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MORPHO-HISTOLOGY OF THE PITUITARY GLAND AND ITS DEVELOPMENT IN *CATLA CATLA* (HAMILTON)

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The development of the pituitary gland in *Catla catla* is studied in 8 successive stages, from the time of the formation of ectophyseal ananage at 5.8 mm long hatchling to the regionation of the different neuroendocrine components of the pituitary at 160 mm long immature under-yearling fish. The pituitary gland in *C. catla* arises as a solid ingrowth of cells from the ectodermal epithelium by the formation of the ectophyseal analage. The pituitary gland is of the leptobasic type having a stalk and is divisible into the adenohypophysis and the neurohpophysis. The adenohypophysis is divisible into the restral pars distalis, proximal pars intermedia. The RPD consists of prolactin cells and the adrenocorticotrophs. The proximal pars distalis consists of the gonadotrophs, somatotrophs and thyrotrophs. The pars intermedia consists of the chromophobes with scantly cytoplasm in the melanophores.

INTRODUCTION

Though voluminous literature is available on the morphology and histocytology of the pituitary gland of adult teleost fishes (De beer, 1926; Ball & Baker; 1969; Sage & Bern, 1971; Holmes & Ball, 1974; Matty, 1985), the development of the teleost pituitary has been studied only in few cases ; trout (De Beer, 1926), *Fundulus heteroclitus* (Mathews, 1937), *Salmo salar* (Woodman, 1939), *Abramis* species (Irikhimovitch, 1948). Some information on the development of the pituitary gland in postembryonal and young stages of *Gasterosteus* (Bock, 1928), *Cyprinus carpio* (Robson, 1938), *Herring* (Buchnan, 1940) and *Chanos* (Tampi, 1951) is also available. However, only information on the development of the pituitary gland of Indian freshwater teleosts is that on *Ophiocephalus* (=*Channa punctatus*) (Belsare, 1962).

The maturity period of *Catla catla* is 2 years. The time at which the development of gonadotrophins secreting cells take place in the pituitary gland is correlated with the stage of development of the larva.

In teleosts, the hypophysis varies in the organisation, arrangement and orientation of the components from species to species. Detailed studies on the pituitary has been studied by Kerr (1942), Pickford & Atz (1957) and Sunderaraj (1981). Studies on the tropical freshwater fishes are comparatively few on the study of the pituitary gland (Baker *et al.*, 1974; Moitra & Sarkar, 1977; Das, 1985 & 1986). Inspite of these works, the information on pituitary cell types are not precise. The present study is undertaken to study in detail the development, morphology and histology of the pituitary gland in *C. catla*, which is one of the commercially important major carp and food fish of India.

MATERIALS AND METHODS

Immature adult and different development stages of *C. catla* were obtained during the breeding season from the Tungabhadra Reservoir Fisheries Unit of the Central Inland Fisheries Institute in India.

The developing stages of the fish were fixed in Bouins fluid. After fixation, they were dehydrated in different grades of alcohol and embedded in paraffin wax (BDH make, 58°C). The sections were cut at 5 microns thick. The brain alongwith the pituitary of later stages of developing fish, fry and that of the immature adult, were fixed in Bouins fluid and processed as above. The sections were stained with Haematoxylin - Eosin and Cleveland Wolfe Trichrome stain.

OBSERVATIONS

Stage 1 : At six hours after hatching, the hatchling did not indicate any trace of the development of the pituitary. During this stage, the eyes were colourless and there was no significant colour pattern developed on the body of the hatchling (Fig. A; Table I).

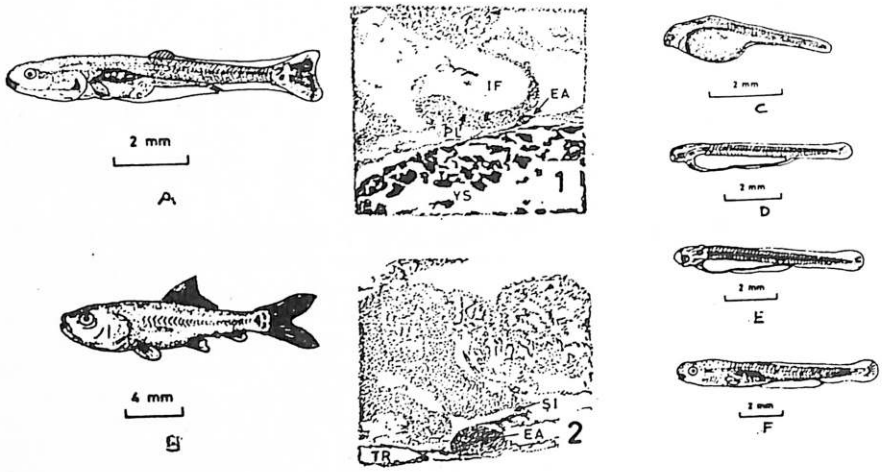


Fig. A-F. Morphological features of different stages of development of *Catla catla*. **A.** Hatchling stage (6 hrs. after hatching); **B**. Larva (12 hrs); **C**. Larva (24 hrs); **D.** Larva (72 hrs); **E**. Post larva (5th day); **F**. Post larva (15th day).
Fig 1-2. Mid sagital section of the larvae brain : **1**. Showing formation of pituitary analage in post-optic lamina region 12 hrs after hatching; **2**. Showing ectophyseal analage 24 hrs after hatching.

Stage 2 : Twelve hours after hatching the begining of the formation of the pituitary analage was noticed. This stage corresponds to the period when pigmented eyes are noticed and the body has a yellowish hue on the dorsal side above the yolk sac. (Fig. B; Table I). The pituitary analage at this stage comprises of an ectodermal epithelial mass, lying in close contact with the floor of the brain, in the region of the diencephalon (Fig. 1). This region is referred to as the post - optic lamina (PL). the pituitary of *Catla catla*, arises as a solid ingrowth of cells from the ectodermal epithelium. the first appearance of this thickened epithelium is noticed at 5-6 mm stage, but a clearly distinguishable ectophyseal analage development is noticed only later (5.8 to 6.0mm stage). there is a progressive development of the analage and a district neuroendocrine structure is noticed only in later stage.

Stage 3 : Twenty - four hours after hatching, the formation of the ectophyseal analage can be seen in the larva (Fig. 2). The ectodermal epithelial mass in the region of the post - optic lamina, shows thickening and hyperplasia. Assumes an ovoid shape. this ectophyseal analage of the pituitary is still not completely separated from the ectodermal epithelium of the pharyngeal roof. This stage corresponds to the morphological appearance of black chromatophores along the edge of the upper margin of the yolk sac. The pigmentation in the eyes become dense and the position of the oral aperture of the larva is discernible as a split (Fig. C, Table I).

Stage 4 : By seventy-two hours after hatching, the formation of the saccus infludibuli and the undifferentiated pituitary are seen. Structural modification take place *viz.* the development of the mouth and its continuity with the foregut cavity (Fig. D; Table I). The ectophyseal analage increases in size and gets separated from the buccal epithelium. From the diencephalic floor a diverticulum (saccus infundibuli) develops and extends upto the tip of the notochord.

Stage 5 : By fifth day after hatching, the analage increases in size and becomes a prominent structure. The cells from the post-optic floor and saccus influndibuli prolifereate to meet the ectophyseal analage and spread along the dorsal side of the latter (Fig. 3). it shows the begining of the neurohypophysis, forming the definitive neuroendocrine components (Fig. 4). However, differentitation among cell types of the adenohypophysis does not appear to have taken place, though the post larva shows characteristic pattern of coloration (Fig E; Table I)

Stage 6 : By the fifteenth day after hatching. The components of the pituitary becomes evident. The pituitary is closely situated near the parasphenoid bone and the nervous tissue encroaches the posterior and dorsal part of the adenohypophysis. The pituitary stalk, is not significantly seen. The distinction between the parts of the adenohypophysis cannot be clearly made out. All cells appear equal in size and show chromophobic reaction (Fig. 5). However, morphologically the post - larva assumes the typical pattern of chromatophores (Fig. F; Table I).

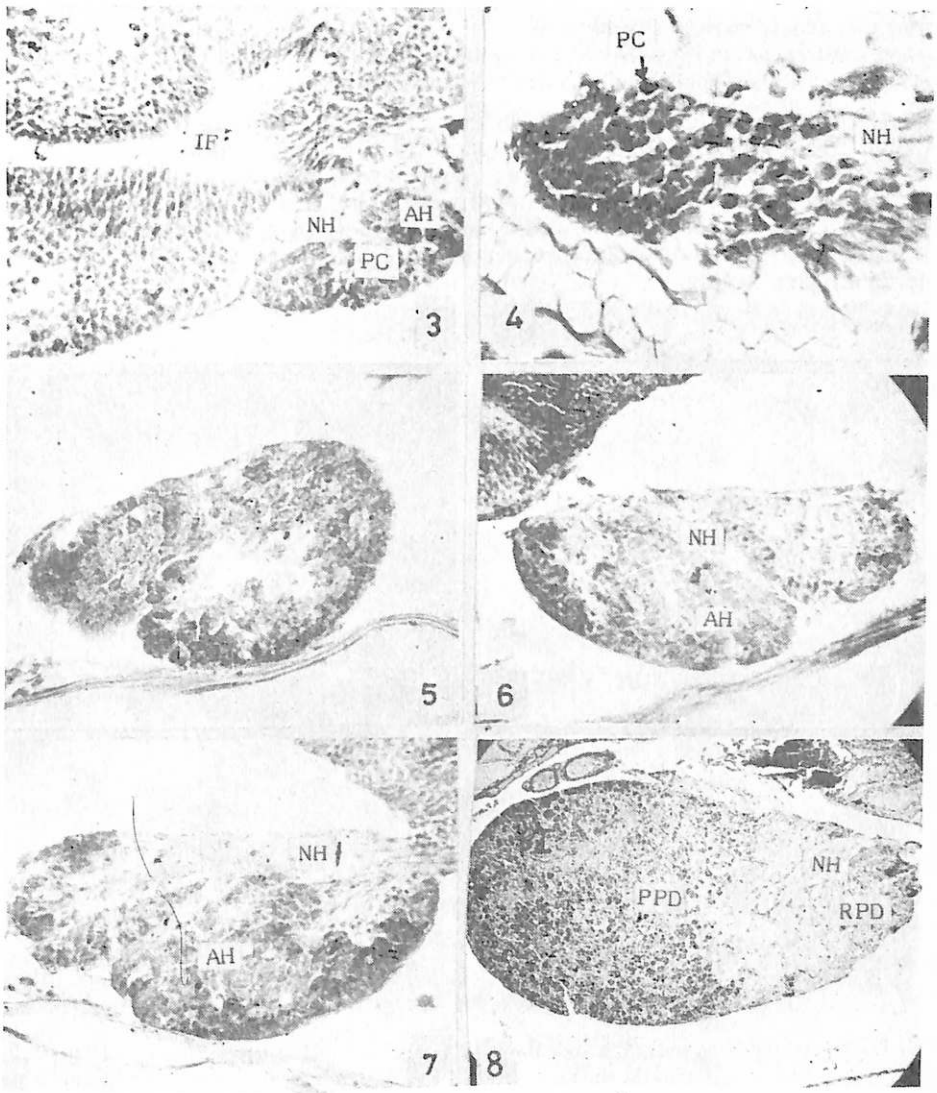
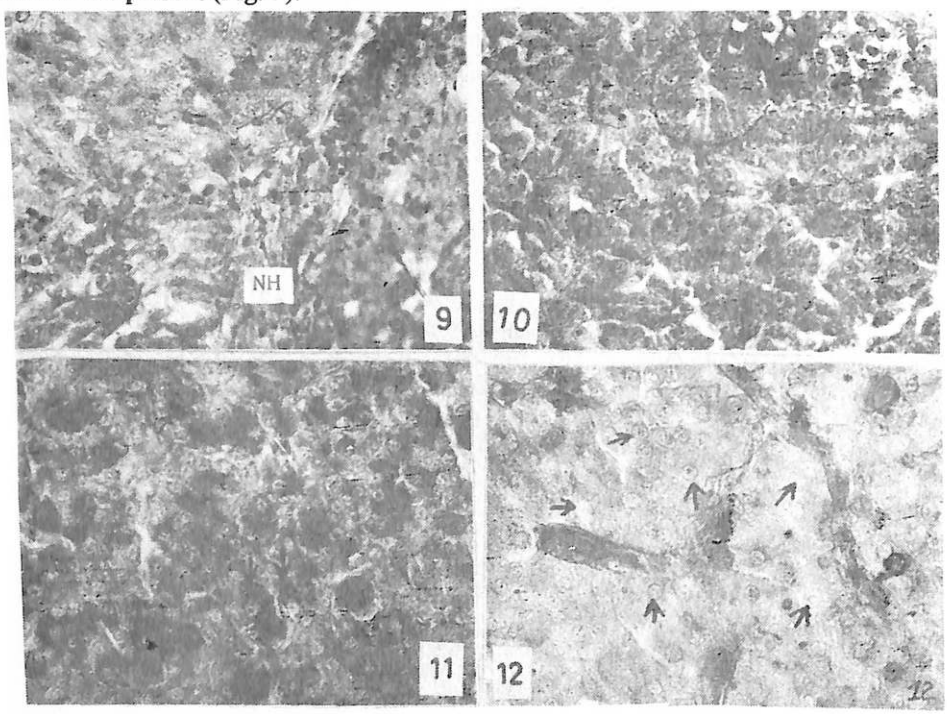


Fig. 3-8. M.S.S. through the brain of a post larva, showing infundibulum and glandular component 5th day after hatching (x 1200); **4.** Showing the beginning of the neurohypophysis (x 1500); **5.** Showing the pituitary of a post larva fifteenth day after hatching. With undifferentiated glandular component of the adenohypophysis, 15th day after hatching (x 150); **6.** Showing Section of the pituitary of a post larva (Fry : Twenty - fifth day after hatching). The neurophypophyseal processes invading the adenohypophysis in fry, 25th day after hatching (x 150); **7**. Showing the pituitary of the fingerling with evident interdigitations of neural (neurohypophysis) and glandular (adenohypophysis) processes (x 150); **8.** Histomorphology of the pituitary gland of an immature adult female (Underyearling) (x 150). (AH = Adenohypophysis; EA = Ectophyseal Analage; F = Follicles; IF = Infundibulum; NH = Neurohypophysis; PA = Pituitary Analage; PC = Pituitary Cells; PI = Pars Intermedia; PL = Post optic Lamina; PS = Pituitary Stalk; PPD = Proximal Pars Distalis; RPD = Rostral Pars Distalis; SI = Saccus infundibuli; TR - Trabeculae; YS = Yolk Sac)

Stage 7 : As a fry, on twenty-fifth day after hatching the pituitary gland becomes more elongated antero - posteriorly. While the invasion of the neurohypophyseal processes into the adenohypophysis is noticed, it is not extensive (Fig. 6). Further, the histological elements of the adenohypophysis remain still indistinct. The fry exhibits distinct morphological resembrance with the adult (Table I).

Stage 8 : In the fingerling stage, two to three months after hatching, the pituitary gland exhibits the typical neuroendocrine features. The adenohypophysis can be distinguishable as a glandular component, different from the neurohypophysis. (Fig. 7). The neurohypophyseal processes intimately interdigitate with the adenohypophysis. The pituitary stalk becomes distinguishable. However, the different regions of the adenohypophysis are still not discernible.

Stage 9 : In an immature adult female (Under - yearling) of *Catla catla*, the distinct features of the adult pituitary are discenible. The pituitary appears like a compact, cylinderical structure situated ventral to the brain to which it is attached by a prominent pituitary stalk (Fig. 8). The pituitary stalk continues into the adenohypophysis as a great trunk - the neurohypophysis which intimately interdigitates with the adenohypophysis components. The neurohypophysis is composed of loosely arranged fibres among which are the mucei of neuroglical cell droplets and irregular patches of colloid like material are present (Fig. 9).



Figs. 1-12. 9. Neurohypophyseal region of the pituitary of the immature adult (x 1050); **10.**-Pitu gland of an immature adult female in the region of the RPD showing the arrangement of pro secreting cells in the form of prominent follicles (x 1050); **11.** Proximal pars distalis showin gonadotrophs (arrows (x 1050); **12.** Region of the pars intermedia showing the intermedical (arrows) (x 1050).

The rostral pars distalis is distinctly regionated and is largely composed of acidophilic cells, few basophilic cells and few chromophobes. The acidophills are arranged in follicles (Fig. 10) which are PAS negative. The cells are identified as the prolactin secreting cells. The chromophobic cells of the rostral pars distalis are not always refractory to stain and normally lie on the region between the prolactin cells and the neurohypophysis. These cells are identified as the adrenocorticotroph cells. At this stage, the proximal pars distalis of *Catla catla* is discernible as a distinct region of the adenohypophysis. It contains well defined acidophils and faintly stained basophils. The basophils may be differentitated into two cell types. The smaller basophils are usually round or oval containing

cytoplasm that is granular. These basophils are regarded as the gonadotrophs. The other type of basophils lie towards the periphery and are the thyrotrophs. Their nuclei are large and rounded. The cells are found in singles or in groups scattered towards the periphery of the proximal pars distalis (Fig. 11). In addition to these two well defined cyanophils, there are smaller cells with contracted nuclei. These cells are the resting cells from which larger and active cells are recruited.

The pars intermedia is a cone like region, with the apex directed anteriorly. This region contains few faintly stained basophils resembling those of the PPD (probably the gonadotrophs), lying adjacent to the latter. The chromophobes appear like naked nuclei due to their scantly non-staining cytoplasm (Fig. 12). These cells are the intermedial cells responsible for the elaboration of the melanophore stimulating hormone. The proximal pars distalis exhibits the presence of acidophilic thyrotrophs and faintly basophilic gonadotrophs. That the latter are not distinct suggest that, the pituitarygonadal axis is not yet estabilised. The pars intermedia shows the presence of chromophobes, which contain little cytoplasm and are regarded as the intermedial cells.

That the pattern of the body surface as well as the pigmentation of the eyes of the fish get established early in the developmental period suggests that, this is probably one of the first adenohypophyseal regions to become functionally established.

DISCUSSION

The pituitary gland of *C. catla* arises as a solid ingrowth of cells from the ectodermal epithelium. This mode of origin corresponds to that of Petromyzon (De Beer, 1926; Mathews, 1937; Woodman, 1939). Ectodermal cells proliferate in the region where ectodermal epithelial covering of the head lies in close association with the diencephalon.

The first appearance of the thickening of the epithelia is seen at 5 mm stage. Ectophyseal analage forms at the stage of 6 mm when the larva develops pharyngeal cavity. In later stages analage becomes separated from the buccal epithelium under the influence of mesodermal tissues. Development of saccus infundibuli take place at 9 mm stage from the diencephalic floor. The base of saccus infundibuli alongwith the post optic floor form the neurohypophyseal part of the pituitary as in other vertebrates.

The neuroecytodermal union is regarded as a definite pituitary. The actual differentiation of the glandular part is recognised on the basis of cell types and penetration of nervous tissue. On the basis of cell types, anterior and posterior regions can be recognised during early stages of the larva. The anterior portion is supplied with nerve fibres and in later stages, cells become differentiated and show deep staining reaction. The present observation strongly support the view of Mathews (1937).

The pituitary gland of *C. catla*, is dorso - basic and arrangement also reported in *Fundulus heteroclitus* (Mathews 1937), *Cirrhina* sp. and *Mystus* sp. (Satyanesan, 1958). it is lodged in the sella turcica. Similar observations were reported in *Polypterus* sp. (De Beer, 1926).

In *Catla catla*. the RPD is distinct as in *Fundulus* (Mathews 1937). The PPD occupies the middle portion as in other teleosts. The PI is known to be associated with the neurohypophysis (De Beer, 1926) is present in *C. catla*. The neurohypophysis ramified in the PI of *Labeo clero* (Das & Daftari, 1967).

The cells of the RPD in *C. catla* consists of abundant acidophils, few cyanophils and chromophobes. Acidophils are the erythrosinophils (prolactin cells) and cyanophils (gonadotrophs). Chromophobes are present in abundance. Cyanophils are present in the RPD. Similar observations have been made in *Salmo trutta* and *Esox lucius* (Kerr, 1942) and in *Puntius sarana* (Rao, 1971).

Cyanophils were absent in the RPD of *Labeo rohita* (Khan, 1962). Gonadotrophs stain immensely with PAS and aniline blue in the Cleveland Wolfe Trichrome Stain. The thyrotrophs stain faint blue with aniline blue, when compared to the gonadotrophs. Somatotrophs stain orange in colour. Thus three types of cells are found in the PPD region of the pituitary gland of *C. catla*.

The PI contain gonadotrophs which stain with PAS and aniline blue. PAS positive cells have been observed in the PI (Satyanesan, 1958). The neurohypophysis ramifies extensively in the PI region. It is loosely arranged fibres with neuroglial cells. The appearance of PAS positive cells in the PPD region shows that the glycoprotain hormones are secreted at this stage for the first time (fingerling stage). As the stage of development of the fish, advances, the gonadotrophs increase in number. The neurosecretory granules appear in the neurohypophysis, when the differentiation of the adenohypophysis takes place. Thus, the development of the pituitary from the time of the formation of the

Table I : *Catla catla* : Characteristic features of different development stages. The numbers in parenthesis indicate the time (hour) after hatching.

	Stage I (6) Hatchling	**Stage II (12) Larva**	**Stage III (24) Larva**	**Stage IV (72) Larva**	**Stage V (5th Day) Post Larva**	**Stage VI (15th Day) Post Larva**	**Stage VII (25th Day) Fry**
Total Length (mm)	4.80	5.60	5.80	7.30	9.00	23.00	30.00
Diagnostic features	Movement of the hatching feeble most of the time at the bottom, on their lateral side and come up to the water surface often. Eyes body more or less whitish in colour (Fig. A).	The body has yellowish hue on the dorsal side above the yolk sac; Pectoral fin rudiment not seen. Eyes bear black pigment at the centre (Fig. B).	Larva exhibits jerky, swift, movements rests laterally, pectoral fin rudiment noticed. Black chromatophores appear along the edge of the upper margin of the yolk sac. Pigmentation in the eyes, dense; caudal fin spatulate with striations. Portion of the oral aperature discernible as a slit (Fig. C).	Larva bright yellow in appearance, particularly in the region of the head. Eyes prominent and deep black. Operculum region with a faint reddish hue. Black chromatophores noticed behind the eyes and continue as a fine streak, through the air bladder region upto the caudal peduncle. Dorsal and ventral embryonic fin folds persist. Dorsal to the notochord in the caudal peduncle region, yellowish pigments are noticed (Fig. D).	Eyes deep black, Dorsal, anal and ventral fin folds present and are devoid of chromatophores, 6 rays in the anterior part of the dorsal fin fold; 18 district fin ray in the caudal fin; notochord sharply turns upwards; terminal part of the fin rays appear to be branching. Black chromatophores are sparsely distributed on the body. Prominent pigmentation below the notochord, in the caudcal regions. Black chromatophores form 2 distinct crescent shaped patches on the caudal region. Lower lip slightly thicker than the upper and projects beyond the latter (Fig. E).	Dorsal fin with 19 rays, of which the first three are unbranched. Anterior margin of the fin bright yellow. Black chromatophores cover the entire margin. 8 rays in the anal, the first two being unbranched; Pelvic with 9 rays. Black triangular patch on the caudal peduncle; posterior to this, two black crescentic pigment concentrations; Black chromatophores distributed on the entire body, which is yellow on the dorsal side; Deep black, prominent chromatopores; Black chromatophores distributed on the entire body, which is yellow on the dorsal side; Deep black, prominent chromatopores on the head. Ventral part of the body pale with fewer chromatophores. The ratio between total body length and length of the dorsal fin at its base is 5:1 (Fig. F).	Body greenish yellow and operculum reddish golden coloured; anterior margin of the eye faintly red; edge of upper lip dark; Margin of entire dorsal fin dark; the upper lobe of the caudal fin slightly longer. Faint diamond shaped area on the caudal peduncle resembles the adult.

ectophyseal analage to the regionation of the different neuroendocrine components of the pituitary is traced by histological evidences, in *C. catla*.

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