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Morphological and cytochemical characterization of cell types of the adenohypophysis of Manjuba, *Anchoviella lepidentostole* (Fowler, 1911) (0steichthyes, Engraulidae).

Caracterização morfológica e citoquímica dos tipos celulares da adeno-hipófise de manjuba, *Anchoviella lepidentostole* (Fowler,1911) (Osteichthyes, Engraulidae).

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SUMMARY

The pituitary gland of *Anchoviella lepidentostole* consists of the neurohypophysis and the adenohypophysis, which is subdivided in *pars intermedia* and *pars distalis*. The *pars distalis* comprises *pars distalis rostralis* and *pars distalis proximalis*. The cell types of the *pars distalis rostralis* are arranged in follicles. In the follicular epithelium, four cell types were cytochemically characterized: **I-PDR** (basophilic), **II-PDR** (lead haematoxylin+/HPb+), **III-PDR** (PAS+, AB pH2.5+ and AF+), **IV-PDR** (acidophilic). The *pars distalis proximalis* has two cell types: I-PDP (PAS+, AB pH 2.5+ and AF+) and **II-PDP** (acidophilic). In the *pars intermedia* there are two cell types: **I-PI** (HPb+) and **II-PI** (chromophobes).

UNITERMS: Anterior pituitary; Osteichthyes; Cytochemistry; *Anchoviella lepidentostole*

INTRODUCTION

The *Anchoviella lepidentostole*, commonly known as manjuba, is a bone *anadromous* fish which inhabits temperate and hot waters, and has a vast geographical distribution from Guianas to Parana State/Brazil. It is specially abundant in the coast of São Paulo State (Lopes *et al.*[26], 1984). Among the *engraulidaes*, manjuba is the species of greatest economical importance in the south-east of Brazil (Figueiredo; Menezes[14], 1978; Suzuki[54], 1983).

The capture of manjuba in the Ribeira do Iguape River from its estuary area and along its course, occurs from October to April. According to Bendazoli; Froschi[4] (1990) the fishing production nowadays reaches 500 tons annually. However, this study also shows that there has been a clear fall in the manjuba fishing in the latter years owing to predatory capture in the estuary region, thus preventing the animals from reaching the areas where spawning takes place.

The evident economical value of *A. lepidentostole*, as well as the imminent extinction rish have led to a great number of researches. Some of them aimed systematic reviews (Ihering[20], 1930; Carvalho[9], 1950; Figueiredo; Menezes[14], 1978) while others are related with the ichthyological, nutritional and fishing aspects (Furuya[15], 1959; Nomura[36], 1962; Nomura[37], 1964; Mandelli; Giamas *et al.*[17], 1984; Paiva Filho *et al.*[43], 1986; Bendazoli; Rossi-Wongstschowski[5], 1990). Nevertheless, there are few studies which dealt with the gonadal characterization and reproductive cycle of this species (Giamas *et al.*[16], 1983; Lopes *et al.*[26], 1984; Giamas *et al.*[18], 1990), and no research on other endocrine features of the manjuba.

As cytochemical methods have been largely used for the characterization of cells from the adenohypophysis in Osteichthyes under normal or experimental conditions (Olivereau[39], 1976; Burns[7], 1991), our goal was to characterize morphologically and cytochemically these cell types in manjuba. By doing so, we would like to contribute to future investigations which relate endocrinology of *A. lepidentostole* with its reproductive cycle. This knowledge and a more adequate fishing technology will allow the survival and reproduction of the manjuba, either in nature or in captivity.

MATERIAL AND METHODS

The pituitary glands used in this study were taken from 40 adult manjubas – *Anchoviella lepidentostole* specimens – of both sexes, captured along the whole Ribeira de Iguape River in São Paulo State/Brazil. A special device named "manjubeira" net was used.

The specimens were sacrificed by asphyxia, decapitated and fixed in Bouin's liquid for 24 hours at room temperature. Then they were decalcified for 15 days in EDTA solution neutralized at 10%, which was daily changed. Afterwards, they were washed in tap water for 24 hours and processed for histological paraffin embedding.

Seven µm serial cephalic sections, oriented in a sagital and frontal manner, allowed localization of the pituitary gland. Sections which contained hypophysial areas for morphological analysis were stained with haematoxylin-eosin (HE) and with Mac Conaill's lead haematoxylin (HPb) (Mac Conaill[27], 1947), Halmi's Trichromic (HT) (Behmer *et al.*[3], 1976) and Mallory's Trichromic (MT) (Mallory[28], 1942). For the cytochemical study the Periodic Acid of Shiff (PAS) (Mc Manus[31], 1946) Alcian Blue pH 2.5 (AB) (Steedman[53], 1950), and Alcian Blue pH 0.5 (Lev; Spicer[24], 1964) methods were used.

In an attempt to have a better control and more details on the polysaccharides cytochemistry, the following methods were used: acetilation+PAS (Mc Manus; Cason[32], 1950) and acetilation+saponification+PAS (Mc Manus; Cason[32], 1950), PAS after salivary amilase treatment (Lison[25], 1960), metilation+AB pH 2.5 (Terner; Lev[55], 1963) metilation and saponification+AB pH 2.5 (Terner; Lev[55], 1963), and acid hydrolysis followed by AB pH 2.5 (Quintarelli *et al.*[46], 1961).

RESULTS

In the manjuba the average diameter of the pituitary gland is about 1 mm, and it is placed in the *sella turcica*, found rostrally to the *saccus vasculosus* and caudally to the palate muscle (Fig. 1).

The pituitary gland is divided in adenohypophysis and neurohypophysis which branches largely as it invades the adenohypophisis in a way that enters all regions.

The adenohypophysis is formed by two regions: the *pars distalis* (**PD**), divided in *pars distalis rostralis* (**PDR**) and in *pars distalis proximalis* (**PDP**), and the *pars intermedia* (**PI**) (Fig.1).

PARS DISTALIS ROSTRALIS: The **PDR** is the most developed adenohypophysary region. It is invaded by large neurohypophysary branches and is organized in follicles which contain stainable material in the lumen. These follicles have irregular shape and are ventrally elongated in the middle of the palate muscles, and turn thinner as they advance farther from the gland.

There are four cell types in the stratified epithelium, according to morphology and stain affinity to the applied methods.

The **I-PDR** cell type covers the lumen of the follicles (Fig. 2, Fig. 3). They are pavimentous cells with elongated nucleus and loose chromatin. The cytoplasm is poor and basophilic. These cells do not show positive reaction for any cytochemical applied method.

The second cell type, **II-PDR**, shows a palisade arrangement and is characterized by having one of its faces turned to the neurohypophisis. They are polyedric, big cells with rounded nucleus, frequently eccentric and with loose chromatin. The cytoplasm is acidophilic when stained by HE and stains in black by HPb method (Fig. 1, Fig. 2).

The **III-PDR** cell type is scattered among the other follicular cell types. They are spindle shaped, the nucleus is rounded, usually central and the chromatin is loose. The cytoplasm shows thin granules which are stained by AB pH 2.5 (Fig. 3), by aldehyde-fucsin of the Halmi's trichromic method, and by PAS. The polysaccharides analysis showed that this cell type is positive for the following methods: PAS after salivary amilase reaction, acetilation followed by saponification+PAS and even by metilation followed by saponification+AB pH2.5.

The fourth cell type from **PDR**, **IV-PDR**, seems to be more abundant. It is characterized by an elongated shape, round and big nucleus which is generally central, and with loose chromatin. The cytoplasm is acidophilic when stained by MT and orange by the HT. These cells are chromophobes when submitted to the other applied methods (Fig. 2).

PARS DISTALIS PROXIMALIS: In this region two cell types can be distinguished according to morphology, staining affinity and topography.

The first cell type, **I-PDP**, prevails in the lateral and ventral portions of the **PDP** (Fig. 4) and it is represented by globular, big cells with rounded and generally eccentric nucleus. The chromatin is loose and the nucleolus is evident. The cytoplasm has rough granules which are stained by aldehyde-fuccsin of the Halmi's trichromic method (Fig. 4), and by blue in Mallory's trichromic. The **I-PDP** cells are still positive for AB pH 2.5 (Fig. 3), PAS (Fig. 5), PAS after salivary amilase action, acetilation followed by saponification+PAS and finally by metilation followed by saponification+AB pH 2.5.

The second cell type of this hypophysary region is distributed mainly in the areas near the **PI**, are arranged in cord-like structures which surround the neurohypophysary branches, and are still scatterly present among the **I-PDP** cells. They are prismatic or pyramidal with oval or round nucleus, generally eccentric. The chromatin is granular. The cytoplasm has delicate granules which are clearly acidophilic by HE and MT methods, and there is no positive reaction for the other applied methods (Fig. 5).

PARS INTERMEDIA: This hipophysary region also has cell types which differ in staining affinity with the HPb method.

The **I-PI** cells are polyedric, the nucleus is oval or rounded and eccentric. The chromatin is loose and the nucleus is conspicuous. The cytoplasm has thin granules which are slightly positive for HPb (Fig. 6). In this cell population there can still appear cells with nucleus and cytoplasm clearly enlarged. They are scatterly distributed in the **PI**.

The second cell type is represented by **II-PI** type cells which appear less than the **I-PI** type and show a polyedrical shape, oval nucleus and are characterized by cytoplasmatic chromophobia (Fig. 6).

Mitosis figures between the two cell types can still be found in the **PI** (Fig. 6).

Figure 1

Anchoviella lepidentostole. Pituitary gland. *Sella turcica* (ST), *Saccus vasculosus* (SV), diencephalon (D), *Pars Distalis Proximalis* (PDP), *Pars Distalis Rostralis* (Dark arrow), where the II-PDR cells are HPB+, *Pars Intermedia* (clear arrow) and the neurohypophysys (N). 130 x Hpb.

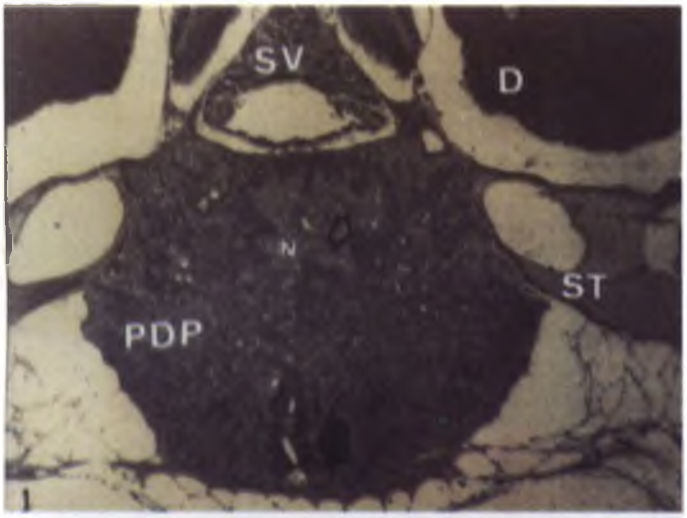


Figure 3

Anchoviella lepidentostole. Pituitary gland. *Pars Distalis Rostralis* (PDR) and *Pars Distalis Proximalis* (PDP). I-PDR cells (arrowhead), III-PDR cells (long arrow) and I-PDP cells (star) 800x Alcian Blue pH2.5.

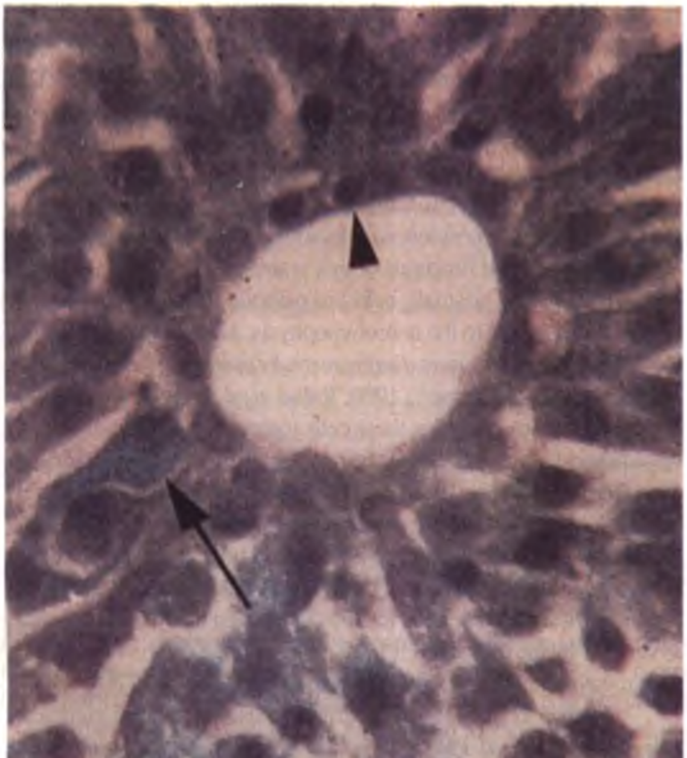


Figure 2

Anchoviella lepidentostole. Pituitary gland. *Pars Distalis Rostralis* (PDR). I-PDR cells (arrowhead), II-PDR cells (dark arrow) and IV-PDR cells (clear arrow). Follicular lumem (L) 800x. Hpb.

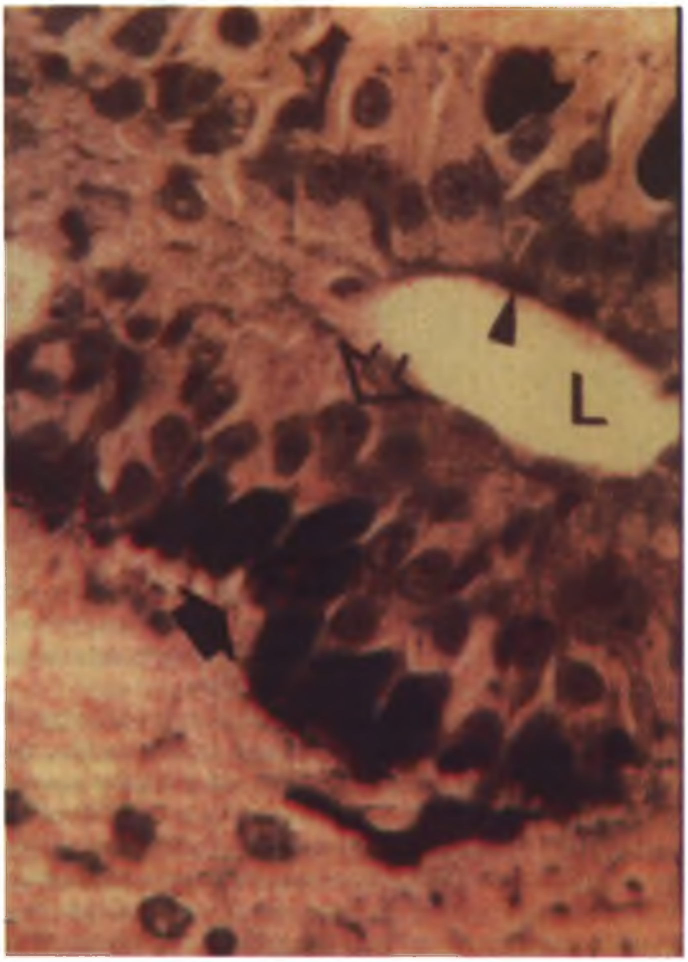


Figure 4

Anchoviella lepidentostole. Pituitary gland. *Pars Distalis Proximalis* (PDP). I-PDP cells (short white arrow) stained by aldehyde fucsin. 130x Halmi's Trichromic.

Figure 5

Anchoviella lepidentostole. Pituitary gland. *Pars Distalis Proximalis* (PDP) I-PDP cells (star) PAS positive; II-PDP cells (white arrow). 800x PAS.

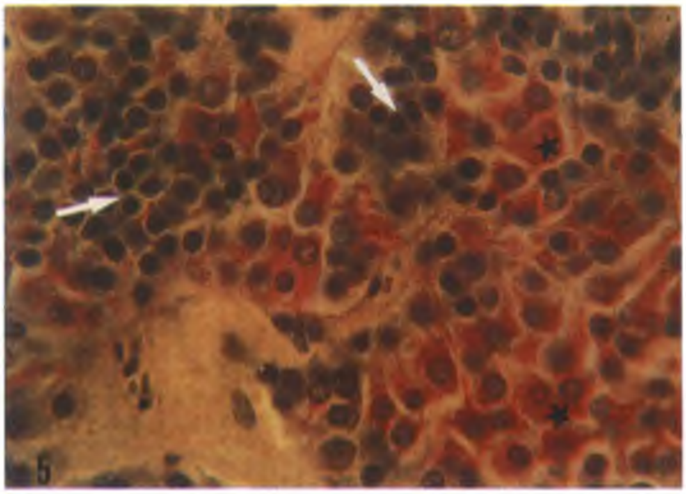
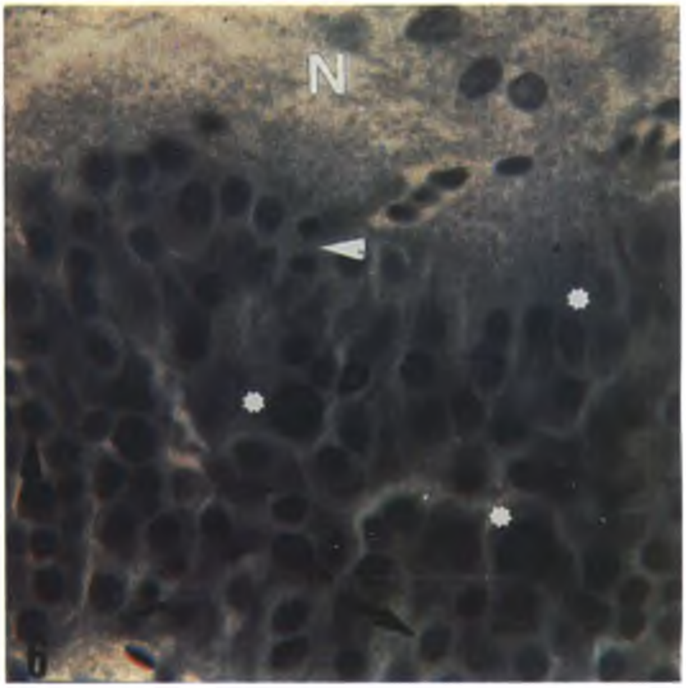


Figure 6

Anchoviella lepidentostole, Pituitary gland. *Pars Intermedia* (PI). I-PI cells (white asterisk) HPb positive; II-PI cells (arrow); mitosis (white arrowhead); neurohypophysis (N). 800X. HPb.



DISCUSSION

Generally the fish neurohypophysis is largely related to the adenohypophysis (Olivereau[38], 1967). In *Poecillia latipinna* (Olivereau; Ball[40], 1964) the neurohypophysis penetrates the hypophysary regions, specially the *pars distalis* as is seen in the manjuba.

In many *teleostei* the adenohypophysis is arranged in compact cell strings, but in more primitive species as *Anguilla anguilla,* the *pars distalis rostralis* is arranged in follicles with a stainable material in the lumen (Olivereau[38], 1967), as is observed in the **PDR** of the manjuba. However, in the *A. lepidentostole* we believe there is only one large follicle in the **PDR**, with an extremely irregular outline which in the histological sections gives the idea of many follicles. This possibility, however, has not been considered in the literature up to now.

In the **PDR** of the manjuba there is an elongated ventral follicle in the middle of the palate muscle, becoming gradually thinner as it goes farther from the pituitary gland. Olsson[42] (1967) comments the existence of a primitive connection from the oral cavity with the rostral region of the pituitary gland in primitive fish. Likely, our observations for *A. lepidentostole* suggest the persistence of Ratke's pouch cavity, or of the cystic rest of the orohypophysary duct.

The **PDP** of *A. lepidentostole* shows its cells arranged in strings intermingled by smooth neurohypophysary branches, as was described for *Pimelodus maculatus* (Fenerich[13], 1975) and for *Prochilodus scrofa* (Borella[6], 1987).

The literature usually refers to the anatomical relation of the neurohypophysis with the *pars intermedia* as in the *Hippocampus* in which the neurohypophysis involves all the faces of the *pars intermedia* (Da Lage[11], 1958) or in the *Prochilodus scrofa* in which the **PI** contains a great number of thin, terminal branches from the neurohypophysis (Borella[6], 1987). In the manjuba this anatomical relation is also observed, but differs clearly from the above species since the **PI** involves the neurohypophysary main branch.

In Ostheichtyes the *pars distalis rostralis* is formed by many cell types characterized according to the *cytochemical* reaction. In the *A. Lepidentostole* four cell types were characterized: **I-PDR, II-PDR, III-PDR, IV-PDR**.

The **I-PDR** cells show negative reaction to the applied cytochemical methods. It is believed that these cells are responsible for the colloid production which occupies the follicular lumen. However, there is no evidence up to now about these cells, as well as about the nature and functions of this secretion.

The **II-PDR** prismatic cells in a palisade arrangement show one of their faces turned to the neurohypophysis, as it is usually described for one cell type in the *pars distalis rostralis* in osteichtyes (Fenerich[13], 1975; Srivastava; Swarup[52], 1980; Rubal *et al.*[48], 1984; Siegmund *et al.*[51], 1987). In the manjuba, these cells were positive for HPb and this was also observed in **PDR** cells from other species as: *Chana marulius* (Srivastava; Swarup[52], 1980) and *Plecostomus albopunctatus* (Rubal *et al.*[48], 1984), but in *Carassius auratus* (Kaul; Vollrath[22], 1974) and *Rutilus rutilus* (Jafri; Ensor[21], 1980) this cell type is chromophobe for HPb. According to Olivereau[38] (1967) and Nagahama[34] (1973) the staining affinity of these cells to HPb depends on their functional state, as verified by the fall of the HPb positivity of these cells in experiments with metapirone, which stimulates the secretion of ACTH.

Cambré *et al.*[8] (1986) described cells which were similar in morphology to the **II-PDR** cell in *D. labrax*, and had the same spatial relation to the neurohypophysis and immunoreactivity to anti-ACTH. Thus, we believe that in manjuba the **II-PDR** cells are possibly corticotrophin secreting cells.

In manjuba the **III-PDR** cells, which are spindled and scatterly distributed among the other cell types, are positive for PAS, AB pH

2.5 and aldehyde-fucsin of Halmi's trichromic. Different authors who used immunohistochemical methods identified tireotropic cells in the **PDR** (Ueda *et al.*[57], 1983; Batten[2], 1986; Cambre *et al.*[8], 1986; Sigmund *et al.*[51], 1987; Toubeau *et al.*[56], 1991) that have morphological and staining characteristics which correspond to the **III-PDR** cell. So, we discuss that this cell type, in *A. lepidentostole*, may possibly secrete the tireotropin hormone.

The **IV-PDR** cells are acidophilic and apparently they outnumber the others in the **PDR** in manjuba. Schreibman *et al.*[50] (1973), Srivastava; Swarup[52] (1980), Zagha; Valsella[60] (1985), analysed specimens in different physiological conditions and verified that the acidophylic cell type and most abundant one in the **PDR** in these fish was the prolactin secreting cell. Batten[2] (1986) and Toubeau *et al.*[56] (1991) used immunohistochemical methods in *Poecilia latipina* and *Barbus barbus*, respectively, and also verified that the most abundant cell type in the **PDR** was immunoreactive to anti-ACTH. By the comparison of the results from the literature with those made for *A. lepidentostole* we can suggest the possible secreting role for **IV-PDR** cells.

Two cell types were observed in the **PDP** in manjuba: **I-PDP** and **II-PDP** cells. The same staining affinity reported for **I-PDP** cells was shown for similar cells in other ostheichtyes species, and they were designated as gonadotropics by the following authors: Aoki; Uemura[1], 1970 *(Oryzyas latipes)*, Chiba; Honma[10], 1974 *(Fugu atictonctus)*, and Srivastava; Swarup[52], 1980 *(Chana marulius)*.

In *A. lepidentostole* we found the same staining affinity in **I-PDP** and **III-PDP** cells for PAS, AB pH2.5 and aldehyde-fucsin of Halmi's trichromic methods, probably because both produce glycoproteic hormones. The two cell types, however, are different in their morphology and location, and this makes the caracterisation easier. Many authors pointed this phenomenon in diferent bone fish species (Olivereau[37], 1976; Ueda *et al.*[57], 1983; Borella[6], 1987) for gonadotropic and tireotropic cells. So, according to what has been just shown and to the immunocytochemical identification of gonadotropic cells (Olivereau ; Nagahama[41], 1983; Fantodji *et al.*[12], 1990) similar to the **I-PDP** of the manjuba, we suggest a probable gonadotropin secreting role for **I-PDP** cells

The **II-PDP** cells of the *A. lepidentostole* are characterised by the prismatic or pyramidal shape, cytoplasmatic acidophily when stained by HE and MT and by the chromophoby to the other cytochemical applied methods. Cells like that have been pointed by other studies as one of the **PDP** cell types and have been considered to be somatotropinsecreting cells after cytochemical and immunocytochemical analyses (Sage; Bern[49], 1971); McKeown; Van Overbeeke[33], 1971; Jafri; Ensor[21], 1980; Nagahama *et al.*[35], 1981; Wagner; McKeown[58], 1983). Then, we suppose that this cell type is the responsible for the somatotropin in the manjuba.

Many studies have pointed the presence of two cell types in the *pars intermedia* in osteichtyes, according to the staining affinity of these cells with Mac Conail's haematoxylinlead and to PAS (Olivereau; Ball[40], 1964; Leatherland[23], 1970). However, these cells show different reactions for these two methods depending on the species studied.

In the manjuba there also were two cell types characterised: **I-PI** cells, positive for HPb, and **II-PI** cells, negative to HPb and PAS. In salmonides, similar results were obtained (Holmes; Ball[19], 1974). Unilikely, in the *Prochilodus scrofa* PAS positive cells and chromopobes to HPb and PAS were also found (Borella[6], 1987). The appliance of anti-β MSH in immunocytochemistry has revealed HPb+ cells in the *pars intermedia* as the melatropin-secreting ones in chondrichtyes (Pelissero *et al.*[44], 1988) and osteichtyes (Cambré *et al.*[8], 1986). Thus, our results suggest that **I-PI** cells are the melanotropic-secreting cells.

Studies on other bone fish have propose different roles for the second cell type of the *pars intermedia*. So, Wendelaar-Bonga *et al.*[59] (1984) consider these cells as hypercalcemic in *Carassius auratus*, while Margolis-Kazan *et al.*[30] (1981) and Batten[2] (1986), studying respectively *Xiphophorus maculatus* and *Poecilia latipinna* suggest the possible gonadotropic role for this cell type.

Nevertheless, Rand-Weaver *et al.*[47] (1991) used a somatolactin anti-hormone in various species of fish and observed that the second cell type in the **PI** described as HPb negative, PAS positive, or chromophobe, depending on the species, is immunoreactive for the anti-hormone. The somatolactin possibly takes part in the reproductive process (Planas *et al.*[45], 1992). So, once we have identified two cellular types in the **PI** of manjuba, and considering that the first one has the same profile as the MSH secretory cells present in others species of fish, we can propose that the **II-PI** cell type has a somatolactin-secreting role in the manjuba, such as the cells described for Rand-Weaver *et al.*[47] (1991). However, more studies are necessary to clarify this question.

RESUMO

A hipófise de *Anchoviella lepidentostole* apresenta-se dividida em neuro-hipófise e adeno-hipófise, sendo que a caracterização morfológica e citoquímica dos tipos celulares desta região foi a proposta deste trabalho. A adeno-hipófise divide-se em *pars intermedia* **(PI)** e *pars distalis* **(PD)**, sendo que esta última se divide em *pars distalis rostralis* **(PDR)** e *pars distalis proximalis* **(PDP)**. As células da **PDR** organizam-se em folículos. No epitélio folicular foram caracterizados quatro tipos celulares: **I-PDR** (basófilo), **II-PDR** (positivo à hematoxilina-chumbo/HPb+), **III-PDR** (PAS+, AB pH2,5+ e AF+), e **IV-PDR** (acidófilas). A **PDP** possui dois tipos celulares: **I-PDP** (PAS+, AB pH2,5+ e AF+) e **II-PDP** (acidófilas). Na **PI** também foram caracterizados dois tipos celulares: **I-PI** (HPb+) e **II-PI** (cromófobo aos métodos empregados).

UNITERMOS: Pituitária anterior; Osteíctes; Citoquímica; *Anchoviella lepidentostole*

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