CHAPTER VI

STUDIES ON THE ADRENOMEDULLARY CELLS(CHROMAFFIN CELLS) OF MIGRATORY H. ILISHA (HAM.) AND NON MIGRATORY H. TOLI (CUV. & VAL.)

It is believed that, throughout the vertebrates, the secretion of hormones by chromaffin cells and cortical cells (interrenal tissues of fishes) is based on similar plan. Adrenaline being the predominant product of adrenomedullary cells in comparison with noradrenaline, later is secreted more in cold blooded vertebrates (Von Euler, U.S., and Fänge,R; 1961). Teleost chromaffin tissues secrete predominantly adrenaline (Von Euler, Fänge, R; 1964). It is also stated that histochemical demonstration of adrenaline and noradrenaline is not so confirmatory, as some other substances (dopamine, dihydroxytryptamine etc.) also react in similar pattern with the chromium salts.(Bertler, A; Fläck, B.; Hillärp, N.-Å.; Rosengren, E; and Torp, A.; 1959). Numerous studies have also demonstrated that a fairly good correlation exist between catecholamine concentration determined by quantitative chemical methods and the intensity of the chromaffin reaction (Eränko, 1955; Hillärp and Hökfelt, 1954, 1955;Coupland and Exley, 1957; Eränko and Hopsu, 1958). Several workers have applied this reaction for the demonstration of chromaffin tissues in the head kidney of teleost fishes (Kräuter,1951,1958; Nandi, 1962; Van Overbeeke, 1960) but satisfactory results for

separate adrenaline and noradrenaline could not be obtained, even with potassium iodate (Hillärp and Hökfelt, 1955) in the present investigations also. Chavin (1966) admitted failure to differentiate epinephrine - from norepinephrine - secreting cells in fishes. It is now established fact that the chromaffin tissues and cortical tissues (interrenal glands) are situated in the head kidney of teleost fishes (Giacomini, 1902 - as cited by Bern and Nandi, 1964; Van Overbeeke, 1960; Nandi, 1962; Honma, 1960a,b; Oguri, 1960a,b; Robertson and Wexler, 1959; Kräuter, 1951, 1958; Rasquin, 1951; Rasquin and Atz, 1952; Rasquin and Rosenbloom, 1954) and are described as cords, clumps or strands of the cells around the post-cardinal veins or along their branches usually within the anterior kidney.

A fairly good deal of literature about chromaffin tissues and interrenal cells of teleost fishes have been published in last few years (Van Overbeeke, 1958; 1960; Honma, 1956a,b; Oguri, 1960a; Nandi, 1962; Kräuter, 1951; 1958; Rasquin, 1951; Rasquin and Atz, 1952; Nandi and Bern, 1959, 1960, 1963,1965; Robertson and Wexler, 1959, 1960, 1962c; Robertson et al., 1963; 1962b; Idler et al., 1959; Schmidt and Idler, 1962; Hane, S. and Robertson, 1959; Chavin, 1966). An attempt is here made to study the activities of the chromaffin cells in migratory (anadromous) fish, Hilsa ilisha (Ham.) and in non-migratory fish Hilsa toli (Cuv. & Val.). This histological

and cytological study may throw light on the probable functions of adrenaline and noradrenaline in these fishes in relation with the changes found in endocrine organs, other visceral organs, neurosecretory activities of nuclei preopticus (NPO) neurohypophysis and in the ion concentrations of river and sea water.

MATERIALS AND METHODS

Firstly, it is felt to state that all the live samples of fishes taken from the fishing nets were sacrificed within two minutes after capture and attention was paid to discard wriggled fish; to avoid the effect of stress. Head kidney was immediately exposed, removed from the viscera and was fixed in Bouin's fixative (Gurr, 1956), half of the same head kidney was fixed in Orth's fluid (Lillie, 1954) 90 ml. of Muller's fluid (1% Na sulfate and 2.5% K dichromate) to which 10 ml. of formalin was added prior to fixation and just before use. The tissues were fixed for 24 hours to 48 hours in both the fixatives and were washed in running tap water for 24 hours. Serial Paraffin sections of 7 to 10 μ were taken and were stained with haematoxylin-eosin. The species, sex and stage of gonad were determined macroscopically in the field. For confirmation of stages of maturity gonads, histologically, Bower's (1954) and Gokhale's (1957) classification of gonads were used. Several head kidneys were fixed in

Orth's fluid also using Potassium iodate (30 gm/1000 ml of water) in place of muller's fluid (Hillärp and Hökfelt, 1954; Eränko and Hopsu, 1958). Mouse adrenals were used as controls, for this method, fixation time, dehydration, embedding time and staining time of which were exactly same as maintained for head kidney of fishes utilized for present investigations.

RESULTS

Due to the application of chromaffin reaction and precautions taken (Fälck and Hillärp, 1959) it was possible to distinguish chromaffin cells from the surrounding hoemopoetic tissues. Kidney tubules and interrenal cells occassionally coming in contact with them. After or this fixation, the chromaffin cells were differentiated by the yellowish brown colouration of the cytoplasm of the cells, which were of more uniform appearance than interrenal cells. These cells did not show any colour after Bouin's fixation. In both, Hilsa ilisha (Ham.) and Hilsa toli(Cuv. & Val.) chromaffin cells were found only interspersed among hoemopoetic tissues and kidney tubules. This type of cells come into type-V of chromaffin cells of teleosts according to Nandi(1962).

Chromaffin cells of Fingerling of Hilsa ilisha (Stage I of maturity) captured from river Narbada:

Chromaffin reaction helped in giving a separate entity to chromaffin cells distributed in head kidney. There were

few cells in the individual groups, these groups were found isolated from each other and were found to be situated in viscinity of interrenal cells and branches of pericardinal veins in the head kidney. These cells had dark brown colour. (Fig 1)

Chromaffin cells of immature Hilsa ilisha (Ham.) captured from sea prior to migrations in the river Narbada:

More cells were found to be added to the individual group of the chromaffin cells. Few small groups or newly formed groups were also observed isolating and separating from the mother groups. The intensity of the chromaffin reaction, as judged by colour intensity remains same. The cytoplasm of those cells showed pale brown perfect colouration due to the application of the chromaffin reaction. In many cells mitotic division were easily observed. In some of the chromaffin cells, nuclei were pycnotic and shrunk. These nuclei did not show perfect round shape, instead showed wavy outlines only. In few cells nuclei were found to be completely destroyed, and only pale brown colour of the cytoplasm was observed. Due to increase in number of groups and clumps of the chromaffin cells, can we presume that adrenaline and noradrenaline secretion is increased prior to migration? (Fig.2).

Chromaffin cells of mature Hilsa ilisha(Ham.) captured during migration from river Narbada:

Macroscopically also the chromaffin cells could be located after utilising chromaffin reaction. There were many groups of

the chromaffin cells in the head kidney and number of cells in each group exceeds far more than the number of cells found in group of chromaffin cells of immature Hilsa ilisha (Ham.) captured from the sea prior to migration. The cells were solidly packed and uniform in appearance. Intensity of chromaffin reaction remained unalterable. In all most all the cells, nuclei showed degenerative changes, pycnosis, and shrunk appearance of the outline. In few cells degenerated nuclei were not observed, leading us to believe the complete destruction of nuclei.

There were no more remarkable changes observed in chromaffin cells of fishes captured from freshwater zone- Maktampur and Zanor than Hilsa ilisha captured from estuarine zone - Bhadbhoot, vide map 2, except in chromaffin cells of former more chromaffin cells were showing degeneration and destruction of nuclei and little separation of the chromaffin cells from the groups. (Fig.3)

From the above mentioned results, it can be stated that due to more secretion of adrenaline and noradrenaline the nuclei of the chromaffin cells exhibited degenerative changes and number of groups of chromaffin cells are multiplied to provide more hormones needed for 'stress' experienced by migrating Hilsa ilisha (Ham.) migrating from hypertonic medium(sea)

to hypotonic media,(estuarine water and fresh water). This might be due to 'stress' due to starvation also as Hilsa ilisha(Ham.) never feeds during migration.

Chromaffin cells of spent Hilsa ilisha (Ham.) from river Narbada:

The groups of chromaffin cells, have increased in number and in size also, as supported by increase in cell height. These groups were loosely arranged and some small groups were seen completely isolated from bigger groups, forming separate groups. The intensity of the chromaffin reaction remained unchanged. The nuclei of all the chromaffin cells showed pycnosis and tremendous degeneration. Many nuclei were not seen in the cells, instead haematoxyline positive materials were observed scattered in the cytoplasm of the cells, might be disintegrated particles of nuclei which were destroyed. In some few cells only outlines of nuclei were observed faintly. These tremendous degenerative changes were exhibited by glomerulii, tubules and interrenal cells also. (Fig.4)

It may be suggested that more adrenaline and noradrenaline is secreted more because of the 'stress' felt by Hilsa ilisha due to hypotonic medium.

Chromaffin tissues of nonmigratory Hilsa toli (Cuv. & Val.) captured from sea:

There was remarkable difference observed in the number of the chromaffin cells than in immature Hilsa ilisha (Ham.) captured from sea prior to migration, the number of chromaffin cells and number of groups of chromaffin cells and number of groups of chromaffin cells was too less though intensity of chromaffin reaction remained the same. The nuclei of these cells are round in shape and filled with finely granular chromatin material and are uniform in shape. Occassionally nuclei appeared to be spindle shaped. (Fig.5)

The difference in number of chromaffin cells and group observed above may suggest that more adrenaline and noradrenaline secretion in migratory Hilsa ilisha (immature) may be to increase cardiac out put for supply of more energy or to provide more energy to the muscles.

Chromaffin cells of nonmigratory mature Hilsa toli (Cuv. & Val.) captured from sea:

Even though Hilsa toli (Cuv. & Val.) were of same stage of maturity of Hilsa ilisha (Ham.) the chromaffin cells of former did not show hyperplasia as exhibited by later. The number of cells in each group and number of groups is more

than that of found in immature Hilsa toli (Cuv. & Val.) captured from sea. The groups of the chromaffin cells are found scattered, with more number of cells, in the head kidney interspersed among interrenals. The nuclei of the cells of chromaffin tissues show pycnosis, but mitotic divisions were also shown by few cells. The intensity of chromatin reaction was found to be similar to that of found in mature Hilsa ilisha (Ham.) captured from river. (Fig.6)

The results mentioned above may indicate that during maturity more adrenaline and noradrenaline is secreted by chromaffin tissues due to stress of gonadal development, whereas migratory Hilsa ilisha (Ham.) has to undergo three types of stress (i) stress due to starvation, (ii) stress due to gonadal development at the cost of starvation and (iii) stress due to change in environment, medium, hypertonic to hypotonic, and it is quite possible that due to above mentioned 'stress' migrating Hilsa ilisha (Ham.) secretes more adrenaline noradrenline than nonmigratory Hilsa toli(Cuv. & Val.) as later has not felt more stress.

Chromaffin tissues of spent Hilsa toli(Cuv. & Val.) captured from sea:

The chromaffin cells were found to be more in number and showed degenerative changes, but number was less than the

chromaffin cells observed in spent Hilsa ilisha captured from river on return to sea after laying eggs in freshwater zone (vide map 2) of river. These chromaffin cells showed little separation in individual group. The intensity of the chromaffin reaction was observed to be less. The nuclei were pycnotic and many cells showed distintegrated and degenerated nuclei. The groups of the chromaffin cells were observed sometimes devoid of nuclei.

The profound, clear and more degenerative changes exhibited by migratory spent Hilsa ilisha of same stage of maturity may support the hypothesis that H. ilisha had to experience more stress than the later due to migration and return migration to sea.

Chromaffin tissues of immature Hilsa toli(Cuv. & Val.) drifted into the estuarine zone (Bhadbhoot) of river Narbada due to force of current of sea felt on the highest high tide day of the year:

The chromaffin cells were very few in number and number of scattered groups was also few. The intensity of the chromaffin reaction remained same. Nuclei of the chromaffin cells were round in shape and were filled with finely granular chromatin. Mitotic divisions were also observed in some of the groups of the chromaffin cells. It

is worthnoting here that some of the chromaffin cells were found to be shrunk and destroyed. These cells were found scattered near blood vessels. This destruction may be due to stress felt by Hilsa toli as it is drifted into the hypotonic medium.

Chromaffin tissues of matured Hilsa toli (Cuv. & Val.) captured from the estuarine zone (Bhadbhoot) of river Narbada due to force of current of sea felt on the highest high tide of the year:

The groups of the chromaffin cells have not increased much as observed in migratory mature Hilsa ilisha of the same stage of maturity but groups have increased number of the cells in them than cells found in groups of immature Hilsa toli, captured from sea of the same stage of maturity. Prominent and profound degenerative changes were observed viz. shrinkage and destruction of chromaffin cells, pycnosis of nuclei in few chromaffin cells, complete destruction of nuclei in many of the cells of the chromaffin tissues. Occassionally single chromaffin cell was observed completely shrunk and was observed scattered near blood vessles. The intensity of the chromaffin reaction remains unchanged.

Chromaffin tissues of spent Hilsa toli(Cuv. & Val.) captured from the estuarine zone Bhadbhoot - on the day of highest high tide of the year:

TABLE I

THE ENHANCED ACTIVITY OF THE CHROMAFFIN TISSUE OF MIGRATORY H. I

OF FAT FROM THE LIVER, ADIPOSE TISSUE AND FATTY LA

SPECIES	SEX AND STAGE OF GONADS	% OF FAT IN DRY WEIGHT OF LIVER	% OF FAT IN DRY WEIGHT OF GONADS	CHROMAFFIN TISSUES
Hils ilisha (Ham.)	I Sex could not be distinguished			Fewcells in few small groups scattered in the head kidney in vicinity of interrenals.
Hilsa ilisha (Ham.)	0 - III	43.6	22.3	Cells are enlarged in size and more number of cells are observed in more number of groups of cells. Individual groups are small and nuclei of these cells show mitotic divisions.
	0 - V	62.7	28.5	
	♀ - IV	64.4	21.4	
	♀ - VII -II	50.5	19.7	
Hilsa ilisha (Ham.)	0 - V	29.3	36.9	Hyperplasia observed. Groups are many and easily seen macroscopically. Cells in individual groups are plenty. Groups are solidly pocked (cells). Nuclei show pycrosis in most of the cells, nuclear degeneration and (few) cells are shrunk. Complete destructions of nuclei are also observed.
	♀ - VI	49.1	55.3	
	♀ - V	26.1	56.8	
Hilsa ilisha (Ham.)	0 - VII) ♀ - VII)	23.4	10.5	Loosely arranged cells in big groups. Number of cells in bigger group has increased few small groups are separating from bigger groups. Nuclei of many cells are completely lost. Nuclear degeneration also observed in many cells.

Almost all the chromaffin cells were shrunk and degenerated. The groups of chromaffin cells were found to be broken and cells were observed scattered in the head kidney, occassionally single shrunk chromaffin cell. Nuclei of the cells of the chromaffin tissue exhibited total destruction and rare pycnosis. The number of cells in individual group of the chromaffin cells and number of group of chromaffin cell was comparatively very less than found in spent Hilsa ilisha of the same stage of maturity. In later, very few cells were shrunk and rarely cells were destroyed. This tremendous change or destructive phase, might be result of stress caused by hypotonic medium and stress of release of hormones from the body cavity.

DISCUSSION

It can be stated that the hyperplasia of the chromaffin tissues in mature and spent, migratory Hilsa ilisha (Ham.) and nonmigratory Hilsa toli (Cuv. & Val.) may be due to 'stress' occasioned by maturity, and starvation. The destruction of few chromaffin cells and degenerative changes shown by the chromaffin tissues of mature and spent Hilsa toli (Cuv. & Val.) drifted into the estuary-hypotonic medium-as shown in the table, on the highest high tide day of the year due to the force of current of sea - may be due to stress

caused by maturity, starvation and predominently by the sudden change into hypotonic medium (Refer table 2). This factor may be also playing major role in formation of hyperplasia of chromaffin tissues in mature H. ilisha (Ham.) during maturation, and migration. After examining several stomachs and intestines we have come to conclusion that both migratory Hilsa ilisha (Ham.) and Hilsa toli (Cuv. & Val.) do not feed during spawning but feeding is resumed after achieving spent stage of maturity, it is also interesting to note that migratory Hilsa ilisha (Ham.) do not feed in river even after spawning,it feeds only in sea after spawning and before spawning. Even after resumption of food in-take and when 'stress' of maturity is not felt, chromaffin tissue persists hyperplasia this may be explained as under:

If the above mentioned 'stress factors' (1) starvation, (2) stress due to maturity (3) and stress due to change into hypotonic medium during migration are eliminated and the role of catecholamines in fat metabolism is considered, it is possible to derive some probable results from the above mentioned tables. It is known that catecholamines increase force of contraction and phosphorylase activity, catecholamines(Epinephrine and norepinephrine especially) produce marked increase in plasma non-esterified fatty acids in fat pads and incubation with epinephrine leads to an increase in glucose uptake into

tissues and release of nonesterified fatty acids (For review, Weiner, N., 1964). Several workers have laid stress on presence of epinephrine for lipolysis (Sidman et al., 1962; Smith et al., 1962; Weiner et al.,1962 - as cited by Weiner, 1964). Vaugham (1960) and Vaugham et al. (1962 - as cited by Weiner, 1964) have also shown that ACTH activity on fat metabolism is dependent on presence of epinephrine. When fat content (percentage of fat content in dry weight of liver) of liver of Hilsa ilisha (Ham.) of mature stage prior to spawning, is compared with that of spawning and migratory Hilsa ilisha of V & VI stage of maturity, a significant decrease in later by 43% and 29% and 64% and 26% in male and female respectively is noticed. This may be due to mobilization of fat from fat depot-liver, for the demand of more energy for migration during starvation and for the development of gonads. It is quite probable catecholamines demand may increase for the mobilisation of fat and hence hyperplasia of the chromaffin tissue is observed in migrating Hilsa ilisha (Ham.). It is also worth noting that the adipose tissue and a fatty layer observed in muscle have disappeared from migrating mature Hilsa ilisha (Ham.) (unpublished work from our laboratory). This may be for the utilisation of stored fat for the development of gonads or for the supply of energy during migration and starvation.

Hyperplasia in chromaffin tissue in migrating mature Hilsa ilisha (Ham.) may be responsible for this mobilisation of fat.

The significant increase of 37% of fat content of liver in spent Hilsa ilisha (Ham.) captured from the sea (returned from spawning grounds of river) than spent Hilsa ilisha (Ham.) captured from river may suggest that fat is again stored in liver as soon as resumption of food intake begins, in absence of the adipose tissue the liver (unpublished work from our laboratory) must be supplying fuel for return migration to sea and mobilization of fat from liver may also need catecholamines, this is evident histochemically.

The little hyperplasia of the chromaffin tissue of mature (Nonmigratory Hilsa toli (Cuv. & Val.) and spent Hilsa toli (Cuv. & Val.) may be due to same reason. For mobilization of fat from liver in absence of adipose tissue and fatty layer from muscles (unpublished work from our laboratory) as this fish also do not feed during spawning but resumes feeding after achieving spent stage.

When the percentage of fat content of liver of migratory Hilsa ilisha (Ham.) of V stage of maturity, captured from sea is compared with that of nonmigratory

Hilsa toli (Cuv. & Val.) of same stage of maturity, a significant difference of about 30% is noticed. This may suggest that comparatively more fat is stored in liver of migratory Hilsa ilisha (Ham.) prior to spawning. For the mobilisation of large quantity of stored fat from liver, more catecholamines from the chromaffin tissues may be secreted in migratory Hilsa ilisha (Ham.) in comparison wit the nonmigratory Hilsa toli (Cuv. & Val.). The percentage of fat in liver of migratory Hilsa ilisha (Ham.) during spawning is approximately similar to the fat content of liver of mature nonmigratory Hilsa toli. This also shows that fat from liver might have been supplied to the organs for the development of gonads or for migration.

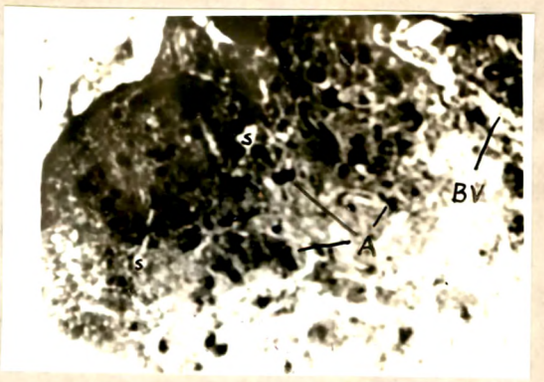

Fig. 1

Small groups of the chromaffin cells fingerling of H. ilisha.
Tissue fixed in Orth's, stained with Haematoxyline and eosin, x63.
A - Chromaffin cells, S - Sinusoids, B.V. - Blood vessels

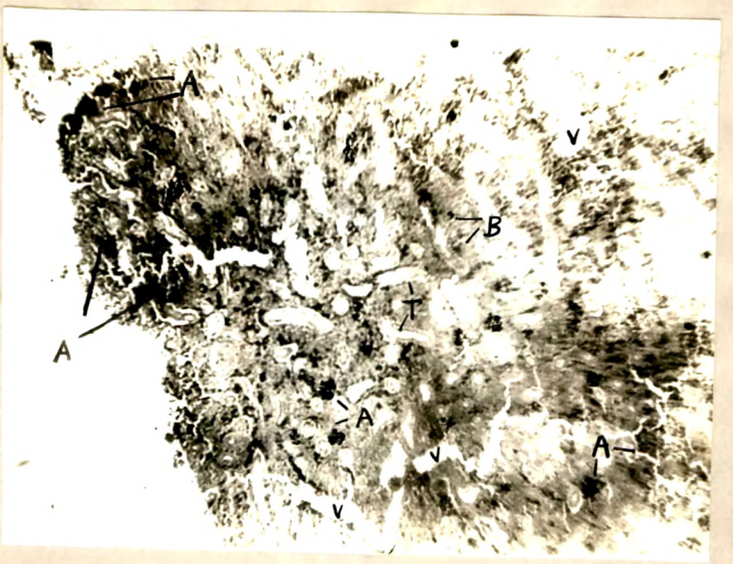

Fig. 2

Chromaffin cells of immature H. ilisha captured from the sea.
Tissue fixed in Orth's, stained with Haemotoxyline, x63
A - Chromaffin cells in large groups, T - Tubule of kidney
B - Chromaffin cells in small groups, V - Venules

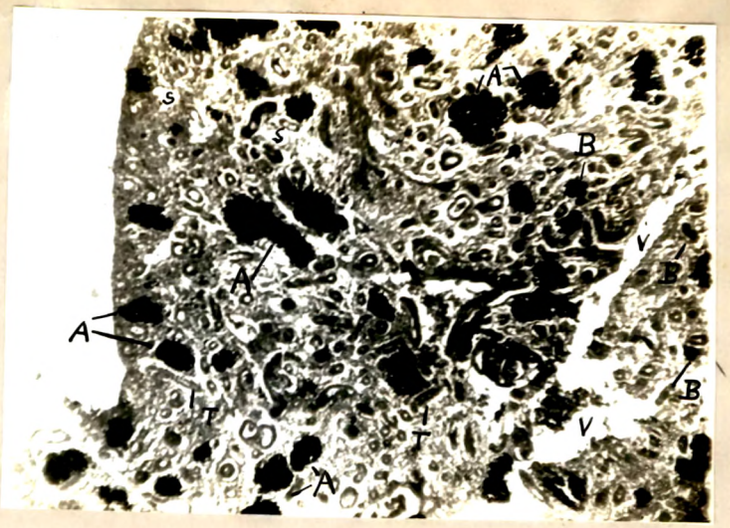

Fig. 3

Chromaffin cells of the migrating, mature H. ilisha.
Tissue fixed in Orth's, Haematoxyline
and eosin stained, x63
A - Chromaffin cells in groups, T - Tubule
B - Small group of chromaffin cells, V - Venule, S -Sinusoid
(Note the tremendous increase in chromaffin cells)

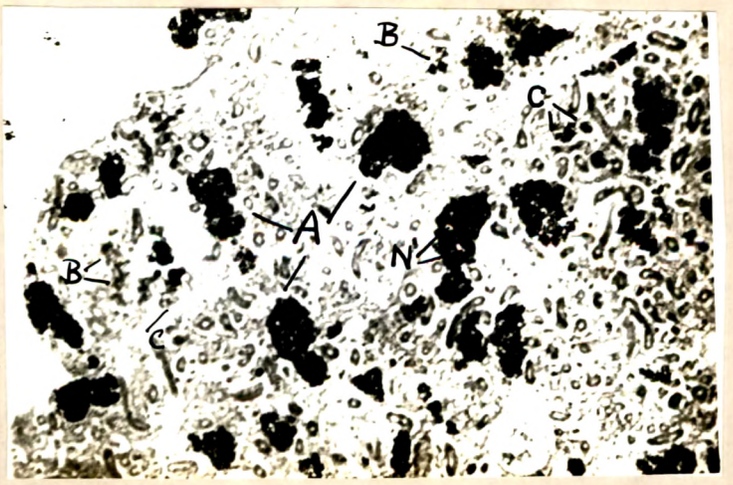

Fig. 4

Chromaffin cells of the spent H. ilisha.
Tissue fixed in Orth's,
Haematoxyline & eosin stained, x63.
A - Shrunk large group of chromaffin cells
N - Large pycnotic nuclei of chromaffin cells,
B - Small isolated group of chromaffin cells
C - Somewhat shrunk and destroyed group of 'A'

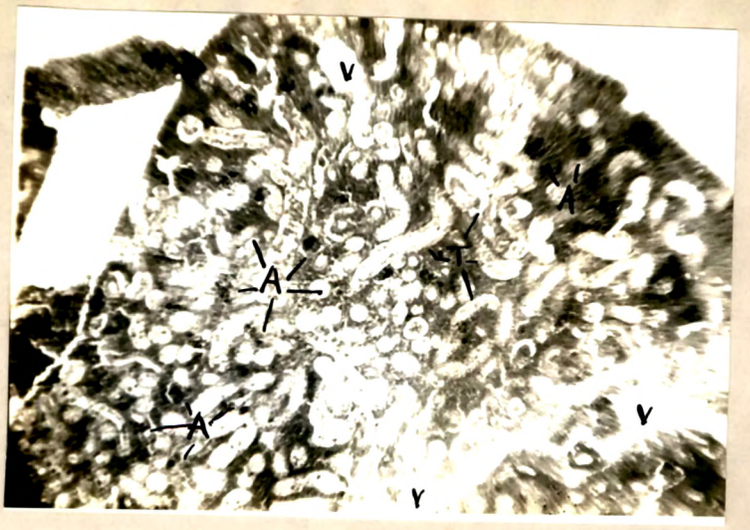

Fig. 5

Chromaffin cells in few small groups of the non-migratory immature H. toli.
Tissue fixed in Orth's stained with Haematoxyline eosin, x63.
A - Small, scattered group of chromaffin cells
T - Tubule, V - Venule

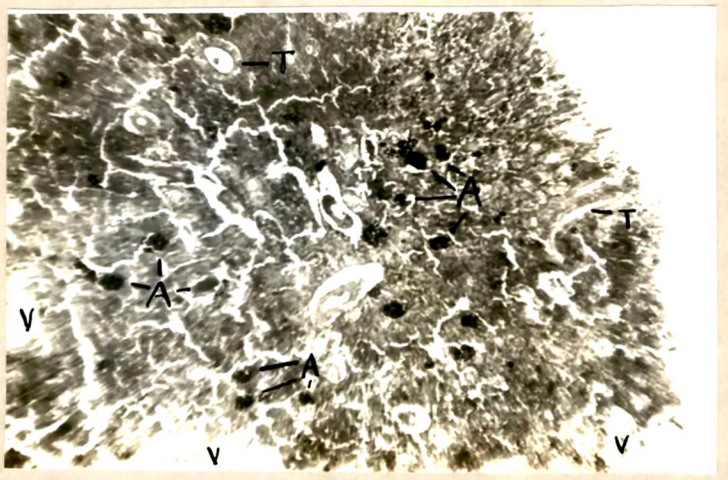

Fig.6

Chromaffin cells in the large groups of the mature non-migratory H. toli.
Tissue fixed in Orth's, stained with Haematoxyline and eosin, x63.
A - Large group of chromaffin cells, T - Tubule, V - Venule
Note mild hyperplasia of chromaffin cells.