Stannius corpuscles and plasma calcium levels during the reproductive cycle in the cichlid teleost fish, *Oreochromis mossambicus*

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**Summary.** The corpuscles of Stannius (CS) of the cichlid *Oreochromis mossambicus* (formerly *Sarotherodon mossambicus*) were studied in relation to sexual maturation and plasma calcium levels. After sexual maturation, the CS are enlarged in female fish, because of an increase in size and number of the type-1 cells. During the ovarian cycle, the size of the CS increases in parallel with the growth of the ovaries. Concurrently, plasma total calcium increases markedly until spawning. This increase is mainly accounted for by calcium bound to proteins (vitelligenins), but the ultrafiltrable calcium fraction is also slightly higher than in males. Ovariectomy is followed by a reduction in the size of the CS, mainly a result of involution of the type-1 cells, and by a reduction in plasma calcium to levels typical for males. Gonadectomy in males does not affect size or ultrastructure of the CS, or plasma calcium levels. Since the type-1 cells of the CS are the presumptive source of a hypocalcemic hormone, we conclude that activation of the CS during the female reproductive cycle is a response to elevated calcium levels that accompany ovarian maturation. We suggest that the CS respond in particular to the elevated ultrafiltrable or ionic calcium levels.

**Keywords:** Stannius corpuscles – Reproductive cycle – Teleost fish – Blood calcium

The corpuscles of Stannius (CS) are small endocrine glands associated with the kidneys in holostean and teleostean fish. In teleosts usually one pair is present, although in some species up to ten CS may occur (Krishnamurthy 1976). Recently it has been suggested that the CS might be homologous to parathyroid glands of terrestrial vertebrates (Milet et al. 1980; Lopez et al. 1984). The CS have been implicated in the endocrine control of calcium metabolism (Fontaine 1964; Pang and Pang 1974), as have the parathyroids (Aurbach 1976). Unlike the latter glands, of which the function in hypercalcemic regulation has been well-defined (Aurbach 1976), the function of the CS has not been fully elucidated. In addition to a role in calcium regulation, the CS may have a special function during gonadal maturation (Subhedar and Rao 1979).

Many authors have attributed a hypocalcemic function to the hormonal products released by the CS. Removal of the glands (stanniectomy) results in a distinct hypercalcemia (Fontaine 1964; Pang et al. 1973; Wendelaar Bonga and Greven 1978; Fenwick and Wendelaar Bonga 1982), whereas injection of homogenates of the glands can reduce the hypercalcemia in stanniectomized fish (Pang 1973; Pang et al. 1973; Dubewar and Suryawanshi 1978; So and Fenwick 1977; Kenyon et al. 1980) or induce hypocalcemia in intact fish (Pang and Pang 1974; Swarup and Srivastava 1982; Laféber et al. 1984).

For some fish species, it has been reported that the CS become activated during gonadal maturation, mostly markedly in female fish (Lopez 1969; Hiroi 1970; Subhedar and Rao 1979). The high CS activity during reproduction has been connected with the changes in calcium metabolism that may accompany gonadal maturation (Subhedar and Rao 1979). Plasma calcium levels are generally raised considerably during gonadal growth in females (Oguri and Takada 1967; Pang 1973; Van Bohemen and Lambert 1982) and occasionally also in males (Balbontín et al. 1978). However, correlative investigations concerning CS structure and plasma calcium levels during gonadal maturation are necessary to explore this possibility. An alternative explanation could be that the CS stimulate gonad development (Hiroi 1970). Both hypotheses may be compatible, however, since in the CS of several fish species two types of secretory cells have been described (Krishnamurthy and Bern 1969; Wendelaar Bonga et al. 1977; Meats et al. 1978). The predominant cell type of the CS (type-1 cells) responds to changes in the calcium concentration of the ambient water and this cell type may produce the hypocalcemic factor of the CS (Meats et al. 1978; Wendelaar Bonga and Greven 1978; Wendelaar Bonga et al. 1980). The function of the second cell type (type-2 cells) is unknown (Olivereau and Olivereau 1978; Wendelaar Bonga et al. 1980). Whether the activation of the CS during gonadal maturation is a result of stimulation of the type-1 cells, the type-2 cells, or both cell types is also unknown.

This paper forms the first part of a study concerning calcium metabolism during reproduction in the freshwater cichlid *Oreochromis mossambicus* Trewavas (formerly *Sarotherodon mossambicus*). The effects of gonadal maturation on CS structure and plasma calcium have been investigated in order to elucidate whether a correlation exists between CS activity and plasma calcium levels in the reproductive period, and to determine which of the cell types of the CS is stimulated. In addition the effect of gonadectomy
on the CS has been examined. If some factor produced by the CS has gonad-stimulating activity, the CS may become activated if the gonads are removed, by analogy with the activation of the gonadotrophic cells of the pituitary gland. If the high CS activity during gonadal maturation is a response to high plasma calcium levels, then ovariectomy may be followed by a reduction in the CS as soon as the plasma calcium concentration has returned to normal values.

Materials and methods
Laboratory stocks of Oreochromis (formerly Sarotherodon) mossambicus (tilapia) were used. The fish were kept at 25°C in 100 l tanks with tap water (2.1 mM Na⁺; 0.5 mM Cl⁻; 0.4 mM SO₄²⁻; 0.8 mM Ca²⁺). Males and females were kept together (about 10 fish per tank).

General procedures. The fish were anesthetized lightly in MS 222. Body weights were determined and the blood collected from the caudal vessels after cutting the caudal peduncle. Plasma was connected by centrifugation. Part of it was ultrafiltered using a Sartorius Membranfilter (membrane cut-off: 10000 Da). Total and ultrafiltrable plasma calcium concentrations were determined by a colorimetric technique with cresalphthalein complexone (Sigma Diagnostics) in a Zeiss PMQ 3 spectrophotometer. Ovaries were dissected, weighed, and the gonadosomatic index (GSI) determined: GSI = ovary weight · body weight⁻¹ · 100. The CS were exposed and the long (l) and short (s) axis of the two glands were measured under a dissecting microscope equipped with an eye-piece micrometer (Olympus OSM: final magnification: 40x). CS size was expressed as CS-index (CSI): CSI = (l₁ + s₁)·(l₂ + s₂)·4⁻¹ (subscripts 1 and 2 refer to each of the two bodies).

Electron microscopy. The CS were prefixed in 3% glutaraldehyde in 0.1 N cacodylate buffer, pH 7.4, for 10–15 min at room temperature. They were then fixed in a solution containing 2% osmium tetroxide, 1.5% glutaraldehyde and 2.5% potassium dichromate in 0.1 N cacodylate buffer, pH 7.4, for 1 h at 0°C. Postfixation followed in 2% uranyl acetate. After dehydration in a graded series of ethanol the tissues were embedded in Spurr's resin. Ultrathin sections were poststained with Reynolds's lead citrate and examined in a Philips EM 200 electron microscope.

CSI and body weight. For determination of the relationship between the size of the CS and body weight, male and female fish were used, varying in body weight between 1.5 and 35 g. The fish were taken randomly from the labora-
Figs. 2, 3. Electron micrographs of Stannius corpuscles of sexually mature fish; Fig. 2: female; Fig. 3: male; t-1 type-1 cells; t-2 type-2 cells; ger granular endoplasmic reticulum; cross sections of the upper-middle parts of the cells in a plane parallel to the basement membrane; note the prominence of the granular endoplasmic reticulum in the cells of the female. × 11000

History stock, and the sample therefore contained female fish at all stages of ovarian maturation.

Ultrastuctural morphometry. The CS of 5 female and 5 male fish (10–12 g body weight) were prepared for electron microscopy. Micrographs at a final magnification of 3000, and representing a sampling area of 500 μm² per cell type per fish, were morphometrically analysed with Kontron Digiplan equipment. The following parameters were determined for type-1 and type-2 cells:

1. Cell area; cells were selected that had been sectioned along their long axis (perpendicular to the basal lamina) and that contained a profile of the nucleus; about 25 cells per cell-type per fish were measured; these cells were also used for determining the other parameters;
2. nuclear area;
3. cytoplasmic area occupied by granular endoplasmic reticulum;
4. cytoplasmic area covered by Golgi areas (Golgi sacules and surrounding area with presecretory granules and vesicles);
5. cytoplasmic area occupied by mitochondria.

Parameters 3 to 5 were expressed as a percentage of the total cytoplasmic sampling area; this is equivalent to the fractional cytoplasmic volume of the organelles concerned.

Gonadectomy. Female fish of about 10 g body weight, and males of about 15 g body weight, were pre-adapted to tap water containing 5 g·1⁻¹ NaCl, 5–7 days before operation. They were kept in the same water after gonadectomy or sham operation, in order to minimize osmotic stress produced by the operation. No effects on plasma calcium, CSI or GSI were observed that might have been caused by the saline. After light anesthesia with MS-222, the gonads were removed through an incision made in the ventro-lateral body wall; this incision was sutured afterwards. Control fish were sham-operated by making similar incisions and sutures. Ten days later the fish were disected. When gonad remnants weighing more than 5% of the original total gonad weight were found at the time of dissection, the fish were discarded. Most of the fish showed no noticeable gonad remnants.

Results
The CS in tilapia are paired flattened oval bodies located on the ventrocaudal surface of the kidneys. They consist of lobes of gland cells, separated by strands of connective tissue that contain blood vessels and nerve tracts. Within the lobes, two cell types are present, type-1 and type-2 cells (Figs. 1–3); these differ in the size of their secretory granules
Fig. 4. Relationship between the size of the corpuscles of Stannius, expressed as the CSI, and body weight in female and male fish and in sexually immature fish; best fitting curve (Ralston and Jenrich 1979) for females: \( y = 435.7 - 425.9e^{-0.100x} \); best fitting curve for males: \( y = 300.9 - 253.5e^{-0.105x} \).

**Table 1.** Morphometric analysis of electron micrographs of CS of sexually mature female (GSI: 4.6-7.8) and male *O. mossambicus* (means ± S.D.; \( n = 5 \))

<table>
<thead>
<tr>
<th>Type-1 cells</th>
<th>Type-2 cells</th>
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<tr>
<td></td>
<td>females</td>
</tr>
<tr>
<td><strong>Cell area</strong></td>
<td></td>
</tr>
<tr>
<td>(( \mu m^2 ))</td>
<td>29.80 ± 3.42*</td>
</tr>
<tr>
<td><strong>Nuclear area</strong></td>
<td>10.37 ± 2.42</td>
</tr>
<tr>
<td>(( \mu m^2 ))</td>
<td></td>
</tr>
<tr>
<td><strong>Mitochondria</strong></td>
<td>3.90 ± 0.95</td>
</tr>
<tr>
<td>(vol. %)</td>
<td></td>
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<tr>
<td><strong>Golgi area</strong></td>
<td>5.13 ± 1.11</td>
</tr>
<tr>
<td>(vol. %)</td>
<td></td>
</tr>
<tr>
<td><strong>Granular ER</strong></td>
<td>17.11 ± 1.96*</td>
</tr>
<tr>
<td>(vol. %)</td>
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* Significantly different from males, \( P < 0.01 \)

and in organization and relative abundance of the granular endoplasmic reticulum. The predominant type-1 cells have large secretory granules and contain large arrays of granular endoplasmic reticulum, concentrated in the middle and apical parts of the cytoplasm, especially in female fish. Type-2 cells have small granules and show small isolated strands of granular endoplasmic reticulum distributed evenly in the cytoplasm. Golgi areas are well developed in both cell types.

1. **CS structure and sexual maturation**

The size of the CS, as reflected by the CSI, increases with body weight in a sex-related fashion (Fig. 4). In fish of less than 6 g, without secondary sexual characteristics, the
Fig. 8. Total plasma calcium and CSI in sham-operated fish (controls) and gonadectomized fish: females; n = 10 (gonadectomized); n = 9 (controls); males: n = 11 (gonadectomized and controls), means ± S.D.

### Discussion

1. **CS and sexual maturation**

In sexually mature *O. mossambicus* the CS are considerably larger in females than in males. This structural difference probably reflects a difference in secretory activity between the sexes. Our studies in vitro have shown that the rate of synthesis of secretory proteins, as determined by incubation of freshly-isolated glands in the presence of tritiated amino acids, is considerably higher in CS from female fish than in those of male fish (in preparation). The sexual difference in CS size becomes evident as soon as sexual maturity is attained. The larger size in female fish is apparently related to reproduction, since ovariectomy reduces the size of the CS of females to that of males. There is no evidence for a reproduction-related function of the CS in male *O. mossambicus*.

A sex difference similar to that in *O. mossambicus* has been described in the catfish *Clarias batrachus* (Srivastava and Swarup, personal communication) and in the mullet *Mugil cephalus* (Johnson 1972). In both species the CS cells are activated during gonad maturation in females only. The Chilean clingfish, *Stichaeus sanguineus*, shows a sexual difference in the number of corpuscles: two in males, and three to four in females (Galli-Gallardo et al. 1977). In the catfish *Heteropneustes fossilis* (Subhedar and Rao 1979) and the Atlantic salmon, *Salmo salar* (Lopez 1969), the CS cells are stimulated in both males and females. In the latter species the CS are activated to a greater extent in males. In the salmon *Oncorhynchus keta* caught at sea during the spawning migration the CS are enlarged in fish with maturating gonads. After the fish entered freshwater, the CS cells regress, even though the gonads are still maturing. Similarly, Heyl (1970) has observed a progressive degeneration of the CS of Atlantic salmon during their spawning migration in freshwater. This regression may indicate that the inhibitory effects on the CS of transfer from seawater to freshwater are more marked than the stimulation that accompanies gonad maturation.

In *Mugil cephalus* and *Heteropneustes fossilis* (Johnson 1972; Subhedar and Rao 1979), the CS are progressively activated during ovarian maturation, and show regression after spawning. Although in *O. mossambicus* the CS are enlarged in all stages of the ovarian cycle when compared to the CS of males, we have observed slight changes in CS size that are positively correlated with the ovariometric index. Thus, the secretory activity of the CS in female *O. mossambicus* is related to ovarian maturation. Full regres-
Fig. 9. Electron micrograph of a Stannius corpuscle of an ovarioctomized fish; the granular endoplasmic reticulum (ger) is reduced; t-1 type-1 cells; t-2 type-2 cells. x 10000

Fig. 10. Electron micrograph of Stannius corpuscle of sham-operated female fish; t-1 type-1 cells; t-2 type-2 cells; ger granular endoplasmic reticulum. x 10000
gonad-stimulating activity, this should also have occurred in males. Although stimulation of the CS in sexually mature females after spawning may be prevented by the rapid succession of the ovarian cycles.

The CS of O. mossambicus contain two cell types. They have the structural characteristics of the type-1 and type-2 cells that we have described earlier in the CS of the stickleback Gasterosteus aculeatus (Wendelaar Bonga et al. 1977), the killifish Fundulus heteroclitus, and goldfish, Carassius auratus (Wendelaar Bonga et al. 1980). Lopez et al. (1984) have recently suggested that type-2 cells may represent a stage in the secretory cycle of type-1 cells. Because of the structural distinctness of the type-2 cells, underlined by the present morphometrical results, we do not consider this explanation likely for O. mossambicus.

From our ultrastructural data we conclude that the sexual difference in CS size in O. mossambicus is mainly produced by type-1 cells. The type-1 cells of the CS in females are larger by 50%, and since the volume of the CS of females is almost twice that of males, these cells are also more numerous than in males. The reduction in size of the CS that follows ovariectomy is probably a result of the regression of type-1 cells, since a reduction in the granular endoplasmic reticulum and an increase in the number of lysosomes is observed in type-1 cells only. Ovariectomy does not affect type-2 cells. Our morphometric data show that the development of type-2 cells is similar in females and males, and thus there is no evidence for a specific function of these cells during reproduction.

2. CS and plasma calcium levels

Why do the CS become activated during the reproductive period in female fish? It could be argued that the CS stimulate the growth of the ovaries. If the CS produce a gonad-stimulating factor, one might expect hyperactivity of the CS after removal of the ovaries. However, we observe involution of the CS in gonadectomized females. If the CS have gonad-stimulating activity, this should also have occurred in males. Although stimulation of the CS in sexually mature males has been reported for some teleost species (Lopez 1969; Subhedar and Rao 1979), this was not found in male O. mossambicus. Removal of the testes did not noticeably affect the size of the CS.

Our observations can be explained more satisfactorily by assuming that CS activation during reproduction in female fish is connected with the elevation in plasma calcium levels that accompanies ovarian maturation in O. mossambicus. High total plasma calcium levels during ovarian maturation are typical of many fish species. Most of the calcium is bound to the calcium-phospholipoproteins (vitellogenins) that are synthesized in the liver, transported via the blood to the ovaries, and incorporated in growing oocytes (Pang 1973; Van Bohemen et al. 1982). This process is under the control of estrogens secreted by the ovaries, and can be induced experimentally, in males as well as in females, by estrogen administration (Oguri and Takada 1967; Mugiya 1982; Van Bohemen et al. 1982). Our hypothesis that the activation of the CS during the reproductive period in female O. mossambicus is connected with the calcium status of the fish is based on the following:

1. Activation of the CS occurs in female fish only. In O. mossambicus, only female, not male fish show elevated plasma calcium levels during sexual maturation cycle.
2. Changes in size of the CS are closely correlated with the changes in plasma total calcium levels during the ovarian cycle.
3. The reduction of the plasma total calcium level that follows ovariectomy is accompanied by involution of the CS; in males, gonadectomy does not affect total plasma calcium levels, or the size or structure of the CS.
4. The activation of the CS in sexually mature female fish is due to stimulation of the type-1 cells. These cells are also stimulated when plasma calcium rises during the exposure of fish to increased calcium levels in their tank water (Wendelaar Bonga et al. 1976; Meats et al. 1978; Wendelaar Bonga et al. 1980). These cells probably represent the source of the hypocalcemic factor, hypocalcin (Pang et al. 1973), of the CS. The reduction in size of the CS after ovariectomy is a result of the change in the type-1 cells; these show signs of cellular involution.

The specific function of type-1 cells during ovarian maturation remains unclear. It is possible that enhanced release of the hormonal product of these cells reduces total plasma calcium, e.g., by stimulating the uptake of calcium-lipoproteins by the oocytes. However, although the marked increase in plasma total calcium is mainly a result of the high protein-bound calcium content, the ultrafiltrable calcium fraction is also elevated in sexually mature females, when compared with sexually mature males. The ultrafiltrable calcium fraction consists of complexed calcium and ionized calcium, in a constant ratio. Ionized calcium is considered physiologically more important and is generally controlled within narrow limits (Chan and Chester Jones 1968; So and Fenwick 1977). We suggest that activation of type-1 cells during ovarian maturation is a response of the CS, contributing to the maintenance of plasma ionic calcium within its physiological concentration range. There are several reports showing that extracts of the CS induce a reduction in the total plasma calcium concentration in sexually immature fish or sexually mature male fish, whether stannie totemized (Pang 1973; Pang et al. 1973; So and Fenwick 1977) or intact (Pang and Pang 1974; Swarup and Srivastav 1982). Bailey and Fenwick (1975), however, were
the first to demonstrate that CS extracts injected in intact *Anguilla rostrata* cause a highly significant reduction in plasma ionic calcium concentration. We have recently confirmed this finding for European eels (Lafeber et al. 1984).

If it is accepted that the activation of the CS during ovarian maturation in fish is a response to the accompanying high plasma calcium levels, the reason that the CS are also activated in sexually mature males of some species (Lopez 1969; Subhedar and Rao 1979) and not in others such as male *O. mossambicus* becomes clear. Whereas ovarian maturation is invariably accompanied by elevated calcium levels, in sexually mature males high plasma calcium levels have been found in only some of the species investigated: e.g. the cod, *Gadus morhua* (Woodhead 1968), and the hake, *Merluccius gayi* (Balbontin et al. 1978). Unfortunately, there are no reports concerning the development of the CS during male sexual development in these fish. More frequently there is no increase in plasma calcium levels in CS during male sexual development in these fish. More frequently there is no increase in plasma calcium levels in sexually mature male fish (Fleming et al. 1964; Oguri and Takada 1967; Balbontin et al. 1978; this study). Correlative studies of CS development and plasma calcium levels in male cod and hake should be undertaken.

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