

Morphological, Histological and Ultrastructural Evidences of the Stannius Corpuscles in Fresh Water Teleost (*Oreochromis niloticus*) and Marine Teleost (*Epinephilus tuvina*)

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Abstract. Morphological, histological and ultrastructural features of the Stannius corpuscles (Stc) in fresh water teleost *Oreochromis niloticus*, and marine teleost, *Epinephilus tuvina* were studied. In *O. niloticus*, the Stc were numerous paired and randomly scattered throughout the trunk kidney as white corpuscles, with the increase in fish size they became concentrated at the dorso-caudal part of the kidney. In *E. tuvina* this gland was observed to be represented only by large pair of corpuscles which were always associated with and often connected to the distal portion of the tubular nephrons. In small fish the Stc consisted of undifferentiated cellular mass attached to the posterior part of the kidney tubules. They became encapsulated and divided into lobules of secretory cells with increase in fish size.

Ultrastructure study of the gland demonstrated two main types (Type 1 and Type 2) of secretory cells in the two teleosts with secretory granules. Type 1 cell predominating in the gland with large granules, numerous free ribosomes, lysosomes and ribosomal endoplasmic reticulum. Type 2 cell with small secretory granules, extensive arrays of smooth endoplasmic reticulum and ribosomal endoplasmic reticulum. Abundant ribosomal endoplasmic reticulum and well developed Golgi suggested the gland role in protein synthesis. It is important to

mention that another non secretory dark cell type was demonstrated in the Stc of *E. tuvina* characterized by cytoplasmic processes penetrate between the main cells, this dark cell may represent a supporting cell. Cells of Stc were innervated by unmyelinated nerve fibers.

Keywords: Stannius corpuscles, gland, fresh water teleost, marine teleost.

Introduction

Stannius corpuscles are tiny endocrine glands associated with the kidney of holostean and teleostean fishes (de Smet, 1962). They are located in the dorso-caudal part of the trunk kidney. Discovery of this gland was made first by Stan-nius, 1839 (de Smet, 1962). Previous researches suggested that Stc are embryologically derived from the pronephric or/and mesonephric ducts (Garret, 1942; Kaneko *et al.*, 1992 and Amemiya *et al.*, 2002). Stanniocalcin is a glycoprotein hormone first discovered in bony fish and mainly secreted from the Stannius corpuscles acts on gills, gut and kidneys to maintain calcium and phosphate homeostasis (Butkus *et al.*, 1987; Sundell *et al.*, 1992 and Lu *et al.*, 1994). The biochemical nature of calcium lowering hormone (stanniocalcin) which regulates calcium fluxes between the fish and it's environment was identified in different species of fishes (Lafeber *et al.*, 1988; Flik *et al.*, 1989 and Wagner & Friesen, 1989). Recently, stanniocalcin has been localized in different mammalian tissues including human tissues (Chang & Reddel, 1998; Luo *et al.*, 2004 and Song *et al.*, 2006). The human stanniocalcin revealed homology in the chemical structure to fish stanniocalcin and the data imply that this hormone regulates the female reproductive system (Mc-Cudden *et al.*, 2001; Ahmad *et al.*, 2002; Varghese *et al.*, 2002; Luo *et al.*, 2005 and Song *et al.*, 2006).

Although previous ultrastructure studies of the gland Stc of several teleosts demonstrated two structurally different cell types (Wendelaar Bonga & Pang, 1986; Chiba, 1990) though it is still unclear whether these reflect different physiological conditions of a single type or represent functionally different cell types. In order to collect more information on the cellular composition of Stc, we have investigated the histological and ultrastructure of the Stc in two teleostean fish, fresh water *O. niloticus* and marine fish *E. tuvina*. Ahmad *et al.* (2002) in their study on Heteropneustes fossils revealed only one cell type with large secretory granules and abundant rough endoplasmic reticulum.

The present study was designed to investigate the distribution, morphology, histology and ultrastructure of this endocrine gland (Stc) in two teleostean fish *O. niloticus* and *E. tuvina*.

Materials and Methods

Animals

Random fish samples of *O. niloticus* were collected alive from Faris fish farm in Dammam (Eastern province of Saudi Arabia) ranging from 5-35cm in total length and 20-625 g/fish in total body weight. Samples of *E. tuvina* fish were collected alive from the Arabian gulf ranging from 28-64cm in total length and 280-3100 g/fish in total body weight. The fish were sacrificed by a sharp knife blow to the head. Dorso-caudal part of the trunk kidney were prefixed in situ for 5-10 minutes in 10% neutral formalin or Bouin's fluid, then removed from body cavity and examined morphologically for the occurrence of Stc, then cut into small pieces and refixed for 24 hours, dehydrated through a graded series of ethanol, embedded in paraffin, cut at 3-5 μ m and stained by routine staining methods (Bancroft & Stevens, 1986). For transmission electron microscopy, the Stc from the kidney were excised and immersed in 4% glutaraldehyde buffered at pH 7.2 with cacodylate at 4°C. After several washes in cacodylate buffer the tissues were post fixed in 1% osmium tetroxide buffered to pH 7.4 with cacodylate. This was followed by dehydration in a graded series of ethanol and embedded in Epon. Ultra thin sections were stained with lead citrate and uranyl acetate (Hayat, 1989) and examined with Jeol Jem-100c \times II.

Results

In *O. niloticus*, Stannius corpuscles are small paired glands located on the dorsal side of the trunk kidney (Fig. 1). In small *O. niloticus* (less than 10cm in total length) they are scattered along the dorsal side of the trunk kidney (Fig. 2) they increased in number and size and became restricted to the dorso-caudal part of the trunk kidney with the increase in fish size. In *E. tuvina* the Stc were represented only by one large pair of organs located superficially on the dorso-caudal part of the trunk kidney (Fig. 3). Their number and location independent on sex or size of the fish. Stannius corpuscles location had no relationship to the position of the venous system and were either superficial or penetrate deeply into the excretory tissue of the trunk kidney.

Histological examination revealed that Stc in small fishes of *O. niloticus* consisted of undifferentiated cellular mass (Fig. 4) located close to the distal renal tubules and collecting duct and gradually increase in size and appeared as a cellular mass surrounded by a thin fibrous capsule which separated the cell from the surrounding renal tissue (Fig. 5 & 6). As with further increase in size, vascularized fibrous connective tissue septa penetrate from the capsule into the gland dividing the cellular mass into many interconnected lobules which is penetrated by sinusoids (Fig. 7). Cells of Stc within the lobule were arranged above thin plasma membrane with their apical surface facing a pseudo lumen

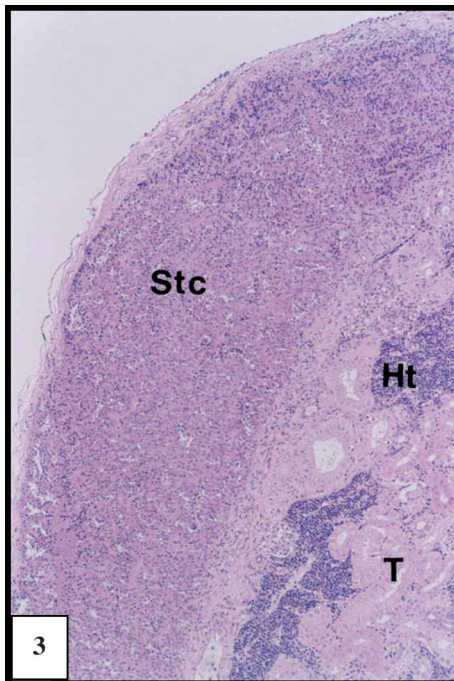
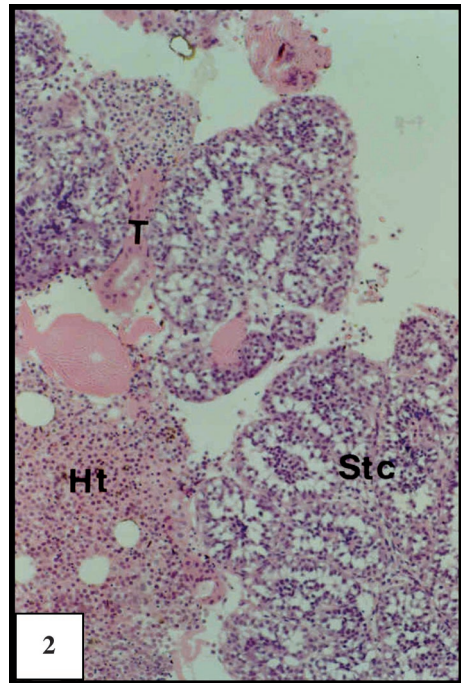
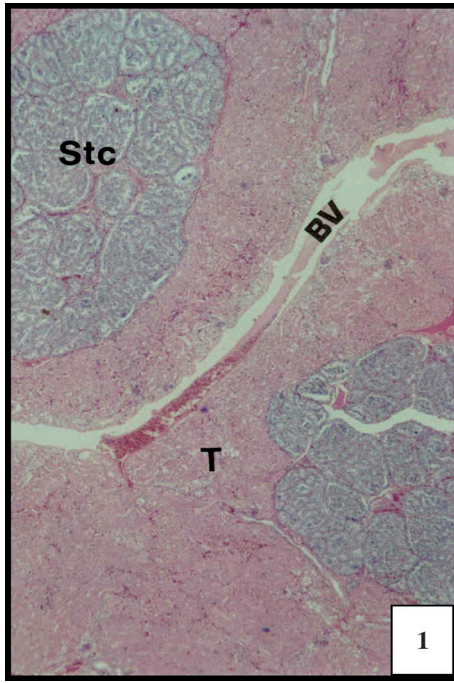


Fig. 1. Light micrograph of Stannius corpuscles (Stc) of large tilapia showing embedding of highly lobulated Stc in the trunk kidney. (BV) large blood vessel, (T) kidney tubules. H&E \times 320.

Fig. 2. Dorsal surface of the trunk kidney showing superficial position of (Stc) and their divisions into lobules. (Ht) haemopoietic tissue, (T) kidney tubules. H&E. \times 250.

Fig. 3. Stannius corpuscle (Stc) surrounded by a thick layer of collagen, (T) kidney tubules, and haemopoietic tissue (Ht). H&E \times 100.

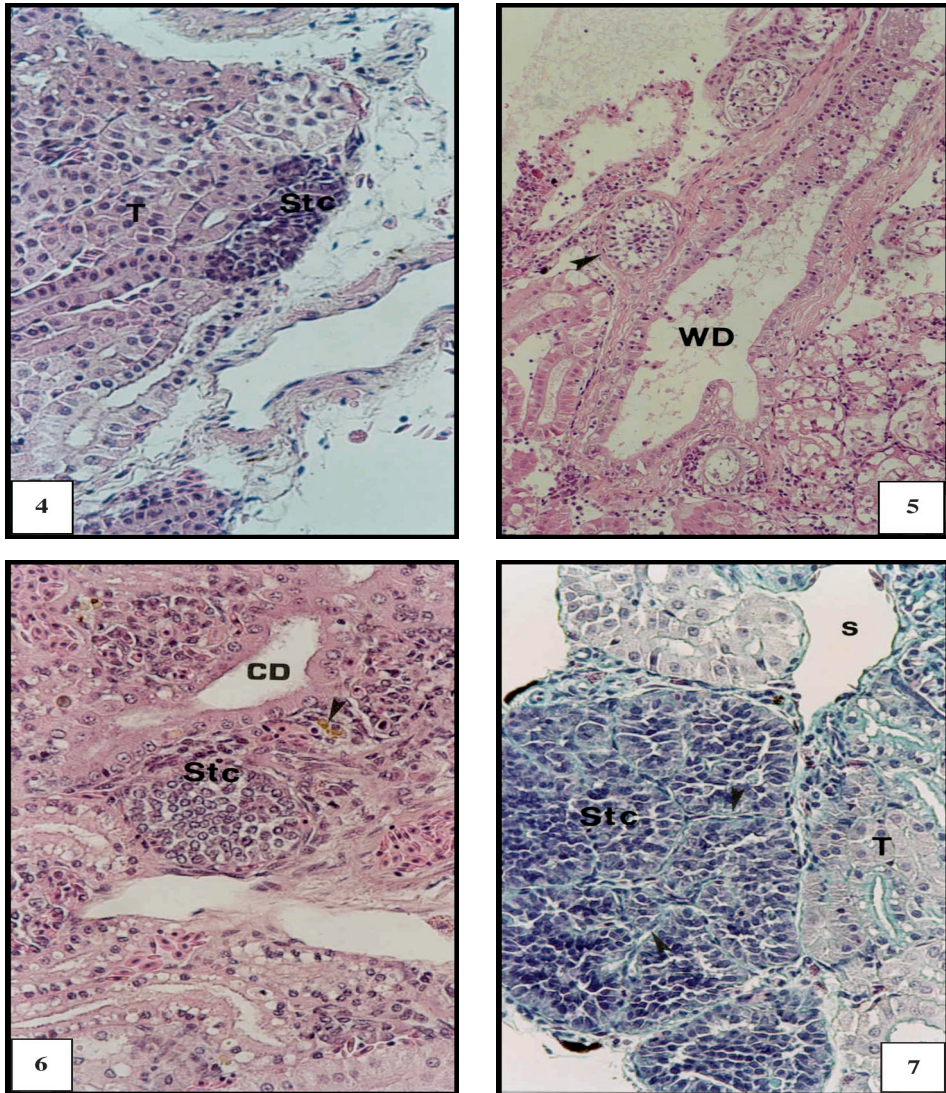


Fig. (4-7). Light micrographs of the trunk kidney in small tilapia showing developing of Stannius corpuscles (Stc):

4. First appearance of (Stc) as a compact mass of undifferentiated cells in contact with the distal portion of the kidney tubules (T). H&E. $\times 400$.
5. Encapsulation of Stc (arrow) within the connective tissue surrounding Wolffian duct (WD). H&E. $\times 250$.
6. Encapsulation of (Stc) and cell differentiation. Note the collecting duct (CD), melano-macrophage cells stain yellow brown (arrow). H&E. $\times 400$.
7. Encapsulation of (Stc) and start of lobulating by penetration of connective tissue (CT) and dividing the cellular mass into lobules. (T) kidney tubules, (S) sinusoid. MTS. $\times 400$.

(Fig. 8 & 9). A consistent feature of each corpuscle was the presence of associated kidney tubules. These kidney tubules were observed to connect to collecting tubules which in turn could be traced to the main excretory duct.

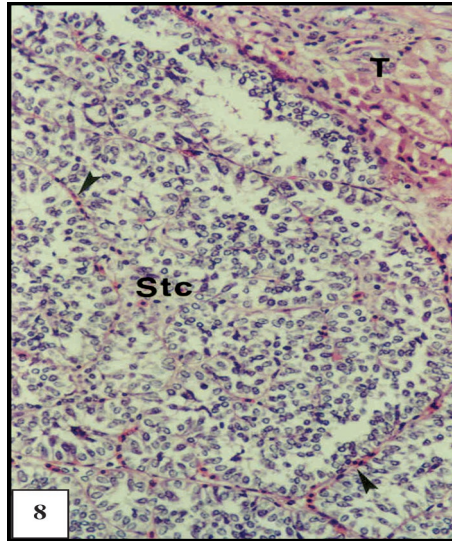


Fig. 8. High magnification from Fig. 1 shows the vascularized connective tissue (arrow) dividing Stc into partially connected lobules without true Lumina. (T) kidney tubules. $\times 400$.

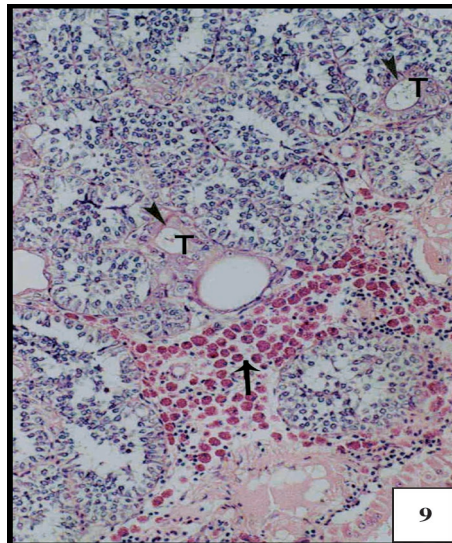


Fig. 9. Note, close association of Stc lobules with distal segments and collecting segments of kidney tubules (T). The lymphocytic and eosinophilic infiltration (arrow). H&E. $\times 250$. 1000.

Ultrastructural study has shown that the lobules were encapsulated in richly vascularized loose connective tissue and containing fenestrated capillaries (Fig. 10 & 11). The Stc cells were arranged as tightly packed cords and possessed variable cytoplasmic densities. At certain points the lateral plasma membranes of the neighboring cells were highly interdigitated. Two structurally different types of cells were clearly distinct in the gland. The predominant cell type (Type 1) was columnar and characterized by high electron density and possessed large homogenous electron-dense granules, large number of free ribosomes scattered in the cytoplasm, abundant flattened cisterns of ribosomal endoplasmic reticulum, Large lysosomes and moderate number of small mitochondria, vesicles and vacuoles (Fig. 12 & 13). Small secretory vesicles were occasionally seen exocytosed into the surrounding capillaries (Fig. 11).

The type-2-cells were fewer and lighter than type-1- cells and tended to be scattered among type -1-cells. Cytoplasmic processes were seen extending into type 1 cells (Fig. 12). The cytoplasm contained fewer and smaller secretory granules than type 1 cells, extensive array of ribosomal endoplasmic reticulum, smooth endoplasmic reticulum, free ribosome and the cells have irregular nuclei with granular chromatin.

In *E. tuvina*, the lobules were encapsulated in highly vascularized connective tissue which contained neuro-vascular elements, fibroblasts, and fibrocytes (Fig. 14). The blood vessels were occasionally associated with unmyelinated nerve fibers containing microfilaments, small clear vesicles, dense cored vesicles and mitochondria (Fig. 15). The capillaries possessed flattened or cuboidal epithelium which bulged into the lumen with numerous fenestrations (Fig. 16). Neurofilaments were emerged into the plasma membrane away from blood vessels (Fig. 17). The gland cells were tightly compact and varied in electron density. The lateral plasma membranes of the adjacent cells were interdigitated at some points leaving narrow intercellular spaces (Fig. 14 & 16).

There were two main types of secretory cells in *E. tuvina* gland. The predominant cell type (Type 1) which was observed as pleomorphic dark cells characterized by a large euchromatic nucleus possessed a rim of heterochromatin and dispersed small blocks and a prominent nucleolus. The cytoplasm possessed numerous large, dense, membrane bound granules, numerous free ribosomes and glycogen. The ribosomal endoplasmic reticulum cisterns were found in flattened short form scattered in the cytoplasm. Small elongated mitochondria with tubulo-lamellar cristae were noticed around the nucleus (Fig. 18). The amount of secretory granules varies from cell to another. Nucleus is ovoid with irregular surface. Some secretory cells showing picnotic nucleus with a paucity of organelles was observed. These may represent degenerating gland cells in the process of apoptosis.

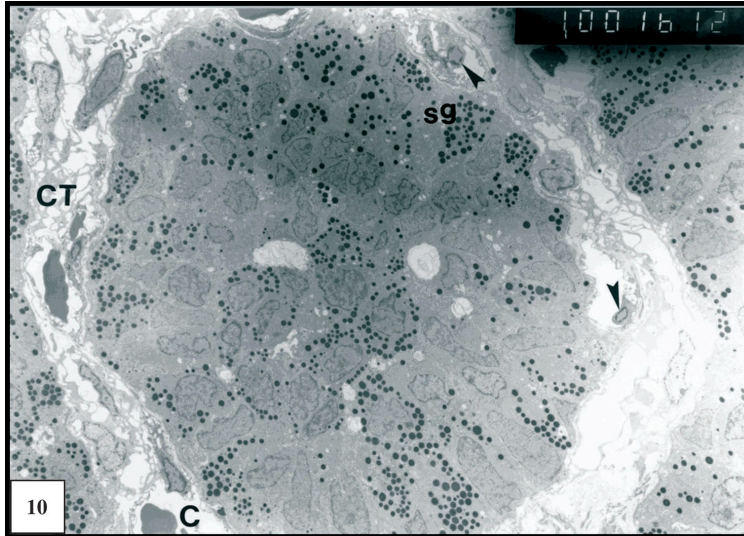


Fig. 10. Electron micrographs of Stannius corpuscles (Stc) at the trunk kidney of *O. niloticus* showing (Fig. 10-13): A lobule of Stc surrounded by highly vascularized loose connective tissue (CT). Note accumulation of secretory granules (sg) in the lobular cells. (C) capillaries, (arrow) endothelial cell. $\times 1000$.

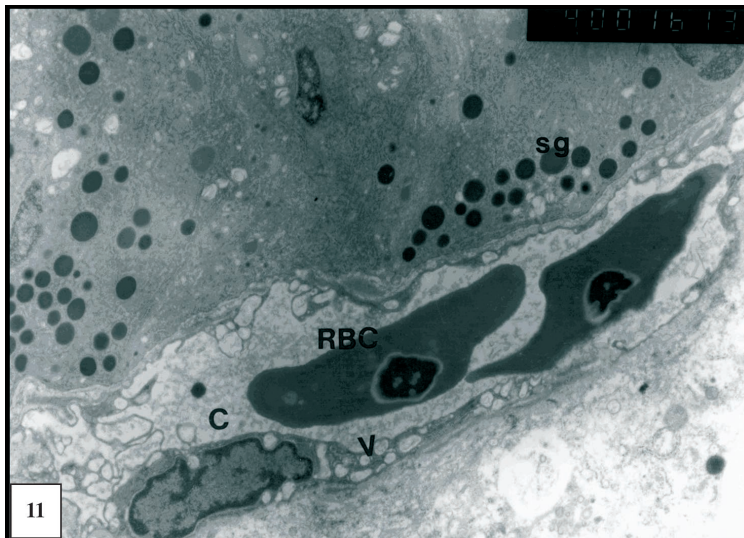


Fig. 11. High magnification from Fig. 10 showing a wide capillary (C) surrounds the gland cells. Note the pinocytotic vesicles (V), red blood cells (RBCs) and secretory granule (sg) within the lumen of the capillary. $\times 4000$.

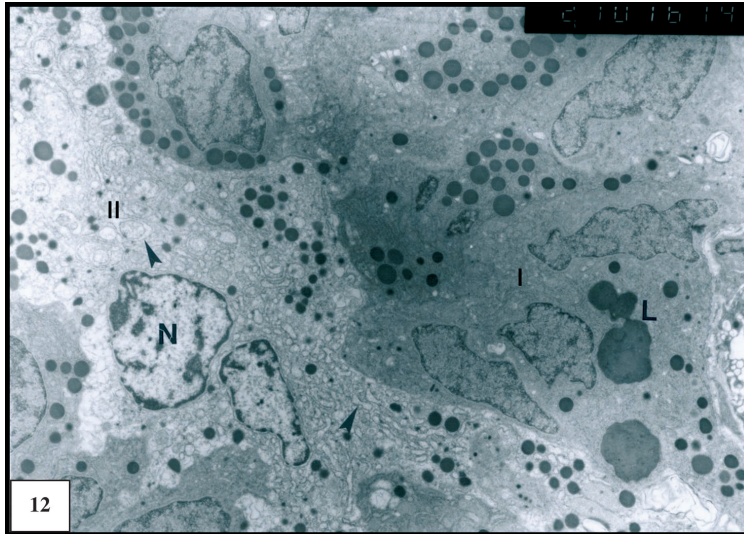


Fig. 12. Two types of Stc cells containing secretory granules. The type I cells are dark and high columnar, light cells (type II) intervene and project between the dark cells. Note, the extensive network of dilated smooth endoplasmic reticulum and ribosomal endoplasmic reticulum in the light cell (arrow), large lysosomes (L), euchromatic nucleus (N). $\times 2700$.

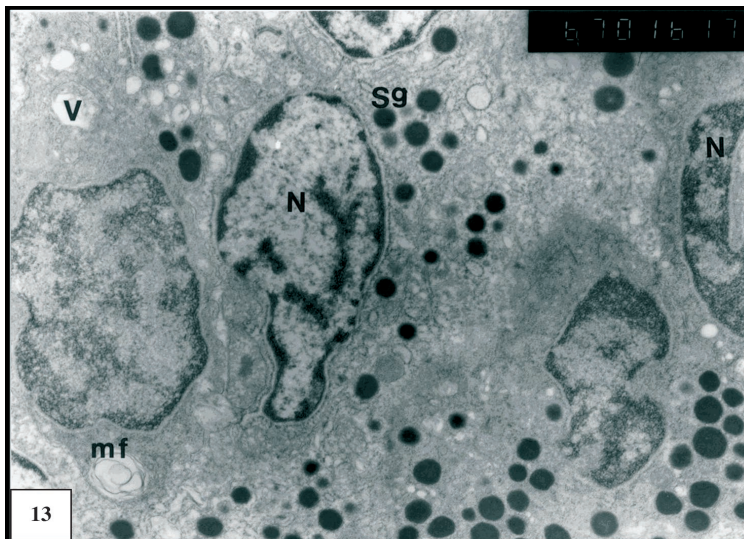


Fig. 13. Stc cells are intermingled and closely packed. Note polymorphism in the nuclei (N), vesicle containing myelin figure (mf), secretory granules (sg). $\times 6700$.

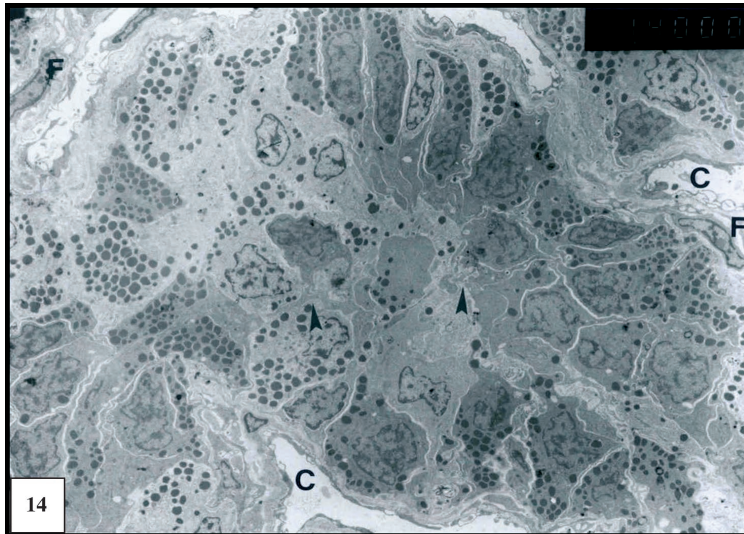
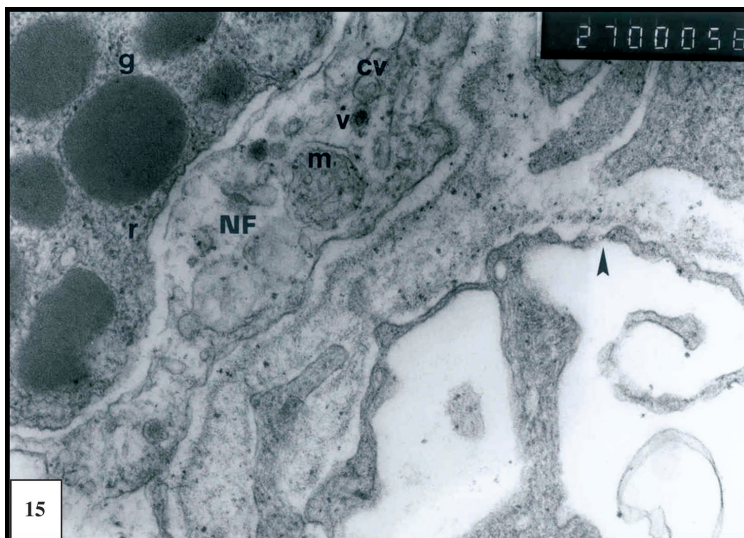


Fig. 14. Electron micrographs of Stannius corpuscles (Stc) in the trunk kidney of *E. tuvina* (Fig. 14-20):

Stc lobules interconnected with each other by vascularized connective tissue. Pseudo stratified epithelium lining the lobules. Note wide capillaries (C) surrounding the lobules, pseudo-lobular lumen, fibroblast (F). Note also the interdigitated plasma membrane (arrow). $\times 1400$.



Figs. 15. High magnification of the Stc shows fenestrated capillary endothelium (arrow), nerve fibers (NF) containing micro-filaments (mf), mitochondria (m), small clear vesicles (cv), dense core vesicles (v). Note the membrane bound dense granules in the dark cell (g) and numerous free ribosomes (r). $\times 27000$.

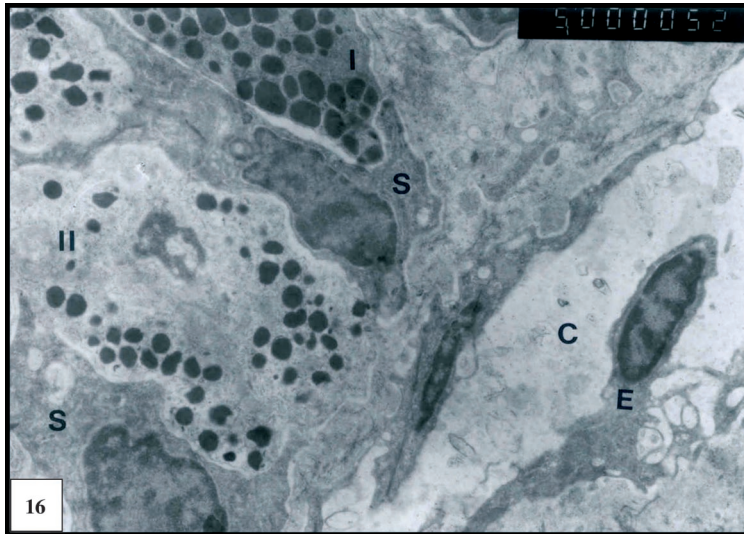


Fig. 16. High magnification of Stc lobule showing two types of cells with numerous dense granules; dark cell (type I) and light cell (type II). A third type with basal large nuclei, long cytoplasmic processes devoided of granules surrounds the light cell (S). Note endothelial cell nuclei (E) bulged inside the capillary lumen. $\times 5000$.

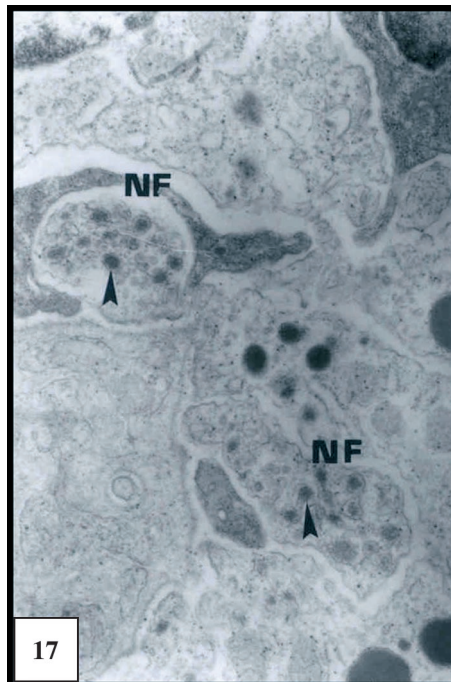


Fig. 17. Unmyelinated nerve fibers embedded in the Stc cells contain large dense core membrane bound granules and small vesicles (arrow). $\times 10000$.

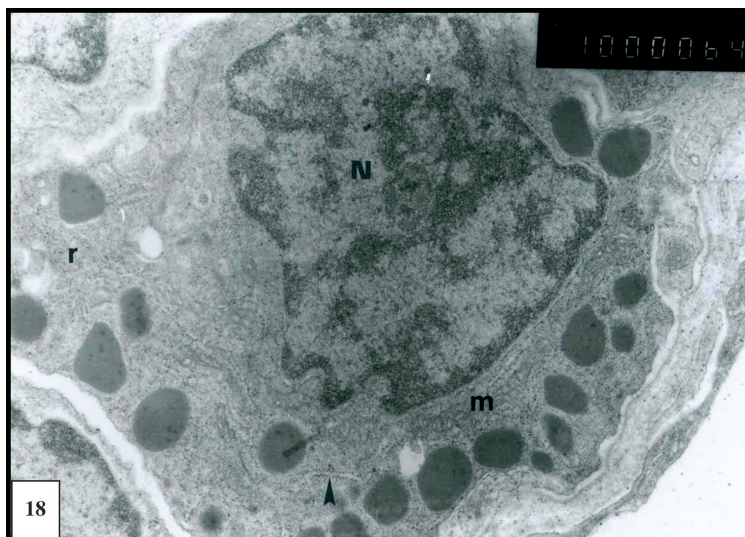


Fig. 18. Dark cell with large nucleus (N) and prominent nucleolus, large secretory granules, elongated mitochondria (m) with lamellated cristae, short flattened cisternae of ribosomal endoplasmic reticulum (arrow) scattered through the cytoplasm, free ribosomes and poly ribosomes (r). $\times 10000$.

The other type of cells (Type 2) was light with irregular outline, contains large nucleus. The cytoplasm possessed dilated cisterns of ribosomal endoplasmic reticulum, secretory homogenous granules with different size and density, mitochondria and well developed Golgi areas with saccules containing electron dense materials (Fig. 19 & 20).

Another type of cells was noticed which characterized by the absence of secretory granules, high electron dense cytoplasm and large basal nucleus (Fig. 16). Ribosomal endoplasmic reticulum and smooth small vesicles were seen. These cells possessed a long apical and basal cytoplasmic projections intervene between the gland cells. This type of cells may represent support cells.

Discussion

The present study demonstrated that Stc of both species *O. niloticus* and *E. tuvina* are located in the posterior part of the trunk kidney as in most teleosts (Krishnamurthy & Bern, 1969; Tomasulo *et al.*, 1970; Cohen *et al.*, 1975 and Oguri, 1966), found in large numbers and small size in *O. niloticus* and distributed throughout the trunk kidney in small fishes (less than 10cm in total length) while the distribution reduced to the doso-caudal part of the trunk kidney in adult fishes (more than 30cm in total length). While in adult *E. tuvina*, Stc were found as a pair of white corpuscles at the caudal part of the trunk kidney. Previ-

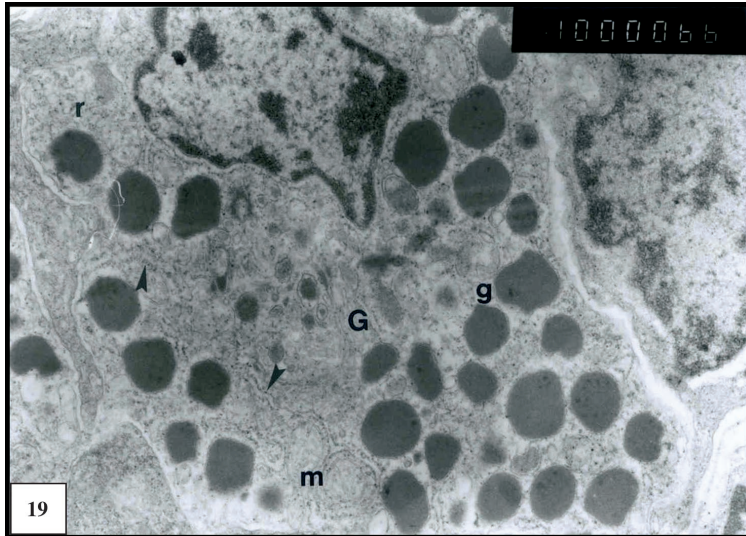


Fig. 19. Light cell with light cytoplasm, numerous dense membranes bound granules, ribosomal endoplasmic reticulum scattered in the cytoplasm (arrow), Golgi (G), mitochondria (m) and free ribosomes (r). $\times 10000$.

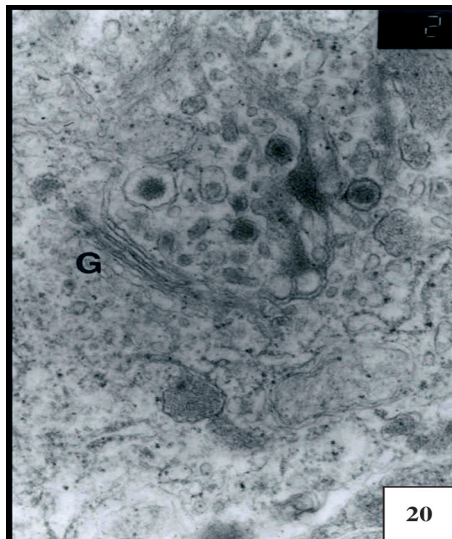


Fig. 20. High magnification of Fig. 19 showing dilated cisternae of Golgi (G) and the secretory vesicles. $\times 20000$.

ous study mentioned that variations in number and distribution of the Stc among fish species may have a phylogenetic significance (Bauchot, 1953 and Wendelaar Bonga & Pang, 1986). Embryological evidence shows that Stc arise as buds from the pronephric or/and mesonephric ducts during their embryonic stages in many species (Garret, 1942; Krishnamurthy, 1969; Belsare, 1973 and Amemiya *et al.*, 2002). In primitive fish, the number of Stc is likely to be high and often located in the middle of the mesonephric kidneys as in *O. niloticus*, while in more advanced fishes the number is generally reduced to one pair of relatively large bodies located in the posterior part of the mesonephric kidney as in group-er. However, it was reported in two teleosts *Gambusia affinis* and *Zeus faber* that Stc were separated from trunk kidney and closely associated with the uterus or mesonephric ducts (Bauchot, 1953).

The Stanniocalcin was originally discovered in fish and was characterized as hypocalcemic hormone to eliminate excess calcium (Wendelaar Bonga *et al.*, 1989 and Amemiya *et al.*, 2002). However, mammalian Stanniocalcin 1 (STC1) was found to have a paracrine action (Luo *et al.*, 2004), but the physiological role of Stanniocalcin 2 (STC2) is unclear (Luo *et al.*, 2005).

The secretory cells within Stc of the studied fish were arranged in lobules, surrounded by a fibrous capsule and possessed a fine structure similar to Stc of other teleosts (Fujita & Homna, 1967; Cohen *et al.*, 1975 and Youson *et al.*, 1976). They were characterized by the presence of abundant rough endoplasmic reticulum, secretory granules and well developed Golgi, which suggesting their role in the protein secretion.

The ultrastructure study demonstrated variable appearance of cells within the Stannius corpuscles suggested a variable degree of metabolic or secretory activity. The cell variations within the Stannius corpuscles was mentioned in certain anadromous teleosts in their fresh water spawning migration (Carpenter & Heyl, 1974) and fishes in calcium deficient seawater (Cohen *et al.*, 1975) or deionized water (Tomasulo *et al.*, 1970) which reflect the response of Stc cells to environmental changes. Experimental evidence indicated that the Stannius corpuscle are endocrine organs secrete hypocalcin which lowers blood calcium levels by unknown mechanism (Wendelaar Bonga *et al.*, 1989 and Wendelaar Bonga & Pang 1991), which show that Stc may be important for teleosts adapted to calcium rich seawater.

Previous investigation indicated that Stc of teleosts residing entirely in fresh water or spending part of their life cycle in freshwater (euryhaline) contain heterogeneous populations of gland cells (*Salmo gairdneri*, Meats *et al.*, 1978, *Fundulus heteroclitus* and *Carassius auratus*, Wendelaar Bonga *et al.*, 1980, and *Oreochromis mossambicus*, Urasa & Bonga, 1987). On contrary, Ahmed *et*

al. (2002) found that Stc of fresh water teleosts of *Heteropneustes fossilis* contain a homogenous cell population similar to those type I cells of other freshwater teleosts which considered to be a source of stanniocalcin. Previous findings of Wendelaar Bonga *et al.* (1976) showed that type 2 cells appear to be active in media of low ionic and osmotic strength.

Previous studies of seawater fishes *Gadus morhua* and *Pleuronectes platessa* (Wendelaar Bonga & Grevens, 1975) and *Opsanus tau* (Bhattacharyya & Buttlar, 1978) confirm the presence of only one type cells similar to Type I in Stc of freshwater teleosts. Many investigators believe that type-I and type-II cells are structurally different forms of one functional type and are responsible for producing the same hormone (Lopez *et al.*, 1984; Lafeber & Perry, 1988; Flik *et al.*, 1989 and Wendelaar Bonga *et al.*, 1989).

The present ultrastructure study confirmed two types of secretory cells in Stc of freshwater fish *O. niloticus* and saltwater fish *E. tuvina*. Type I cells numerically predominate in *E. tuvina* and characterized by high electron density, dense and large secretory granules, abundant free ribosomes, moderately developed lamellar ribosomal endoplasmic reticulum, small mitochondria and long cytoplasmic projections extending between type II cells. Whereas type II cells characterized by low electron density, few and small secretory granules, extensive network of ribosomal endoplasmic reticulum scattered in the cytoplasm, large mitochondria with light matrix and few tubular cristae and well developed Golgi. Interestingly, the present ultrastructure study on *E. tuvina* demonstrated the presence of a third type of cells depleted of secretory granules and possessed a high electron dense cytoplasm. These cells have long apical and basal cytoplasmic projections extending between the two types of secretory cells. This type may represent supporting cells.

The present study provided ultrastructural data to show that type I and type II cells release their secretory products into the intercellular spaces by exocytosis. Evidence of exocytosis has been reported in the Stc of other species as well (Aida *et al.*, 1980). By using the tannic acid method, Wendelaar Bonga and Pang (1986) successfully demonstrated exocytosis in type-I cells of the Stc immersed in a medium containing a high calcium concentration. It has been well documented that a high calcium concentration, in either the internal or external media, stimulates the release of secretory products from the Stc (Wendelaar Bonga & Pang, 1986).

In accord with previous findings (Bhattacharyya & Butler, 1978 and Wendelaar Bonga & Pang, 1991) the present study mainly in *E. tuvina* demonstrated that Stc were innervated by unmyelinated nerve fibers, which occurred largely in the areas adjacent to the vascular walls in the interlobular connective tissue,

as previously shown in other species. The neural actions were primarily on the blood vessels, and that the actions on the gland cells, if they occur, are indirect, *i.e.*, mediated by the local circulation or vascular mechanism (Bhattacharya & Butler, 1978 and Chiba, 1990).

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دلائل مورفولوجية ونسجية وتركيبية دقيقة على كريات ستانيوس في أسماك المياه العذبة (البطي) وأسماك المياه المالحة (الهامور)

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المستخلص. تم في هذا البحث دراسة الشكل الظاهري والتركيب النسيجي والدقيق لكريات ستانيوس في كل من أسماك المياه العذبة (البطي) وأسماك المياه المالحة (الهامور). ولقد وجد أن كريات ستانيوس تكون في صورة أزواج من الكريات البيضاء وبأعداد كبيرة في سمك البطي، ومنتشرة على طول جذع الكلية في الأسماك الصغيرة، بينما انحصرتواجدها في الجزء الخلفي في الأسماك الكبيرة. واحتوت أسماك الهامور على زوج واحد من كريات ستانيوس كبيرة الحجم تقع في الجزء الخلفي من جذع الكلية، وتكون عادة متصلة بالجزء البعيد من النفرون. كما ظهرت كريات ستانيوس في صورة أزواج غير متميزة ومتصلة بالجزء الخلفي من الأنبيبات البولية في أسماك البطي الصغيرة، ومع نمو الأسماك ظهرت كريات ستانيوس محاطة بمحفظة ليفية نفذت إلى الداخل لتقسمها إلى فصوص من الخلايا الإفرازية.

أوضح الفحص المجهرى الدقيق وجود نوعين من الخلايا الإفرازية في كريات ستانيوس في كلا نوعي الأسماك. النوع الأول وهو السائد يحتوي على حبيبات إفرازية كبيرة والعديد من الرايوسومات الحرة والليسوسومات والشبكة الإندوبلازمية الخشنة، أما النوع الثاني من الخلايا فهو يحتوي على حبيبات إفرازية صغيرة وشبكة إندوبلازمية كثيفة خشنة وملساء وجهاز جولجي متميز مما يقترح دور (الغدة) كريات ستانيوس في تصنيع البروتين. ومن الجدير بالذكر أن كريات ستانيوس في

أسماك الهامور البحرية احتوت على نوع آخر من الخلايا تميزت بكثافة إلكترونية عالية ولم يظهر بها حبيبات إفرازية وظهر بها زوائد سيتوبلازمية امتدت بين الخلايا الأساسية وهي ربما تمثل خلايا دعامية. كما استقبلت خلايا الغدة إمداداً عصياً بألياف عصبية غير ميلينية.