CHAPTER II

STUDIES ON THE CYCLIC CHANGES IN THE HYPOTHALMO NEURO-HYPOPHYSIAL NEUROSECRETORY SYSTEM OF (ANADROMOUS) MIGRATORY HILSA ILISHA (HAM.) AND IN THE NON-MIGRATORY HILSA TOLI (CUV. & VAL.) DURING DIFFERENT PHASES OF THE LIFE-CYCLE

INTRODUCTION

The extensive literature on the hypothalamo-neurohypophyseal system of fishes has appeared in last few years (For review, Pickford and Atz, 1957; Bern and Nandi, 1964; Dodd and Kerr, 1963; Sathyanesan, 1965a,b; Jasinski and Gorbman, 1966). Recently submitted electron microscope studies on neurosecretory cells of fish (Palay, 1960; E. Scharrer, 1962) and on the neurosecretory material in neurohypophysis and in adenohypophysis of fish, have provided good deal of informations to the Neuroendocrinologists (Follenius and Porte, 1962; K. Lederes, 1964; Follenius and Porte, 1960, 1961a,b; Legait, E. and Legait, H. 1957, 1958). The pharmacological studies carried out on the neurohypophyseal hormones of fishes have given some ideas about the role of these hormones in water balance and on Na metabolism in fish. Recently identified and studied arginine vasotocin from the pituitaries of fish (teleosts) have exposed the secrets of the molecular structure of this hormone (Heller and Pickering, 1960; Acher et al.,1961; Rasmussen and Craig, 1961). Whether it contains the Vasopressor-antidiuretic-water balance principles is not yet clearly established (Sawyer, 1961a, Sawyer,1963 in book Hormones and Kidney). A 'fast moving '(Oxytocic principle) neurohypophyseal peptide is also recorded and isolated from teleost pituitaries (Heller, Pickering, Maetz, and Morel, 1961). Sawyer (1963) named it as teleost oxytocin-like principle, Heller (1963) called it as 'Ichthyotocin', whereas Acher et al. (1962) described it as 'Isotocin' on basis of their pharmacological studies. This 'fast moving peptide', one of the neurohypophyseal peptides, obtained from teleosts, is believed to have very high specific oxytocic activities (Sawyer et al., 1960; Sawyer, 1963). In their wonderful review, Heller and Bentley (1963) suggested that, on the basis of the conflicting results obtained by several workers (Holmes, 1961; Maetz, 1963; Carlson and Holmes, 1962), that neurohypophyseal hormones of fish have effect on Na balance, but their effects on the water metabolism in aquatic vertebrates are not yet known; though it is believed that these hormones have an effect on glomerular filteration rate (GFR). He put forward a new statement, that the neurohypophyseal hormones in fish have two way complex physiological system on Na balance.

Several histological studies indicate a probable role of neurohypophyseal hormones in osmoregulation, as many workers have noted depletion of neurosecretory material (NSM) from the hypothalamo-neurohypophyseal neurosecretory system of marine teleosts and of fresh water teleosts when fish were studied in hypotonic medium and in hypertonic medium respectively (Arvy et al., 1954; 1955, 1959; Favro, 1960; Fridberg and Obssen, 1959). Other school of workers, on basis of their observations and results, suggested the close association of neurohypophyseal hormones with gonadotropic activities. As the gonadal maturity correlated with secretory cycle of Nucleus lateralis tuber (NLT) and cyclic changes in NSM (Öztan,1963; Pickford and Atz, 1957, for review; Hild, 1951; Vön Brëhm, 1958; Juan Kruslovic, 1963; Bern and Nandi, 1964, for review). Recently, Sawyer and Pickford (1964), supporting the observations of Sokol (1961) on the role of NSM in reproductive cycle of *Fundulus heteroclitus*, stated that depletion noted in oxytocin-like principle, 8-serine, 8-isoleucine oxytocin principle in female pituitaries, during reproductive season, may participate in regulation of reproduction in that fish. This hypothesis was advanced by K. Lederis (1964), after carrying out electron microscope studies on neurohypophysis of Rainnow trout exposed to sea water. He reported, that, the two hormones can be released independently of each other and Ichthyotocin may be concerned with reproductive activity; whereas Arginine vasotocin may be associated with water and electrolyte metabolism.

Though, our present investigations are within the bounds of histological studies and our interpretations are based on them, if the comparison of cyclic changes in the Hypothalamo-neurohypophyseal neurosecretory systems of migratory and nonmigratory, H. ilisha and H. toli, is made, it is probable that it may throw some light on the probable role of NSM in maturity and in osmoregulation.

MATERIALS AND METHODS

The fishes were captured live, directly from the nets, from the various places as shown in the map 1 and map 2. Enough care was taken to discard wriggled fish during collection of the samples. The head was removed and the brain was exposed by removing the bony cover within few minutes after capture and was fixed in Bouin's fixative for 6 hours. After that the brain was completely exposed to the fixative for 4 to 6 days. Bouin's fixative was found best of all other fixatives tried, Zenker's formol, 10% neutralized formalin. 4 μ to 6 μ paraffin serial sections were taken and were stained for the neurosecretory cells (Nucleus Preopticus-NPO, Nucleus Lateralis Tuberis-NLT) and the neurosecretory substance (NSM). The following staining procedures were adopted.

(1) Halmi's (1952) aldehyde-fuchsin method as modified by Dawson (1953) (A.F.)

(2) Chrome-Alum-Haematoxyline-Phloxine(CAHP) method (Pearse, 1960).

(3) Paget's Aldehyde fuchsin-Thionin-PAS as narrated by Stahl and Leray (1962).

(4) Silver nitrate, Gold chloride method for demonstration of lipofuchsin granules (after Nassar, Issidorides and Shanklin, 1960; Gurr, 1962).

Out of all above mentioned staining methods, A.F. method was best felt for the demonstration of NSM in NPO, in preoptico-neurohypophyseal pathways (Pathways) and in neurohypophysis (neural lobe as mentioned in Chapter IV) but it could not differentiate basophils from NSM. For this differentiation, Paget's method was applied and was found much successful for the demonstration of NSM in NPO, pathways and in neurohypophysis. CAHP method, successfully demonstrated Nucleus lateralis tuberis (NLT), NPO and NSM also, and was utilized for NLT in the present study.

OBSERVATIONS AND RESULTS

GENERAL MORPHOLOGY AND PLAN OF THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF H. ILISHA AND H. TOLI:

The entire system resembles each other in H.ilisha and H. toli so that they need not be described here separately. It can be divided into three components (1) the neurosecretory cells of Nucleus Preopticus (NPO) (2) The neurosecretory cells of Nucleus Lateralis Tuberis (NLT) (3) The hypothalamo-neurohypophyseal and (H.N. tract) and (4) The neural lobe or neurohypophysis. All these components are shown in Camera Lucida drawing in Fig. 1. The different regions, are marked with letters in Fig. 1.

THE NEUROSECRETORY CELLS - NUCLEUS PREOPTICUS(NPO):

This group was located on the either side of the third ventricle, anterior to the optic nerve and below the ependymal cells. Two subgroups were noticed in this group, on the basis of morphology and slight change in position (Fig. 1 - NPO). The group of neurosecretory cells (NS cells) which was situated in the anterior part of the region, just below the ependyma, was termed as PARS MICROCELLULARIS or PARS PARVOCELLULARIS (Nayar and Menon, 1960; Öztan, 1964; Dodd and Kerr, 1963). Large number of cells were observed. Their, somewhat fusiform shape, helped in distinguishing them. The other group of NS cells known as PARS MAGNOCELLULARIS consisted of large sized cells. They were arranged in the posterior part of the entire region. Actually, there was no clear demarkation existed in between two groups of the cells; they were classified on basis of morphological characteristics only (Fig. 1).

The different stages of cells were classified by Nair and Menon (1960) and Öztan, (1964) , on the same basis, the classification of cells of NPO is mentioned under. These different stages are represented by alphabetic letters (in Figs. 28, 35 and 48). In running chapter to show the stages of the secretion cycle, letters will be mentioned.

(A) The NS cells with comparatively larger nuclei (Paget's A.F. positive) with very little amount of cytoplasm. The A.F. positive granules were distributed in the form of needle like threads or granules in the nucleus. Rarely A.F. positive granules were visible in cytoplasm.

(B) A.F. positive granules were noticed in voluminous cytoplasm, in which, few vacuoles were also observed. Sometimes A.F. positive granules were surrounding the vacuoles at the Golgi zone. Nucleus remained large.

(C) Nucleus was still larger, but cytoplasm increased in volume. Many A.F. positive granules around Golgi zone were noticed, which were more than observed in type 'A' and type 'B'.

(D) No A.F. positive granules were visible in the large sized nucleus, instead, all A.F. positive granules were concentrated around the golgi zone in perinuclear area of the cell. Many granules were also noticed at the axonal beginning of the cells.

(E) Maximum number of somewhat large sized granules were observed throughout the cytoplasm. The nucleus showed reduction in size. This stage was regarded as 'storage phase'.

(F) This stage showed discharge of the NSM in the form of granules. Due to which many vacuoles appeared in the cytoplasm. There were no A.F. positive granules in the cytoplasm. This phase may be called as "Exhaust Phase".

All the NS cells stained beautifully with A.F. staining and Paget's A.F. Thionin-PAS method. CAHP staining also demonstrated good results, especially for nuclear contents. Shanklin's method gave positive results for these cells.

NUCLEUS LATERALIS TUBERIS:

The NS cells of this group were comparatively smaller and were observed in the lateral and latero-ventral wall of the hypothalamus above the pituitary gland. Three different groups of NS cells could be classified on the basis of their distribution. The entire region of NLT as shown in the figure can be classified into anterior, lateral and posterior regions (Fig. 1). NS cells distributed in the particular region were known according to their location e.g. NS cells distributed in the anterior region of hypothalamus were called as NLT anterior. These cells were A.F. negative, CAHP positive, Paget's A.F. Thionin-PAS positive. The cells of antero-ventro-lateral region and posteroventro-lateral regions were of larger size. The cells observed in dorsal region were smaller. They are plenty in numbers and are polygonal in shape (Fig. 1 - NLT).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT (HN TRACT):

The axons carrying A.F. positive granules were traced from both NPO microcellularies and NPO magnocellularies.

They extend anteriorly and take turn towards pituitary and form a compact mass of axon fibres carrying A.F. positive granules. In CAHP preparations, axons carrying NSM from NLT could not be distinguished from axons of NPO. The entry of pathways coming from NPO could easily be seen going down in the neurohypophysis. Plenty of the capillaries were noted in the region of patnways, coming from NPO, very intense NSM was observed concentrating around the walls of the blood vessels. Sometimes axons were noticed directly terminating on the walls of the blood vessels. This pathway could easily seen in spent <u>H. ilisha</u> prior to its migration to sea (Fig. 1 - pathways). This pathway is divided in the five segments as shown in Fig. 1.

THE NEUROHYPOPHYSIS (OR NEURAL LOBE):

It consisted of glial tissues, connective tissues and blood vessels plenty of cells with large round nuclei and scanty cytoplasm were noted (Pituicytes ?). It gave an appearance of a branched tree, as many branches penetrated deeply in all the regions of the pituitary gland. The pathways coming from NPO were easily noticed entering in the dorsal region of neurohypophysis. The NSM in the form of A.F. positive small granules of redish purple colour, large globules of deep purple colour and colloid like purple materials were seen distributed in this region. Very often NSM was observed surrounding the blood vessels, in proadenohypophysis and in mesoadenohypophyseal basophilic cells. NSM was also seen in the cells of follicles of proadenohypophysis, surrounding the nuclei. With Paget's A.F.-Thionin-PAS staining technique, it was possible to distinguish NSM from basophils (Fig. 1 - Neurohypophysis).

Dodd and Kerr (1963) have already discussed all the different components of the hypothalamo-neurohypophyseal neurosecretory systems of vertebrates.

THE HYPOTHALAMO-NEUROHYPOTHYSEAL NEUROSECRETORY SYSTEM OF FINGERLING OF H. ILISHA CAPTURED FROM RIVER NARBADA:

NUCLEUS PREOPTICUS:

The cells of this group were small and were closely packed. A rich vascular bed was noticed in this region (Fig. 2). Few cells were observed terminating their axon fibres full of NS granules on the wall of blood vessels. Many of the axons full of reddish purple fine granules were noticed travelling towards the preoptico hypophyseal pathways (Figs. 3 and 4). Most of the cells were in 'E' and 'F' phases of the secretory cycle. Their nuclei were perfectly round and exhibited orange G positive and phloxino-philic nucleolus in A.F. staining and CAHP staining preparations, respectively (Fig. 3).

NUCLEUS LATERALIS TUBERIS:

The cells of NLT - anterior were of larger size and were compactly placed. Their cytoplasm was with scanty granules, but observed to be voluminous. The nucleolus stained predominantly with phloxine in CAHP staining. Some of these cells were noticed with long axons travelling towards the pituitary gland. Many capillaries were noted in this region (Fig. 10).

The cells of NLT-lateralis were smaller in size and were scattered in lateral region of the hypothalamus. Few but long capillaries were observed in this region. Their nuclei were round and were with nucleolus and scanty cytoplasm (Fig. 11).

The NS cells of NLT posterior were of larger size and were with large round nuclei (Fig. 10). On the whole, all the cells of NLT were faintly stained by CAHP staining. Their cytoplasm was noticed with very few granules. It appeared,that, these cells were not yet in functional state as cells of the NPO were (Fig. 10).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT:

The axons from NPO carrying reddish purple granules were visible upto very short distance from the NPO. As the tract proceeded ahead, few small A.F. positive reddish purple granules were seen scattered throughout the tract. Due to less NS granules in the tract, the entire tract appeared of very small in width (Figs.4,5 and 7).

THE NEUROHYPOPHYSIS:

The neurohypophysis in the dorsal region was filled with very few scattered (A.F. positive) small granules, but in the branches penetrating the pituitary, plenty of round, deep purple, granules were noticed. Many of them were surrounding blood vessels. Above mentioned study suggest that in fingerling, NS cells have discharged NSM in the neurohypophysis and they have stored NSM in them (Fig. 8). NLT were inactive and they are silent, Few cells of NPO were also observed in 'A' - 'B' phases indicating active synthesis of NSM (Figs. 8 & 76. Fig.5 of Chapter IV).

PROBABLE THIRD GROUP OF NEUROSECRETORY CELLS IN FINGERLING OF H. ILISHA:

These CAHP positive, A.F. positive, probable NS cells were observed on the dorsal side of the 4th ventricle in the region of medulla oblongata. They were in rows above the 4th ventricle cavity (Fig.12). They exhibited a secretory cycle similar to that of NS cells of NPO (Figs. 3, 14 and 15). A remarkable blood supply was noticed in this part. Some of these cells were sending their long axons towards the cavity of the 4th ventricle (Fig. 16). Frequently of the base of densely stained axons, a mass (darkly stained by CAHP staining) was observed above the layer of cavity (Fig. 16). Some of these cells probably 'F' phase of secretory cycle showed 'irregular shape and phloxinophilic cytoplasm. In some irregular shaped cells nuclei were not visible.

The secretory phases and the rich blood supply suggest that synthesized NSM (?) may be discharged either in capillaries or in the cavity of 4th ventricle (Figs. 13 and 14).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF IMMATURE (STAGE III) HILSA ILISHA CAPTURED FROM THE SEA:

The cells were not compactly placed with each other. Most of the NS cells were in 'E' and 'F' phase of secretory cycle. Some cells showed 'A', 'B' and 'C' phase too. The NSM in the form of fine, reddish purple granules were also noticed in the axons of NS cells travelling upto little distance (Figs. 17 and 18). Some of the axons were terminating on the walls of blood vessels and capillaries. Abundant blood supply was noticed in this region. Dark purple coloured globules were also noticed in the distant axons which were joining the tract. Some of these globules were noticed on the wall of the capillaries. Large form of these globules may be arranged together in number of 3 to 4 or may be arranged keeping little distance from each other in an axon fibre. Fine reddish purple grannules were also visible on the axon fibres carrying large globules. Larger globules were visible only in the axons of NPC and not in the pathway or tract (Fig.19). This may be considered as beginning of discharge of NSM from the cells of the NPO.

NUCLEUS LATERALIS TUBERIS:

Plenty of capillaries were noticed in the region of this group of NS cells. The NS cells had increased in size. Very little CAHP positive granules were noticed in each cell of different groups. NLT-posterior cells were larger with voluminons cytoplasm. Their axons could be traced upto certain distance. Their nuclei were comparatively larger and were with fine chromatin materials and a nucleolus. Few NS cells of this region were observed terminating axon fibres on the capillaries (Figs. 24, 25 and 26).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT:

As the discharge of NSM from NPO was not much, the tract was observed to be thinner in size consisting of few fibres, carrying light purple fine granules. These granules were noticed concentrating around the blood vessels and capillaries which comes in way. Throughout the tract fine reddish purple granules were visible scattered in groups or in number of one or five (Figs. 19, 20, 21, 22 and 23).

THE NEUROHYPOPHYSIS:

On the whole, in this region, few A.F. positive reddish purple and purple granules were observed. In few sections, the branches penetrating mesoadeno. and metaadenohypophysis were loaded with dark purple granules. These granules were surrounding the blood vessels in large numbers, but in all the sections, the branches distributed in proadenohypophysis and some branches of mesoadenohypophysis were completely devoid of NSM. This suggests that, little amount of NSM was discharged into the neurohypophysis. The tract was also with few NS granules indicating the NSM is not yet supplied (Fig. 23. Fig.9 of Chapter IV).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF MATURE (STAGE IV) HILSA ILISHA CAPTURED FROM SEA PRIOR TO MIGRATION INTO THE RIVER NARBADA:

NUCLEUS PREOPTICUS:

Almost all the NS cells exhibited 'E', 'D' and 'F' stages of the secretion cycle. The presence of many cells of 'F' stage was significant and in this NPO- NS cells differ from NPO-NS cells of immature H. ilisha. Orange-G positive nucleolus with prominantly and large size, was visible in all large sized nuclei. Many axons emerging out and travelling towards the ventral side, were noticed with deep purple coloured,mostly, large globules. Fine granules of reddish purple colour were also visible in the axons (Figs. 27, 28 and 29). The axons, which met the capillaries on their way, were seen globules depositing (?) NSM on the capillaries. Few capillaries were noticed in the cell mass of NPO. The entire pathway beginning from NPO upto neurohypophysis could be detected.

NUCLEUS LATERALIS TUBERIS:

The NS cells of all the subgroups have increased notably in their size. Fine, dust like, accumulation of CAHP positive granules, throughout the cytoplasm were observed. Tremendous increase in nuclear size was also noticed. A prominant phloxinophilic, fairly large sized nucleolus, was always present in these cells. The granules were concentrated more towards the axons. Many of the axons were noticed terminating on the capillaries. A remarkable increase in number of blood vessesls was noted. Axons of NLT-posterior were noted travelling away from the pituitary glands, whereas the axons of NLT-lateralis were seen going towards neurohypophysis. In few sections the axons were easily noted entered in the neurohypophysis. In few of the cells, nuclei multiplied and resulted into large two to three nuclei, with one or two nucleolus in them. These cells were of irregular in shape and their nuclear region was also filled with fine granules. On the whole, tremendous increase in number of NLT cells, remarkable increase in the volume of the cytoplasm and secretory activities of NLT cells was noticed. So far remained silent NLT-NS cells have entered into the field of secretion.

THE HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT:

Due to the presence of many A.F. granules and globules, the detection of the entire pathway or tract was easily made.

Fine, reddish purple granules were observed scattered in plenty of numbers, throughout the tract. They were also noted concentrating in large number around the blood vessels, which come in their contact, during distal migration. In the region of neurohypophysis, accumulation of NSM was observed clearly with A.F. staining. Large, deep purple globules, reddish purple small granules and colloid like deep purple NSM, were noticed in the dorsal region of the neurohypophysis. The entire tract was thicker and prominent than that of immature <u>Hilsa</u> <u>ilisha</u> (Figs. 29,30,31,32 and 33).

NEUROHYPOPHYSIS:

The branches penetrating meso. and metaadenohypophysis were full of deep purple globules and granules. Few branches, going in mesoadenohypophysis, were devoid of NSM. NSM was always noticed concentrating around the blood vessels. Sometimes, it gave an appearance of deep purple clump around the blood vessels. Large, deep purple and colloid like NSM was also noticed. Comparatively, scantly in branches penetrating the region of proadenohypophysis. In some of the follicular cells of proadenohypophysis, NSM was noticed around the nuclear mass (Fig. 13 of Chapter IV).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF MATURE (STAGE V AND VI) HILSA ILISHA CAPTURED FROM RIVER NARBADA DURING MIGRATION FOR SPAWNING:

NUCLEUS PREOPTICUS:

On the whole, NS cells appeared loose due to exhibition of 'F' stage by many NS cells. They were shrunk and completely devoid of NS granules. The cells, which were dominating showed 'E' phase of secretory cycle. The NS cells of 'F' stage stained faint with light green and their nuclei did not clearly showed nucleolii. NS cells in 'E' phase showed round nuclei and prominant nucleolus in them. The long axons of NS cells of 'E' stage, were full of fine granules and were noticed travelling upto long distances. From the axons, large, dark purple A.F. positive globules were also noticed descending down towards pituitary gland. In some samples, about 80% of the NS cells of preopticus group were in 'F' phase (Figs. 34 and 35). Many capillaries were noticed in this region, the blood supply may be considered maximum here. This may be for the supply of blood for the resynthesis of NSM.

PREOPTICO NEUROHYPOPHYSEAL-PATHWAYS:

The axon fibres were densely filled with A.F. positive, small, dark purple and red purple granules. The width of the pathway was much more than observed in mature H. ilisha captured from the sea, prior to spawning, but these granules were concentrated in the beginning portion of this region only, rest of the pathway, was noticed with fine few and scattered granules, due to which the pathways upto neurohypophysis, could not be traced (Figs. 34, 36 and 37).

NEUROHYPOPHYSIS:

The branches, penetrating metaadenohypophysis were fully packed with deep purple NS granules. These granules were concentrated around the blood vessels, filled with blood cells. The branches of mesoadenohypophysis, were moderately filled with NSM, but the deeper branches of this region were densely packed with dark purple granules and globules (Fig. 77). The dorsal region of neural lobe was noticed filled with colloid like deep purple NSM along with deep purple globules. Paget's A.F.-Thionin-PAS staining wonderfully contrasted basophilic cells of mesoadenohypophysis from NSM, by giving blue colour to the former and green to the later. Branches travelling in the proadenohypophysis were observed filled with few red purple granules. These granules were surprisingly noticed in the follicular cells of proadenohypophysis. Granules of deep blue and deep purple colour were seen around the nuclei of follicular cells and in the basement membrane of the follicular cells also(Figs. 15 and 16 of Chapter IV). Generally NSM was surrounding the blood vessels of neural lobe in proadenohypophysis.

NUCLEUS LATERALIS TUBERIS:

Remarkably noticed was tremendous increase in volume of cytoplasm and nuclei of these NS cells. Nuclei of irregular shapes, were also noticed in these cells. Inter nuclear space was also filled with fine CAHP positive granules. Nucleolii were prominantly stained by phloxine and in many nuclei of cells, nucleolii were more than one (Figs. 40 and 41). On the whole, multiplication of number of cells, presence of densely stained granules in the cytoplasm, enlargement of cytoplasm and nucleus, division of nuclei into two or three, presence of more number (2 to 3) of nucleoli in the nucleus, indicate an increase in functional cycle (Figs.40 and 41), which may be correlated with increase in secretory activities of basophils of the pituitary gland. The much abundance of vascular beds was a noticeable feature(Figs. 38,39,40,41,42 and 43).

GLOBULAR PROTRUSIONS FROM EPENDYMAL CELLS,PROJECTING IN THE CAVITY OF THIRD VENTRICLE :

Especially in mature H. ilisha, some apical portions of the ependymal cells were noticed with globular protrusions. These long protoplasmic processes (?) of the ependymal cells stained deep blue with CAHP, dark brown with Nassar, Issidories and Shanklin's method and light green with A.F. With CAHP, some of these globular protrusions and vesicle like structures, showed presence of fine granules in them (Figs.44,45 and 46). The NLT cells were also seen in near viscinity of these protrusions, it is quite probable, that, the NSM from the NLT might have reached to the globular protrusions through the ependymal cells. The means of which is not yet known. Similar features were noted by Stahl and Leray (1962) in Mugil capito and in mammals by Löftgreen (1959,1960).

At some places, long protoplasmic processes had a small bulb and a curved stalk, later in contact with the cells of 3rd ventricle. The bulb stained densely with CAHP and Nassar, Issidorides and Shanklin's method. These curved stalk like processes exhibited a line-stained by above mentioned two methods (Fig. 45).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF SPENT (STAGE VII & VII-II) OF MIGRATORY H. ILISHA ON RETURN MIGRATION TO SEA:

NUCLEUS PREOPTICUS:

Due to the presence of many NS cells of 'F' stage of secretion cycle, the entire region appeared to be loose. NS cells of 'F' stage stained faint green with A.F. and were of irregular in shape. Their nuclei were of round shape and cytoplasm of irregular nature, which was completely devoid of NS granules. Long capillaries were noticed forming a network in this region. Most of the cells were 'discharing' NSM in the pathways by their much widened axons (Fig. 48). Some cells did showed 'E' phase of the secretion cycle. Large deep purple globules were visible in plenty of numbers in the axons of NS cells travelling upto long distances (Figs. 47 and 48). Few of them were terminating on the blood vessels also. Due to many axons filled with dark purple large globules and fine reddish purple granules, a very thick mass of globules arranged in linear fashion was noticed clearly (Fig. 49). These axons after forming an obtuse angle, proceeded to join the pathways.

THE HYPOTHALAMO-NEUROHYPOPHYSEAL PATHWAYS:

The axons coming from NPO, when joined together to form a pathway consisting of individual axon fibres, form a compact mass filled with A.F. positive deep purple large globules, granules of moderate size and fine reddish purple granules. This tract, rather entire tract upto neurohypophysis, was filled with fine dark purple and large dark purple granules due to which it could be traced upto the neurohypophysis. Entire pathway was of much width due to more number of fibres carrying NSM from the NPO (Figs. 47,48,49,50,51,52 and 53). Many blood vessels were seen in this region. NSM in the form of fine granules was also surrounding these blood vessels.

NUCLEUS LATERALIS TUBERIS:

All the NS cells appeared empty and devoid of granules as evident by very faint staining by CAHP method. The voluminous cytoplasm had aquired irregular shape (Figs. 55, 56, 57, 58 and 59). Long capillaries were noticed. NLT of anterior region had shrunk, giving rise to an empty space between the surrounding cells and NLT cells of anterior region. This may be due to loss of NSM from the cells, during spawning. Much blood supply may be for resumption of synthesis NSM. The decrease in secretory activity of NLT may be correlated with degranulation of basophils of central mesoadenohypophysis and shrunk and empty gonads. Many NS cells exhibited few granules (CAHP positive) inside nuclei and in between the nuclei - that is in internuclear space

(Figs. 55,56,57,58 and 59). The nucleolii of all these cells were densely stained by phloxine.

THE NEUROHYPOPHYSIS:

In most of the specimens it appeared with scanty of NSM. Entire region was loose and with many blood vessels. Very little NSM was noted around blood vessels, unlike noted in mature H. ilisha on the way to migration. The little quantity of NSM present in this region was in the form of purple coloured colloid,(C) and in the form of fine deep purple coloured droplets(E) and granules (Figs. 28 and 29 of Chapter IV). A significant decrease noticed in the entire region of the neurohypophysis, indicated the utilization of hormones during migration and spawning. Very little quantity of NSM was observed in the branches of proadenohypophysis. The presence of NSM in proadeno. follicles was observed without any remarkable change. This suggests that the hormones were utilized during spawning and during migration due to increased demand to adjust the body in hypotonic medium.

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF IMMATURE (STAGE III) HILSA TOLI CAPTURED FROM THE SEA:

NUCLEUS PREOPTICUS:

Most of the NS cells were in 'D' and 'E' of stage of secretion cycle. Their nuclei were round and cytoplasm was full of purple coloured granules. The axons travelling upto long distance were filled with purple coloured globules, arranged in linear fashion in the axons. Fine reddish purple granules were homogenously distributed throughout the axon fibres. These axons after making an obtuse angle were noticed proceeding towards the pituitary gland, to join the pathways. Large globules and fine reddish purple granules were visible in this region. The entire region including NPO-NS cells, axons coming from NPO, was densely supplied with blood vessels. Few axons carrying fine reddish purple granules were noticed terminating on the capillaries. Few cells exhibited 'A' and 'B' phases of secretion cycle also (Fig. 60).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL PATHWAYS:

The entire pathway could be traced roughly in the region near NPO cells, few large globules were noticed. Entire pathway, when observed under oil immersion, was found filled with fine, small reddish purple, A.F. positive granules, but in the region near the pituitary, granules were scanty (Figs. 60 and 61). The thickness or width of the pathways was of considerable size, but less than that of spent H.ilisha, but can be compared with mature H. ilisha captured from sea.

THE NEUROHYPOPHYSIS:

The entire region is filled with colloid like NSM, deep purple globules and fine reddish purple granules. In other respect it resembled with that of mature H. ilisha captured from sea.

THE HYPOTHALAMO NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF MATURE (SPAWNING) H. TOLI CAPTURED FROM THE SEA:

NUCLEUS PREOPTICUS:

All the NS cells resemble closely in their activities to the NPO-NS cell groups of mature H. ilisha captured from sea prior to spawning (Fig. 62).

NUCLEU LATERALIS TUBERIS:

Due to close resemblances noticed with NLT of mature migrating H. ilisha the description is not mentioned here.

THE PREOPTICO-NEUROHYPOPHYSEAL PATHWAYS:

The width of pathway was much reduced and few A.F. fine deep purple and reddish purple granules were noticed in the entire pathway. There were very few large, deep purple globules in the beginning portion of the pathways (Figs. 62, 63, 64 and 65).

THE NEUROHYPOPHYSIS:

Most of the branches, except the branches of metaadenohypophysis, were filled with scanty of NSM. The branches of metaadenohypophysis were with moderate amount of NSM in the form of reddish purple granules, deep purple granules and deep purple colloid like NSM, concentrated especially around the blood vessels. It suggests that hormones utilized during spawning period, whereas neurohypophysis of mature migrating H. ilisha was noticed densely filled with NSM (Fig.15 of Chapter IV).

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF SPENT H. TOLI CAPTURED FROM THE SEA:

NUCLEUS PREOPTICUS:

The cells of NPO exhibited characteristics similar to those of shown by spent H. ilisha. Many of them were discharging NSM in to the pathways by their axons. NSM was noticed in the form of large globules, and deep purple granules in the axons travelling upto the pathways. Frequently large globules were with empty spaces also (Figs. 66,67 and 68).

THE PREOPTICO-NEUROHYPOPHYSEAL PATHWAYS:

Except the presence of less number of globules, granules in the entire pathway, this region closely resembled to that of spent H. ilisha captured from river. The pathway was comparatively thiner in size, but could be traced upto the neurohypophysis (Figs.66, 67,69 and 70).

THE NEUROHYPOPHYSIS:

This region is filled with NSM which was in the form of dark purple globules, purple granules and reddish purple granules. The dorsal region was noticed with colloid like NSM. This suggests that NSM is supplied, as it was exhausted during spawning (as observed in mature spawning H. toli). This picture is in contrast with the picture described of spent H. ilisha.

THE HYPOTHALAMO-NEUROHYPOPHYSEAL NEUROSECRETORY SYSTEM OF DRIFTED H. TOLI CAPTURED FROM ESTURY ON THE HIGHEST HIGH TIDE DAY:

NUCLEUS PREOPTICUS:

The most of the NS cells were destroyed leaving empty spaces behind. In few specimens, these empty spaces were noticed with light green positive cytoplasmic structures filled with large, deep purple globules and large globules distributed around them. This may be the remnants or 'Debris' of affected NS cells of NPO. The NS cells situated near the preoptico-neurohypophyseal pathways and on the ventral side of brain nearer to optic nerve, had remained healthy, except, the enlarged nuclei in them. These cells showed 'E' and 'F' phase of the secretory cycle and were (E phase) filled with dark purple fine small granules. The destruction of NPO may be due to their inability to cope up with the sudden increase in demand of neurohypophyseal hormones arose due to sudden change (hypotonic medium) in medium. The NS cells had aquired dark light green colour and were noticed with enlarged nuclei and cytoplasm filled with large dark purple globules and droplets in few specimens. The NS cells of immature (Stage III of maturity) mature (Stage IV of maturity) and spent (Stage VII of maturity) *H. toli*-drifted - into the estury of river Narbada - showed above mentioned pecularities, irrespective of stages of maturity (Figs.72 and 75).

PREOPTICO-NEUROHYPOPHYSEAL PATHWAYS:

In immature and in mature *H.toli* the entire pathways could not be traced due to destruction of certain regions of the brain which might be due to drifting, but in spent H. *toli* the pathway, though, not a continuous, but some region of it could be traced. It was composed at few axons filled with NSM (Figs. 73 and 74).

THE NEUROHYPOPHYSIS:

In all the stages of maturity of drifted H. *toli* it was fully laden with NSM. At several regions, it was noticed to be broken and destroyed, leaving empty spaces behind. The shrunk appearance was also a remarkable feature. NSM was observed in the form of deep purple droplets, globules and granules. NSM in the branches of proadenohypophysis and in acidophilic follicles of the same region was also observed.(Figs. 33, 39 of Chapter IV).

DEGENERATIVE CHANGES AND DESTRUCTIVE CHANGES OBSERVED IN THE BRAIN OF DRIFTED H. *TOLI* (ALL STAGES OF MATURITY):

Several portions of the brain were affected, probably, due to change in medium due to drifting, as these changes were not noticed in brains of mature and spent migratory H. *ilisha* nor in mature and spent H. *toli* captured from the sea. Offactory lobe region appeared loose and shrunk. Many lacunae gave rise to empty spaces.

TABLE I

HILSA - ILISHA

STAGE OF GONADS	NUCLEUS PREOPTICUS	HYPOTHALAMO-NEUROHYPOPHYSEAL TRACT
I FINGERLING	Small group and closely packed cells; with rich vascular bed. Axons full of granules travelling towards pathways. 'E' & 'F' phase in majority Mean Nuclear diameter G.	Few small reddish purple granules throughout the tract. Width is too small (of tract)
III	Region increased. Cells not compactly placed. Most of them in 'E' & 'F' phase. Fine purple granules in axons travelling upto short distance towards pituitary.	Tract thin. Few fibres with light purple fine granules generally concentrating around the blood vessels. Granules throughout the tract visible.
IV & V	All in E-D-& F stages. Many in 'F'. Axons filled with deep purple large globules & reddish purple fine granules few capillaries noted.	Increase in width remarkable. Full of fine reddish purple granules. Entire Pathway could be detected. NSM concentrated around the blood vessels. Pathway is prominent.
VI	Many cells in 'F' phase,'E' phase dominating. Presence of many globules and fine granules in axons travelling upto long distances towards pituitary gland. Many capillaries present.	Width considerably increased. Globules & Granules in the beginning portion of pathways, Rest of the entire tract with reddish purple fine granules. Entire pathway could not be traced.
VII	Many in 'F' stage. Loose mass. Ample of blood supply. Discharged NSM in the form of large deep purple gobules in axons travelling upto long distances forming a compact mass. Axons filled with fine granules and large globules.	Thickest in all compact mass filled with deep purple large globules & fine reddish purple granules upto neurohypophysis. Entire pathway could be traced upto neurohypophysis. Many blood vessels come in contact with axons of pathways and were surrounded by granules. Large globules exhibited peculiar characteristics.

TABLE II

H I L S A - T O L I

TAGE OF ONADS	NUCLEUS PREOPTICUS	PREOPTICO-NEUROHYPOPHYSEAL TRACT
III	'D' and 'E' phase in majority. Axons travelling upto long distances and filled with large deep purple globules and fine reddish purple granules Much blood supply.	Roughly entire pathways could be trace Large deep purple globules in region nearer to NPO Fine reddish granules distributed in large numbers except in the region nearer to neurohypophysi width somewhat similar to Mature ilisha (Sea).
ATURE PAWNING V	Similar to mature H.ilisha captured from the Sea.	Width much reduced. Few A.F. +ve deep purple & reddish purple granules. Large deep purple globules in region nearer to NPO in very few numbers.
VII PENT	Similar to spent H.ilisha captured from river after spawning. NSM in axons less than sp.ilisha Discharge is 'moderate' and not 'heavy'.	Similar to sp. ilisha captured from river after spawning but thickness of pathway is less. Large globules lesser. But filled in axon fibres upto neurohypophysis.

DRIFTED HILSA TOLI ON THE HIGHEST HIGH TIDE DAY OF THE YEARS 1963.64

TAGE OF ONADS	Nucleus Preopticus	Hypothalamo-Neurohypophyseal Tract
II I II	Most of the NS cells destroyed leaving empty spaces behind. Few shrunk cells with large deep purple globules were seen. Healthy NS cells were noticed with enlarged nuclei and were in 'E' & 'F' phase, Empty space frequently exhibited, filled with 'debris' of disintegrated cells.	Complete pathway could not be traced due to destroyed portions of the brai as result of drifting- along with disintegration of several other regio (Refer description). In spent thwough was broken at few places, thin pathwa upto pituitary and neurohypophysis could be noticed filled with fine A.F +ve deep purple granules.

NEUROHYPOPHYSIS	WATER ANALYSIS - Mequi/Litre			
	Na	K	Ca	Chloride
Similar to mature *H. ilisha* captured from Sea. Colloid like NSM and deep purple globules many in numbers.	SEA:			
	610.4	14.32	22.46	646.0
	608.2	14.32	22.46	650.6
	608.2	13.82	21.96	658.0
	592.6	13.82	22.46	668.5
Very scantly NSM seen. Nearly empty. Moderate NSM in branches of metaadenohypophysis noticed in few specimens.				
Region nearer to pathways filled with colloid like NSM, large globules, many granules. Deeper branches with moderate amount of NSM and not scanty as noticed in sp. ilisha captured from river.				

Neurohypophysis	Water Analaysis-Mequi/Litre.			
	Na	K	Ca	Chloride
Irrespective of stages of maturity, this region was noticed fully laden with NSM in the form of dark deep purple large globules, granules and reddish purple granules. At several places this region was destroyed and lost leaving empty spaces behind.	ESTUARY			
	165.3	3.633	7.236	183.9
	177.9	3.581	7.734	186.9

Optic nerves appeared to distorted and their fibres appeared wavy and loose. In several sections it appeared as cut completely from the brain, may be due to destroyed fibres. In cerebellum, Purkinje cell layer was completely destroyed and lost; instead, large lacunae were noticed. In medulla oblongata zone, disintegration and destruction of nerve fibres were noticed. In short, throughout the brain, many lacunae and shrunk fibres were observed.

DISCUSSION

The increase in number, alongwith the enhanced secretory activities of the NLT cells in mature *H. toli* and in mature *H. ilisha* suggest a role for NLT in stimulation of gonads. As the secretory activities of the NLT increase, the basophils of the pituitary gland multiply and become granulated. After spawning, significant decrease in activities in NLT was noted in correlation with the degranulation of basophils of the pituitary gland of spent *H. ilisha* and spent *H. toli*. The inactive group of NLT of fingerling and immature *H. ilisha* and immature *H. toli* may be considered in relation with the presence of a few and comparatively very less granulated, basophils of the pituitary gland (Chapter IV). Several investigators have suggested a role of the NLT of fishes in the development of the gonads based on histological studies (Öztan, 1963, Stahl and Leray, 1962; Stahl, 1954; 1957; Stahl, Seite and Leray, 1960; Stahl and Leray, 1962; K.D. Juan, 1963; for review; Pickford and Atz, 1957; Bern and Nandi, 1964). Our findings based on the histological studies of the secretory cycle of NLT of H. ilisha and H. toli support the views of the above mentioned workers.

Stahl and Leray (1962) suggested that the globular protrusions of the ependymal cells, lying around the infundibular recess of Mugil capito, may act as the carrier of NSM from NLT to the cerebrospinal fluid, which may act in the absence of a portal system in fishes, as a vehicle for the transport of NSM to the pituitary gland for the stimulation of basophils. The globular protrusions noticed in mature, migrating H. ilisha and mature H. toli stained densely with CAHP method and with Shanklin's method (1962), which confirms the presence of NSM. Several axons of NLT cells were also noticed in very close contact with the walls of the capillaries. The abundance of the vascular beds in the region of NLT, during maturity, may be taken to indicate that NSM from NLT may also be discharged directly into the blood stream and may stimulate the basophils for the discharge of the gonadotropic hormones.

The significance of the presence of the NSM in the follicles of the proadenohypophysis of mature and spent *H. ilisha* and *H. toli* is discussed in Chapter IV.

It is known that teleost neurohypophyseal hormones contain 8-Arginine-Vasotocin and Isotocin or Ichthyotocin or teleost oxytocic principle (Heller, 1963; Sawyer, 1963; Acher *et al.*, 1962). However, it is still a matter of dispute whether these hormones contain a vasopressor-antidiuretic-water-balance principle (Sawyer, 1963). The exact function of 8-Arginine-Vasotocin is not yet clearly understood (For review, Sawyer, 1963). The presence of these two hormones in *H. ilisha* and in *H. toli* may be taken for granted on the basis of A.F. staining and Paget's A.F.-Thionin-PAS staining for NSM.

Pickford and Sawyer (1964) have recorded a depletion in Isotocin content of the pituitary gland of *Fundulus heteroclitus* during spawning and they concluded that Isotocin may be concerned with maturity and not 8-Arginine-Vasotocin as latter did not show any remarkable difference during reproductive period of F. heteroclitus. The depletion of NSM from the neurohypophysis of F. heteroclitus during reproductive season was reported by Sokol (1961). The depletion of NSM from the neurohypophysis of H. ilisha and H. toli may suggest depletion of Isotocin content of the pituitary during reproductive season.

Lederis (1964), on the basis of his studies on the neurohypophysis and neurohypophyseal hormones content of the trout Salmo irideus noted depletion of only 8-Arginine-Vasotocin content of the neurohypophysis by 50%, when Salmo irideus was exposed to sea water for 8 hours. He also noted depletion of osmiophilic material from the elementary granules of some nerve swellings on basis of electron microscope studies, when trout was exposed to sea water for 2 hours. On basis of his studies, he suggested that the two hormones, 8-Arginine-Vasotocin and Ichthyotocin or Isotocin are released independently from each other. Former may be concerned with electrolyte metabolism and latter may be concerned for the sexual maturity. The depletion of NSM from the NPO and utilization of NSM from the neurohypophysis of mature H. ilisha may speak for the release of both the hormones, 8-Arginine-Vasotocin and Ichthyotocin or Isotocin, as both were utilized during maturity and migration. Former may be for adjustment in hypertonic medium-sea, and later may be for next reproductive cycle. The depletion of the NSM from the neurohypophysis of the spent *H. toli* suggest that the hormone Isotocin might have been consumed during the spawning period and NPO, hence might have resumed the supply of new quota of hormone by discharging NSM in the hypothalamo-neurohypophyseal tract. As there is no change of salinity only Isotocin might have been utilized during spawning in *H. toli*.

The partial destruction of NPO of drifted *H. toli* of all stages of maturity (immature, mature and spent) may be due to (i) "stress" caused by sudden change in the medium and (ii) Inability of NPO to supply the suddenly increased demand for the neurohypophyseal hormones, arising due to change in the medium. The remaining NPO showed enlarged nuclei and 'E' phase of secretion cycle, which support the above mentioned view. In addition to this, the neurohypophysis of drifted *H. toli*, of all the stages of the maturity were partially lost or destroyed and the remaining portions were densely filled with NSM. This picture is contrary to that one noticed in *H. toli* captured from the sea.

The CAHP positive, A.F. -ve secretory product(NSM?) of the probable third group of NS cells (?) was noticed as if it is discharged in the cavity of 4th ventricle and in the blood stream. It is evident from our studies based on histological observations of the caudal neurosecretory system (CNS) of *H. ilisha* and *H. toli* that the CNS of fingerling of *H. ilisha* is feebly developed (unpublished work from our laboratory). The discharge of the product of the 3rd group of NS cells (?) may be carried to the end portion of the spinal cord by the cerebrospinal fluid. One may presume that the NSM from these cells may play a role in osmoregulation in the absence of a well developed Caudal Neurosecretory System in the fingerling. In the mature *H. ilisha* and *H. toli* no NS cells(?) of such third group were noticed. Scharrer (1965) has already pointed out the possibility of cerebrospinal fluid as carrier of the NSM, in certain mechanisms of the neuroendocrine mechanisms.

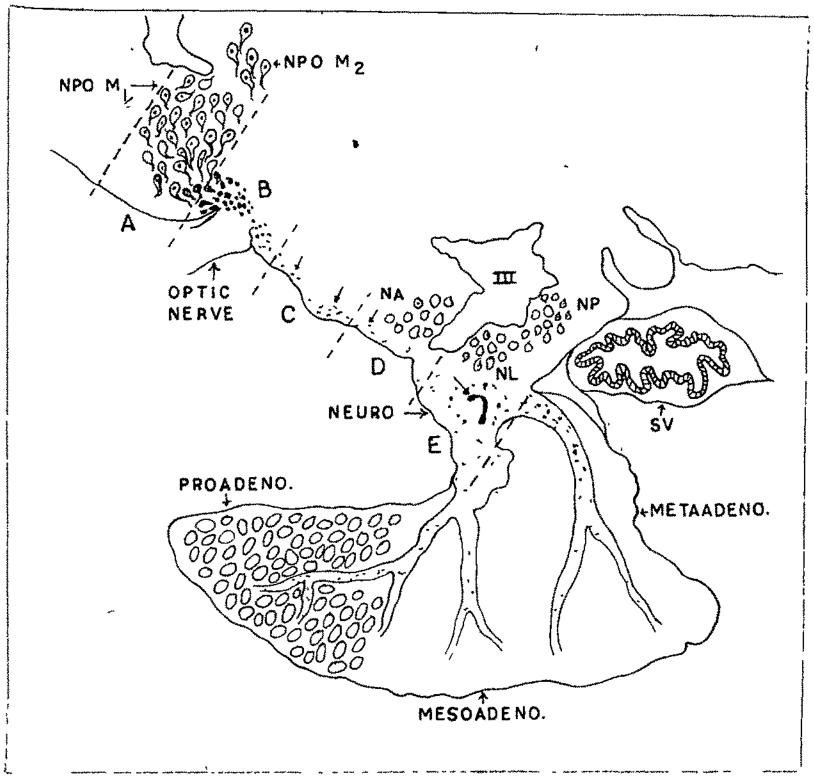


Fig. 1

Camera lucida drawing of the Hypothalamo-Neurohypophyseal Neurosecretory System of <u>H. ilisha</u>.

NPO M_1 - Nucleus Preopticus. Microcellularis, NPO M_2 - Nucleus Preopticus Magnocellularis, NA - Nucleus Lateralis Tuberis Anterior, NP - Nucleus Lateralis Tuberis Posterior, NL -Nucleus Lateralis Tuberis Lateral, Proadeno - Proadenohypophysis, Mesoadeno. - Mesoadenohypophysis, Metaadeno. - Metaadenohypophysis, Neuro - Neurohypophysis or Neural Lobe, III - Cavity of third ventricle, SV - Saccus Vasculosus.

Letters, A, B, C, D, E represent different region of the Hypothalamo-Neurohypophyseal Neurosecretory System by thin lines.

Arrow indicate neurosecretory granules in pathways and in neurohypophysis.

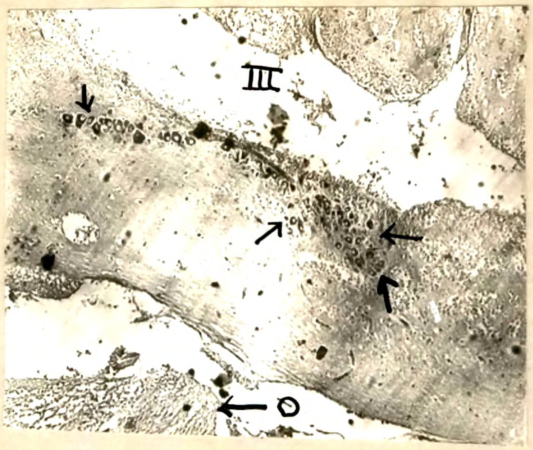


Fig. 2

The NPO of the fingerling of H. ilisha (indicated by arrow). Region 'A' and 'B' of Fig. 1.
Chrome-Alum-Haematoxyline-Phloxine, x63.
III - Cavity of the 3rd ventricle.
O - Optic Nerve.

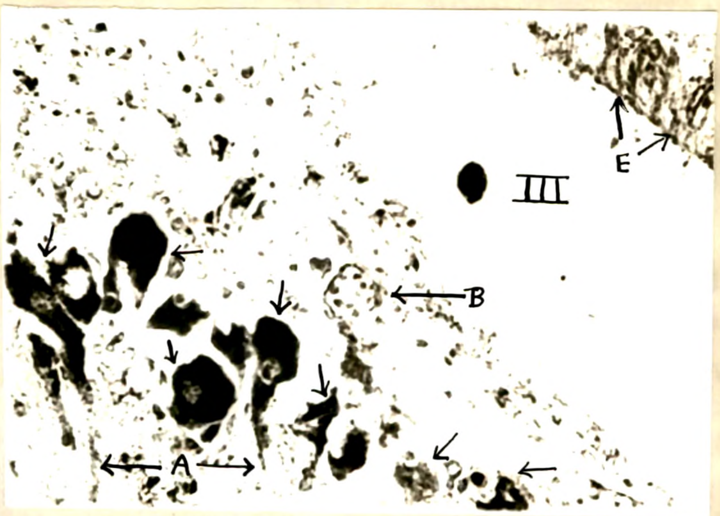


Fig. 3

The NPO of the fingerling of H. ilisha (indicated by arrow).
Chrome-Alum-Haematoxyline-Phloxine, x400.
III - Cavity of 3rd ventricle, B - Blood vessel
E - Ependymal cells, A - Axons of the NPO.

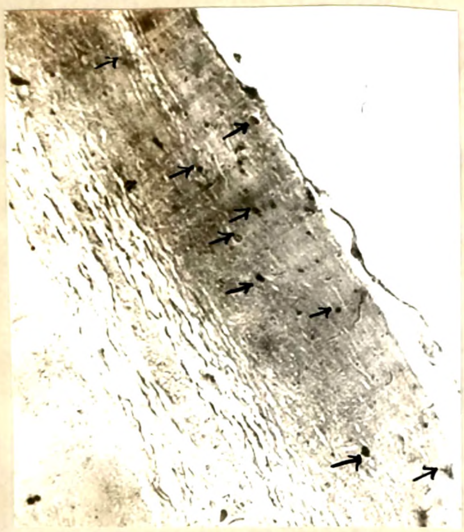


Fig. 4

Fig.5

Region 'A' of the pathways. Region 'B' of the pathways.
(Both) Chrome-Alum-Haematoxyline-Phloxine, x63.
Arrow indicates neurosecretory granules.

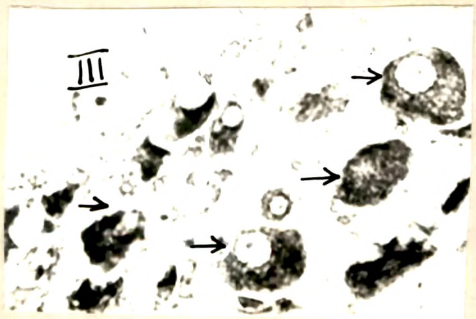


Fig. 6

NPO-Pars microcellularis of the fingerling of H. ilisha, indicated by arrows. Chrome-Alum-Haematoxyline-Phloxine, x400.

III - Cavity of the third ventricle.

(Note densely filled NPO)

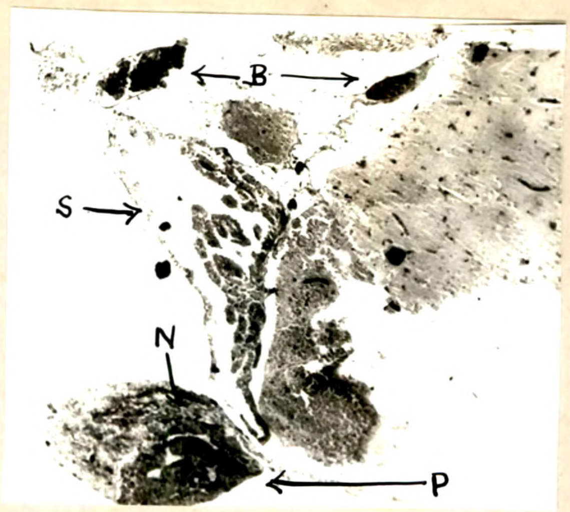


Fig. 7

Region 'D' & 'E' of the neurosecretory system of the fingerling of H. ilisha.

Aldehyde Fuchsin, x63.

P - Pituitary gland, B - Blood Vessel.
S,- Saccus Vasculosus, N - Neurohypophysis.

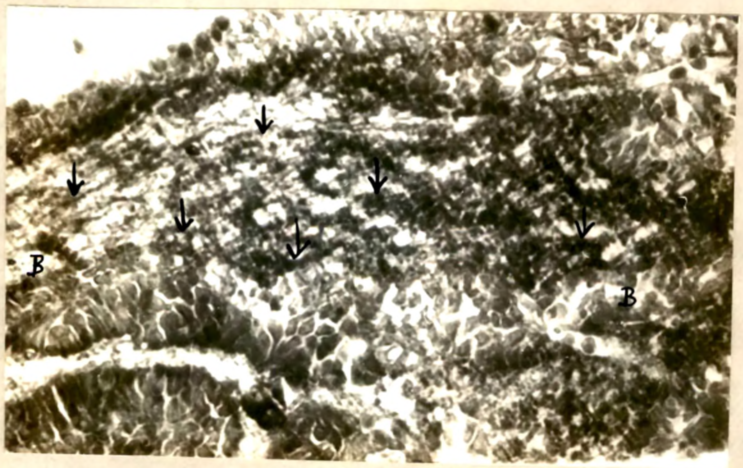


Fig. 8

A Region of the Neurohypophysis of the fingerling of H.ilisha, densely filled with Neurosecretory material.

Aldehyde Fuchsin, x1000.

NSM indicated by arrow.
B - Blood vessel
A - Acidophils.

Fig. 9 Fig.10

The Nucleus Lateralis Tuberis, anterior(A), B - Posterior, and L - Lateral of the fingerling.
Chrome-Alum-Haematoxyline-Phloxine
(Fig. 9 magni. 400) (Fig.10 Nucleus Lateralis Tuberis posterior magnified by 1000)
B - Blood vessel

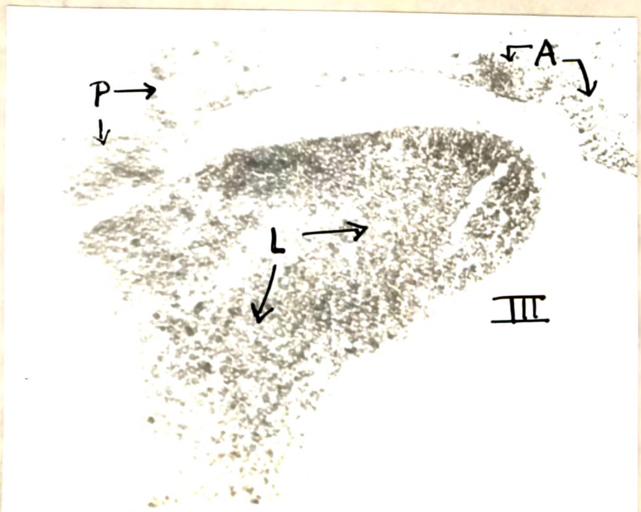


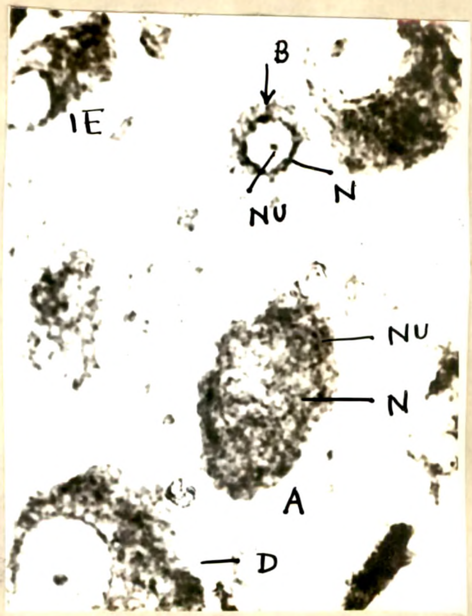
Fig.11

Nucleus Lateralis Tuberis, anterior(A), Posterior(B) and lateral(C). Chrome-Alum-Haematoxyline-Phloxine, x400.
III - Cavity of the third ventricle.

Fig. 12

The (supposed) third group of the neurosecretory cells (indicated by arrow) in vicinity of the cavity of the 4th ventricle(IV).

Chrome-Alum-Haematoxyline-Phloxine, x40



Fig, 13

Fig.14

Both the figures showing the different phase(a,b,c,d,e) of the secretion cycle in the third group of the neurosecretory cells of the fingerling. Letters, a,b etc. represent different phases of secretion cycle.

Chrome-Alum-Haematoxyline-Phloxine, x 1000.

N - Nucleus, NU - Nucleolus, G - Granules.

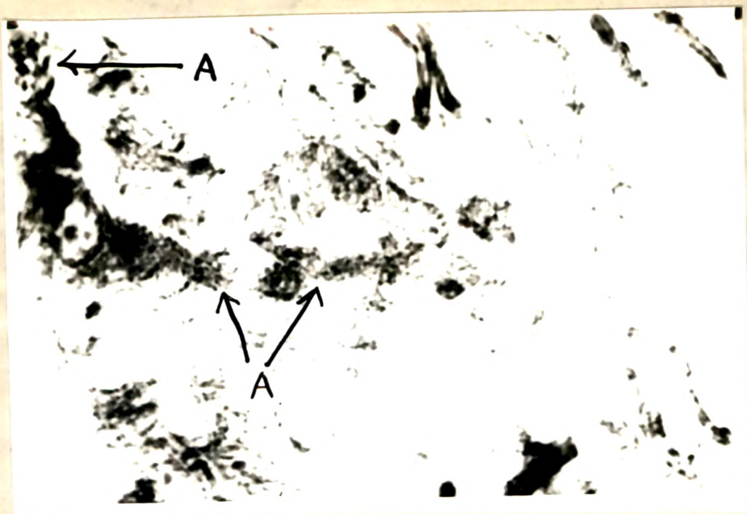


Fig. 15

The bipolar Neurosecretory cell of the third group of the fingerling of H. ilisha, showing axons(A) fully loaded with the CAHP +ve granules.
Chrome-Alum-Haematoxyline-Phloxine, x1000.

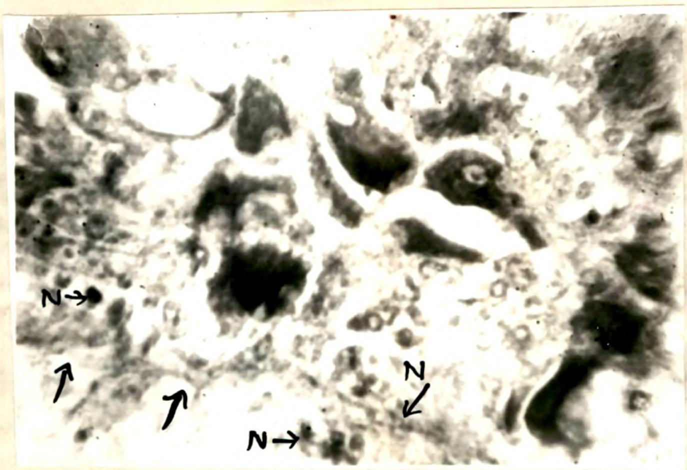


Fig. 16

The discharge of the neurosecretory material(N) by the neurosecretory cell in the cavity of the 4th ventricle (indicated by arrow).
Chrome-Alum-Haemotoxyline-Phloxine, x1000.

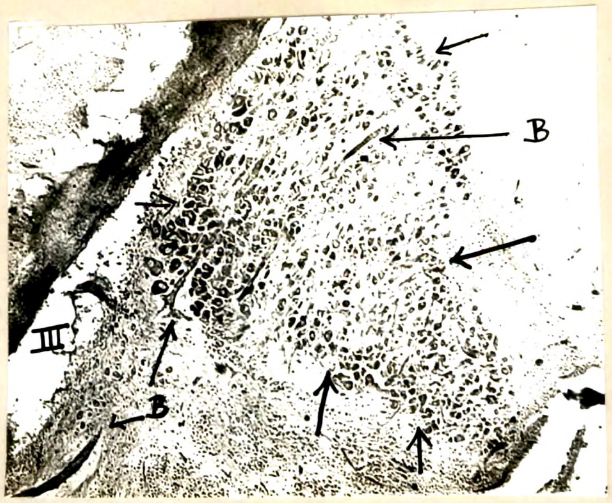


Fig. 17

The Nucleus Preopticus (indicated by arrow) of the immature H. <u>ilisha</u> captured from the sea.
Aldehyde Fuchsin, x 63.
III - Cavity of the third ventricle, B - Blood vessel.

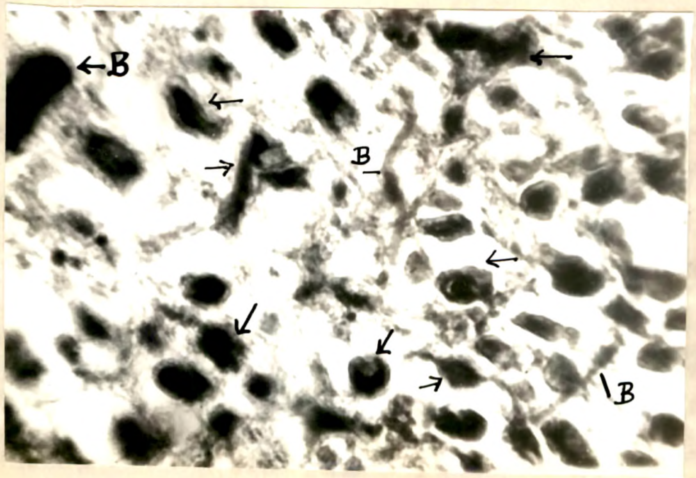


Fig. 18

The Nucleus Preopticus of Fig. 17 magnified.
Aldehyde Fuchsin, x400.
All NS cells (indicated by arrow) with blood vessels(B).

Fig. 19

The Nucleus Preopticus and region 'B' of the Neurosecretory system of immature H. ilisha.
Aldehyde Fuchsin, x63.

Fig. 20

Region 'C' of the neurosecretory system of immature H. ilisha.
Aldehyde Fuchsin, x63.

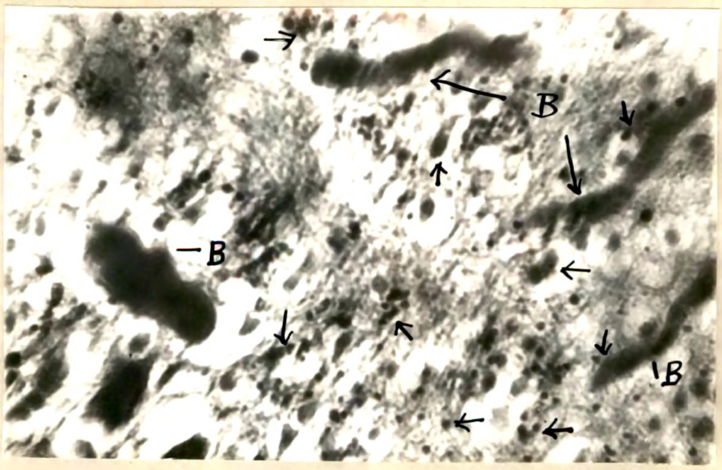


Fig. 21

Region 'C' of Fig. 20 magnified. Aldehyde Fuchsin, x400.

Arrow indicate neurosecretory granules, B - Blood vessel, A - Axon, III - Cavity of 3rd ventricle.
(Legend same for Figs. 19, 20 and 21).

Fig. 22

Region 'D' of the neurosecretory system of immature <u>H</u>. <u>ilisha</u>.

Aldehyde Fuchsin, x63.

Arrow indicate neurosecretory granules,
B - Blood vessel.

Fig. 23

Region 'E' of the neurosecretory system of immature <u>H</u>. <u>ilisha</u>.

Aldehyde Fuchsin, x63.

A - Neurohypophysis, I - Infundibulum,
C - Colloid like neurosecretory material
Arrow indicate granular neurosecretory material.

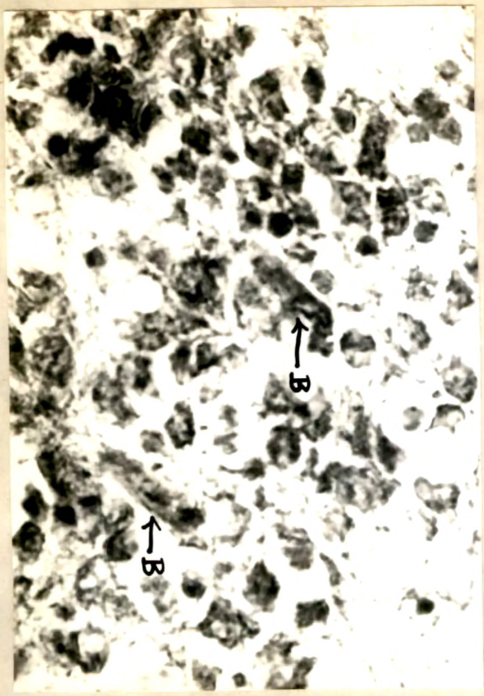


Fig. 24

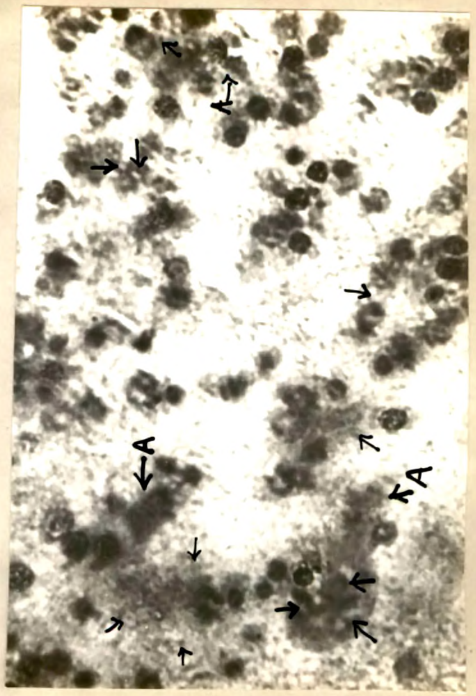


Fig. 25

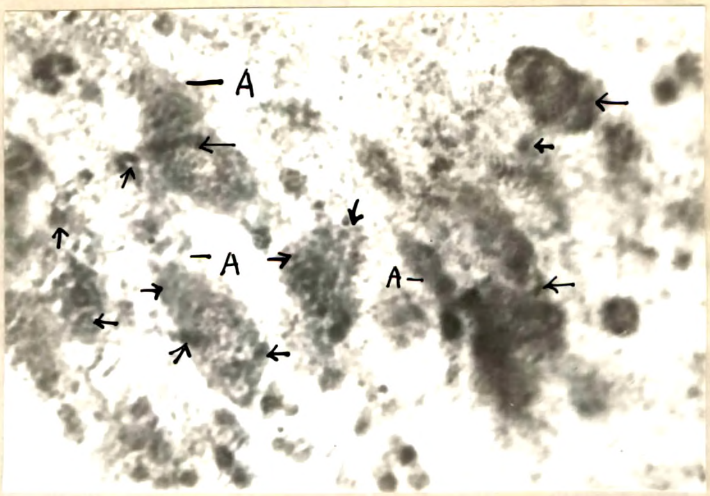


Fig. 26

Fig.24: The Nucleus Lateralis Tuberis of the immature, <u>H</u>. <u>ilisha</u> captured from the sea. x400.

Fig.25 & 26: The same region magnified. x1000.

(All stained by chrome-Alum-Haematoxyline-Phloxine)
B - Blood Vessels, A - Axons, arrow indicate neurosecretory granules.

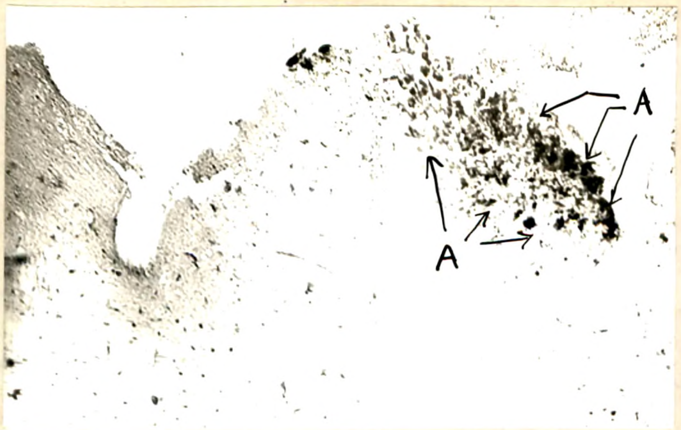
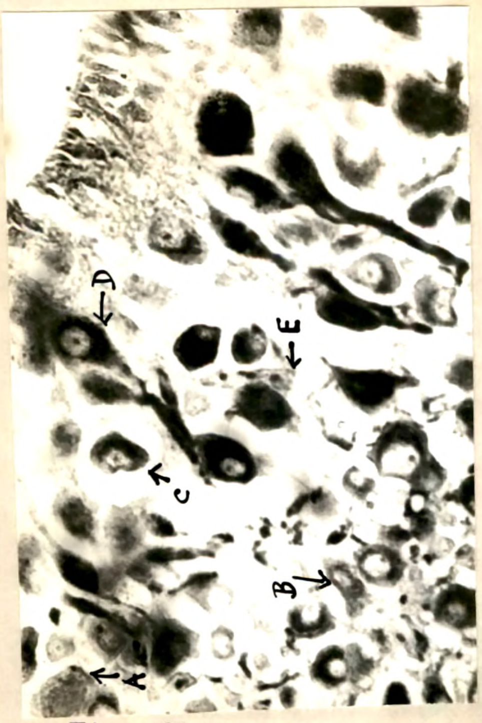


Fig. 27



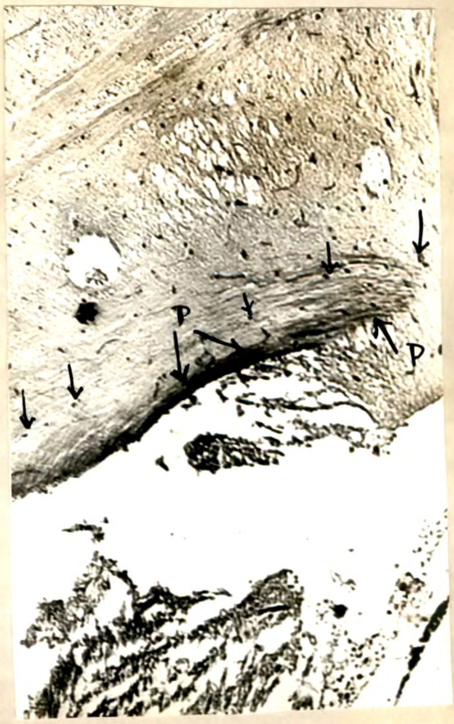


Fig. 28

Fig. 29

Fig. 27: NPO of mature H. ilisha (Sea).
Aldehyde Fuchsin, x63.

Fig. 28: NPO of Fig.24 magnified. Aldehyde Fuchsin, x400.
(Letters denote stages of secretion cycle).

Fig. 29: Region 'A' of the neurosecretory system of mature *H. ilisha* (Sea.). Aldehyde Fuchsin, x63.
A - Cells of NPO, B - Blood vessels, P - Pathways.
Arrow indicate neurosecretory granules.

Fig. 30

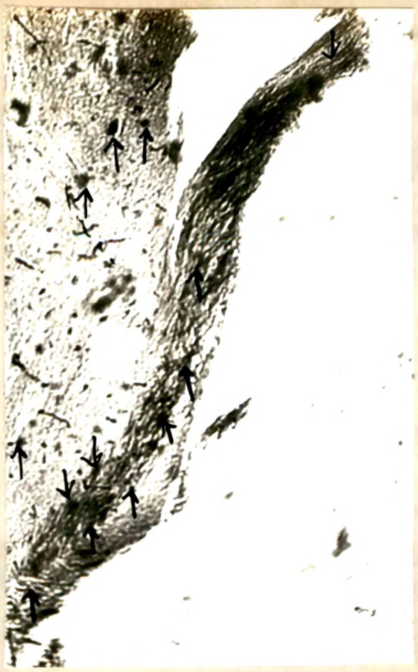


Fig. 31

Fig.32

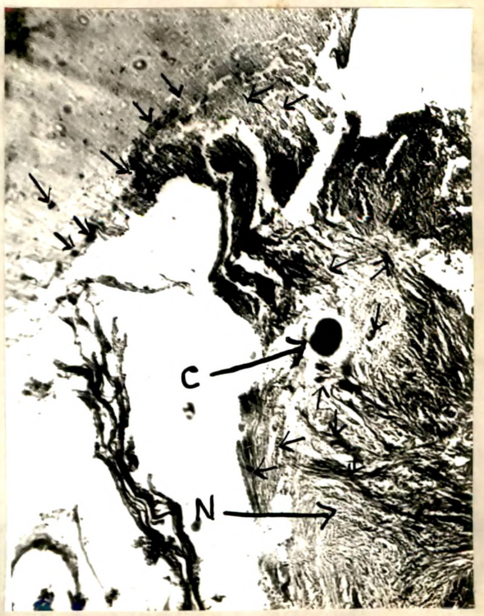


Fig. 33

Figs. 30, 31 show the region C.D. and Figs. 32,33 show region E of the neurosecretory system of mature H. ilisha (Sea).
(For all Figs., Aldehyde Fuchsin, x 63).
N - Neurohypophysis, C - Colloid-like neurosecretory material, arrow indicate neurosecretory material in pathways.

Fig. 34

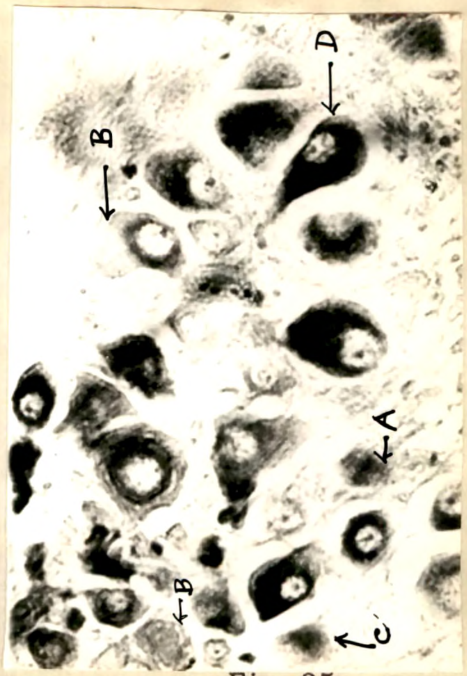


Fig.35

Fig. 36

Fig.37

Fig. 34 : The NPO of the mature, migrating <u>H.ilisha</u> (river). x63.
Fig. 35 : Region of Fig.34 magnified. x400.
Fig. 36 : Region 'B' of the neurosecretory system of <u>H. ilisha</u> (river). x63.
Fig. 37 : Region 'C' of the neurosecretory system of <u>H. ilisha</u> (river). x63.
All stained by Aldehyde Fuchsin. Arrow indicate the neurosecretory granules, O - optic Nerve, B - Blood vessel, Letters indicate stages of the secretion cycle.

Fig. 38

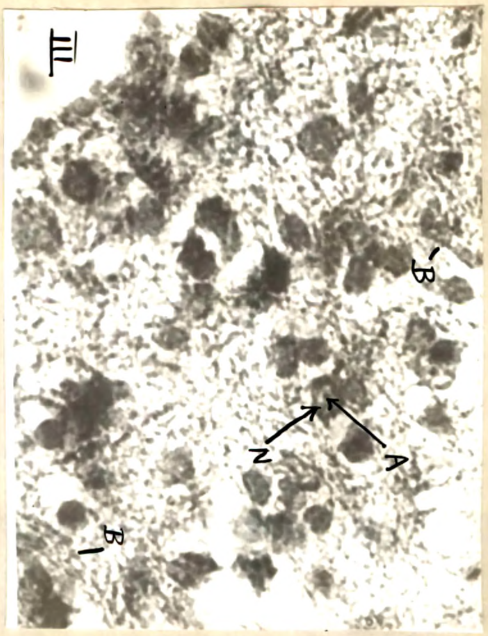
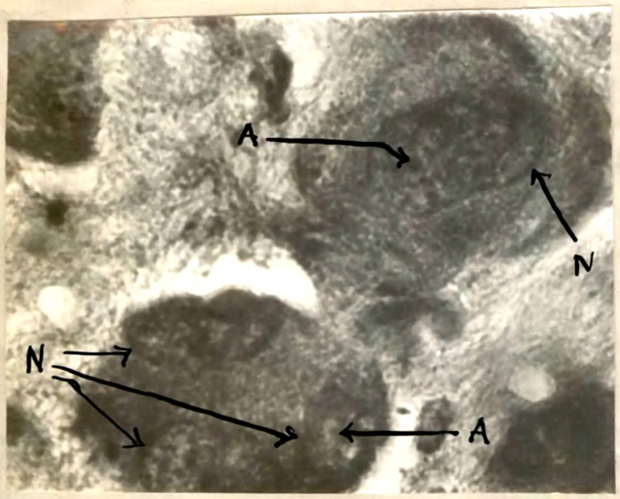


Fig.39



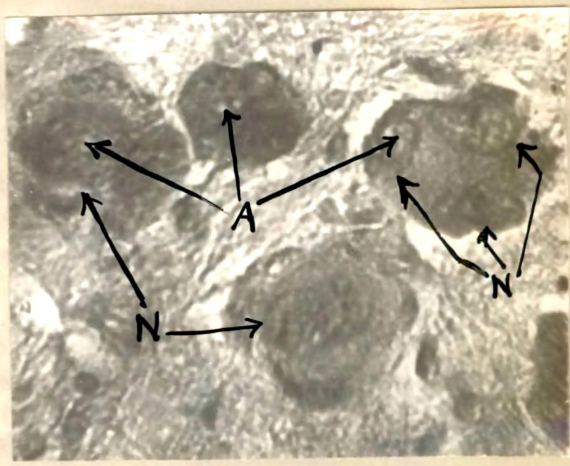


Fig.40

Fig.41

Figs.38 & 39: The Nucleus Lateralis Tuberis of mature, migrating <u>H. ilisha</u>. x 63, & x400 resptly.

Figs.40 & 41: The region of Figs.38 & 39 magnified respectively. x1000

All stained by Chrome-Alum-Haematoxyline-Phloxine.
N - Nucleus, A -Nucleolus, B - Blood Vessel,
III - Cavity of 3rd ventricle.

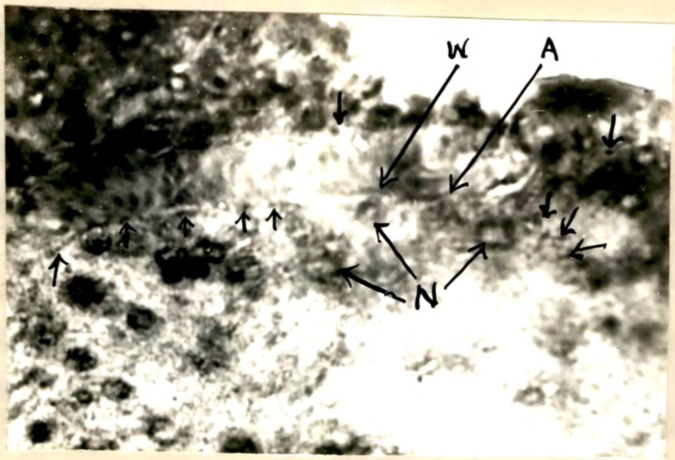


Fig. 42

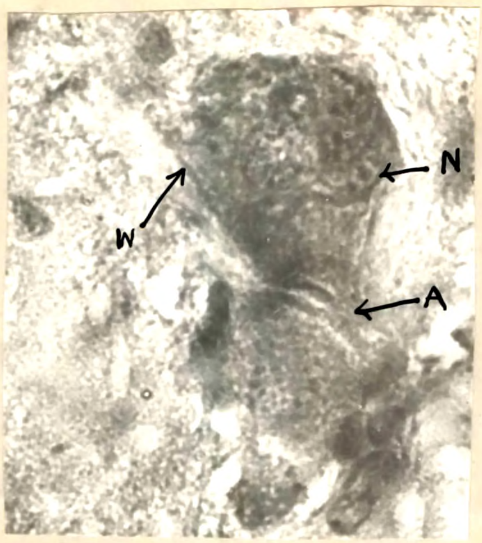


Fig. 43

Fig.42 : The Nucleus Lateralis Tuberis of mature H.ilisha(river), terminating on the wall of the capillary.
x1000

Fig.43 : Same region magnified. x1000

(Both stained by Chrome-Alum-Haematoxyline-Phloxine).

W - Wall of capillaries, N - Nucleus of neurosecretory cells of NLT
A - Axon.
Arrow indicate neurosecretory granules.

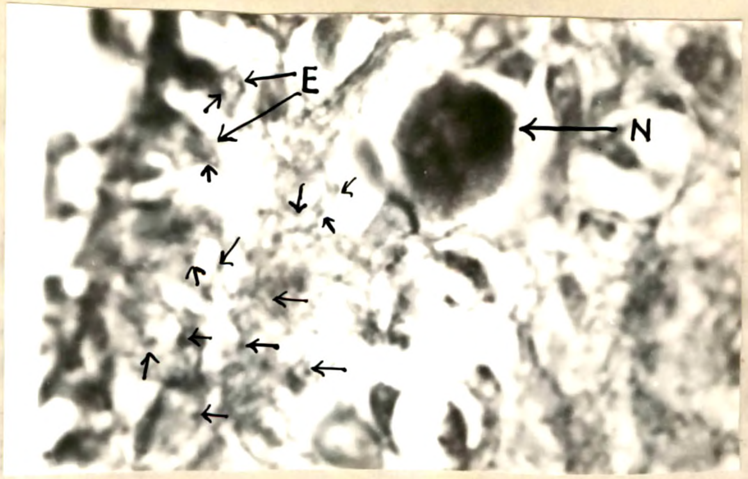


Fig. 44

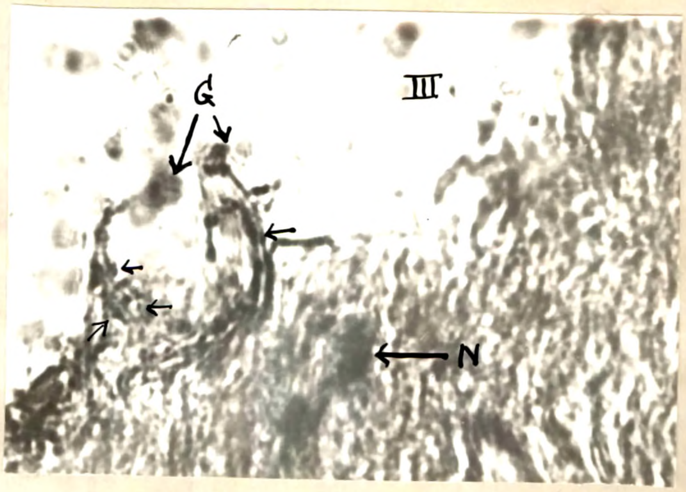


Fig. 45

Fig.44 : The discharge of the neurosecretory material in the cavity of 3rd ventricle through the ependymal cells.

Fig.45 : The discharge of neurosecretory material in the cavity of the third ventricle by globular protrusions.(G)

Both stained by Chrome-Alum-Haematoxyline-Phloxine, x1000. N - Neurosecretory cell, arrow indicate the neurosecretory material, E- Ependymal cells

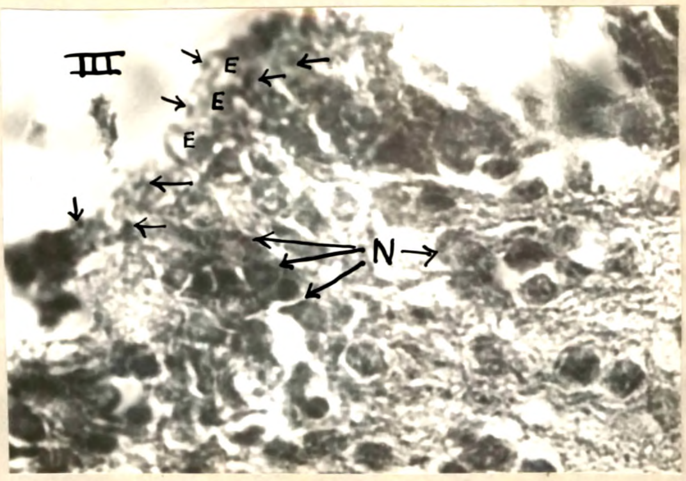


Fig. 46

The discharge of neurosecretory material (indicated by the arrow) in the cavity of 3rd ventricle (III) through the ependymal cells(E) by Nucleus Lateralis Tuberis(N).
Chrome-Alum-Haematoxyline-Eosin,x1000.

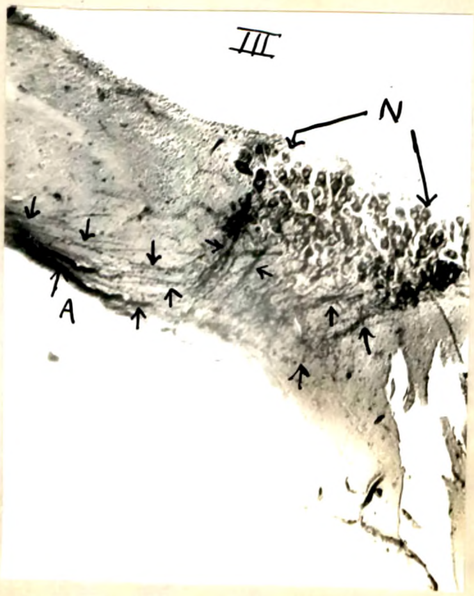


Fig. 47

The heavy discharge of neurosecretory material (as shown by arrow) by Nucleus Preopticus(N) Region 'A' of the neurosecretory system of spent H. ilisha. III - Cavity of the 3rd ventricle, A - Pathways full of NS granules.
Aldehyde Fuchsin, x63.

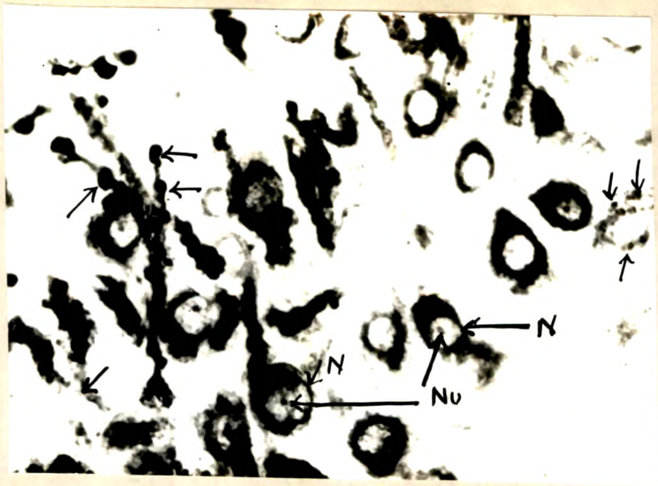


Fig. 48

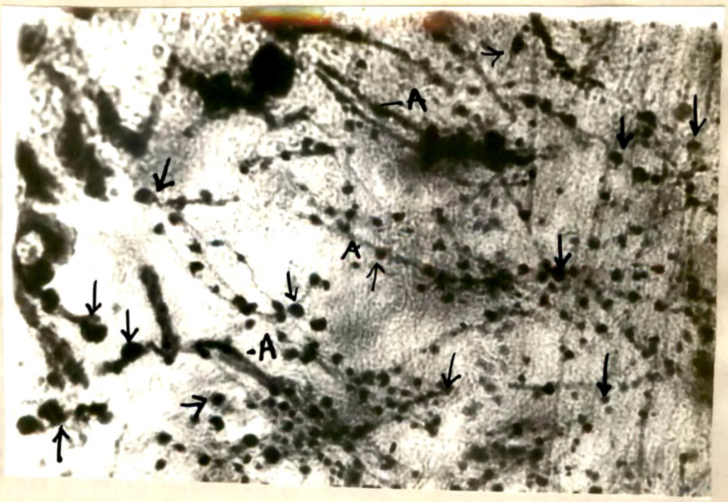


Fig. 49

Fig. 48: The Nucleus Preopticus of Fig. 47 magnified.
Aldehyde Fuchsin, x400.

Fig. 49 :The axons(A) of the Nucleus Preopticus of Fig.47 magnified.
Aldehyde Fuchsin, x400.
N - Nucleus, Nu - Nucleolus.
Arrow indicate neurosecretory granules.

Fig. 50

Fig.51

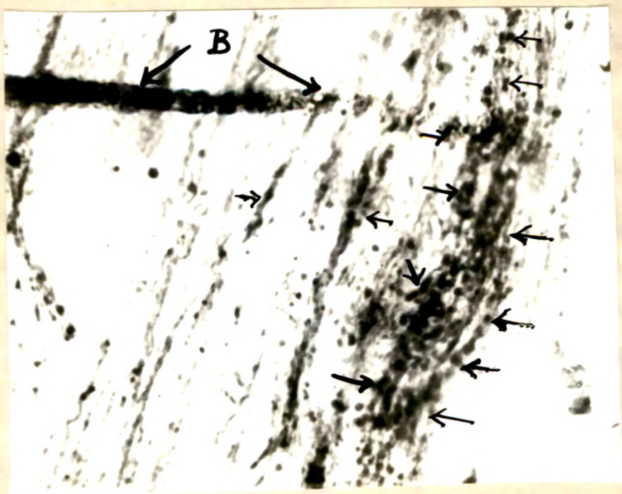


Fig. 52

Figs. 50 & 51: Part of region and region'B' of neurosecretory system of spent H. ilisha.

Aldehyde Fuchsin, x63.

Fig. 52 : Part of Fig.51 magnified.

x 400.

III - Cavity of 3rd ventricle, C - Droplet of neurosecretory material, B - Blood vessel, Arrow indicate neurosecretory granules in the pathways.

Fig. 53

Fig.54

Figs.53 & 54: Region 'E' of the neurosecretory system of spent <u>H</u>. <u>ilisha.</u> Aldehyde Fuchsin, x63.

N - Neurohypophysis, C - Colloid like Neurosecretory material.
Arrow indicate neurosecretory material in pathways.

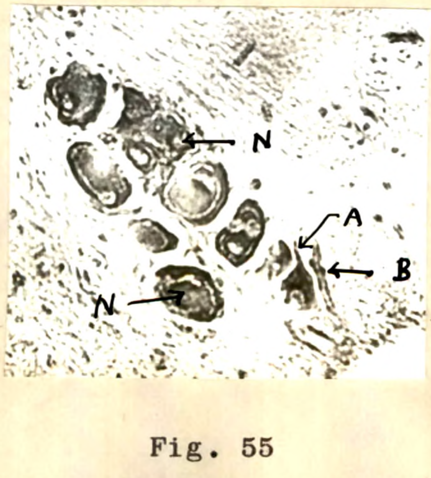


Fig. 55

The Nucleus Lateralis Tuberis of spent <u>H</u>. <u>ilisha</u>, showing exhaust phase of secretion cycle.
Chrome-Alum-Haematoxyline-Phloxine, x63.
N - Nucleus, A - Axon, B - Blood vessel.
(Compare with Figs. 38)

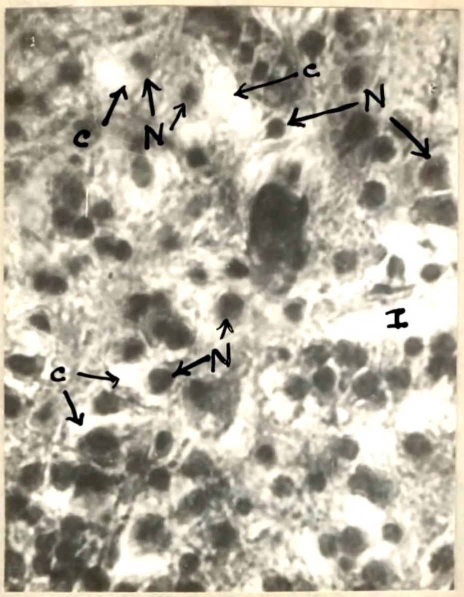


Fig. 56

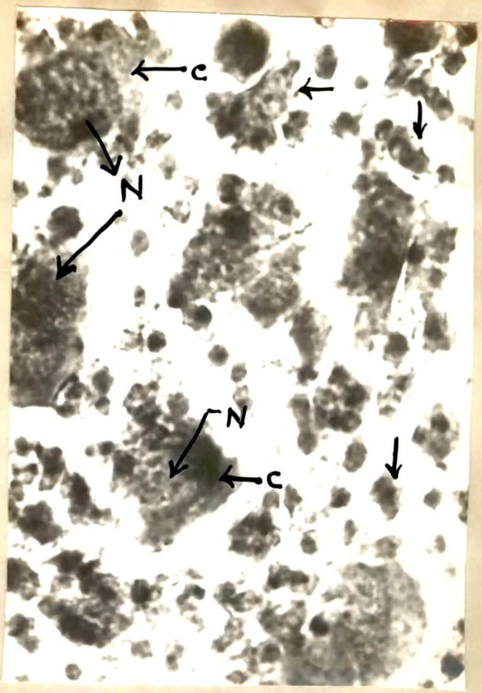


Fig.57

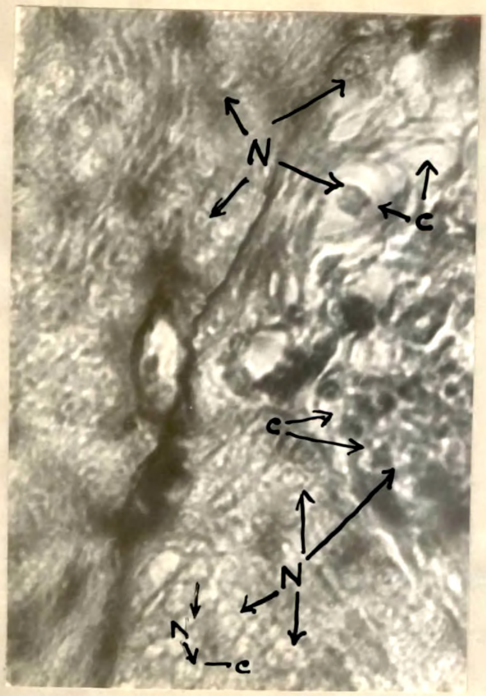


Fig. 58

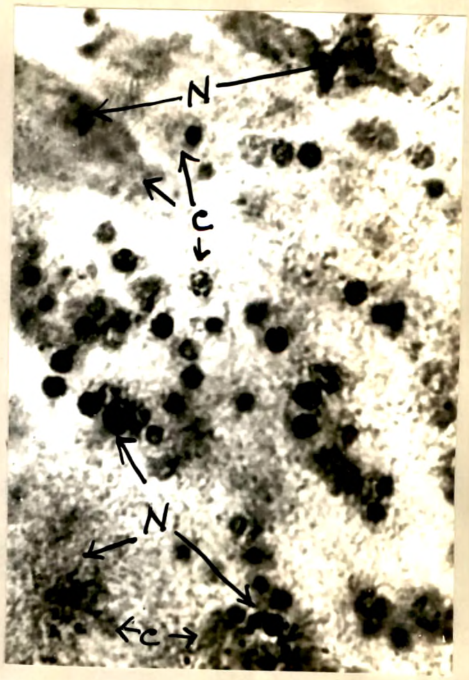


Fig. 59

Figs.56,57,58 & 59: The Nucleus Lateralis Tuberis of spent <u>H</u>. <u>ilisha</u> exhibit 'Exhaust phase'.

N - Nucleus, C - Cytoplasm , I - Inter cellular space.
Arrow indicate shrunk cells.
(Compare with Figs. 25,26, 38, 40 and 39).
Chrome-Alum-Haematoxyline-Phloxine,x1000.

Fig.60

Fig.61

Fig. 62

Figs.60 & 61: The Nucleus Preopticus of immature H. toli region 'A' (in Fig.60), and region 'B' and part of region 'C' in Fig.61.
Aldehyde Fuchsin, x63.

Fig. 62 : The Nucleus Preopticus of mature H. toli, showing region 'A' of the neurosecretory system.
Aldehyde Fuchsin, x63.
Arrow indicate neurosecretory granules in pathways.
A - Nucleus Preopticus, C - Droplet of neurosecretory material.

Fig.63

Fig.64

Fig. 65

Figs. 63,64 & 65 represent region 'C', 'D' and 'E' of the neurosecretory system of mature H. toli, respectively.
Aldehyde Fuchsin, x 63.

N - Neurohypophysis, A - Nucleus Lateralis Tuberis cells, B - Blood vessel. Arrow indicate neurosecretory granules in the pathways.

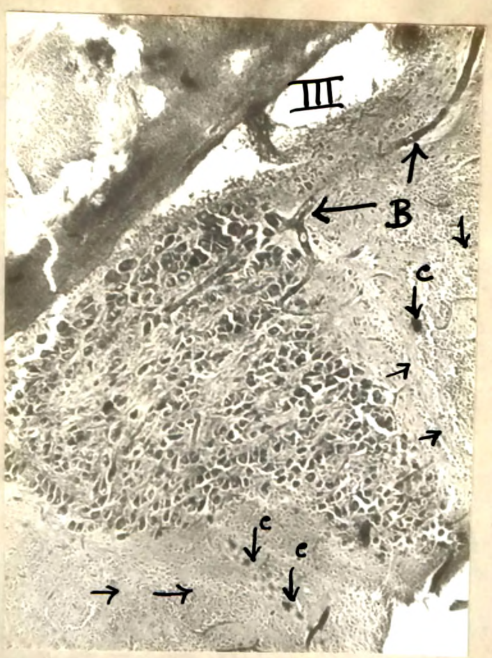


Fig.66

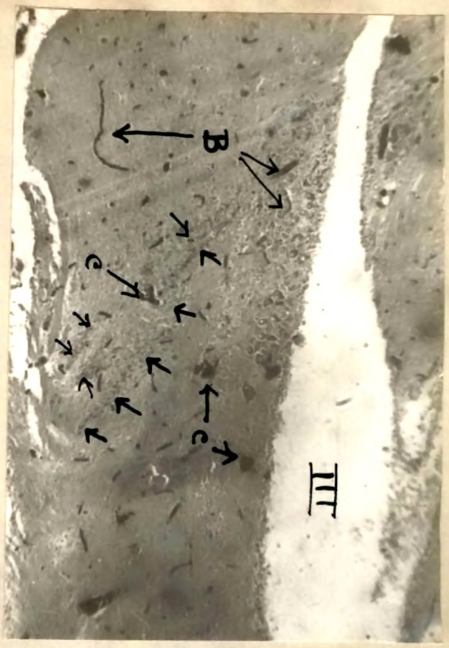


Fig.67

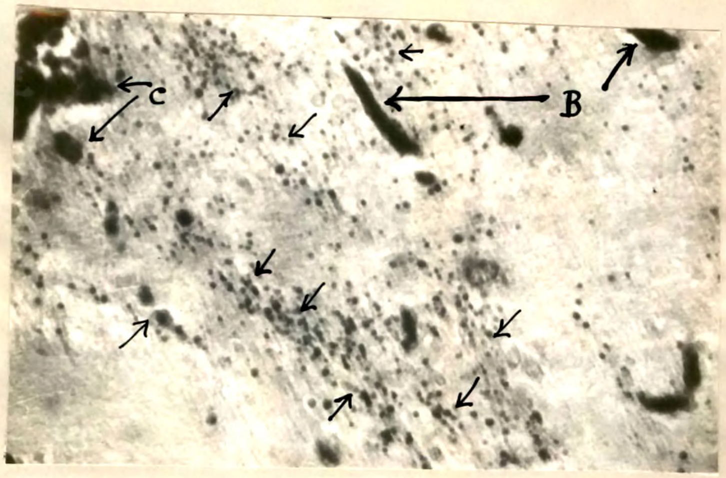


Fig. 68

Figs.66 & 67 showing the Nucleus Preopticus of spent H.toli.
Aldehyde Fuchsin, x63.

Fig. 68 : Region of Fig. 67 magnified.
Aldehyde Fuchsin, x400.

B - Blood vessel, III - Cavity of 3rd ventricle,
C - Globules and droplets of neurosecretory material.
Arrow indicate neurosecretory granules in the pathways.

Fig.69

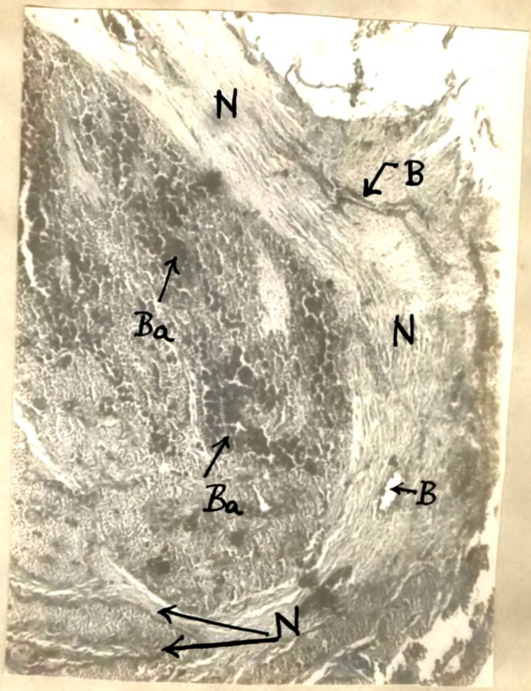


Fig.70

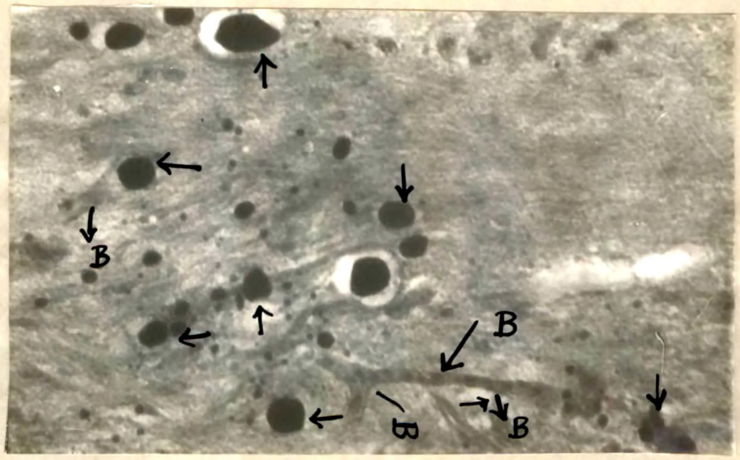


Fig. 71

Figs. 69 & 71 represent region 'D' and 'E' of the neurosecretory system of spent H. toli.

Aldehyde Fuchsin, x63

Fig. 70 shows magnified view of region shown in Fig. 69.

Aldehyde Fuchsin, x400

B - Blood Vessel, N - Neurohypophysis, Ba- Basophils, Arrow indicate neurosecretory granules.

Fig. 72

Fig. 73

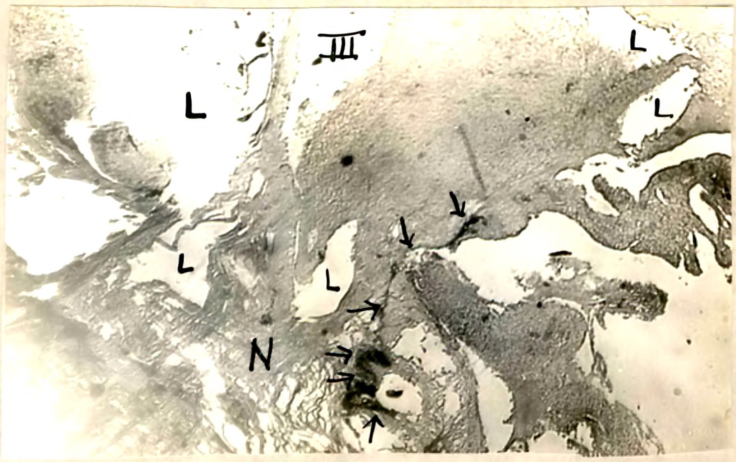


Fig. 74

Fig.72 shows partially destroyed Nucleus Preopticus(N) and partially destroyed pathways(P), and a part of region 'A' of the neurosecretory system of drifted H.toli.
Aldehyde Fuchsin, x63

Fig.73 shows region 'B' and 'C' of the neurosecretory system of same fish.
Aledhyde Fuchsin, x63

Fig.74 shows region 'D' and 'E' of the neurosecretory system of the same fish.
Aldehyde Fuchsin, x63

Arrow indicates neurosecretory granules in pathways.
N - Neurohypophysis, III - Cavity of 3rd ventricle, L -Lacunae.

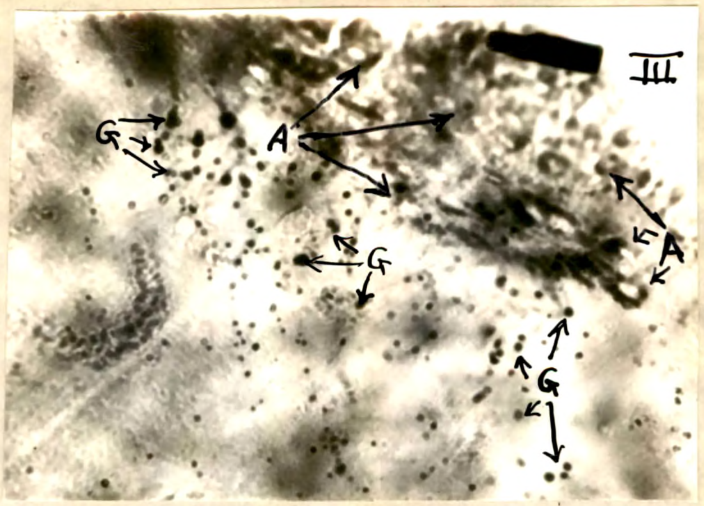


Fig. 75

The Nucleus Preopticus (A) of drifted H. toli.
Aldehyde Fuchsin, x400
III - Cavity of 3rd ventricle, G - Neurosecretory granules,
B - Blood vessel.
(Note hypertrophied NS cells)

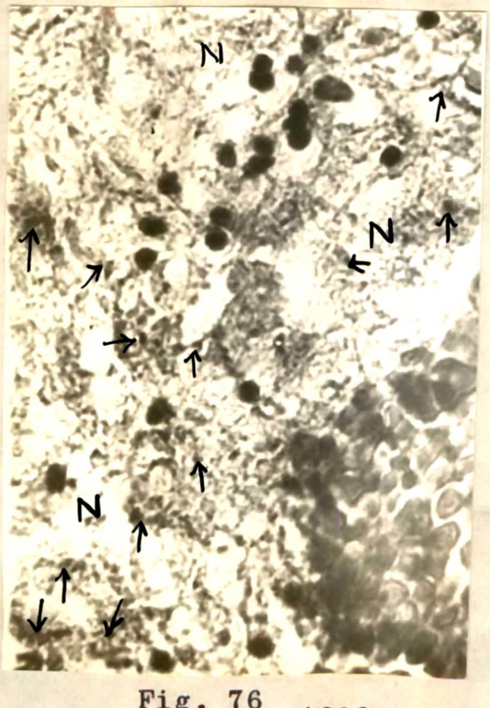


Fig. 76 x1000

Fig. 77 x400

Figs. 76 and 77 represent a region of the neurohypophysis of Fingerling H. ilisha and mature, migrating H. ilisha.
Both stained by Aldehyde Fuchsin.
Arrow indicate neurosecretory granules, L - Lacunae,
N - Nervous tissue.