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PITUITARY HISTOMORPHOLOGY OF THE GRASS CARP *CTENOPHARYNGODON IDELLA* (VALENCIENNES)

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The adenohypophysis of *C. idella* is divisible into rostral pars distalis (RPD), proximal pars distalis (PPD) and pars intermedia (PI). Aldehyde fuchsin (AF) positive neurohypophysial fibres penetrate into the pars intermedia region. On the basis of tinctorial and histochemical properties seven cell types of chromophils and 8th cell type of chromophobes have been identified. The RPD contains cell type 1, 2 and 3. The PPD contains type 4 acidophils and type 3 and 5 cyanophils. The PI is composed of two cell types PbH positive and PAS positive cells. Chromophobes take no stain and constitute the 8th cell type located in all the component parts of the adenohypophysis. Cell types described in the adenohypophysis of other teleosts are also discussed.

INTRODUCTION

Eversince the appearance of the first reference text on the physiology of the pituitary gland of fishes by Pickford & Atz (1957), many publications and reviews have dealt with the general structure and cellular composition of the teleost adenohypophysis (Olivereau & Ball, 1964; Ball & Baker, 1969; Holmes & Ball, 1974; Fontaine & Olivereau, 1975) Out of about 30,000 known species of fishes only in a small fraction of them detailed microanatomy of the pituitary is studied adopting more recent techniques. During recent years better technical standardization to distinguish the different cell types of pituitary of teleosts was achieved by extending the methods adopted in mammalian tissue (Herlant, 1960). Detailed studies on the pituitary of tropical fishes inhabiting Indian waters are comparatively few (Baker *et al*, 1974; Maitra & Sarkar, 1976; Joy & Sathyanesan, 1979; Latey & Rangneker, 1982; Das, 1985 & 1987; Das & Sinha, 1988). Inspite of these works the information about the pituitary cell types are not precise.

In the present study an attempt has been made to make a detailed study of the cell types to yield precise and accurate information about the histomorphology of the different pituitary cells in a culturable carp *Ctenopharyngodon idella* to have a background to study the functional aspects of the cells in subsequent studies.

MATERIAL AND METHODS

Over 20 mature adult specimens of grass carp were used in this investigation and were collected from ponds of Dhauli Fish Farm of Central Institute of Freshwater Aquaculture, Kausalyaganga. They were sacrificed through decapitation and after removing the brains, the pituitaries alone were taken out with care and immediately immersed in Bouin's sublimate fixative for 24 hours.

Paraffin sections were cut at 3 and 5 μm thickness in sagittal and transverse planes and the following staining procedures were used :

1. Aldehyde fuchsin (AF) technique preceded by acid permanganate oxidation using Halmi's fast green–chromotrope 2R–orange G combination as counterstain. Ehrlich's haematoxylin was used as nuclear stain when needed.

2. Aldehyde thionin (ATh)–Periodic acid Schiff (PAS)–orange G (OG) procedure.

3. Alcian blue (AB) (pH : 0.2 and 3.0)–PAS–OG technique using acid permanganate oxidation. Mayer's hemalum was used as nuclear stain.

4. Lead haematoxylin (PbH) technique preceded by PAS.

5. Herlant's tetrachrome technique.

6. Cleveland & Wolfe's trichrome.

7. Mallory's triple stain.

8. Heidenhain Azan's staining method.

OBSERVATIONS

The adenohypophysis of *C. idella* is divisible into three components : RPD (rostral pars distalis), PPD (proximal pars distalis) and PI (pars intermedia). The structure and distribution of cell types are comparable with many other teleostean pituitaries (Fig. 1). Eight cell types could be differentiated in

the hypophysis of this fish based on their colour affinities to different staining reactions which are recorded in Table 1.

Among the three cell types evidenced in the RPD, the type 1 cells are predominant and arranged in compact mass. The cells are round or oblong and their stainable cytoplasmic granules show strong affinity to orange-G, erythrosin, acid fuchsin and azocarmine. They are termed as acidophils (Fig. 2) and are negative to PAS, AB, AF, PbH and aniline blue stains. Some cells bordering the neurohypophysis and its branches impact dark grey colour showing positiveness to PbH stain These cells constitute the type 2 cells of the RPD and are fewer in numbers They show amphiphilic reaction when stained with various trichrome and tetrachrome techniques. They are scattered in the RPD having elongated and irregular shapes. The cell type 3 of the RPD which also evident in the anterodorsal region of the PPD are cyanophilic in nature. They are strongly positive to PAS (Fig. 2), AF, AB and Aniline blue. They do not show any sign of reactions for the acid dyes.

Apart from the type 3 cells of the RPD which are present also in the PPD, the latter component has two other types of cells : type 4 acidophils and type 5 cyanophils. The type 4 cells are distributed throughout the PPD in groups without any specific pattern of arrangement. They are stained with acid fuchsin,

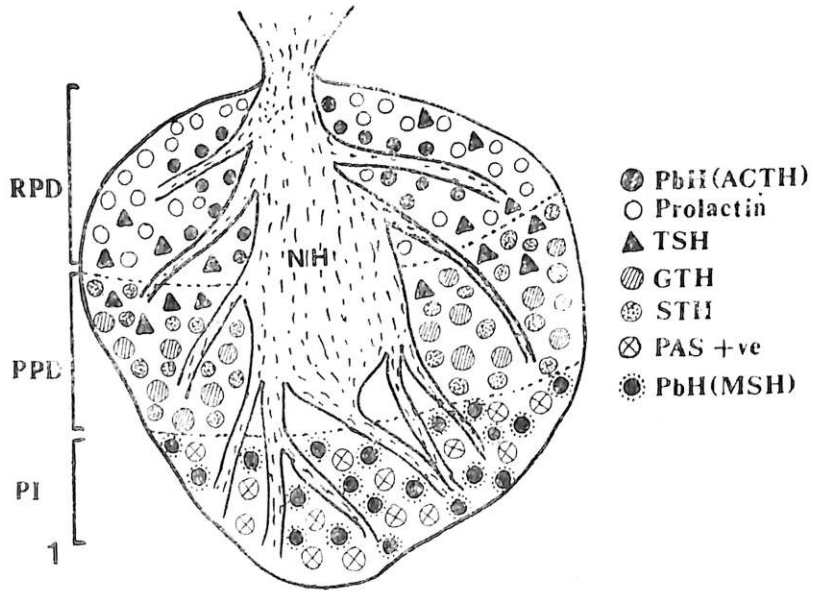
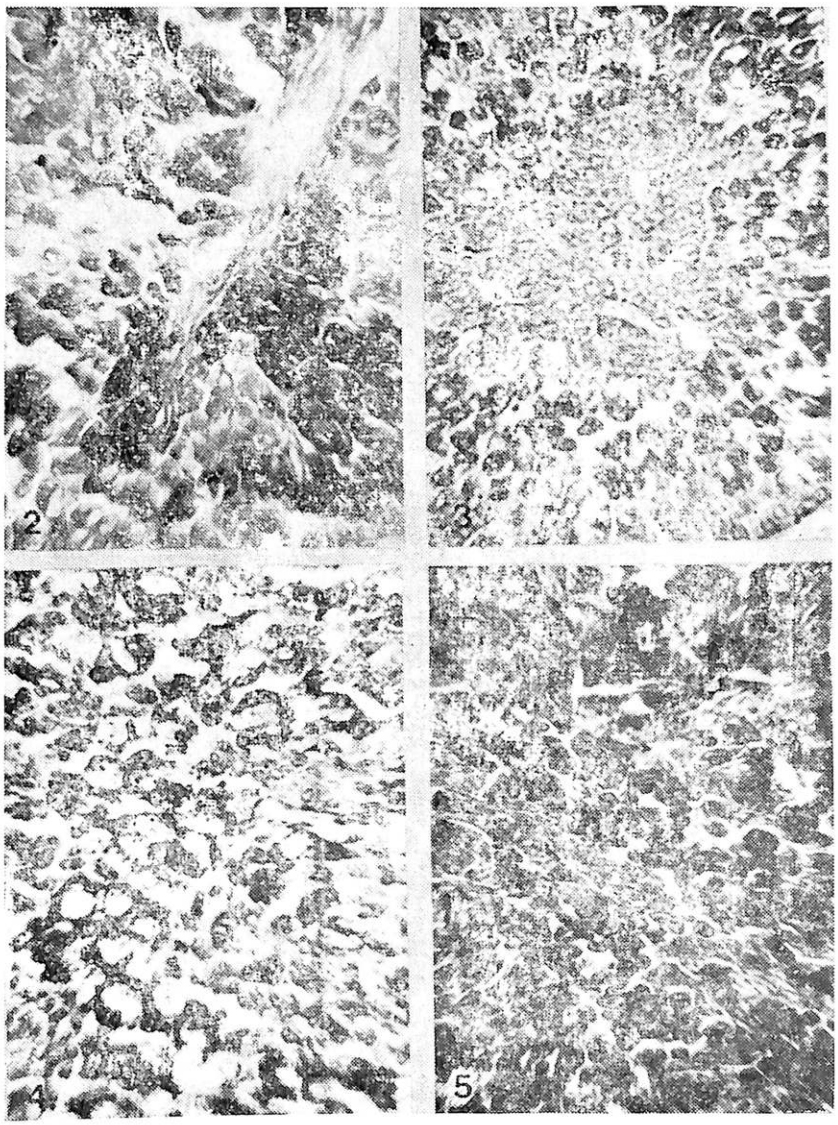


Fig. 1. Diagrammatic representation of pituitary of *C. idella* at mid segittal plane showing different component parts and cellular distribution.

azocarmine, orange-G and erythrosin (Fig. 3). Type 5 cyanophils show strong affinity towards PAS, AB, AF and Aniline blue and are deeply stained with PAS and AB imparting purple and bluish colouration respectively (Fig 3-arrows). They are differentiated from the type 3 cyanophils in respect of their large size



Figs. 2—5. **2.** RPD of the fish showing type-3 cyanophils (dark cells) and type-1 acidophils. Cleveland Wolfe's trichrome. × 600 **3.** PPD showing deeply stained type-5 cyanophils and lightly stained acidophils (type-4 cells). Herlant's tetrachrome × 600. **4.** PPD of the fish pituitary showing almost vacuolated and hypertrophied type-5 cells. AB-PAS-OG × 600. **5.** PI of the fish showing type-7 PAS positive cells and type-6 amphiphilic cells. AB-PAS-OG × 450.

Table I. Tinctorial affinity of cells of the adenohypophysis of *Ctenopharyngodon idella.*

Cell types	Rostral pars distalis (RPD)			Proximal pars distalis (PPD)			Pars intermedia (PI)		Neurohypophysis
	Type–1	Type–2	Type–3	Type–3	Type–4	Type–5	Type–6	Type–7	NSM
Heidenhain Azan's stain	Azocarmine ++	Azocarmine + Aniline blue +	Aniline blue ++	Aniline blue ++	Azocarmine +++	Aniline blue +++	Azocarmine +	Azocarmine + Aniline blue	Aniline blue +
AB-PAS-OG	Orange–G ++	—	Alcian blue +++	Alcian blue +++	Orange–G ++	Alcian blue +++	—	PAS +	Alcian blue ++
PAS–PbH	—	PbH ++	PAS ++	PAS ++	—	PAS +	PbH ++	PAS +	—
AF-FG-C2R -OG	Orange–G ++	—	AF ++	AF ++	Orange–G ++	AF ++	—	—	AF ++
Mallory's triple stain	Acid fuchsin ++	Acid fuchsin + Aniline blue +	Aniline blue +	Aniline blue +	Acid fuchsin +++	Aniline blue ++	Acid fuchsin +	—	Aniline blue +
Cleveland Wolfe's trichrome	Erythrosin ++	Erythrosin + Aniline blue +	Aniline blue ++	Aniline blue ++	Erythrosin +++	Aniline blue +++	Erythrosin +	Erythrosin + Aniline blue +	Aniline blue +

++++ very intense, +++ intense, ++ moderate, + weak, — absent.

of oval, round or oblong shapes. These cells contain dense cytoplasmic granules and do not show any affinity for acid fuchsin, orange–G, and azocarmine. During sexual maturation phase, these cells show hypertrophy and almost vacuolated probably after discharging their hormone contents to the blood (Fig. 4).

The pars intermedia is constituted of type 6 PbH positive cells and type 7 PAS positive cells (Fig 5). The PbH positive cells are fewer in number and scattered within the type 7 cells. Their cytoplasmic granules are readily stainable dark with PbH in PAS–PbH reactions, whereas the other cell type (type 7) is lightly stained with PAS. In tetrachromes and trichrome stains, the type 6 cells are found to be lightly acidophilic and the type 7 cells amphiphilic in their colour reactions The 8th cell type are the chromophobes and are evident in all the component parts of its adenohypophysis and they may be the degranulated chromophils.

DISCUSSION

In many species of teleosts the RPD is reported to have PbH positive ACTH cells arranged in groups bordering the neurohypophysis (Ball & Baker, 1969; Olivereau, 1970; Sage & Bern, 1971; Das, 1985 & 1986). In *Tinca tinca* (Olivereau, 1970) the PbH cells are irregularly scattered in the RPD In minnow, *Phoxinus phoxinus* these cells could not be identified in the RPD (Benjamin, 1975). In *C idella*, these cells are distinctly stained with PbH and were noticed among other component cells of the RPD bordering the neurohypophysial branches.

The type 1 acidophilic cells predominent in the RPD of *C. idella* are stainable with acid fuchsin, azocarmine, orange–G and erythrosin. In several teleosts the prolactin cells are packed with azocarmine or acid fuchsin or erythrosin staining granules (Schreibman *et al.*, 1973). The acidophils and PbH positive cells of the RPD in different teleostean species are described as the prolactin and ACTH cells respectively (Val Sella & De Moraes, 1979; Srivastava & Swarup, 1980; Benjamin, 1982; Das, 1985). On the basis of colour reactions cell types 1 and 2 of *C. idella* may be comparable to the prolactin and ACTH cells respectively of the teleostean pituitary literature.

Sage & Bern (1971) observed that TSH cells are angular or polyhedral in comparision to somewhat similarly staining gonadotrophs that are usually rounded. The distributional pattern and site of TSH cells seem to vary among different teleostean species. They may be in the RPD or PPD (Srivastava & Swarup, 1980; Das, 1985 & 1986) or even in between RPD and PPD (Jafri & Ensor, 1980). In *C. idella*, these cells are located in an area comprising of anteroventral RPD and anterodorsal PPD. On the basis of their staining reac-

tions, shape and distributional pattern, these cells may be comparable to the TSH cells described by earlier investigators.

The gonadotrophs are known to be stained with PAS, AB, AF, ATh and Aniline blue (Ball & Baker, 1969). Similar observations were also made recently in *Labeo rohita* (Jose & Sathyanesan, 1977), in *Rutilus rutilus* (Jafri & Ensor, 1980) and in *Labeo bata* (Das, 1986). In some teleosts, two types of gonadotrophs were reported based on the colour differences, cell and granular size, distributional position (Olivereau, 1970; Ueda, 1981), whereas in several other teleosts only one type of gonadotrophs are reported (Baker *et al.*, 1974; Joy & Sathyanesan, 1979; Das, 1986). In *C. idella* only one type of gonadotrophs (type 5 cells) are evidenced based on their tinctorial affinities. But they could not be differentiated from type 3 cells on the basis of their staining reactions except during breeding season when gonadotrophs show hypertrophy.

The acidophils of the PPD are reported to synthesize somatotropin (Ball & Baker, 1969; Sage & Bern 1971). The arrangement of STH cells varies in different species. In *C idella* the acidophils of the PPD are scattered irregularly in groups without any specific pattern. Das (1986) reported a similar observation of arrangement and staining reactions of type 4 cells in *Labeo bata* recently. The type 4 cells observed in the PPD of *C. idella* might be the STH cells of the teleostean literature.

Two cell types are generally discribed in the PI of teleosts which are reported to be acidophilic, basophilic or amphiphilic in response to trichrome and tetrachrome techniques (Joy & Sathyanesan, 1979; Srivastava & Swarup, 1980; Das, 1986). The PAS positive cells bordering the neurohypophysis in *Perca fluviatilis* are described as basophilic (Matty & Matty, 1959) or acidophilic (Follenius & Porte, 1961) but in some other teleosts they are stainable with only PAS (Olivereau, 1964). In *C. idella* at least some of the cells which are acidophilic to trichrome and tetrachrome stains are apparently PbH positive and those that are amphiphilic are PAS positive.

Thus, on the basis of colour reactions, the cell types 1, 2, 3, 4, 5 of *C. idella* are comparable to the eta (prolactin), epsilon (corticotrophs), delta (thyrotrophs), alpha (somatotrophs), beta and gamma (gonadotrophs) cells described in the teleostean pituitary literature. These comparisions are tentative and need further experimental verifications.

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