CHAPTER VII

HISTOLOGICAL AND HISTOCHEMICAL STUDIES OF ADRENOCORTICAL TISSUES OF MIGRATORY (ANADROMOUS) HILSA ILISHA(HAM.) AND NON MIGRATORY HILSA TOLI (CUV. & VAL.)

The interrenal gland-adrenocortical tissue-mammalian adrenal cortex homologue- was located in the head kidney of Hilsa ilisha and Hilsa toli in the form of scattered groups of cells and cords of cells surrounding branches of pericardinal veins of the head kidney. Many workers have described the interrenal glands or adrenocortical tissues of teleost fishes as diffuse tissue lying alongside of post-cardinal veins or scattered in the head kidney (Kräuter, 1951,1958; Olivereau and Fromentin, 1954; Overbeeke, 1960; Oguri, 1960a; Hona,1960a; Robertson and Wexler, 1959; Robertson et al., 1961b). Numerous studies have shown that the adrenocortical tissue of fishes is homologus to the adrenal cortex of the mammals (Review by Pickford and Atz, 1957; Chester Jones, 1957; Chester Jones et al., 1959; Chester Jones and Phillips, 1960; Bern and Nandi, 1964; Nandi and Bern, 1959, 1960,1963,1965; Chavin and Kovacevic, 1961; Chavin, W., 1966). The results of the work done by several workers have confidently suggested that corticosteroids are secreted by adrenocortical tissue of teleost fishes and they are similar to the higher mammals (Nandi and Bern,1959,1960,1963, 1965; Ogawa, 1963).

Several workers have noted very high content of corticosteroids in blood, during spawning migration of fishes (Hane and Robertson, 1959; Schimidt and Idler,1962) and during peak of maturity also. Robertson et al. (1961b) have noted significant rise in level of 17-Hydroxycorticosteroids in migratory (anadromous) Rainbow trout (Salmo gairdnerii) during sexual maturation and spawning and in nonmigratory Pacific salmon (Genus Oncorhynchus) during maturity accompanied with hyperplasia of adrenocortical tissues and several other changes in other organs (Robertson and Wexler, 1957,1959,1960). Oguri (1960b) also noticed hyperplasia in adrenocortical tissues in migrating up Chum salmon, during migration into the river for spawning. Hene, Robertson, Wexler and Krupp (1966) reported the effect of stress and ACTH in Pacific salmon and steelhead trout; stating that stress of holding Pacific salmon in confined space and repeated bleeding, resulted into change in concentration of 17-OHCS levels in blood plasma, steelhead trout showed a higher rise in plasma spawning corticosteroid levels than did spawning salmon following ACTH injection.

MATERIALS AND METHODS

The head kidney of the fish was cut into small pieces. Small pieces were fixed in Bouin's fluid for two days and in 10% cold neutral formalin for 12 to 18 hours. After washing

the tissues in tap water Bouin's fixed tissues were dehydrated, 5 μ paraffin sections were taken and stained by haematoxylin-eosin. Formalin fixed tissues were embedded in 15% gelatin at 37°C for 3 hours and blocks were prepared in cold 6% neutral formalin. Sections were cut on a freezing microtome and rest of the procedure was followed by Khanolkar et al. (1958). Sections were washed in distilled water and transferred into phenylhydrazine-acetic acid mixture (10 ml of phenylhydrazine with 0.5 ml of 1% acetic acid), for 5 to 10 minutes to block plasmal reaction. The sections were then washed three to four times in water and transferred in 5% $FeCl_3$ maintained at 60°C for half an hour. The sections thus treated were washed in water, kept in Schiff's reagent for 30 to 40 minutes, in SO_2 water and then washed in tap water to be finally mounted in glycerine jelly. Counterstaining with haematoxylin was not found necessary as separate paraffin sections were stained by haematoxylin and eosin. Corticoids stained pink. To confirm the reaction the adrenal of rat was used as control.

Paraffin sections revealed good results. Interrenal tissue distinctly separated from the kidney tubules by light eosin stain in H. ilisha and dark eosin stain in H. toli.

RESULTS

Adrenocortical tissue of fingerling of Hilsa Ilisha:

It consists of very small and few cells in the head kidney. They were situated in vicinity of venules. These cells had a nucleus. Nuclei were small and perfectly round and filled with fine granular chromatin (Fig.1). The cytoplasm of these cells showed unclear few granules. These few granules stained pink with Khanolkar et al. (1958) method in gelatin sections.

Adrenocortical tissue of immature Hilsa ilisha captured from sea prior to spawning migration in river Narbada:

The number of groups have increased when compared with that of fingerling. These groups of cells were found around the branches of veins and also in the form of scattered groups in the hoempoetic tissues of the head kidney. These cells were 1 to 3 cell thick and were with round nuclei, filled with finely granular chromatin. A prominant nucleolus was also observed in each nucleus. These cells were less eosinophilic than kidney tubules. The granules were seen moderately distributed evenly in the cytoplasm in paraffin sections (Fig. 3).

After application of Khanolkar's method for corticoids, (Khanolkar et al., 1958) pink, small granules were observed in cytoplasm of few cells distributed evenly. Few groups of cells only showed pink granules (Fig. 2).

Adrenocortical tissue of sexually mature Hilsa ilisha captured from river Narbada during migration:

The adrenocortical tissue of Hilsa ilisha captured from Bhadbhoot, Maktampur and Zanor did not show any significant difference. Hyperplasia of adrenocortical tissue accompanied with following remarkable changes was noticed. Two to three cell thick groups had increased in number and were found scattered in hoemopoetic tissues of the head kidney. These cells were also found around the veins and their branches. An increased in size of the cells was also noticed. They were separated by sinusoids, which sometimes carry blood cells. The nuclei were round. Pycnosis were also observed in them. The big granules were seen distributed densely in the cytoplasm of the cells (Fig. 6).

In gelatin sections, stained for corticoids, pink, big granules were found in all cells, the cells were distributed throughout the head kidney and were of big size (Figs. 4 and 5).

Adrenocortical tissue of spent Hilsa ilisha captured from Bhadbhoot, Zanor and Maktampur during return migration to sea:

The cells were loosely arranged and occassionalyy small groups or individual cell seen separated by sinusoids.

Cells were shrunk and newly formed sinusoids had broken the entire group. Nuclei of many cells exhibited pycnotic retaining round shape (Fig. 9). In few cells, nuclei appeared shrunk and gave ununiform shape. Very few granules were noticed in the cells. Vacuolization was prominent in most of the cells. Staining effinity for eosin was lost and cells were stained very faintly with eosin (Figs.8,10).

In gelatin sections, when stained for corticoids, it was difficult to distinguish adrenocortical tissue from the tubules, in few cells few pink granules were observed (Fig. 7).

In short, profound degenerative changes were noticed.

Adrenocortical tissue of nonmigratory immature Hilsa toli captured from sea:

There was no remarkable difference observed between adrenocortical tissue of immature migratory Hilsa ilisha and that of nonmigratory immature Hilsa toli. The cells were sparse in number and showed similar details of the structures as was observed in H. ilisha (Figs. 11 and 12).

Adrenocortical tissue of mature nonmigratory Hilsa toli captured from sea:

Little but marked hyperplasia was noticed in comparison with mature migratory Hilsa ilisha. Number of groups of cells

was less than migratory fish (Figs. 13 and 14).

Adrenocortical tissue of spent nonmigratory Hilsa toli captured from sea:

The marked and severe degenerative changes as observed in spent migratory were not noticed, though little degeneration was seen. Occassionally vacuolization of the cytoplasm accompanied by pycnosis of nuclei was marked. Very few cells were shrunk and showed complete destruction. The granules were sparse but cells did retained original shape. Staining affinity for eosin was found to be lost, as cells took very faint eosin staining (Figs. 7,8,9,10). In short, degeneration was pronounced,but not upto extent of degeneration noticed in migratory Hilsa ilisha and Hilsa toli drifted into the river on the highest high tide day of the year (see below).

Adrenocortical tissue of immature Hilsa toli drifted into the estuary on the highest high tide day of the year:

Very few groups of cells were scattered around the venules and in the head kidney. It was remarkable to notice few cells destroyed in these groups of the tissue. The empty space left by destroyed cells were occassionally occupied by remnants of destroyed nuclei or only nuclei, debris of curdled, shrunk, destroyed cytoplasm. In some cells, nuclei were not round in shape but they showed uneven shape, though chromatin

material was present in the form of finely granular form. Vacuolization evident and sometimes much pronounced also. Sinusoids did appear among the cells (Figs.15,16,17 and 18).

Adrenocortical tissue of nonmigratory, mature Hilsa toli captured from estuary on the highest high tide day of the year:

The degenerative and predominantly destructive changes were exhibited at climax by this fish. The hyperplasia due to maturity is not doubt can be presumed, but simultaneously destruction causes surprise. Groups of the cells appear shrunk loose and separated from each other. Cell boundaries were destroyed in many cells and in some they were present in the form of wavy outlines only. In other cells many vacuoles were observed and complete vacuolization was also noticed. Few cells showed nuclei in contact with cell membrane only by means of thin cytoplasmic stripes. Few destroyed cells exhibited extrusion of nuclear material into the empty space caused by destruction and dissolution of cytoplasm. Many destroyed cells had left behind the empty spaces; very often, wandering nuclei and remnants of destroyed cytoplasm were observed in empty spaces. Few intact cells exhibited densely packed granules in their cytoplasm (Figs. 15,16,17,18).

In gelatin sections, after staining for corticoids few cells showed uniform, big, pink granules. This may be due to destruction observed in paraffin sections.

Adrenocortical tissues of spent, nonmigratory Hilsa toli captured from estuary on the highest high tide day of the year:

The destructive and degenerative changes exhibited by mature, nonmigratory Hilsa toli drifted into the estuarym persisted and reached peak in this fish. Most of the cells were destroyed and few showed similar degenerative changes as observed in mature drifted Hilsa toli. Very few cells remained normal, when studied, they exhibited degranulation and pycnotic nuclei (Figs. 15,16,17,18).

In gelatin sections, few cells showed the pink few granules in the cytoplasm, when stained for corticoids. This may be due to destruction of adrenocortical cells.

DISCUSSION

It is obvious that H. ilisha has to face tremendous changes of ion concentrations of water. The downfall in ion concentration and starvation during migration and spawning may cause 'stress'. These two factors may be responsible for the hypertrophy of adrenocortical cells of both, mature and

spent H.ilisha. The feeding is stopped during migration and spawning (Our studies on the stomach contents of H. ilisha and H. toli during different periods of life cycle, unpublished). It is believed strongly that Interrenal or adrenocortical glands of lower vertebrates do respond to 'stress' as reacted by adrenal cortex of higher vertebrates (Robertson and Wexler, 1957,1959; Hane et al., 1966). It was also observed that enlargement of the adrenal glands or cytological evidence of stimulation occurs in lower vertebrates, when stress is felt due to administration of ACTH (Hane et al.,1966). The hypothesis put forward by Olivereau and Fromentin (1954) states that high salinities inhibit interrenal function and low salinities stimulates it, is supported by our results, as far as hyperplasia observed in migratory Hilsa ilisha, mature and spent, both are concerned.

Our histological studies pointed out that no remarkable and severe hyperplasia or destructive changes were exhibited by nonmigratory Hilsa toli when mature stage and spent stage are attained. This may be due to the stress caused by change in salinity is not felt but stress due to starvation experienced during spawning. We studied stomach contents of several mature and spent Hilsa toli, but we failed to find any food materials in them (unpublished work from our laboratory). Robertson et al. (1961b) also observed similar changes in anadromous

migratory and nonmigratory Rainbow trout (Salmo gairdnerii) accompanied by elevated concentrations of 17-hydroxycorticosteroids, depletion of lymphocytes from spleen, degeneration of many internal organs etc. The hyperplasia of adrenocortical tissue in Pacific salmon (Onchorhyncus) and Rainbow trout (Salmo gairdnerii) was also observed by Robertson and Wexler (1959) accompanying maturation and spawning (Hane et al.,1966). Oguri (1960[a,b]) also obtained similar results in migrating up Chum salmon. The results obtained by us support the observations of above mentioned workers.

The tremendous degenerative changes exhibited by the adrenocortical cells of nonmigratory immature Hilsa toli drifted into the estuary on the highest high tide day of the years, may be due to unexpected sudden change from hypertonic medium to hypotonic medium-estuary, as stress due to starvation is not felt. Immature Hilsa toli feeds regularly (from our studies on stomach content of Hilsa toli of all stages of maturity - unpublished work), whereas pronounced significant and severe degenerative and dominantly destructive changes of adrenocortical tissues of drifted nonmigratory mature and spent Hilsa toli may be the result of stress occassioned by change of salinity and due to starvation during spawning. It may also be stated that due to sudden increase in demand of corticosteroids during drifting the cells of adrenocortical tissues were

destroyed. Chester Jones, Henderson and Butler (1965a) have reported the significance of role of adrenocortical hormones in Na and K balance in European Eel(Anguilla anguilla L.) and have stressed that adrenocortical hormones do play important role in Na and K balance.

Robertson and Wexler (1959) suggested that cells of the adrenocortical tissues may be differentiated according to their state of activities during spawning and they resemble different zones of the adrenal cortex of mammals. Chavin and Kovacevic (1961) on basis of many histochemical reactions carried out on adrenocortical tissue of Goldfish (Carassius auratus, L.) suggested that adrenocortical tissues of Goldfish are comparable to those of zona fasciculata and zona reticulata of the mammalian adrenal cortex. Chavin(1966) also stated, after studying adrenal histochemistry of adrenal tissue of marine and fresh water fishes that adrenocortical tissue of all species were positive for all components normally reported in the vertebrate adrenal cortex. According to them, relatively small cell types densely granulated cells of immature Salmon may be considered as zona glomerulosa homologue of mammals and larger cells may resemble zona fasciculata of adrenal cortex of mammals as hyperplasia is shown by these cells during maturity. The degenerated cells of spent fish may be similar to zona reticulata.

If andrenocortical tissue of drifted, immature Hilsa toli is observed, one can judge from the groups of adrenocortical tissue, that, some cells are destroyed and shrunk, remaining cells were not affected much. The destroyed cells and cells exhibited pronounce degeneration may possibly be concerned with mineral metabolism and may be considered mammalian glomerulosa homologue. The large and enlarged cells-hyperplastic-of adrenocortical tissues of mature migratory and nonmigratory, Hilsa ilisha and Hilsa toli respectively, may be similar to zona fasciculata of mammals, whereas degenerated cells of adrenocortical tissues of spent Hilsa ilisha and Hilsa toli may be considered zona reticulata homologue. Can we be permitted, on basis of above mentioned observations, to support the hypothesis of Chester Jones (1957) that adrenal cortex is similar throughout the vertebrates?

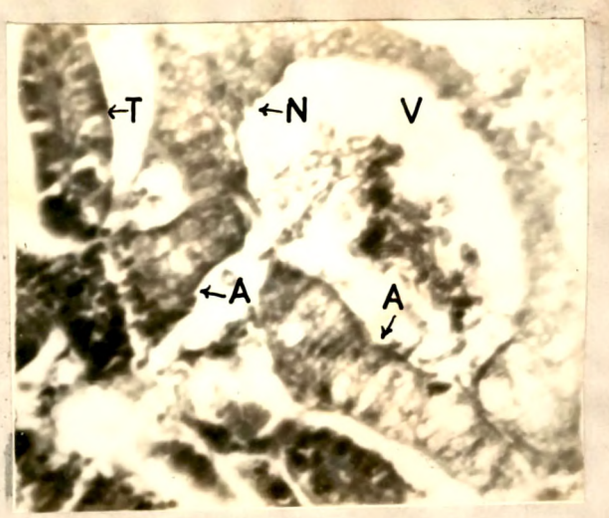


Fig. 1

Adrenocortical tissue(A) of fingerling of H. ilisha.
Haematoxyline Eosin, x630

V - Venule, N - Nucleus, G - Granules,
T - Tubule of the head kidney.

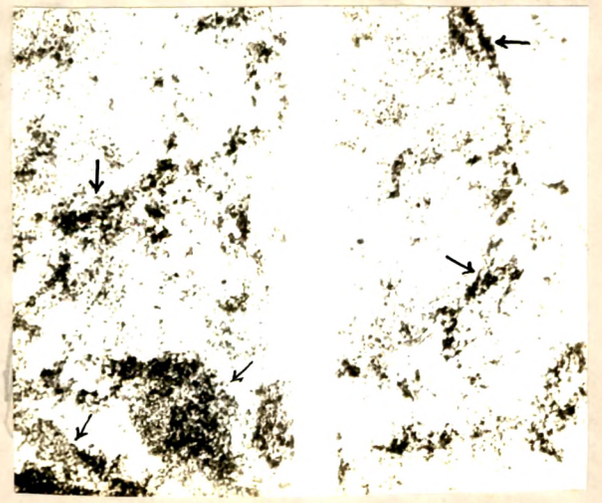


Fig. 2

Demonstration of the corticoids in the adrenocortical tissue of immature H. ilisha.
According to method of Khanolkar, et al., x400.

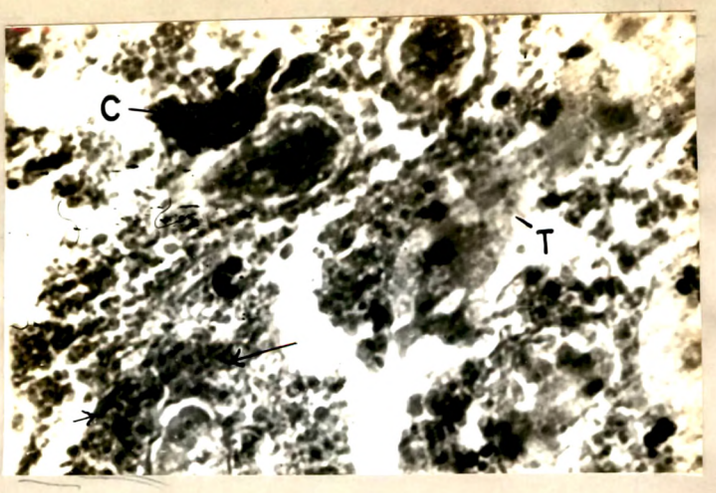
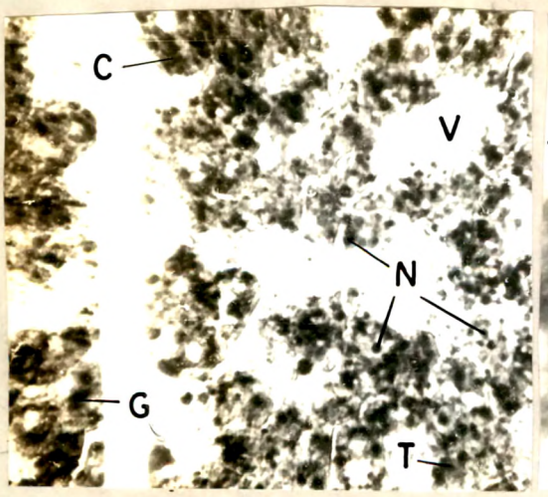


Fig. 3

Adrenocortical tissue of immature H. ilisha.
Haematoxyline & Eosin, x400.
N - Nucleus, G - Glomerulus, C - Chromaffin cells,
V - Venule, T - Tubule of the head kidney.
(Note small groups of the adrenocortical tissue)



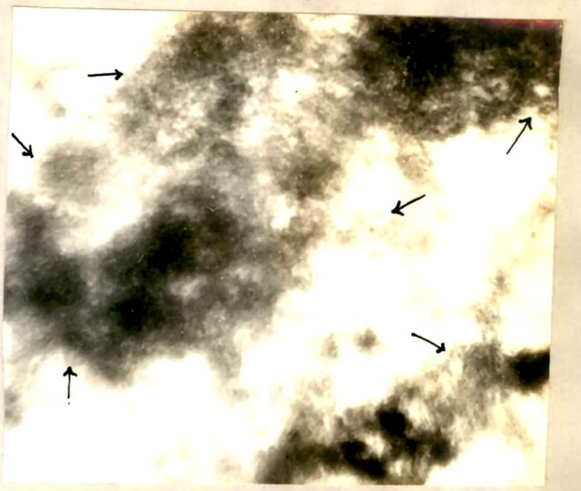


Fig. 4 Fig.5

Fig.4: Demonstration of corticoids in the adrenocortical tissue of mature H. ilisha captured from the sea. According to Khanolkar et al. method, x 400.
Fig.5: Magnified view of Fig. 4. x1000
Note abundance of granules and increased cell groups of the adrenocortical tissue.

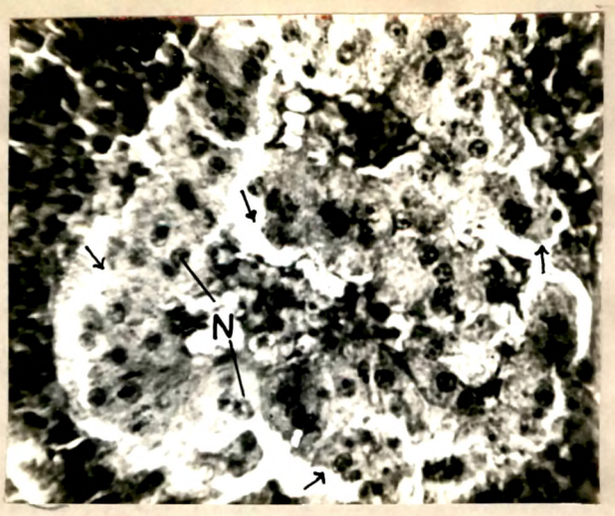


Fig. 6

Adrenocortical tissue of the mature H. ilisha captured from the sea.

Haemotoxyline & Eosin, x630.

N - Nucleus.
Arrow indicate adrenocortical tissue with plenty of granules.

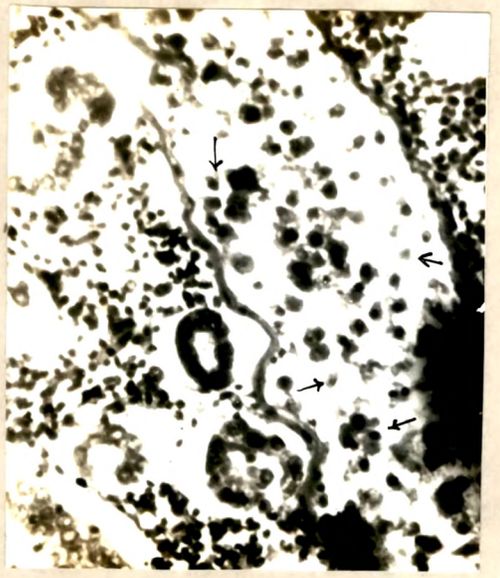


Fig. 7 Fig.8

Adrenocortical tissue of the spent H. ilisha.

Haematoxyline & Eosin, x630.

Arrow indicate shrunk, partially destroyed, loose cells.
Note the pycnotic nuclei and degeneration.

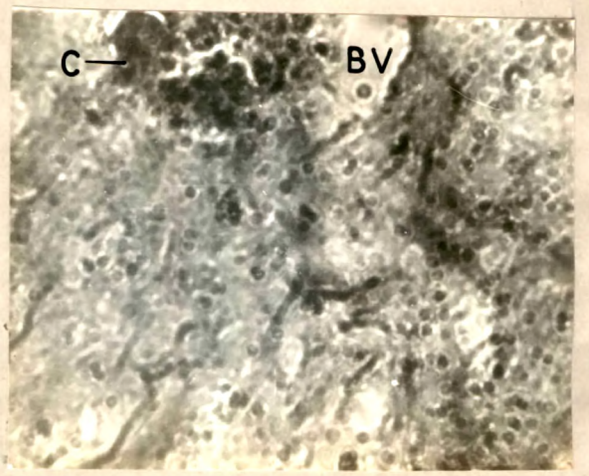


Fig. 9

Adrenocortical tissue of spent H. ilisha.
Haematoxyline & Eosin, x630.

Note the cells devoid of granules.
B - Blood vessel, C - Chromaffin tissue.

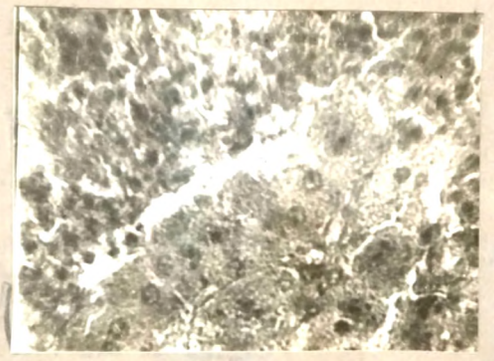


Fig. 10

Demonstration of corticoids in the adrenocortical tissue of spent H. ilisha.
According to Khanolkar, et al. method, x630.

Fig. 11

Demonstration of corticoids in adrenocortical tissue of immature H. toli.
According to Khanolkar et al. method, x400.

Arrow indicate adrenocortical tissue.

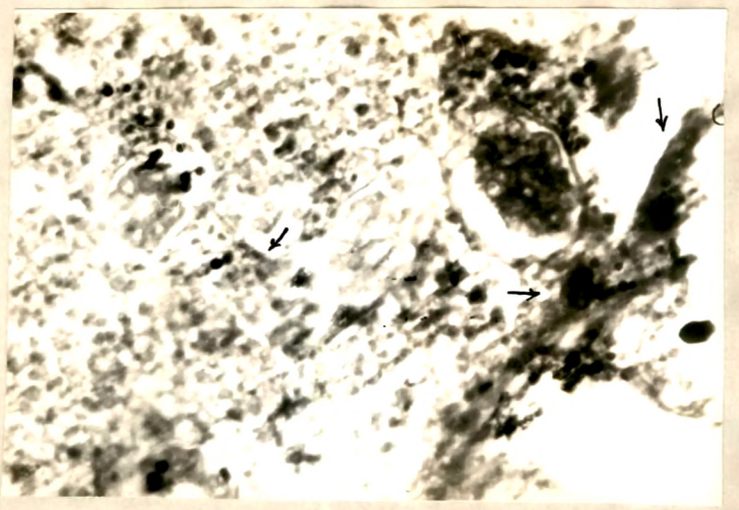


Fig. 12

Adrenocortical tissue of immature H. toli.
Haematoxyline & Eosin, x630.

Arrow indicate the adrenocortical tissue.
T - Tubule of head kidney, G - Glomerulus
C - Chromaffin tissue.

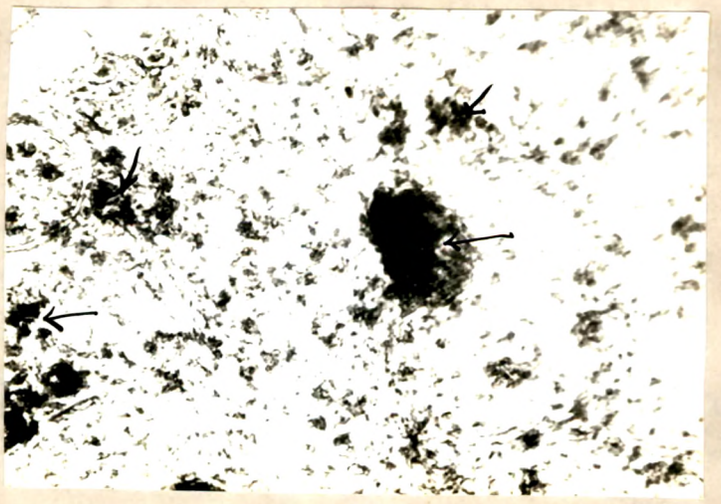


Fig. 13

Demonstration of corticoids in the adrenocortical tissue of mature H. toli.
According to Khanolkar et al. method, x400.

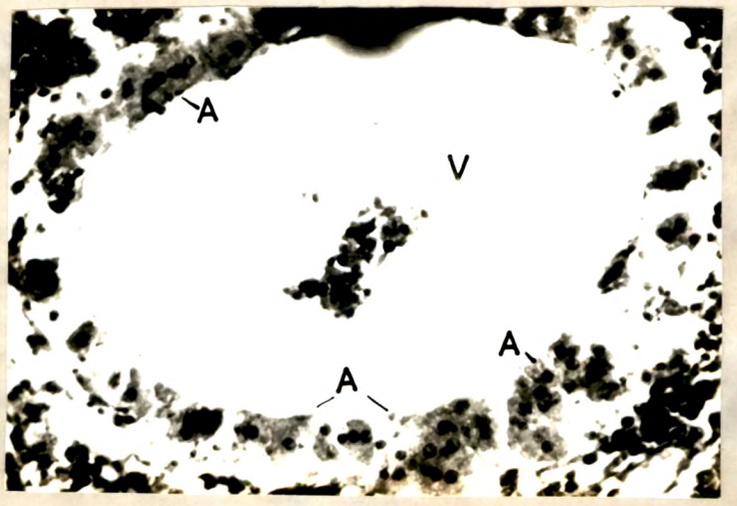


Fig. 14

Adrenocortical tissue of mature H. toli.
Haematoxyline & Eosin, x630.

A - Adrenocortical tissue, V - Venule.

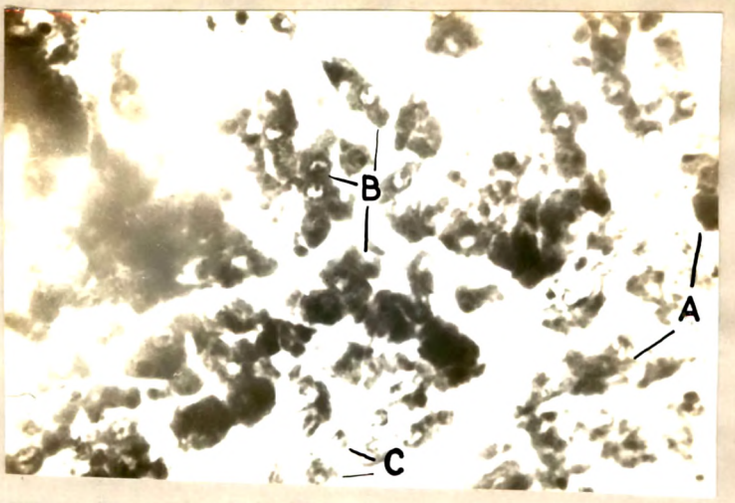


Fig. 15

Adrenocortical tissue of drifted H. toli.
Haematoxyline & Eosin, x630.

Arrow indicate adrenocortical tissue.
A - Cells exhibiting vacuolization
B - Cells showing pycnotic nuclei
C - Destroyed cells.

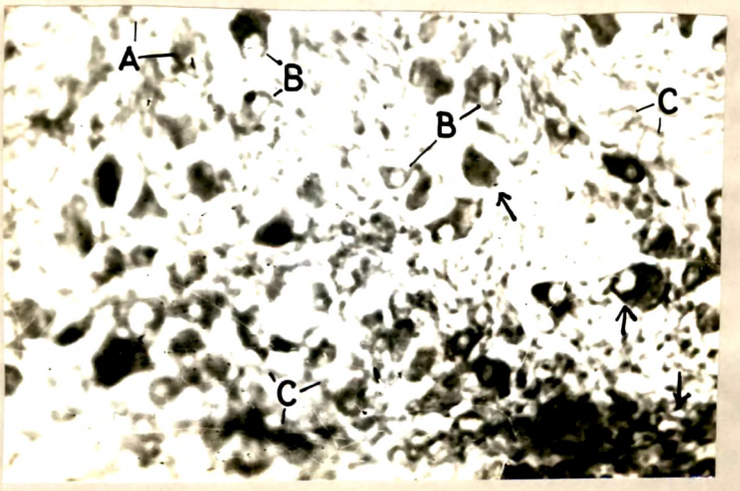


Fig. 16

Adrenocortical tissue of drifted, mature H. toli.
Haematoxyline & Eosin, x630.
(Legend as in Fig. 15).

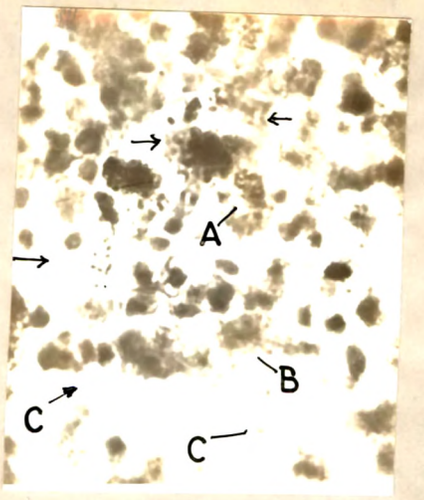


Fig. 17

Adrenocortical tissue of drifted, mature H. toli.

Arrow indicate the cells of adrenocortical cells.
Note pronounced destruction of the cells.
A - Cells with pycnotic nuclei
B - Cells exhibiting vacuolization
C - Lost and destroyed cells.

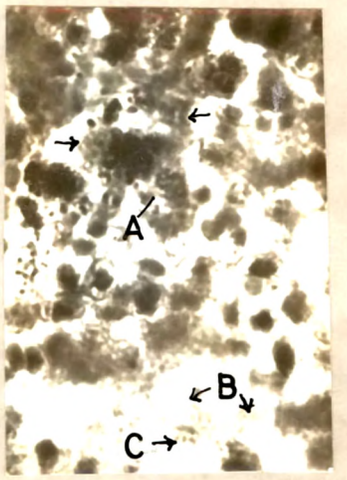


Fig. 18

Adrenocortical tissue of drifted, spent H. toli.
Haemaloxyline & Eosin, x630.
Note pronounced destruction of cells.
(Legend same as in Fig. 17)