CHAPTER VIII

HISTOLOGICAL STUDIES ON THE CAUDAL NEUROSECRETORY SYSTEM OF MIGRATORY HILSA ILISHA(HAM.) AND MARINE HILSA TOLI(CUV. AND VAL.)

No concrete data has yet appeared reporting the role of Caudal Neurosecretory system (CNS) of fishes in osmoregulation; but, substantial work have favoured the positive side; (Bern et al.,1965; Enami, 1956,1959; Enami et al.,1956; Takasugi and Bern, 1962; Maetz et al.,1964; Fridberg et al., 1966), few data have gathered on the other side too (Imai et al., 1965). Similar to the neurosecretory centres of the brain, the depletion of neurosecretory material (NSM) was noticed in the urophysis by several workers (Enami, 1956; Imai, 1959; Takasugi and Bern, 1962), whereas, Sano (1961) could not demonstrate, the same. The histological and cytological work on the CNS of few teleosts by Bern and Takasugi (1962) and Roy (1962) yielded very informative results. Recently, carried out histological, cytological and ultrastructural studies on the CNS of teleost Albula Vulpes, by Fridberg, Bern and Nishioka, has provided interesting results. They suggested on basis of their studies, that there exist two types of Neurosecretory cells, which could be differentiated on basis of staining reactions and on presence of elementary granules in them. Experimental work carried out by Yagi and Bern (1965) also indicated the presence of

neurosecretory units which were different from each other, one group is sensitive to Na^+ concentration, whereas other group responds Na^+ free solution. The above mentioned work has suggested an osmoregulatory role for the CNS of fishes. The ultrastructural studies of the CNS of fishes have opened to a new chapter in the field of cytology and several workers suggested the origin of NS granules to be Golgi bodies(Sano and Knoop, 1959; Bern and Takasugi, 1962; Fridberg, Bernand Nishioka, 1966; Bern, Nishioka and Hagadorn, 1962). The organization of NS granules of CNS is similar to that of NS granules of hypothalamic area (Bern, Nishioka and Hogadorn, 1962).

The studies carried out on CNS of Albula vulpes by Fridberg et al., 1966, reported a probable correlation between light microscope observations and electronmicroscope studies, suggesting loss of electrodense granules from the urophysis is similar to the deplation of neurosecretory material (NSM) observed in CNS of Albula obtained from pond.

The experimental work on the CNS of several Indian fishes, carried out by Roy (1962) suggested a corticotropin-releasing factor (CRF) - like activity in CNS of fishes. He also supported the work of earlier workers in favour of an osmoregulatory role for CNS of fishes.

Electrophysiological studies on the CNS of Tilapia mossabica reported presence of two kinds of neurosecretory units, one activated by high Na^+ concentrations and another activated by Na^+ free solutions (Yagi and Bern, 1965).

MATERIALS AND METHODS

Hilsa ilisha and H. toli were captured from the area marked with "X" cross and marked with dotted (in map I and map 2 of Chapter I). The tail region was cut from the body keeping 6" length from the beginning of the caudal fin, muscles were rapidly removed and spinal cord alongwith bulb was exposed to the fixative - Bouin's fluid. After bringing to the laboratory at shore, entire spinal cord alongwith bulb was completely removed from the vertebrae and were imersed in the Bouin's fluid for 4 to 6 days. After dehydration, paraffin blocks were prepared and 4 to 6 µsections were taken. Staining procedure of Takasugi and Bern (1962) was adopted, as NSM of the CNS of fishes is Gomori negative (Bern and Takasugi, 1962).

OBSERVATIONS

The CNS of H. ilisha and H. toli may be considered as a "typical teleostean" CNS and can be put in "Generalized teleostean CNS" - as per Bern and Takasugi (1962).

BASIC ANATOMY:

(1) Caudal Neurosecretory cells and
Neurosecretory fibre tracts.

(2) Extraurophysieal Neurohemal regions.

(3) Urophysis.

Caudal Neurosecretory Cells:

The large irregular shapes NS cells were located in an area of the spinal cord of the last eight vertebrae, anterior to the urophysis. The can be easily recognised and their axons may be lodged on the edge of the central canal of the spinal cord. Variety of shapes were exhibited by their nuclei - many cytoplasmic invaginations were observed. All these cells were positive to Acid violet staining (A.V. staining). The NS granules were fairly large in size and at several occassions were observed arising from nuclei and cytoplasmic invaginations also (Figs. 10 and 11).

Neurosecretory fibre tract:

These fibres run from the cell bodies either to the nearby blood capillaries or run directly to the urophysis, where, they surround the blood vessels. Very often they were observed ending at the ependymal cells of the central canal of the spinal cord (Figs. 1 and 7).

Urophysis or the storage bulb:

Urophysis were somewhat oval in shape and consisted of roughly, two regions, (inner) medulla and (outer) cortex.

Cortex was consisting of neurohemal area, whereas,medulla was made up of neurosecretory fibres and blood capillaries. The extensive blood supply was prominent (Fig. 3 as in Fig. 13).

Extra Urophysial Neurohemal regions:

This area was also frequently supplied with blood vessels. Alongwith many NS fibres, many of ependymal and glial fibres were noticed in this region, which extend from the area (where NS cells located) up to the junction of urophysis with the spinal cord. The parivascular regions around the capillaries was observed to be of varying thickness and very often were with numerours collegenous fibres. Several NS fibres terminated at the walls of capillaries (Fig. 4).

SECRETION CYCLE OF THE NEUROSECRETORY CELLS OF THE CAUDAL NEUROSECRETORY SYSTEM (CNS) OF H. ILISHA AND H. TOLI:

The different four phases of the secretion cycle of NS cells were studied by Scharrer et al., 1945. Oztan (1962) has also reported the secretion cycle of Nucleus preopticus of Platypoecilus maculatus of the CN system. They are classified into 5 different phases as under:

(a) Stage:

Nuclei large, prominent. Nucleolus not noticed. Acid violet (A.V.) positive fine granules in the middle of the

nuclei may be in the form of very thin threads. Cytoplasm very thin, about in the form of a rim around big nucleous. Rarely A.V. positive granules noticed in the cytoplasm (Fig. 10).

(b) Stage:

No change in size of nuclei. A.V. positive granules found in the cytoplasm near the poles. Cytoplasm increased in volume- and was with few small vacuolas (Fig. 10).

(c) Stage:

Increase in volume of nucleus. Nucleolii large and prominent. Many A.V. positive granules scattered in the cytoplasm. Cytoplasm in many cases fully loaded with A.V. positive granules. Few A.V. positive granules in the nucleus and fine chromatin net work is seen in the nucleus (Fig.10).

(d) Stage:

The migration of A.V. positive granules towards and in the axons of NS cells is conspicious. Many vacuoles in the cytoplasm. Very often the finger like projections of the nucleus were observed to discharge A.V. positive fine granules in the cytoplasm. Axons' distal end with many A.V. positive granules. This stage may be called as "Exhaust phase" of the secretion cycle (Fig. 10).

(e) Stage:

Nucleus completely empty. Many vacuoles in the cytoplasm. Axons with few granules or devoid of granules. Few granules in the nucleus too. This stage may be called as "Regaining phase" of the secretion cycle (Fig. 10).

In the running chapter the different phases of the NS cells of CNS will be mentioned by letters as indicated above.

THE CAUDAL NEUROSECRETORY SYSTEM OF H. ILISHA CAPTURED FROM THE SEA:

Caudal Neurosecretory cells:

Large irregular shaped NS cells were observed with light green positive cytoplasm and were noticed in 'a', 'b' and 'c' phases of the secretion cycle. Some few cells were observed in 'd' and 'e' phases also. Large NS granules - A.V. positive - were noticed. Large NS cells in intimate contact with capillary exhibited axons terminating on the capillary wall - Axons were laden with A.V. granules (Fig.2).

Neurosecretory fibre tract:

Long densely stained fibres were noticed in the form of wavy fibres. Many A.V. positive two types of large granules were noticed: (1) A.V. positive densely stained granules and (2) somewhat empty granules (Fig. 1).

Extra Urophysial Neurohemal area:

The ependymal cells adjacent to the central canal exhibited dense A.V. staining granules in them and also showed small projections bathing in the canal, densely stained with A.V. The glial fibres were uniformly stained with light green. Many capillaries walls were densely stained with A.V. staining (Figs. 7,8 and 9).

Urophysis:

The densely (A.V.) stained cortex is a remarkable feature. The capillary walls of this region were intensely stained with A.V. staining. The axons - heavily stained with A.V. - probably from NS cells of the spinal cord carrying NSM for deposition in urophysis-giving wavy appearance, were noticed in plenty (Fig. 3).

A.V. stained pale axons were noticed in medulla. Few A.V. positive granules were noticed scattered in medulla (Fig. 4).

The NS cells of the ventral region of the urophysis, exhibited 'd' and 'e' phase of secretion cycle.

THE CAUDAL NEUROSECRETORY SYSTEM OF - MATURE, MIGRATING INTO RIVER NARBADA - H. ILISHA:

Caudal Neurosecretory cells:

Almost all NS cells were in 'e' phase of secretion cycle. Few did exhibited 'd' phase. At very few places

NS cells were shrunk and lost leaving empty spaces behind. The presence of 2 to 3 nuclei in many of NS cells was clear, as these nuclei were with prominant nucleolus and thread like chromatin (as in Fig. 15).

Neurosecretory fibre tract:

Few axon fibres could be traced up long distance as they stained heavily with A.V. staining. The faintly stained walls of capillaries were worthnoting.

Occassionally these fibres were noticed diverting towards the central canal and terminating on the ependymal cells. The ependymal cells showed A.V. granules in them. The protoplasmic processes bathing in the central canal also exhibited A.V. granules, suggesting a transport of A.V. granules into the cerebrospinal fluid (as in Figs.7,8, & 9).

Extra Urophysial Neurohemal area:

This region showed many lacunae. The nervous tissue appeared to have suffered due to the change in medium. At several places wavy appearance of glial tissue gave coarse view of the entire organization (as in Fig. 15).

Urophysis:

Cortex gave completely different histological picture than of sea ilisha. Extremely faint A.V. staining in the

cortex, presence of faintly stained walls of capillaries of cortex and extremely pale stained axons fibres, suggested utilization of NS material from the urophysis due to the entry of H. ilisha in hyportonic medium-river water. NS cells of this region appeared very active and exhibited 'd' and 'c' phase of secretion cycle. The same group was inactive in H. ilisha captured from sea. The loss of some region and presence of many lacunae suggest, that, this part of CNS had suffered probably due to change of medium (as in Fig. 13).

Few faintly stained axon fibres were observed in medulla of urophysis. Except loss of some region medulla appeared to be compact.

THE CAUDAL NEUROSECRETORY SYSTEM OF SPENT H. ILISHA ON RETURN MIGRATION TO SEA - CAPTURED FROM RIVER NARBADA:

Caudal Neurosecretory cells:

The loss of some NS cells, as evident by empty spaces filled with remnants of nuclei, was remarkable feature. The large cells which were functioning, showed 'a' and 'b' phase of secretion. Few inactive, light green positive cells were also noticed. They may be chromophobes.

Neurosecretory fibre tract:

The tremendous blood supply, as evident by the appearance of plenty of the capillaries, was remarkable feature. This may be for the resynthesis of NSM - for which a blood supply is necessary. The NSM carrying, pale stained NS fibres, generally were observed concentrated near the central cannal. The ependymal cells also were loaded with NS granules - the surface of them, facing the cannal, was with fine A.V. granules (as in Figs.7,8 and 9). On the whole, NS fibres could be traced upto considerable distance on basis of A.V. staining.

Extraurophysial Neurohemal area:

The appearance of plenty of capillaries, was believed to be for the increase of blood supply for the resynthesis and production of NSM. At many places, many lacunae were noticed. Empty spaces were very often found in the glial tissue, NS fibres and nuclei. This region exhibited degenerative changes (Figs. 10 and 11).

Urophysis:

It appeared to receive densely stained NS fibres from the spinal cord, which penetrated deeply in urophysis. Cortex was completely devoid of NSM, except at the peripheri of the end portion of it. Large A.V. positive droplets were occassionally noticed in the end portion of the urophysis. The

walls of the blood vessels were stained with A.V. staining. At few places the cortex appeared to have lost certain region. Medulla did exhibit empty spaces caused by loss of certain regions. The NS cells were in 'a', 'b' and 'c' phases of secretion cycle. Many chromophobes were observed. Noticeable increase in blood supply was exhibited (Fig. 13).

THE CAUDAL NEUROSECRETORY SYSTEM OF IMMATURE - NON-MIGRATORY, MARINE H. TOLI:

Caudal Neurosecretory cells:

The NS cells were small in size and few in number. They showed light green positive cytoplasm and stage 'a' and 'b' of secretion cycle. Other NS cells completely devoid of NSM and uniformly stained with light green were also noticed (Fig. 23). This picture is unlike picture obtained from immature migratory H. ilisha.

Neurosecretory tract:

Thin and faintly stained NS fibres were observed in the spinal cord, but, at the junction of the spinal cord and of urophysis. These NS fibres stained densely and could be traced upto long distance in the urophysis. Plenty of the capillaries intercepted the neurosecretory tract. The walls of capillaries in few cases stained faintly with A.V.

Extraurophysial Neurohemal area:

The presence of numerous capillaries was worthnoting. Some of the NS fibres were noticed travelling towards the ependymal cells and were found terminating on them (as in Figs. 7,8 and 9).

Urophysis:

It was smaller in size. The NS fibres, densely stained by A.V. appeared penetrating deeply in the medulla of urophysis. These fibres - densely stained by A.V. stain-surrounded the walls of the capillaries and the walls of the capillaries were also stained deeply by A.V. staining. In urophysis of H.ilisha more number of the NS cells were noticed however, in urophysis of H. toli were very few and were observed. NS cells were completely inactive. From this histological picture, it seems that NSM is stored in the urophysis and NS cells of the spinal cord has resummed the production of NSM.

THE CAUDAL NEUROSECRETORY SYSTEM OF MATURE, NON-MIGRATORY MARINE, H. TOLI CAPTURED FROM THE SEA:

Caudal Neurosecretory cells:

The NS cells were in active state of secretion - 'd' phase of secretion cycle. Few cells did exhibit 'e' phase and 'a' phase of secretion cycle. Many NS cells situated on the 'a' phase of secretion cycle. Many NS cells situated on

the dorsal peripheral region of the spinal cord, near the junction of urophysis, showed light green positive cytoplasm and were inactive. They were arranged in a row. Few chromophobes were also noticed.

Neurosecretory fibre tract:

Few NS fibres were noted travelling towards the bulb and were densely stained. This tract was noticed in the middle of the spinal cord, whereas near the peripheral region few pale stained fibres were noticed. Very few capillaries were observed and their walls were not stained with A.V. staining. These NS fibres made a concentrated pathway and were deeply stained with A.V. As soon as they entered in urophysis, they penetrate deeply in the cortex and medulla of the urophysis.

Neurohemal region:

This region was somewhat loose on the peripheral side. Glial tissue was intermingled with pale staining NS fibres.

Urophysis:

Plenty of capillaries had appeared and their walls were densely stained with A.V. stain. Cortex periphery was devoid of NSM. Many empty spaces were noticed in the medulla.

THE CAUDAL NEUROSECRETORY SYSTEM OF NON-MIGRATORY MARINE SPENT H. TOLI CAPTURED FROM THE SEA:

Caudal Neurosecretory Cells:

Large NS cells showed exhaust phase and 'a' phase of secretion cycle. These NS cells were fewer in number than those found in H. ilisha spinal cord. Few of them gave shrunk appearance and showed an empty space between surrounding tissues and NS cells. Few chromophobes were also noticed (Fig. 23).

Neurosecretory fibre tract:

Very rarely, very pale staining NS fibres were observed. They could not be traced for a long distance.

Extraurophysial Neurohemal area:

The appearance of numerous blood capillaries was a noticeable feature. The walls of capillaries were not stained by A.V. staining. The region was compact in appearance (as in Fig. 22).

Urophysis:

At very small region of the periphery of the cortex, A.V. positive NSM was noticed; in almost all the other region of the cortex and medulla, no NSM was observed, not even on the walls of the capillaries present in the urophysis. Almost

all NS cells were in 'e' phase of secretion, whereas few exhibited 'a' stage of secretion cycle.

THE CAUDAL NEUROSECRETORY SYSTEM OF DRIFTED NON-MIGRATORY MARINE IMMATURE H. TOLI:

Caudal Neurosecretory cells:

The NS cells were destroyed leaving empty spaces behind, very few cells present, showed shrunk appearance, no nucleus and light green positive cytoplasm (Fig. 17 and as in Fig. 20).

Neurosecretory fibre tract:

Very few axon fibres were found carrying NSM and in very little quantity as evident by the pale A.V. staining.

Extraurophysial Neurohemal area:

Fibres gave a wavy appearance, many lacunae were also noticed. Disintegrated remnant of fibres were observed in many empty spaces. Large number of capillaries were noticed.

Urophysis:

Very few NS cells appeared in 'a' phase of secretion, most of them were destroyed, completely leaving empty spaces behind. NSM was noticed in the form of A.V. positive rim only. Medulla as well as cortex were affected severely, as evident by loss of fibres and shrinkage of fibres. Light

green affinity was also reduced due to the loss of cytoplasmic contents of the fibres (Fig. 18).

THE CAUDAL NEUROSECRETORY SYSTEM OF DRIFTED NON-MIGRATORY MARINE MATURE H. TOLI:

Caudal Neurosecretory cells:

The degenerative and destructive changes exhibited by the CNS of immature drifted H. toli were also noticed. Rarely NS cell was observed intact. Mostly, NS were lost and destroyed leaving empty spaces and remnants of cytoplasm and nuclei behind. Those cells present, were found shrunk and in 'a' phase of secretion. The cells at the extreme end of the spinal cord, situated in the dorsal peripheral region, were in 'a' and 'b' phase of the secretory cycle (Fig. 20).

Extraurophysial Neurohemal region:

This region was affected severely as evident by loss of fibres, presence of many lacunae, empty spaces filled with remnants of fibres, and cytoplasm and nuclei. The walls of the capillaries were not stained by A.V. staining and were fully packed with blood cells.

Urophysis:

Urophysis exhibited presence of many lacunae, most of them were filled with remnants of cytoplasm and nuclei. The

edge of the cortex was densely stained by A.V. stain, especially in the end of the urophysis. Many deeply stained axon fibres were observed in the cortex. The appearance of many NS cells in the cortex was an interesting feature. All these cells exhibited 'a' and 'b' phase of secretion cycle. The capillaries, filled compactly with blood cells did not show A.V. staining on their walls (as in Fig. 18).

THE CAUDAL NEUROSECRETORY SYSTEM OF THE NON-MIGRATOY, MARINE SPENT H. TOLI:

Caudal Neurosecretory Cells:

In the spinal cord, except few, NS cells were completely destroyed leaving large empty spaces behind. The destruction had reached at the peak here. Very few cells showed 'd' phase of secretion, whereas some of them exhibited 'a' phase. The NS cells, situated at the end of the spinal cord were normal and exhibited 'a' phase of secretion, however, a few were in the 'e' phase of the secretory cycle (as in Fig. 20).

Neurosecretory fibre tract:

Except few, scattered, densely stained fibres, there were no NS fibres present. The destruction was at the climax. At the junction of the spinal cord, these fibres were densely stained by A.V. stain (as in Fig. 20).

UROPHYSIS	REMARKS
Very few NS cells in 'A' phase, most of NS cells, were destroyed leaving empty spaces behind. NSM was noted only on the peripheri of the urophysis. Loss of fibres and tissues gave empty spaces in the medulla and in the cortex. Very few faintly A.V. stained fibres were noticed in the urophysis.	Destruction and Exhaustion probably due to sudden change to hypotonic medium caused by drifting.
Many lacunae in the cortex and in the medulla, filled with remnants of destroyed NS cells and fibres. Wandering nuclei and cytoplasm of destroyed cells were observed in lacunae. Deep stained NS fibres appeared concentrated at the junction of the spinal cord and the urophysis. The walls of capillaries were not stained with A.V.	Same as above
Only peripheri of the urophysis was densely stained by A.V. staining. In the cortex and in the medulla, destruction of some regions was frequently observed. The capillaries, densely packed with blood cells, were observed with A.V. stained walls. Colloid like NSM was observed in the lumen of several capillaries. NS cells were active.	Destruction at peak, probably due to sudden change in medium.

WATER ANALYSIS mequi/litre				NPO
Na	K	Ca	Chlorides	
610.4	14.32	22.48	646.0	Immature H.ilisha(Sea): Most of them were in 'e' & 'f' phase. NS tract full of granules. Neurohypophysis filled partially with NSM.
608.2	14.32	22.46	650.0	
608.2	13.82	22.96	658.0	
592.6	13.82	22.46	668.5	
ESTUARY:				Mature H. ilisha: Most of them were in 'e' phase and rest in 'f' phase. Axons full of NSM. Pathway increase in thickness. Neurohypophysis densely packed with NSM.
168.3	3.633	7.236	183.9	
177.9	3.581	7.734	186.9	
RIVER:				
1.74	0.1023	0.0991	1.9	
1.75	0.1279	0.0981	2.051	
1.52	0.1023	0.0995	1.748	
RIVER:				Spent Hilsa ilisha: All are in 'e' phase. Discharge of NSM in the form of large globules & droplets. Pathway thick and full of NS globules and granules. Neurohypophysis with very scanty NSM.
1.74	0.1023	0.0981	1.9	
1.75	0.1229	0.0981	2.051	
1.52	0.1023	0.0995	1.748	

WATER ANALYSIS

Mequi/Litre

Na	K	Ca	Chloride
610.4	14.32	22.46	646.0
608.2	14.32	22.46	650.0
608.2	13.82	21.96	658.0
592.6	13.82	22.46	668.5

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NUCLEUS PREOPTICUS	CAUDAI CAUDAL NEUROSECRETORY CELLS
Immature Hilsa toli: 'D' & 'E' phase of secretion. In most of axons A.F. +ve granules noticed. Pathway full of A.F. granules. Neurohypophysis full of NSM	NS cells smaller and fewer than those of observed in migratory H. ilisha. All in 'A' and 'B' phase of secretion. Light green +ve cells noticed
Mature Hilsa toli: Similar to mature H.ilisha captured from the sea. All NS cells in 'E','D' &'F' phase of secretion. Axons full of NS granules. Neurohypophysis with scanty of NSM	All NS cells in active state of secretion in 'D' & 'E' phase. NS cells of the dorsal peripheral region of the spinal cord were inactive. Few chromophobes noticed.
Spent Hilsa toli: Many in the 'F' stage of secretion. 'E' phase is dominating. Thick pathways full of A.F. +ve granules and globules. Neurohypophysis with moderate quantity of NSM.	Large NS cells in 'Exhaust phase' of secretion and few in 'A' phase. On the whole fewer NS cells were noticed than observed in the CNS of H.ilisha. Few chromophobes noticed.

Extraurophysial Neurohemal area:

The presence of many empty space filled with lost part of glial tissue, muscle fibres and broken or entire nuclei was characteristic.

Urophysis:

The edge of urophysis was densely stained by A.V. in the form of a rim. Several fibres, densely stained by A.V., were scattered in the cortex region. Many empty spaces were noticed in the cortex and in the medulla. The walls of few capillaries were not stained by A.V. (Fig. 19). Few of these capillaries were densely packed with blood cells. The appearance of numerous active NS cells in the urophysis was worthnoting. Colloid-like A.V. positive masses were observed in the lumen of the blood vessels (Fig. 19). Most of the NS cells showed 'd' phase of secretion. Their NS granules loaded axons were noticed terminating on the walls of nearest capillary(Fig.19).

DISCUSSION

The caudal neurosecretory system of fish represents an endocrine area and it is similar to the hypothalamo-neurohypophysial neurosecretory system anatomically and his tologically, but about its physiological role, our knowledge is still in fairly elementary state. In the last few years extensive literature have been published on the CNS of fishes (Enami,

1955a, 1956, 1959; Enami et al., 1956, 1958; Sano and Knoop, 1959; Holmgren, 1958, 1961a, 1961b, 1964; Sano, 1958a,b, 1961; Bern and Takasugi, 1962; Fridberg, 1962a,b, 1963; Takasugi and Bern, 1962, 1963; Bern and Takasugi, 1962; Roy, 1962; Nishioka and Bern 1964; Kobayashi et al., 1963; Oota, 1963; Peyrot, 1964; Imai et al., 1965; Yagi and Bern,1965; Friberg, et al.,1966; Nishioka and Bern,1964).

Hilsa ilisha, a migratory fish, faces severe environmental hazzards, the CNS of this fish showed several histological changes.

It is evident that there is production and synthesis of NSM by the NS cells of the spinal cord and the NSM is transported to the urophysis. The CN cells of the spinal cord of migrating H. ilisha show a totally different picture; the NSM stored in the urophysis is utilized, as visualized by faintly stained urophysis and very few NS fibres stained deeply by A.V. The NS cells of the spinal cord exhibited 'e' phase. This suggests that NSM was already sent to the urophysis. Large cells, with many lobate nuclei and with plenty of granules suggest that these active cells are engaged in synthesis of NSM. Due to exhaustion few NS cells were found destroyed, and many others appeared to have resumed synthesis of NSM. This is evident by 'a' stage of secretion. This picture is different from that of H. ilisha captured from

the sea. The neurohormones might have been utilized in hypotonic medium, for the osmoregulation and as more quantity is required; the NS cells of the spinal cord might have resumed the synthesis of NSM. A similar function can be attributed to the NS cells of the urophysis proper also. Large number of NS cells of the spinal cord of spent H. ilisha were destroyed and few other NS cells exhibited 'a' and 'b' phase. They may have resumed synthesis of neurohormones probably to maintain ion levels of the body fluid in hypotonic medium. The deeply stained NS fibres noted at the junction of the urophysis and in the medulla of urophysis probably indicate that the shrunk and destroyed NS cells might have transported neurohormone(s) to the urophysis and after that they have been destroyed due to exhaustion. Large droplets of NSM in the urophysis supports the above mentioned view. Simultaneously NPO also has started transporting NSM to empty neurophysis; probably suggesting the role of both NPO and CNS in osmoregulation (Table I). Takasugi and Bern (1962) have suggested a direct or indirect relation between CNS and osmotic or ionic regulatory mechanism in relation with NPO of brain. Roy (1962) on the basis of his experimental works on the CNS of some Indian fishes have suggested the ion regulatory role of the CNS. The present observations are concerent with those of the previous investigators.

It is clear that H. toli faces negligible change in the ion contents of sea water (Table II). The histological changes observed in the CNS of H. toli therefore suggests its possible role in maturity. The NS cells of immature H. toli were in 'a' and 'b' phases of secretion, whereas the NS fibres were with little of NSM. Urophysis was full of NSM. This picture speaks that NSM produced by NS cells was stored in the urophysis and it was not utilized at all.

The NS cells of the CNS of mature H. toli exhibited (Table II) 'd' and 'e' phase, the NS fibres were deeply stained by A.V. staining. The urophysis was faintly stained by the A.V. This picture suggests that NSM from urophysis was utilized. NSM was resynthesised and was being transported to urophysis. It may be interpreted that NSM of CN system was utilized during growth and maturity in H. toli.

The urophysis of spent H. toli was with little of NSM suggesting consumption of neurohormones. Most of the NS cells exhibited 'e' phase or 'Exhaust phase' of secretion. Probably they might have sent NSM to the urophysis. The resumption of synthesis of NSM is evident by 'a' phase of secretion. The presence of few chromophobes might be the result of complete loss of the NS granules from the NS cells. All these observations suggest that neurohormones were consumed during

maturity. The resumption of synthesis of NSM by NS cells suggest resupply of the neurohormones to the urophysis, as urophysis was with little NSM. The results of the present study suggests the possible role of CNS in maturity and ageing as reported by Nishioka and Bern (1964).

The CNS of drifted H. toli presents a very informative picture (Table III). Irrespective of the stages of maturity, the CNS of drifted H. toli showed almost all destroyed NS cells. Few healthy NS cells exhibited 'a' and 'b' phase of secretion. Deeply A.V. stained, broken NS fibres reveals transport of NSM from NS cells to the urophysis. The urophysis in immature, mature and spent drifted H. toli showed complete utilization of neurohormones. It may be concluded that the destroyed NS cells may be the result of (1) sudden increase in the demand of neurohormones to maintain osmotic balance in hypotonic medium; the estury, and (2) the inability of NS cells to cope up with the sudden increased demand of neurohormones due to drifting. The pronounced degenerative changes exhibited by all components of CN system of drifted H. toli may be considered the result of drifting, as the same are not noticed in the CNS of H. toli captured from sea of the same stages of maturity.

These observations suggest an osmoregulatory role for the CNS and support the view of several previous investigators

(Enami, 1955a, 1956, 1959; Bern and Takasugi, 1962; Sano, 1961; Fridberg, 1962a; Takasugi and Bern, 1962, 1963; Nishioka and Bern, 1964; Yagi and Bern, 1965; Fridberg *et al*., 1966).

The CNS of *H. toli* may play role in sexual maturity and aging, the same system showed heavy pronounced destruction, when same fish was dfirfting in the estury on the highest high tide day. It can be presumed from the observations obtained from the study of CNS of marine *H. toli*, that, this system may have a dual role (1) in ageing and sexual maturity and (2) in ion regulation or osmoregulation. The probable role of CNS in sexual maturity and ageing was suggested by Nishioka and Bern (1964). The present observation on the CNS of *H. toli* supports the view of Nishioka and Bern (1964) that CNS may participate in sexual maturity and ageing.

Roy (1962) noticed hyperplasia of the adrenocortical tissue of dog after injecting the extracts of the CNS obtained from several Indian fishes and he suggested that the hyperplasia noticed in dog might be due to a corticotropin-releasing factor (CRF) present in the extracts. It is tempting to suggest that the strong hyperplastic adrenocortical tissue of mature migrating *H. ilisha* and mild hyperplasia of marine mature *H.toli* (Chapter VII) may be the result of an activation of CRF of the CNS.

The 'active cytoplasmic invaginations' of the nuclei of NS cells of Albula vulpes captured from the pond, reported by Bern and Nishioka (1962), Fridberg et al. (1966), were observed 'very active' in the CN cells of migrating, mature and in the spent H. ilisha and in few healthy NS cells of the drifted H. toli (Figs. 21, 22). The previous workers considered these 'invaginations' to be the active centres for the production of NS granules, while favouring the osmoregulatory role for the CNS, they reported these centres to be active in Albula collected from ponds. They attributed these activities in 'invaginations' to the frequent changes in salinity of pond water. The present observations on 'active cytoplasmic invaginations'noticed in mature, spend migrating H. ilisha and drifted H. toli (Figs. 21 and 22), supports the view held by Nishioka and Bern (1964) and Fridberg et al. (1966) that these 'centres' are active when H. ilisha migrates in and H. toli is drifted in the fresh water, where fish has to face changes in salinity. Indirectly these observations support the ion regulatory role or osmoregulatory role for CNS.

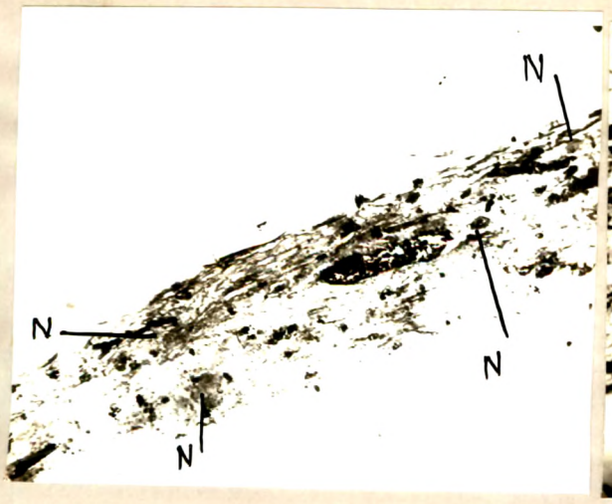
It is interesting to note the termination of NS fibres on the ependymal cells of the central canal of the spinal cord of immature, mature and spent H. ilisha (Figs.7,8 and 9), the presence of A.V. positive granules in the ependymal cells and in the protoplasmic projections bathing in cerebrospinal fluid

of the central canal (Fig. 9). The present observations are concurrent with those reported by Fridberg et al. (1966). The probable role of the cerebrospinal fluid as vehicle for the hormones is suggested by Scharrer (1965). The discharge of NSM in the central canal in the above mentioned fish may be for the transport of the neurohormones, if Scharrer's (1965) views are implemented.

The termination of NS fibres on the walls of blood vessels and the presence of A.V. colloidal NSM in the migrating H. ilisha, non-migratory H. toli and in spent drifted H. toli respectively (Fig. 14), speaks that some NSM may be discharged in the blood vessels also. Similar observations were also reported in the hypothalamo neurohypophyseal neurosecretory system of H. ilisha and H. toli, where the NS fibres from NPO and NLT were noticed terminating on the walls of the blood vessles (Chapter II).

It is interesting to observe the termination of NS fibres near the central canal of the spinal cord of immature, mature and spent H. ilisha, the presence of A.V. positive granules in the ependymal cells lying on the central canal and the A.V. positive granules in the protoplasmic projections bathing in the cerebrospinal fluid (Fig.9). Fridberg et al. (1966) have reported such observations in the CNS of

Albula vulpes. Scharrer (1965) has suggested the possible role of transport of hormones by cerebrospinal fluid. The probable discharge of NSM in the central canal may be for the transport of neurohormones.



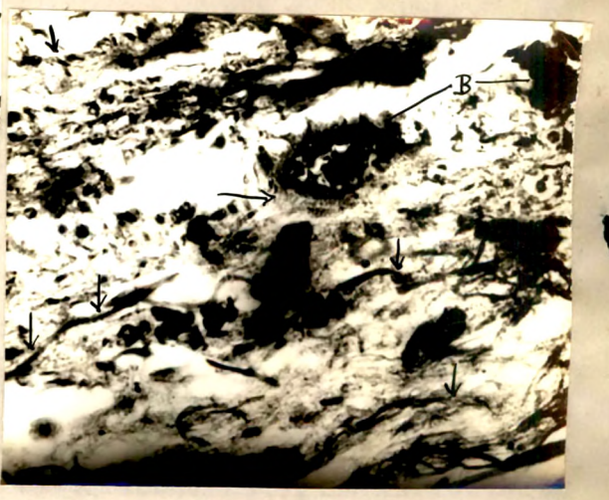


Fig.1 Fig.2

Fig.1: Caudal Neurosecretory Cells(N) in the spinal cord of immature H. ilisha.
Acid Violet, x63.

Fig.2: Region of Fig.1 magnified Acid Violet, x630.

Axons carrying Neurosecretory granules indicated by arrow,
B - Blood vessel.

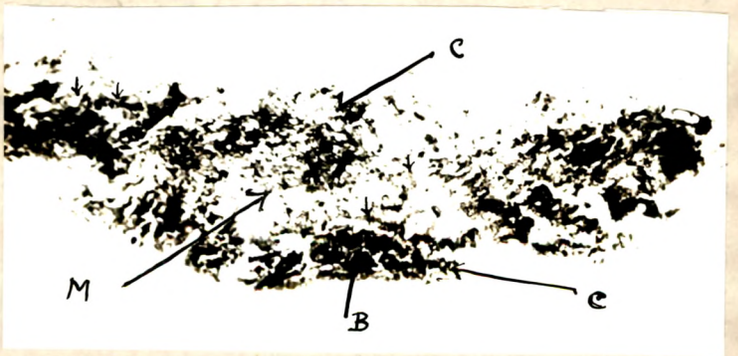
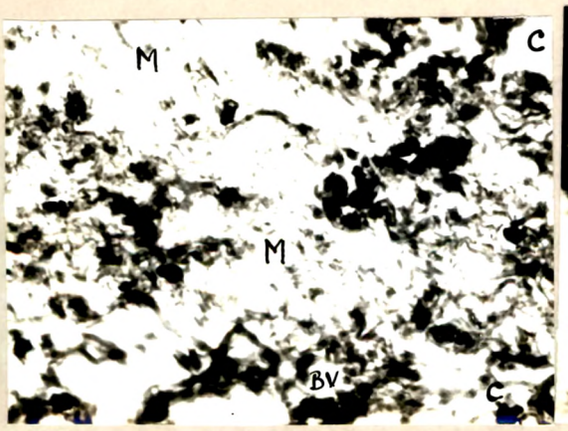


Fig. 3

The urophysis of immature H. ilisha.
Acid Violet, x63.

C - Cortex, M - Medulla
(Legend same as in Fig. 1 & 2)
Note NSM loaded urophysis.



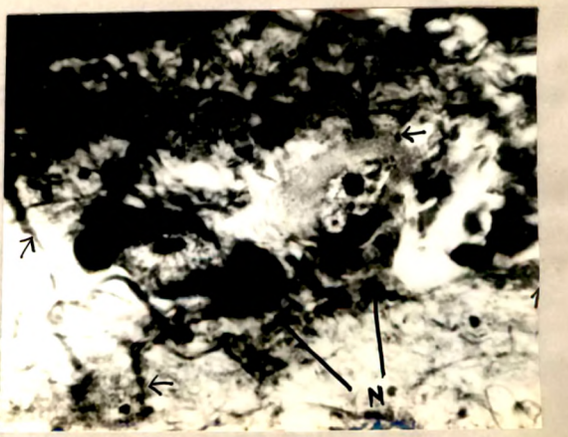


Fig. 4 Fig.5

Fig.4: Cortex(C) and Medulla(M) of the Urophysis of immature H. ilisha. Acid Violet, x63.

Fig.5: Region in Fig. 4 magnified. Acid Violet, x630.

N - Neurosecretory cell, B - Blood vessel.
Arrow indicate neurosecretory granules in axon.

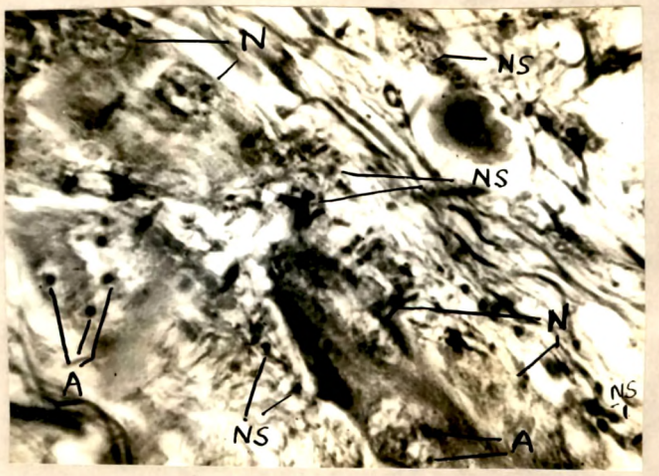


Fig.6

The Caudal Neurosecretory cell (indicated by arrow) from the spinal cord of immature H.ilisha, showing three to four nuclei(N) with nucleolii(A). Acid Violet, 1000.
NS - Neurosecretory granules.
Compare 'Inter nuclear Cytoplasm' with Fig.22.

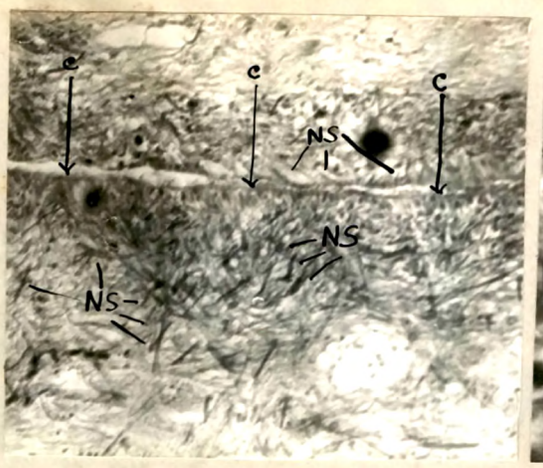


Fig. 7

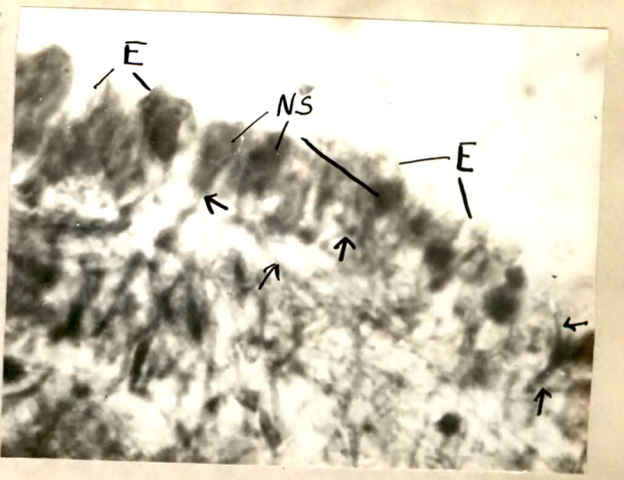


Fig.8

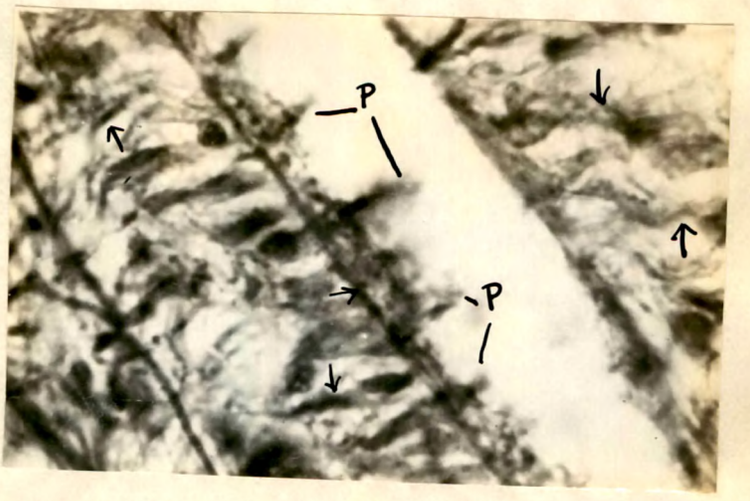


Fig.9

Fig.7: Region of the spinal cord of immature H. ilisha show concentration of neurosecretory fibres(NS) in vicinity of the central canal(C).
Acid Violet, 160.

Fig.8: Region of Fig.7 magnified to show neurosecretory material(NS) in the ependymal cells(E).
Acid Violet, x1000.
NS bearing axons end on the ependymal cells are marked by arrow.

Fig.9: Region of Fig.7 magnified to show globular protoplasmic processes(P) carrying NSM. NS bearing axons (marked by arrow) seen carrying NS granules.
Acid Violet, x1000.

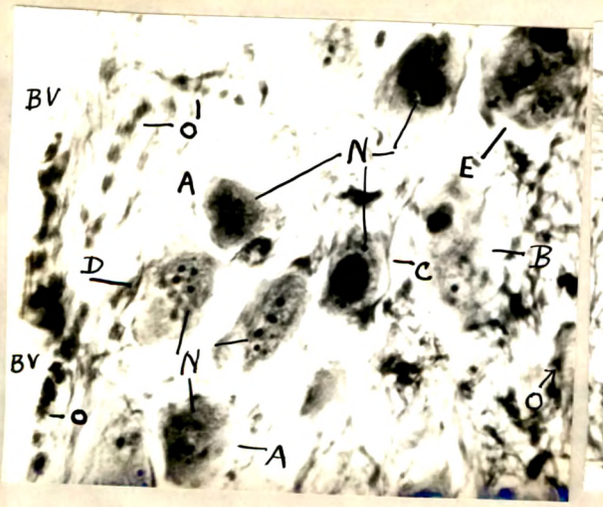


Fig.10

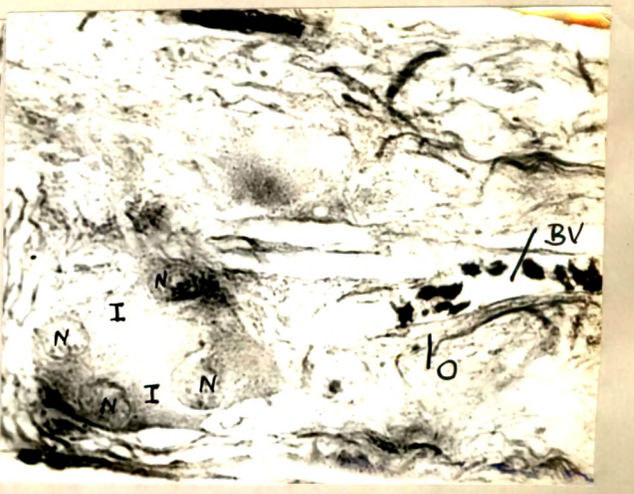


Fig.11

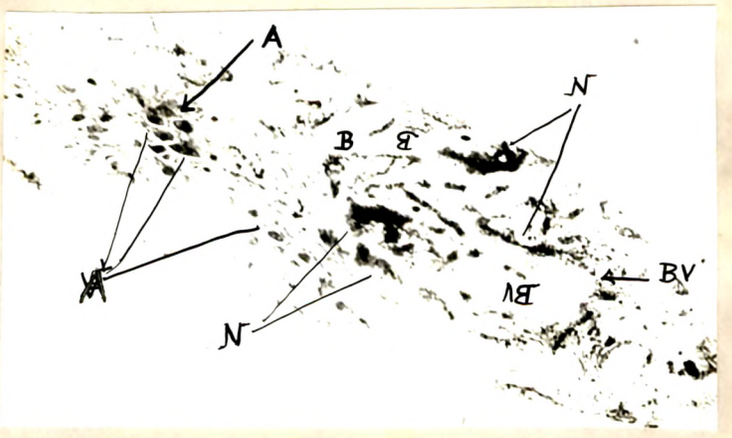


Fig.12

Fig.10 & Fig.11: The Caudal Neurosecretory cells of the spinal cord of spent H. ilisha.
Acid Violet, x400.
Letters'A','B','C','D' & 'E' represents stage of secretion cycle.
BV- Blood vessel, O - Axon carrying NSM,N - Nucleus, Note multinucleared NS cells in Fig.11. Note abundance of granules in 'Internuclear cytoplasimic invaginations'.(I)

Fig.12: Urophysis of spent H. ilisha, with little of neuro secretory material(N). Acid Violet, x63.
A - Neurosecretory Cells, BV- Blood vessel.

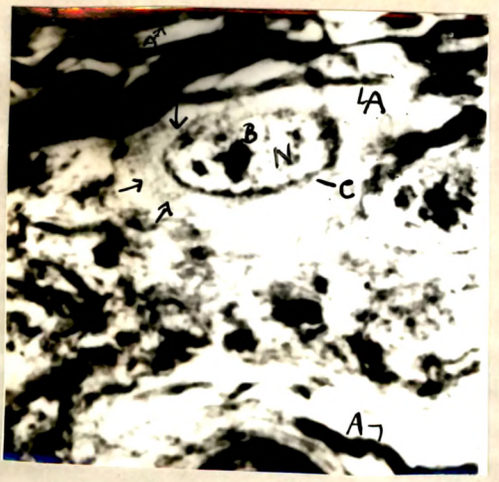


Fig. 13

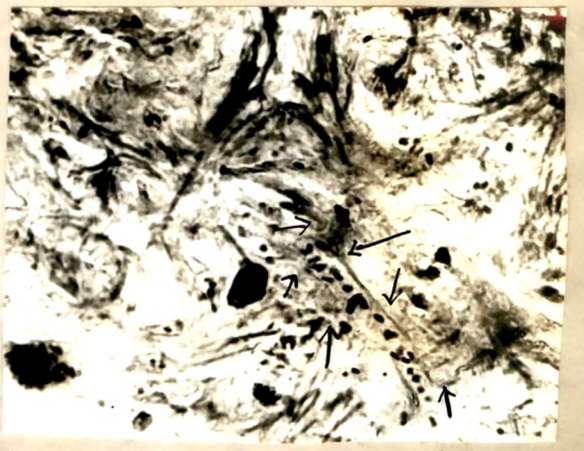


Fig.14

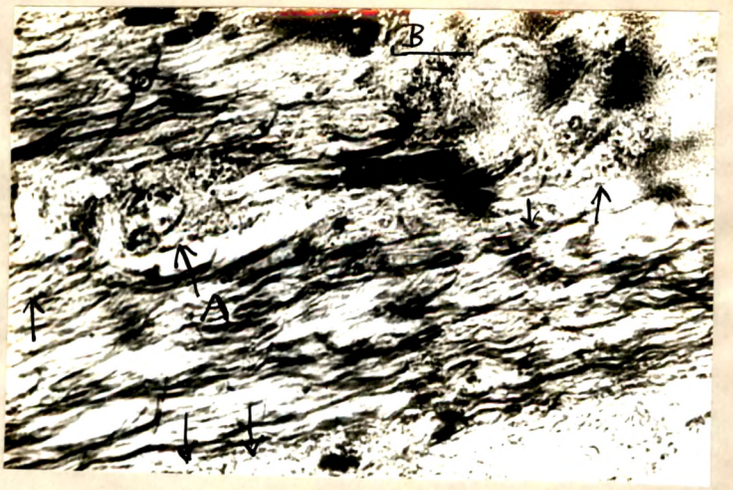


Fig.15

Fig. 13: Caudal Neurosecretory cell from the spinal cord of H. ilisha showing extrusion of neurosecretory material (shown by arrow) from the nucleus(N). Acid Violet, x1000
A - Axons carrying neurosecretory granules, B - Nucleolus
C - Nuclear membrane.

Fig. 14: Termination of NS bearing axons on the wall of blood vessel indicated by arrow. Acid Violet, x1000.

Fig. 15: Caudal Neurosecretory cells from the spinal cord of spent H. toli in 'exhaust phase' (A) and in 'D' phase (B). Acid Violet, x1000.
Arrow indicate NS granules in the axons.

Fig.16

Fig.17

Fig.18

Fig. 16: Urophysis of spent H. toli with little of neuro-secretory material(as shown by arrow). Acid Violet, x63.

Fig. 17: Caudal Neurosecretory cells seen destroyed and shrunk (A) in the spinal cord of drifted H. toli. Acid Violet, x63.

Fig. 18: Urophysis of drifted H. toli showing destruction, shrinkage etc. degenerative changes. Arrow indicate neurosecretory material. B - Blood Vessel, L - Lacunae, A - Axon.

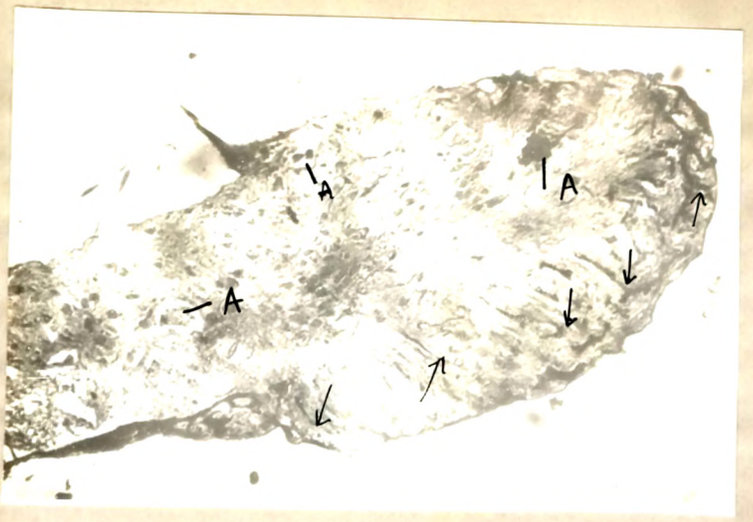


Fig. 19

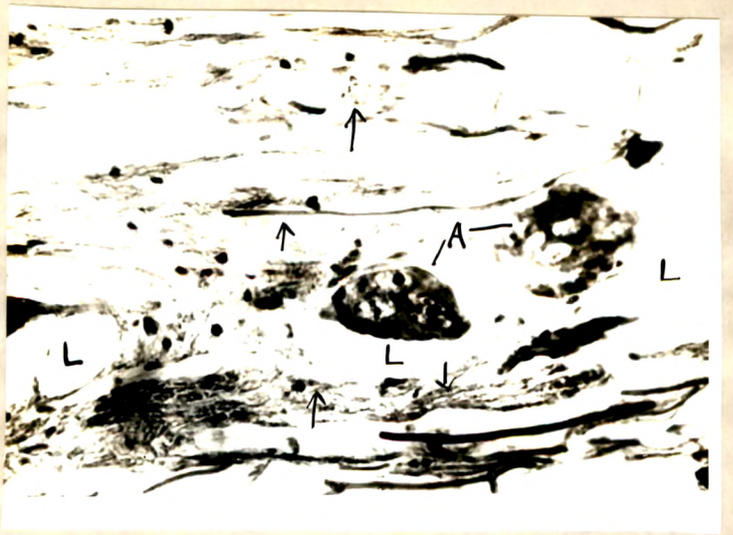


Fig. 20

Fig. 19: Some part of spinal cord and Urophysis of drifted, spent H. toli. Acid Violet, x63.
Note colloid like Neurosecretory material(A) and axons travelling toward peripheric(indicated by arrow).

Fig. 20: Caudal Neurosecretory cells from the spinal cord of drifted, mature H. toli. Acid Violet, 400.

Note NS cells(A) and lacunae(L).
Arrow represents neurosecretory granules carrying axons.

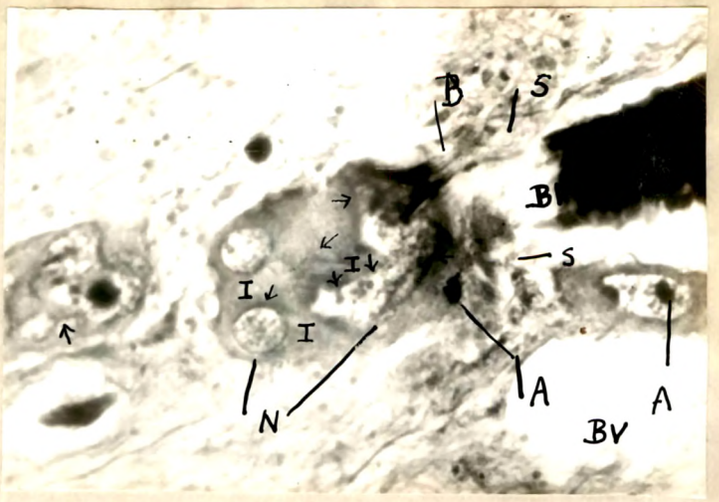


Fig. 21

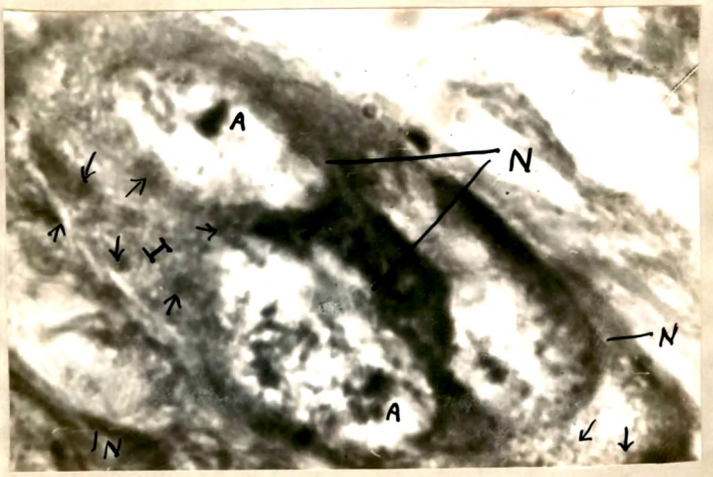


Fig. 22

Fig. 21 & 22: Caudal Neurosecretory cells from the spinal cord of the spent H. ilisha.
(Both stained by Acid Violet, x400 and 1000 resptly.).

N - Nucleus, A - Nucleolus, B - Axon, BV-Blood vessel.

Arrow indicate neurosecretory granules in internuclear cytoplasmic invaginations(I). Note the termination of the axon on the wall of blood vessel in Fig. 21 as shown by leter 'S'.
Compare with Fig.6.

Fig. 23

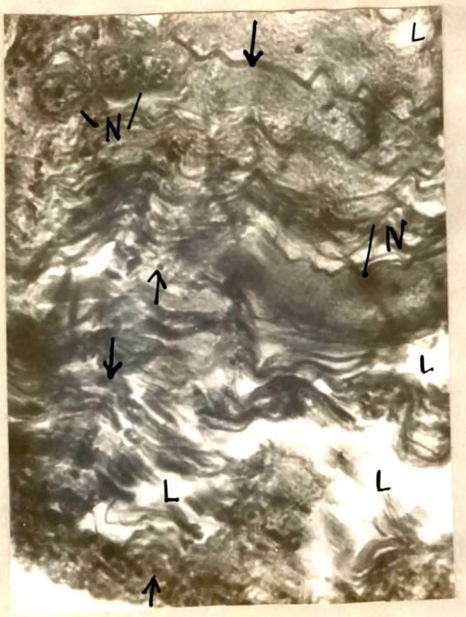


Fig. 24

Fig. 23: Caudal Neurosecretory cells(N) and light green positive cells - chromophobes(C) from the spinal cord of immature H. ilisha. Acid Violet, x400. N - Nucleus, B - Blood Vessel, arrow indicate axons carrying NS granules.

Fig. 24: Region of the spinal cord of spent H. toli. Acid Violet, x400. Note loose structure, presence of lacunae(L). Arrow indicate axons carrying NS granules.