CHAPTER III

HISTOCHEMICAL STUDIES ON THE DISTRIBUTION OF CHOLINESTERASES IN THE NUCLEUS PREOPTICUS, HYPOTHALAMO-NEUROHYPOPHYSEAL PATHWAYS AND IN NUCLEUS LATERALIS TUBERIS OF MIGRATOARY HILSA ILISHA (HAM.) AND OF NON-MIGRATORY HILSA TOLI(CUV. & CUL.) IN RELATION WITH THE CYCLIC CHANGES IN HYPOTHALAMIC NEUROSECRETORY SYSTEM

The direct or indirect involvement of cholinesterases in the secretory activities of the neurosecretory cells is suggested by several workers. (Arvy, 1962; Kobayashi and Farner, 1964). As the literature regarding the cholinesterases in the neurosecretory centres is reviewed by Kobayashi and Farner (1964) very little will be mentioned here. Their studies noted strong activities of acetylcholinesterase(AchE) in the perikarya of the supra optic paraventricular nuclei and in the infundibular nucleus, whereas, weak AChE activity was observed by them in the pars nervosa. Though little is known about the significance of the presence of acetylcholinesterase in the neurosecretory cells, but its presence is well established (Haruka, Uemura, 1965). Several workers have suggested a role for acetylcholinesterase in the release of neurohormones from the neurosecretory cells (Uemura, Kobayashi and Ishii, 1963; Pearse, 1958 - as cited by Kobayashi & Farner, 1964). With a view to throw light on the probable function of Acetylcholinesterases and Butyrylcholinesterase(BChE) in release of neuro-

hormones from Nucleus preopticus and Nucleus lateralis tuberis of migratory H. ilisha and of nonmigratory H. toli, the present work was undertaken and was compared with the cyclic changes observed in the Hypothalamo-neurohypophyseal neurosecretory system of above mentioned fishes (Chapter II).

MATERIALS AND METHODS

The fishes captured alive were obtained directly from the nets. Enough care was taken to discard wriggled fish and to expose brain alongwith pituitary within 2 minutes. Head was separated and entire brain alongwith pituitary was fixed in chilled 10% neutral formalin and Formal saline (Gurr, 1956). Fixation time was for 6 to 8 hours. Gelatin blocks were prepared, were fixed in 1% neutralized formalin and were preserved for 30 hours in freeze, 15 μ to 20 μ Gelatin sections were taken on freezing microtome. Sections were washed throughly with water to remove formalin. They were then incubated. The method adopted for the demonstration of cholinesterases in present study was of Koelle and Fridenwald (1949) as modified by Coupland and Holmes (1957). Sections were incubated separately to demonstrate two enzymes, acetyl (AchE) and butyrylcholinesterases (BchE) in the Nucleus

preopticus (NPO), in Hypothalamo-neurohypohyseal pathways (Pathways) and in Nucleus lateralis tuberis (NLT). Acetylthiochl$\overset{\circ}{\underset{\wedge}{\phi}}$ineiodide and butyrlthiocholineiodide were two substrates used for the demonstration of specific and non-specific enzymes. Enough precaution was taken to maintain pH of the incubation medium between 5.6 and 6.0 as optimal precipitation of the crystalline deposits of copper thiocholine sulphate is believed to occur at that pH (Malmgren and Sylven, 1955). The temperature 37° was maintained during incubation period, later was found to vary according to the concentration of enzymes in relation with the secretory cycle of NPO and NLT.

It is known that eserine sulphate inhibits both the enzymas. 3x10 M solution of Eserine sulphate was used at 37°C for 3 to 4 hours to inhibit both enzymes. Sections for control, were treated with Eserine sulphate for 3 to 4 hours at 37°C.

It may be noted that unfixed tissue Brain,could not give successful results and hence fixed tissues were used.

RESULTS

The results showing activity of both enzymes, time of incubation in relation with the neurosecretory activities of NPO and NLT are tabulated under.(Refer Table I).

DISCUSSION

The results tabulated above strongly suggest the presence of AchE and BchE in the perikarya of NS Cells and in the axons of NS Cells of group NPO and NLT.

The NPO of spent H. ilisha, on way to return migration to sea after spawning, were noticed fully engaged in discharing NSM into neurohypophysis through the pathways (Chapter II), strong activity for AchE and BchE was localized in the perikarya of NPO and in axons of NPO within 10 hours of incubation (++++ - 10 hours of incubation, Figs. 1 & 2).

In immature H. ilisha and in immature H. toli, strong AchE and BchE activity was observed in NPO and in axons after 12 hours and 13 hours of time of incubation. The NPO were noticed synthesizing NSM and transporting NSM into neurohypophysis as revealed by paraffin section (++++ activity in 12 hours and in 13 hours time of incubation).

The strong activity for AchE and BchE was noted in the perikarya of NPO and axons of NPO neurosecretory cells of spent H. toli. The discharge of NSM by NPO into neurohypophysis was observed in paraffin section study (++++ activity in 10 hours of time of incubation).

When the transport of NSM from NPO to the neurohypophysis is somewhat declined as observed in mature migrating H. ilisha

and mature nonmigratory H. toli by paraffin section, the activity for AchE in NPO and in axons of NPO was obtained after 13 hours of time of incubation (++++ after 13 hours) simultaneously weak activity for BchE was demonstrated in NPO and in axons of NPO after 20 hours of incubation in mature H. ilisha, feable activity for BchE was obtained in NPO and in axons of NPO after 24 hours of time of incubation. A strong AchE activity was localized in perikarya of NPO and in axons of NPO of drifted nonmigratory H. toli (++++ activity in 10 hours of time of incubation). Weak and feable BchE activity was observed in NPO and in axons of NPO after 20 hours of time of incubation (++ activity) and after 24 hours of time of incubation (+ activity) respectively.

From these results we may suggest that when there is a remarkable transport of NSM from NPO, AchE activity is obtained after short time of incubation, e.g. in spent H.ilisha, spent toli, immature H.ilisha and in the immature H. toli. Several authors have suggested a role for AchE in release of neurohormones from the neurosecretory cells (Uemura,Kobayashi and Ishii, 1963; Kobayashi and Farner, 1964; Arvy,1962;Abrahams and Pickford, 1954; Koelle, 1961), our observations support the hypothesis of Koelle that AchE may be involved in transport or in release of NSM from the NS cells.

Similar observations supporting the above mentioned view may be available from the following. It is somewhat on established fact that NLT is engaged in maturity of fishes (Öztan, 1963; Scharrer, 1954; Chapter II). Stronge AChE activity is localized in NLT of mature H. ilisha and mature H. toli within 12 hours of time of incubation (+++ activity in 12 hours of time of incubation, Figs.3 & 4). When NLT are inactive, as in immature H. ilisha and in immature H. toli, weak activity for AChE was noted in NLT after 18 hours of time of incubation(++ activity in 18 hours). Similarly exhausted NLT of spent H. toli and spent H. ilisha exhibited weak AChE activity (++ in 18 hours). This may suggest a role for AChE in the release of neurohoromones from the neurosecretory cells.

The strong activity (++++) for BchE noticed in the axons of spent H. toli may suggest a close association of this enzyme with AchE in release of NSM from NS cells.

Fig. 1

AChE of the NPO of the spent H. ilisha.
Substrate, AThCh, x400
P - Perikarya of NS cells, A - Axon of NS cell
W - Wall of blood vessel , B - Blood cells

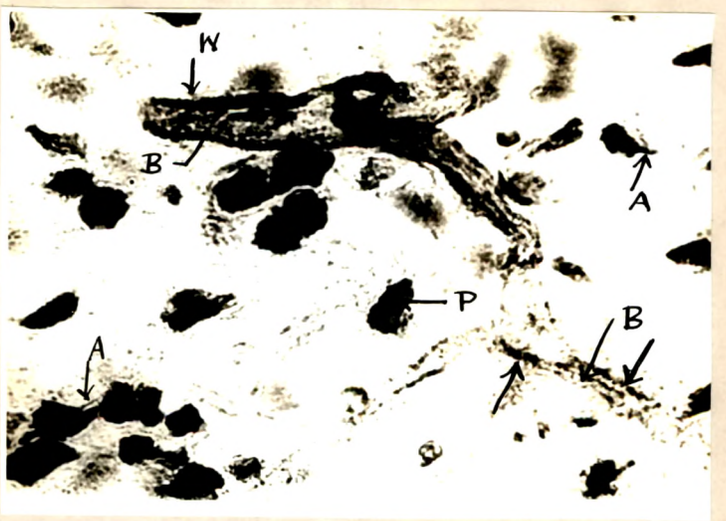

Fig. 2

BChE of the NPO of the spent, H. ilisha.
Substrate, BuThCh, x400

(Legend as in Fig. 1. Note the termination of the axon on the wall of the blood vessel, as indicated by arrow)

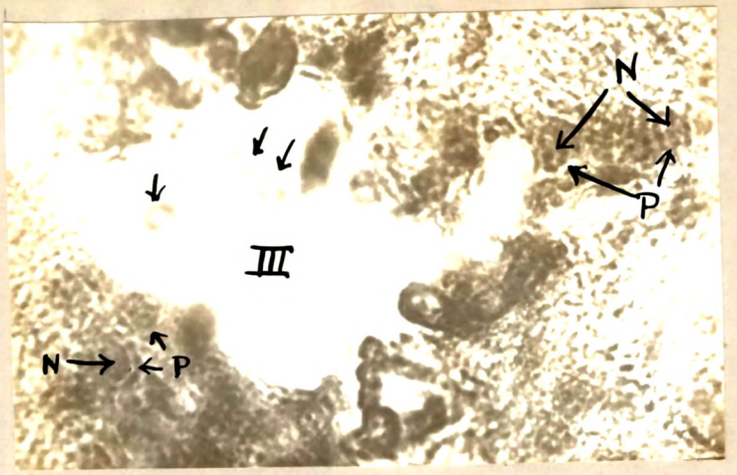

Fig. 3

AChE of the NLT of the mature H. ilisha.
Substrate, AThCh, x400.
III - Cavity of the third ventricle
P - Perikarya of the NS cells
N - Nucleus of the NS cells
A - Axon of the NS cell
(Arrow indicates phagocytes(?) in the cavity of the third ventricle)

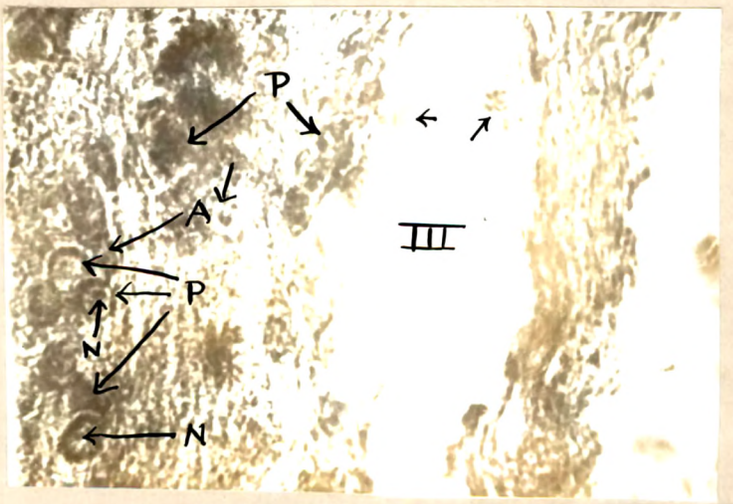

Fig. 4

BChE of the NLT of the mature H. ilisha.
Substrate, BuThCh, x400.

(Legend as in Fig. 3)