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Source: Zoological Science, 15(2) : 263-276

Published By: Zoological Society of Japan

URL: <https://doi.org/10.2108/zsj.15.263>

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Immunocytochemical and Ultrastructural Identification of Pituitary Cell Types in the Protogynous *Thalassoma duperrey* during Adult Sexual Ontogeny

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ABSTRACT—Protogynous wrasses (*Thalassoma duperrey*): females (F), primary males (PM) along with a few terminal-phase males (TM) and sex-changed males (SM), were used to characterize the topographical organization of the pituitary. In general, immunocytochemical and ultrastructural features of the adeno-hypophyseal cell types of the saddleback wrasse pituitary resemble those of other teleosts. In the rostral pars distalis (RPD), corticotropic cells were found bordering the neurohypophysis (NH) and surrounding the centroventrally located prolactin cells. Thyrotropic cells formed a small group in the anteriodorsal part of the rostral and proximal pars distalis (PPD). The somatotropic cells were distributed in large clusters, mostly organized in cell cords around the interdigitations of the NH of the dorsal PPD. Cells containing gonadotropin I β subunit were localized in the dorsal parts of the PPD, in close association with somatotropic cells and gonadotropin II β subunit containing cells were seen in the centroventral parts of the PPD and along the periphery of the pars intermedia (PI). The pars intermedia was composed of melanotropic cells and somatolactin cells that lined the neurohypophysis.

Distinct ultrastructural differences in corticotropic and somatotropic cells were not observed between the four groups. In all groups, prolactin cells in the ventral-most RPD could be immature cells or actively secreting prolactin. Gonadotropic II cells of PM and F had relatively higher incidence of “nuclear budding” and cell organelles compared to TM and SM. Besides gonadotropic, the active melanotropic and somatolactin cells might be associated with some aspect(s) of reproduction.

INTRODUCTION

Several groups of coral reef fish, including many species of wrasse (Labridae), are sequential hermaphrodites. Various external factors (Ross, 1981; Shapiro, 1990) have been found to induce sex change, but little is known of the internal processes which mediate their effects. A change in steroid levels has been observed during sex change in the saddleback wrasse *Thalassoma duperrey* (Nakamura *et al.*, 1989; Hourigan *et al.*, 1991). The effects of sex steroids on behavioral or gonadal sex change could operate via the hypothalamo-hypophyseal axis. Using techniques which include histochemistry (Nagahama, 1973; Bern *et al.*, 1974), immunocytochemistry (Munro, 1985; Quesada *et al.*, 1988; Toubeau *et al.*, 1991;

Garcia-Hernandez *et al.*, 1996) and ultrastructure (Bern *et al.*, 1974; Batten, 1986; Quesada *et al.*, 1988; Garcia-Ayala *et al.*, 1997) the different cell types in the pituitary of teleosts have been shown to segregate between three zones of the adeno-hypophysis. The prolactin cells and the corticotropic cells are located in the rostral pars distalis. The somatotropic cells and the gonadotropic cells are found in the proximal pars distalis. The somatolactin and the melanotropic cells are present in the pars intermedia. The neurohypophysis, on the other hand, consists of peptidergic, aminergic and GABAergic axons innervating from the hypothalamus (Kah *et al.*, 1987; Batten *et al.*, 1990; Holmqvist and Ekstrom, 1995). Thus, the adeno-hypophyseal cell activity in teleost is under a direct hypothalamic control (Peter *et al.*, 1990). To our knowledge, the pituitary of only two sex-changing fishes (*Crenilabrus melops*: Benjamin, 1979; *Monopterus albus*: O and Chan, 1974) have been studied at light microscopic level.

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Thalassoma duperrey is found in abundance on Hawaiian coral reefs. This protogynous hermaphrodite is diandric, i.e. primary males (PM) are born as males and mature to become terminal-phase males (TM) without the prior existence of a female (F) phase, whereas functional secondary males (SM) are derived from mature F that undergo sex change (Ross, 1981). F, PM and TM are easily caught and identified from the reefs. SM, however, can only be ascertained histologically (after death) or experimentally by rearing caged F (Ross *et al.*, 1983).

In the present study, pituitaries from F, PM and TM caught in the field, as well as a small sample of transformed secondary males (F induced to change sex), were included to observe the immunocytochemistry and the ultrastructure of the adenohypophyseal cells. This study will provide a basis for future research on the relationship between hypothalamo-hypophyseal control of various physiological processes including sex change.

MATERIALS AND METHODS

Immunocytochemistry

Thalassoma duperrey (males and females; $n = 9$) collected from the Kaneohe Bay around the Hawaii Institute of Marine Biology were rapidly decapitated, pituitaries were removed and fixed in Bouin's solution. All the tissues were then dehydrated through a graded series of ethanols, cleared in *n*-butanol and embedded in paraffin (Paraplast Plus; Oxford Labware, USA). Serial sections (8 μm) in sagittal, horizontal and coronal planes were cut and processed for immunocytochemistry. Localization of pituitary cell types was carried out by immunocytochemical procedures, as previously described by Parhar *et al.* (1995).

The rabbit anti-coho salmon gonadotropin I β -subunit (Lot #8510, diluted 1 : 2,000), anti-coho salmon gonadotropin II β -subunit (Lot #9010, diluted 1 : 2,000), anti-chum salmon growth hormone (Lot #8502, diluted 1 : 2,500), anti-chum salmon prolactin (Lot #8208, diluted 1 : 2,000), and anti-cod fish somatolactin (Lot #9105, diluted 1:900) were provided by Dr. H. Kawauchi, Kitasato University, Japan. The rabbit anti-rat thyrotropin β -subunit (Lot #HAC-RT29-01RBP86, diluted 1 : 2,000) was provided by Dr. K. Wakabayashi, Gunma University, Japan. All dilutions were made with 0.01 M phosphate buffered saline (PBS; pH 7.6).

The sections were deparaffinized in xylene, rehydrated through graded ethanols, washed in phosphate buffered saline, and incubated in a solution of gelatin (0.75%) and a solution of normal goat serum (1%), each for 10 min.

After 48 hr incubation with primary antiserum at 4°C, sections were incubated in biotinylated anti-rabbit IgG followed by avidin-biotin-horseradish peroxidase complex (Vectastain "ABC" Elite Kit, Vector Labs.). Following incubation in the "ABC" complex, the sections were thoroughly washed in PBS followed by a wash in 0.05 M Tris buffer, pH 7.6 (Sigma). The sections were then immersed in 0.05% DAB (3,3'-diaminobenzidine tetrahydrochloride) or 4-chloro-naphthol as a chromogen, with 0.001% H₂O₂ in 0.05 M Tris buffer, washed thoroughly in water, dehydrated through graded ethanols, cleared in xylene and coverslips applied with Permount (Fisher Scientific, USA).

The specificity of the antisera and validation for localization of pituitary hormones was determined at the time of its production by Prof. H. Kawauchi and co-workers (Naito *et al.*, 1983; Nozaki *et al.*, 1990; Rand-Weaver *et al.*, 1991). These pituitary antisera have been used in our previous studies (Parhar and Iwata, 1994; Parhar *et al.*, 1995) and have been shown to be highly specific.

Ultrastructure

Thalassoma duperrey were collected by hook and line from Kaneohe Bay, Oahu, Hawaii between May and July, 1987. Fish were either decapitated at sea and their pituitaries fixed for electron microscopy (F: $n = 13$; PM: $n = 8$; TM: $n = 4$) or they were brought back to the Hawaii Institute of Marine Biology (HIMB) for experimental study.

Males were classified as PM or TM males based on size (standard length) and morphology (Hourigan *et al.*, 1991). PM(s) ranged in size from 80–90 mm. These individuals had large testis. TM(s) ranged from 130–140 mm. These individuals had thin, "thread-like" testis. Females, like PM were 80–90 mm in standard length.

Female wrasses were identified for experimental sex change by either cannulation (insertion of a small diameter tubule into the reproductive tract for gamete extraction) or by gently squeezing the abdomen to exude gametes as in Ross (1984). Females thus identified were placed in submerged pens constructed of 12.7 mm² wire mesh with approximate dimensions of 1 m³. Females induced to change sex after 9 weeks in these pens (Ross *et al.*, 1990) were used as secondary males (SM: $n = 2$; standard length 90 and 101 mm) for this study. The probability of sex change in females of this fish is a function of their relative size in the social group (Ross *et al.*, 1990).

For ultrastructural study, pituitaries were fixed in Karnovsky's formaldehyde-glutaraldehyde fixative (1–3 hr) and then washed overnight in 0.1 M sodium cacodylate buffer (pH 7.2). After post-fixation in cacodylate-buffered 1% osmium tetroxide and subsequent dehydration through a graded series of alcohols, the pituitaries were embedded in Spurr resin (Sigma, USA). Sections 1 μm thick were stained with 1% toluidine blue in 1% borax. Ultrathin sections picked on copper grids (100–150 mesh) were stained with uranyl acetate/lead citrate. A JEOL 100CX transmission electron microscope was used for observations.

For any one cell type, a random sample of about 10 electron micrographs were photographed from mid-sagittal sections of the pituitary gland. Electron micrographs printed at an initial magnification of $\times 7,200$ and the final, magnification of $\times 14,000$ were used to determine the diameter of the secretory granules. Using the limiting membrane as the boundary, the major (a) and minor (b) axes of secretory profiles were measured. Each secretory profile was then considered to be a circle whose diameter was $(a + b)/2$. An estimate of mean secretory diameter was obtained from several measurements per cell type. But for prolactin and thyrotropic cells all granules present in each cell were measured. From this, the mean and the standard deviation of the granule diameter was estimated.

The functional morphology of each cell type was classified according to the extent of rough endoplasmic reticulum (RER), the amount of secretory granules, presence or absence of mitochondria and Golgi zones. These were then, subjectively, represented as scores on a five-point scale as follows: –, absent; +, few/scarcely; ++, moderate; +++, many; +++++, very many.

RESULTS

As in the majority of teleost fish, the pituitary of *T. duperrey* consists of the adenohypophysis and the neurohypophysis. The adenohypophysis can be subdivided into three distinct regions: rostral pars distalis (RPD), the proximal pars distalis (PPD) and pars intermedia (PI). Nerve fibers innervating the pars distalis and pars intermedia form the neurohypophysis (NH). The distribution of the different endocrine cell types is shown in Fig. 1.

Rostral pars distalis (RPD)

Corticotropic (ACTH) cells

In 1 μm thick, toluidine blue stained sections, ACTH cells

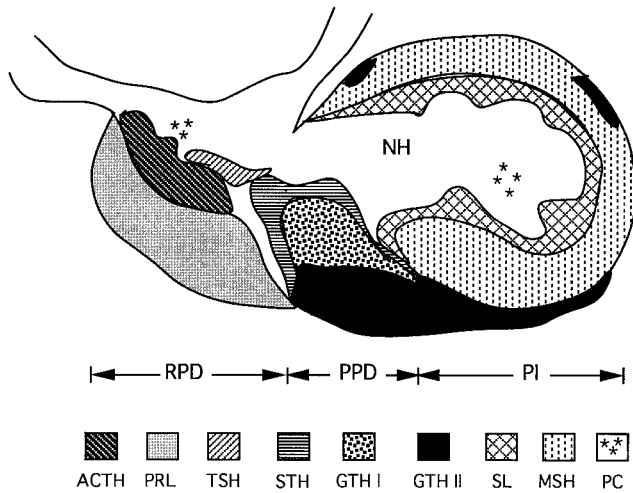


Fig. 1. Diagrammatic pituitary of *Thalassoma duperrey*, showing the distribution of endocrine cell types. RPD, rostral pars distalis; PPD, proximal pars distalis; PI, pars intermedia; NH, neurohypophysis; ACTH, corticotropic cells; PRL, prolactin cells; STH, somatotropic cells; GTH I, gonadotropin I cells; GTH II, gonadotropin II cells; TSH, thyrotropic cells; MSH, melanotropic cells; SL, somatolactin cells; pituicytes (**).

were distributed as a compact mass of chromophilic cells lining the interdigitation of the NH of the rostral pars distalis. These cells formed a layer between the NH and the prolactin cells (Fig. 2a–c).

At the ultrastructural level ACTH cells were irregular in shape. The cytoplasm contained numerous secretory granules with electron-dense center separated by a clear ring (Fig. 3). Granules averaged 150.1 ± 7.2 nm in diameter. Some RER, dilated into small cisternae were scattered throughout the cytoplasm. The mitochondria were moderately developed and the Golgi zones were rarely observed.

The ACTH cells of all groups were similar (Table 1).

Prolactin (PRL) cells

In one micron thick, toluidine blue stained sections, PRL cells appeared chromophobic. The prolactin cells, revealed by anti-chum salmon PRL, were located throughout the RPD and were numerous compared to ACTH cells located dorsally (Fig. 2b).

At the ultrastructural level PRL cells were polygonal with slightly indented nuclei. Their cytoplasm was pale, with a small number of secretory granules (Fig. 5a). The average granule diameter (153.8 ± 6.7 nm) was almost the same as that of

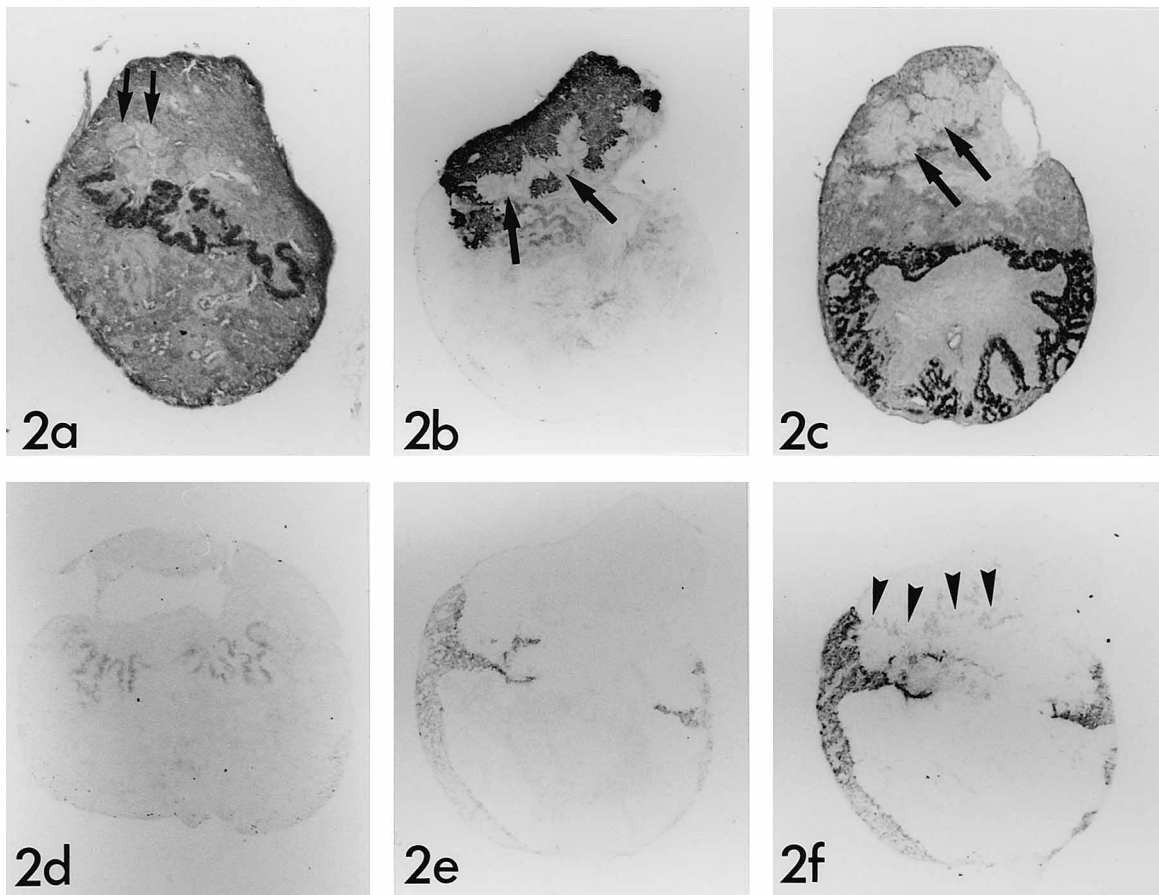


Fig. 2a–f. Photomicrographs of *Thalassoma duperrey* pituitary, showing the distribution of immunoreactive endocrine cell types (dark regions). (a) Somatotropic cells; (b) prolactin cells; (c) somatolactin cells; (d) GTH I cells; (e) GTH II cells; (f) arrowheads indicate cells immunoreactive to anti-rat thyrotropin but not to anti-gonadotropin β or IIF; (a–c) arrows indicate ACTH cells. $\times 40$.

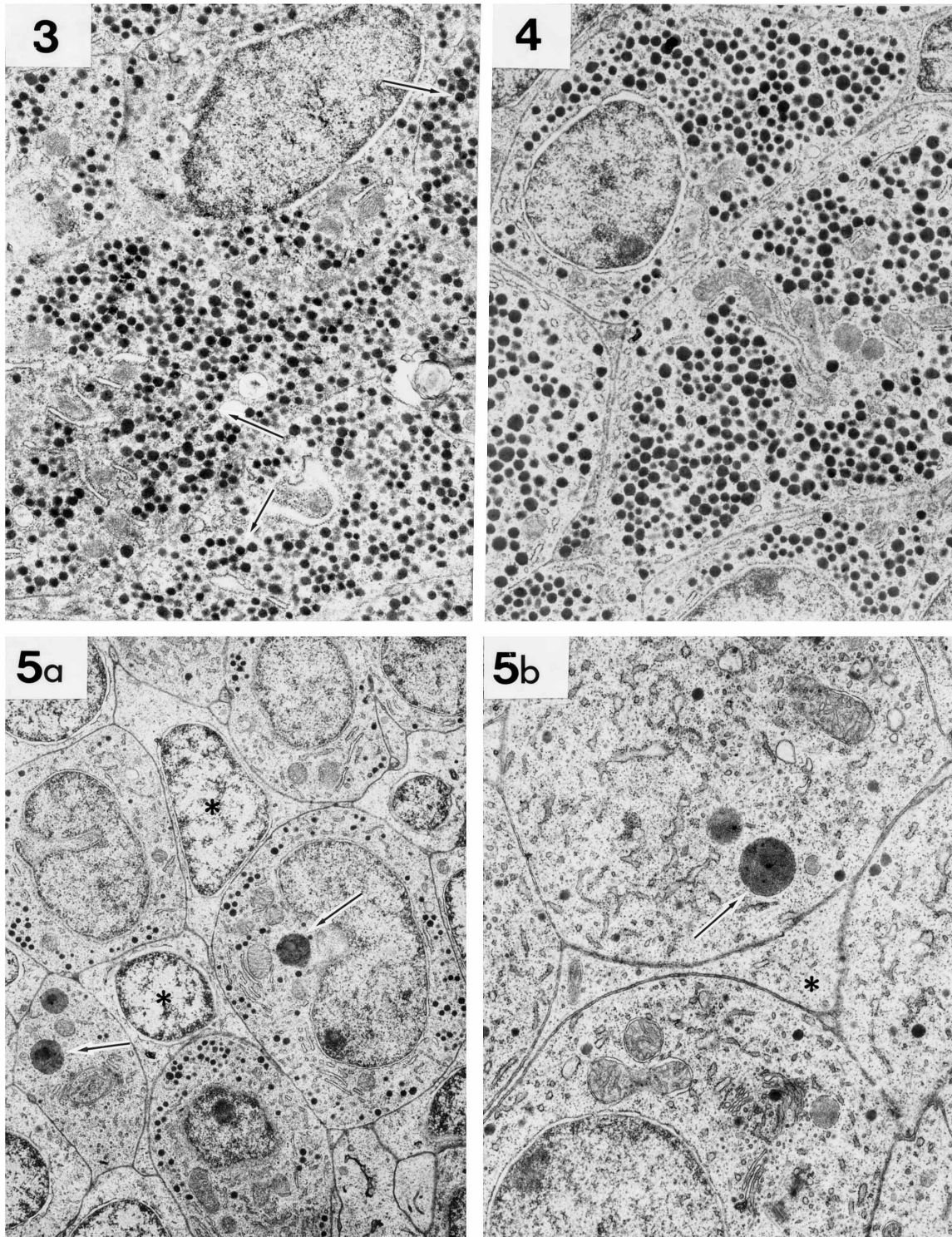


Fig. 3. ACTH cell of primary male full of granules. Note (arrow) granules with electron-dense centers separated by a clear ring. $\times 18000$.

Fig. 4. STH cells of female filled with numerous electron-dense granules. $\times 9000$.

Fig. 5a–b. PRL cells of female: (a) dorsally located cells having many secretory granules ($\times 6900$) relative to; (b) ventrally located cells. $\times 12000$. Lysosomes (arrow) seen in some cells. Stellate cells (*) interspaced among PRL cells.

ACTH granules (150.1 ± 7.2 nm). More ventrally located prolactin cells, near the peripheral zone of the RPD, were either almost devoid of or had few mature granules (Fig. 5b). A few

cells had electron-dense globular structures (lysosomes-like structure). The RER was fairly dilated to form small cisternae, and remained moderate in number. The mitochondria and the

Table 1. Sizes of granules and scores of organelles in endocrine cells of *T. duperrey*, during various reproductive phases

Cell types	Granule size (nm) Mean \pm S.E	Sexual phase	Globule number	Granule number	Golgi zones	Mitochondria	RER	
ACTH	150.1 \pm 7.2	F	–	+++	+	++	+	
		PM	–	+++	+	++	+	
		TM	–	–	+++	+	++	+
		SM	–	–	+++	+	++	+
PRL	153.8 \pm 6.7	F	–	++	++	++	++	
		PM	–	+	++	++	++	
		TM	–	+	++	++	++	
		SM	–	+	++	++	++	
TSH	135.2 \pm 4.1	F	–	+	+	++	+	
		PM	–	+	+	++	+	
		TM	–	+	+	++	+	
		SM	–	+	+	++	+	
STH	391.8 \pm 21.5	F	–	+++	++	++	+	
		PM	–	+++	++	++	+	
		TM	–	–	+++	++	++	+
		SM	–	–	+++	++	++	+
GTH I	283.5 \pm 13.4 660.7 \pm 31.7 (globule)	F	++	++	++	++	++	
		PM	+	++	++	++	++	
		TM	+	+++	++	++	++	
		SM	+	+++	++	++	++	
GTH II	320.5 \pm 18.9	F	–	+++	++	++	++	
		PM	–	+++	++	++	++	
		TM	–	–	+++	+	+	+
		SM	–	–	+++	+	+	+
MSH	322.7 \pm 13.7	F	+	+++	++	++	+++	
		PM	+	+++	++	++	+++	
		TM	+	+++	++	++	++++	
		SM	+	+++	++	++	++++	
SL	158.0 \pm 5.8 (round) 234.9 \pm 9.4 (Oval) 1081.3 \pm 64.4 (globule)	F	+	+++	+++	+++	+++	
		PM	–	–	+++	+++	+++	
		TM	+	+	++	+++	+++	
		SM	–	–	+++	+	+	

Score scale: –, absent; +, few/scarce; ++, moderate; +++, many; +++++, very many. RER, rough endoplasmic reticulum.

Golgi zones were moderately developed.

In females, the more dorsally located PRL cells had many mature granules relative to other groups (Fig. 5a, b; Table 1).

Stellate cells

Ultrastructurally many of these cells, with a very pale cytoplasm, were present among the prolactin cells. These cells did not contain granules but had mitochondria and large processes spreading between PRL cells, in a honeycomb-like form (Fig. 5a, b).

Proximal pars distalis (PPD)

Somatotropic (STH) cells

The somatotrophic cells, revealed by anti-chum salmon STH were distributed in large clusters in the dorsal regions of the proximal pars distalis. These cells were mostly organized in cell cords around the interdigitations of the NH of the proximal pars distalis (Fig. 2a).

Ultrastructurally, STH cells were columnar in shape and had an oval nucleus. An eccentrically placed nucleolus was seen in a few cells (Fig. 4). The cytoplasm contained numerous electron-dense granules of varying diameter (391.8 \pm 21.5 nm) and shapes. The RER was dilated into small cisternae. The well developed mitochondria, round or rod-shape were scattered throughout the cytoplasm. The Golgi zones were moderately developed.

STH cells of all groups were similar (see Table 1).

Gonadotropin (GTH) I cells

Cells in the wrasse pituitary were not immunoreactive to anti-coho salmon GTH I β . However, in one specimen GTH I β immunoreactivity was seen located in the dorsal proximal pars distalis similar in distribution to somatotrophic cells but distinct from GTH I β immunoreactivity (Fig. 2d, e).

At the ultrastructural level GTH I cells appeared polyhedral in shape with a cytoplasm paler than the adjacent STH

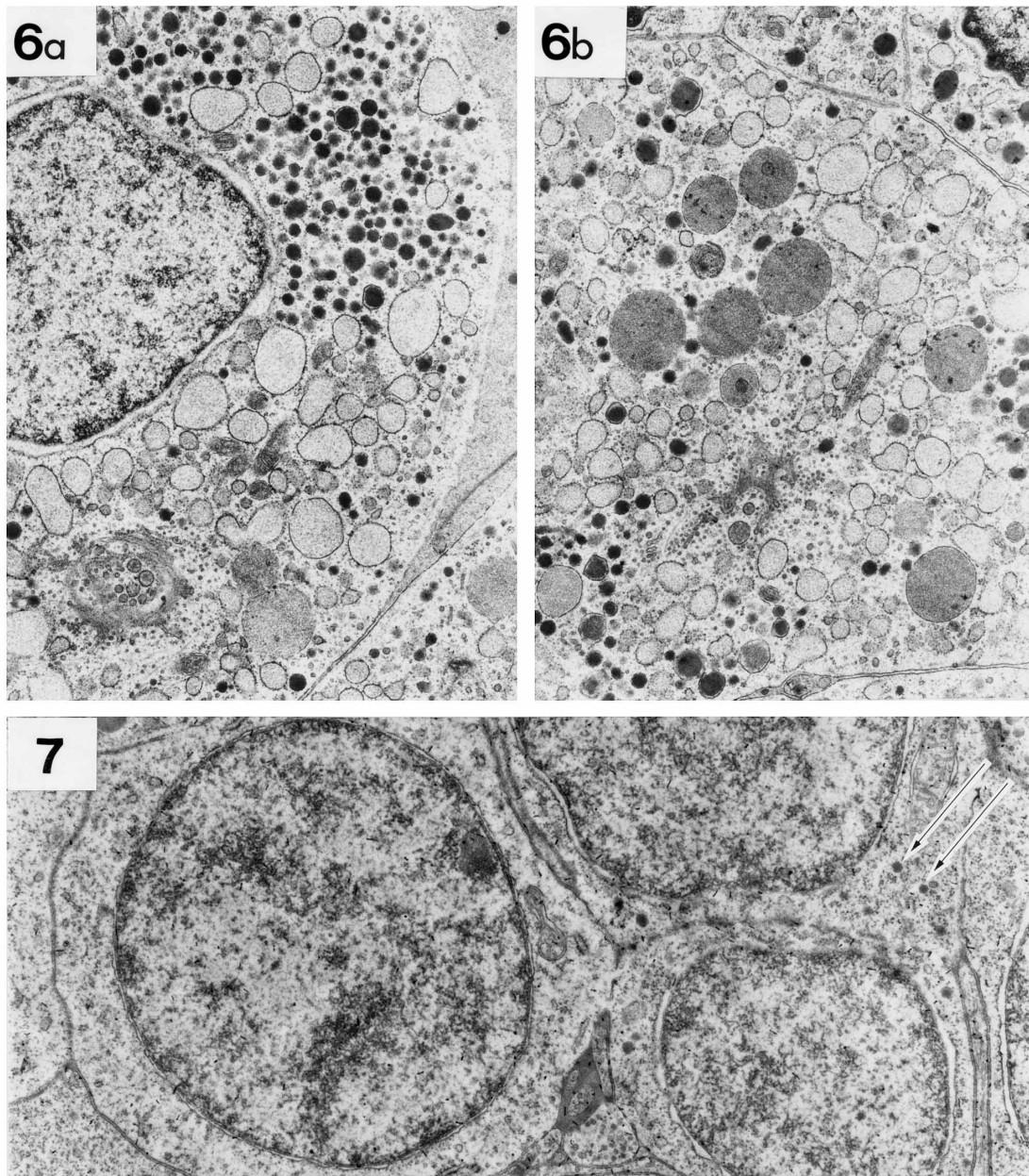


Fig. 6a–b. GTH I cell: (a) with large amount of dilated cisternae of RER in the primary male ($\times 14000$) and; (b) with numerous electron-dense globules in the female. $\times 14000$.

Fig. 7. A cluster of TSH cells. These cells have a large nucleus and very few small granules (arrow). $\times 16000$.

cells. They had an indented nucleus. A small number of secretory granules and globules (semi-dense/pale matrix) were present. The secretory granules of GTH I cells were less electron dense than those of STH cells; they were mostly spheric, of varying sizes. The granules were 283.5 ± 13.4 nm and the globules were 600.7 ± 31.7 nm in diameter. The cisternae of RER coalesced to form small vacuoles and occupied a large part of the cytoplasm (Fig. 6a). The mitochondria and the Golgi zones were moderately developed.

TM and SM had more granules (Table 1) and the F had more semi-dense membrane-bound globules per cell than the PM (Fig. 6a, b).

Gonadotropic (GTH) II cells

Cells immunoreactive to anti-coho salmon GTH II β were located within the ventral regions of the proximal pars distalis and surrounding the pars intermedia. The location and distribution of GTH I β and GTH II β immunoreactivity was different (Fig. 2d, e).

In electron micrographs GTH II cells, found in the ventral part of the PPD, had an unusual nuclear morphology. Margination of chromatin along the nuclear membranes was prominent (Fig. 8a–e). Prior to nuclear protrusion development, the portion of the inner nuclear membrane immediately adjacent to the developing protrusion became thickened (Fig. 8a, e).

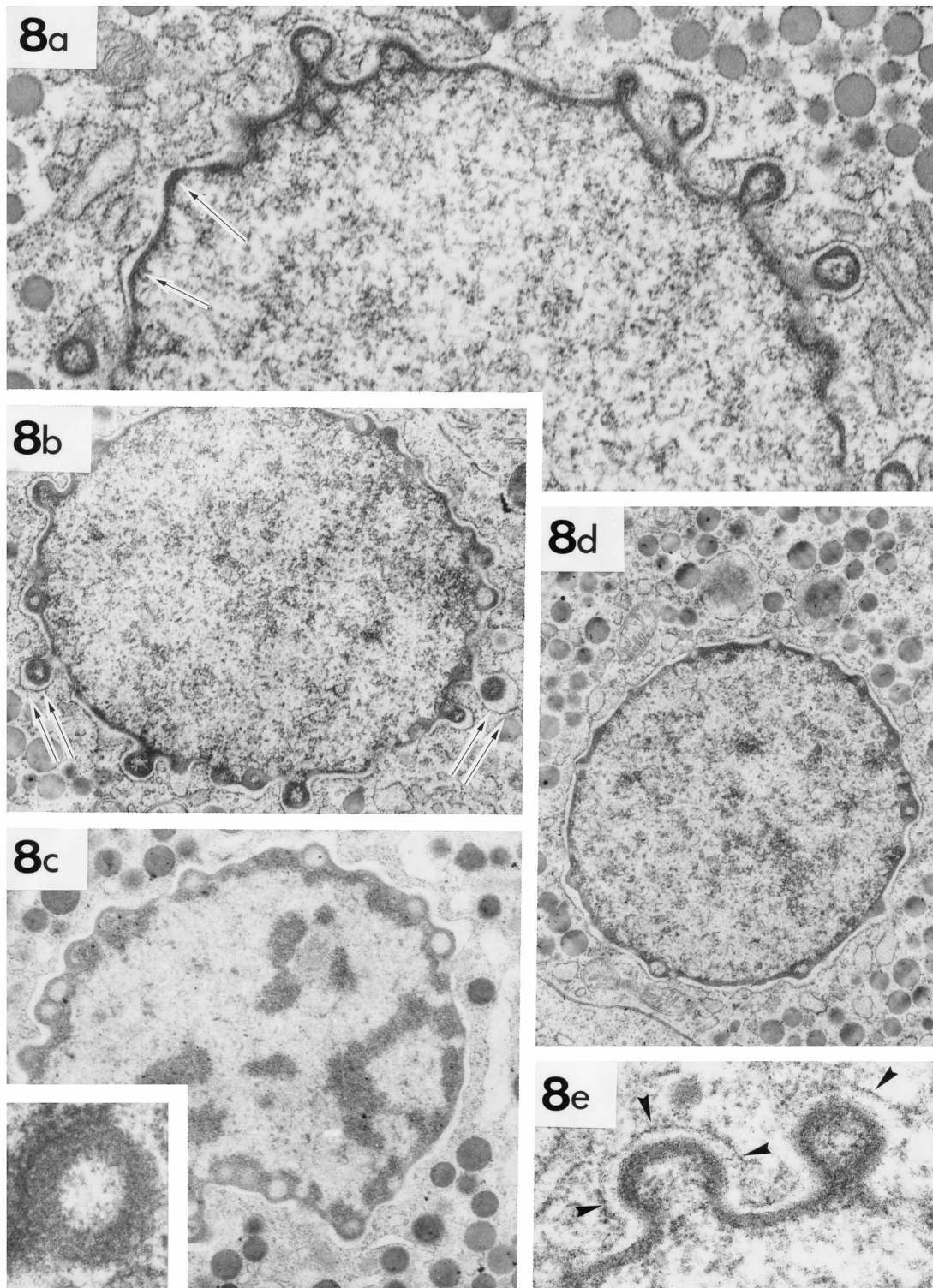


Fig. 8a–e. GTH II cells in the female showing clusters of nuclear protrusions in the process of development/pinching-off, within dilations of perinuclear cisterna. (a) Inner nuclear membrane thickening seen prior to protrusion formation (arrow: $\times 28000$); (b) a pinched-off nuclear protrusion (double arrow: $\times 16000$); (c) An extensive cluster of nuclear protrusions line the periphery of the nucleus in the primary male ($\times 21000$). These spherical particles have an electron-lucent center, surrounded by an electron-dense amorphous material (see insert: $\times 120,000$); (d) less pronounced budding seen in terminal-phase male ($\times 14000$); (e) a developing nuclear protrusion in a female, seen as a thickening of the nuclear membrane within the nuclear envelope (arrowheads). $\times 53000$.

Clusters of protrusions were seen within dilations from the perinuclear cisterna (Fig. 8a–d). Protrusions, in the final stages

of “budding” and those that eventually “bud-off” had four distinct morphological features: Each individual particle was oval

or spherical. These particles had an electron-lucent center, surrounded by an electron-dense amorphous material (Fig. 8c). There was evidence that some particles acquired their envelope from the cell's nuclear membrane (Fig. 8e). The secretory granules (320.5 ± 18.9 nm) were more regular, round, and less electron dense than the STH granules. Many small cisternae of RER were present. The mitochondria and the Golgi zones were moderately developed.

In the TM and SM (Fig. 8d), the extent of budding, vacuolization and the number of RER, mitochondria or Golgi zones were not as pronounced as in the PM and F (Fig. 8a–c; Table

1).

Thyrotropic (TSH) cells

Anti-rat TSH showed a distribution similar to that of GTH II cells. However, cells immunoreactive to anti-rat TSH were also present in the dorsal RPD-PPD interface, along the neurohypophysis. These cells showed no immunoreactivity to anti-GTH I β , anti-GTH II β , anti-GH, anti-PRL or anti-SL (Fig. 2f).

Ultrastructurally, TSH cells present in the dorsal RPD-PPD interface appeared polyhedral in shape. They had a large nucleus. The cytoplasm contained only a few granules. Each

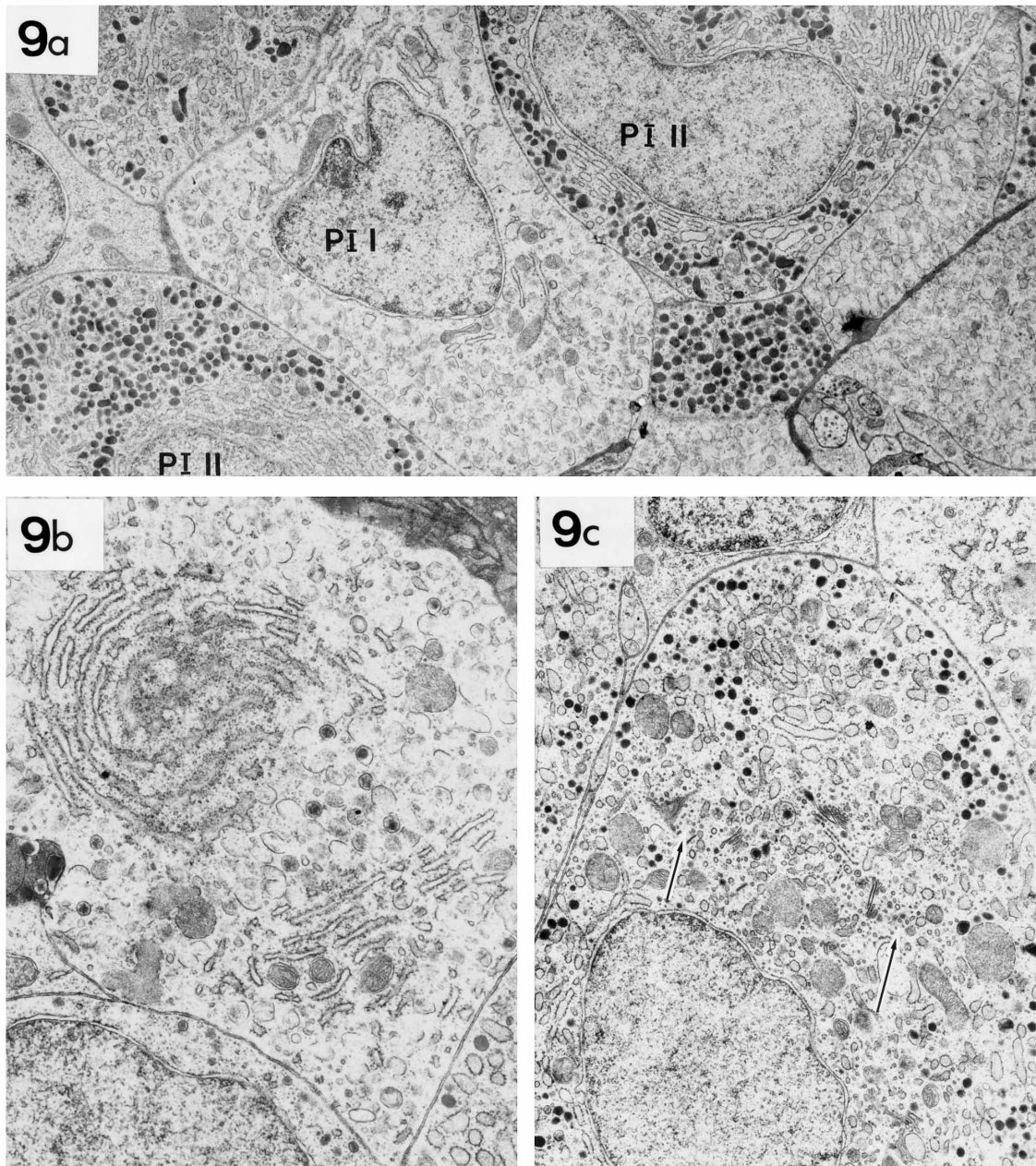


Fig. 9a–c. MSH and SL cells in the pars intermedia of primary male: (a) Vesicles with incomplete limiting membrane are typical of MSH cells $\times 8400$; (b) dilated cisternae of RER in the MSH cells of the terminal-phase male, filled with an amorphous substance and vesicles with incomplete membrane ($\times 15000$); (c) SL in the female with numerous immature granules (arrow), Golgi zones, many small dilated cisternae of RER and globules. $\times 9000$.

granule had an electron-dense center with a clear ring of space. Although they were similar to ACTH granules, they appeared smaller in size (135.2 ± 4.1 nm). Immature granules were seen around the many Golgi zones. The cells had few small cisternae of RER and moderately developed mitochondria (Fig. 7; Table 1). These cells were few in number and were therefore difficult to detect.

Pars intermedia (PI)

Melanotropic (MSH) cells

In one micron thick, toluidine blue stained sections, these cells were chromophobic. In electron micrographs, these cells appeared polyhedral in shape. The oval nucleus was sometimes deeply indented. The cytoplasm was paler than the somatolactin cells but was full of vesicles (322.7 ± 13.7 nm) whose limiting membrane appeared incomplete and opened into the cytoplasm (Fig. 9a, b). A small number of electron-dense membrane-bound granules were present. Large globular structures with a semi-dense matrix, probably lysosomes, were present in some cells. A few profiles were active (Fig. 9b). A large part of their cytoplasm was filled with a slightly dilated parallel lamellae of RER. Many dilated cisternae of RER were often filled with an intracisternal material. The mitochondria were moderately developed and the Golgi zones were observed with some immature vesicles.

The cells in the female were similar to PM but those in the TM and SM had a higher frequency of very active profiles (Fig. 9b; Table 1).

Somatolactin (SL) cells

A large number of cells immunoreactive to anti-cod fish somatolactin were found in close proximity to the interdigitations of the neurohypophysis of the pars intermedia (Fig. 2c). Some cells were scattered amongst melanotropic cells.

In electron micrographs, somatolactin cells appeared almost spherical. The nucleus was generally oval and placed towards the periphery of the cell (Fig. 9a). In some cells the nucleus had a distinct eccentrically-placed nucleolus. The electron-dense cytoplasm was full of rounded (158.0 ± 5.8 nm), often ovoid (long axis 314.8 ± 13.5 nm; short axis 155.0 ± 9.0 nm) granules (Fig. 9a; Table 1).

Cells in the F and PM had numerous immature granules, several Golgi zones, many mitochondria and RER (Fig. 9c). TM had relatively fewer granules but as in the F, these cells contained an average of 2–4 large globules (1081.3 ± 64.4 nm; Table 1). In the SM these cells were full of mature granules but contained fewer RER, Golgi zones and mitochondria.

The cytoplasm of some cells scattered among the somatolactin and melanotropic cells was intensely electron-dense, these cells could be degenerating.

Neurohypophysis

The PPD and the RPD had a dense and a highly interdigitating, although smaller, zone of NH than the PI. The NH of the PI was devoid of axonal granules but had few rounded

cells as the NH of the RPD and the PPD (Fig. 10a). The axonal types could be identified (Table 2) as: type A i.e granules with an electron dense core having either a closely (Ac: 82.4 ± 2.6 nm) or loosely (Al: 128.6 ± 6.8 nm) adhering limiting membrane (Fig. 10a, c). The type B (82.1 ± 3.7 nm) were similar to type Al but their electron-dense centers were smaller (Fig. 10b). Axons type B and Al were seen to form direct contacts with STH (Fig. 10b) and GTH cells (Fig. 10c).

DISCUSSION

The different endocrine cells of the adenohypophysis, identified by immunocytochemistry and ultrastructure, were found to be segregated in one of the three divisions of the pituitary. The location and general characteristics of adenohypophyseal cells of *T. duperrey* are comparable to those of similar cells in other teleosts. (Bern *et al.*, 1974; Munro, 1985; Cambre *et al.*, 1986; Quesada *et al.*, 1988; Garcia-Hernandez *et al.*, 1996).

Corticotropic cells. The secretory granules of cells lining the invaginating NH into the RPD have a central dense core surrounded by a clear narrow halo. This is a characteristic feature of ACTH cells in other teleosts (Bern *et al.*, 1974; Quesada *et al.*, 1988). Likewise, cells in a similar position in the RPD of various other teleosts are immunoreactive with ACTH antisera (Munro, 1985; Quesada *et al.*, 1988; Toubeau *et al.*, 1991; Garcia-Hernandez *et al.*, 1996). Thus, the ACTH cells identified here correspond both topographically and ultrastructurally with those identified in other teleosts. There were no apparent differences between the ACTH cells of PM, TM, F and SM derived from caged F.

Prolactin cells. The anti-chum salmon PRL antiserum specifically immunostained PRL cells but weakly cross-reacted with GH and SL cells in the *T. duperrey*. The cross-reactivity of PRL antiserum with GH and SL cells is probably due to the molecular similarities between PRL/GH/SL genes (Ono and Kawachi, 1994). PRL cells have been characterized on the basis of immunocytochemistry (Cambre *et al.*, 1986; Quesada *et al.*, 1988; Huang and Specker, 1994; Garcia-Hernandez *et al.*, 1996), electron-microscopic immunocytochemistry (Specker *et al.*, 1993; Garcia-Ayala *et al.*, 1997) and recently PRL mRNA localization by in situ hybridization (Nishioka *et al.*, 1993). As in most other fish, PRL cells are the principle component of the RPD. Unlike the follicular arrangement in some teleost (Naito *et al.*, 1983; Parhar and Iwata, 1994; Quesada *et al.*, 1988), in the *T. duperrey*, PRL cells are chromophobic and arranged as a compact mass (Naito *et al.*, 1983; Cambre *et al.*, 1986; Huang and Specker, 1994). However unlike another wrasse (*Crenilabrus melops*: Benjamin, 1979), there was no evidence for vacuolization. The moderately developed Golgi zones and mitochondria and relatively few secretory granules, in more ventrally located PRL cells, may indicate that these are immature/undifferentiated cells. Alternatively, the presence of a few small secretory granules has been related to high-salinity environment (Nagahama, 1973; Quesada *et al.*, 1988) and therefore these cells could be actively secreting PRL.

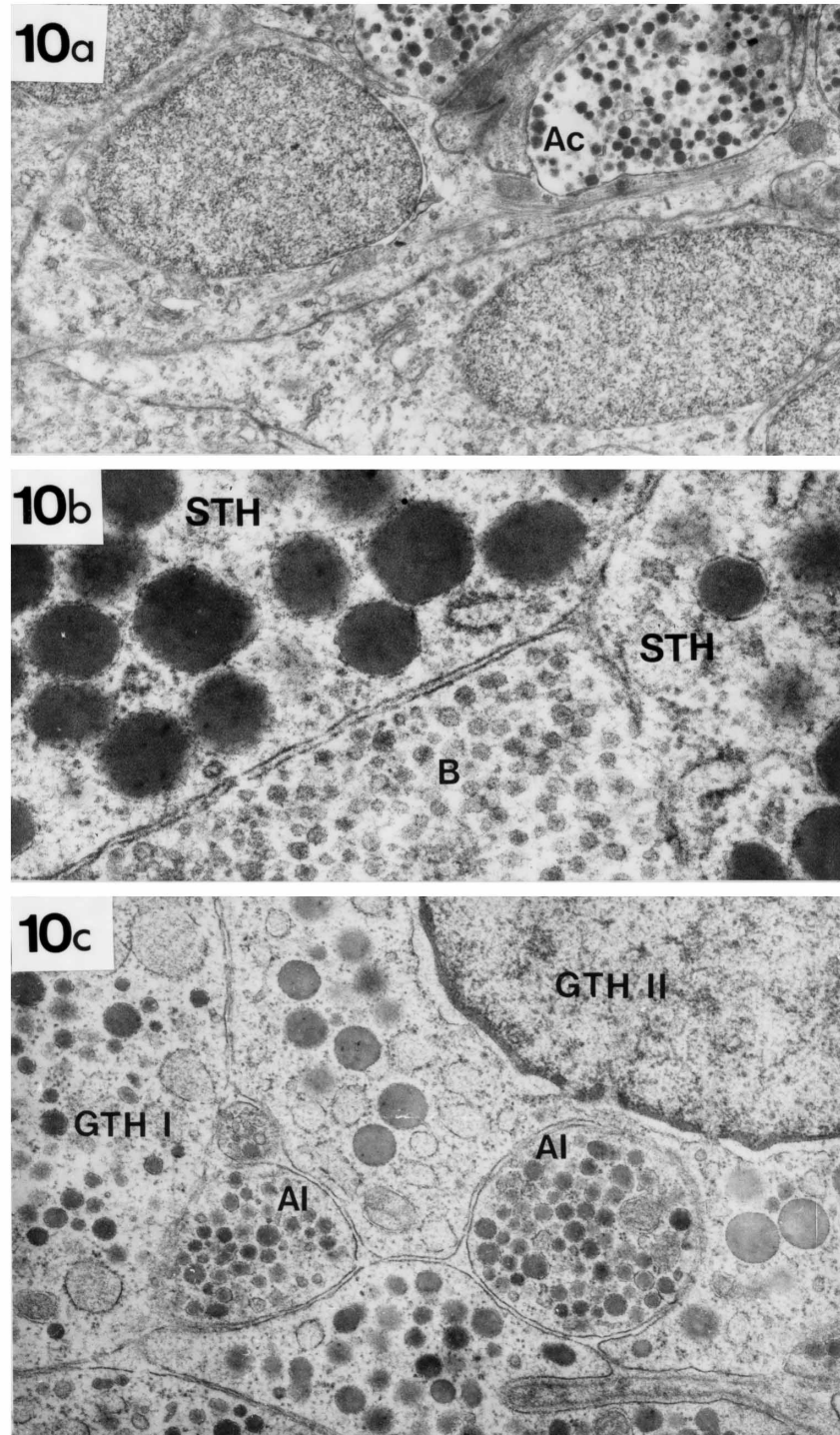


Fig 10a–c. Axonal profiles with : (a) Ac type granules and cells (probably pituicytes) in the NH ($\times 13000$); (b) type B axons in close contact with STH cells ($\times 58000$); (c) type AI axons seen in direct contact with GTH I and GTH II cells. $\times 20000$.

Table 2. Axon types in the neurohypophysis and their mean vesicles diameters in *T. duperrey*

Axon type	Vesicles diameters Mean \pm S.E. (nm)
A1 ¹	128.6 \pm 6.8
Ac ²	82.4 \pm 2.6
B	82.1 \pm 3.7

¹Loose limiting membrane; ²Closely adhering membrane

Stellate cells. Except for their non-electron-dense cytoplasm, the stellate cells of *T. duperrey* are similar to those in other teleosts (Bern *et al.*, 1974; Chiba and Honma, 1988; Quesada *et al.*, 1988). Stellate cells might help in phagocytosis, support of the pituitary gland and transporting hormone from the endocrine cells to the blood vessels (see Chiba and Honma, 1988; Garcia-Ayala *et al.*, 1997).

Somatotropic cells. The columnar cells in the dorsal regions of the PPD along the neurohypophyseal boundaries resemble the STH cells described by immunocytochemistry, electron-microscopy and in situ hybridization in other teleosts (Nagahama *et al.*, 1981; Ueda *et al.*, 1985; Quesada *et al.*, 1988; Toubreau *et al.*, 1991; Nishioka *et al.*, 1993; Parhar and Iwata, 1994; Garcia-Hernandez *et al.*, 1996; Garcia-Ayala *et al.*, 1997). Two forms of somatotrophic hormones and two distinct STH cell populations differing in shape, immunostaining intensity and organization have been reported (Huang and Specker, 1994; Garcia-Hernandez *et al.*, 1996). However, in the *T. duperrey* STH cells contained a heterogeneous population of secretory granules, but there was no morphological distinction between cells.

Gonadotropin I cells. Two GTHs, structurally and functionally different and localized in distinctly separate cells have been reported in salmonids (see Nozaki *et al.*, 1990; Naito *et al.*, 1991, 1993). In the *T. duperrey*, cells present in the dorsal PPD resemble the GTH cells identified immunocytochemically and ultrastructurally in other teleosts (see Van Oordt and Peute, 1983; Kaneko *et al.*, 1986; Nozaki *et al.*, 1990). The dilated RER cisternae (vacuoles) might contain secretory products or by-products of degraded secretory granules (see Kaneko *et al.*, 1986). The large globular inclusions contain lytic enzymes and the β -subunit of the GTH molecule (Naito *et al.*, 1995; Sharp-Baker *et al.*, 1996). The large amount of dilated cisternae of RER, semi-dense globules but fewer granules in the GTH I cells of PM and F, relative to SM and TM, presumably represents active hormone synthesis and secretion. In the female wrasse, GTH may be necessary for initiation of interstitial tissue proliferation and testicular lobule formation, as has been demonstrated in the female *Monopterus* (Tang *et al.*, 1974) with exogenous mammalian LH. Active synthesis of GTH I hormone during the onset of vitellogenesis and, in males, during the early phases of spermatogenesis (see Nozaki *et al.*, 1990; Naito *et al.*, 1991) suggests the important role GTH I β plays during early stages of gonadal development, and therefore explains the lack of GTH I β immunoreactivity in our adult specimens.

Gonadotropin II cells. Ultrastructurally, a second cell type was observed adjacent to GTH I cells in the ventral PPD in all groups used in the present experiment. These cells had budding nuclear protrusions. There is no evidence to indicate that the nuclear protrusions are fixation artifacts or just aberrant structures. One possibility is that these cells are in the initial phase of apoptosis. However, it is also possible, the nuclear protrusions may represent the "budding-off" of the nucleus into the cytoplasm. The structural components of the budding protrusions i.e., an electron-lucent center surrounded by an electron-dense amorphous material (tegument), which separates the center from the envelope and the "budding-off" appearance of nuclear protrusions is a characteristic feature of herpes virus (Fleckenstein and Desrosiers, 1982; Hay *et al.*, 1987; King *et al.*, 1974). On the contrary, these viral-like particles are too large to qualify as herpes virion (generally 150–300 nm). These particles could be the product of a very

highly active cell, where the message for protein synthesis is being delivered in its genomic form. The extensive nuclear protrusions seen in both the PM and the F could be a marked increase message for oocyte maturation, ovulation and spermiation. Thus, as in salmonids, GTH II hormone could be a maturational GTH hormone (Naito *et al.*, 1991).

If, on the other hand, these nuclear protrusions are virus of some form, then they may be some non-pathogenic agents harbored in the GTH II cells in an incomplete form, since they show no episodes of infections. However, like some herpes virus, they may have the capacity to establish latent infections (Stevens, 1980). Clearly, further work is required to determine the processes underlying nuclear protrusions formation, their subsequent fate and their functional significance.

Although we have classified the second cell group as GTH II cells, this distinction is based on immunocytochemical localization and their distinct nuclear morphology from GTH I cells. However, it remains to be tested whether these two cell groups (GTH I and GTH II) in the *T. duperrey* synthesize chemically distinct gonadotropins, GTH I and GTH II (see Kawauchi *et al.*, 1989; Xiong *et al.*, 1994) or they are different functional phases of a single cell type (see Van Oordt and Peute, 1983; Kaneko *et al.*, 1986)

Thyrotropic cells. Isolated TSH cells in the PPD have been reported in some teleost species (see Quesada *et al.*, 1988). However, immunoreactive thyrotropic cells seen as a discrete cell population distinct from the GTH cells at the dorsal RPD-PPD interface in the *T. duperrey* are similar to those described in the pituitary of other teleost (Ueda *et al.*, 1983; Garcia-Hernandez *et al.*, 1996). In electron micrographs, thyrotropic cells in the same location, with their characteristic small electron-dense secretory granules have been described in the *Oreochromis mossambicus* (Bern *et al.*, 1974). In the *T. duperrey*, anti-rat TSH β antiserum did not specifically immunostain TSH cells but also cross-reacted with GTH cells. Similarly, using anti-human TSH β antiserum a specific (Munro, 1985; Garcia-Hernandez *et al.*, 1996) and also a weak cross-reaction with GTH cells has been reported (Ueda *et al.*, 1983; Yan and Thomas, 1991).

Melanotropic cells. The predominant cell-type in the pars intermedia of all fish was the polyhedral osmiophobic cell, which has been described as lead haematoxylin-positive in many teleosts (Bern *et al.*, 1974; Benjamin, 1979; Quesada *et al.*, 1988). The melanotropic cells are the source of proopiomelanocortin, the precursor of melanophore stimulating hormones (Naito *et al.*, 1984). Melanotropic cells specifically immunostain with α -MSH antisera but also cross-react with anti-ACTH 1-24 (Munro, 1985; Quesada, 1988; see Garcia-Hernandez *et al.*, 1996). The melanotropic cells have been implicated in melanogenesis and in background colour adaption (Van Eys, 1980; Van Eys and Peters, 1981) in other teleosts. In TM, and SM these active melanotropic cells could be associated with the development and 'flashing' of a lateral bar during courtship. In TM, it could also be a stress response while defending territory since some types of stress can activate melanotropic cells (Sumpter *et al.*, 1985).

Somatolactin cells. Somatolactin cells identified by ultrastructure, immunocytochemistry and in situ hybridization (Rand-Weaver *et al.*, 1991; Kaneko *et al.*, 1993; Parhar and Iwata, 1994; Garcia-Hernandez *et al.*, 1996; Garcia-Ayala *et al.*, 1997) are found mainly in close proximity to the interdigitations of the neurohypophysis of the pars intermedia, and correspond to the periodic-acid-Schiff (PAS)-positive cells of other teleosts (Bern *et al.*, 1974; Benjamin, 1979; Quesada *et al.*, 1988). As in the *Seriola dumerilii*, somatolactin cells with predominantly round secretory granules and large local dilations of endoplasmic reticulum cisternae seem to be undergoing an intense process of hormone synthesis and storage, whereas cells with very irregular granules, which seemed to result from the fusion of other secretory granule populations, might represent an active stage of granular release (Garcia-Ayala *et al.*, 1997). The function of these cells is not clear: in other teleosts, they have been implicated in ion regulation, adaptation to stressful environment and dark background (Ono and Kawauchi, 1994; Kakizawa *et al.*, 1995; Rand-Weaver *et al.*, 1993; see Kaneko *et al.*, 1993; Zhu and Thomas, 1996). In some teleost, the presence of gonadotropin-releasing hormone immunoreactive fibers in close association with somatolactin cells (Parhar and Iwata, 1994; Parhar *et al.*, 1995), their activation by gonadotropin-releasing hormone (Kakizawa *et al.*, 1997), and their role in gonadal maturation, gonadal recrudescence and gonadal steroidogenesis (Schreibman *et al.*, 1973; Planas *et al.*, 1992; Olivereau and Rand-Weaver, 1994) suggests that somatolactin cells might be functionally important for reproduction.

Dark somatolactin cells showing ultrastructural features of involutive cells have been reported in the *Seriola dumerilii* (Garcia-Ayala *et al.*, 1997). The large number of cell deaths (cells with intensely electron-dense cytoplasm) and relatively inactive (or hyperactive) cells in SM may be a stress-induced response to the unnatural environment in the cages. Elevated plasma somatolactin has been observed during stress (Rand-Weaver *et al.*, 1993; Kakizawa *et al.*, 1995). However little is known about stress and sex change.

Neurohypophysis. Both A- and B-type axons found close to adenohipophyseal cells in the pars distalis is an evidence of direct innervation as in other teleosts (Batten *et al.*, 1990; Holmqvist and Ekstrom, 1995). The cells in the NH are probably pituicytes which may help in the disposal of neurosecretory products (Leatherland, 1972).

In conclusion, the topographical organization of immunocytochemically and ultrastructurally identified adenohipophyseal cells in the *T. duperrey* is similar to that of other teleost (see introduction). An increased gonadotropic cell activity in PM and F, and an increased somatolactin and melanotropic cell activity in TM and SM indicates the possible role of these cell types at different stages of reproduction. However, any opinion drawn must be tentative because of the small sample size of sex changed males and our subjective method of analysis of cell activity.

ACKNOWLEDGEMENT

We wish to thank Drs. G. Losey and E. G. Grau, Hawaii Institute of Marine Biology, The University of Hawaii, for organizing the summer program on the biology of sex change in tropical marine vertebrates. Thanks are due to Drs. C. Schreck, M. Nakamura, K. Yamauchi, T. Hourigan, C. Hunter, S. Adachi, B. Howerton, J. Borski, D. Okimoto and G. Weber for their help during the program and the Edwin W. Pauley Foundation (USA) for providing financial support. We also thank Dr. A. D. Munro and Prof. T. J. Lam for their encouragement and the use of electron microscope facilities, at the National University of Singapore.

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(Received October 27, 1997 / Accepted December 24, 1997)