CHAPTER 5

DISTRIBUTION OF CHOLINESTERASE IN THE LIVER AND STOMACH OF MIGRATORY H. ILISHA AND NON-MIGRATORY H. TOLI

Two main types of cholinester hydrolases, acetylcholinesterase (AChE) and cholinesterase (ChE) have been experimentally studied in various tissues. Histochemical studies of liver and alimentary canal showing regional distributions and types of enzymes are available in literature (Gerebtzoff, 1959). Hepatic origin of plasma ChE and the role of ChE in the assimilation of food have been suggested. The role of cholinesterases in the alimentary canal has not yet been clearly established. Various functions like hydrolysis of acetylcholine, protection of mucosa, digestion and assimilation of food, metabolism of choline etc. have been suggested. Thus the variations in their licalization and the type of cholinesterases have remained without any plausible explanations.

With the above information at hand, the liver and stomach of H. ilisha and H. toli were examined histochemically for the localization of cholinesterases. In addition, the alterations of enzymic activity, which occur in the above organs when H. ilisha is starving during migration and spawning were studied. Enzymic alterations were also studied in drifted H. toli captured from river.

MATERRIALS AND METHODS

The fishes were collected from places as mentioned

in Chapter 1. Live fishes were decapitated immediately after removal from the net. Small pieces of tissue were fixed in cold 10% formol saline. Gelatin blocks were prepared and sections, 20-30μ were cut on a freezing microtome. These were incubated separately for acetyl and butyryl cholinesterases activity. Acetylthiocholine iodide and butyryl thiocholine-iodide were used for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) respectively. The method of Coupland and Holmes (1957) was followed. Control sections were treated for 30 minutes with $3x10^{-5}$ M solution of eserine sulphate solution to inhibit the enzymes.

RESULTS

The results of the present study are presented in three groups as follows: The cholinesterases activity and site of localization showed no difference in the mature and immature H. ilisha and H. toli. Also all these fishes collected from sea are actively feeding. Hence the results are presented under a group called sea Hilsa. The second group comprises of H. ilisha collected from river. These represent non feeding fishes undergoing migration and spawning. Under the third group are presented the observations made on the drifted H. toli captured from river.

The results concerning the type of cholinesterases and their localizations are summarized in Table I.

SEA HILSA

Corpus (Cardiac stomach)

AChE and BuChE were present in the epithelium as revealed by fine granular material. Both these enzymes were present in equal concentration. Neck cells of gastric glands also showed equal concentrations of both enzymes as noted in the epithelium (Fig. 1). The glands proper showed negligible (±) AChE but no BuChE activity. In the striated muscles the sarcoplasmic AChE activity was present in negligible (±) concentration whereas BuChE showed little (+) activity. Neuromuscular junctions showed both enzymes in equal moderate concentrations (Fig. 2). The blood vessels showed moderate AChE concentration.

Pyloric stomach

Epithelium showed slightly more of BuChE activity than AChE, the latter showing a more or less equal intensity as observed in the epithelium of corpus of sea Hilsa. The neck cells also showed similar concentrations of both enzymes (Fig. 3). The glands proper showed little (+) activity of AChE but very less (±) activity of BuChE. The smooth muscle fibres of the circular muscle layer showed no cytoplasmic activity. The BuChE activity was slightly greater than AChE at neuromuscular junctions (Fig. 4). The intensity was more or less same as at endplates in corpus of sea Hilsa. In the subepithelial connective tissue the cholinergic nerve fibres and plexuses showed maximum intensity of BuChE (Fig. 5). AChE activity was little less than BuChE.

Liver

In the liver, in all the fishes studied AChE and

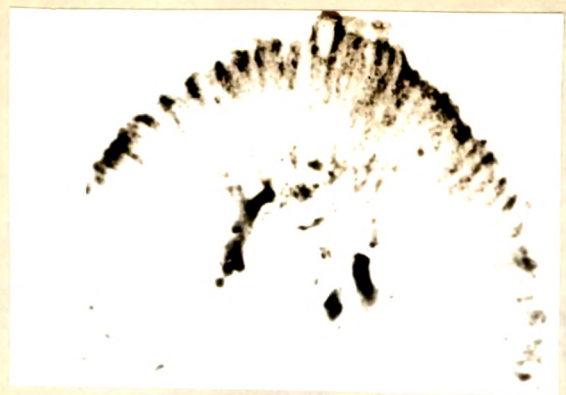

Fig. 1

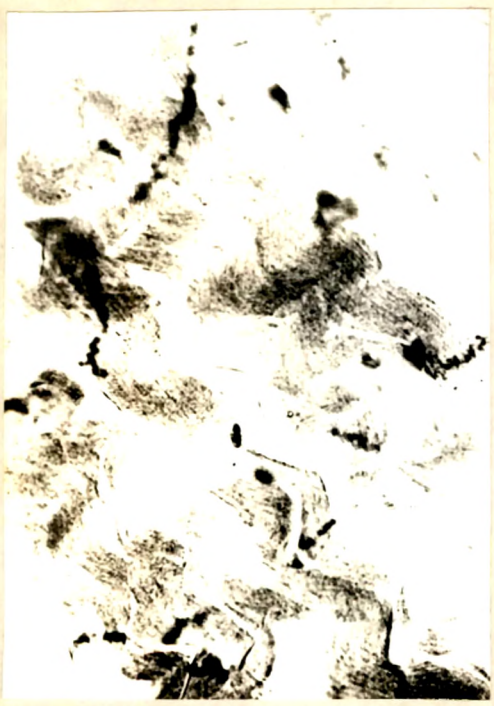

Fig. 2

Fig. 3

Fig. 1. Corpus of sea Hilsa showing AChE activity in the epithelium and neck cells . X 63

Fig. 2. Corpus of sea Hilsa showing AChE activity at neuromuscular junctions of striated muscle fibres. X 400

Fig.3. Pyloric stomach of sea Hilsa showing AChE activity in the epithelium, neck cells and glands. X 160

BuChE activities were revealed in the form of minute granules localized along the sinusoids and the walls of blood vessels. In the sea fish, the AChE and BuChE activity along the sinusoids and blood vessel walls was most intense. Cells surrounding these regions showed moderate activity. Away from the blood vessels and sinusoids the activity gradually decreased (Fig. 6). Fine nerves in the liver showed moderate AChE and BuChE activity in the form of fine beaded elongated structures (Fig. 7).

RIVER HILSA ILISHA

Corpus (Cardiac stomach)

The stomach showed differences in enzyme activity from those observed in sea Hilsa.

Epithelium showed AChE activity more than BuChE. Both these enzyme reactions were much less than the one observed in sea Hilsa. Same was true for enzyme activity in the neck cells of gastric glands. The AChE activity was little (+) whereas BuChE was negligible (±). Glands showed distinct activity. Sarcoplasm of striated muscles forming circular layer showed very less reaction. Neuromuscular junctions showed moderate enzymic reactions both, AChE and BuChE, similar to the reactions given by striated muscles in the corpus of sea Hilsa. Blood vessels of serosa showed AChE activity.

Pyloric stomach

Epithelium and glands showed no cholinesterase activity. Neck cells of glands showed slightly more BuChE activity than AChE. Both these enzymic reactions showed less intensity than

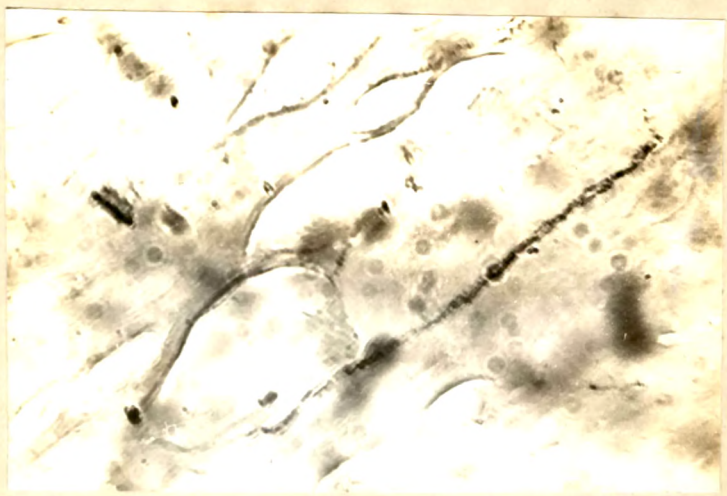

Fig. 4

Fig. 5

Fig. 6

Fig. 4. Pyloric stomach of sea Hilsa showing AChE activity at neuromuscular junctions of smooth muscle fibres. X 160

Fig. 5. Pyloric stomach of sea Hilsa showing AChE activity in nerve fibres in subepithelial connective tissue. X 160

Fig. 6. Liver of sea Hilsa showing AChE activity around sinusoids and blood vessels. X 63

those observed in the pyloric stomach of sea Hilsa. No cytoplasmic activity was noticed in smooth muscles of the circular muscle layer. BuChE in the nerves in this muscle layer was absent, whereas AChE activity was moderate showing similar reaction as shown by the smooth muscles of the pyloric stomach of sea Hilsa. Blood vessels showed AChE and BuChE activity.

Liver

In the liver of river H. ilisha and drifted H. toli, the localization and intensity of reactions for AChE and BuChE were similar to that of sea fish. However, the enzyme activity was comparatively more restricted to the immediate region surrounding the blood vessel and sinusoids (Fig. 10). Away from the blood vessels the activity decreased more rapidly than it was observed in the liver of sea Hilsa. Secondly the cholinesterases activity was totally absent in the nerves.

DRIFTED H. TOLI

Corpus (Cardiac stomach)

Only the neck cells and neuromuscular junctions showed moderate activity comparable to sea Hilsa.

Pyloric stomach

In case of the pyloric stomach, a few blood vessels of serosa showed AChE activity and a little BuChE activity. All the other layers showed no reactions for either of the cholinesterases (Figs. 8 and 9).

Liver

The liver of drifted H. toli showed similar results as noted in the case of river H. ilisha.

Table. I. Histochemical observations on the cholinesterase activity in the stomach.

Stage	Region		Incubation time (hr)	Epithelium	Glands	Neck cells	Muscles CY	Muscles NMJ
SEA HILSA	Corpus	AChE	38	+++	±	+++	±	+++
		BuChE	38	+++	-	+++	+	+++
	Pyloric	AChE	38	+++	+	+++	-	+++
		BuChE	38	++++	±	++++	-	+++
RIVER HILSA ILISHA	Corpus	AChE	40	++	-	+	±	+++
		BuChE	40	+	-	±	±	+++
	Pyloric	AChE	42	-	-	++	-	+++
		BuChE	42	-	-	+++	-	-
DRIFTED H. TOLI	Corpus	AChE	42	-	-	+++	-	+++
		BuChE	42	-	-	-	-	-
	Pyloric	AChE	42	-	-	-	-	-
		BuChE	42	-	-	-	-	-

CY- Cytoplasmic. NMJ- Neuromuscular junctions.

The intensity of staining is denoted by '+' signs.

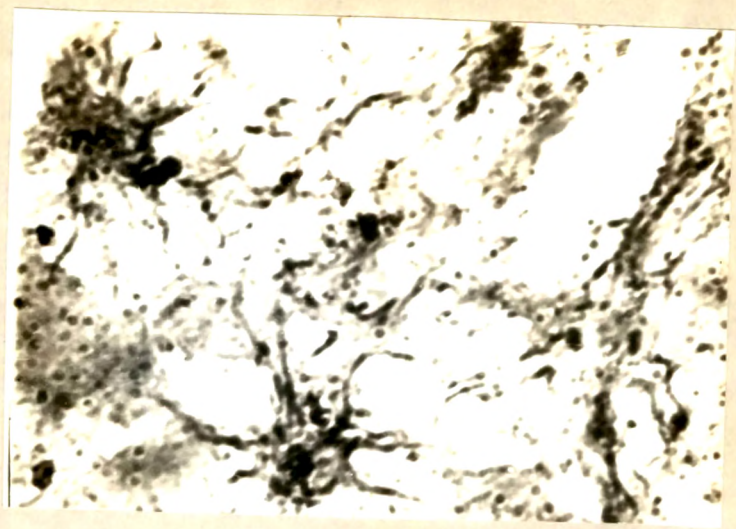

Fig. 7

Fig. 8

Fig. 7. Liver of sea Hilsa showing AChE activity in the nerves in form of fine beaded structures. X 400

Fig. 8. Pyloric stomach of drifted H. toli. Note the absence of cholinesterases in the epithelium and glands. X 63

Fig. 9

Fig. 10

Fig. 9. Pyloric stomach of drifted H. toli.Note the absence of cholinesterases in the smooth muscle layer. X 63

Fig. 10. Liver of river H. ilisha showing AChE activity around sinusoids and blood vessels. X 400

DISCUSSION

The morphological structure of the nerve ending in the striated muscles of the stomach was similar to the multiple nerve endings in the lateral red muscle of the same fish, Hilsa ilisha (Joseph, 1967). This type of nerve endings have been described by Bone (1966) as "en grappe" type in the red muscles of dogfish. Barets (1961) referred to such type of nerve endings as "termination distribuee". Only sea Hilsa showed little sarcoplasmic activity in the striated muscle. No cytoplasmic cholinesterase activity was discernible in smooth muscles whereas nerve fibres which course through this muscular layer showed AChE and BuChE activity only in sea Hilsa and river H. ilisha. Gerebtzoff (1959), on the other hand, has reported the presence of intense cholinesterase activity in smooth muscles of the stomach. Also he could not detect nerve fibre in the smooth muscles in guinea-pig and rat.

Epithelium of sea Hilsa showed moderate AChE and BuChE activity, which showed a decrease in the river H. ilisha when the fish is starving. In guinea-pig and rat no such activity has been reported by Gerebtzoff (1959).

In the sea Hilsa, the glands showed very less activity of AChE and in the river H. ilisha the activity was completely absent. Gerebtzoff (1959) could notice the activity in chief gland cells of guinea-pig but not in the gland cells in the glandular part of the stomach of rat. Neck cells in Hilsa showed moderate reaction for AChE and BuChE. Such reports are not

available in other animals studied by various workers. Thus the epithelium and neck cells showed both AChE and BuChE activity besides localization of both enzymes in nerves. Gerebtzoff (1959) found only cholinesterase activity in the epithelium of intestine and glands of stomach except rabbit. Koelle et al.(1950) found AChE activity in the above sites. Thus it seems that there is a species difference in the localization and type of enzymes.

As mentioned in chapter 2 there is a marked atrophy and degeneration of the alimentary canal of H. ilisha during migration and spawning, when it is not feeding. Parallel alterations showing reduction of enzymic activity has also been observed. These reduction of activity was noticed in epithelium and neck cells of glands. Neuromuscular junctions in muscles, however, showed no reduction of enzyme activity.

Marked destructive changes in the alimentary canal of drifted H. toli captured from river are also reflected in the enzymic activity. Only the neck cells of glands and neuromuscular junctions in the corpus showed moderate AChE activity.

From the results obtained by various workers on cholinesterases in the alimentary canal it is evident that great variability in the activity, localization and type of enzymes exist. This has been reviewed by Gerebtzoff (1959) and Svensmark (1965). The present study could not add any conclusive proof as far as the role of cholinesterases in the alimentary canal is concerned.

The localization of cholinesterases in liver along the sinusoids and blood vessels finds support in the work of

Rogister (1956), who has found a mixture of ChE abd AChE in the liver of a teleost fish, *Uranoscopus scaber*. In other two teleosts, *Anguilla vulgaris* and *Scyllium canicula*, on the other hand, he could not find any histochemical localization of cholinesterases. In the starving *H. ilisha* from river, the difference noted was that enzymic activity is more restricted mostly to the region immediately surrounding blood vessels. In feeding *Hilsa* from sea, the activity was comparatively more spreaded. This so to say resembles the peripheric and centrolobular localizations of enzymes in mouse feeding actively and mouse in inaition respectively (cf. Gerebtzoff, 1959). The fine nerves in the liver showed enzymic activity only in sea *Hilsa*. Ban (1965) has shown the nerve fibres in relation to bile production in rabbit liver and suggested hypothalamic influence upon hepatic enzymes of a neural nature rather than humoral. *H. ilisha* in river is starving and hence it can be assumed that no bile is produced. Degenerative alterations were observed in liver (Chapter 3). These might be the possible reasons for the absence of enzymic activity in nerves in the liver of river *H. ilisha*.

Speculations regarding the rôle of cholinesterases in the liver are many. Hepatic origin of plasma cholinesterase has been suggested. Assimilation of food also has been considered (as reviewed by Gerebtzoff, 1959). Cholinesterase may play a role in fat metabolism which is actively taking place in the liver, in feeding as well as starving fish (Chapter 4). This speculation is based on the report of Clitherow *et al.* (1963) who reports that "possibly enzyme acts by inactivating butyryl-

choline which might be synthesized by the choline acetylase system from butyryl coenzyme A formed in the course of fatty acid metabolism".