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Morpho-histology of the pituitary gland of the estuarine teleost fish, *Valamugil cunnesius* (Valenciennes)

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Abstract. Six different cell types were identified in the pituitary gland on the basis of their characteristic arrangement, distribution and staining properties. The lactotrops and thyrotrops were identified in the rostral pars distalis and the corticortrops in the interphase between the neurohypophysis and the rostral pars distalis. The somatotrops and the gonadotropic cells were distinguished in the proximal pars distalis and the melanotrops in the pars intermedia.

Keywords. Pituitary gland; *Valamugil cunnesius*; morpho-histology.

1. Introduction

The identification of different cell types in the pituitary gland is possible by the characteristic arrangement, distribution and the staining properties of different cell types. The organisation of the teleostean pituitary gland has been the subject of several reviews (Sage and Bern 1971; Holmes and Ball 1974). Cook and van Overbeeke (1969) undertook a detailed study of the fine structure of eta cells in the pituitary gland of the adult migratory Sockeye Salmon *Oncorhynchus nerka*. Satyanesan (1971) scrutinised the significance of the structure of neurohypophysis and the tetrapod-like characteristics of its hypothalamo-hypophysial vascularisation in *Clarius batrachus*. Haider and Satyanesan (1973) undertook histophysiological studies on the adrenocorticotropic and pars intermedia cells of the teleost fish *Rita rita* (Ham). Satyanesan and Joy (1976) studied the functional cytology of the pituitary gland of *Clarias batrachus*. Recently the pituitary cytology of the fish *Tilapia mossambica* (Peters) was investigated by Joy and Satyanesan (1980).

In this study an attempt has been made to investigate the morphological and histological characteristics of the pituitary gland of *Valamugil cunnesius*.

2. Material and methods

The fish (12–18 cm in total length and in stage II of sexual maturity) collected from the brackish waters of Cochin, were anaesthetised, chilled and prepared for dissection to avoid postmortem changes in the pituitary gland. The dorsal surface of the skull was opened and part of the brain excised. The details of the morphological features were examined under the microscope. The pituitary gland along with a portion of the brain was fixed in aqueous Bouin's fixative and Helly's fluid. Sagittal and transverse sections of the whole pituitary gland were taken (6–8 μm) using a rotary microtome. The

sections were stained using the following techniques (i) periodic acid schiff (PAS)-orange G with celestine blue haemalum sequence (Mc Manus 1946, modified by Pearse 1959). (ii) chromium-haematoxylin-phloxine (Gomori 1941) (iii) Orange-fuchsin-green OFG (Slidders 1961) (iv) Mallory Heidenhain's (modified after Koneff 1938) and (v) Cameron and Steele method (1959).

Microphotographs showing the different topographical regions and the cell types of the pituitary were taken (using Olympus photomicrographic system—Model PM 10 AD).

3. Results

3.1 *Morphology*

The pituitary gland of *Valamugil cunnesius* is a compact, slightly cone-shaped structure located in the sella turcica, of the sphenoid bone. The gland is encapsulated in dura mater and is situated ventral to the brain, immediately behind the optic chiasma and above the capillary network, the rete mirabile. The gland is of the cranio-leptobasic type, having a short stalk entering the gland from the anterior side and is vascularised by the branches of the internal carotid artery (figure 1).

Microscopic observation of the whole pituitary gland showed that it is composed of four well-defined regions *viz* the frontal lobe, the rostral pars distalis, the middle lobe, the proximal pars distalis, the distal lobe, the pars intermedia and the neurohypophysis innervating the pars intermedia. The topographical relations of the regions are shown in the sagittal sections of the whole pituitary gland (figure 2).

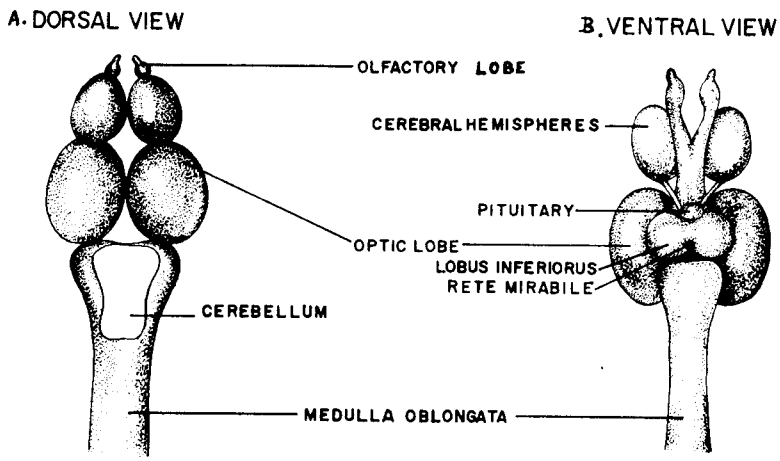
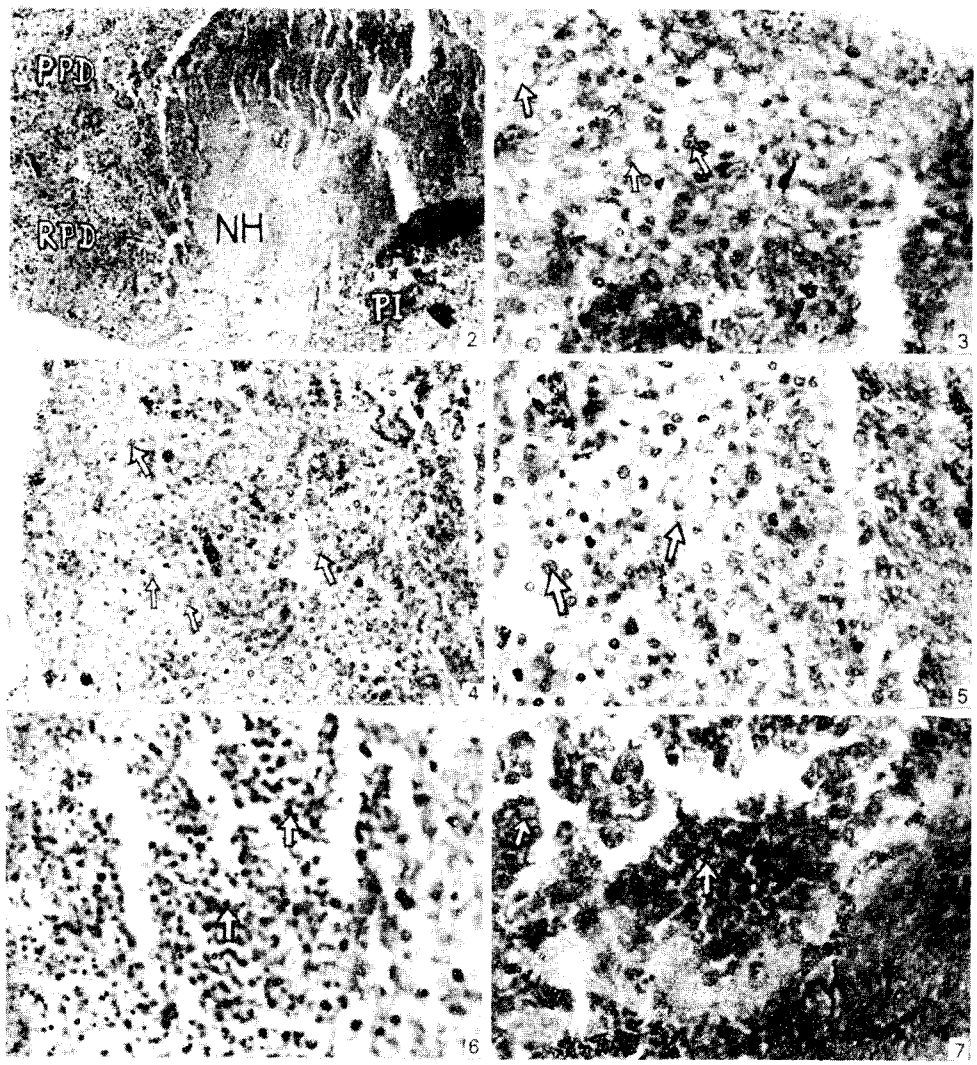


Figure 1. Diagrammatic representation of the brain of *Valamugil cunnesius* showing the **A.** dorsal view and **B.** ventral view and the position of the pituitary gland.

3.2 *Histology*

3.2a *Rostral pars distalis* (RPD): It is the anteriormost region of the pituitary gland occupied by acidophils and basophils. The acidophils showed affinity to aniline blue and acid fuchsin and were negative to PAS orange and CHP. These cells were identified as the lactotrops (figure 3). The basophils in this region were identified as the thyrotrops



Figures 2–7. **2.** Sagittal section of the pituitary of *V. cunnesius* showing its component parts. OFG (× 160); **3.** A part of the RPD showing the lactotropic and thyrotropic cells. The larger paler cells are the lactotrops and the smaller darker cells are the thyrotrops. OFG (× 640); **4.** A part of the RPD and PPD showing the corticotrops and somatotrops. CHP (× 320); **5.** A part of the RPD and PPD showing corticotrops and somatotrops. The pale cells with clear nuclei are the corticotrops and the darker ones are the somatotrops. CHP (× 640); **6.** A part of the PPD showing the gonadotrops. CHP (× 640) **7.** A part of the PI showing the deeply stained melanotrops. Mallory Heidenhain's (× 640).

(figure 3). The cells lying at the interphase between the lactotrops of the RPD and neurohypophysis were identified as the corticotrops. The cells stained only faintly with PAS orange G and were negative to aldehyde fuchsin and aniline blue (figures 4 and 5).

3.2b *Proximal pars distalis* (PPD): There is no line of clear demarcation between the RPD and PPD, but the regions could be distinguished by the differential staining of their cells. The basophils found in this region were identified as the gonadotrops (figure 6). These cells are strongly PAS-positive, aldehyde fuchsin positive and aniline blue positive. The acidophilic cells were identified as the somatotrops (figure 5). These cells stained positively with PAS orange G, Mallory Heidenhain's and acid fuchsin and the granules were negative to aldehyde fuchsin and CHP. They were found more abundantly in the peripheral regions of the neurohypophysis.

3.2c *Pars intermedia* (PI): This distal region surrounding the neurohypophysis is the smallest region which was extensively innervated by the neurohypophysial tracts and was mainly constituted by the deeply stained basophils. These cells which stained positively with PAS orange G and OFG were identified as the melanotrops. The melanotrops were negative to Mallory Heidenhain's, CHP and aldehyde fuchsin (figure 7).

3.2d *Neurohypophysis*: The major components of this region were the neurosecretory nerve fibres arising in the preoptic nucleus. The neurohypophysial processes that interdigitate with the pars intermedia have a central canal continuous with the brain cavity. Lining the canal were the pituicytes which were surrounded by neurosecretory nerve fibres that synapse with the pituicytes (figure 2).

The reactions of the different cell types to the five different staining methods are indicated in table 1.

4. Discussion

The pituitary gland of *V. cunnesius* belongs to the cranio-leptobasic type. The lactotrops of *V. cunnesius* which did not show any definite pattern of arrangement were identified in the RPD. This observation agrees with those of Olivereau and Ball (1964) in *Poecilia*. The lactotrops of *V. cunnesius* were negative to PAS orange G. The thyrotrops were identified between the lactotrops in *V. cunnesius* and this finding is in accordance with Olivereau (1967) who also made similar observations in the RPD of *Anguilla anguilla*. The thyrotrops of *V. cunnesius* stained positively with PAS orange G, aldehyde fuchsin and Mallory Heidenhain's, but negatively with CHP and acid fuchsin. In *Tilapia mossambica* the thyrotrops were identified in the proximal pars distalis along with the gonadotrops. The thyrotrops and gonadotrops in *T. mossambica* stained alike with PAS, aldehyde fuchsin and aniline blue; those cells which showed significant hypertrophy during breeding were identified as the gonadotrops (Joy and Satyanesan 1980).

In this study the corticotrops were found in rostral pars distalis adjacent to the neurohypophysis and this agrees with the observation of Olivereau (1967). The corticotrops stained only faintly with PAS orange G in *V. cunnesius* and were negative to aldehyde fuchsin and aniline blue.

The acidophilic cells observed in the PPD were reported as somatotrops by Sage and Bern

Table 1. Staining reactions of the cytoplasmic granules in the secretory cell types of different parts of the pituitary gland of *V. cunnesius*.

Stain reaction	Rostral pars distalis			Proximal pars distalis		Pars intermedia	Neurohypophysis pituicytes	Remarks
	PRL*	TSH	ACTH	STH	GTH**	MSH		
PAS orange G celestine blue haemalum sequence	− −	+ +	+ −	+ + +	+ + +	+ + +	+	Pituitary cells were stained purple and the cells orange yellow
Chromium haematoxylin phloxine	+ +	− −	+ +	+ −	− −	− −	+	Acidophils stained red and basophils blue. Gonadotrops did not stain at all.
Mallory Heidenhain's	+ +	+ +	+ + +	+ −	+ +	− −	−	Acidophils stained red and basophils blue
OFG (Slidder's method)	+ −	− −	− −	+ +	+ +	+ + +	−	MSH, STH and GTH stained positive
Cameron and Steele method	+ +	+ +	−	−	− −	− − −	−	Prolactin and TSH cells stained positive.

Note: *Prolactin cells; **Gonadotrops; $^{+++}$Strongly positive; $^{++}$Positive; $^{+-}$Pale. $^{-}$Weak staining; $^{--}$Very weak staining; $^{---}$Did not stain.

(1971). It was observed that the somatotrops in *V. cunnesius* stained positively with PAS orange G and acid fuchsin and negatively with CHP and aldehyde fuchsin. A clear prominent nucleus could be recognised in these cells. In *Oncorhynchus kisutch* the somatotrops are arranged as cords of cells running roughly dorso-ventrally, separated by clearly defined sinusoidal channels (Leatherland and Sonstegard 1981).

Gonadotrops usually lie in the proximal pars distalis in the ventral and lateral regions. In *O. kisutch* gonadotrops were located in the PPD as cords of cells (Leatherland and Sonstegard 1981). The gonadotrops of *V. cunnesius* stained positively with PAS orange G and aniline blue. PAS, aldehyde fuchsin and aniline blue positive granules were observed in the gonadotrops of *O. nerka* (Van Overbeek and Mc Bride 1967).

The melanotrops are the basophilic cells located in the pars intermedia. The melanotrops of *V. cunnesius* stained positively with PAS orange, G. PAS positive cells have been described in the pars intermedia of several teleosts (Joy and Satyanesan 1979). The salmonids however appeared to lack the PAS positive cells as observed by Leatherland and Sonstegard (1981).

The structure of the neurohypophysis in teleosts has been reviewed by Sage and Bern (1971). The major components of the neurohypophysial region of *V. cunnesius* consisted of the neurosecretory nerve fibres arising in the preoptic nucleus.

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