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Vascularization, Innervation, and Ultrastructure of the Endocrine Cell Types of Stannius Corpuscles in the Teleost *Gasterosteus aculeatus*

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**ABSTRACT**

The blood circulation of the Stannius corpuscles, like that of the kidneys to which the corpuscles are attached, represents a portal system. The corpuscles receive blood from the dorsal caudal vein and from a vein coming from the hypaxial musculature. They are drained by veins which enter the caudal parts of the kidneys and therefore endocrine substances released by the corpuscles pass through the kidneys before they enter the general body circulation. The corpuscles are penetrated by sympathetic nerves coming from a small subvertebral ganglion. It is likely that these nerves innervate the muscular coat around the blood vessels. The muscular coat surrounding the renal blood vessels, the collecting tubules and part of the ureters, is innervated by nerves from the same ganglion. The secretory activity of the gland cells appears to be controlled by blood borne factors, because neither synaptic contacts with these cells, nor gap junctions among the cells, have been found in thin sections and freeze-etch replicas of the corpuscles.

The corpuscles contain two cell types, both presumed to have endocrine function. Histochemical and ultrastructural data indicate that the gland cells produce glycoproteins. It is likely that the contents of the secretory granules are released by exocytosis. One cell type is structurally similar to the cells described in many other teleosts and thought to be engaged in the synthesis of a hypocalcemic hormone. The ultrastructure of the second cell type resembles cells described only in other migratory species: salmonids and eels. It may be involved in the control of monovalent ions.

The corpuscles of Stannius, endocrine glands typical of holostean and teleostean fish, are likely to be involved in the control of ionic metabolism (Krishnamurthy, '76). There is evidence that these glands produce a hormone, called hypocalcin, which lowers plasma ionic calcium levels (Pang et al., '74; Pang and Pang, '74). Removal of the Stannius corpuscles leads to a marked rise of the blood levels of ionic calcium and a decrease of inorganic phosphate (Fontaine, '64, '67; Chan et al., '67; Butler, '69, '72). The Stannius corpuscles have been implicated in the endocrine control of bone metabolism (Butler, '69; Lopez, '73; Chan, '72), of calcium influx in the gills (Fontaine et al., '72; Fenwick and So, '74; Fenwick, '76) and of renal calcium metabolism (Fenwick, '74). In eels stannectomies also disturb sodium and potassium equilibrium between intra- and extracellular body fluids (Chan et al., '69; Chan, '72; Butler, '69, '72).

Although the physiological importance of the Stannius corpuscles is unquestionable, knowledge of several structural aspects of these glands is limited. Although the corpuscles are known to be richly innervated, at least in the few species studied (Krishnamurthy and Bern, '71), it is not known whether the nervous innervation concerns the gland cells, the vascular elements, or both. Furthermore, the vascular and nervous connections between the corpuscles and the kidneys deserve attention. The connections with the kidneys are of interest, not only since the kidneys are presumptive target organs of the corpuscular endocrine substances (Rankin et
al., '67; Chester Jones et al., '69; Butler, '72; Fenwick, '74), but also because there is a close topographical and embryological relationship between the kidneys and the Stannius corpuscles. The corpuscles arise as buds which become detached from the pronephric (De Smet, '62) or the mesonephric ducts (Krishnamurthy, '67; Belsare, '73).

Another aspect of the Stannius corpuscles which deserves attention concerns the composition of the gland cell population. In most of the many histological studies of Stannius corpuscles only one glandular cell type has been described (Krishnamurthy, '76). In a number of species, however, two structurally different cell types have been reported (Nadkarni and Gorbman, '66; Krishnamurthy and Bern, '69; Lopez, '69; Heyl, '70). We have reported preliminary evidence that in sticklebacks and eels two secretory cell types are present, which react differently to modified environmental calcium and potassium levels (Wendelaar Bonga and Greven, '75). Accordingly, it is questionable for at least some species whether there is only one hormone produced in the corpuscles of Stannius. A detailed investigation of the cellular composition of the corpuscles is indicated.

In this study of the migratory form of the three-spined stickleback, vascularization and innervation of the Stannius corpuscles are described, with special reference to their relationship with the vascular and nervous supply of the kidneys. Also the results are reported of a light and electron microscopic study of the structure of the gland cells of the corpuscles and of the histochemical nature of the secretory substances produced by these cells.

MATERIAL AND METHODS

Immature female three-spined sticklebacks (Gasterosteus aculeatus, form trachurus) were collected during their upstream migration, in January, in streams in the northern parts of The Netherlands. They were maintained at 15°C in aquaria with running tap water under eight hours light for at least six weeks. The body length varied between 60 and 65 mm.

Vascularization and innervation of the Stannius bodies were reconstructed from serially cut sections of 10 μm thickness. Decapitated fish were fixed in Bouin-Holland fluid for 48 hours, decalcified in 5% HNO₃ solution, and embedded in paraffin. To facilitate the observation of blood vessels, some specimens were perfused with a solution of India ink in saline (0.6% NaCl). This solution was injected slowly into the conus arteriosus of animals anesthetized with MS 222 (Sandoz; 80 mg/l). The tissue sections were stained with hemalum and eosin. The pattern of vascularization of the kidneys, as well as the direction of blood flow, were examined in anesthetized fish with a dissection microscope.

For light microscopic examination Stannius corpuscles and kidneys were dissected out and fixed in Bouin-Holland fluid for 24 hours and embedded in paraffin. Serial sections of 7 μm thickness were stained as a routine with hemalum and eosin. For selective staining of the nervous tissue paraffin sections were mounted in gelatin, as recommended by Albrecht ('54), and treated with the ammoniated silver carbonate technic for nervous tissue after Van Campenhout ('53). For analysis of the nature of the secretory products the following procedures were applied: paraldehyde fuchsin after Gabe, periodic acid-Schiff (PAS) with acetylation and de-acetylation after McManus, alcian blue (pH 1.0) combined with alcian yellow (pH 2.5) with or without previous permanganate oxidation (for details see Wendelaar Bonga, '70) and Bowie's stain (Bowie, '36).

The method of Baillie et al. ('66) with dehydroepiandrosterone as a substrate was used for the demonstration of 3 β-hydroxy steroid dehydrogenase, the method of Karavolas et al. ('70) for 17 β-hydroxy steroid dehydrogenase with 5 α-dihydrotestosterone as a substrate, and the method of Rudolph and Klein ('64), at pH 8.0, for glucose-6-phosphate dehydrogenase. These methods were applied to 15 μm thick unfixed cryostat sections. The interrenal cells of the head kidneys were used as standards.

For electron microscopy the Stannius bodies and parts of the kidneys were prefixed in cacodylate buffered (0.1 M, pH 7.2) glutaraldehyde solution (2%) for ten minutes at room temperature, and fixed in a similarly buffered solution of 1% osmium tetroxide, 1.5% potassium dichromate and 1.5% glutaraldehyde, for two hours at 0°C. The tissue blocks were embedded in Epon. Freeze-etch replicas were prepared as described before (Wendelaar Bonga and Veenhuis, '74a). The terminology used for labeling the membrane faces and surfaces was adopted from Branton et al. ('75). Ultrathin...
sections and freeze-etch replicas were examined in a Philips EM 300 electron microscope.

RESULTS

Vascularization and innervation

The Stannius corpuscles are small oval structures, about 0.5 × 0.7 mm in size in adult animals. One pair of these glands is present in each fish. It is located dorsocaudally to the trunk kidneys (figs. 1, 3). The Stannius corpuscles, like the kidneys, are supplied with blood by the caudal veins and by small segmental vessels coming from the hypaxial musculature. The dorsal caudal vein sends branches to the caudal part of the right kidney. Its main trunk is continuous with the right posterior cardinal vein, which collects blood from the right kidney. The ventral caudal vein is the afferent vessel for the left kidney. The latter is drained by the left posterior cardinal vein. Each Stannius corpuscle receives two afferent vessels. One is a side-branch of the dorsal caudal vein, the other is a small venous vessel coming from the hypaxial musculature (fig. 1). An extensive network of blood capillaries is present in between the gland cells (fig. 2). A small efferent vein runs from each of the glands to the most caudal part of the kidneys, one to the right, and the other to the left kidney (fig. 3). Both veins open into the renal blood circulation. Thus the vascular supply of the Stannius corpuscles, like the blood circulation to the kidneys, represents a portal system. The direction of blood flow ensures that the blood coming from the corpuscles passes through the kidneys.

The Stannius bodies and the kidneys are innervated by a sympathetic nerve. The left and right sympathetic chain are fused in the region above the kidneys, as is common for teleosts (Bertin, '58a). The unpaired sympathetic nerve arises from this fused region and runs ventrocaudally towards the Stannius corpuscles and enters the kidneys, passing in front of both glands. In the area close to the corpuscles this nerve shows a ganglionic swelling (figs. 1, 4). It contains several groups of large neuronal cell bodies. From this swelling, which we qualify as a subvertebral ganglion, two small nerves arise, each of which penetrates a Stannius corpuscle, passing along with the branches of the caudal vein. After entering the bodies, the nerves ramify. The major branches are found in association with the larger blood vessels. The minor branches escape further observation in the light microscope. Ganglion cells were not found in the corpuscles. With the electron microscope, unmyelinated axons as well as an occasional myelinated axon were seen in the connective tissue septa which separate the glandular cell cords. The nerve fibers are found in association with the smooth muscle cells around the blood vessels and the larger capillaries. They contain large numbers of microtubules and some dense cored vesicles with a diameter varying between 60 and 90 nm (fig. 5). These vesicles, with a small electron transparent halo, are typical for aminergic fibers (Thureson-Klein et al., '73). Synapse-like contacts, although scarce, were exclusively found with the smooth muscle cells. The gap between axon and muscle cell was never less than 80 nm. A single nerve runs from the ganglionic mass and innervates the kidneys. It penetrates the connective tissue around the kidneys and ramifies frequently. The major branches are associated with the muscular coat around the blood vessels and the ureters.

Fig. 1  Diagrammatic lateral view of the caudal region of the trunk kidneys (TK). SC, Stannius corpuscles; SN, sympathetic nerve; SG, sympathetic ganglion, sending two nerve branches to the Stannius corpuscles and a major nerve to the kidneys; U, ureter; DCV, dorso-caudal vein; VCV, ventro-caudal vein; HV, vein from the hypaxial musculature; arrows, direction of blood flow; the venous connections between Stannius corpuscles and kidneys are not shown.
The minor branches were hard to follow in the light microscope. Extensive examination of the kidneys at the ultrastructural level, in the course of a morphometric study (Wendelaar Bonga, '73, '76), did not reveal a single synaptic contact with the epithelia lining the renal tubules. Many small nerves and single axons, predominantly unmyelinated, were found in the coat of smooth muscle cells which surrounds the larger blood vessels, the collecting tubules, and the ureters. The axons contain many microtubules and some dense-cored vesicles of the same size as found in the Stannius corpuscles.

No indications were found for the presence of parasympathetic innervation of the Stannius corpuscles or the trunk kidneys. Small nerves of presumably parasympathetic origin were found only in the bladder area. These nerves innervate the muscular coat covering the epithelial lining of the bladder, the distal parts of the ureter, and the larger blood vessels in these areas. With the electron microscope many unmyelinated axons were seen among the muscle cells. Some contained small vesicles of the type found in the muscle cells. Some contained small vesicles of the type found in the Stannius corpuscles and the kidneys. An occasional axon showed granules of a different type. These granules varied in diameter from 120 to 180 nm. Their contents were moderately electron dense.

The gland cells

The gland cells are arranged in branching and anastomosing cords. The thickness of the cords is usually limited to two cell layers. The cords are covered by a thin basal lamina and are separated from each other by thin layers of connective tissue penetrated by many small capillaries. The walls of the capillaries consist of endothelial cells with many fenestrations (fig. 6), a thin collagenous capsule, and irregularly arranged smooth muscle fibers.

The predominant gland cell type in the Stannius corpuscles, referred to as type 1, is ovoid in shape. The nuclei are rounded, often with a deep infolding of the nuclear envelope. They are located in the center of the cells. The cytoplasm contains secretory granules with a diameter up to 0.5 μm (fig. 7). In paraffin sections, these granules react positively to mercury bromphenol blue and, after oxidation, to PAS as well as to paraldehyde fuchsin. The PAS-positivity is unaffected by diastase, which demonstrates that it is not accounted for by glycogen. Acetylation prevents PAS staining, while de-acetylation restores the positive reaction. Without oxidation, no staining was observed with the Schiff solution. The secretory substance is stained green after the combined alcian blue/alcian yellow procedure following permanganate oxidation. Without oxidation it has a low affinity for the yellow stain only. It reacts weakly to Bowie's stain.

Electron microscopical examination of thin sections reveals membrane arrays of granular endoplasmic reticulum, a few relatively large mitochondria, many microtubules and microfilaments, and some Golgi areas. These cell structures are rather evenly distributed in the cytoplasm. The secretory granules are large and have electron-dense contents. It is likely that they arise by constriction from the Golgi membranes, since electron-dense material is present within some of the Golgi sacules (fig. 8).

The base of the cells is almost invariably in contact with the connective tissue. In these areas indentations of the outer cell membrane are occasionally found. Their presence points to release of secretory material by exocytosis. Some small clear vesicles, often with irregular outlines, occur in the cytoplasmic zone bordering on the connective tissue. No intercellular connections such as desmosomes or gap junctions, were observed.

Since gap junctions may escape attention in ultrathin sections, their absence was verified in freeze-etch replicas. The freeze-etch technique facilitates the examination of surfaces and fracture faces of cellular membranes. If present, the plaques of regular arrays of membrane-associated particles which characterize gap junctions are prominent in freeze-etch replicas (Raviola and Gilula, '73). A few particles (ϕ: 6-10 nm) are uniformly distributed over the surfaces of the outer cell membranes. The fracture faces are densely covered by such particles (figs. 12, 15). But extensive examination of these membranes did not reveal a single gap junction.

Among the type 1 cells, another cell type is present. It is less numerous and its structure differs completely from that of the former cell type. It is referred to as type 2. The cells are long and slender and most cells possess several cytoplasmic processes, which penetrate between the type 1 cells (figs. 2, 7). Most of the nuclei of the type 2 cells are cylindrical. This facilitates the recognition of this type in the light microscope. The cytochemical characteristics of the secretory material were difficult
to establish in the light microscope. This substance is mainly located in the distal parts of the cytoplasmic processes. We found that it reacts positively to PAS and paraaldehyde fuchsin after oxidation. The PAS-positivity is diastase resistant, disappears after acetylation, and is restored after de-acetylation. It shows a deep green color after alcin blue/alcian yellow with permanganate oxidation. Without oxidation the material remains unstained. It does not take Bowie's stain.

In ultrathin sections examined in the electron microscope the cytoplasm of the type 2 cells shows a few strands of granular endoplasmic reticulum. Golgi areas (fig. 11) are more frequently observed than in type 1 cells, whereas the Golgi saccules are smaller than in type 1 cells (figs. 13, 14). There are many small mitochondria, some microtubules and, especially in the cytoplasmic processes, many microfilaments (fig. 9). An occasional lysosome-like body as well as some multivesicular bodies are found in the perinuclear region. The secretory granules are small, with a diameter never surpassing 0.2 μm and with electron-dense contents (fig. 7). Some Golgi saccules contain electron-dense material. A few presecretory granules are occasionally present in the Golgi areas, together with many small coated as well as uncoated clear vesicles.

The type 2 cells show a definite polarity as far as the distribution of the cytoplasmic organelles is concerned. In the perinuclear area of the cells secretory granules are scarce. Here the Golgi areas are concentrated (fig. 11). In the cytoplasmic processes Golgi areas are not found. Secretory granules are mainly found in dense accumulations in the distal parts of the processes (figs. 7, 12). These endings are extended where they border on the basal lamina. Membrane indentations as well as small clear vesicles are common in this area (fig. 10). These indications for release of secretory material were never found in areas bordering on other glandular cells. They are characteristic for the distal endings of the processes. Even in areas where the perinuclear cytoplasm is in contact with the basal lamina phenomena indicative of exocytosis were never found. It is likely that the secretory granules, after being formed by constriction from the Golgi membranes in the perinuclear area, are transported to the swollen ends.
of the cytoplasmic processes. Here the secretory material is extruded in the connective tissue in the vicinity of blood capillaries. The structural data are incorporated in the diagram of figure 2.

In ultrathin sections and freeze-etch replicas neither desmosomes nor gap junctions were observed between type 2 cells or between type 1 and type 2 cells (fig. 15).

The enzyme histochemical tests for the demonstration of steroid synthesizing or metabolizing capacity failed to show any activity of $3\beta$ and $17\beta$-hydroxysteroid dehydrogenases or glucose-6-phosphate dehydrogenase.

**DISCUSSION**

**Vascularization and innervation**

The present results show that the vascularization and innervation of the Stannius bodies and the kidneys are closely related. The blood circulation of the stickleback kidneys is comparable to the *Gadus*-type of circulation in the classification of Bertin ('58b). The main afferent vessels of the kidneys are the ventral and dorsal caudal veins. The main blood supply of the corpuscles of Stannius is provided by a small branch of the dorsal caudal vein. The circulatory system of the corpuscles, like that of the kidneys represents a portal system. Another characteristic the Stannius bodies and kidneys have in common is that they are both supplied with venous blood by small vessels coming from the hypaxial musculature. Since the corpuscles are drained by small veins which run to the kidneys and open into the renal portal system, the hormonal substances produced in the corpuscles pass the kidneys before they enter the main body circulation.

The corpuscles as well as the caudal areas of the kidneys are innervated by small nerves originating from a small ganglion located in the proximity of the corpuscles. Similarly located ganglia have been described in Atlantic salmon, *Salmo salar* (Heyl, '70) and in eight other teleost species (Krishnamurthy and Bern, '71). In these species, as in sticklebacks, small nerves run from these ganglia and penetrate the Stannius corpuscles along with blood vessels. Krishnamurthy and Bern could not determine if they were sympathetic or parasympathetic. Our reconstruction of silver stained paraffin sections showed that in the sticklebacks the nerves are sympathetic in nature. Size and appearance of the granules present in the axons are typical for post-ganglionic adrenergic fibers of the vertebrate sympathetic nervous system (Thureson-Klein et al., '73). A sympathetic type of innervation of the corpuscles of Stannius has been described before by Young ('31) in *Uranoscopus scaber*. We consider the ganglionic mass to be a subvertbral ganglion. Our observations show that the nerves running from this ganglion innervate the smooth muscle cells associated with the blood vessels of the Stannius bodies and the kidneys, and with the muscular coat of the larger renal ducts. It is likely that these nerves are involved in the nervous control of the blood flow in these organs, and the regulation of the peristaltic contractions of the renal ducts. The stickleback kidneys are poorly innervated, especially in comparison with the kidneys of higher vertebrates (Barger and Herd, '73). Similar as in sticklebacks the renal nervous supply in the higher vertebrate groups is confined to the perivascular smooth musculature and is not directed to the nephric tubules.

A relationship between the juxtacorpuscular ganglion and the control of blood flow has been postulated before by Heyl ('70) for *Salmo salar*. Heyl suggested that the ganglion was part of a mechanism for systemic blood pressure regulation through a renin-like pressor substance as found in the Stannius corpuscles of eels by Chester Jones et al. ('66). The latter authors found that stannicuteomy is accompanied by marked hypotension, while injection of corpuscular extracts had a hypertensive effect (Chester Jones et al., '65, '66; Chan et al., '69). A renin-like substance was also found in the Stannius corpuscles of three other teleost species, although its concentration was extremely low (Sokabe et al., '70). Recently Bailey and Penwick ('75) convincingly argued that the hypotension following stannicuteomy is not necessarily related to a specific pressor substance produced by the Stannius corpuscles, but can be accounted for by an effect on the cardiovascular system of the high blood calcium levels found in stannicuteomed fishes.

Krishnamurthy and Bern ('71) suggested that the small nerves running from the ganglion to the Stannius corpuscles innervate the gland cells. It is premature to conclude an absence of nervous innervation merely on the basis of our failure to detect synapses on these cells. The possibility remains that the cells are electrically coupled by gap junctions, which makes the presence of a single synapse
sufficient to control large numbers of cells. This situation is probably present in the mammalian pancreatic islands (Orch, '74). In sticklebacks, gap junctions are located in large numbers between the epithelial cells lining the renal tubules (Wendelaar Bonga and Veenhuis, '74b). The absence of gap junctions between the gland cells of the Stannius corpuscles, as appears from this study, supports our conclusion that the gland cells are devoid of synaptic nervous control. The presence of control by nervous substances released in the connective tissue at some distance from the gland cells, as has been reported for teleost adenohypophysis (Bern et al., '71), cannot be excluded. But structural evidence in favour of this possibility was not found. Presumably, the secretory activity of these cells is controlled by blood-borne substances. Schreiberman and Pang ('75) reached the same conclusion for the killifish Fundulus heteroclitus. They established that the Stannius corpuscles continued active secretion after denervation, since autotransplanted glands retain their ability to influence serum calcium levels. However, autotransplantation experiments do not rule out the possibility that the cells are normally under inhibitory nervous control.

We did not find indications for the presence of parasympathetic innervation of the Stannius corpuscles and the kidneys. Parasympathetic tracts were only observed around the bladder and the distal parts of the ureters. Ultrastructural examination of these nerve fibers revealed the presence of secretory granules which differ from the type found in the Stannius corpuscles and the kidneys. Their size and appearance resemble granules of purinergic nature (Burnstock, '72). Their presence supports Burnstock's opinion that the parasympathetic supply of the urinary bladder in several vertebrate groups, including fishes, is purinergic rather than cholinergic. The small clear vesicles characteristic for cholinergic nerves were not observed in this study.

The gland cells

The structural data presented in this study show that the corpuscles of Stannius of sticklebacks contain two types of secretory cells. Both have endocrine characteristics as judged by cytological criteria. The shape of the cells differs markedly. The structure of the type 2 cells, with many long cytoplasmic processes containing distal accumulations of secretory granules, is unusual for gland cells and shows some resemblance to neuro-endocrine cells. In addition to the differences in the shape of the cell bodies, both cell types show differences in the shape of the nuclei, the size of the secretory granules and the size of the mitochondria. Moreover, in the type 2 cells the Golgi membranes and vesicles are more numerous than in the type 1 cells, while the reverse holds for the membrane profiles of the granular endoplasmic reticulum.

It is likely that the secretory granules in both cell types arise by constriction from the Golgi membranes and release their contents by exocytosis. The presence of membrane indentations as well as small clear vesicles in the cytoplasmic area bordering on the connective tissue point to the occurrence of this mode of release. During exocytosis the limiting membrane of the granules is incorporated in the outer cell membrane. For several secretory cell types experimental evidence has been obtained that excess membrane material is removed from the cell membrane by endocytosis (Geuze and Kramer, '74). The clear vesicles we found in the gland cells probably arise by the latter process.

In the Stannius corpuscles of other species, including eels, light microscopic observations interpreted as holocrine and apocrine modes of extrusion of secretory material have been reported (c.f. Krishnamurthy, '76). Our unpublished electron microscopic observations on freshwater eels (Anguilla anguilla) revealed that also in this species exocytosis does occur, especially when the dominant cell type was activated by transfer of the animals to sea water. This may be the principal mode of extrusion in this species, since well documented ultrastructural reports on holocrine and apocrine modes of secretion are scarce and, to our knowledge, do not include protein secreting endocrine glands.

The staining characteristics of the secretory material in both cell types show some similarity. Both substances are PAS-positive, which indicates the presence of carbohydrates containing dialdehyde groups. The green color adopted after oxidation and treatment with alcian blue/alcian yellow is indicative of the presence of carbohydrates associated with cysteine or cystine containing proteins (Peute and Van de Kamer, '67). The proteinaceous nature of the secretory substances is further indicated by their affinity for mercury bromophenol blue and by the presence of arrays of
granular endoplasmic reticulum, which are numerous in the type 1 cells. It is likely therefore that both cell types produce glycoproteins. The differences observed between both cell types in the relative amounts of granular endoplasmic reticulum, in the extent of the Golgi apparatus, and in the intensities of the staining reactions point to differences between the secretory materials of the cell types in carbohydrate and protein content.

Steroid synthesizing or metabolizing capacities have been attributed to the Stannius corpuscles, especially in older literature (see for reviews Idler and Freeman, '66; Krishnamurthy, '76). Although the present histochemical data on the secretory material allows conclusions about carrier substances or hormone precursor molecules rather than about the nature of the biologically active factors, it is unlikely that the corpuscles of sticklebacks are important centers of steroid production. Steroidogenic cells are in general characterized by large amounts of agranular endoplasmic reticulum and by mitochondria with tubular cristae. These features are absent from the corpuscular cells of sticklebacks, and have never been observed in other species under physiological conditions (Krishnamurthy, '76). Our structural and histochemical observations in both cell types point to the formation and release of proteinaceous secretory material, and cannot be satisfactorily interpreted as the machinery for the production of enzymes necessary for the synthesis or transformation of steroids, as has been suggested by Krishnamurthy ('76) for structural data on other species. Furthermore, our failure to demonstrate 3β- and 17β-hydroxysteroid dehydrogenases, both key enzymes in steroid synthesis and steroid metabolism (Lofts and Bern, '72; Gouder et al., '75), and glucose-6-phosphate dehydrogenase, an enzyme generally found in steroid metabolizing cells (Savard et al., '63), make steroid synthesis of any importance unlikely. The extensive biochemical analysis of the corpuscular tissue of the trout (Salmo gairdneri) by Colombo et al. ('71) has demonstrated that the steroid synthesizing capacity of the Stannius corpuscles is negligible. This conclusion is supported by our observations on sticklebacks, although on the basis of our data a role of the corpuscles in steroid transformation, in addition to the secretion of proteinaceous material, cannot be excluded.

The Stannius corpuscles of many teleost species have been described, mostly in light microscopic studies. In most of these reports only one cell type has been mentioned. It shows similarity with the type 1 cells of sticklebacks. The secretory material is mostly characterized as PAS and/or pararaldheyde fuchsin positive. In the electron microscope the material appears as large osmiophilic granules. The ultrastructure of these cells invariably points to the formation of proteinaceous substances (Krishnamurthy, '76).

Ultrastructural evidence for the presence of a second cell type, as presented for sticklebacks in this study, is limited so far to two other species: the rainbow trout Salmo gairdneri (Krishnamurthy and Bern, '69) and the eel Anguilla anguilla (Wendelaar Bonga and Greven, '75). The cells of the Stannius bodies of these species show some resemblance to the type 1 and type 2 cells of the sticklebacks. One cell type is characterized by large secretory granules, the other by relatively small granules. The electron micrographs of the Stannius bodies of the rainbow trout Oncorhynchus kisutch (Hiroi, '70), of Atlantic salmon Salmo salar (Carpenter and Heyl, '74) and of the guppy Lebistes reticulatus (Tomasula et al., '70) suggest that also in these species two cell types are present. In several salmonids and in two other species, Gymnothorax flavimarginatus and Hypsurus caryi, Krishnamurthy and Bern ('69) discriminated with the light microscope between granular, usually PAS-positive, and non-granular PAS-negative cells. For an interpretation of this observation additional ultrastructural data are essential.

Although the above mentioned studies show that the occurrence of two structurally different cell types in the Stannius corpuscles is probably not restricted to sticklebacks, eels and salmonids, it is unlikely to be typical for teleosts in general. Ultrastructural examination of plaice, Pleuronectes platessa, cod (Gadus morrhua) and goldfish (Carassius auratus) revealed only one cell type, which was structurally similar to the type 1 cells of the stickleback (Wendelaar Bonga and Greven, '75; Wendelaar Bonga et al., '76b).

Experiments involving exposure of sticklebacks and eels to media of different ionic composition have indicated that the type 2 cells are engaged in the control of monovalent ions, especially sodium and potassium (Wendelaar Bonga et al., '76a,b). This hypothesis is consistent with the observation that stannectomy in sticklebacks (our unpublished re-
sults) and in eels (Fontaine, '64; Chan et al., '67; Butler, '69, '72) leads to changes in plasma sodium and potassium levels.

ACKNOWLEDGMENTS

The authors are indebted to Miss C. Mein and Mr. J. Zagers for technical assistance.

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PLATES
EXPLANATION OF FIGURES

3 Paraldehyde fuchsin stained section of Stannius corpuscle (CS), showing its close topographical relation to the kidney; KT, kidney tubules; arrows: small vein running from corpuscle to kidney. × 120.

4 Sympathetic ganglion located frontally of the Stannius corpuscles; CN, nerves running to Stannius corpuscles; KN, nerve running to kidney; N, neuronal cell bodies; ammoniated silver carbonate stain. × 260.

5 Axons (AX) in the muscular coat around a blood vessel in a Stannius corpuscle, with neurotransmitter granules (G). × 68,000.

6 Detail of Stannius corpuscle, with part of type 2 cell (T-2), connective tissue (CT) and the endothelial wall, with fenestrae (F), of a blood capillary; E erythrocyte. × 30,000.

7 Type 1 cells (T-1), with large secretory granules, and cytoplasmic processes of type 2 cells (T-2), with small secretory granules; CT, connective tissue; FC, fibrocyte. × 14,500.
8 Golgi area of type 1 cell. Electron-dense material is present in the Golgi saccules (arrows); CV, clear vesicles. × 62,000.

9 Detail of figure 7, showing a bundle of microfilaments (F) in the cytoplasmic process of a type 2 cell (T-2); T-1, type 1 cell. × 42,000.

10 Detail of figure 7. Membrane indentation indicative of exocytosis (arrow) in the distal ending of a type 2 (T-2) process. CV, clear vesicles; CT, connective tissue. × 60,000.

11 Part of the perinuclear area of a type 2 cell (T-2). GA, Golgi areas; C, centriole; (T-1), type 1 cell. × 49,000.
PLATE 4

EXPLANATION OF FIGURES

Figs. 12-15  Electron micrographs of freeze-etch replicas; arrows in the left upper corners indicate direction of metal shadowing.

12 Type 1 cells (T-1) and cytoplasmic processes of type 2 cells (T-2); N, cross-fractured nucleus; P, nuclear pores; PS, protoplasmic surface of outer cell membrane; PF, protoplasmic fracture face of outer cell membrane; G, secretory granules; MI, mitochondria. × 21,500.

13 Golgi area of a type 1 cell, with saccules (S) and vesicles (V); MI, mitochondrium. × 38,000.

14 Golgi area in a type 2 cell, with saccules (S) and many vesicles (V); MI, mitochondrium. × 38,000.
PLATE 5
EXPLANATION OF FIGURE

15 Part of a type 1 cell (T-1) with secretory granules (G) and a crossfractured nucleus (N), and the perinuclear part of a type 2 cell (T-2) with a cytoplasmic process (CP). The cytoplasm of the latter cell contains small secretory granules (G) and mitochondria (M1). The nuclear envelope (N) shows nuclear pores (P). Many small particles are associated with the protoplasmic face (PF) of the outer cell membranes of both cells. × 35,000.