph. 28; 329, 1917), Doyle (Amer. J. Anat. 111; 223, 1962) and Bulger (Anat. Rec. 147; 95, 1963) have been generally confirmed, revisions being primarily ascribable to advances in techniques and to the ability to control the rate of secretion in the isolate perfused gland. The principal new features are t response of the excretory duct and the finding of a muc in the secretory cells.

Isolated glands from *Squalus acanthias*, perfused b P. Silva, were fixed by arterial perfusion with a modifie Karnovsky fixative containing 2% glutaraldehyde, and 4 formaldehyde in 0.13 M cacodylate buffer, pH 7.2. Slice of fixed tissue were posted fixed in 1% osmic tetroxide embedded in Epon and thin sections were stained wi lead and uranium for electron microscopy. Glands we fixed immediately after excision (zero time controls), af 30 minutes perfusion with elasmobranch saline (perfus controls), after 30 minutes perfusion with theophyllin 0.2 mM (stimulated gland), after 30 minutes perfusion wit ovabain 10^{-4} M, and after 30 minutes of theophyll followed by ovabain perfusion. The unstimulated isolate perfused glands produced about 0.2 ml/hour for th average 2.5 gram gland. The stimulated glands delivere 2.0 to 6.0 ml per gland per hour. Secretion of th stimulated gland was reduced within one minute aft administration of ovabain to the unstimulated level.

Histological findings: Vascularization of the rect gland is similar to that of the mammalian adrenal. Cortic arteries give rise to thin-walled sinusoids surrounding th secretory tubules with only a few small (40 m diamete arterial vessels running to the central canal. The sinusoi fuse at least twice and the secretory tubules branch a least twice in giving rise to the large sinuses whic surround the excretory ducts in the central canal. W consider the excretory duct to be a stratified cuboid epithelium rather than a transitional epithelium as terme by Hoskins and Bulger. Our electron micrographs show basal layer of cells, an intermediate layer and a surfac epithelium with a specialized apical surface. (Figure 1.) the theophyllin stimulated gland this epithelium sho

onsiderable distension of the intra-ellular space (Figure 2) which is ab-ent in the perfused control. We inter-ret this to indicate appreciable reab-orption of the tubular secretion by e duct which is surrounded by the enous sinus in the central canal. In ectron micrographs of the central inal we have observed unmyelinated erves with densecored vesicles and rminams associated with the smooth uscle of the venous sinus.

Despite the twentyfold difference rate of secretion of rectal gland fluid etween the perfused control and the eophyllin stimulated gland the fine ructural differences in the cells of e secretory tubules are minor. There e slight differences between zero me (unperfused) controls which indi-ate slight cell swelling. The changes specimens treated with 10^{-4} ovabain e those to be expected after 30 min-es, namely, cell swelling but only nor mitochondrial damage.

The fine structure of the secre-ory cell has many features in ommon with the chloride cell of the *Squalus* gill reported in this Bulletin Doyle). The apical surface abuts on he lumen rather than being exposed o seawater and the lateral borders of he cell are more extensively interdigi-ated with their neighbors. In both here is an accumulation of small (0.15 .05 μm diameter) vesicles in the api-al cytoplasm interspersed with tub-lar profiles of like diameter. The small esicles approach the apical cell membrane. A previously undescribed eature of these secretory cells is the presence of relatively large (1.0 μm di-meter) slightly electron-dense (gray) acuoles. These have the appearance f mucin. These gray vacuoles are in-ermingled with electron lucent ones f similar size and with others whose ontent appears diluted. We interpret hese to represent the secretion of a roteic mucoid substance which is dis-ributed via the endoplasmic reticulum nd Golgi to the small tubular and ves-ular components in the apical region nd subsequently discharged to the umen in a manner similar to that of ther protein exporting glands. The difference here is that the product in he apical secretory vesicles is not lectron dense. Other features of the ell are consistent with active synthe-is of a mucin. The nucleolus is sharp-y delineated, there is appreciable ranular endoplasmic reticulum and prominent Golgi lamellae and cisterns n addition to the large numbers of

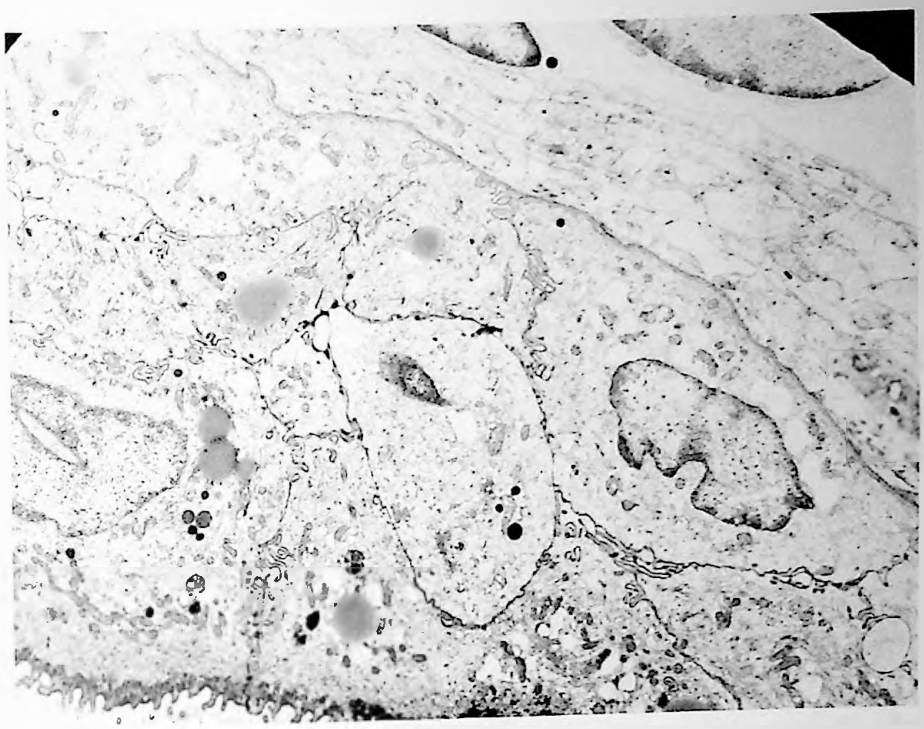
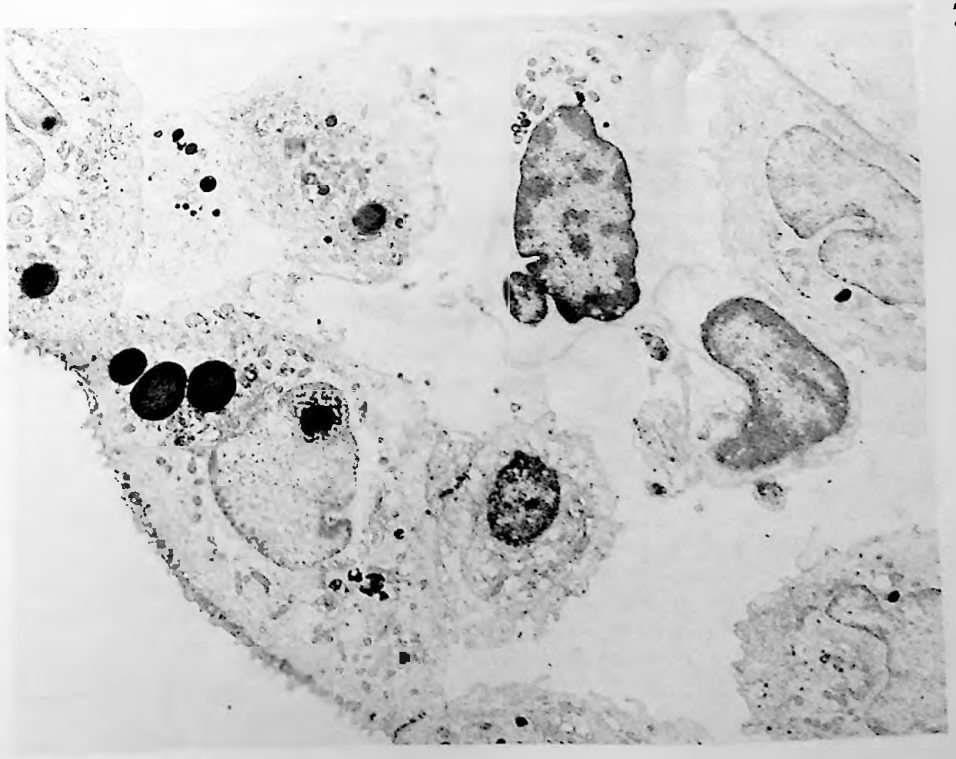


Figure 1 (above)
Rectal gland – excretory duct
Control (4,200 X)

Figure 2 (below)
Rectal gland – excretory duct
Stimulated (4,200 X)



small apical vesicles.

In all of our preparations there are numerous membrane bound profiles in the lumens of the secretory tubules. These may be artefacts of the fixation procedure or errors in the fusion of apical vesicles with plasma membrane. They are present in zero time control preparations and after ovabainperfusion as well as in the stimulated and control perfused glands. We do not suggest that a large portion of the secretory product is discharged by pinching off of apical membrane. If, however this process occurs normally, some residue of membrane as well as the secreted mucin should be detectable in rectal gland fluid.

The occurrance of mucin synthesis in these cells, and in the *Squalus* chloride cell invites consideration of its possible relation to salt secretion. These cells are quite unlike the ordinary mucous cells which occur in gills or the very large mucous cells scattered in the excretory duct of the rectal gland. The presence of a polyanionic substance in the Golgi of pancreatic acinar cells has been suggested by Palade (Science 189, 347, 1975) as a condensing mechanism, trapping cationic proteins and eliminating their osmotic effect, thereby allowing water to return to the cytoplasm. Polyanions have also been reported within the tubular reticulum of teleost chloride cells (Philpott and Copeland, J. Cell Biol. 18, 389, 1963).

Although a very large amount of polyanion would be required to trap the amounts of sodium present in rectal gland fluid, one wonders why this substance is synthesized in these situtions and whether it may play an auxiliary role in the production of hyperosmotic saline secretions. Further work on the detection and characterization of a mucin in rectal gland fluid is warranted. The evidence for reabsorption of tubular secretion by the excretory duct also indicates the need for micro puncture analyses. Supported in part by a grant from the National Science Foundation BMS 74-03529.

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The Effect of Phlorizin and its Derivatives on the sugar uptake by teased renal tubules of the winter founder (*Pseudopleuronectes americanus*).

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Phlorizin and its aglycone derivative phloretin have been used for some time in investigating the characteristics of carrier-mediated sugar transport systems. Accordingly, the effects of phlorizin, phloretin, and 2-deoxy-glucosyl phloretin on the sugar transport systems of teased flounder tubules were investigated. The uptake of sugars in this preparation predominately reflects transport at the basolateral face. Past research (Kleinzeller & Mullin, *Bull. MDIBL 15,* in press) has indicated the existence of three separate sugar transport systems: one shared by glucose, 2-deoxy-glucose and mannose (Glc-2dGlc); a second shared by galactose and 2-deoxy-galactose (Gal-2dGal); and a third system for the transport of φ-methyl-glucoside and glucose. Each system has been shown to be passively-equilibrating and to possess a characteristic structural specificity.

Teased tubules were incubated with 0.5 mM labelled sugar and 0.05 mM, 0.10mM, and 0.50mM concentrations of the three inhibitors. Phloretin, phlorizin, and 2-deoxy-glucosyl phloretin produced significant inhibition of the uptake of glucose, 2-deoxy-glucose, galactose, and 2-deoxy-galactose. In all cases, it was a decrease in the phosphorylated sugar which primarily accounted for the inhibition of sugar uptake; even at high concentrations of the inhibitors the apparent concentrations of cellular free sugars did not vary significantly from control values (Figure 1). Only in the case of 2-deoxy-galactose did the inhibitors cause any appreciable decrease in free sugar.

In the Gal-2dGal system, 0.05mM phloretin was more inhibitory than phlorizin or 2-deoxy-glucosyl phloretin. At high concentrations all three inhibitors had approximately the same effect on the transport of both galactose and 2-deoxy-galactose. The same relative potency of the inhibitors was seen in the Glc-2dGlc system. In both systems 0.05mM phlorizin significantly inhibited glucose and galactose uptake while it required 0.10 mM phlorizin to appreciably inhibit the uptake of the 2-deoxy derivatives. None of the inhibitors produced significant changes in the extracellular (PEG) space.

To assess the affinity of the inhibitors for the sugar transport sites, the tissue was pre-incubated with and without 0.5mM phlorizin for 30 minutes, washed in physiological saline for 2 minutes, and then incubated with or without 0.5mM phlorizin and with 0.5 mM labelled 2-deoxy-glucose. Tubules incubated with phlorizin showed the characteristic decreased sugar accumulation. Conversely, tubules which were only pre-incubated with phlorizin and then washed showed no inhibition of sugar uptake as compared with the control values (See Table II).

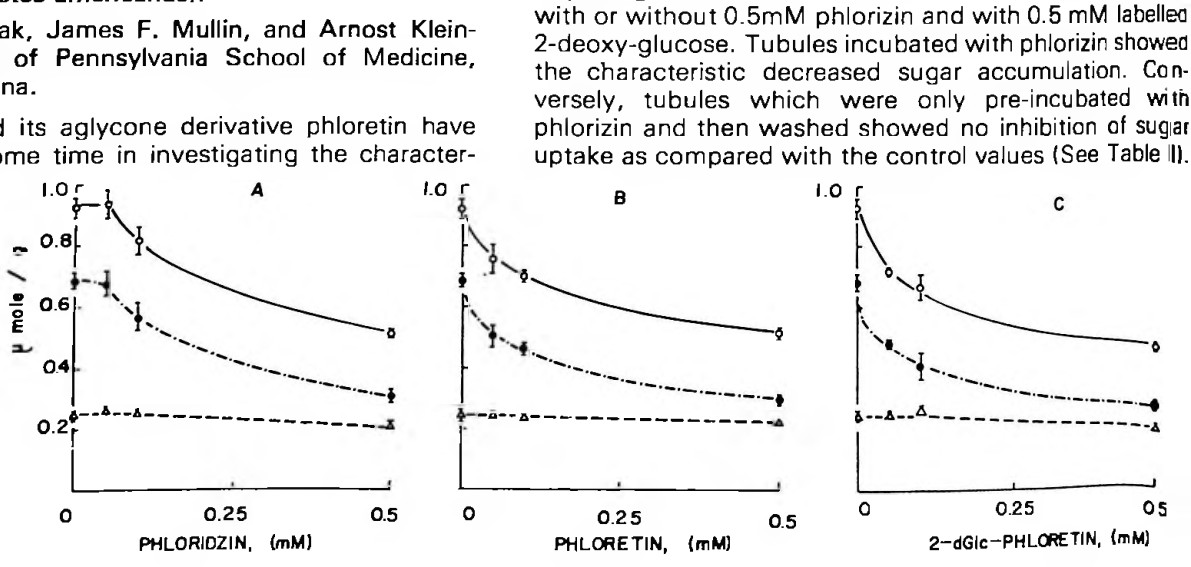

Figure 1