CHAPTER 11

HISTOLOGICAL STUDIES OF SECRETORY CYCLE OF CATECHOLAMINE SECRETORY CELLS OF HEART OF HILSA ILISHA AND HILSA TOLI

The demonstration of catecholamine secreting cells in the hearts of primitive vertebrates by several workers has opened up the possibility of attributing some endocrine function to the heart. The reports of the work done on these catecholamine secreting cells, at present do not throw light on their functional significance but the release of products by these cells and the nature of the secretion are wellknown (Augustinsson et al., 1956; Ostlund,et al., 1961; Bloom et al., 1962). The electron microscope studies of these cells have confirmed the presence of catecholamine granules in the main body of the cells as well as in the protoplasmic processes of the cells (Bloom et al., 1962). The role of catecholamine, especially epinephrine, as stimulator of contraction in isolated mammalian heart was demonstrated by Hoffman et al. (1945). Thus catecholamines of heart now attract the increased attention of research workers.

In the present investigation, the changes in the secretory cells of the heart of migratory H. ilisha and non-migratory H. toli were studied.

MATERIALS AND METHODS

Live specimens of H. ilisha and H. toli were collected

from the net. They were sacrificed immediately by decapitation and the heart was fixed in Bouin's fixative. Wax sections of 15-20 μ thickness were cut. Haematoxyline-eosin and chrome alum haematoxylin-phloxine staining (Pearse, 1961) were employed. The following staining procedure as modified by Bloom et al. (1962) was adopted for the specific staining of catecholamine secretory cells of the heart.

Procedure:

1. Deparaffinize and pass through ammoniated alcohol (1% ammonia in 70% alcohol) to distilled water.
2. Impregnate in 10% silver nitrate in N/1000 HNO_3 at 37°C for 24 hours.
3. Rinse for two minutes in several changes of distilled water.
4. Treat sections with 2% Borax in N/20 NaOH for one minute.
5. Develop for 5 minutes at 37°C in a solution containing 1 g. hydroquinone and 5 g. anhydrous sodium sulphite in 100 ml of borax solution.
6. Rinse in several changes of 50% alcohol for 5 minutes.
7. Tone in solution of gold chloride (1g. $HAuCl_4$ and 6 drops glacial acetic acid in 200 ml of distilled water) for 30-60 seconds.
8. After rinsing again in water, treat for 3 minutes with a solution of 5 g. oxalic acid in 1000 ml of 50% alcohol at 37°C.
9. Rinse in tap water and fix in 5% aqueous sodium thiosulphate for about 10 seconds.
10. Rinse again in tap water, dehydrate and mount in DPX.

RESULTS

Examination of silver-impregnated sections with light microscope showed a large number of dark brown and light reddish brown cells scattered throughtout the ventricle. The cells were polygonal, long and slender. They were bipolar or multipolar, with long slender processes. These processes were observed to be branching, often coiles, and usually tapering at ends. Very often they came in contact with neighbouring cells and adjascent muscle fibres.

These cells were not observed in sections stained with haematoxyline-eosin but were stained bluish when stained with chrome- alum haematoxylin-phloxine staining.

These cells were also observed in the wall of the blood vessels, with the processes ramifying in the wall of the blood vessel and often travelling towards the lumen, ending in fine, tapering ends near the lumen.

Catecholamine granular secretory cells (CGS cells) were very small and few in number in the heart of fingerlings of H. ilisha returning to the sea. The protoplasmic processes were found traversing considerable distance, but were few in number (Fig. 1).

CGS cells were numerous in the hearts of immature, migratory H. ilisha, before spawning migration, captured from sea. They were deeply stained giving a dark brown colour not only to the cell body proper but to the long, coiled protoplasmic processes also. Very often darkly stained bulb-like structures

were observed in the processes. Many of the CGS cells were bipolar, while a few were unipolar (Fig. 2). CGS cells were found in the wall of the blood vessels with processes ramifying in all directions and some of these endings near the lumen.

In sections of the heart of mature migrating H. ilisha captured from river the CGS cells were few in number and were not easily visible unlike those in the heart of immature H.ilisha from sea prior to migration. CGS cells were also found in the wall of blood vessels, with processes spreading in all directions though staining affinity was feeble.

When sections of heart of spent H. ilisha captured from river were examined, very rarely the CGS cells were observed. However, long, faintly stained protoplasmic processes were seen. The staining affinity was found to be the weakest of all the different stages of maturity. Faint CGS cells were seen around the lumen in heart (Fig. 3).

In the sections of the heart of drifted non-migratory H. toli collected from esturine zone, many dark,brown and reddish brown cells were observed scattered throughout the heart (Fig. 4). They had long, comparatively thick process traversing very long distances. At several places bulb-like structures were also observed on the long processes (Fig. 5). The cells foumd around the lumen showed certain interesting features. Their comparatively thick protoplasmic processes were travelling towards the lumen of the blood vessel and ending in the close viscinity of the lumen (Fig. 6). The protoplasmic processes near the end were tapering and pointed. There were

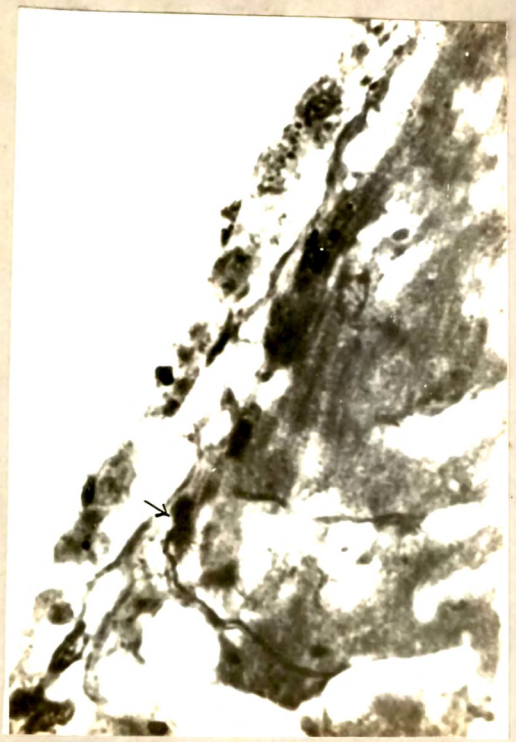

Fig. 1

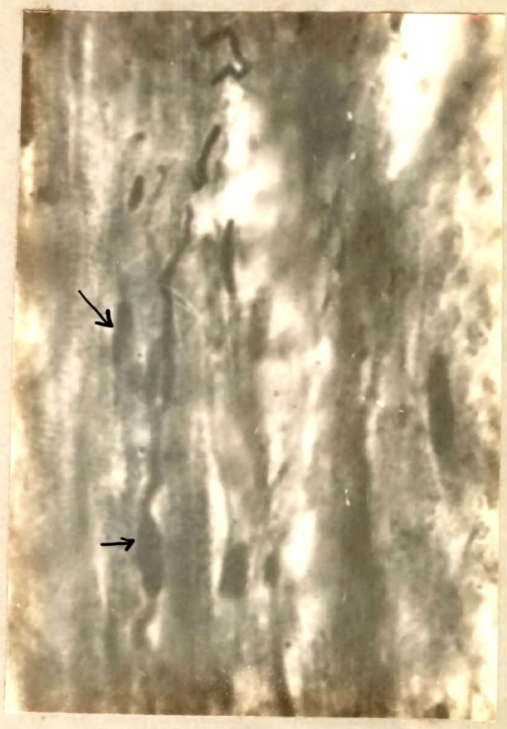

Fig. 2

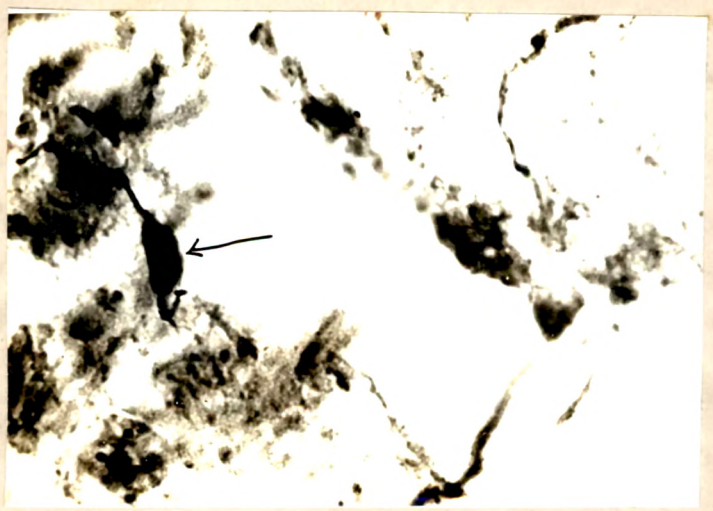

Fig. 3

Fig. 1.CGS cells (arrow) with process in the heart of fingerling of H. ilisha. X 1000.

Fig. 2.Bipolar CGS cells (arrow) in the heart of immature H, ilisha. X 1000.

Fig. 3.CGS cell (arrow) in the heart of mature H. ilisha. X 1000.

Fig. 4

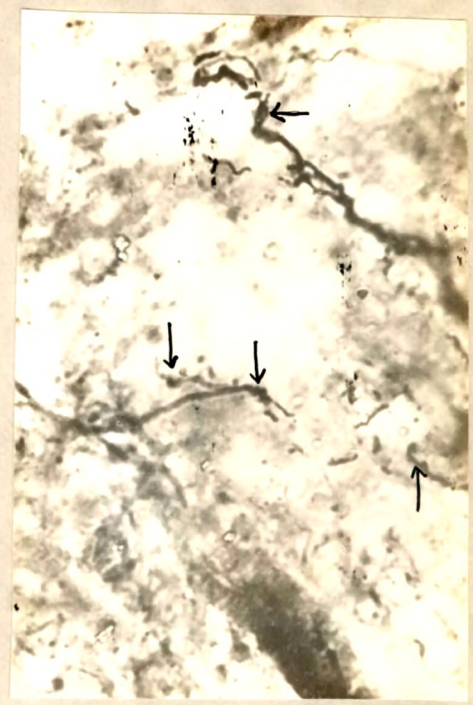

Fig. 5

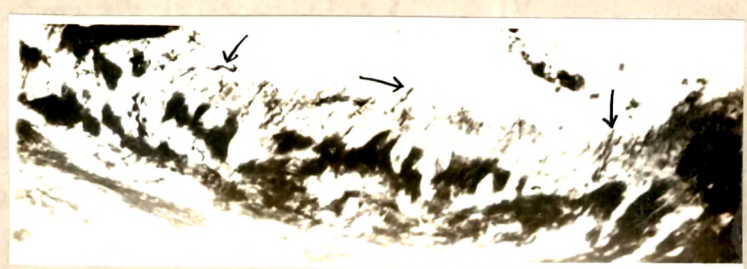

Fig. 6

Fig. 4. CGS cells (arrow) in the heart of drifted H. toli. X 1000.

Fig. 5. Beaded processes (arrows) in the heart of drifted H. toli. X 1000.

Fig. 6. Processes (arrows) traversing towards lumen in the heart of drifted H. toli. X 1000.

many processes from the CGS cells, situated at a little distance from the blood vessel, traversing towards the lumen. These types of protoplasmic processes were observed prominently in drifted non-migratory H. toli only, which were captured from esturine zone.

The CGS cells from the heart of immature H. toli, captured from sea, were deeply stained and their processes were with bulb-like structures. These processes extended upto long distances and were found intensely coiled.

Upon examination of sections of hearts of mature non-migratory H. toli from sea, it was found that CGS cells were less in number and staining affinity was poor. Mostly faintly stained cells were observed. The CGS cells in the wall of blood vessels were also faintly stained.

CGS cells in the heart of spent H. toli captured from sea were very few in number and visible rarely. A large number of faintly stained protoplasmic processes were seen. The general staining affinity was much resuced.

DISCUSSION

From the results obtained by several workers (Östlund et al., 1960; Bloom et al., 1962) there remains little doubt of the nature of secretion of the cells. But the functional significance of these cells has not yet been clearly understood. Treatment with reserpine has been shown to decrease the staining affinity of these cells (Bloom et al., 1962) and cause a decrease in the number of cells.

In the present investigation, in almost all the specimens of different stages of maturity, CGS cells were found in the walls of blood vessels and protoplasmic processes from these cells were observed traversing towards the lumen. This may indicate that catecholamines are discharged into the blood stream.

It was also observed that, when the migratory fish ascends the river for spawning during maturity, and in the non-migratory fish at the time of spawning and maturity, the number of CGS cells were very few and the staining affinity was markedly low. Similar but more pronounced changes were observed in the spent migratory and non-migratory fishes, This may be due to increase in demand of catecholamines when metabolic activities are high during migration and spawning and more energy is needed during migration and spawning, especially when the fish is starving. This is supported by the work done (Desai, 1967) on the increased activities of chromaffin tissues of H. ilisha and H. toli during migration and spawning.

The presence of secretory cells at the peak of the storage phase in drifted non-migratory H. toli may be due to the stress to which the fish is subjected due to sudden changes in salinity, from hypertonic sea water to hypotonic esturine water.

The discharge of catecholamine for lipolysis or fat mobilization from liver is essential during migration and spawning (Chapter 4). As the energy is needed the fat is to be transported from the liver . From the quantitative estimations of

the fat content of liver and gonads (dry weight) of both migratory and non-migratory fishes, it is presumed that fat from liver is utilized during starvation period.

As the swimming activities are increased during spawning and migration, more blood supply is needed and the heart has to work more. It has been shown that more catecholamines may increase the force of contraction and the phosphorylase activity of the heart muscles of mammals(Mayer and Moran, 1960, cf. Weiner, 1964).

In short we may suggest the following probable functions for the CGS cells of the heart of migratory and non-migratory H. ilisha and H. toli.

1. Their discharge of products in mature and spent fishes may help to increase the force of concentration of heart to supply more blood to the various organs during migration.
2. The catecholamines discharged in mature and spent fishes may stimulate lipolysis and induce mobilization of fat from liver.