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Structure and Fine Structure of the Hypophyseal Pars distalis in Endigenous African Species of the Genus *Tilapia*

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Summary. The structure and fine structure of the pars distalis hypophyseos was examined in five species of *Tilapia* fishes (*T. alcalica, T. grahami, T. leucosticta, T. zillii, T. nigra*) which were collected from lakes of a wide range of salinities. The pars distalis in all the species is composed of 5 granulated ("secretory") and 1 chromophobic cell types. The rostral pars distalis prolactin cells appear most numerous and active in the fresh water species and smaller and least active in the "soda" lake fish. The evidence from nuclear measurements suggests that the species adapted to hyposmotic media have compensated for the freshwater environment (and the subsequent need for greater prolactin secretion) by increasing the number of prolactin cells rather than by increasing the synthetic activity of individual cells.

In "soda" lake species which were acclimated to fresh water the prolactin cells are markedly hyperactive and degranulated when compared with any other group.

The ACTH cells appear more active in the "soda" lake species than in the fresh water groups, however, these cells are maximally active in "soda" lake fish acclimated to fresh water.

The rostral pars distalis stellate cells are described and discussed in relation to their possible involvement in the release of hormone from the pars distalis "secretory" cells.

The proximal pars distalis somatotrophs appear active in all the species investigated although they were maximally active in fresh water acclimated "soda" lake species. The structure of the proximal pars distalis gonadotrophs and thyrotrophs is variable both within the same animal and between the species but the variation is not consistent with environmental salinity parameters.

The means by which granules are released from the different cell types is discussed.

Key words: Adenohypophysis — *Tilapia* — Salinity — Cell types — Light- and electron microscopy — Teleost fish.

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Introduction

The involvement of the pars distalis in osmo(iono) regulation in teleosts is well established (see reviews by Olivereau and Ball, 1970; Ball and Baker, 1969; Ensor and Ball, 1972; Lam, 1972; Schreibman, Leatherland and McKeown, 1973). In this investigation the comparative structure and fine structure of the pars distalis is examined in 5 species of *Tilapia* collected from lakes and ponds in Kenya in an attempt to relate histological differences to the different ambient environments. Some of the lakes, so-called "soda" lakes (Natron, Magadi and Nakuru), contain high concentrations of sodium carbonate whereas other lakes and ponds (Naivasha and Sagana) are freshwater (Leatherland, Hyder and Ensor, 1974). The *Tilapia* collected from the "soda" and freshwater lakes are closely related species; they may thus provide information of the endocrine mechanism of long-term acclimatization (adaptation) to the hyperosmotic (non-sodium chloride) "soda" lake environs.

Recent work has shown that two species from the "soda" lakes (*T. alcalica* and *T. grahami)* can tolerate freshwater conditions for periods of several days whereas *T. zillii* and *T. nigra*, collected from fresh water were not able to withstand salinities greater than a 60% sea water equivalent (Leatherland, Hyder and Ensor, 1974). In the work described here the effect of decreased salinity on the activity of the pars distalis cells in *T. alcalica* and *T. grahami* is examined in order to determine in these species the endocrine response to reduced ambient salinities.

Materials and Methods

The fish used in these investigations were those used for plasma Na^+ and K^+ studies described before (Leatherland, Hyder and Ensor, 1974). Specimens of *Tilapia* were collected from a number of sources: *T. leucosticta*, Lake Naivasha (fresh water); *T. zillii* and *T. nigra*, Sagana hatchery ponds (fresh water); *T. grahami*, Lake Nakuru (dilute "soda" lake) and Lake Magadi, (concentrated "soda" lake); *T. alcalica*, Lake Natron (concentrated "soda" lake). The concentrations of Na^+ and K^+ in the lakes are shown in Table 1.

Specimens of *T. grahami* (Lake Magadi) and *T. alcalica* (Lake Natron) were maintained in the laboratory in 100% artificial Magadi lake water; this was prepared by dissolving the precipitated lake salts in sufficient quantities in Nairobi tap water. One group of 12 fish of each species was maintained in this medium, while 2 further groups of 12 fish were placed in separate aquaria which contained 50% artificial Magadi lake water. The water in 1 group of each species was subsequently diluted each day by the addition of tap water so that the final ambient medium was tap water; they were killed 5 days after placing them in tap water. The fish in 50% and 100% artificial Magadi lake water were killed at the same time.

Electron Microscopy: Each fish was killed by transection of the nerve cord and the pituitary rapidly dissected from the skull. The gland was fixed for 1–2 h in ice cold phosphate buffered (pH 7.4) 5% glutaraldehyde, post-fixed for 1–2 h in ice cold phosphate buffered (pH 7.4) 1% osmium tetroxide, dehydrated in a graded series of ethanol, finally dehydrated in propylene oxide and embedded in Araldite resin (CIBA).

Sections were made by means of glass knives, they were then mounted on uncoated nickel or copper grids and stained with uranyl acetate alone or uranyl acetate and lead citrate. The sections were examined on a J.E.M. 7A, Philips 100 or Philips 200 electron microscope.

Sections (1 μ thick) were taken from each block and stained for examination by light microscope methods for purposes of orientation (Leatherland, 1970).

Light Microscopy: The fish were killed as described above, the brain exposed dorsally and laterally and the cranium placed in Bouin Hollande sublimate. The brains were fixed for 7–10 days, dissected from the skull and embedded in "Paraplast" for light microscope histology, 4–7 μ sections were made and stained with Herlant's tetrachrome, alcian blue-periodic acid Schiff (PAS)—orange G or lead haematoxylin-PAS. Nuclear measurements were made under

Table 1

Source	Species	Ambient concentrations mEq/l	
		Na^+	K^+
Lake Natron	*T. alcalica*	306.0	1.0
Lake Magadi	*T. grahami*	215.3	2.8
Lake Nakuru	*T. grahami*	39.8	0.8
Lake Naivasha	*T. leucosticta*	0.9	0.3
Sagana hatchery ponds	*T. zillii* *T. nigra*	0.4	<0.1
Artificial lake Magadi water			
100%		168.0	0.5
50%		82.8	0.5
Nairobi tap water		3.2	0.3

oil immersion by means of an ocular micrometer. Measurements of the major and minor axes were made on 10 randomly chosen nuclei lying in one plane of section in 5 animals in each group. The nuclei were assumed to be elliptical in order to calculate the nuclear area.

Statistics: The nuclear area data for prolactin and ACTH cells were compared by one-way analysis of variance. Individual means were compared by Tukey's W test (Steel and Torrie, 1960).

The nuclear area data for STH cells in *T. grahami* and *T. alcalica* were compared by Students "t" test.

Results

The pars distalis in all the *Tilapia* species examined may be divided into 2 zones, rostral (RPD) and proximal (caudal) (PPD) by virtue of the cellular components (Figs. 2a, b, c). The RPD is composed of three cell types, epsilon (putative ACTH), eta (putative prolactin) and non-secretory "stellate" cells. The PPD is composed of large acidophils and two types of basophils (putative thyrotrophs and gonadotrophs).

Rostral pars distalis (RPD)

Epsilon (ACTH) Cells

"Soda" Lake Species: Epsilon cells are columnar or cuboidal cells lining the junction of the RPD and the neurohypophysis (NH). They have rounded nuclei and contain cytoplasmic granules which stain grey or dark blue with lead haematoxylin and dark red or purple with Herlant's tetrachrome procedure. The sizes of the nuclei in the different species are shown in Fig. 1; all 3 groups of fish have ACTH-cell nuclei of similar size which is suggestive of a similar level of hormone synthesis.

In electron micrographs (Figs. 4a, b) the cells are seen to be separated from the NH by a "basement membrane" (see review by Leatherland, 1972) and to contain cytoplasmic granules which characteristically have a central electron-dense core separated from the limiting membrane by a clear space. In addition, the cells

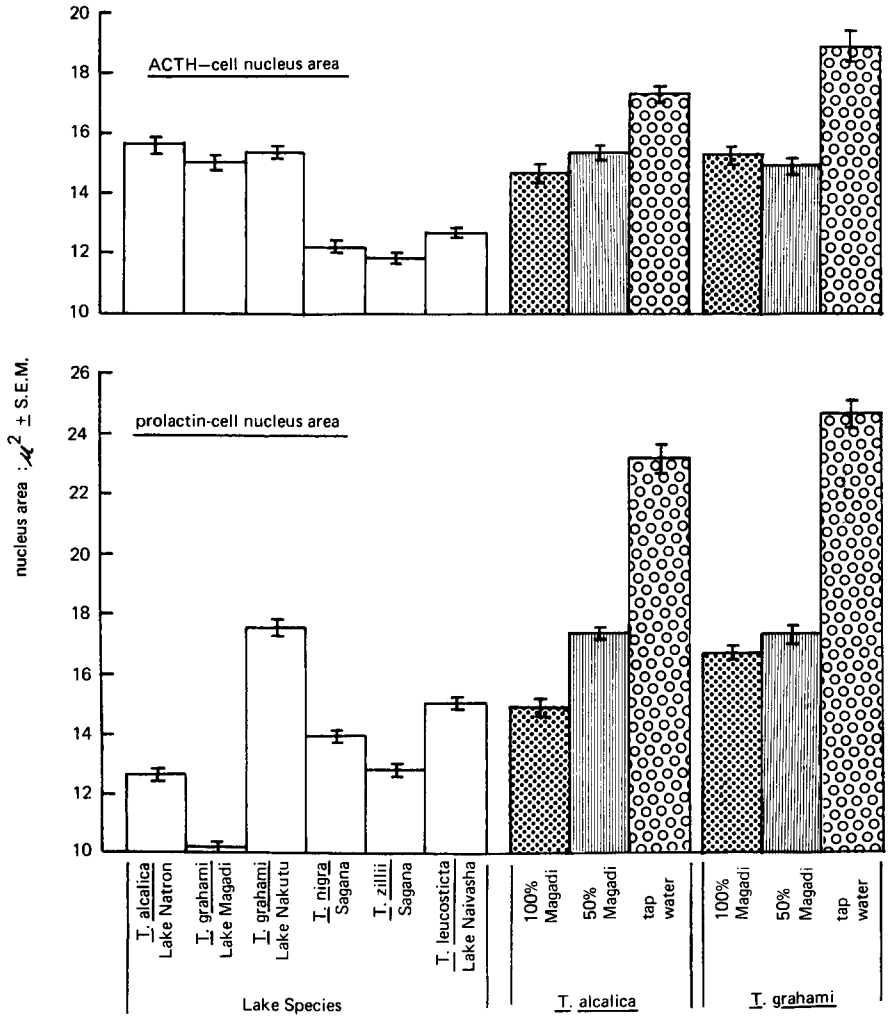


Fig. 1. Nuclear areas of ACTH and prolactin cells in lake species of *Tilapia* and *T. alcalica* and *T. grahami* acclimated to three ambient salinities. The ACTH cell data from *T. alcalica*, *T. grahami* (Lake Magadi) and *T. grahami* (Lake Nakuru) were all significantly different from *T. nigra*, *T. zillii* and *T. leucosticta* ($p<0.01$); *T. leucosticta* was significantly different from *T. zillii* ($p<0.05$). *T. grahami* and *T. alcalica* in tap water were significantly different from similar groups in 50% and 100% artificial Magadi lake water ($p<0.01$). The prolactin cell data from the Lake species were all significantly different ($p<0.01$) except for the comparison of *T. alcalica* and *T. zillii* ($p<0.05$) and *T. nigra* and *T. leucosticta* ($p<0.05$). The *T. alcalica* in the three ambient salinities all differed significantly ($p<0.01$). *T. grahami* in tap water was significantly different from similar groups in 50% and 100% artificial Magadi lake water ($p<0.01$)

contain varying numbers of mitochondria, plentiful endoplasmic reticulum and Golgi bodies. The ACTH cells in *T. alcalica* appeared the most active of all the lake samples ("soda" and freshwater).

Table 2

Species	Treatment	Epsilon (ACTH)	Eta (prolactin)
T. alcalica	Lake Natron, 100% and 50% artificial Lake Magadi water	1900–2000 Å	2200–2900 Å
	fresh water	2300–2400 Å	2400–3000 Å
T. grahami	Lake Magadi, Lake Nakuru, 100% and 50% artificial Lake Magadi water	1950–2100 Å	2400–2600 Å
	fresh water	1950–2300 Å	2200–3000 Å
T. zillii and *T. nigra*	fresh water	1200–1500 Å	2000–2500 Å
T. leucosticta	fresh water	1200–1500 Å	2400–2600 Å

Fresh Water Species: The epsilon cells in these groups are generally similar in appearance to those in the "soda" lake species. The nuclear size is significantly smaller ($p < 0.01$) than in the 3 "soda" lake groups (Fig. 1). Furthermore, that of *T. leucosticta* is significantly larger than in *T. zillii* ($p < 0.05$). The granule diameters are smaller in the fresh water species (*T. zillii*, *T. nigra*, *T. leucosticta*) (1200 to 1500 Å) than in the "soda" lake fish (1900 to 2100 Å) (see Table 2).

Effect of salinity changes: There are significant ($p < 0.01$) increases in epsilon cell nuclear area in *T. grahami* and *T. alcalica* acclimated to fresh water when compared with the "soda" lake species and those acclimated to 100% or 50% artificial Magadi lake water (Fig. 1); the nucleoli appear larger and more pronounced in the fresh water acclimated animals. In both light and electron micrographs the epsilon cells in tap water acclimated *T. grahami* and *T. alcalica* appear more degranulated and have all the indications of high cellular activity (increased endoplasmic reticulum, more mitochondria and Golgi bodies) in the tap water groups (Fig. 4b). In addition, the epsilon cell granules in tap water acclimated *T. alcalica* are larger than in other groups of the same species (Table 2).

Eta (prolactin) cells

"Soda" Lake Species: Eta cells compose the major part of the RPD in all the *Tilapia* species examined (Figs. 2a, b, c). In *T. alcalica* (from Lake Natron) and *T. grahami* (from Lake Magadi) they constitute 1/7 or 1/8 of the volume of the whole pituitary. In *T. grahami* they are small, sperical or oval cells with small spherical (Fig. 1), dark nuclei and very small nucleoli (Fig. 3a). The cytoplasm contains numerous erythrosinophilic granules and little cytoplasmic RNA. In *T. alcalica* the cells are similar in appearance although the cell nuclei were significantly larger ($p < 0.01$) than in *T. grahami* (Lake Magadi) (Fig. 1). Conversely, in *T. grahami* from Lake Nakuru the prolactin cells constituted a larger proportion of the adenohypophysis and appear more active and with significantly larger ($p < 0.01$) nuclei when compared with both *T. grahami* from Lake Magadi and *T. alcalica* (Figs. 1, 3a, b, c).

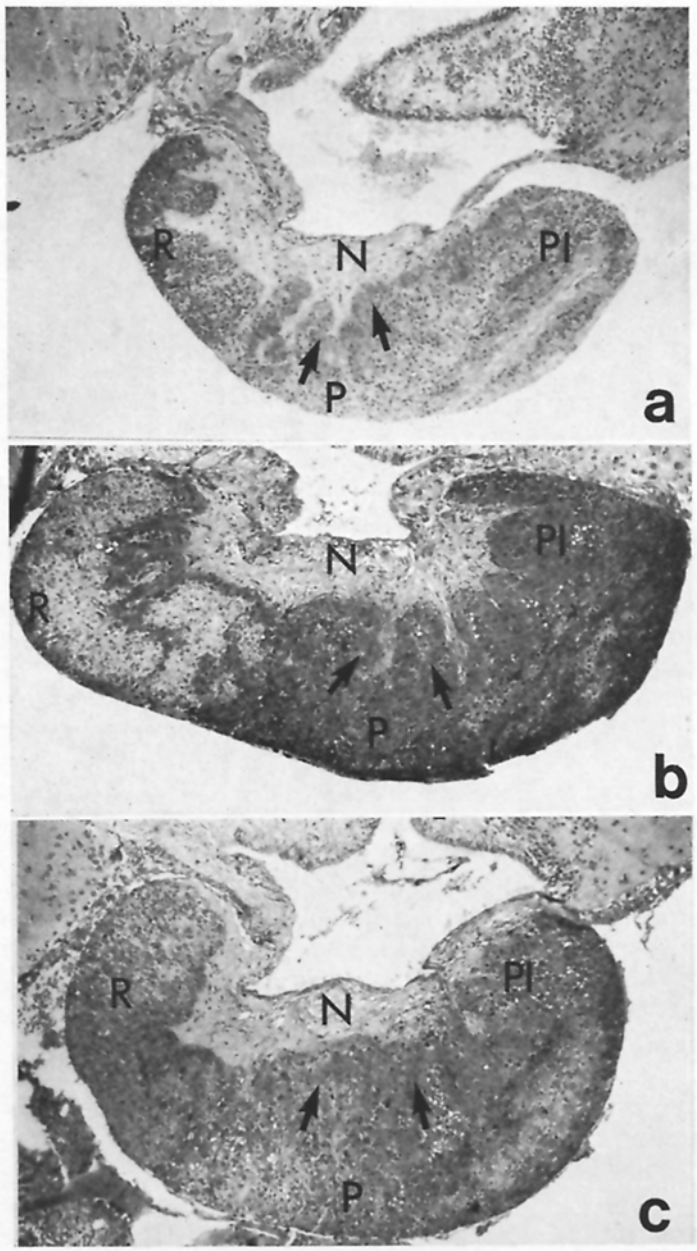


Fig. 2a—c. Light micrographs of sagittal sections of pituitary gland in *Tilapia grahami*. a. *T. grahami* cought in Lake Magadi; b) *T. grahami* acclimated to tap water; c) *T. grahami* acclimated to 100% artificial Magadi lake water. Note the enlargement of the rostral pars distalis region (*R*) in b and c compared with a. Note also the enlargement of the putative somatotrophs (arrows) in b and c) *P*, proximal pars distalis; *PI*, pars intermedia, *N*, neurohypophysis. Herlant's tetrachrome ($\times 100$)

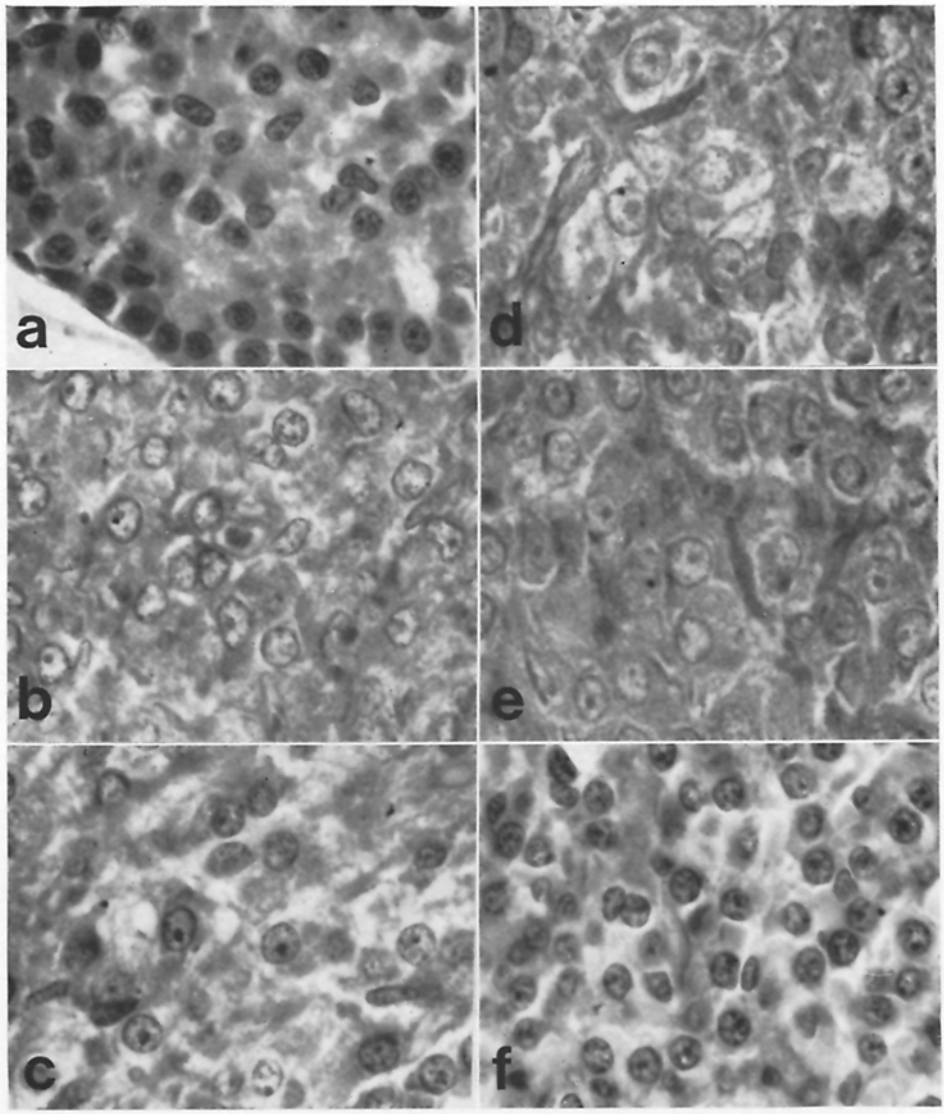


Fig. 3a—f. Light micrographs of putative prolactin cells in rostral pars distalis. a) *T. grahami* from Lake Magadi; b) *T. grahami* from Lake Makuru; c) *T. grahami* acclimated to 100% artificial Magadi lake water; d) *T. grahami* acclimated to tap water; e, *T. alcalica* acclimated to tap water; f, *T. zillii* from Sagana hatchery ponds, The cells in "soda" lake species (a) are similar to those in the fresh water species (f). In *T. grahami* adapted to (b) or acclimated to a lowered salinity (c and d) the cells are markedly more active than in the Lake Magadi fish. Maximal activity is found in *T. alcalica* (e) and *T. grahami* (d) acclimated to tap water. Herlant's tetrachrome (×1000)

In electron micrographs (Figs. 4a, 5a), the intensely electron dense- membrane-bound cytoplasmic granules are 2200 to 2900 Å in diameter (see Table 2). The sparse endoplasmic reticulum is in the form of cytoplasmic and perinuclear lamellae.

Golgi bodies and mitochondria are small and few in number. In *T. grahami* from Lake Nakuru the eta cells appear markedly larger and more active than in *T. grahami* from Lake Magadi. They appear at an intermediate state of activity between the Lake Magadi fish and *T. grahami* in tap water.

Fresh Water Species: In these species the eta cells form a larger mass than in the "soda" lake species. The cells are larger and in light micrographs the cytoplasm appears partially degranulated; Golgi bodies are clearly visible. The cells in *T. leucosticta* appear larger, more active and with significantly larger nuclei than *T. zillii* ($p<0.01$) or *T. nigra* ($p<0.05$) (Fig. 1). Furthermore the nuclear area in *T. zillii* was significantly smaller ($p<0.01$) than in *T. nigra* ($p<0.01$) and was not significantly different from that of *T. alcalica*, one of the "soda" lake species (Fig. 1).

In electron micrographs, the eta cells in *T. zillii*, *T. nigra* and *T. leucosticta* are indistinguishable. They contain abundant endoplasmic reticulum both as perinuclear and cytoplasmic lamellae. Golgi bodies and mitochondria are numerous throughout the cytoplasm. The cytoplasm appears lobulated with interlocking stellate-like projections (Figs. 5b, c, 6c, d).

Effect of Salinity Changes: In light micrographs, prolactin cells in „soda" lake species maintained in the laboratory in artificial lake Magadi media appear more active and partially degranulated compared with the lake samples; in both species the nuclei are markedly larger (Figs. 1, 3), more regular in outline and have a more pronounced nucleolus than in the lake fish. *T. grahami* and *T. alcalica* in 50% artificial Magadi lake water have eta cells which appear larger and more active than in both the lake fish and those acclimated to the artificial Magadi medium. In *T. alcalica* the nuclear size is significantly greater in the fish acclimated to 50% when compared with the 100% artificial Lake Magadi acclimated animals (Fig. 1). In both species in 50% artificial Magadi lake water the prolactin cell cytoplasm contains moderate amounts of RNA and is partly degranulated.

Maximal prolactin cell activity is found in *T. grahami* and *T. alcalica* in tap water (Figs. 3d, e). The eta cell region of the RPD is considerably enlarged and more vascularized not only when compared with similar species in hyperosmotic environs (lake samples and animals maintained in the laboratory) but also when compared with hyposmotic lake species. This apparent hyperactivity is reflected in significant enlargement of prolactin cell nuclei in tap water adapted *T. alcalica* and *T. grahami* compared with artificial Magadi lake water adapted specimens (50 and 100%) ($p < 0.01$) and all the lake samples of *Tilapia* (Fig. 1). The prolactin cell nuclei in tap water acclimated *T. grahami* and *T. alcalica* have smooth outlines and prominent nucleoli; the cytoplasm contains abundant RNA and is partially or wholely devoid of erythrosmophilic granules. The prolactin cells in tap water acclimated *T. alcalica* appear more degranulated than those in *T. grahami* in similar conditions.

In electron micrographs the prolactin cells in lake samples of *T. grahami* and *T. alcalica* and those acclimated to 100% artificial Magadi lake water were indistinguishable. In fish acclimated to the 50% artificial Magadi medium the endoplasmic reticulum is markedly increased in volume. In fresh water acclimated *T. grahami* and *T. alcalica* the cells appear greatly enlarged. The degranulated cytoplasm contains massive endoplasmic reticulum developments, numerous polyribosomes, Golgi bodies and large mitochondria. In *T. alcalica* the prolactin

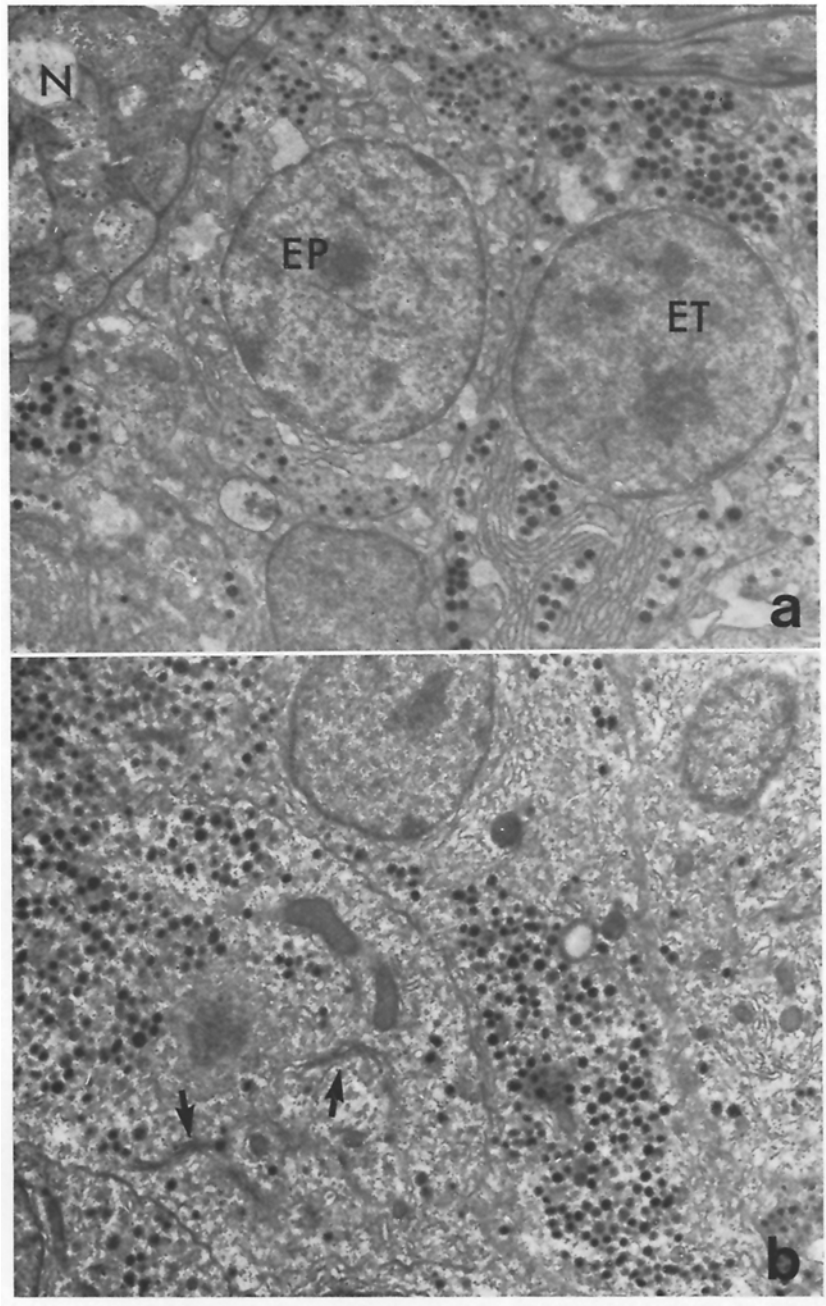


Fig. 4. a) RPD in *T. grahami* from Lake Magadi showing eta (putative prolactin) (*ET*) and epsilon (putative ACTH cells) (*EP*). Note close proximity of epsilon cells with neurohypophysis (*N*) (×5600). b) Epsilon cells in *T. grahami* acclimated to tap water. Note increase in the size of cells and active Golgi apparatus (arrow) (×600)

cell cytoplasm appears more voluminous and more degranulated than in *T. grahami* and contains more ribosomes (both free and attached to the extensive endoplasmic reticulum) and large mitochondria with numerous cristae (Figs. 6a, b).

Non-secretory "Stellate" Cells

"Stellate" cells are found in all the species examined. They are small cells, visible in light micrographs but more readily identifiable in electron micrographs. The cytoplasm has numerous projections between the eta cells although the nucleus and cell body are most commonly at the periphery of the gland. Small amounts of endoplasmic reticulum, moderate numbers of unattached ribosomes and bundles of microfibrils are commonly present in the main cell body (Figs. 5a–c, 6c, d, 7a).

The cells are more numerous in the fresh water species (*T. zillii*, *T. nigra* and *T. leucosticta*) than in the "soda" lake species acclimated to dilute media. However, in *T. alcalica* in fresh water cytoplasmic extensions were not found between the eta cells.

Proximal Pars Distalis (PPD)

The region is composed of 3 cell types, 1 acidophil (putative somatotrophs (STH)) and 2 basophils (putative thyrotrophs (TSH) and gonadotrophs (GTH)).

The middle of the PPD is formed by columns or cords of cells running roughly perpendicular to the central longitudinal axis of the gland. The region is interpenetrated by processes of the NH. The bulk of the columns is formed by STH acidophil cells, which form a nearly continuous border to the NH tissue (Figs. 2a–c). STH cells are generally cuboidal (sometimes neatly arranged perpendicular to the NH/adenohypophysial interface); they contain orangeophil secretory granules which may be so densely packed as to appear a homogeneous mass. The nuclei are large and elliptical, with 1 or 2 nucleoli. When very active, there is an obvious halo which appears dark purple-grey in the sections stained with Herlant tetrachrome (possibly RNA) around the nucleus. The Golgi image shows as clear tubules or crescents among the granules.

In electron micrographs the granules in the putative STH cells are generally more electron dense and larger in diameter than in the RPD prolactin cells; the STH granule diameters (2600–3900 Å) do not appear to differ in the different species investigated. The cells have large mitochondria, active Golgi bodies and small amounts of granular perinuclear endoplasmic reticulum. They form close connections with the basement membrane both in the vicinity of blood vessels and the NH. The cytoplasmic granules appear to be released into the matrix of the basement membrane (Figs. 7b, c).

The PPD acidophil cells are large and active in all of the lake species investigated; in electron micrographs they appear more active (with increased endoplasmic reticulum, Golgi bodies and mitochondria) in *T. grahami* and *T. alcalica* acclimated to tap water than in other groups. Similarly, in light micrographs the STH cells in tap water acclimated fish were clearly more active (clearer nuclear outline, prominent nucleoli, significantly larger nucleus ($p < 0.01$) (Fig .2), more prominent Golgi profiles than in animals from hyperosmotic media.

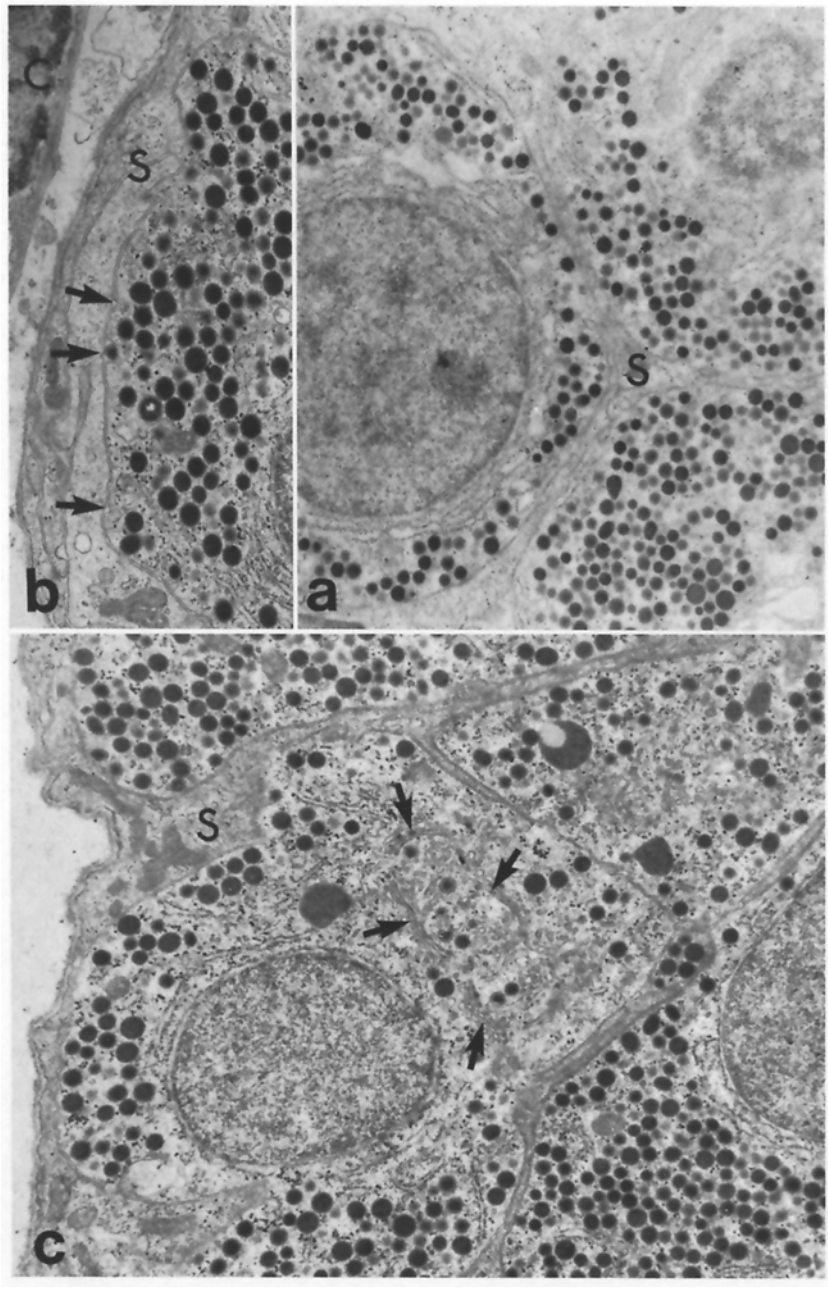


Fig. 5. a) Eta cell in T. *grahami* from Lake Magadi. Note relatively small inactive cells. *S*, stellate cell (×10000). b) Peripheral eta cell in *T. zillii* from Sagana fish pond showing granule-release profiles (arrow). *S*, stellate cell, *C*, blood capillary (×15000). c) Peripheral eta cells in *T. zillii* from Sagana fish ponds. Note active appearance of cells and extensive Golgi bodies (arrows). *S*, stellate cell (×11000)

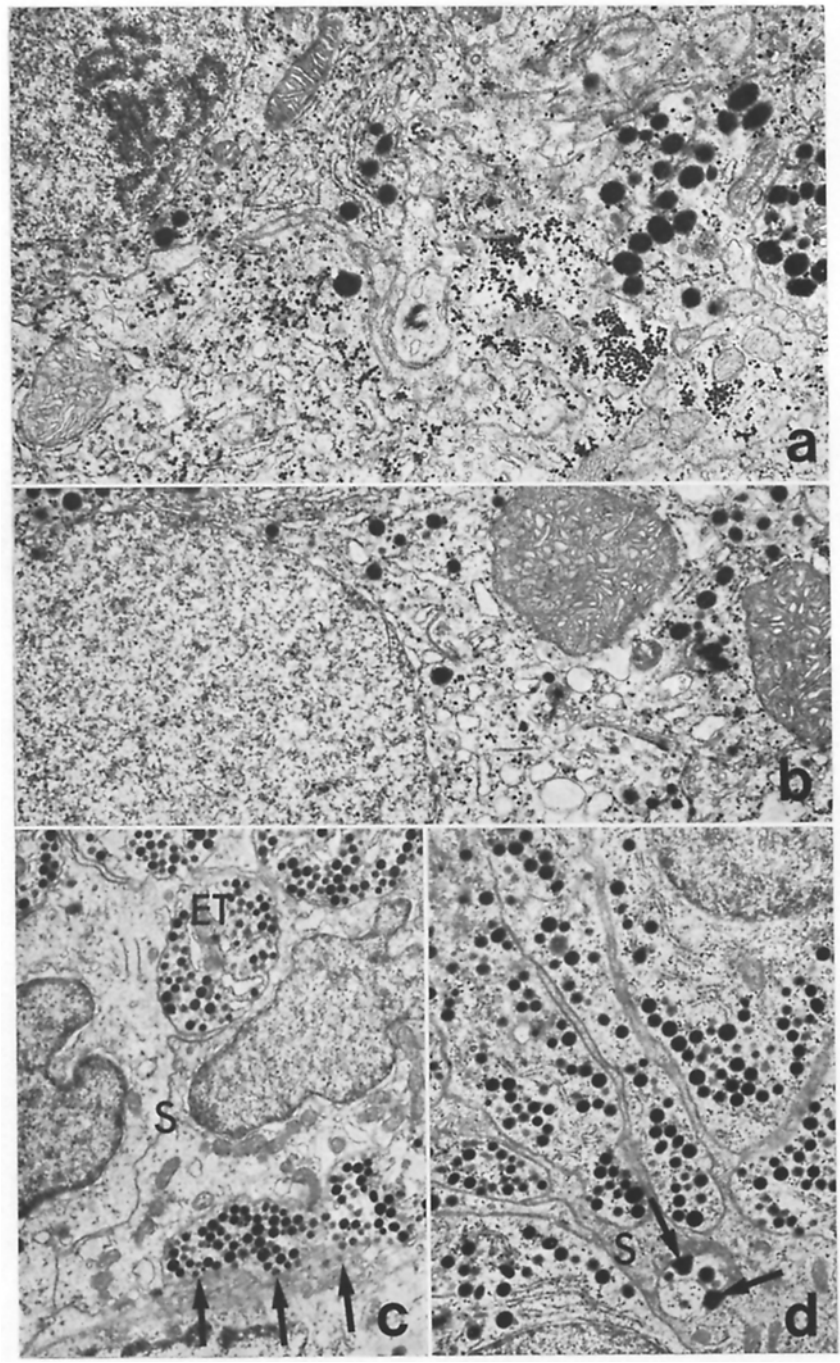


Fig. 6. a) Part of eta cell in *T. alcalica* acclimated to tap water showing the numerous ribosomes and markedly degranulated cytoplasm when compared with Fig. 4a and 5a (×10500). b) Similar to 6a showing enlarged mitochondria with multiple cristal (×10000). c) Peripheral

Among the STH cells are small basophils (putative TSH). They occur singly or in small clumps and in some fish are rarely found. They occur predominantly in the antero-lateral regions of the PPD, less commonly in the sagittal region. However, in some species (notably *T. alcalica*) they occur in the ventral PPD also amongst the putative GTH cells. The TSH cells have relatively large nuclei and little cytoplasm. The nuclei lie to one side of the cell and appear crescentic in outline. The cells contain cytoplasmic granules which stain a clear (pale) blue in Herlant's tetrachrome technique. The granules are frequently aggregated into rod-shaped, crystalline-like bodies. The cytoplasm sometimes contains granules which stain red in Herlant's ("R-granules").

Crystalline cytoplasmic inclusions appear to be fewer in "soda" lake species acclimated to tap water whereas the "R-granules" appear to increase in abundance in these groups.

In electron micrographs TSH cells appear as large ovoid cells with ovoid nuclei. The cytoplasm contains numerous mitochondria and Golgi bodies but only small amounts of endoplasmic reticulum. The types of cytoplasmic inclusions include small, membrane-bound, electron dense granules (1300–2000 Å diameter), vesicles, of widely different sizes, containing finely granular homogenous material and large, rod-shaped, electron-dense bodies (1500–25000 Å in length) (Figs. 8a, b). The electron-dense bodies appear to arise as aggregations of the smaller electron-dense granular and in many cases have a uniform rectangular appearance akin to that of the protein crystal inclusions in yolk platelets (Fawcett, 1969).

One pole of the TSH cells abuts the basement membrane around blood vessels in the PPD and at these points of contact granules appear to be released into the basement membrane structures which surround the vessels (Fig. 8a). Putative TSH cells are found in all the lake species and in electron micrographs did not appear to differ in any of the groups investigated. However, since there is a great deal of variation in the appearance of these cells within the same animal, the possibility that cells are at different levels of activity in the different groups cannot be discounted.

Ventral and lateral to the STH/TSH cell mass is found a mass of large basophils (putative GTH). In sagittal or parasagittal sections they may penetrate *en masse* amongst the STH and TSH cells to reach the border of the NH. Occasionally, isolated GTH cells are present in the RPD. The nuclei of these cells are large and rounded with 1 or 2 prominent nucleoli. The appearance of the cells varied greatly between individuals, possibly concomitant with different levels of gonad activity.

The GTH cells contain fine secretory granules which stain slateblue or purple-blue with Herlant's technique. The cytoplasm often contains huge "empty" intracellular vesicles of vacuoles which are bordered by large, orange G stainable, granules; the latter are refractile, variable in size and similar to the "R-granules" in GTH cells in other teleosts (Ball and Baker, 1969). "R-granules" can also be

eta (*ET*) and stellate cells (*S*) in *T. leucosticta* from Lake Naivasha. Note granule-release from eta cell (arrows) (×9000). d) Apparent "budding" of eta cells in *T. zillii* from Sagana fish ponds. Note the granule-release profile (arrow) between "bud" and stellate cell (*S*) and the fine projections of stellate cell cytoplasm between eta cells (×8500)

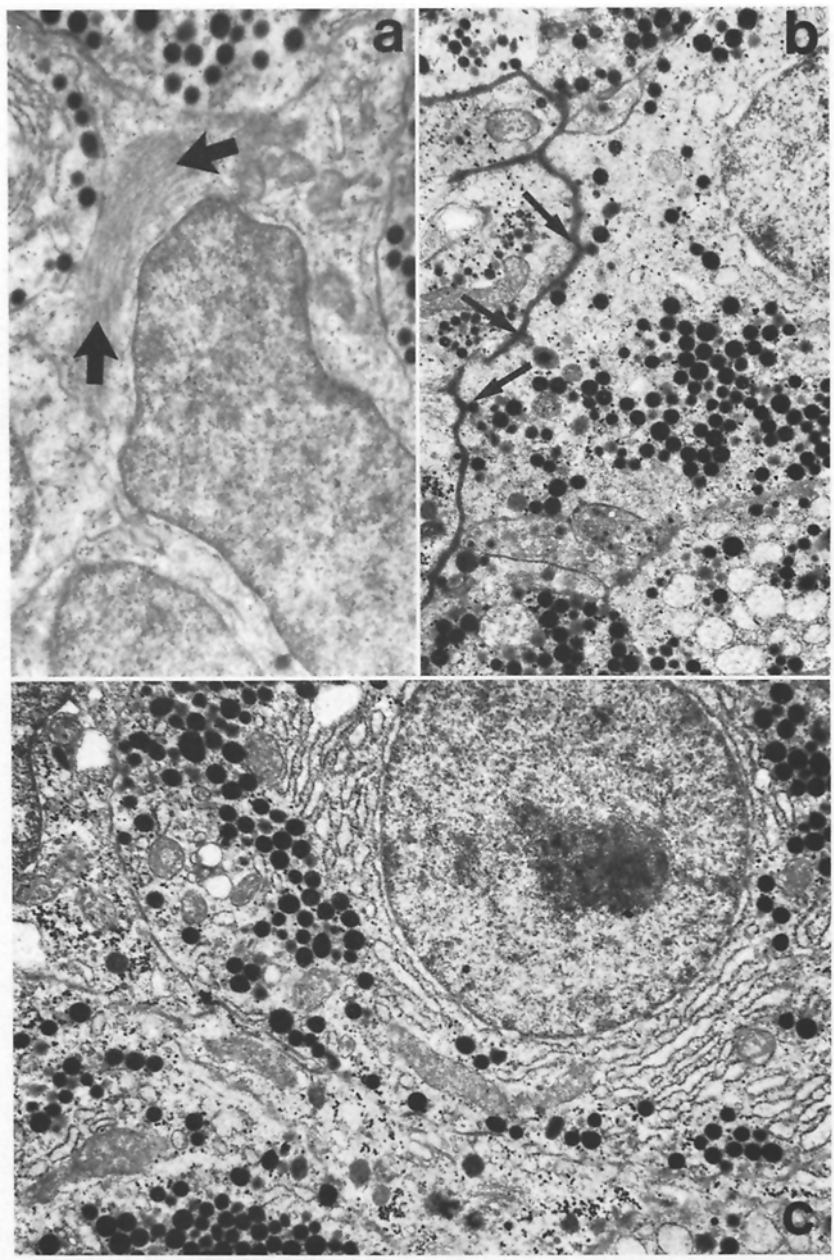
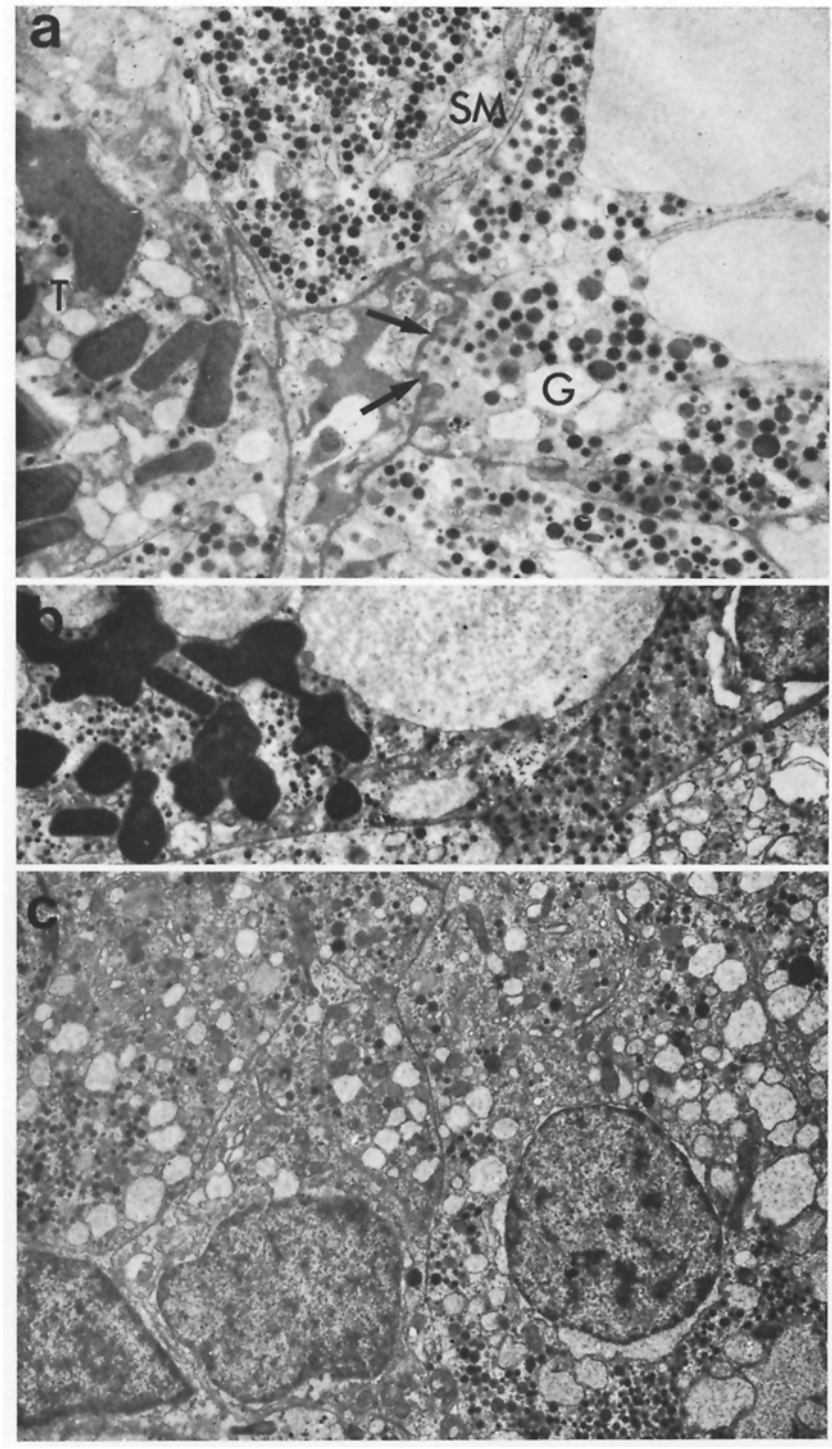


Fig. 7. a) Microfibril organelles (arrow) in stellate cell of *T. grahami* from Lake Magadi (×10000). b) Putative somatotroph in *T. alcalica* in 100% artificial Magadi lake water showing electron-dense basement membrane in regions of granule release (arrows) (×10000). c) Similar to 7b showing characteristic endoplasmic reticulum formation of somatotroph (×12500

Fig. 8. a) Putative thyrotroph (*T*), gonadotroph (*G*) and somatotroph (*SM*) in PPD of *T. grahami* from Lake Magadi. Note the large vesicles in the gonadotrophs and the apparent release of granules from these cells into a basement membrane structure (arrows) (×6500).



b) Putative thyrotrophs in *T. grahami* from Lake Magadi showing crystalling inclusions (×6900). c) Putative gonadotrophs in *T. zillii* from Sagana hatchery ponds showing smaller cytoplasmic vecuoles (×6700)

seen among the secretory granules giving a marked purple tinge to the cells, and are particularly prominent when the GTH cells are otherwise degranulated. The "R-granules" may take the form of rods bordering the large vacuoles.

In electron micrographs (Figs. 8a, c) the cells appear rather elongated with one cell pole touching the basement membrane around blood vessels in this region. The cells contain membrane-bound, cytoplasmic granules of a wide range of diameters up to 4500 Å which vary in electron density from very electron-dense to virtually electron-translucent. The large vesicles within the cell cytoplasm (which take the form of numerous small structures) containing finely granular, amorphous material. The large vesicles appear to be formed by aggregations. of smaller vesicles The "R-granules", evident in light micrographs appear in electron micrographs as large electron-dense organelles bordering the cytoplasmic vacuoles.

The putative GTH basophils vary markedly in appearance both within the same animal and between fish of the same species. Cells of similar appearance are found in all species investigated and no evidence was found of a variation in the structure of these cells in the five different species used here.

Granule Release

The eta cells appear to release their granules by exocytosis in the manner described in other species (Weiss, 1965; Leatherland, 1970; 1972; Nagahama, Nishioka and Bern, 1973). The release occurs most commonly at parts of the cell membrane which are in contact with the stellate cells or adjacent to blood vessels. The extracellular granules appear to retain their electron-density at least temporarily (Figs. 5b, 6c).

Granule release from the RPD epsilon (putative ACTH) cells and the PPD acidophil (putative STH) cells is less distinct. Granules appear to be released into the basement membrane (Fig. 7b) which separates the NH and RPD. The granules appear to change in composition and become less electron-dense prior to their release.

The fate of the cytoplasmic vesicular and crystalline inclusions in the putative TSH basophils is not known. The electron-dense granules appear to be released by exocytosis into the basement membrane adjacent to capillaries in the PPD (Fig. 8a). A similar phenomenon is found in the putative GTH basophils; the granules being released most commonly contain "pale" material.

In all cases, the region of basement membrane adjacent to an area of granule release appears markedly more electron-dense than in other zones (Figs. 7b, 8a).

Discussion

The apparently greater secretory activity of prolactin cells in the *Tilapia* species from freshwater environments when compared with the species from the "soda" lakes is consistent with the hypothesis that prolactin is required by *Tilapia* species for hydromineral regulation in freshwater. The findings are in agreement with studies in other species of euryhaline or stenohaline freshwater teleosts (see reviews by Ball, 1969; Ball and Baker, 1969, ; Lam, 1972; Ensor and Ball, 1972; Schreibman, Leatherland and McKeown, 1973; also Dharmamba and Nishioka, 1968; Dharmamba, 1970; Dharmamba, Handin, Nandi and Bern, 1967; Dhar-

mamba and Maetz, 1971). However, one of the criteria of cellular activity, that of nucleus size, was found to be similar in both *T. alcalica* and *T. zillii* (Fig. 1). Furthermore, although the nuclear sizes in the fresh water species *(T. zillii, T. nigra, T. leucosticta)* were significantly *larger* ($p < 0.01$) than in *T. grahami* from Lake Magadi (a concentrated "soda" lake) they were significantly *smaller* ($p < 0.01$) than in *T. grahami* from Lake Nakuru (a dilute "soda" lake) (Table 1) and *T. grahami* acclimated to 100% and 50% artificial Magadi lake water (Fig. 1). This may suggest that the long term acclimatization (adaptation) of the fresh water species to the dilute media has caused them to be less sensitive than that "soda" lake species to dilution of the ambient media. The mechanisms of adaptation possibly include an increase in the number of prolactin cells and adaptive changes at the sites of osmotic exchange similar to those that have occurred in other freshwater stenohaline species. This is supported by studies of mortality rates and plasma electrolytes levels in *T. zillii* and *T. nigra* subjected to hyperosmotic environments which clearly demonstrated that these species had, at best, a limited euryhaline capability (Leatherland, Hyder and Ensor, 1974).

The marked hyperactivity of the prolactin cells in *T. alcalica* and *T. grahami* subjected to tap water environments also clearly indicates the involvement of the hormone in hydromineral regulation in dilute media. Clarke (1973) demonstrated a 50% drop in the pituitary prolactin content of *T. grahami* transferred for 24 hours to tap water; this finding is supported by the histological appearance of the gland. The very marked activity of the prolactin cells in "soda" lake species adapted to fresh water compared with the species collected from fresh water (Fig. 1) may be due to a number of factors. Firstly, the freshwater species may be genetically better suited than the "soda" lake species to the dilute media. Thus, the "soda" lake species may require more prolactin in order to maintain themselves in the "foreign" environment. Secondly, the hyperactivity of prolactin cells in "soda" lake species in dilute environs may reflect the need for the production of large amounts of the hormone by relatively (relative to the freshwater species) small numbers of cells. However, in *T. grahami* from Lake Nakuru which were introduced into the lake from Lake Magadi 10–12 years before the collection, and which therefore represent long-term acclimatization of "soda" lake fish to a dilute media, the prolactin cells have a significantly larger nucleus ($p < 0.01$) and are thus probably more active than in the freshwater lake species (Fig. 1). This suggests that even prolonged acclimation of the "soda" lake species would not increase their freshwater regulatory capabilities and that genetic selection processes are involved in the success of the fresh water species.

Prolactin cells in *T. grahami* and *T. alcalica* from the concentrated "soda" lakes (Lake Magadi and Lake Natron respectively) although apparently inactive were not totally regressed and granules were occasionally seen to be released from the cells. In sea-water adapted *T. mossambica*, exogenous prolactin appears to inhibit Na^+ extrusion and results in an elevated plasma Na^+ level (Dharmamba, Mayer-Goslan, Maetz and Bern, 1973). This would be of obvious disadvantage to the "soda" lake species and thus the continued prolactin secretion in these species may be tolerated either to accomodate for possible infrequent dilutions of ambient media (during periods of rain) or play a role as yet not known. A possible role is that of regulating Ca^{++} metabolism (see review by Pang, 1973), especially since

Lakes Magadi and Natron have only low concentrations of this ion (Leatherland, Hyder and Ensor, unpublished data). The prolactin cells in *T. alcalica* and *T. grahami* transferred to tap water showed marked increases in activity, thus indicating that whatever their possible role in fish in hyperosmotic media, they are still involved in osmo(iono) regulation in hyposmotic environs. The markedly greater responses of the eta cells in tap water acclimated *T. alcalica* compared with those in similarly treated *T. grahami* may be correlated with the different plasma Na^+ homeostatic capabilities of the two species: the plasma Na^+ concentrations in tap water *T. alcalica* acclimated was 40–50% lower than in fish killed immediately on removal from the lake while *T. grahami* lost only 10–15% of plasma Na^+ (Leatherland, Hyder and Ensor, 1974). Thus *T. grahami* appears to be less susceptible than *T. alcalica* to Na^+ loss in fresh water.

The epsilon (ACTH) cells in *T. mossambica* were briefly described by Dharmamba and Nishioka (1968). The same cells in the various native *Tilapia* species were of similar general morphology. The high secretory activity of the epsilon cells in *T. alcalica* may be associated with the high salinity of Lake Natron water. This would agree with findings in other euryhaline teleosts in which the ACTH-interrenal axis is thought to be involved in ionic homeostasis in hyperosmotic ambient media (Chester Jones, Chan, Henderson and Ball, 1969). The increased activity of epsilon cells in tap water-acclimated *T. alcalica* and *T. grahami* when compared with lake species may reflect a non-specific stress response to the hyperosmotic medium. Conversely, the response may be associated with ionic homeostasis in the dilute ambient medium (Chester Jones, Chan, Henderson and Ball, 1969) as distinct from the osmotic homeostatic role of prolactin (see the foregoing).

The functional significance of epsilon cell granules of different sizes in different species or within the same species exposed to different physiological conditions is not fully understood. The size differences may reflect changed rates of synthesis, "maturation" or release of granules from the epsilon cells in the different groups. This may explain the larger epsilon cell granules in the more active fresh water-acclimated *T. alcalica* compared with the lake specimens. Similar hypotheses have been postulated to explain differences in the diameter of granules in prolactin cells in fish acclimated to, or collected from, environments of different salinity (Abraham, 1971; Dharmamba and Nishioka, 1968; Holtzman and Schreibman, 1972; Nicholl, 1972). Such differences in prolactin cell granules are not apparent in the *Tilapia* species examined here.

Non-secretory cells are present in the RPD in a number of teleosts (see review by Schreibman, Leatherland and McKeown, 1973). Weiss (1965) postulated that in *Xiphophorus* they may facilitate "budding" of eta cells which he proposed as a means of hormone release. This phenomenon may also occur in *Gasterosteus aculeatus* form *trachurus* (Leatherland, 1970) and in the freshwater *Tilapia* species used in this investigation. However it is not known whether the apparent "buds" of eta cell cytoplasm are attached in another plane of section. In *Tilapia* as in *G. aculeatus* (Leatherland, 1970) the cytoplasm of the peripheral non-secretory cells contains many mitochondria particularly in the regions of the cell membrane which abut the connective tissue that surrounds the RPD. In addition the cells contain bundles of actin-like microfibrils (Bloom and Fawcett, 1968) (possibly

indicating a degree of motility) and form very close associations with projections of the basement membrane in the RPD. Thus the non-secretory cells appear to play an active role (so far not determined) in the functioning of the secretory cells possibly in facilitating release of prolactin-cell granules (Leatherland, 1970). Stellate cells appear to be a common feature of the adenohypophysis in a number of vertebrate classes other than teleosts (see review by Vila-Porcile, 1972) where they also appear to be involved in the release of material from the "secretory" adenohypophysial cells (Vila-Porcile, 1972). Similar cell types are also found in the adenohypophysis in cyclostomes (Percy, 1973; Percy and Leatherland, 1973) which is indicative of an ancient association between "secretory" and "secretory aid" cells in the pituitary.

In tap water-acclimated *T. alcalica* the cytoplasmic projection of the non-secretory cells between the prolactin cells were not apparent. This may have been due to the hyperplasia and hypertrophy of the prolactin cells which physically excluded the non-secretory cell cytoplasm from this region. A similar phenomenon was found in *G. aculeatus* at the time when the prolactin cells were rapidly enlarging prior to the fish entering fresh water; at this stage of migration the percentage chromophobe cell area in the RPD was significantly reduced compared with sea water-acclimated fish (Leatherland, 1970).

The PPD acidophils (putative STH cells) have many of the characteristics of STH cells in other teleostean species (Ball and Baker, 1969; Schreibman, Leatherland and McKeown, 1973). There is a paucity of information regarding the physiological role(s) of STH cells in teleosts. They appear to be hyperactive in "soda" lake *Tilapia* species which were placed in tap water (see results) and therefore may be involved in osmo(iono) regulation in these species. The evidence for the involvement of STH in hydromineral regulation in other teleosts is not clear (Olivereau and Ball, 1969). Chartier (1959) suggests that the hormone may be involved in the retention of K^+ in muscle tissue of fresh water trout. However, in kokanee salmon, the plasma levels of STH were similar in distilled water-acclimated and sea water-acclimated fish even though the plasma and muscle K^+ values were markedly different in these groups (Leatherland and McKeown, 1974). It seems unlikely that the marked increase in activity of the STH cells in tap water acclimated *T. grahami* and *T. alcalica* could be due to K^+ maintenance since the concentration of K^+ in Lakes Magadi and Natron, in the artificial Magadi water and in tap water differed at most by only 2.5 mEq/l (Table 1).

It is possibly that in some teleosts STH synergises with other hormones in the response to a dilution of the ambient medium since these cells become hyperactive in thyroxine-treated *T. zillii* and *T. nigra* (unpublished data). Similar synergisms between the TSH-thyroid axis and STH have been found in *G. aculeatus* (Leatherland and Lam, unpublished data) and *Poecilia reticulata* (Pandey and Leatherland, 1970). In *Oncorhynchus nerka* STH appears to be involved in the mobilization of lipids (Leatherland, McKeown and John, 1974; McKeown, Leatherland and John, unpublished data).

The dorsal basophils in the PPD are probably TSH cells, They are found in the same region of the PPD as thyrotrophs in other teleosts (see review by Ball and Baker, 1969), and have similar staining characteristics. Furthermore in *T. nigra*, *T. zillii* and *T. mossambica* they are activated by thiourea and inhibited by thyr-

oxine (unpublished data). In electron micrographs the cells are vesiculated and similar to thyrotrophs in *O. keta* (Nagahama and Yamamoto, 1970), *Zoarces viviparous* (Oztan, 1966), thiourea-treated *Carassius auratus* (Nagahama and Yamamoto, 1969) and some mammals (Barnes, 1960). The functional significance of the large crystalline inclusions that are found in *Tilapia* is not known. They may be lytic bodies involved in the degradation of granule material (this phenomenon was shown in eel and molly (*P. latipinna*) prolactin cells (Hopkins and Baker, 1968; Hopkins, 1969). However, since the bodies have the same staining characteristics as the TSH cell granules they may also represent aggregates of TSH possibly associated with the storage of the hormone.

The ventral PPD basophils have some of the characteristics of gonadotrophs in other species (Ball and Baker, 1969). The globular, hyaline cytoplasmic inclusions have parallels in the gonadotrophs in *C. auratus* (Nagahama and Yamamoto, 1969; Leatherland, 1972), *O. nerka* (Cook and van Overbeeke, 1972) and *O. keta* (Nagahama and Yamamoto, 1970). Similarly, the large vesicles have characteristics which are like the gonadotroph II cells in sexually mature *O. nerka* (Cook and van Overbeeke, 1972). However, the single vacuoles constituting $^1/_3$ to $^1/_2$ of the cell have not been described in other teleosts. The functional significance of these vesicles is not known. They may contain the degradation products of the electron dense granules although direct secretion of the granules into the vesicles was not found. However, some of the granules appear less electron-dense than others, possibly indicating intra-granular changes in their chemistry, the end results of which might be their release into the cytoplasmic vesicles. The electron-dense granules are seen to be released around capillaries thus whatever the role of the large vesicles, they do not appear to contain material for immediate release unless the release occurs in such a way as to be undetected by the methods employed here.

The question of one or two gonadotroph cell types in teleosts has been discussed in recent reviews (Ball and Baker, 1969; Schreibman, Leatherland and McKeown, 1973). Only one gonadotroph cell was apparent in the *Tilapia* species investigated. However, since there was great variability in the activity of the gonadotrophs both within the same gland and between individuals the possibility of a second, undetected gonadotroph in *Tilapia* cannot be discounted.

References

Abraham, M.: The ultrastructure of the cell types and of the neurosecretory innervation in the pituitary of *Mugil cephalus* L. from fresh water, the sea, and a hypersaline lagoon. I. The rostral pars distalis. Gen. comp. Endocr. **17**, 334–350 (1971)

Ball, J. N.: Prolactin (fish prolactin or paralactin) and growth hormone. In: Fish Physiology (Hoar, W. S. and Randall, D. J. eds.). New York: Academic Press 1969

Ball, J. N., Baker, B.: The pituitary gland: Anatomy and histophysiology. In: Fish Physiology (Hoar, W. S. and Randall, D. J. eds.). New York: Academic Press 1969

Barnes, B. G.: Comparative cytology of the anterior pituitary of the male and female mouse. Eur. Reg. Conf. Electron Micr., Delft (1960)

Bloom, W., Fawcett, D. W.: A Textbook of Histology. Philadelphia-London-Toronto: W. B. Saunders 1968

Chartier, M. M.: Influence de l'hormone somatotrope sur les teneurs en eau et en électrolytes du plasma et du muscle de la truite arcen-ciel (*Salmo gairdnerii*). C. R. Soc. Biol. (Paris) **153**, 1757–1761 (1959)

Chester Jones, I., Chan, D.K.O., Henderson, I. W., Ball, J. N.: The adrenocortical steroids, adrenocorticotropin and the corpuscles of Stannius. In: Fish Physiology (Hoar, W. S. and Randall, D. J., eds). New York-London: Academic Press 1969

Clark, W. C.: Disc-electrophoretic identification of prolactin in the cichlid teleosts *Tilapia* and *Cichlasoma* and densitometric measurement of its concentration in *Tilapia* pituitaries during salinity transfer experiments. Canad. J. Zool., **51**, 687–695 (1973)

Cook, H., Overbeeke, A. P. van: Ultrastructure of the pituitary gland (pars distalis) in sockeye salmon (*Oncorhynchus nerka*) during gonad maturation. Z. Zellforsch. **130**, 338–350 (1972)

Dharmamba, M.: Studies on the effects of hypophysectomy and prolactin on plasma osmolarity and plasma sodium in *Tilapia mossambica*, Gen. comp. Endocr. **14**, 256–269 (1970)

Dharmamba, M., Handin, R. I., Nandi, J., Bern, H. A.: Effect of prolactin on freshwater survival and on plasma osmotic pressure of hypophysectomized *Tilapia mossambica*. Gen. comp. Endocr. **9**, 295–302 (1967)

Dharmamba, M., Maetz, J.: Effects of hypophysectomy and prolactin on the sodium balance of *Tilapia mossambica* in fresh water. Gen. comp. Endocr. **19**, 175–183 (1971)

Dharmamba, M., Mayer-Gostan, N., Maetz, J., Bern, H. A.: Effect of prolactin on sodium movement in *Tilapia mossambica* adapted to sea water. Gen. comp. Endocr. **21**, 179–187 (1973)

Dharmamba, M., Nishioka, R. S.: Response of "prolactin-secreting" cells of *Tilapia mossambica* to environmental salinity. Gen. comp. Endocr. **10**, 409–420 (1968)

Ensor, D. M., Ball, J. N.: Prolactin and osmoregulation in fishes. Fed. Proc. **31**, 1615–1623 (1972)

Fawcett, D. W.: The Cell. Its Organelles and Inclusions. Philadelphia-London: W. B. Saunders, 1969

Holtzman, S. and Schreibman, M. P.: Morphological changes in the "prolactin" cell of the freshwater teleost, *Xiphophorus hellerii*, in salt water. J. exp. Zool. **180**, 187–196 (1972)

Hopkins, C. R.: The fine structural localization of acid phosphatase in the prolactin cell of the teleost pituitary following the stimulation and inhibition of secretory activity. Tiss. and Cell **1**, 653–671 (1969)

Hopkins, C. R., Baker, B. I.: The fine structural localization of acid phosphate in the prolactin cell of the eel pituitary. J. Cell Sci. **3**, 357–364 (1968)

Lam, T. J.: Prolactin and hydromineral regulation in fishes. Gen. comp. Endocr., Suppl. **3**, **3**, 328–338 (1972)

Leatherland, J. F.: Seasonal variation in the structure und ultrastructure of the pituitary in the marine form (*trachurus*) of the threespine stickleback. *Gasterosteus aculeatus* L. I. Rostral pars distalis. Z. Zellforsch. **104**, 301–317 (1970)

Leatherland, J. F.: Histophysiology and innervation of the pituitary gland of the goldfish,, *Carassius auratus* L.: A light and electron microscope investigation. Canad. J. Zool. **50**, 835–844 (1972)

Leatherland, J. F., Hyder, M., Ensor, D. M.: Regulation of plasma Na^+ and K^+ concentrations in five African species of *Tilapia* fishes. Comp. Biochem. Physiol., in press (1974)

Leatherland, J. F., McKeown, B. A.: Effect of ambient salinity on prolactin and growth hormone secretion and on hydro-mineral regulation in kokanee salmon smolts (*Oncorhynchus nerka*). J. comp. Physiol., in press (1974)

Leatherland, J. F., McKeown, B. A., John, T. M.: Circadian rhythm of plasma prolactin, growth hormone, glucose and free fatty acid in juvenile kokanee salmon, *Oncorhynchus nerka*. Comp. Biochem. Physiol., **47 A**, 821–828 (1974)

Nagahama, Y., Nishioka, R. S., Bern, H. A.: Responses of prolactin cells of two euryhaline marine fishes, *Gillichthys mirabilis* and *Platichthys stellatus* to environmental salinity. Z. Zellforsch. **136**, 153–168 (1973)

Nagahama, Y., Yamamoto, K.: Basophils in the adenohypophysis of the goldfish (*Carassius auratus*) Gumma Sym. Endocr. **6**, 39–55 (1969)

Nagahama, Y., Yamamoto, K.: Morphological studies on the pituitary of the chum salmon, *Oncorhynchus keta* (1). Fine structure of the adenohypophysis. Bull. Fac. Fish. Hokkaido Univ. **20**, 293–302 (1970)

Nicholl, C. S.: Some observations and speculation on the mechanism of "depletion", "repletion", and release of adenohypophyseal hormones. Gen. comp. Endocr. Suppl. **3**, 86–96 (1972)

Öztan, N.: The fine structure of the adenohypophysis of *Zoarces viviparous* L. Z. Zellforsch. **69**, 699–718 (1966)

Olivereau, M., Ball, J. N.: Pituitary influences on osmoregulation in teleosts. Mem. Soc. Endocr. **18**, 57–85 (1970)

Pandey, S., Leatherland, J. F.: Comparison of the effects of methallibure and thiourea on the testis, thyroid and adenohypophysis of the adult and juvenile guppy, *Poecilia reticulata* Peters. Canad. J. Zool. **48**, 445–450 (1970)

Pang, P.K.T.: Endocrine control of calcium metabolism in teleosts. Amer. Zool. **73**, 775–792 (1973)

Percy, R.: Structure and fine structure of the pituitary gland in the sea lamprey (*Petromyzon marinus*). M. Sc. Thesis, University of Guelph (1973)

Percy, R., Leatherland, J. F.: Fine structure of the pituitary gland in larval sea lampreys (*Petromyzon marinus* L.). J. Endocr. **59**, XL—XLI (1972)

Steel, R.G.D., Torrie, J. M.: Principles and Procedures of Statistics. New York: Mc Graw Hill 1960

Vila-Porcile, E.: Le réseau des cellules folliculo-stellaires et les follicules de l'adénohypophyse du rat (pars distalis). Z. Zellforsch. **129**, 328–369 (1972)

Weiss, M.: The release of pituitary secretion in the platyfish *Xiphophorus maculatus* (Guenther). Z. Zellforsch. **68**, 783–794 (1965)

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