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LIGHT AND ELECTRON MICROSCOPIC STRUCTURE OF CORPUSCLES OF STANNIUS OF THE FRESH WATER FISH, *NOTOPTERUS NOTOPTERUS*

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ABSTRACT

The corpuscles of *Notopterus. notopterus* spherical in shape, the cells of the gland are usually arranged in strands, separated by septa of connective tissue continuous with outer fibrous capsule. The cyto architecture of the CS in *Notopterus notopterus* shows that four types of arrangement of connective septa and cells .Two principle types of secretory cells are observed. Type-l (PAS+ve) and Type-2 (PAS-ve). Type-1 cells are round with clear vesicular nucleus and stainable material in the cytoplasm Type-II cells are slender, irregular cell bodies that may contain cytoplasmic processes extending between the type-I cells, A third type-III cells was also noticed. Presence of lipids was detected by Sudan Black-B staining method. The presence of proteins was observed by Mercury-bromophenol-blue staining method. The ultrastructural observation of corpuscles of Stannius in the fish, *Notopterus notopterus* shows basically three types of cells. The identification of three different cells is based on the nuclear structure, morphology of secretary granules and other cytoplasmic components.

KEYWORDS: Electron Microscopic**,** *Notopterus notopterus,* Teleostean Fishes

INTRODUCTION

The corpuscles of Stannius (CS) are enigmatic glands which vary in number and are usually embedded in the kidneys of teleostean fishes. The CS contain one to three cell types, two of which are loaded with secretory granules that, on first inspection, remind one of zymogen granules in the cytoplasm of pancreatic exocrine cells.

In all species examined, the majority of CS cells are of the type-I. In the bowfin, toadfish and a number of other species (Youson and Butler, 1978; Wendelaar Bonga and Greven, 1975; Bhattacharya and Butler, 1978). The CS is composed almost exclusively of type-I cells. The other cell type is type-II cells tend to be narrower with smaller irregularly shaped granules and sparsely distributed endoplasmic reticulum and fewer Golgi bodies (Wendlaar Bonga *et al.,* 1977). There is evidence that the two cell types come from a single lineage whereby their histological differences may reflect different stages of development or different states of activity (Bhattacharya *et al.,* 1978). It has even been suggested that type-II cells may represent type-I cells that are undergoing programmed cell death or aptosis (Wyllie *et al.,* 1980). The detailed cytology of CS through histology, histochemistry and ultra structural studies have been undertaken

MATERIALS AND METHODS

Collection of Corpuscles of Stannius and Fixation for Electron Microscopic Studies

Fish were killed with a blow to the head. Then a longitudinal incision was made through the ventral body wall to expose the kidneys. The CS or white bodies were clearly visible along most of the length of the kidney, each CS together

with the surrounding kidney was flooded with ice-cold glutaraldehyde (icg) buffered to pH 7.3 with phosphate and fixed for 5 min. CS were excised from the adjacent kidney tissue and placed individually, in separate 2 ml glass vials containing additional ice-cold fixative for 2 hr. In the fish, *N. notopterus,* there are two white oval corpuscles, approximately 0.5 to 2.00 mm in diameter, which are embedded in the ventral surface of the anterior position of the posterior functional kidney at the point where the right post cardinal vein leaves it. Both CS were transferred to the Ice cold Glutaraldehyde (ICG) fixative for 2 min then removed, cut into about four pieces, and placed in a vial of ICG for 2 h.

Fixation

The corpuscles of Stannius (CS) were primarily fixed in Glutaraldehyde (because its capacity to stabilize most of the protein without coagulation) following fixation in the primary fixative, the tissues were washed in 0.1 M buffer. The tissues were post fixed in 1%, Osmium (OsO_4) for 1-2 hours at 4^0C. They are dehydrated using absolute alcohol (glass distilled) at 4^0C.

The tissues were washed with 0.1 M buffer after post fixing in OSO_4.

70% ethanol 1 hr

80% ethanol 1 hr

The tissues are Enblock stained with uranyl acetate 95^0C ethanol at 4^0C for 1-2 hrs.

Enblock staining gives better fine structural preservation membrane structures and cell joints, protein, myofibrils and mitochondira. Absolute ethanol 2 changes 30 min. each.

Clearing

The tissues are cleared with a clearing agent propylene oxide to facilitate infiltration. 2 changes – 15 min, each at room temperature.

Infiltration and Embedding

The tissue is embedded in embedding medium (Epon 812/Araldite Cy212). Infiltration involves gradual replacement of the dehydrating agent with the embedding medium and embedding consists of complete impregnation of the interstices of the tissue specimen with the medium. It also attaches the tissue block sufficiently strongly, enabling it to be handled to obtain ultrathin sections.

Infilteration is carried out at room temperatures with a liquid resin with which embedding of tissues are carried out.

Semithin Sections for Light Microscopy

Before proceeding to ultrathin sectioning 1 µ thick section are cut for scanning the tissue under the light microscope. The semithin sections floated on the water are lifted with a thin glass rod on a clean glass slide. The slide is placed on a hot plate at about 80^0C and dried. The sections are stained using 1% toludine blue for 1 minute. Washed in running water, dried and mounted with dibutylpthalate plasticizer xylene (DPX). The slides are observed under light microscope.

Pieces of CS in each vial were washed several times with phosphate buffer and post-fixed for 2 hr in chilled 1% O_SO_4 buffered to pH 7.3 with phosphate followed by dehydration in a graded series of chilled ethanol, then propylene oxide and embedded in araldite. Ultrathin sections were cut at various levels through the tissue blocks using a diamond knife on a Reichert OM U3 ultra-microtome, the sections were mounted on copper grids, stained with uranyl acetate and lead citrate and examined using an electron microscope. Representative photomicrographs of these tissue samples were taken. All sections were viewed at a magnification of 5500. As noted above, sample areas (500 lm2) were selected randomly and photographed.

OBSERVATION

The fish selected for the present study is *Notopterus notopterus* (Figure 1) locally available in the freshwater aquatic bodies of Gulbarga.

The general organization of the corpuscles of Stannius in *Notopterus notopterus* shows that, it has a spherical structure and are found lying on or embedded in the anterior portion of posterior part of the kidney (Figure 2, 3, 4 and 5). Only one pair of the corpuscles was observed (Figure 3). The gland is highly vascularised with rich innervation. The diameter of the corpuscles ranges from 0.5-2.0 mm which varies during different phases of the reproductive cycle. The corpuscles of Stannius of *Notopters notopterus* are whitish (Figure 6) and have a fibrous capsule and are composed of short columnar cells, closely packed in columnar groups, (Figure 7).

Histology of Corpuscles of Stannius

The corpuscles of *N. notopterus* spherical in shape, the cells of the gland are usually arranged in strands, separated by septa of connective tissue continuous with outer fibrous capsule, (Figure 8). The septa contain all the vascular and nervous elements. The vascularization of the Stannius corpuscles is rich, which results in a better access of the gland cells to the blood circulation. The capillaries are associated with renal arteries and cardinal veins. The Stannius corpuscles probably also have a rich nervous supply. The nerves are found in close association to the corpuscles. This indicates rich innervations.

The cyto architecture of the CS in *Notopterus notopterus* shows that four types of arrangement of connective septa and cells as reported by Subhedhar and Rao (1976). The first type which is common in which cells are arranged in the form of cords lined by single layer of cells, along thin septa exhibiting circular appearance (Figure 8). In the second type, a thin penetrating connective tissue septa divide the corpuscles into several incompletely delimated lobes. The third type septa are better developed and more prominent then the previous type. The union of ramifying connective tissue causes same groups of cells to become separated from the rest, forming smaller lobular from the completely delimited lobes (Figure 8). In the fourth type corpuscle is composed of aggregates of small lobes (Figure 8). Each of which consist of a number of complete and incomplete lobes. The penetration of connective tissue septa into single corpuscles makes the corpuscles bi-lobed, tri-lobed or even multi-lobed condition.

Two principle types of secretary cells are observed. Type-l (PAS+ve) and Type-2 (PAS-ve). Type-1 cells are round with clear vesicular nucleus and stainable material in the cytoplasm Type-II cells are slender, irregular cell bodies that may contain cytoplasmic processes extending between the type-I cells, A third type-III cells was also noticed.

Histochemical Studies

Histochemical studies for the demonstration of lipids, proteins and dichromate reaction for the localization of any adrenaline and nor-adrenaline containing cell was carried out.

Presence of lipids was detected by Sudan Black-B staining method. The corpuscle was fixed in calcium formol and stained with Sudan Black-B. In presence of Sudan black color in some portion of the Stannius corpuscles indicate the presence of small amount of lipid, (Figure 9).

The presence of proteins was observed by Mercury-bromophenol-blue staining method. The corpuscles showed the blue color, which indicated the presence of proteins. The cells of corpuscles are well-equipped for protein synthesis and secretion. The positive reaction indicates presence of glycoproteins which may be stanniocalin as reported in other fishes, (Figure 8).

To detect the presence of adrenaline and nor-adrenaline containing cells, the corpuscles of Stannius was fixed in potassium dichromate solution. The presence of some dark brown and light yellow pigments shows the presence of pigment cells, (Figure 10). The positive reaction to dichromate was observed in between the CS cells indicating innervation.

Ultrastructural Studies

The ultrastructural observation of corpuscles of Stannius in the fish, *Notopterus notopterus* shows basically three types of cells. The identification of three different cells is based on the nuclear structure, morphology of secretary granules and other cytoplasmic components (Figure 11).

The semithin sections prepared for ultrastructural studies shows the cellular organization (Figure 12). At the ultra structural level, cells of three types are located in the CS of *Notopterus notopterus.* These secretary cells as type-1 are arranged in follicles (Figure 8). They are characterized by oval or round nuclei with uniform chromatin material (Figure 7), and numerous large membrane bound electron dense secretary granules scattered all over the cytoplasm. Abundant ribosomal endoplasmic reticulum (RER), arranged as lamellar arrays in the vicinity and nucleus as well as in the rest of the cytoplasm are also visible. A few elongated filamentous mitochondria with lamellar or tubular cristae are present in the cytoplasm. The Golgi areas are well developed with large vacuoles containing electron dense materials and some are completely empty. The type-II cells are characterized by electron dense cytoplasm, few membrane bound secretary granules, the nucleus has chromatin patches and these cells have cytoplasmic extensions amongst the type-I cells, the type-III cells are smaller in size and lesser in number with uniform cytoplasm having less secretary granules. These cells have broader nucleus with chromatin material. Amongst the three types of cells clearly identified, the type-I cells are prominent and found to be the major cell type probably secreting the hormone of the corpuscles of Stannius. Amidst the gland cells, a mast cell packed with membrane bound electron dense bodies of variable size and a large irregular shaped nucleus is also conspicuous (Figure 13).

DISCUSSIONS

In a light microscope study of eight teleost species, (Krishnamurthy and Bern 1971) demonstrated rich autonomic innervations of corpuscles of Stannius, but they were unable to show whether it was sympathetic or parasympathetic. In all species examined, nerves and ganglia were found in close proximity to the corpuscles. Ganglia like accumulation of

neurons usually in close proximity to the corpuscles but occasionally within the corpuscles capsule, further it has been described by (Heyl, 1970; Belsare 1973; Schreibman and Pang 1975; Wendlaar Bonga *et al.*, 1977).

From this ganglion, nerve fibers penetrate the bodies along the blood vessels and branch into small fibers that run into the septa in close association with the capillary network. In many species the septa also contain single neuron (Krishnamurthy and Bern, 1971; Belsare, 1973; Unsicker *et al.*, 1977). The nerve fibers are always confined to the septa. They do not seem to penetrate between the glandular cells and synaptic nerve endings on gland cells have never been reported. The lack of evidence for direct nervous innervation of the gland cells together with close association invariably found between the nervous and vascular supply of the corpuscles of Stannius have led most authors to conclude that the nervous supply of the CS is from a primarily connected with the control of the blood flow through the bodies any effect on the glands may be indirect (Heyl 1970; Krishnamurthy and Bern, 1971; Wandelaar Bonga *et al.*, 1977; Bhattacharya and Butler, 1978).

In the most detailed study of the nervous supply of the CS available so far, (Unsicker *et al.*, 1977) came to the same conclusion for rainbow trout (*Salmo irideus*). With flourimetric technique they showed that the corpuscles of Stannius contain considerable amount of catecholamines and 5-Hydroxytryptamine they also shown that the nerve fibers running in the septa are adrenargic, although the fibers also might contain some 5-hydroxy tryptamine. The neurons dispersed in the connective tissue appeared to produce nor-adrenaline. These cells were sympathetically innervated by cholinergic nerves.

The ultrastructural observation of CS cells in the *Notopterus notopterus* shows three types of cells. The first type cells are type-I cell are large in number having electron transient cytoplasm with vacuolation, large secretary granules and abundant mitochondria and ribosomal endoplasmic reticulum, the second type of cell known as type-II are less in number characterized by electron dense cytoplasm, few membrane bound secretary granules, these cells have cytoplasmic extensions amongst the type-I cells. The third type of cells also have been observed laying small in size having broader nucleus, well marked nuclear membrane and has uniform cytoplasm with very less secretary granules. The most conspicuous feature of these cells particularly type-I cells is the presence of a large number of vacuoles of irregular shape which gives exhausted appearance. The secretary granules are large in type-I cells whereas, they are smaller in type-II cells. The Golgi areas are seen, the mitochondria are large and more in number in every cell particularly in the type-I cells the nuclei of all the three types of cells are clear with prominent nucleolus. The chromatin particles are seen in the type-II and type-III cells, which are not seen in the type-I cells. Hence, based on the structure of nucleus and cytoplasm, the three types of cells can be easily distinguished.

The electron microscopic observations have been carried out in CS cells of other fishes and it has been reported that the freshwater fish species possess two types of cells (type-I and II) in their corpuscles which the marine fishes have just one type (Firoz Ahmad *et al.*, 2004). However, exceptions have been reported with regard to types of cells in the CS of teleostean fishes (Firoz Ahmad *et al.*, 2002) in the fish, *Notopterus notopterus*, and three types of cells are clearly visible based on the cytoplasmic state, nuclear structures and secretary granules. The prominent cell type is type-I cells which have large secretary granules with abundant mitochondria and extensive vacuolations suggesting that major hormone of the CS may be secreated by these cells in comparison to other two types of cells (type-II and III). The CS of teleosts residing entirely in freshwater or euryhaline species, spending part of their life cycle in freshwater have been shown to contain heterogeneous populations of gland cells, unlike the marine forms where the CS contain cells of only one type

(Wendelaar Bonga and Grevens 1975), *Onchorhynchus mykiss* (Krishnamurthy and Bern 1969; Meats *et al.,* 1978) *Fundulus heteroclitus* and *Carassius auratus* (Wandelaar Bonga., 1980), *Oreochromis mossambicus.*

CONCLUSIONS

The light and ultrastructural observation of corpuscles of Stannius in the fish, *Notopterus notopterus* shows basically three types of cells. The identification of three different cells is based on the nuclear structure, morphology of secretary granules and other cytoplasmic components.

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APPENDICES

Figure 1: Showing the Photograph of the Fish, *Notopterus notopterus* Selected for the Present Study

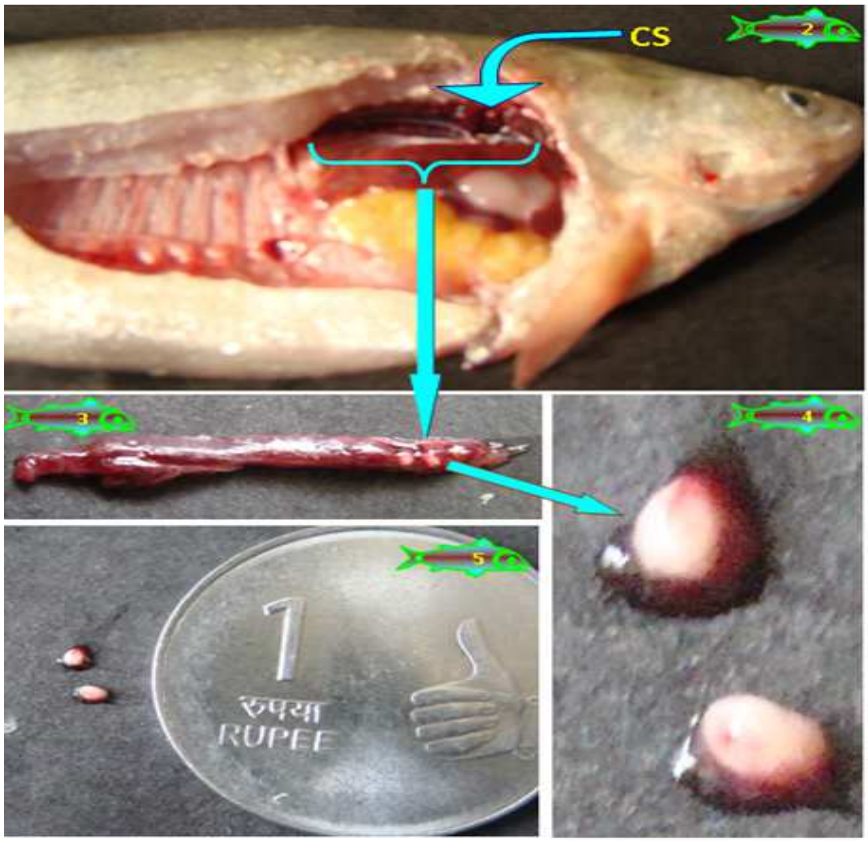

Figure 2: Showing Photograph of Fish Dissected for Exposing Corpuscles of Stannius Embedded in the Kidney
Figure 3: Photograph Showing Corpuscles of Stannius Embedded in the Kidney
Figure 4 & 5: Photograph Showing a Pair of Corpuscles of Stannius

Figure 6: Photograph Showing the Whitish Corpuscles of Stannius of Notopterus Notopterus

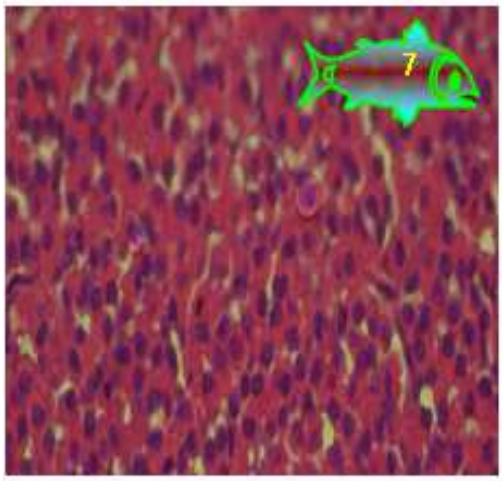

Figure 7: Showing the Section of Corpuscles of Stannius Composed of Columnar Cells, Closely Packed in Groups H & E × 1200

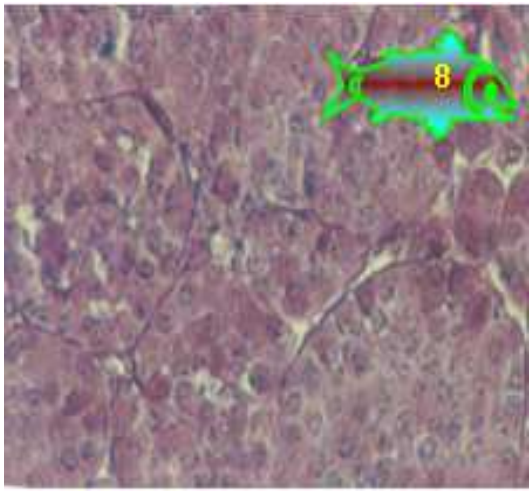

Figure 8: Section of Corpuscles of Stannius Showing the Presence of Proteins by Mercury-Bromophenol Blue Method × 1200

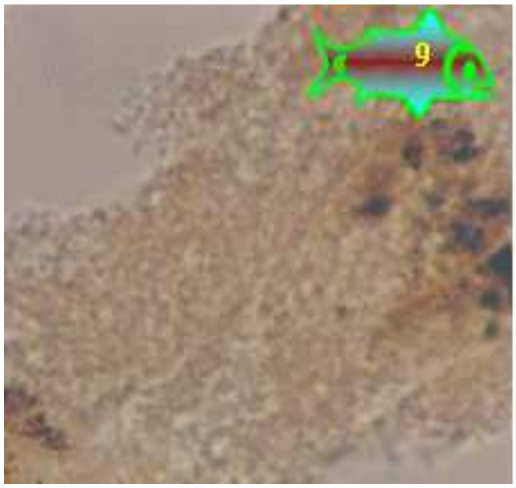

Figure 9: Section of Corpuscles of Stannius Showing Dichromate Reaction Indicating Positive Dark Brown and Light Yellow Colour Indicating Innervations × 1200

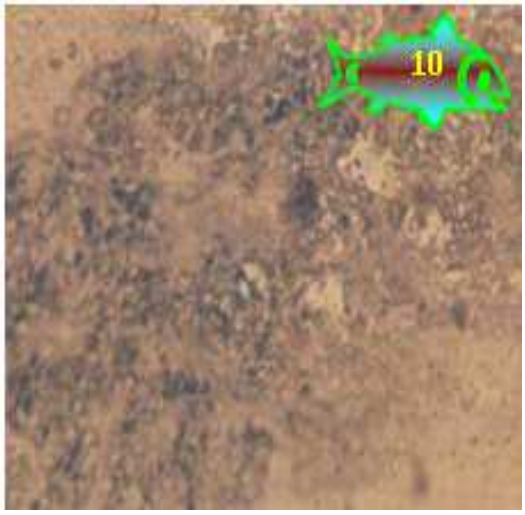

Figure 10: Section of Corpuscles of Stannius Showing of Weak Sudanophilic Reaction in the Corpuscles of Stannius, Sudan Black-B Stain × 1200

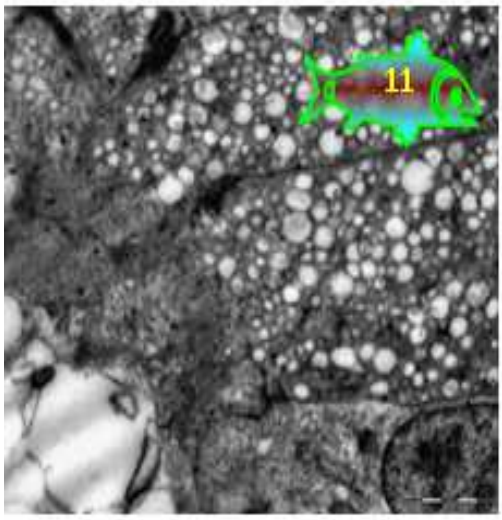

Figure 11: Ultrastructure of Corpuscles of Stannius in the Fish, Notopterus Notopterus Showing Highly Vacuolated Type-1 Cells × 9300

Figure 12: Semithin Section of Corpuscles of Stannius Showing the Cellular Organization × 1200

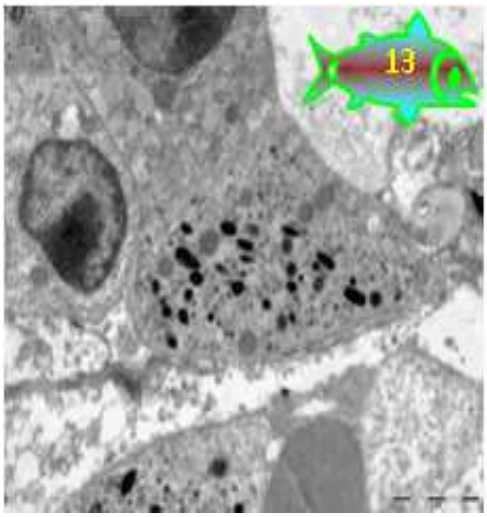

Figure 13: Showing the Section of Corpuscles of Stannius Composed of Columnar Cells, Closely Packed in Groups H & E × 1200