

showed some signs of mitochondrial damage characterized by the formation of whorls.

The specimens in the current series were examined the third day after transfer to fresh water. In comparison with chloride cells of eels fully adapted to seawater, these chloride cells after 3 days in fresh water show a less well developed tubular reticulum, the cytoplasm is as dense as expected for a seawater specimen but a larger proportion of the cells are below the surface epithelium (i.e., not exposed to the seawater). In those cells in which the tubular reticulum is moderately well developed it is less branched than in animals fully adapted to seawater. What is new in these specimens is the presence of a greatly increased vesicular component in the apical third of most cells. The following figure is at 12,300 magnification. The mitochondria instead of being evenly distributed from the base almost to the apex of the cell are absent from the apical half to one third of the cell.

In this experiment the numbers of mitochondria per cell and the numbers of chloride cells in the filament appeared unchanged from that in seawater conditions. Despite a change in configuration of the tubular the total area of this membranous component did not differ significantly from that in specimens adapted to seawater. These morphological observations are consistent with the findings of Epstein et al. (this Bulletin) in seawater adapted animals. However we found 1) many chloride cells located beneath the surface epithelium rather than exposed to the water and 2) alterations in the tubular reticulum from the

normal branched configuration in close apposition to mitochondria to a population of vesicles no longer in such close apposition to mitochondria. These results are consistent with the observed decrease in salt efflux.

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The Chloride-Cell in *Squalus* Gill

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The rate of sodium transport in the elasmobranch gill has been reported to be about one-tenth of that in teleosts. Boylan (Bulletin MDIBL 5, 1962) found the gill almost impermeable to water and urea. Burger and Horowicz (Bulletin MDIBL 6, 1966) found sodium efflux of the head region to be 2.2 m moles/sq.m/hour, efflux being about one-fifth of influx. There have been only occasional and fragmentary reports of mitochondrial-rich cells in elasmobranch gills and no detailed comparison with chloride cells of teleosts.

The dogfish, *Squalus acanthias*, was studied with histological sections used to orient samples used for electron microscopy. In cross sections of gill filaments there is an artery and vein at the free edge, a central blood sinus and collecting vessels at the attached side of the filament. The respiratory (secondary) lamellae are found at each side of the primary filament. The epithelium of the respiratory lamellae consists of a single cell type characterized by surface ridges, a dense apical cytoplasm with a layer of small ellipsoidal mucous vesicles in a microfilamentous web. The epithelium of the primary filament consists of similar cells, but in two regions one finds much larger cells interspersed among them. At the free edge the large cells are present with some regularity, but it is at the attached edge of the filament that they predominate, being almost as numerous as the ordinary epithelial cells. Failure to observe these large cells in previous electron microscopic studies can be ascribed to their absence from the respiratory lamellae and to their location at the attached edge of the filament. In the electron microscope the large cells show considerable superficial resemblance to the chloride cells of teleost gills in that the mitochondria are very numerous and the cytoplasm is filled with spherical and elongate vesicles and tubules, the lateral and basal borders of the cell have elaborate interdigitating processes and a conspicuous intercellular space. The apical portion of the cell is free of filaments and many of the vesicles are close to the apical membrane. At higher magnifications (Figure 1, pg. 28) it can be seen that there is no regular branching of the tubular reticulum so characteristic of the teleost chloride cell, nor can we find instances of tubular connections to the lateral cell membrane. In the teleost (eel), however, transformation of the branching tubular reticulum to a vesicular form has been observed under some conditions of adaptation (Doyle — 18

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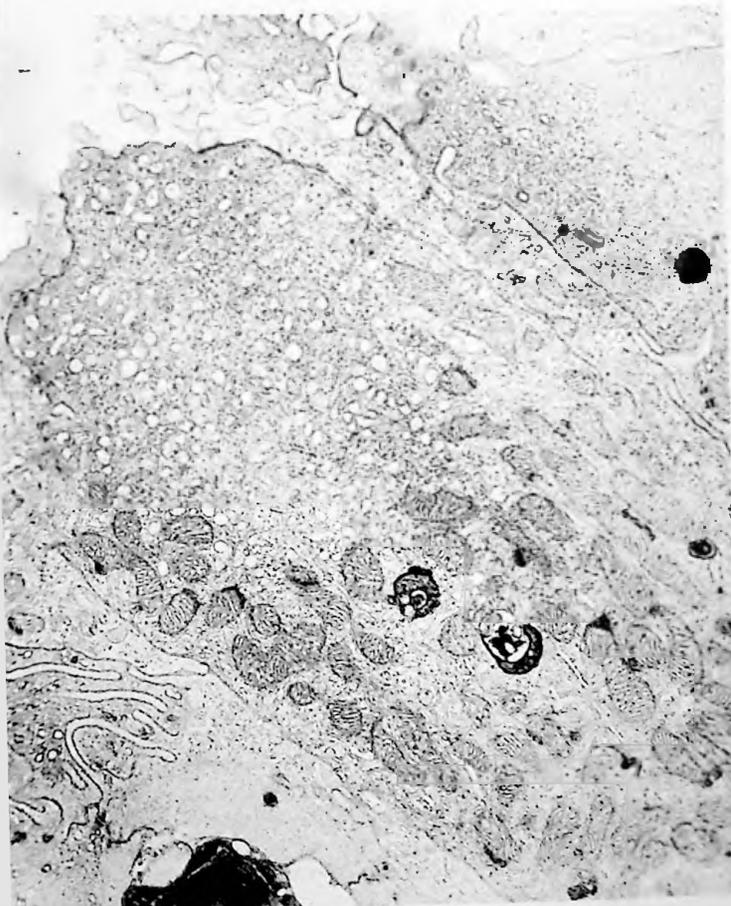


Figure 1
Anguilla gill (12,300 X)

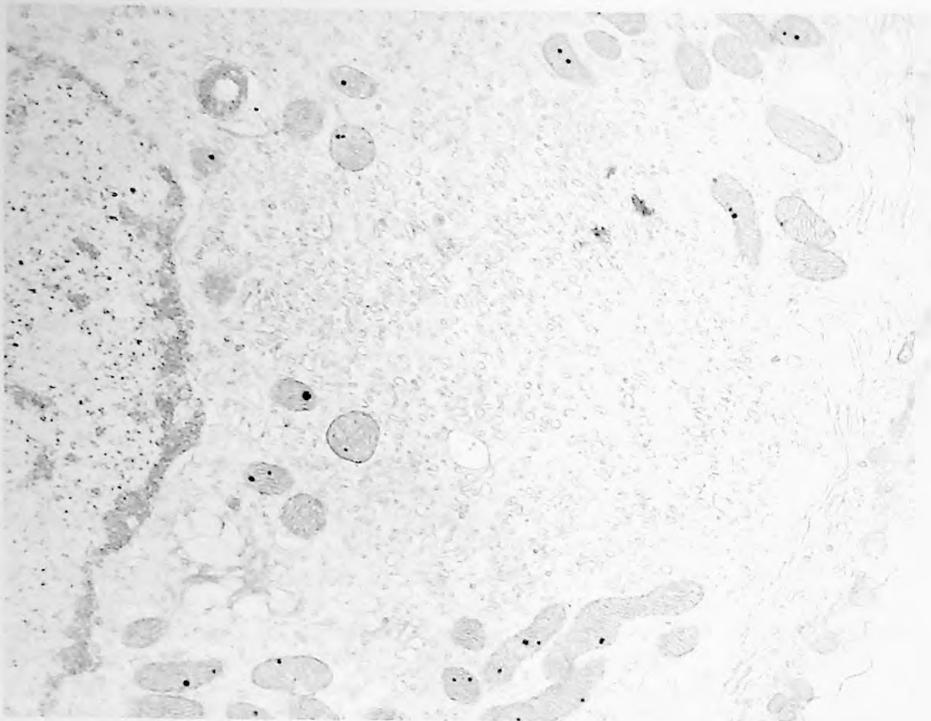


Figure 1 A portion of the supranuclear cytoplasm of the "chloride cell" in *Squalus* (12,300 X). Nucleus at the left and adjacent respiratory epithelial cell at the right with dense mucous vesicles beneath the surface ridges. Between the two cells a prominent intercellular space and interdigitating cell processes. The small vesicles and elongate tubules in the center of the micrograph are closely packed and represent the branching tubular reticulum 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 secretory (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 reticulum are 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 occurrence at a definite location on the primary gill filament. Figure 1 illustrates the significant 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 the response of the excretory duct and the finding of a mucin in the secretory cells.

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

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