STUDIES ON THE PITUITARY GLAND OF SELECTED CULTURABLE FINFISHES

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C E R T I F I C A T E

This is to certify that this Dissertation is a bonafide record of work carried out by Kum. ASHA NARAYAN under my supervision and that no part thereof has been presented before for any other degree.

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C O N T E N T S

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P R E F A C E

Among the endocrine organs, the pituitary gland or the hypophysis is both structurally and functionally the most complex. It occurs in all the vertebrates, including cyclostomes, fish, amphibians, reptiles, birds and mammals. The gland shows remarkable similarities in its overall morphology among the different vertebrate classes; but structurally it is highly specialised showing considerable differences in details.

In most vertebrates, the pituitary lies in a depression in the floor of the cranium with the brain above and the buccal cavity below and by this very situation the pituitary was a difficult subject for study. Smith (1926) described a technique for the hypophysectomy of rats thus paving the way for large scale experimental studies of pituitary glands.

The various physiological activities of the vertebrates are controlled by the pituitary gland. The endocrine organs are important in regulating the multitude of events in the life of the organism and their activity in turn is subject to the careful control of the central nervous system. The secretory activity of the adenohypophysis to a large extent is under the direct control of the nervous system. The pituitary portal vascular system acts as the major functional link between the adenohypophysis and the central nervous system. In addition, the external environmental factors can also regulate pituitary activity via the hypothalamo-portal-adenohypophysial link. The gland's activity is also controlled by the level of the adenohypophysial hormones by their feedback mechanisms.

The pituitary gland offers special advantage for endocrine research since the cells of different types are grouped together. The cells can be identified after experimental treatments although their morphological appearance may have been altered.

The development of staining techniques and the characterisation of the cell types were the contributions of Flesch (1884) who reported his findings on the anterior pituitary of the horse, dog and man. The major limitations in the cytological studies of the pituitary gland were the lack of exact knowledge of the functions of the gland and the crude histological techniques then in use. Flesch (1884) was able to identify two distinct cell types viz. the larger cells with granulation which coloured strongly with his dyes and the smaller cells which took up no stain.

A good deal of progress was made in pituitary cytology with the publication of a new method of staining by Cleveland and Wolfe(1930) staining the anterior lobe of the pituitary gland. Wolfe and his co-workers using this method were able to identify four different cells types.

With the recent advances in research methodology and the invention of electron microscope, it has been possible to locate and identify the different cell types based on their fine morphology. The ultrastructural details of the nucleus and the granulation of the cells, have been made possible and this has greatly helped in correlating the structure and hormonal activity of the different cell types. The techniques of autoradiography have also been used in investigating the structure and function of the pituitary gland.

Hormonal manipulations in controlling the reproductive cycles of economically important organisms have been successfully tried in the fields of poultry and animal husbandry. The reproductive behaviour of many fishes is influenced by the environmental changes sensed by the nervous system. This neural information, on reaching the hypothalamus, determines the activity of the pituitary gland. Based on this, induced breeding techniques for fishes have been developed by injecting crude pituitary extract.

Once the detailed chemical compositions of the various hormones produced by the different parts of the pituitary gland are known one can go in for the production of synthetic hormones in large scale to meet the increasing requirements of the gland extracts.

In the present study an attempt has been made to investigate the morphological details of the pituitary glands of certain economically important finfishes such as *Mugil cephalus*, *Liza parsia*, *Valamugil cunnesius* and *Tachysurus maculatus*. Efforts have also been made to localise and identify the different cell types of the pituitary glands by using the latest staining techniques.

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I N T R O D U C T I O N

The vital endocrine organs of the vertebrates playing dominant role in the physiological activities of the organisms are the pituitary gland, the thyroid, the adrenal, the parathyroid, the urohypophysis and the pancreatic islets. Early ideas relating to the function of the pituitary stemmed from Aristotle's view that the brain served to cool the body, secreting one of the four humours, the phelgm or pituita. The position of the gland lying in a hollow in the base of the skull completely overlaid by the brain inevitably makes it difficult to approach in the living animal. The earliest attempts of hypophysectomy were fatal; so it was thought that pituitary was essential for life. Cushing (1909) studied the inter-relationship between the pituitary and other ductless glands and enhanced the understanding of the gland by a combination of clinical observations and experimental studies. Aschmer (1912) published results of removal of the pituitary of dogs and noted that cessation of growth and genital infantilism followed the operation. Large scale experimental studies on mammals were not started until Smith (1926) described a technique for hypophysectomy of rats, a readily available animal which also offered the possibility of inbred colonies allowing controlled comparative studies.

Our present understanding of the functions of the pituitary gland has been achieved through an enormous amount of observational and experimental work. Development of appropriate surgical techniques to allow hypophysectomy with survival enabled the study of the deficiencies resulting from the operation. Smith (1930) indicated the relationship between the pituitary, the other endocrine glands and the reproductive system and this study was followed by the purification of the active principles and their analysis with the ultimate aim of synthesis. With the application of the modern cytological research aids like the electron microscope it has become possible to understand to a large extent the morphological basis of function in the various parts of the gland. Furthermore it has become now apparent that the role of the endocrine system in general, and of the pituitary gland in particular is not limited to post-natal life and that the gland plays a major role in the embryonic development also. During the thirties Houssay and his students in Argentina, working on the amphibians obtained information on the metabolic role of the endocrine glands including the pituitary, in the toad Bufo arenarum.

As the studies on pituitary glands progressed attention was drawn to the avian pituitary also. Riddle (1933) showed that the pituitary principle which initiated the milk secretion in mammals was the same as that which caused growth of pigeon crop sac. The factor was identified as prolactin (lactotropic hormone), the first pituitary hormone to be identified as a pure protein.

During the thirties information was accumulating about the fish pituitary, some of it coming from the practice of injecting fish pituitary material to the cultivable fishes for inducing them to spawn. In Russia similar methods were tried in sturgeons, to improve the yeidl of sturgeon fisheries. In France and Canada, impetus was given to the study of the pituitary and other endocrine glands of salmon.

In India, aquaculture plays a dominant role in augmenting food production. The technique of induced breeding using pituitary gland extracts has been successfully tried in a variety of Indian fishes for the production of seeds from captive brood stock.

In teleosts, the pituitary gland is both structurally and functionally the most complex of the organs of internal secretion. In the class Teleostei the gland shows remarkable similarities in its overall morphology; but it is structurally highly specialised and there are considerable differences in detail between the different classes and even between closely related species. Structural complexity is equalled by that of the gland's secretions and of the mechanisms by which they are controlled.

The pituitary glands of the teleosts secrete a number of chemically different hormones. To get a thorough knowledge of how induced spawning is brought about in fishes by the injection of pituitary extracts, a study of the hormones secreted by the gland and of the different cell types secreting them is essential.

The organisation of the teleostean pituitary gland has been the subject of many reviews (Pickford and Atz, 1957; Olivereau, 1954, 1963; Stahl, 1963; Wingstrand, 1966). Kerr(1942) made a broad comparative study of the teleost pituitaries. The appearance, ultrastructure and distribution of neurosecretoy material in the pituitary glands of Lebistes reticulatus and Perca fluviatalis were investigated by Follenius and Porte (1962). Satyanesan (1963) carried out a detailed study of the structural peculiarities in the pituitary of clupeioid fishes together with their probable evolutionary significance. The functional anatomy of the pituitary of Labeo rohita (Ham.) was studied by Khan (1962). Lal (1964) undertook morphological, histological and histochemical studies of the pituitary gland of Cirrhina reba. The neurosecretory innervation of the pituitary of the eel Anguilla anguilla and the structure of the pars distalis in different stages of life cycle were investigated by knowles and Vollrath (1966). Morphological and histological studies of the pituitary gland of a catfish Clarias batrachus were carried out by Lehri (1966). The entire organisation and cytophysiology of the pituitary gland in teleosts have been reviewed by Sage and Bern (1971). The morphological structure, adenohypophysial structure and the changes of the prolactin cells during the life cycle were revealed by the studies of Nagahama and Yamamoto(1970).

A better understanding of the relationship between the structure and function of the different regions of the gland has been made possible by cytological studies with the aid of the electron microscope which provided a means of more certain identification of types of cells based on their fine morphology. The shape and range of size of secretory granules are often characteristic for a given functional cell type. Knowledge of these have enabled the functional attributions to be made more precisely. Observations on the seasonal changes in the PAS positive cells in the pituitary of the catfish Mystus vittatus (Bloch) was reported by Satyanesan et al.,(1962). The ultrastructure of the prolactin cells in the pituitary gland of Tilapia mossambica and their response to environmental salinity were studied by Dharmamba and Nizhioka (1968). The various modifications of the prolactin cells in seawater were investigated by Olivereau (1966). Cook and Van Overbeeke(1969) undertook a detailed study of the fine structure of the eta cells in the pituitary gland of adult migratory sockeye salmon Oncorhynchus nerka. Nelly and Abraham (1969) studied the influence of

environmental salinity on the prolactin and gonadotropin secreting regions in the pituitary of Mugil spp.
The pituitary innervation and control of colour change in the skates Raja naevus and R.clavata and others were investigated by Chevins and Dodd (1970). Satyanesan (1971) scrutinised the significance of the structure of neuro-hypophysis and the tetrapod like characteristics of its hypothalamohypophysial vascularization. The functional similarities between the different cell types-the cortico-cotrops and the pars intermedia cells-in the catfish Clarias batrachus were analysed by Haider and Satyanesan (1975). The fine structure of the adenohypophysis in immature sockeye salmon Oncorhynchus nerka was studied by Mc Keown and Van Ove (1973). Haider and Satyanesan (1973) undertook histophysiological studies on the adrenocortico-tropic and pars intermedia cells of the teleost Rita rita (Ham.).

The ultrastructure of the cell types and of the neurosecretory innervation in the pituitary (the proximal pars distalis) of Mugil cephalus from the freshwater, the sea and a hypersaline lagoon were studied by Abraham(1974). Baker et al., (1974) carried out a study of the cell types in the adenohypophysis of the Indian catfish Heteropneustes fossilis (Bloch). The proximal pars distalis andits innervation of the roach Leuciscus rutilus was studied by

Bage *et al.*, (1974). Studies on the endocrine glands of the salmonid fish, *Plecoglossus altivelis* relating to the seasonal changes in the cells of the adenohypophysis with special reference to the prolactin secreting cells were made by Homma and Yoshie (1974). The morpho-histology of the pituitary glands in two freshwater major carps of India, *Labeo rohita* (Ham.) and *Cirrhinus mrigala* were investigated by Moitra and Sarkar (1976). Joy and Satyanesan (1976) undertook a study of the functional cytology of the pituitary gland of *Clarias batrachus*. Jose and Satyanesan carried out cytological studies of the pituitaries of the Indian carp *Labeo rohita* (1977), *Anabas testudineus* (1980a) and *Tilapia mossambica*(1980b). Matty and Matty (1959) made a histochemical investigation of the pituitary gland of the teleosts. Contributions of Beach (1959), Sundararaj (1959, 1963) Lagios (1965), Goswami (1966), Overbeeke and Mc Bride (1967), Sundararaj and Goswami(1968, 1972) Higgs *et al.*,(1976),Olivereau and Chambolle (1979), Nath and Sundararaj (1979), Stuart *et al.*,(1981),Pandey and Pandey (1981) and Thomas and Satyanesan (1981) have dealt with the pituitary gland and its relationship with the gonad in teleostean fishes.

In the present study an attempt has been made to investigate the morphological and histological characteristics of the pituitary glands of three species of mullets viz. *Mugil cephalus*, *Liza Parsia* and *Valamugil cunnesius* and the catfish *Tachysurus maculatus*.

MATERIAL AND METHODS

1. SPECIES SELECTED.

Three commercially important mugilids viz. Mugil cephalus Linnaeus 1758, Liza parsia (Mugil parsia Hamilton 1822) and Valamugil cunnesius (Valenciennes 1836) and the catfish Tachysurus maculatus (Thunberg 1792) have been selected for the study. The mullets were collected from the Chinese dipnets operated in the Cochin bar mouth and the catfish from the Fisheries Harbour, Cochin. Live specimens immediately on capture were brought to the laboratory. The length range and stage of sexual maturity of the fishes used for the study are given in table 1.

TABLE - 1.

Sl.No.	Species	Length range (cm).	Stage of sexual maturity
1.	Valamugil cunnesius	12 - 18	II stage
2.	Liza parsia	15 - 21	I and II stage
3.	Mugil cephalus	40 - 50	I and II stage
4.	Tachysurus maculatus	25 - 30	I stage.

2. PREPARATION FOR MORPHOLOGICAL STUDY.

Immediately on reaching the laboratory, the fishes were anaesthetised, chilled and prepared for dissection to avoid post mortem changes in the pituitary gland. They were dissected under the dissection microscope to expose the cranial cavity as the pituitary was located in a depression at the base of the skull. To avoid damaging the delicate tissue, the dorsal surface of the skull was opened and part of the brain excised. The now exposed pituitary gland and adjacent hypothalamic part of the brain were taken out. Rough sketches of the brain showing the different parts and the position of the pituitary gland were drawn using the camera lucida. The details of the morphological features of the pituitary glands were examined closely under the microscope.

3. PREPARATION OF TISSUE SECTIONS.

The pituitary gland along with a portion of the brain was carefully taken and fixed in aqueous Bouin's fixative and Helly's fluid. The period of fixation ranged from 24-48 hours. After fixation, the tissues were washed thoroughly in 30% alcohol and dehydration was done by transferring the tissues to the higher grades of alcohol in sequence. Finally the tissues were subjected to cold impregnation with xylene (saturated with wax) for about 30 minutes. Embedding and blocking procedures were done using paraffin wax of melting point 58-60°C. The embedding was done using shallow watch-glasses after applying a layer of glycerine facilitating the easy removal of the blocks.

Transverse sections of the whole pituitary glands were taken at 6-8 µ using a Rotary Microtome.

4. STAINING PROCEDURE:

The staining properties of the cytoplasm and its elements were used to characterise and differentiate the cells of the pituitary. The glandular cells of the pituitary are classified as either acidophils or basophils.

For the identification of the different cell types the following staining procedures were adopted:-

i) Periodic Acid Schiff (PAS-Orange G with celestine blue haemalum sequence (Mc Manus 1946, modified by Pearse 1959).

ii) Chromium- haematoxylin-phloxine (Gomari 1941).

iii) Mallory Heidenhain's (Modified after Koneff 1938).

iv) Orange-Fuchsin-Green O.F.G. (Slidders 1961) and

v) Cameron and Steele method (1959).

In the chromium-haematoxylin-phloxine method, the sections were kept in haematoxylin solution for 45 minutes until the beta cells became deep blue. In the PAS- Orange G procedure with celestine blue haemalum sequence, instead of Meyer's haematoxylin, Cole's haematoxylin was used. While following the PAS procedure, rinsing the sections three times in 0.5% aqueous sodium metabisulphite was not done, but instead a thirty minutes washing under the running tap was given. For Mallory Heidenhain's method, the slides were stained in azocarmine solution at 52°C in oven for two hours. In the O.F.G. Method of Slidders, a 0.2% solution of light green was used instead of 1.5% light green.

5. MICROPHOTOGRAPHY.

Microphotographs showing the different topographical regions and the cell types of the pituitary were taken using Olympus Photomicrographic system- Model PM 10AD. The film used for taking the black and white microphotographs was ORWO NP27 400 ASA. Colour photographs were taken using Kodacolor II color negative film of speed 100 ASA.

RESULTS

A. Morphology

The structure of the teleost pituitary is variable in different species and different nomenclatures have been proposed by various workers to designate the parts. In the present study, the terminology used by Green (1951) has been adopted.

1. Adenohypophysis- the part of the pituitary derived from the stomodaeum.
2. Neurohypophysis- the part of the pituitary derived from the diencephalon.
3. Subdivisions of adenohypophysis:
   - i) Pars distalis-the rostral and the proximal parts constituting the main bulk of the adenohypophysis.
   - ii) Pars intermedia- the distal region of the adenohypophysis where the innervation of the neurohypophysis is maximum.

a) Valamugil cunnesius.

The pituitary gland of Valamugil cunnesius is a compact and slightly cone shaped structure located in the sella turcica, a concavity of the spheneid bone. The gland is situated ventral to the brain, immediately behind the optic chiasma and above the capillary network, the rete mirabile. The gland is encapsulated by the dura mater. It is attached to the floor of the infundibulum by a very short stalk and hence the pituitary gland is of the lepto-basic type. Since the stalk enters the gland from the anterior side it can be described as cranio-leptobasic. The pituitary gland in this case, is dull white in colour and is surrounded by connective tissue and fat. The pituitary is vascularised by the branches of the internal carotid artery.

Microscopical observation of the whole pituitary section shows that it is composed of four well defined regions viz. the frontal lobe the rostral pars distalis, the middle lobe, the proximal pars distalis and the distal lobe, the pars intermedia (together constituting the ade-nohypophysis) and the neurohypophysis above innervating more in pars intermedia. The topographical relations of these regions are shown in the transverse sections of the whole pituitary gland. (plate 2(a)). A diagrammatic representation of the morphology of the brain (dorsal and ventral views) is shown in Fig.1.

b) <u>Liza parsia.</u>

The basic arrangement of the pituitary gland of <u>Liza parsia</u> is similar to that of <u>Valamugil cunnesius</u>. The pituitary gland of <u>L.parsia</u> is a pinkish and cone-shaped structure situated ventral to the brain, immediately behind the optic chiasma and in front of the capillary network, the rete mirabile situated in the middle region of the lobus inferiori. The gland is located in the sella turcica, a concavity of the sphenoid bone. A definite stalk is present for the gland and hence it is of the leptobasic type; and since the stalk enters the gland from the anterior side it can also be described as cranio-leptobasic. The gland is surrounded by connective tissue and fat.

In L.parsia, the pituitary gland, when examined closely under the microscope showed four definite regions: the rostral pars distalis, the proximal pars distalis, the pars intermedia and the neurohypophysis. The major portion of the gland is occupied by the pars distalis; neurohypophysis occupies a comparatively lesser area and the remaining portion is occupied by the pars intermedia. The neurohypophysis is seen in the sections as a triangular region composed of a meshwork of nerve fibres and contain comparatively few cells. This region is seen to be surrounded by the pars distalis and the pars intermedia. The position of the different regions in the pituitary gland is shown in plate 4 (b). The ventral and lateral views of the whole brain along with the pituitary gland are shown in plates 1(c) and (d). A diagrammatic representation of the morphology of the brain (dorsal and ventral views) is shown in Fig.2.

c) <u>Mugil cephalus</u>.

The general pattern of the enlodgement of the pituitary gland is the same as in the other two species. The pituitary gland of <u>Mugil cephalus</u> is much larger in size when compared to the other two species and is dull pink in colour, typically cone shaped and compact, with a small depression on the dorsal side. It is located in the sella turcica, the concavity of the sphenoid bone. It is situated on the ventral side of the brain in between the lobus inferiori, below the optic chiasma and above the rete mirabile. The gland is of the cranio-leptobasic type and is found to be surrounded by connective tissue and fat.

In the transverse sections, the pituitary gland appears to be heart shaped and four distinct regions could be distinguished as described for earlier fish. The neurohypophysis is very narrow and the majority of the area is occupied by the pars distalis (proximal and rostral). The distal end of the pituitary gland is occupied by the pars intermedia. A diagrammatic representation of the morphology of the brain (dorsal and ventral views) is shown in Fig.3.

d) <u>Tachysurus maculatus</u>.

When compared to the pituitary glands of <u>Valamugil</u> sp, <u>Liza</u> sp., and <u>Mugil</u> sp., in this species it is more evolved. The brain is larger in size than that of the other species studied. The deep pink, oval pituitary gland is enlodged in the concavity of the para-sphenoid. It is situated just behind the optic chiasma in a notch between the two inferior lobes and above the rete mirabile. The gland is of the cranioleptobasic type, with a short stalk with which it is attached to the floor of the indundibulum. The pituitary gland and the parts of brain surrounding it is found to be covered by layers of fat and connective tissue. The gland lies in close approximation with the brain and its dorsally raised region fits into a notch in the brain formed between the two inferior lobes. The pituitary is bilaterally symmetrical with a slightly convex and smooth ventral surface.

Three different regions could be recognised in the transverse sections of the entire pituitary gland.

i. a centrally located neurohypophysis.

ii. surrounding the neurohypophysis and innervated by its nerve fibres the pars intermedia.

iii. the distally located pars distalis.

Majority of the area is occupied by the pars distalis, a comparatively lesser area is occupied by the pars intermedia and the rest of the area is occupied by the neurohypophysis.

The ventral and lateral views of the whole brain along with the pituitary gland are shown in plate 1(a) and (b). A diagrammatic representation of the morphology of the brain (dorsal and ventral views) is shown in Fig.4.

PLATE 1

a) Brain of <u>Tachysurus maculatus</u>- ventral view.

b) Brain of <u>T. maculatus</u> - lateral view.

c) Brain of <u>Liza parsia</u> - ventral view.

d) Brain of <u>L. parsia</u>. - lateral view.