

University of Groningen

**The Relationship between the Ionic Composition of the Environment and the Secretory Activity of the Endocrine Cell Types of Stannius Corpuscles in the Teleost *Gasterosteus aculeatus***

Wendelaar Bonga, S.E.; Greven, J.A.A.; Veenhuis, M.

*Published in:*  
Cell and Tissue Research

*DOI:*  
[10.1007/BF00218708](https://doi.org/10.1007/BF00218708)

**IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.**

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
1976

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Wendelaar Bonga, S. E., Greven, J. A. A., & Veenhuis, M. (1976). The Relationship between the Ionic Composition of the Environment and the Secretory Activity of the Endocrine Cell Types of Stannius Corpuscles in the Teleost *Gasterosteus aculeatus*. *Cell and Tissue Research*, 175(3). DOI: 10.1007/BF00218708

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

## The Relationship between the Ionic Composition of the Environment and the Secretory Activity of the Endocrine Cell Types of Stannius Corpuscles in the Teleost *Gasterosteus aculeatus*

S.E. Wendelaar Bonga, J.A.A. Greven, and M. Veenhuis\*

Department of Zoology and Laboratory for Ultrastructural Biology, University of Groningen, The Netherlands

**Summary.** The corpuscles of Stannius of threespined sticklebacks contain two glandular cell types of presumed endocrine nature. To elucidate the function of both cell types the secretory activity of the cells was studied in fully adapted seawater and freshwater fishes and in specimens transferred from sea water to fresh water or adapted to media of various ionic composition. The secretory activity was established, in tissue sections and freeze-etch replicas, by estimating the volume of the nuclei, the density of the nuclear pores, and the frequency of exocytotic phenomena.

The type-1 cells, ultrastructurally comparable to the predominant or only cell type described in many other teleosts, are more active in sea water than in fresh water. The activity of the type-2 cells, whose ultrastructural appearance is known only for salmonids and eels, is higher in fresh water. Transfer of seawater fishes to fresh water results in reduction of type-1 cells and activation of type-2 cells. The factors responsible for these changes were analyzed by exposure of fishes to solutions of various salts in fresh water and to artificial sea water with a reduced content of one of its components. The high activity of type-1 cells in sea water proved to be related to the high calcium content of this medium. These cells probably produce a substance comparable to hypocalcin, the endocrine factor isolated from the Stannius corpuscles of some other teleost species. The high activity of type-2 cells in fresh water appeared to be connected with the low sodium and potassium levels of this medium. Type-2 cells possibly produce a hitherto unknown hormone involved in the control of sodium and/or potassium metabolism.

**Key words:** Corpuscles of Stannius – Endocrine control – Ionic regulation – Morphometry – Teleosts.

---

Send offprint requests to: Dr. S.E. Wendelaar Bonga, Department of Zoology, University of Nijmegen, Toernooiveld, Nijmegen, The Netherlands

\* The technical assistance of Miss C. Mein and Mr. J. Zagers is gratefully acknowledged

## Introduction

The corpuscles of Stannius, small endocrine glands characteristic of bony fishes, are known to be involved in the control of calcium metabolism. Extirpation of the corpuscles leads to prolonged hypercalcemia (Fontaine, 1964, 1967; Chan and Chester Jones, 1968; Pang, 1971; Fenwick, 1974). A substance with a hypocalcemic effect (*hypocalcin*) has been isolated from the Stannius corpuscles of some teleosts (Pang and Pang, 1974).

Removal of the corpuscles does not affect solely the ionic calcium levels. In eels it also results in changes in the plasma levels of sodium, potassium, chloride (Fontaine, 1964, 1967; Chan et al., 1969; Butler, 1972) and magnesium (Butler, 1969; Chan et al., 1969). The latter changes have been interpreted as indirect effects of the operation (Leloup-Hatey, 1970; Chan, 1972; Fleming et al., 1973; Pang et al., 1975). Undoubtedly, the high calcium levels following operation will affect the hydromineral balance, whether directly or indirectly. But the possibility must also be considered that the hypocalcemic substance is not the only endocrine factor produced in the corpuscles of Stannius.

The supposition that these glands secrete more than one type of hormone is substantiated by histological data. There is ultrastructural evidence for the presence of more than one glandular cell type. Up till now this evidence is limited to a few teleost species. In the Stannius corpuscles of most teleosts studied only one cell type has been described (Oguri, 1966; Fujita and Honma, 1967; Ogawa, 1967; Krishnamurthy and Bern, 1969; Cohen et al., 1975). When cells different from the predominant cell type have been mentioned, these were usually interpreted as immature or exhausted stages of the principal cell type (Nadkarni and Gorbman, 1966; Tomasulo et al., 1970). But in some of the species examined by light microscopy, Krishnamurthy and Bern (1969) found indications of a second cell type. In one of these species, *Salmo gairdneri*, the occurrence of two structurally different cell types was confirmed by electron microscopy. Wendelaar Bonga and Greven (1975), studying the ultrastructure of the Stannius corpuscles of the three-spined stickleback *Gasterosteus aculeatus* and the eel *Anguilla anguilla*, reported the presence of two structurally and probably also functionally different cell types in both species.

In the present study, which deals with the Stannius corpuscles of sticklebacks, the first results are presented of our attempts to elucidate the functions of the two cell types. The sticklebacks concerned, the euryhaline form *trachurus*, migrate in late winter and early spring from the sea to fresh water and return to the sea in summer and autumn, after the reproductive period.

Preliminary examination of sticklebacks and eels has indicated that the secretory activity of both cell types in seawater fishes differs considerably from that of freshwater fishes (Wendelaar Bonga et al., 1976). This observation suggests that these cells play a role in the endocrine control of ion regulation and possibly in the ion-regulatory adjustment during migration. In this paper the difference in the secretory activity between the Stannius corpuscles of freshwater and seawater fishes was analyzed in more detail and in part quantitatively. In addition the effects were studied of transfer of seawater fishes to fresh water. In order to define the environmental factors accounting for the differences between seawater

and freshwater sticklebacks, the Stannius corpuscles were studied in fishes exposed to solutions of various salts in fresh water, and to modifications of artificial sea water.

The volume of the nuclei, the density of the nuclear pores, and signs of release of secretory material (exocytosis) were used as parameters for secretory activity.

## Materials and Methods

Adult immature female sticklebacks, with a body length varying between 60 and 65 mm, were caught in late winter in canals and along the coast of the Wadden Sea. They were kept in containers with running tap water or natural or artificial sea water (Wimex®), at 15°C and a daily light regimen of 8L16D, for at least 3 weeks. Concentrations of the main electrolytes in these media (in mmol/l):

	Na <sup>+</sup>	K <sup>+</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Cl <sup>-</sup>
Tap water	2.13	0.06	0.05	0.21	1.75
Artificial sea water	460.0	10.0	10.0	53.0	535.0

These values, as well as the values for the Na<sup>+</sup> and K<sup>+</sup> concentrations given in the Discussion, were determined by atomic absorption spectrophotometry.

*a) Comparison of Stannius Corpuscles of Fully Adapted Freshwater and Seawater Fishes.* The freshwater specimens studied were kept for 2 months in tap water, the seawater specimens for 2 months in natural sea water (obtained off shore in the North Sea) under the conditions described above.

*b) Adaptation of Seawater Specimens to Fresh Water.* Fully adapted seawater sticklebacks were transferred to tap water and examined 24, 48 and 72 h afterwards.

*c) Adaptation to "Sea Water" of Limited Electrolyte Composition, Modified after Hale (Lockwood, 1963).* Seawater specimens were transferred for 16 days to a solution of distilled water containing the following salts (mmol/l): NaCl (410.0); KCl (9.7); CaCl<sub>2</sub> (10.2); MgCl<sub>2</sub> (54.0); Na<sub>2</sub>SO<sub>4</sub> (28.2); NaHCO<sub>3</sub> (2.3). This solution will be referred to as Hale's sea water.

*d) Adaptation of Freshwater Fishes to Different Salt Solutions.* Freshwater sticklebacks were adapted during 6 days to daily increasing solutions in tap water of one of the salts mentioned under c, or to MgSO<sub>4</sub>. Afterwards they were kept for 10 days at the final concentration, which was similar to the concentration of the respective salt in Hale's sea water. The final concentration of the MgSO<sub>4</sub> solution amounted to 28.2 mmol/l. Some mortality, although less than 20%, occurred in the first week in the solutions containing KCl, CaCl<sub>2</sub> and NaHCO<sub>3</sub>. The remaining fishes were able to survive for at least 6 weeks in these solutions.

*e) Adaptation to Hale's Sea Water with Reduced Content of One of the Salts.* Seawater fishes, kept in Hale's sea water for at least 2 weeks were adapted during 6 days to modified Hale's sea water. The concentration of one of the 6 salts was gradually reduced during these 6 days, to a final concentration of 10% of the normal concentration. The fishes were kept for 10 days at this final concentration. Some mortality, around 20%, was observed in the first week in the low-calcium and low-potassium solutions. The surviving fishes were able to live for at least 6 weeks in these solutions.

For light microscopic examination the Stannius bodies were fixed for 24 h in Bouin-Hollande. Paraffin sections were stained as a routine with Mayer's heamalum and eosin. Nuclear size was determined in the light microscope; 25 nuclei of each cell type were measured per animal. The nuclei of the type-2 cells were only measured if their long axis (mostly orientated perpendicular to the basal

lamina) was located in the plane of sectioning. Nuclear volumes were calculated by considering the nuclei of type-1 cells as spheres, those of the type-2 cells as cylinders. The results were analyzed by Wilcoxon's test.

For electron microscopy the Stannius bodies were prefixed for 10 min in cacodylate buffered (0.1 M, pH 7.2) glutaraldehyde solution (2%) at room temperature, and subsequently fixed in a similar buffered solution of 1% osmium tetroxide, 1.5% potassium dichromate and 1.5% glutaraldehyde, for 2 h at 0°C. Ultrathin Epon sections were stained with lead citrate.

Freeze-etch replicas of Stannius corpuscles were prepared after infiltration for 15 min in 25% glycerol in 0.1 M phosphate buffer (pH 7.2). The corpuscles were frozen in liquid Freon-22 and stored in liquid nitrogen. Replicas were made in a Balzers BA 360M Freeze Etch apparatus. Freshly cleaved corpuscles were etched for 3 min at -100°C and shadowed with platinum and carbon. The replicas were cleared in saturated solution of  $K_2Cr_2O_7$  in 70%  $H_2SO_4$  for some hours and afterwards in 40% NaOH for 1 h. They were rinsed in distilled water and examined on unsupported grids. For estimating the density of the nuclear pores, nuclear membrane areas of  $25 \mu^2$  per animal were sampled in micrographs at a final magnification of about 20.000 $\times$ . The results were analyzed for significance by Student's *t*-test.

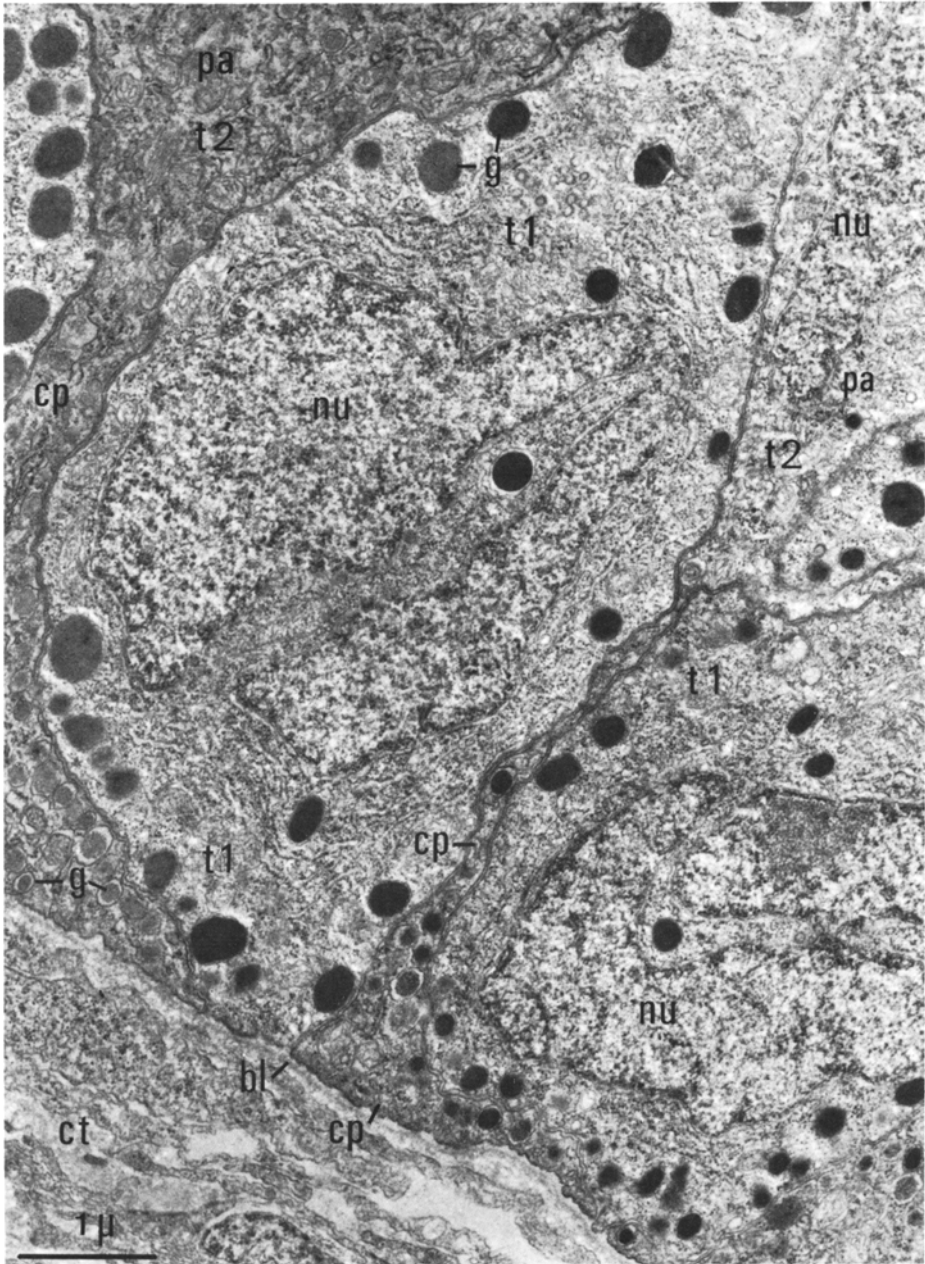
Thin sections and replicas were examined in a Philips EM 300 electron microscope.

## Results

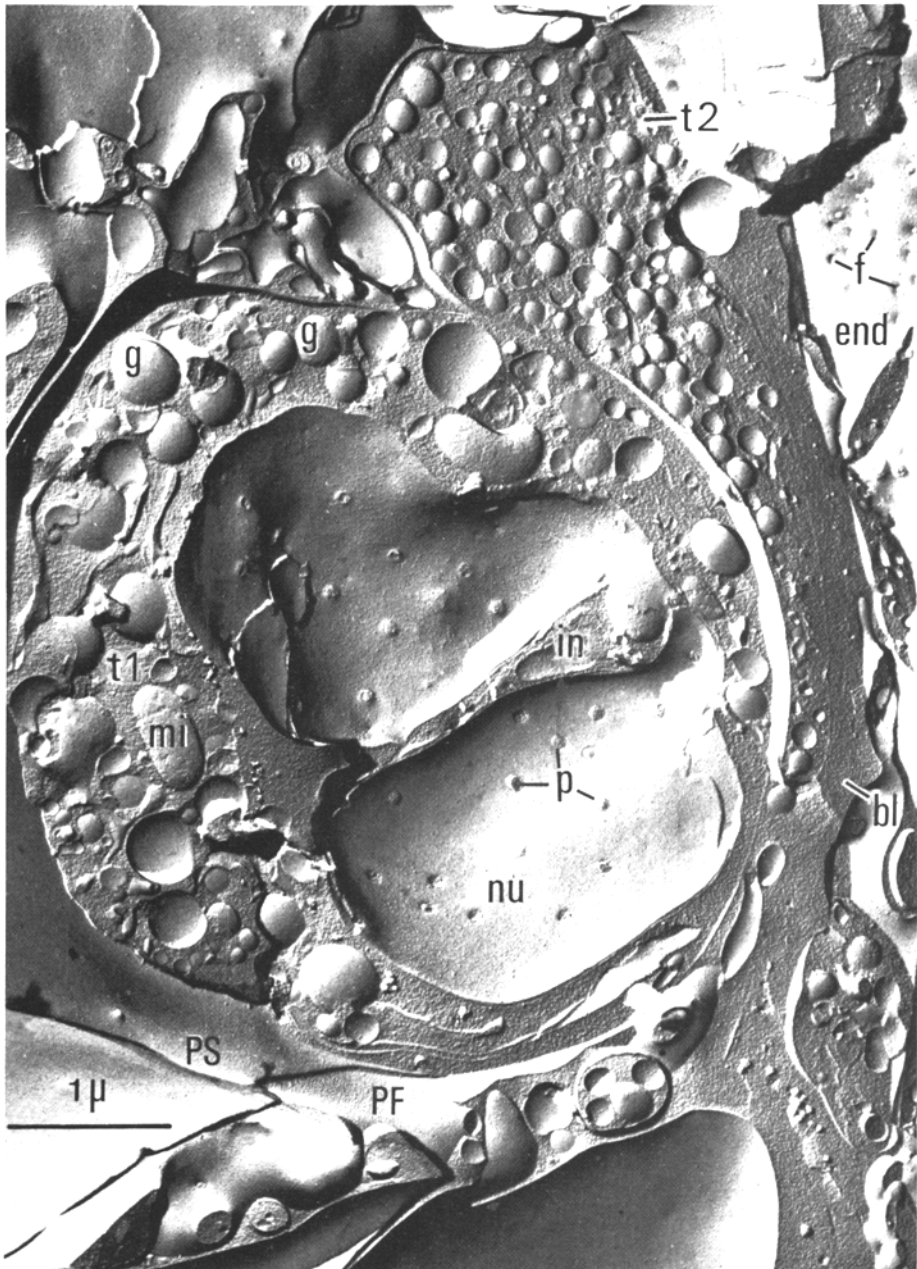
The corpuscles of Stannius are small oval endocrine glands. One pair of these glands is present in each fish. They are located dorsocaudally to the kidneys. The glandular cells are arranged in branching and anastomosing cords, separated by layers of connective tissue and a finely branched venous capillary network. The predominant cell type, referred to as type-1, is characterized by large electron dense secretory granules with a diameter up to  $0.5 \mu$  (Fig. 1). The cells are oval in shape. In areas where the cells are in contact with the connective tissue, the outer cell membrane shows indentations characteristic of release, by exocytosis, of the contents of the secretory granules. Strands of granular endoplasmic reticulum, some Golgi zones and several large mitochondria are evenly distributed in the cytoplasm.

The cells of type-2 are very different. They are long and slender and most show one or more cytoplasmic projections penetrating between the type-1 cells (Fig. 1). The secretory granules are electron dense and small, with a diameter less than  $0.2 \mu$ . Most nuclei are cylindrical. Their long axis is located perpendicular to the basal membrane. In the light microscope they are easily distinguished from the oval nuclei of type-1 cells. The cytoplasm contains some strands of granular endoplasmic reticulum, some Golgi zones and many small mitochondria. In contact areas with the connective tissue, the outer cell membrane occasionally shows membrane indentations indicative of release of secretory material by exocytosis (Fig. 3).

The volume of the nuclei, which reflects cellular activity and hence the secretory status of gland cells, was determined in paraffin sections. The density of the nuclear pores was estimated in freeze-etch replicas. The number of nuclear pores indicates the intensity of interaction between nucleus and cytoplasm (Lott et al., 1972; Scheer, 1972) and represents therefore another parameter for cellular activity. Changes in cellular activity may be reflected by changes in the density of the nuclear pores long before changes in nuclear volume become apparent (Wendelaar Bonga and Veenhuis, 1974). The envelope of the nucleus and the nuclear pores are clearly revealed by the freeze-etch procedure, as this method facilitates the exposure of large membrane surfaces and fracture faces (Fig. 2).



**Fig. 1.** Fresh water. Type-1 cells (*t1*), characterized by large secretory granules (*g*), and type-2 cells (*t2*), with perinuclear area (*pa*), and cytoplasmic processes (*cp*) containing small secretory granules (*g*); *bl* basal lamina; *ct* connective tissue; *nu* nucleus



**Fig. 2.** Fresh water, freeze-etch replica. Type-1 cell (*t1*) and distended ending of a cytoplasmic process of a type-2 cell (*t2*), separated by a basal lamina (*bl*) from the endothelial wall of a blood capillary (*end*). The nucleus (*nu*) of the type-1 cell shows a deep cytoplasmic indentation (*in*) and the nuclear envelope shows many nuclear pores (*p*); *f* fenestrae of the endothelium; *g* secretory granules; *mi* mitochondrion; *PS* protoplasmic surface of the outer cell membrane; *PF* protoplasmic fracture face of the outer cell membrane

**Table 1.** Volume of nuclei and density of nuclear pores of type-1 and type-2 cells of fully adapted seawater and freshwater sticklebacks. Means ( $\pm$ S.E.) of 5 fishes per group

		Sea water	Fresh water
Nuclear volume ( $\mu^3$ )	type-1:	34.60 $\pm$ 2.29	25.52 $\pm$ 1.27
	type-2:	26.33 $\pm$ 2.25	39.17 $\pm$ 4.95
Density of nuclear pores/ $\mu^2$	type-1:	6.2 $\pm$ 0.3	4.9 $\pm$ 0.3
	type-2:	5.1 $\pm$ 0.2	6.4 $\pm$ 0.4

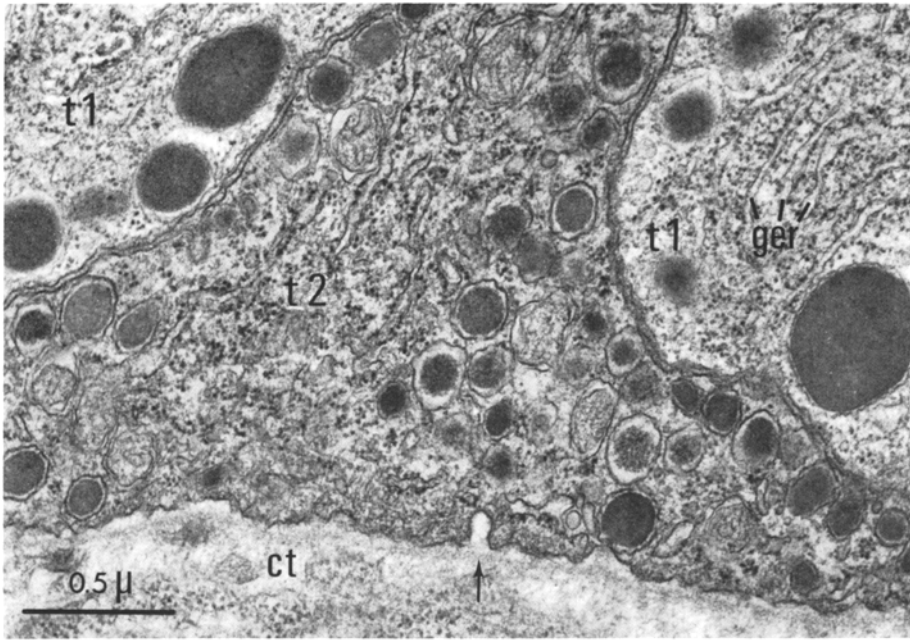
*a) Comparison of Stannius Corpuscles of Seawater and Freshwater Fishes.* Microscopic analysis of the Stannius corpuscles of fully adapted seawater and freshwater fishes leads to the conclusion that type-1 cells are more active in sea water and type-2 cells in fresh water (Table 1). In sea water, the mean volume of the nuclei of the type-1 cells is higher by about 35% than in fresh water ( $p < 0.001$ ). The number of nuclear pores, per surface area of nuclear envelope, shows a similar difference ( $p < 0.001$ ). There are almost twice as many secretory granules in the cytoplasm of type-1 cells of seawater fishes as in the cells of freshwater fishes. The granular endoplasmic reticulum is more extensive and the Golgi zones are more prominent in the seawater group. A higher secretory activity of type-1 cells in seawater is further indicated by the high incidence of phenomena of exocytosis. These were 3 to 5 times more frequently found, per unit length of the outer cell membrane contacting the connective tissue, than in the freshwater group.

For the type-2 cells the above mentioned indications for high secretory activity were found in freshwater animals. Compared to the seawater group, the nuclear volume was higher by 50% ( $p < 0.001$ ), the density of the nuclear pores by 25% ( $p < 0.001$ ). The cytoplasmic processes of the type-2 cells are more pronounced in fresh water. The distal ends of these processes are often extended and cover the type-1 cells over large areas, separating the latter cells from the connective tissue. In these distended extremities secretory granules are found in large numbers (Figs. 1, 3). In seawater fishes, the cytoplasmic projections of the type-2 cells are thin and small, and extensions covering the type-1 cells are seldom found. The cytoplasmic areas of the type-2 cells bordering on the connective tissue are markedly reduced when compared to the freshwater fishes. In some areas of the glands type-2 processes are hardly found (Fig. 4). Phenomena of exocytosis are frequently found in freshwater fishes, but seldom in the seawater specimens.

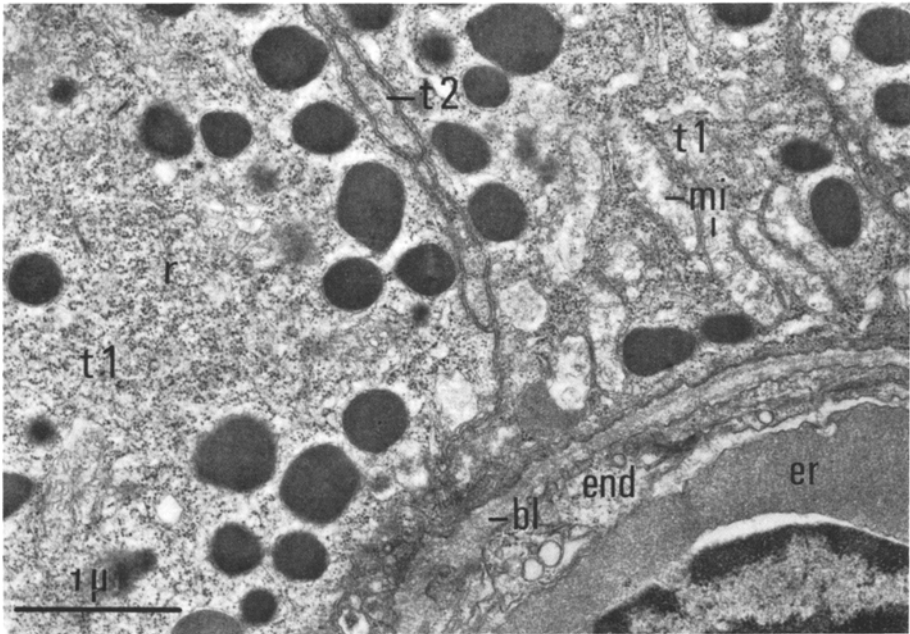
The frequency of phenomena of exocytosis, as expressed per unit length of the outer cell membrane in contact with the connective tissue, most directly reflects the intensity of secretory activity in the gland cells. However, comparison of type-2 cells in freshwater and seawater fishes shows that the length of the outer cell membrane bordering on the connective tissue is a function of the activity of the cells. Therefore, the frequency of exocytotic phenomena cannot be established with great accuracy.

*b) Adaptation of Seawater Sticklebacks to Fresh Water.* Transfer of seawater adapted fishes to fresh water leads to reduction of the activity of type-1 cells and





3



4

**Fig. 3.** Fresh water. Distended ending of a cytoplasmic process of a type-2 cell (*t2*), located between type-1 cells (*t1*). The cell membrane shows an indentation characteristic of exocytosis (arrow); *ct* connective tissue; *ger* granular endoplasmic reticulum

**Fig. 4.** Sea water. Type-1 cells containing secretory granules, many ribosomes (*r*) and some mitochondria (*mi*). Small profiles, without granules, of cytoplasmic processes of type-2 cells (*t2*) are located between the type-1 cells; *bl* basal lamina; *end* endothelial wall of blood capillary; *er* erythrocyte

to activation of type-2 cells, as judged by cytological criteria. After 24 h in fresh water, type-1 cells show accumulation of secretory granules. This is probably due to an abrupt reduction of the release of secretory material, since phenomena of exocytosis are scarce. The mean volume of the nuclei is unchanged after 24 h (Fig. 5a) but the density of the nuclear pores has decreased significantly already after 12 h ( $p < 0.01$ , Fig. 5b). After 48 h the cytoplasm of type-1 cells is still crowded with granules. Focal autolysis is indicated by the presence of membrane-limited vesicles containing cytoplasmic constituents (autophagosomes). Nuclear volume has decreased by more than 30% in comparison to the seawater value ( $p < 0.01$ ; Fig. 5a).

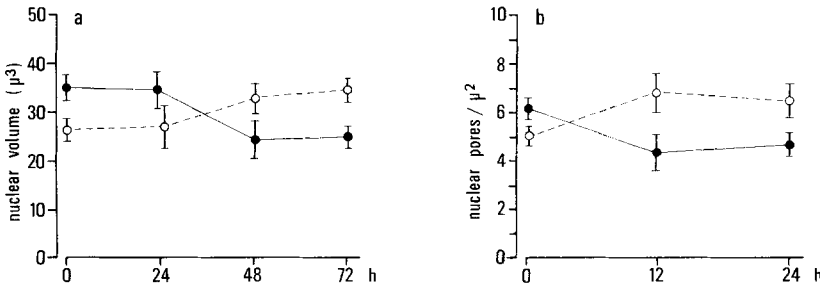
In type-2 cells activation is indicated already 24 h after transfer to fresh water. The number of secretory granules has decreased and, in spite of this degranulation, indications of exocytosis are commonly found. The volume of the nuclei, unchanged after 1 day, has increased by 48 h and reaches values characteristic for fresh water after 72 h ( $p < 0.01$ ; Fig. 5a) which indicates an increase of about 30%. The density of the nuclear pores is already significantly higher after 12 h ( $p < 0.01$ ; Fig. 5b), but does not change further.

*c) The Effects of Hale's Sea Water.* Seawater sticklebacks were adapted to modified Hale's artificial sea water (Lockwood, 1963). This solution of six salts approximates natural sea water as far as osmolality and concentration of the quantitatively most important ions are concerned. After a stay for 16 days in Hale's solution, the secretory cells of the Stannius bodies were structurally similar to those of seawater controls.

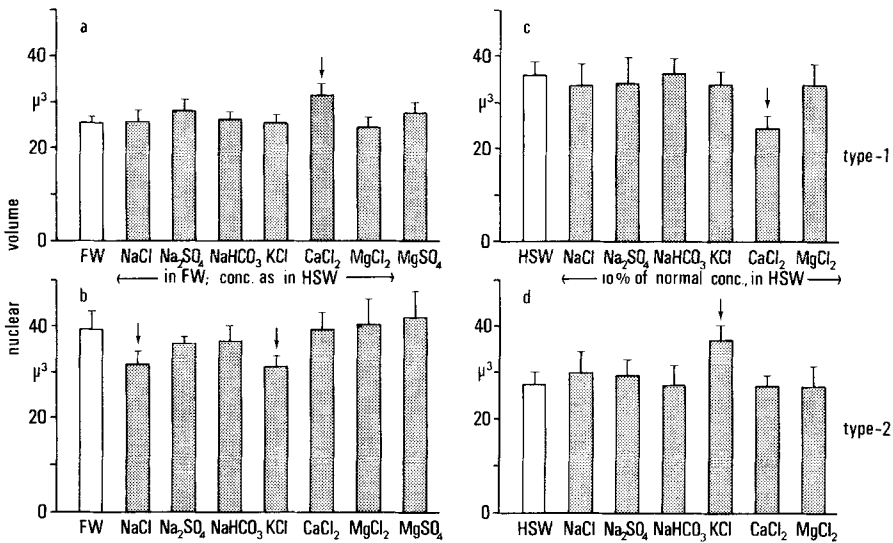
*d) Adaptation to Salt Solutions in Fresh Water.* Freshwater sticklebacks were adapted to solutions in fresh water of one of the 6 salts composing Hale's sea water. The concentrations were the same as in Hale's solution. The results (Fig. 6a, b) show that the volume of the nuclei of the type-1 cells approximates the freshwater value in all solutions, except for the calcium chloride solution. In this medium the nuclei have increased in size significantly ( $p < 0.01$ ). They are slightly smaller than the nuclei of seawater fishes (see Table 1). The growth of the nuclei is apparently due to the high calcium concentration in the medium, and not to chloride. In the sodium chloride solution, with a considerably higher chloride concentration than in the calcium solution, the size of the nuclei of type-1 cells is unaltered.

The nuclei of the type-2 cells decrease in size in both the sodium chloride and the potassium chloride solutions ( $p < 0.01$ ; Fig. 6b) but not in the other media. The values in both solutions are only slightly above the low value characteristic for seawater fishes (see Table 1). The reduction of the nuclear volumes in both solutions is clearly related to the high sodium and potassium levels, and not to the high chloride content. In the solutions with a chloride concentration surpassing that of the potassium chloride solution (magnesium and calcium chloride) the nuclear volumes are unchanged.

The volume of the nuclei in both cell types is apparently unrelated to the osmolality of the ambient medium.

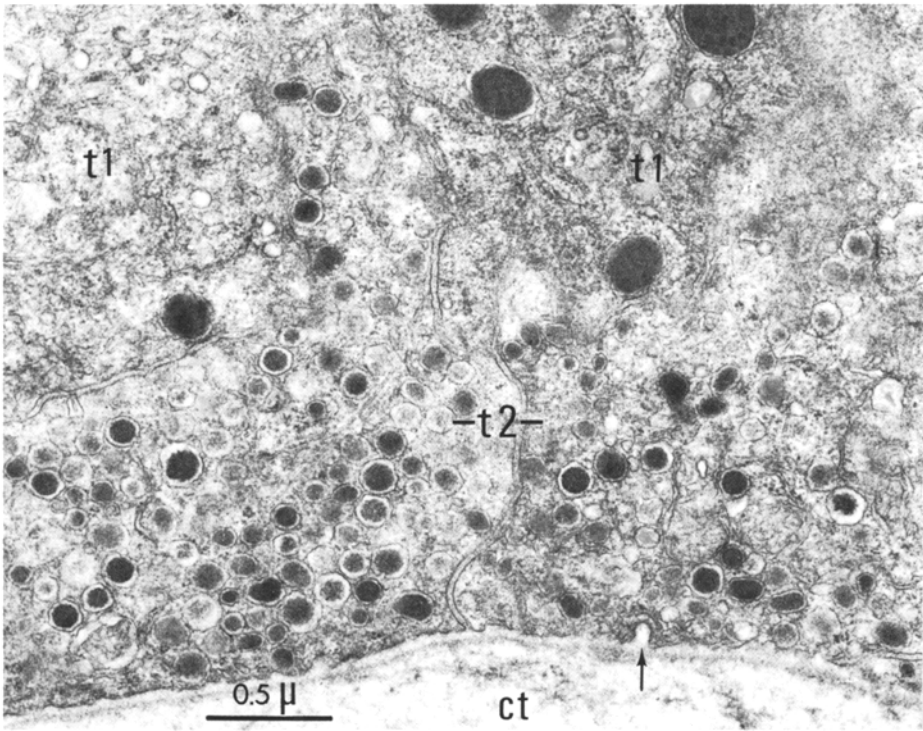


**Fig. 5a and b.** Volume of the nuclei **a** and density of the nuclear pores **b** of type-1 cells (dots) and type-2 cells (circles) of the corpuscles of Stannius of sticklebacks transferred, at 0 h, from sea water to fresh water. Means  $\pm$  S.E. of 5 specimens are given



**Fig. 6a-d.** Volume of the nuclei of type-1 cells (a, c) and of type-2 cells (b, d) of sticklebacks adapted to media of different ionic composition. Arrows: values significantly different from controls (white bars). Means (+ S.E.) refer to 5 animals. **a and b.** Specimens adapted to fresh water (FW; white bars) or to solutions of salts in fresh water (shaded bars). The salts, except for MgSO<sub>4</sub> all components of Hale's artificial sea water (HSW), are indicated below the bars. The concentrations are similar to those in Hale's solution (MgSO<sub>4</sub>: 28.2 mmol/l). **c and d.** Specimens adapted to Hale's artificial sea water (HSW; white bars) or to Hale's sea water with reduced content (10% of normal concentration) of one of its components (indicated below the bars)

Specimens of the groups exposed to calcium, potassium and sodium chloride were examined in the electron microscope. The ultrastructural observations are in line with the results of the light microscopical measurements. The type-1 cells of the fishes exposed to calcium chloride show signs of increased activity. Presecretory granules are frequently found in the Golgi areas, while phenomena of exocytosis are commonly observed. In the type-2 cells no changes are apparent.



**Fig. 7.** Hale's artificial sea water with low potassium concentration. Large accumulations of secretory granules in distended endings of processes of type-2 cells (*t2*); *t1* type-1 cells, *ct* connective tissue; arrow: membrane indentation characteristic of exocytosis

In the groups exposed to potassium chloride the type-1 cells are unaltered, while the type-2 cells show signs of reduced activity. Accumulations of secretory granules in the cell processes, common for freshwater fishes, are scarce. Indications of exocytosis, although not scarce, are less frequently found than in freshwater controls. The corpuscles of the group adapted to sodium chloride appeared similar.

*e) Adaptation to Modified Hale's Sea Water.* Sticklebacks adapted to Hale's sea water were exposed for 10 days to Hale's solution modified by lowering the concentration of one of the component salts to 10% of its normal value. The results of nuclear measurements are presented in Figure 6c, d. The volume of the nuclei of type-1 cells is similar to the control value (Hale's sea water) in all solutions, with the exception of the low-calcium medium in which a decrease occurs. The calcium concentration of this medium (1 mmol/l) approximates that of fresh water. The volume of the nuclei is also similar to the value normally encountered in freshwater fishes. Compared to the controls the difference is statistically significant ( $p < 0.01$ ). Thus, it is likely that the activity of the type-1 cells is related to the calcium content of the medium. The chloride concentration in the low calcium solution is only slightly lower than in the controls.

The volume of the nuclei of the type-2 cells in most solutions is similar to that of the control seawater group, except for the value found in the low-potassium medium, which represents a marked exception. Nuclear volume in this group shows an increase of 30% ( $p < 0.01$ ), and is almost as high as in freshwater fishes.

There is no obvious relationship between the nuclear volume of the gland cells and the osmolality of the media. In the low calcium and low potassium solutions, which induce marked changes in cellular activity, the osmolality is reduced by less than 5% compared to normal sea water.

The groups exposed to low calcium and potassium levels were examined ultra-structurally. The results confirm the conclusions drawn from the changes in nuclear volume. In the low calcium solution the type-1 cells show signs of decreased secretory activity. Lysosome-like bodies, rarely found in normal seawater fishes, occur in low numbers. Indications of exocytosis are extremely scarce. The density of the nuclear pores is reduced by about 30% ( $4.3 \pm 0.4/\mu^2$ ) compared to the value found for seawater fishes (Table 1,  $p < 0.001$ ). The structure of the type-2 cells is unchanged. The density of the nuclear pores ( $5.2 \pm 0.4 \mu^2$ ) is similar to the value of the seawater group ( $p > 0.25$ ).

In the low potassium solution the type-1 cells are unaltered, but the type-2 cells show marked changes. The cytoplasmic processes of these cells are very extensive. They are even more prominent than in freshwater fishes. The distended endings of these processes contain large accumulations of secretory granules (Fig. 7). The density of nuclear pores, unchanged in the type-1 cells ( $6.3 \pm 0.6/\mu^2$ ;  $p > 0.25$ ) amounts to  $6.7 \pm 0.4/\mu^2$  in the type-2 cells, an increase of about 30% compared to the seawater controls (Table 1;  $p < 0.001$ ).

## Discussion

The present results show that the Stannius corpuscles of sticklebacks contain two cell types which are not only structurally different, but which also behave differently in experimental situations.

We found that the secretory activity of the type-1 cells is higher in fishes from the sea than in animals from fresh water. The reverse was found for the type-2 cells. The results of the transfer experiments demonstrate that the composition of the ambient medium accounts for these differences. If sticklebacks are transferred from sea water to fresh water, type-1 cells are inhibited, while type-2 cells are stimulated.

The effect of environmental salinity on the structure of the Stannius corpuscles has been studied in some other species. These studies, concerning the european eel *Anguilla anguilla* (Olivereau, 1964; Hanke et al., 1965; Fontaine and Lopez, 1967), atlantic salmon *Salmo salar* (Fontaine and Lopez, 1965; Heyl, 1970; Carpenter and Heyl, 1974), and the killifish *Fundulus heteroclitus* (Cohen et al., 1975) have shown that in general the cells are more active in sea water than in fresh water. Ogawa (1967) observed an increase in cell size and hypertrophy of Golgi apparatus and endoplasmic reticulum in Stannius corpuscles of freshwater goldfish, *Carassius auratus*, during adaptation to diluted sea water. These observations obviously concern the predominant (or only) cell type described in the

species mentioned, i.e. the cell type which is structurally comparable to the type-1 cell in the stickleback (Wendelaar Bonga and Greven, 1975). Thus, there is not only a structural resemblance but also a similarity in the response to sea water between the type-1 cells of the stickleback and the principal cell type found in other species.

The results of experiment *d* and *e* show that it is mainly the calcium concentration of the medium which accounts for the high secretory activity of type-1 cells in sea water and the low activity in fresh water. Involvement of Stannius bodies in calcium regulation has been demonstrated frequently since Fontaine (1964) first showed that extirpation of the bodies in european eels leads to prolonged hypercalcemia. This observation has been confirmed in the same species by Chan and Chester Jones (1968), in american eels (*Anguilla rostrata*) by Fenwick (1974), and in killifish by Pang and coworkers (Pang, 1971; Pang et al., 1973). Normal blood calcium levels can be obtained by reimplantation or by injection of homogenates of the glands (Chan et al., 1969; Lopez, 1970; Fenwick and Forster, 1972; Pang et al., 1973). Pang et al. (1974) have suggested the name hypocalcin for the hypocalcemic factor they demonstrated in homogenates of Stannius bodies of cod (*Gadus morrhua*) and killifish (*Fundulus heteroclitus*). The hypocalcemic effect is most marked in a calcium rich environment such as sea water or calcium enriched fresh water. Probably, in sticklebacks the type-1 cells produce a hypocalcin-like substance.

The type-2 cells do not react to variations in calcium content of the ambient medium. As our data show, these cells respond instead to changes in the concentrations of sodium and potassium ions of the environment. A low concentration of these ions, as in fresh water, stimulates the secretory activity of these cells, whereas high concentrations as found in sea water are inhibitory. The sodium and potassium concentrations in the fresh water used in the experiments (2.13 and 0.06 mmol/l, respectively) are considerably lower than those of the blood plasma ( $\pm 175$  and  $\pm 5$  mmol/l). Accordingly, in fresh water, fishes have to cope with losses of these ions due to outward diffusion and excretion. To maintain the high levels in blood plasma and intracellular fluid, uptake of both ions from the outer medium is essential. We suggest that these cells produce a hormone that stimulates the uptake and/or reduces the losses of sodium and potassium ions. The high secretory activity in type-2 cells of freshwater adapted fishes, as well as the increase of the activity in Hale's sea water with reduced potassium content (1 mmol/l, i.e. lower than the plasma potassium level in sea water,  $\pm 5$  mmol/l), are in line with this hypothesis. The activity of the type-2 cells in Hale's sea water with low sodium content was not noticeably altered, which does not support our interpretation. However, the absence of a notable effect may be due to the fact that the difference between the sodium concentration of this medium (100 mmol/l) and of the plasma sodium level in seawater fishes (188 mmol/l) is relatively small. But the possibility cannot be excluded that type-2 cells are primarily involved in the control of potassium metabolism. The effects of sodium on these cells may be indirect, since sodium and potassium metabolism, especially at the level of the gills (Evans et al., 1973; Maetz, 1974), are closely linked. Our results are open for other interpretations, however, since for instance changes in sodium and potassium levels in the environment also affect the acid-base balance (Maetz, 1974).

A relationship between Stannius corpuscles and monovalent ions, as found in the present study, has been reported before. Removal of the corpuscles in eels not only leads to an increase in ionic calcium in the blood but also to changes in sodium and potassium levels, as has been observed in the european eel, *Anguilla anguilla* (Fontaine, 1964, 1972), the american eel, *A. rostrata* (Butler, 1969, 1972), and the asiatic eel, *A. japonica* (Chan, 1972). These effects have been interpreted as indirect consequences of the operation, caused by the high plasma calcium levels on interrenal cells (Leloup-Hatey, 1970), on ultimobranchial bodies (Chan, 1972), on the prolactin producing cells in the hypophysis (Pang et al., 1975), or on ion permeability of the gills (Fleming et al., 1973). The high calcium levels certainly will affect many body functions. But the possibility cannot be ignored that the changed sodium and potassium levels after removal of the corpuscles in eels are partly due to the disappearance of a cell type comparable to the type-2 cells in sticklebacks. The corpuscles of european eels contain a second cell type which resembles the type-2 cells of sticklebacks, not only structurally (Wendelaar Bonga and Greven, 1975), but also in its response to high potassium levels. Addition of potassium chloride to the medium induces structural signs of inactivation in the type-2 cells of freshwater eels (unpublished observations), as in freshwater sticklebacks. This reaction points to a role of the type-2 cells in the maintenance of high blood and tissue potassium levels in fresh water, i.e. a medium with an extremely low potassium content. This interpretation is not supported by the results of removal of the corpuscles in eels, which is followed by a rise of potassium and a fall of sodium plasma levels. Butler (1969) concluded that the operation leads to a shift of potassium from the muscles into the extracellular fluid. Such an effect may obscure the presumed function of the corpuscles in the control of total body sodium and potassium.

Indirect evidence for the involvement of type-2 cells in the control of sodium and potassium is offered by the results of corpuscle removal in two other teleost species, goldfish (Ogawa, 1968) and killifish (Pang et al., 1975). The removal of the corpuscles in these fishes leads to a marked hypercalcemia, but in contrast to the results obtained with eels, consistent effects on plasma sodium and potassium levels could not be demonstrated. Ultrastructural examination of the Stannius corpuscles of goldfish (Oguri, 1966; Ogawa, 1967; our unpublished results), and killifish (Cohen et al., 1975) revealed only one secretory cell type, comparable to the type-1 cells of sticklebacks and eels. The presence of type-2 cells in the european eel (Wendelaar Bonga and Greven, 1975) and the absence of a similar cell type in goldfish and killifish, may explain the differences found between these species in the effects of removal of the corpuscles.

## References

- Butler, D.G.: Corpuscles of Stannius and renal physiology in the eel (*Anguilla rostrata*). J. Fish. Res. Bd. Canada **26**, 639–654 (1969)
- Butler, D.G.: Failure to observe changes in selected metabolites following removal of the Stannius corpuscles from the freshwater North American eel, *Anguilla rostrata*. J. Fish. Res. Bd. Canada **29**, 1362–1364 (1972)
- Carpenter, S.J., Heyl, H.L.: Fine structure of the corpuscles of Stannius of Atlantic salmon during the freshwater spawning journey. Gen. comp. Endocr. **23**, 212–223 (1974)

- Chan, D.K.O.: Hormonal regulation of calcium balance in teleost fish. *Gen. comp. Endocr.*, Suppl. **3**, 411–420 (1972)
- Chan, D.K.O., Chester Jones, J.: Regulation and distribution of plasma calcium and inorganic phosphate in the European eel (*Anguilla anguilla*). *J. Endocr.* **42**, 109–117 (1968)
- Chan, D.K.O., Rankin, J.C., Chester Jones, J.: Influences of the adrenal cortex and the corpuscles of Stannius on osmoregulation in the European eel (*Anguilla anguilla* L.) adapted to fresh water. *Gen. comp. Endocr.*, Suppl. **2**, 342–354 (1969)
- Cohen, R.S., Clark, N.B., Pang, P.K.T.: Ultrastructure of the corpuscles of Stannius of the killifish, *Fundulus heteroclitus*, and its relation to calcium metabolism. *Gen. comp. Endocr.* **27**, 413–424 (1975)
- Evans, D.H., Mallery, C.H., Kravitz, L.: Sodium extrusion by a fish acclimated to seawater: physiological and biochemical description of a Na- for-K exchange system. *J. exp. Biol.* **58**, 627–636 (1973)
- Fenwick, J.C.: The corpuscles of Stannius and calcium regulation in the North American eel (*Anguilla rostrata* Lesueur). *Gen. comp. Endocr.* **23**, 127–135 (1974)
- Fenwick, J.C., Forster, M.E.: Effects of stanniectomy and hypophysectomy on total plasma cortisol levels in the eel (*Anguilla anguilla* L.). *Gen. comp. Endocr.* **19**, 184–191 (1972)
- Fleming, W.R., Brehe, J., Hanson, R.: Some complicating factors in the study of the calcium metabolism of teleosts. *Amer. Zool.* **13**, 793–797 (1973)
- Fontaine, M.: Corpuscules de Stannius et régulation ionique. (Ca, K, et Na) du milieu intérieur de l'anguille (*Anguilla anguilla* L.). *C.R. Acad. Sci. (Paris), Sér. D* **259**, 875–878 (1964)
- Fontaine, M.: Intervention des corpuscules de Stannius dans l'équilibre phosphocalcique du milieu intérieur d'un poisson téléostéen l'anguille. *C.R. Acad. Sci. (Paris), Sér. D* **264**, 736–737 (1967)
- Fontaine, M., Lopez, L.: Endocrine function of corpuscles of Stannius with special reference to the physiological preparation for catadromic migration of two migratory teleosts (*Salmo salar* L. and *Anguilla anguilla vulgaris* L.). XXIII. *Int. Congr. Physiol. No.* **244** (1965)
- Fujita, H., Honma, Y.: On the fine structure of corpuscles of Stannius of the eel, *Anguilla japonica*. *Z. Zellforsch.* **77**, 175–187 (1967)
- Hanck, W., Bergerhoff, K., Chan, D.K.O.: Histological observations of pituitary ACTH-cells, adrenal cortex, and the corpuscles of Stannius of the european eel (*Anguilla anguilla* L.). *Gen. comp. Endocr.* **9**, 64–75 (1967)
- Heyl, H.I.: Changes in the corpuscles of Stannius during the spawning journey of the Atlantic salmon (*Salmo salar*). *Gen. comp. Endocr.* **14**, 43–52 (1970)
- Krishnamurthy, V.G., Bern, H.A.: Correlative histologic study of the corpuscles of Stannius and the juxtaglomerular cells of teleost fishes. *Gen. comp. Endocrinol.* **13**, 313–335 (1969)
- Leloup-Hatey, J.: Influence de l'ablation des corpuscules de Stannius sur le fonctionnement de l'inter-rénal de l'anguille (*Anguilla anguilla* L.). *Gen. comp. Endocr.* **15**, 388–394 (1970)
- Lockwood, A.P.M.: Animal body fluids and their regulation. London: Heinemann 1963
- Lopez, E.: L'os cellulaire d'un poisson téléostéen, *Anguilla anguilla* L. II. Action de l'ablation des corpuscules de Stannius. *Z. Zellforsch.* **109**, 566–572 (1970)
- Lott, J.N.A., Larsen, P.L., Whittington, C.M.: Frequency and distribution of nuclear pores in *Cucurbita maxima* cotyledons as revealed by freeze-etching. *Canad. J. Bot.* **50**, 1785–1787 (1972)
- Maetz, J.: Aspects of adaptation to hypo-osmotic and hyper-osmotic environments. In: *Biochemical and biophysical perspectives in marine biology*, Vol. 1 (D.C. Malins and J.R. Sargent, eds.), pp. 1–167. London-New York-San Francisco: Academic Press 1974
- Nadkarni, V.B., Gorbman, A.: Structure of the corpuscle of Stannius in normal and spawning pacific salmon. *Acta zool. (Stockh.)* **47**, 61–66 (1966)
- Ogawa, M.: Fine structure of the corpuscles of Stannius and the interrenal tissue in goldfish, *Carassius auratus*. *Z. Zellforsch.* **81**, 174–189 (1967)
- Ogawa, M.: Osmotic and ionic regulation in goldfish following removal of the corpuscles of Stannius or the pituitary gland. *Canad. J. Zool.* **46**, 669–676 (1968)
- Oguri, M.: Electron microscopic observations on the corpuscles of Stannius in goldfish. *Bull. Jap. Soc. Sci. Fish.* **32**, 903–908 (1966)
- Olivereau, M.: Corpuscles of Stannius and osmoregulation in eels. *Amer. Zool.* **4**, 326–327 (1964)
- Pang, P.K.T.: The relationship between corpuscles of Stannius and serum electrolyte regulation in killifish, *Fundulus heteroclitus*. *J. exp. Zool.* **178**, 1–8 (1971)
- Pang, P.K.T., Pang, R.K.: Environmental calcium and hypocalcin activity in the Stannius corpuscles of the channel catfish, *Ictalurus punctatus* (Rafinesque). *Gen. comp. Endocr.* **23**, 239–241 (1974)



- Pang, P.K.T., Pang, R.K., Griffith, R.W.: Corpuscles of Stannius: lack of direct involvement in the regulation of serum sodium, potassium, and chloride in the teleost *Fundulus heteroclitus*. Gen. comp. Endocr. **26**, 179–185 (1975)
- Pang, P.K.T., Pang, R.K., Sawyer, W.H.: Effects of environmental calcium and replacement therapy on the killifish *Fundulus heteroclitus*, after the surgical removal of the corpuscles of Stannius. Endocrinology **93**, 705–710 (1973)
- Pang, P.K.T., Pang, R.K., Sawyer, W.H.: Environmental calcium and the sensitivity of the killifish (*Fundulus heteroclitus*) in bioassays for the hypocalcemic response to Stannius corpuscles from killifish and cod (*Gadus morrhua*). Endocrinology **94**, 548–555 (1974)
- Scheer, U.: Nuclear pore flow rate of ribosomal RNA and chain growth of its precursor during oogenesis. Develop. Biol. **30**, 13–28 (1972)
- Schreibman, M.P., Pang, P.K.T.: The histophysiology of transplanted corpuscles of Stannius in the killifish *Fundulus heteroclitus*. Gen. comp. Endocr. **26**, 186–191 (1975)
- Tomasulo, J.A., Duane Belt, W., Russell Hayes, E.: The fine structure of the Stannius body of the guppy and its response to deionized water. Amer. J. Anat. **129**, 307–328 (1970)
- Wendelaar Bonga, S.E., Greven, J.A.A.: A second cell type in Stannius bodies of two euryhaline teleost species. Cell Tiss. Res. **159**, 287–290 (1975)
- Wendelaar Bonga, S.E., Greven, J.A.A., Mein, C., Veenhuis, M.: Morphometrical study with light and electron microscope of the role in ionic regulation of two endocrine cell types in Stannius bodies of sticklebacks. Gen. comp. Endocr. **29**, 282 (1976)
- Wendelaar Bonga, S.E., Veenhuis, M.: The effect of prolactin on the number of membrane-associated particles in kidney cells of the euryhaline teleost *Gasterosteus aculeatus* during transfer from sea water to fresh water: a freeze-etch study. J. Cell Sci. **16**, 687–701 (1974)

*Accepted April 14, 1976*