CHAPTER V

HISTOLOGICAL STUDIES ON CYCLIC CHANGES OF THYROID GLAND OF MIGRATORY (ANADROMOUS), HILSA ILISHA (Ham.) AND NON-MIGRATORY HILSA TOLI (Cuv. & Val.)

Many workers have described the structure and development of the teleostean thyroid gland (For review; Pickford and Atz, 1957; Hoar, 1957; Bern and Nandi, 1964; Gorbman, 1959; Baker, 1958) which occurs usually in the form of uncapsulated, scattered follicles in subpharyngeal tissues or may be observed in the form of wellcapsulated structures in separate organ. The electron microscope studies on the thyroid gland of a teleost, *Seriola quinqueradiata* (Fujuta and Machino, 1965) suggested that thyroid of teleost differ slightly in structure than the thyroid of higher vertebrates. Several workers have also noted the presence of noncapsulated thyroid follicles in head kidney, ventral aorata, basibranch, spleen etc. (Baker,1958a,1958b; Baker-cohen,K.F., 1961; Chavin, 1956,b-c; Baker *et al*.,1955).

The role of thyroid - putuitary axis in development of fishes is not as clear as established in mammals and other higher vertebrates. The results obtained by workers are conflicting. Olifan (1945 - as cited by Pickford & Atz,1957) reported the presence of thyroid gland at the time of hatching in Sevrivga (Acipenser stillatus) and activity of thyroid gland was found to be increasing on 13th and 16th day. Somewhat similar results were also reported by Fontaine (1953), Baker (1964) reported the presence of aldehyde fuchsin positive faint cells in the pituitary of some fish from 2nd day of hatching, while conducting experiments on fish, *Herichthys cyanoguttatus*, with thiourea solution. He stated that a pituitary-thyroid relationship is existing during the development. Whereas Buchmann (1940) and Rasquin (1955 - as cited by Pickford & Atz, 1957) reported that thyroid may be independent of pituitary during early development of fishes. Results obtained by Fortune (1955 - as cited by Pickford & Atz, 1957), Kajishima (1960) throw light on pituitary control on thyroid in early development. Belsare (1965) reported that hypophysectomy in *O. punctatus* resulted into the atrophy of gonads and low activity of thyroid gland, suggesting an influence of the pituitary gland over thyroid gland as found in higher vertebrates. In migratory juveniles and larvae of fish, the activity of thyroid was correlated with the commencement of migrations (Hoar & Bell, 1950; Baggerman,1958). Buchman(1940) suggested that in absence of thyrotrops, the colloid of proadenohypophysis may act as stimulator of thyroid glands. Fontaine, Leloup and Olivereau (1955 - as cited by Pickford and Atz, 1957) have suggested a correlation between hyper-activity of thyroid with degranulation of basophils indicating hypersecretion. The iodine metabolism of thyroid follicles of teleosts follows the same pattern as in higher vertebrates, not only this, but, the role of teleostean thyroid follicles is similar to higher vertebrates in fat metabolism, in sexual maturation, in growth, and in promotion of hoemopoetic tissues (Baker Cohen,K.F., 1961). Radiothyroidectomy reduced the rate of growth and development in juvenile rainbow trout (La Roche, Woodall, Johnson and Halver, 1966). Thyroid in fish acts similarly as thyroid of higher vertebrates, this was confirmed by La Roche <u>et</u> <u>al</u>. (1965) as radiothyroidectomized fish expressed (i) reduction in growth rate (ii) extensive deposition of abdominal fat and (iii) considerable difference in red blood cells etc.

The role of thyroid in migration of fishes, attracts the attention of many research workers, especially, when osmoregulation comes into the picture. Many workers(Baggerman, 1957; 1958; 1960; 1962; Fontaine and Leloup, 1960; Leloup and Fontaine, 1960; Hoar, 1958; Woodhead, 1963) reported about the activation of thyroid pituitary axis prior to migration. It is believed that thyroid first, stimulates central nervous system and causes increase in locomotor activities, results of which, fish may seek a new environment. Osmoregulatory role of thyroid is also suggested by Hickman Jr., (1959). Other school of thought considers low salinities responsible for increase in the activities of thyroid (Pickford and Atz, 1957; Fontaine, 1956). Woodhead (1966) reported that migration and thyroid activity were closely related in adult female of ovoviviparous elasmobranch, Spurdogs, Squalus acanthias. He also noted the annual changes in thyroid activity corresponded with the period of annual migration in male of Spurdog. His work also suggested a close relation of thyroid activity with migration as Juveniles of Spurdog, who normally do not make regular seasonal migrations, showed no marked seasonal flucutations in thyroid activity.

During spawning, it is also noted that the activity of thyroid is enhanced (Swift, 1955 - as cited by Pickford and Atz, 1957; Hoar, 1952,1957; Buchmann, 1940; Fortune,1955 - as cited by Pickford and Atz, 1957; Barrington and Matty, 1954).

MATERIALS AND METHODS

Fishes captured from different places were sacrificed and pharyngeal area, basibranch, ventral aorata, head kidney and stomachs were fixed in Bouin's fluid for 4 to 6 days. Tissues were dehydrated and embedded in paraffin wax. Sections were cut at 5 μ and stained with the following: (a) Haematoxy-line-Eosin (b) Heidanhain's Azan trichrome (c) Halmi's aldehyde fuchsin and (d) PAS haemalum.

Follicles were smaller in size and epithelial cell heights, were too small to measure and hence; when follicles, stained blue with azan, were considered to be active and red as inactive (Pickford, 1953; 1954 - as cited by Pickford and Atz, 1957; Pickford and Atz, 1957). According to Eales (1963) cell height is only a morphological characteristic, which is probably controlled by TSH and does not necessarily reflect the rate of iodine metabolism through the thyroid in poikilotherms, where temperature plays important role in stimulation of thyroid gland, Olivereau (1955 - as cited by Pickford and Atz, 1957) had also demonstrated in Salmo gairdneri. The percentage of the follicles with blue colloid was assessed. More than 500 follicles were observed and then activity of the thyroid gland was judged. Haematoxyline-eosin was found best for the demonstration of nuclei; where as PAS haemalum was adopted for the demonstration of thiroglobulin. Heidanhain's azan was best among all stains for the demonstration of changes in colloid. The percentage of follicles with active colloid was assessed by counting from all the follicles present in the pharyngeal area.

RESULTS

The thyroid follicles of fingerlings of H. ilisha captured from river on way to migration to sea:

The percentage of active follicles with azan blue colloid, varied from 85% to 88%. They exhibited vacuoles at the peripheri and in centre of the follicles. The nuclei of these follicles were flattened and stained deep blue, sharply, with haematoxylin-eosin. Inactive follicles, with azan red and pinkred eosinophilic colloid, had large nuclei with chromatin materials. The colloid was devoid of vacuoles and was perfectly homogenous in nature. Few follicles were found broken also, where faint blue colloid with many small vacuoles were observed (Fig. 2). This indicates discharge of thyroid hormone in intercellular spaces (Baggerman, 1939 - as cited by Pickford and Atz, 1957).

The thyroid follicles of H. ilisha captured from the sea prior to migration:

The active follicles with azan blue colloid was found to be in majority (80 to 82%), but, comparatively follicles were many in numbers and were larger in size. The nuclei were larger and with chromatin material. Nuclei number in epithelium of the follicles varied from 5 to 8. Vacuolization was prominent feature and in most of the active follicles, was concentrated at peripheri in some of the active follicles big two to three vacuoles were observed in the center of the lumen also. The colloid stained deep blue or mauve coloured with A.F., and blue or precipitated blue with haematoxylin-eosin. Few inactive follicles with azan red colloid and eosinophilic colloid showed homogenous nature of colloid and nuclei of epithelial wall larger and fewer than that of active follicles. These inactive follicles were of small size (Fig. 3).

The thyroid follicles of mature, migrating H. ilisha captured from river:

Almost all follicles (90 to 93%) showed azan blue colloid and flattened nuclei, vacuoles were many and were also found at peripheri as well as in the center of the lumen. The number of follicles was increased. Few follicles gave appearance of discharge of hormone as evident by rupture of colloid by breaking of epithelial wall, as noticed in Fig.6. Rarely observed, small sized, inactive follicles were found to be with azan red, eosinophilic, homogenous colloid. Nuclei of such follicles were fewer and larger when compared with nuclei of active follicles (Fig. 4). When A.F. stained sections were studied more follicles (probably active ones) exhibited dark blue colour with vacuoles, suggesting presence of thyroglobulin and few larger ones mauve coloured, colloid indicating discharge of colloid from the follicles.

The thyroid follicles of spent H. ilisha captured from river:

Most of the follicles were enlarged and were found concentrated in groups in the pharynx floor. The nuclei of these follicles were flattened and cellheight also was reduced.

Very often the epithelium gave an appearance of a faint line with nuclei. With Azan almost all follicles (80 to 85%) showed very faint blue (azan) colloid, with many vacuoles distributed throughout the lumen (Fig. 5). The colloid gave precipitated appearance in some follicles when stained with hae.-eosin. It was worthnoting, the breaking of epithelium in follicles situated in the centre of the group of follicles. This lead to degeneration of some follicles, probably after discharge of hormone in the intercellular spaces (Fig.6). Occassionally empty space in the centre of the group was found with degenerated cells and nuclei presumably of degenerated epithelial cells of follicles. The appearance of small-sized follicles with azan-red colloid was an interesting feature, number of such follicles was very less (Fig. 6). These follicles showed pinkish red colloid with hae.-eosin strain and gave blue precipitated appearance with A.F. staining. The nuclei were larger and were found to be with chromatin material. The observations lead us to conclude that thyroid activity is reduced in spent H. ilisha and was at peak before migration to the river.

The thyroid follicles of immature H. toli captured from the sea:

Among all the follicles scattered in pharyngeal region in small groups, few follicles were in active state of secretion (75 to 85%) and stained blue with azan. The nuclei were larger and with chromatin material. The number of follicles was lesser than migratory H. ilisha. Granular colloid was also exhibited by few follicles (Fig. 7).

The thyroid follicles of mature H. toli captured from the sea:

The number of follicles was found to be increased when compared with immature H. toli captured from sea. Most of the follicles were in active state of secretion and were found with azan blue colloid with many vacuoles at peripheri. With A.F. bluish precipitated colloid was observed in them. The nuclei of such follicles were flattened (70 to 80%). Few follicles were observed broken discharging hormone in the intercellular spaces as observed in mature migrating H. ilisha. About 7 to 15% of follicles, in a few thyroid follicles with azan red homogenous colloid were noticed. Their nuclei were larger and were laden with chromatin material (Fig. 8).

The thyroid follicles of spent H. toli captured from the sea:

Few follicles with bluish (azan) colloid and flattened nuclei were observed. The same follicles stained bluish (precipitated) with A.F. staining and bluish with hae.-eosin stain. Many vacuoles were seen in the colloid, when azan blue colloid was studied. As observed in spent, migratory H. ilisha, follicles situated in the centre of the groups were noticed with broken epithelial cells and frequently completely degenerated, leaving empty space behind(Fig.9).

The thyroid follicles of H. toli drifted into the river on the highest high tide day of the year:

The goitorous thyroid follicles were observed in submucosal region of stomach of this fish. The epithelial cells were enlarged and entire follicle was expanded. The nuclei of the epithelium were large and found with nucleolii. Many granules were noticed in the cytoplasm of the cells. When stained with Heidanhain's azan, colloid stained red and gave homogenous appearance. When stained with H.E.-stain, colloid stained pink (Fig. 9). This goitorous condition may be due to iodine poor river water in which H. toli is drifted due to strong current of sea felt on highest high tide day of the year.

DISCUSSION

The presence of many active follicles in the fingerlings of H. ilisha can be correlated with existance of few A.F. positive cells (TSH cells) in the pituitary of fingerling (Chapter on Pituitary gland, Chapter IV). These cells may be considered as TSH secreting cells, which might be responsible for the activity of thyroid follicles. Baker (1964) observed few A.F. positive cells in the pituitary of Herichthys cyanoguttatus on the second day of hatching, he also considered A.F. positive cells to be thyrotrops. The activated thyroid follicles of fingerlings might have induce nuclei Preopticus to discharge NSM in the hypothalamo-hypothalamic pathways for migration to sea (Chapter III). Baggerman (1958) suggested that in juvenile migrants of Pacific Salmon (Onchorhynchus), when they migrate from fresh-water to sea, thyroid may be the main factor. This active thyroid induces central nervous system and hence a release of NSM is observed. Our work supports the findings of Baggerman; as in fingerlings of H. ilisha, on migration of sea, NSM from nuclei preopticus(NPO) is observed as released (Chapter II). Hoar and Bell (1950) reported that, increased activity of thyroid in juveniles of Salmonids, is related to change from fresh water to salt water. We may interpret that, the increased thyroid activity in fingerlings of H. ilisha may cause discharge of thyroxine to stimulate NPO to discharge NSM (Chapter II). Several workers also have obtained similar results.(Arvy, Fontaine and Gabe,1955b,1959). Woodhead (1966) noted lack of any marked seasonal variations in thyroid activity of juveniles of spurdogs, he interprets, as there is no regular seanonal migrations by juveniles of spurdogs, there is no marked change in the thyroid of juveniles of spurdogs. The increased activity of thyroids of fingerlings of H. ilisha may be for the migration in the sea.

The thyroid follicles of migratory H. ilisha captured from the sea, prior to migration to river, are in active state of secretion, as evident by histological results obtained, whereas the activity is reduced as migration proceeds and breakdown, degeneration of follicles are observed in spent H. ilisha. Thus there a gradual decrease of thyroid activity is noticed. The active thyroid in H.ilisha prior to migration to river may be for the stimulation of the central nervous system for (i) Increase in muscular activity and (2) discharge of neurosecretory material for hypotinic medium - river. H. ilisha captured from sea with active thyroid follicles did show all NPO in storage phase, hypothalamo-hypothalamic pathway devoid of NSM and neurointermediate lobe without NSM (Chapter II), but H. ilisha captured from the mouth of river Bhadbhoot (refer map 2), showed all NPO discharing NSM in hypo-hypoth. pathway, neurointermediate lobe was also noticed to be full of NSM. This may be considered as result of active thyroid observed in H. ilisha captured from sea prior to commencement of migration. Baggerman (1957, 1958, 1960, 1962) correlated the increased thyroid activity with the migratory behaviour in all teleost fishes. He also stated that thyroid hormone plays important role in behaviour and salinity preference, which usually, is observed before the migration starts. Robertson and Wexler (1960) also observed maximum activity of thyroid in migratory(anadromous) Pacific Salmon in sea and noted gradual decrease in thyroid activity during migration. The work of Woodhead (1966) on thyroid gland of spurdog, an ovo-viviparous elasmobranch Squalus acanthias, also have shown the changes in activity of thyroid gland of male and female spurdog, which are closely related with the migrations. The activity of thyroid is decreased in migrating H. ilisha and decreased notably in spent H. ilisha, in later, almost complete discharge of colloid is noticed, accompanied by appearance of few inactive follicles and degeneration of old follicles. This may be explained as under. H. ilisha never feeds during migration, naturally increased muscular activity during migration will ask for more supply of energy, which results in increase in metabolism. On return migration to sea, NSM is noticed discharging in hypo-hypotha. pathways, and neurointermediate lobe is filled with scanty NSM (Chapter II). This suggests that NSM is utilised and for return migration more NSM from NPO is supplied. This might be stimulated by discharged hormone from the follicles broken as evident from the histological picture of thyroid follicles presented by spent

H. ilisha. Bargman (1939 - as cited by Pickford and Atz, 1957) had already reported that by breaking the follicles, hormones are discharged, which is observed in migrating and spent *H. ilisha* and also in spent non-migratory *H. toli*. Thus, it can be interpreted that gradual discharge of thyroid hormone during (i) prior to spawning and migration stage (ii) during migration period and (iii) after spawning and on return migration to sea period, may be for stimulation of central Nervous system, for increase in muscular activity, for sexual growth and for increase in metabolic demands during starvation. Many workers agree with the view(Baggerman, 1960; Fontaine, 1960; Woodhead, 1959a,b,1966; Fontaine and Leloup, 1958) that thyroid may not play an osmoregulatory role, but it induces migratory behaviour. The results obtained in present investigation support the view.

It was noted by many research workers that thyroid is very active during spawning (Olivereau, 1954b - as cited by Pickford and Atz, 1967; Swift, 1955). It is believed that due to increased muscular activity and increase in metabolic demands, the thyroid is stimulated and is active. Buchmann (1940), Barrington and Matty (1954), Fortal (1955) also reported discharge of colloid after spawning. The results obtained from the histological study of thyroid follicles of H. toli of all stages of maturity lead us to state that thyroidial colloid is discharged after spawning in spent H.toli and thyroid follicles are active before spawning. The breakdown and degeneration of thyroid follicles in spent H. toli and azan blue colloid in mature H. toli supports the view held by above mentioned workers.

Comparatively fewer follicles found in nonmigratory fish may ask us to interpret as under: More thyroid hormone may be required for migration (due to increase in metabolic demands during starvation, continous swimming, experiencing hypotonic medium and return migration, etc.) and hence the more number of follicles have been noticed in migratory H.ilisha than in nonmigratory H. toli.

The goitorous thyroid observed in nonmigratory H. toli, drifted into the river Narbada due to the strong current of sea felt only on the highest high tide day of the year may be due to sudden transfer of H. toli from hypertonic, iodine rich sea water to hypotonic, iodine poor river-esturine (medium) water. Goitorous tissue was observed in stomach and not in the pharynx, fish thyroid is found in various organs by several authors (Baker, 1958a, 1961; Chavin, 1956a,1956b; Baker et al., 1955) and the follicles found in other organs like, headkidney etc., extrapharyngeal heterotropic thyroid tissues are considered similar to the thyroid follicles found in pharynx or in ventral aorata. Baker (1958b) also observed thyroid tumours and goitors in headkidney of Platy-fish, when fishes were kept in iodine poor environment. Robertson and Chaney (1953) showed hyperthyroidism in Lake Michigan Trout(Salmo gairdnerii) in comparision with California sea run from due to low iodine content of lake water.

It is also observed that thyroid of fishes play, significant role in growth, sexual maturation, and in promotion of hoemopoetic tissues (K.F. Baker-Cohen, 1961). Can we attribute the active thyroid in mature H. ilisha and H. toli for above mentioned roles? Hoemopoetic tissue in mature,migratory H. ilisha and in nonmigratory H. toli was observed much developed, (unpublished work from our laboratory). This may be due to active thyroid follicles observed in same fishes.

The role of thyroid in fat metabolism cannot be discarded here. La Roche et al. (1966) on basis of their work on the radithyroidectomized rainbow trout (Salmo gairdnerii Rich.) reported that in absence of thyroid, growth rate of fish is reduced, extensive deposition of fat is noticed, significant number of red blood cells was reduced etc. This suggests that thyroid in fish behaves similarly as thyroid behaves in higher vertebrates. Unpublished works from our laboratory have observed transport of fat from fat-depot like liver, adipose tissues in migratory H. ilisha and also in nonmigratory H.toli during spawning migration and spawning respectively.

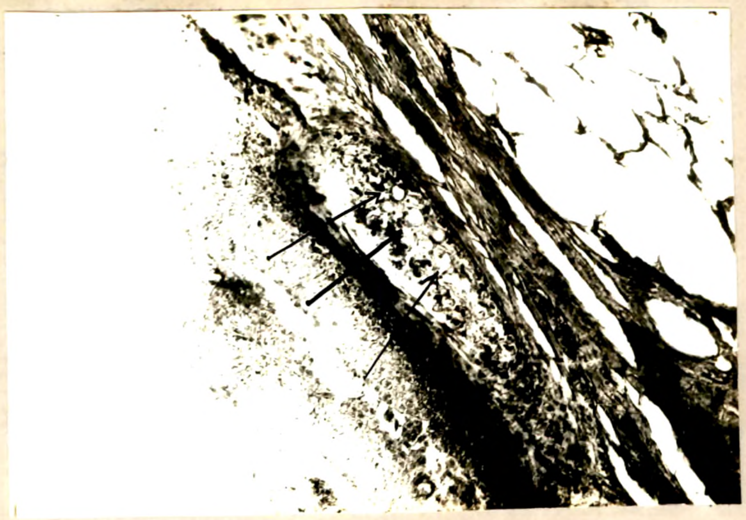

Fig. 1

Thyroid follicles (TF) in the pharyngeal area of <u>Hilsa ilisha</u>.

Heidanhain's Azan, x160

(Follicles showed by arrow)

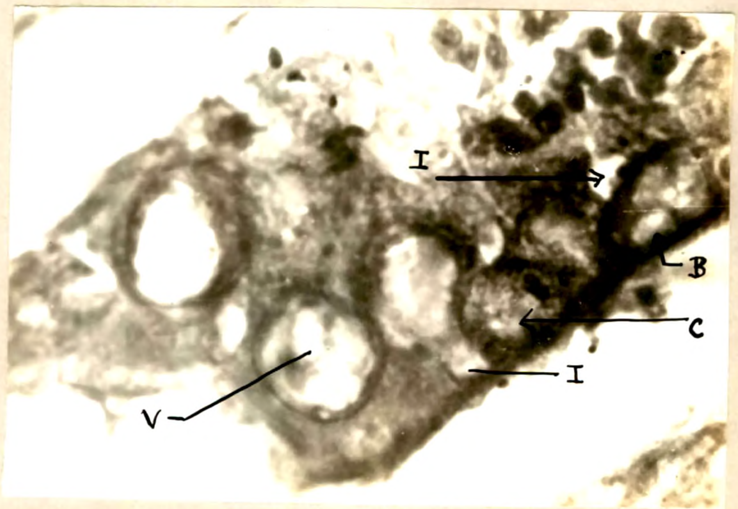

Fig. 2

Thyroid follicles from the pharyngeal area of the fingerling of <u>H. ilisha</u>.

Azan, x630

C - Colloid devoid of the vacuoles.
V - Vacuoles
B - Broken follicle
I - Intercellular space

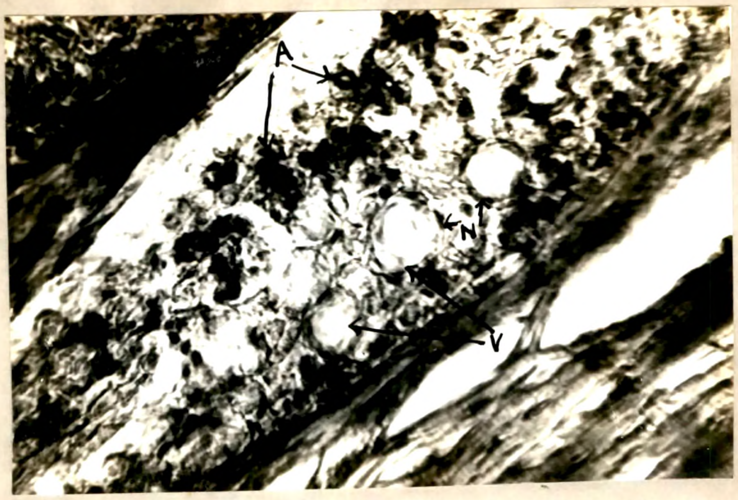

Fig. 3

Thyroid follicles from the pharyngeal floor of <u>H. ilisha</u>, captured from the sea, prior to migration.
Azan, x630

N - Nuclei
V - Vacuole
A - Inactive follicles

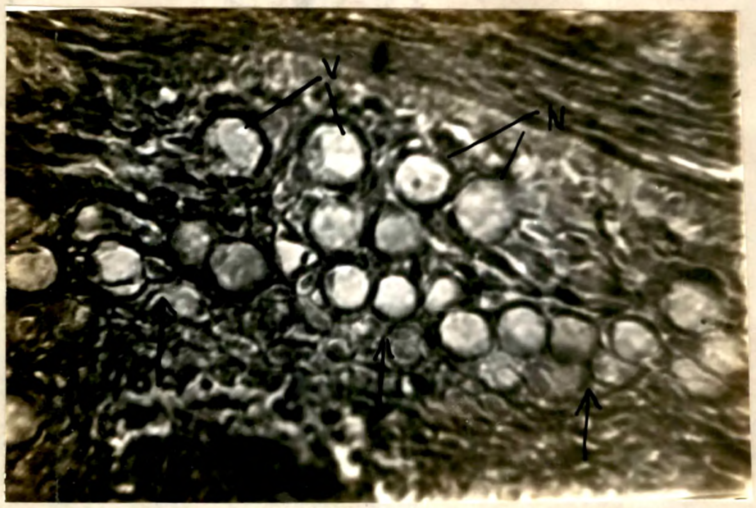

Fig. 4

Thyroid follicles from the pharyngeal floor of migrating, mature <u>H. ilisha</u>.
Azan, x630

N - Nuclei ; V - Vacuole
Arrow indicates rupture of follicular wall and discharge of the hormone in the intercellular space.

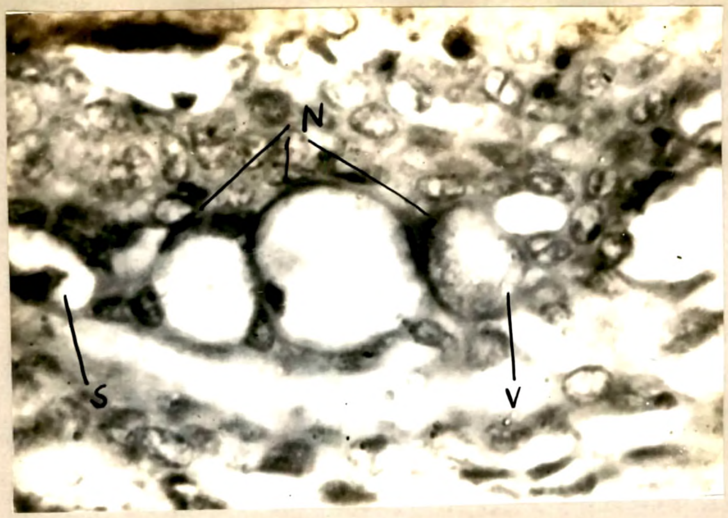

Fig. 5

Thyroid follicles from the pharyngeal floor of the spent H. ilisha.

Azan, x630

N - Nuclei, V - vacuole, S - Small follicles with colloid

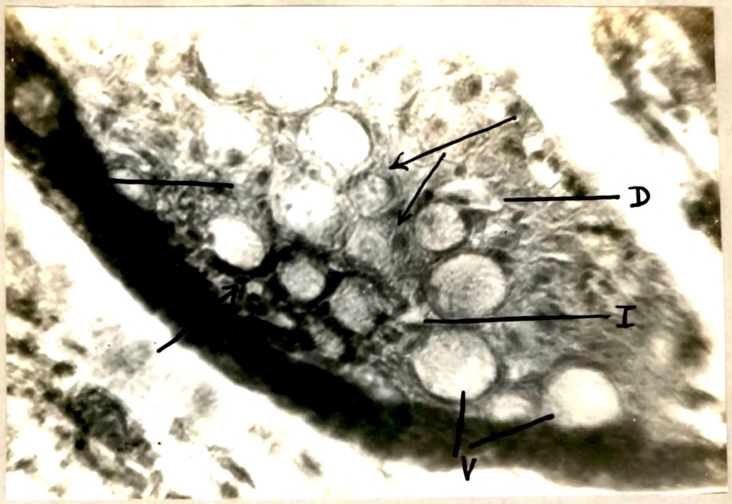

Fig.6

Thyroid follicles of the spent H. ilisha, showing degeneration of the follicles.

Azan, x630

D - Degenerating follicles, I - Intercellular space
V - Vacuole
Arrow indicate newly appeared small follicles.
(Note the presence of small, new follicles)

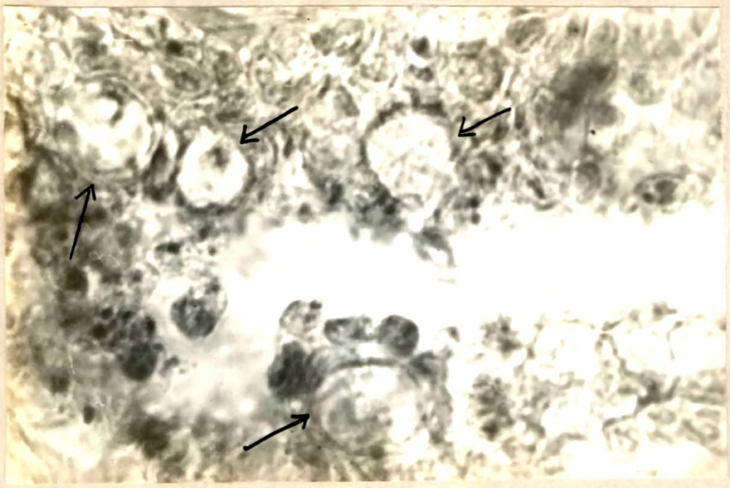

Fig. 7

Thyroid follicles from the pharyngeal floor of the immature, marine H. toli.

Azan, x630

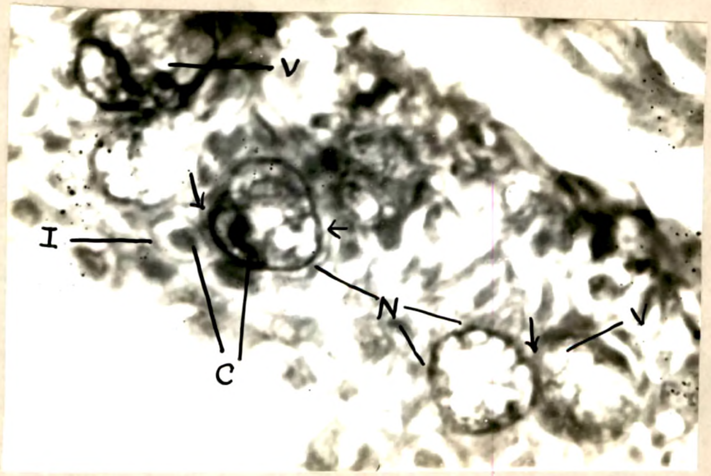

Fig. 8

Thyroid follicles from the pharyngeal floor of the mature, marine H. toli.

Azan, x630

N - Nuclei
V - Vacuoles
I - Intact, active follicles
C - Colloid
Arrow indicates intercellular space.

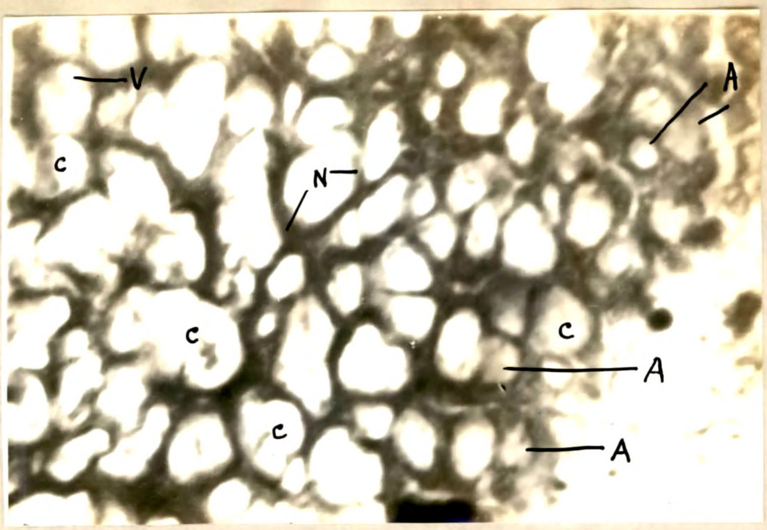

Fig. 9

Thyroid follicles from the pharyngeal floor of the spent, marine H. toli.

Azan, x630

N - Nuclei, C - Colloid, V - Vacuoles ,
A - Small sized follicles
(Arrow indicates ruptured and degenerated follicles)

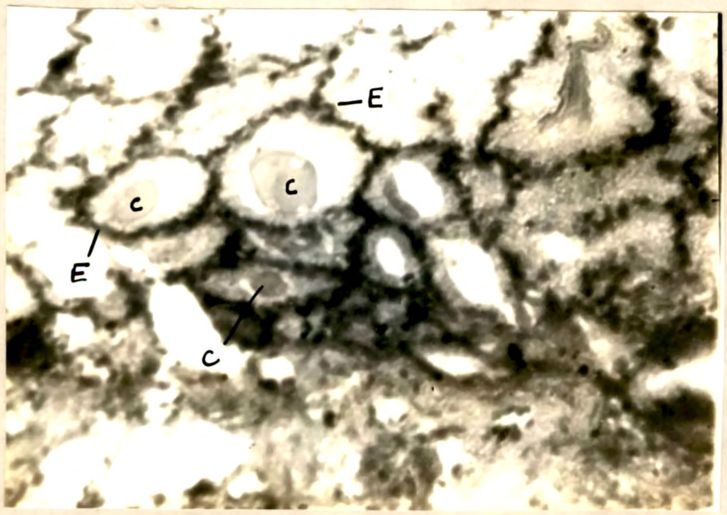

Fig. 10

Thyroid follicles from the submucosal region of the stomach of drifted mature, H.toli.

Azan, x16

E - Epithelium , C - Colloid
(Note the goitorous condition. Compare with Figs. 4 and 9)