#### CHAPTER 2

STUDIES ON THE HISTOLOGICAL CHANGES IN THE ALIMENTARY CANAL OF <u>H. ILISHA</u> AND <u>H. TOLI</u> DURING DIFFERENT PHASES OF LIFE CYCLE

Considerable literature on the anatomy and histology of the alimentary canal of fishes has accumulated since the pioneering work of Green (1912) (Blake, 1930, 1936; Dawes, 1929; Al-Hussaini, 1949; Vanajakshi, 1938; Weinreb and Bilstad, 1955; Ishida and Sato, 1960; Hale, 1965).

Histological alterations in the alimentary canal during migration of salmon have been studied by Greene (1926, cf. Robertson and Wexler, 1960) and Robertson and Wexler (1960). <u>H. ilisha</u> is an anadromous migratory fish but unlike salmon, no post spawning death is reported. The present study was undertaken to study the histological alterations in the alimentary canal of <u>H. ilisha</u> during different phases of life cycle and to compare the same with those of non-migratory <u>H. toli</u>.

#### MATERIAL AND METHODS

The fishes were collected from different habitats as described in Chapter 1. Live fishes were removed from the net and decapitated immediately. Visceral organs were removed and small pieces of the different parts were fixed in Bouin's and 10% neutral formalin. Paraffin sections of 5-6 /µ thickness were cut for the histological studies. Gelatin sections were prepared from material fixed in neutral formalin and the distribution of lipid in the various parts of the alimentary canal was demonstrated by staining with Sudan Black B.

For histological studies the following staining methods were employed. 1. Haematoxylin - eosin, 2. Heidenhain Azan, 3. Aldehyde fuchsin.

#### RESULTS

The histological features of the various parts of the alimentary canal of the two fishes of different stages are presented in a concise form in Tables I & II at the end of the chapter. A detailed description is given below. <u>General account of the alimentary canal of H. ilisha</u>:

The alimentary canal can be grossly divided into the following regions in addition to the buccal cavity, pharynx, oesophagus, corpus (cardiac stomach), pyloric stomach and intestine with pyloric caecae.

The region from the pharyngeal pads to the narrow constriction which is also visible externally is the pharyngeal region. The constriction marks the anterior boundary of the oesophagus. The posterior limit of the oesophagus is indeterminable externally. However, when the alimentary canal is opened the oesophagus and the stomach could be made out because of the appearance of the gastric mucosal folds which are quite distinct from the numerous longitudinal folds of the oesophagus.

The stomach is an U shaped organ. The first limb extending from the oesophagus is the corpus or the cardiac stomach. When empty, the corpus is cylindrical in form and shape. Pyloric stomach or pylorus is strongly developed due to preponderence of its muscular wall and has a more or less globular appearance.

The pylorus continues as a much convoluted intestine. Numerous cacae exist in the form of clusters and tufts. The are studded immediately hehind the pylorus and extend to some distance over the first limb of the intestine .

The alimentary canal of H. toli showed no difference from the above in the gerneral morphology.

<u>Pharynx</u>. The mucosa of pharynx consisted of stratified epithelium. The mucosa is gently corrugated. The internal folds were running transversely in the anterior region and longitudinally in the posterior region. The outermost layer was formed of striated muscles arranged in circular fashion. Internal to this layer, longitudinal muscles were located in the subepithelial connective tissue- the region between epithelium and outer circular muscle layer. No stratum granulosum and stratum compactum were noted.

## Pharynx of fingerling of H. ilisha captured from river:

The entire region was compactly arranged (Fig. 1). The nuclei of the cells of the epithelium were situated basally and stained deeply with haematoxylin. Taste buds were observed in large numbers (Fig. 2°). Giant cells stained deeply with eosin. Their nuclei were prominent. The mucus secreting cells showed basally situated nucleus and bluish mucus when stained with Azan stain (Fig. 2). The ducts could be clearly seen discharging the contents. Sub epithelial connective tissue was compact and nuclei of this region stained faintly with haematoxylin . Many blood vessels were present.

Muscle layers showed normal structure with blood vessels traversing the layers.

## Pharynx of immature H. ilisha captured from sea:

The thickness of all layers had increased considerably. The epithelial cells were uniformly arranged. Tall mucus secreting cells showed prominent basal nuclei. Many taste buds were also noticed (Fig. 3). Giant cells were few and were in active state of division.

The sub epithelial connective tissue, richly supplied with blood vessels, was compactly arranged below the epithelial layer (Fig. 3) but somewhat loosely arranged above the muscle layers. Many empty cells storing fat were also present.

Muscle layers exhibited normal structure with fat storing cells (Fig. 4).

#### Pharynx of H. ilisha captured from sea before migration:

Except the muscle layers, pharynx of <u>H</u>. <u>ilisha</u> before migration showed normal structure as described under immature <u>H</u>. <u>ilisha</u>. In some cases, the central region of the muscle cell was lost. This probably was due to the loss of central fibrils. Very faint eosin staining was noticed here.

<u>Pharynx of migrating mature H. ilisha captured from river during</u> <u>spawning period</u>:

The epithelium remained uniform except at a few places where it was ruptured. Epithelial cells at such broken regions





Fig. 3

Fig. 4

- Fig. 1. T. S. of Pharynx of fingerling of <u>H</u>. <u>ilisha</u>. HE X 63.
  Fig. 2. T. S. of Pharynx of fingerling of <u>H</u>. <u>ilisha</u> showing taste buds (T) and mucus cells (M). HE X 400.
  Fig. 3. T. S. of Pharynx of immature <u>H</u>. <u>ilisha</u> showing compact subepithelial connective tissue and taste bud (T). Azan X 400.
- Fig. 4. T. S. of Pharynx of immature H. <u>ilisha</u> showing normal muscle layer with fat cells. HE X 63.

were noticed separating from the surrounding intact cells and connected to the latter by a layer of few cells in thickness. The number of mucus cells appeared to have increased, many of them showing discharged phase. Taste buds were scanty. The giant cells exhibited deeply stained nuclei.

Subepithelial connective tissue exhibited loose structure, at some places separated from epithelium. The entire region was well vasculdrised. Frequently empty spaces were noted. The presence of empty fat cells was a prominent feature.

The longitudinal muscle gave a wavy appearance and many lacunae were seen. At many places the fibres were broken and found lost. This was a notable feature.

## Pharynx of spent H. ilisha captured from river:

The degenerative alterations noted in mature <u>H</u>. <u>ilisha</u> were more conspicuous in spent <u>H</u>. <u>ilisha</u> (Fig. 5).

The epithelium showed further degenerative changes. Many of the epithelial cells were destroyed, leaving empty spaces at several places. The complete separation of epithelium from the layers beneath was frequently observed. Such regions were faintly stained. In addition to this many lacunae were noticed in intact portions. The mucus secreting cells appeared shrunk as if the content was discharged completely. In some cases they were completely destroyed.

The subepithelial connective tissue was very faintly stained and was not compact (Fig. 6). Many fat cells were observed along with rich blood supply.

In the region of muscle layers, marked degenerative

changes were observed (Figs. 7 & 8). The entire layer took very faint eosin stain. In the longitudinal muscle fibres, loss of fibrils, and also sometimes of muscle fibre, was observed. The nuclei of such fibres were large and with very little chromatin, in the form of granules.

## Pharynx of immature H. toli captured from sea:

The histological structure was found to be similar to that of the immature <u>H</u>. <u>ilisha</u>.

### Pharynx of mature H. toli captured from sea:

Cells of the epithelial layer remaind normal except at few places where they were either missing or found separating from underlying tissue. Numerous mucus secreting cells were observed.

The subepithelial tissue was well vascularized and loosely arranged with many lacunae. It was faintly stained by eosin. Many empty fat storing cells were observed. The alterations observed were not so marked as seen in the case of mature <u>H. ilisha</u>. Very few parts showed loss of fibrils.

# Pharynx of H. toli captured from sea:

The histological structure was more or less similar to that observed in mature <u>H</u>. <u>toli</u> except for the presence of few prominent taste buds and numerous empty mucus secreting cells.

#### Pharynx of drifted mature H. toli captured from river:

The complete epithelial layer seemed to have detached from the subepithelial connective tissue (Fig. 9). Mucus





13

Fig. 5

Fig. 6





Fig. 7

Fig. 8

- Fig. 5. T.S. of Pharynx of spent H. <u>ilisha</u> showing broken epithelium (arrow) and other degenerative changes. HE X 63.
  Fig. 6. T.S. of Pharynx of spent H. <u>ilisha</u> showing degenerative alterations of epithelium and subepithelial connective tissue. HE X 400.
  Fig. 7. T.S. of Pharynx of spent H. <u>ilisha</u> showing degeneration of longitudinal muscles. HE X 400.
  Fig. 8. T.S. of Pharynx of spent H. <u>ilisha</u> showing degeneration of circular muscles. HE X 400.

secreting cells stained faintly with haematoxylin-eosin. Their basally situated nuclei of irregular shape were conspicuous when stained with haematoxylin. Some of them were empty with small vacuoles inside, probably due to discharge of mucus. At many places the region of epidermal cells was missing and the remaining parts of the detached broken cells were found in the nearby area. The giant cells stained very faintly with eosin. Their nuclei were with very fine granular chromatin.

The subepithelial connective tissue region also showed pronounced degenerative changes. The nuclei of this region were mostly of irregular shape. Many were deeply stained with haematoxylin, others were dusted with finely granular chromatin material. The longitudinal muscle layer was stained deeply with eosin but showed loose arrangement and loss of cytoplasm from some part of the muscle fibre. Their nuclei were of elliptical, spindle or irregular shape and were deeply stained with haematoxylin. Fat storing empty cells were also numerous. Lacunae caused by disintegrating muscle fibre showed small fragments of fibre. Sometimes only muscle fibre membrane with nuclei had remained. Blood cells were frequently found scattered in the space between the muscle fibres.

#### Oesophagus:

It showed projections of epithelium forming warty tubercles in the anterior region, whereas posteriorly it showed longitudinal folds of varying width. These folds were more in number in comparison with the number of folds in the corpus. The layers found in the cesophagus were (1) epithelium,

(11) subepithelial connective tissue with longitudinal muscles and (111) outermost circular striated muscle layer. Stratum granulosum and stratum compactum were found absent. Oesophagus of fingerling of H. ilisha captured from river:

Oesophagus showed normal structure (Fig. 10). In the anterior region of oesophagus, presence of large mucus secreting cells in the epithelium were noted. These cells had bluish cytoplasm when stained with Heidenhan's Azan stain. The nucleus was basally situated with few chromatin granules and was faintly stained with haematoxylin. Giant cells and taste buds were found in the anterior region only. The epithelial cells were with oval prominent nucleus and nucleolus.

Well vascularised subepithelial connective tissue was found to be normal. Their nuclei were elliptical, dusted with chromatin material and deeply stained with haematoxylin. Fat cells were present.

The muscularis region was stained with eosin. In the longitudinal muscles, nuclei were very few and strongly haematoxylin positive. In the region of circular muscles some fibres showed irregular faintly stained nucleus whereas the remaining nuclei were spindle shaped and well stained.

Serosa was with many blood vessles and thin subof serosa connective tissue fibres.

#### Oesophagus of immature H. ilisha captured from sea:

The epithelium was normal (Fig. 11) and made up of tall columnar cells interspersed with mucus secreting cells. The oval nuclei with fine chromatin were basally situated.

Mucus glands were large, with basally situated deeply stained nucleus.

Subepithelial connective tissue was compact. The nuclei of this region were spindle shaped and with granular chromatin material. The layer was well vascularised.

Both the miscle layers appeared normal. They were compact and well stained with eosin (Fig. 12).

Serosa layer was thin having a compact subserosa of connective tissue.

# <u>Oesophagus of maturing H. ilisha captured from sea before</u> <u>migration</u>:

Epithelial lining was formed of tall columnar cells and mucus cells. Columnar cells had apically situated oval nuclei, the latter with fine chromatin material. Mucus glands were smaller in size and most of them were empty.

Much vascularised subepithelial connective tissue was found to be compact with deeply staining nuclei. Empty cells representing the fat cells were noticed in this region also.

Longitudinal muscle layer was compact. Nuclei were deeply stained. Circular muscle layer was mostly uniform with darkly stained nuclei. Very few exhibited loss of fibrils, broken fibres etc.

# Oesophagus of migrating mature H. ilisha captured from river:

All layers in general, showed degenerative alterations (Fig. 13). At certain places epithelium was found partially separated and hanging in the lumen and in certain other



Fig. 9

Fig. 10







Fig. 12

- Fig. 9. T.S. of Pharynx of drifted <u>H. toli</u> showing degenerative alterations of epithelium. <u>HE.X 63</u>.
  Fig.10. T.S. of Oesophagus of fingerling of <u>H. illisha</u>. <u>H.E.X 63</u>.
  Fig.11. T.S. of Oesophagus of immature <u>H. illisha</u> showing normal villi. <u>HE. X 400</u>.
- Fig.12. T.S. of Oesophagus of immature <u>H</u>. <u>ilisha</u> showing normal muscles. HE. X 63.

parts completely lost (Fig. 13). The intact epithelium also showed the cells completely shrunk and crumpled. Hence it was not possible to study the detailed structure..

Occasionaly deep indentations were noticed in the subepithelial connective tissue due to loss of epithelial tissue. In the lower layer many lacunae were observed. Most of the nuclei of this region were empty and of irregular shape. A few were of rounded or oval shape with moderate staining affinity.

Fat cells were also present, showing thin cytoplasmic membrane and irregular nuclei, Longitudinal muscles in subepithelial connective tissue showed degenerative alterations (Figs. 14 & 15) and were faintly stained. Vacuolisation was seen with the remnant of fibres ie. scattered crumpled nuclei and muscle cells with only cell membrane. The circular muscles were irregularly and loosely arranged. Many lacunae were noticed. The inner region showed more alterations than the outer layers. Many fibres had shrunk and were faintly eosinophilic. In some fibres the central region was lost. Fragmentation was prominent and the nuclei of such regions were irregular in shape.

Serosa at some places was separated from muscle layer and occasionally was completely destroyed.

## Oesophagus of spent H. ilisha Captured from river:

The epithelium showed alterations more or less as observed in mature <u>H</u>. <u>ilisha</u>. Epithelial cells were cuboidal with prominent nuclei. At certain places epithelial cells were separated from giant or undifferentiated cells. These undifferentiated cells showed mitotic division. The number of such cells

had increased only in spent <u>H</u>. <u>ilisha</u>. Mucus cells showed faint staining with reticular cytoplasm.

The subepithelial connective tissue was loosely arranged with many large lacunae. The entire region was faintly stained with eosin. The nuclei also were faintly stained and were irregular shape. Fat cells and many large blood vessels were present.

The degenerative alterations exhibited by muscle. layers and serosa were more pronounced than those observed in mature <u>H</u>. <u>ilisha</u> captured from river.

Oesophagus of immature H. toli captured from sea:

The histological structure was more or less similar to that of immature <u>H</u>. <u>ilisha</u>.

Oesophagus of mature H. toli captured from sea:

The mucus cells were tall with deeply stained basally situated oval nuclei. The cytoplasm was reticular. Epithelial cells were cuboidal having scanty cytoplasm and deeply staining nuclei.

Subepithelial connective tissue was some what loosely arranged and well vascularised. Nuclei were deeply stained. Few lacunae were noticed in this region. Fat cells were also present.

Inner longitudinal muscles were loosely arranged when compared with immature <u>H</u>. <u>toli</u>. Longitudinal and circular muscle layers showed very less alterations when compared to mature <u>H</u>. <u>ilisha</u> from river.

#### Oesophagus of drifted H. toli collected from river:

The major portion of the epithelium showed marked alterations (Fig. 16). The columnar cells appeared to have shrunk. Some intact cells showed flattened nuclei situated in the middle of the cell and with granular chromatin. Details regarding mucus cells could not be studied.

Few areas only showed normal subepithelial connective tissue. Otherwise marked destruction and alterations were prominent (Fig. 17). Normal area showed moderately stained nuclei but other regions had irregular shaped nuclei with faint staining and very little chromatin. Large lacunae with remnants of destroyed tissue were observed.

The longitudinal muscle layer showed different affinities for eosin. Some regions were faintly stained whereas the others took normal staining. Vacuolisation was prominent. Fibres with normal staining showed spindle shaped deeply staining nuclei, whereas nuclei of the faintly staining regions were elongated and devoid of chromatin.

On the whole circular muscle fibres appeared normal. However, they were separated from each other and a few fibres showed fragmentation exhibiting loss of fibrils or breaking of fibres.

Serosa was thin. It was not uniform in thickness and was separated from the muscle layer and disintegrated in several regions. The nuclei were deeply stained.

## <u>Corpus: (Cardiac stomach)</u>

Internally it shows prominent longitudianal folds or



Fig.	13.	L.S. of Oesophagus of mature <u>H. illisha</u> showing degenerative alterations. Note the broken epithelium. HE. X 63.
Fig.	14.	L.S. of Oesophagus of mature <u>H. illisha</u> showing degeneration and dissolution of muscle fibres. HE. X 63.
Fig.	15.	Same as Fig. 14. X 400.
Fig.	16.	T.S. of Oesophagus of drifted <u>H. toli</u> showing degeneration of epithelium. HE. X 63

ridges. The corpus wall consisted of (i) epthelium formed of columnar cells and (ii) a region of areolar connective tissue external to the epithelial layer which extends upto the stratum compactum layer. Mucosal and submucosal parts could not be differentiated. Muscularis mucosa was also absent. Hence the region between the epithelium and stratum compactum has referred as 'subepithelial connective tissue'. In this region, on the out side of the epithelium are situated numerous digestive glands (iii) In between the subepithelial connective tissue and circular muscle layer ie. on the out side of the former, are present stratum compactum and stratum granulosum. Layer of stratum compactum was found to be absent in the corpus of H. toli. (iv) Muscular layers: longitudinal striated muscles in the form of bundles, were present in the subepithelial connective tissue, on the inner side of stratum granulosum (in case of H. toli) or stratum compactum (in case of H. ilisha) Circular muscle layer, outer most layer was composed of striated muscle fibres. The longitudinal muscle layer generally found on the outer side of the circular muscle layer was absent in the corpus of both H. ilisha and H. toli. (v) Serosa was found to be formed of a thin layer of connective tissue (called the subserosa) and a simple epithelium.

Corpus of fingerling of H. ilisha captured from river:

Epithelium was formed by closely packed columnar cells with basally situated nucleus containing finely granular chromatin.

Glands were compactly arranged. Gland cells were granular and nuclei basally situated with fine granular chromatin. Neck cells were also granular. Crypt cells were stained with eosin.

Sub-epithelial connective tissue between glands was prominent, staining deep with eosin. The remaining region was not deeply stained. The nuclei were spindle shaped. Many blood vessels were visible.

Stratum compactum and stratum granulosum were not found in juvenile corpus.

The thickness of the muscle layer was much less as compared to the inner layers.

Serosa showed a few blood capillaries and deeply stained nuclei.

#### Corpus of immature H. ilisha captured from sea:

Mucosa showed normal folded villi (Figs. 18 & 19). Tall columnar epithelial cells had oval nuclei at the basal region. Nuclei showed nucleolus within, had little chromatin and took faint staining, Epithelial cells were compactly situated.

Cells of the gastric glands were compactly situated (Fig. 20) and were roughly polygonal, with basal nucleus and cytoplasm filled with zymogen granules. Nucleus showed prominent nucleolus and were moderately stained. Cells lining the neck of the glands were also full of granules, with round basal nuclei containing the nucleolus within. Cells lining the crypt



Fig. 17

Fig. 18



Fig. 19

- Fig. 17. T.S. of Oesophagus of drifted <u>H. toli</u> showing marked degeneration of epithelium. HE. X 400.
  Fig. 18. T.S. of Corpus of immature <u>H. ilisha</u> showing normal epithelial folds. HE. X 63.
  Fig. 19. Same as Fig. 18. X 400.

were faintly stained with eosin. These cells were roughly cuboidal, with round nucleus and plenty of granules. The glands were supported by connective tissue septa.

Sub-epithelial tissue was made up of connective tissue fibres with numerous blood capillaries. The nuclei of this region were spindle shaped and showed little of granular chromatin. The striated longitudinal muscles in the sub-epithelial muscles layer showed normal structure.

Stratum compactum was made up of a hyaline compact mass without prominent nuclei. Though the region was well marked, no further details were seen.

Stratum granulosum was formed of densely granulated cells, well differentiated from the durrounding tissues. The nucleus was oval in shape and stained moderately.

Circular muscle layer was normal, formed of deeply stained striated muscle fibres. A few blood vessels were also noticed. The nuclei were spindle shaped and deeply stained with haematoxylin. The serosa was thin, with connective tissue fibres.

Corpus of maturing H. ilisha captured from sea before migration:

Atrophy of epithelium had begun and normal villi were absent (Fig. 21). At some places the epithelial region was found to be broken. Epithelial cells appeared to have shrunk and had acquired a cuboidal shape. Nuclei of these cells were smaller in size, and deeply stained with haematoxylin. Nucleolus was not discernible. Gland cells were somewhat tall and devoid of granules. These cells possessed basally situated deeply stained nucleus. Intercellular spaces were observed. Cells lining the neck were faintly stained with eosin and some cells possessed a few granules and stained moderately with eosin. Oval deeply stained nuclei were centrally placed. Intercellular spaces were also visible.

31

Sub-epithelial connective tissue appeared to have shrunk showing many lacunae. Empty cells storing fat were found in this region (Fig. 22). Presence of fat in these cells was revealed by Sudan Black B staining (Fig. 23). Some regions in immature <u>H</u>. <u>ilisha</u> was occupied by longitudinal muscles. Prominent blood vessels were observed.

Stratum compactum was of homogenous hyaline material showing spindle shaped deeply stained nuclei.

Cells of the stratum granulosum were somewhat shrunk and deeply stained with eosin. Cells were devoid of granules. Nuclei were situated on one side and were deeply stained.

In the circular muscle region, in some fibres empty areas with faint membrane was discernible. This might indicate the loss of fibrils from the muscle fibre. At certain places fibres were loosely arranged. Nuclei were spindle shaped and moderately stained.

Serosa was/numerous blood wessels. Here and there a few fibres of connective tissue were lost leaving faint eosinophilic mass with nuclei.



- Fig. 20. T.S. of Corpus of immature <u>H</u>. <u>ilisha</u> showing normal gland cells. <u>HE</u>. X 400.
  Fig. 21. T.S. of Corpus of maturing <u>H</u>. <u>ilisha</u> prior to migration showing atrophy of epithelium. Note absence of villi. <u>HE</u>. X 63.
  Fig. 22. T.S. of Corpus maturing <u>H</u>. <u>ilisha</u> prior to migration showing longitudinal muscles and fat cells in subepithelial connective tissue. <u>HE</u>. X 63.

#### Corpus of migrating mature H. ilisha captured from river:

During migration, due to starvation, the stomach had contracted and the lumen much reduced (Fig. 24).

At many places epithelium was broken and partly lost (Fig. 25). Remnants of broken cells were found in groups. Epithelial cells found at few places were shrunk and cuboidal. Their irregular shaped nuclei were densely stained with haematoxylin.

Entire glands appeared to have shrunk (Fig. 25). The cells of the gland were completely degra nulated, their cytoplasm staining faintly with eosin. Nuclei were with very little chromatin. Cells of the neck were also degranulated and lightly stained. Basally situated nuclei were with little chromatin. Crypts were not visible clearly as the epithelial tissue had shrunk considerably. A few places showed complete eosinophilic collagenous mass above the crypt. In some cases the epithelium with gastric glands and part of subepithelial connective tissue were broken and lost.

Subepithelial tissue showed advanced degenerative changes. Connective tissue fibres appeared to have broken leaving lacunae. Occasionally in these lacunae were found shrunk nuclei with fragments of connective tissue fibres. Fat staining cells were numerous. Fat was found to be present in large quantity as compared with maturing  $\underline{H}$ . <u>ilisha</u> before migration (Figs. 24 & 23).

Stratum compactum and stratum granulosum could not



Fig. 23

Fig. 24



Fig. 24-A



Fig. 25

- Fig. 23. T.S. of Corpus of maturing <u>H</u>. <u>ilisha</u> prior to migration showing some fat loaded cells in subepithelial connective tissue. Sudan Black. B. X 17.
- Fig. 24. T.S. of Corpus of mature <u>H. ilisha</u> showing fat loaded subepithelial connective tissue. SBB. X 17.
  Fig.24A. T.S. of Corpus of mature <u>H. toli</u>. No deposition of fat in subepithelial connective tissue. SBB. X 22.
- Fig. 25. T.S. of Corpus of mature H. ilisha showing marked atrophy of epithelium and gastric glands. HE. X 63.

be differentiated clearly as both the layers appeared to have mixed together forming a thick eosinophilic band. In the outer side of this region, probably stratum compactum, nuclei as described above, but of a smaller size, were discernible in the eosinophilic band.

The circular muscle layer showed wavy fibres and different intensity oc staining. Faintly stained areas showed degenerative changes such as loss of fibrils, shrinkage, fragmentation etc. (Fig. 26).

Serosa was found to be discontinous being broken at many places. It showed faint eosinophilic mass with nuclei and blood vessels. Corpus of spent H. ilisha captured from river:

The corpus showed similar structure and alterations as described in the case of migrating mature <u>H</u>. <u>ilisha</u>. In some cases additional degenerative changes such as loss of neck cells of the gland, gland cell proper, or complete shrinkage and dissolution of glands were noticed.

Corpus of immature H. toli captured from sea:

The histological structure resembled that of the immature <u>H</u>. <u>ilisha</u>. The following points are noteworthy. The subepithelial tissue was compact with the longitudinal muscle bundles cut in a transverse plane, showing deeply stained nuclei at the periphery of muscle bundle. These muscle bundles were penetrated by connective tissue fibres which on the outer side extended upto the stratum granulosum.

The stratum compactum, as mentioned already, was

found to be absent in <u>H</u>. toli.

Stratum granulosum was present between the longitudinal muscles of the subepithelial connective tissue and circular muscles. The layer was one to three cells in thickness. Cells were full of granules, with large oval nuclei containing fine chromatin granules.

Corpus of mature H. toli captured from sea:

Epithelial cells were trumpet shaped, with scanty granules and faintly stained nuclei.

Gastric gland cells showed normal appearance in a few cases. Many however, showed a distorted shape. Some were broken also. The nuclei were large, empty and with prominent nucleolus. Granules in cell were scanty. Debris of broken cells was observed. Neck cells also showed a varying degree of destruction like the gland cells. The few intact cells showed centrally placed round nuclei with granular chromatin material.

In between the glands the connective tissue septa showed deep eosin staining and contained large nuclei with chromatin. Blood cells were also found in this region. Subepithelial connective tissue exhibited structural alterations similar to those noted in mature <u>H</u>. <u>ilisha</u>. The only difference was that the longitudinal muscle bundles were absent. These muscles showed slight vacuolisation.

Stratum granulosum layer showed marked changes. The layer had become thick and eosinophilic and the cells were

shrunk, showing vacuolisation of the cytoplasm and the presence of very few granules. The nuclei were irregular in shape, some were curdled and were devoid of chromatin. This layer appeared to be detached from the surrounding layer.

Circular muscles and serosa were similar to those observed in mature <u>H</u>. <u>ilisha</u>. Serosa had become thick with more connective tissue fibres.

#### Corpus of spent H. toli collected from sea:

Except at a few places the faintly stained, trumpetshaped epithelial cells were intact. The nuclei were oblong and basally situated.

Most of the gland cells were normal, with basally situated nuclei containing little of chromatin. These cells contained many granules. At few places the gland cells were lost leaving empty spaces. Neck cells also were destroyed or lost at many places. The intact neck cells possessed numerous granules and an oval nucleus devoid of chromatin.

The remaining layers showed similar structure as observed in mature <u>H</u>. <u>toli</u>.

# Corpus of drifted H. toli collected from river:

At many places the epithelial tissue, cells of crypts etc. were completely destroyed (Fig. 27). The normal appearing gastric folds showed frequent loss of epithelial cells, detached epithelial tissue from the underlying subepithelial connective tissue. The remaining epithelial cells were trumpet-shaped, having basally situated oval nuclei containing little chromatin. Glands appeared to be destroyed. The cells were scattered and appeared separated. Nuclei of some of these cells were 'pycnotic', curdled and of irregular shape. Neck cells also showed same degree of changes.

Subepithelial connective tissue was faintly stained. Nuclei also were faintly stained, of irregular shape and were curdled.

Longitudinal muscles were also found missing from certain regions. Those present showed normal staining of both fibres and nuclei. Stratum granulosum though maintained its entity, its cellular structure was not clear. Faint eosinophilic layer with scattered deeply stained nuclei were only discernible. Presence of many empty spaces in this layer was a remarkable feature.

The circular muscle layer showed marked disintegration and destruction (Fig. 28). In one sample the circular muscle layer was broken and subepithelial layer was exposed to the exterior. In others it was cut in transverse, longitudinal and oblique sectional planes. This was presumably due to uneven contractions and twisting of the muscle layer.

The serosa was not a continous layer. It was mostly broken, exposing the circular muscle layer.

## PYLORIC STOMACH

Internally the surface mucosa ie. the epithelium and the subepithelial connective tissue was thrown into a net work of folds. The stomach wall is much simple here, consisting of (i) epithelium (ii) subepithelial connective tissue containing glands of pyloric stomach or gastric glands (iii) outer circular muscle layer formed of smooth muscles and (iv) serosa, covering these layers. Outer longitudinal muscle layer as well as layers of stratum compactum, stratum granulosum and muscularis mucosa were absent.

Pyloric stomach of fingerling of H. ilisha captured from river:

Epithelium is formed of cuboidal cells with faintly stained nuclei.

Glands showed basally situated nuclei containing a small quantity of chromatin. The cells contained a large number of granules towards the lumen. Neck cells with basally situated nuclei were faintly stained. Crypt cells possessed granules and were faintly stained with eosin.

The circular muscles showed normal structure and staining. Serosa was thin and contained blood vessels. <u>Pyloric stomach of immature H. ilisha captured from sea</u>:

Epithelium showed normal folds (Figs. 29 & 30). The epithelial cells were columnar with round nuclei basally situated. They were faintly stained and possedsed granules. The basal portion of the cell showed a comparatively deeper staining.

The bulb like glands were deeply situated below the epithelium (Fig. 29 & 31). Their neck showed a clear opening, between the gastric folds into the lumen of the stomach. The cells possessed basal round nuclei with little chromatin. The granules were situated towards the base.

The subepithelial connective tissue appeared to be compact between the glands. The whole region was moderately



- Fig. 26. T.S. of Corpus of mature <u>H</u>. <u>ilisha</u> showing marked degeneration of muscle layer. Note fat cells in subepithelial connective tissue. HE. X 63.
  Fig. 27. T.S. of Corpus of drifted <u>H</u>. <u>toli</u> showing broken epithelium. HE. X 63.
  Fig. 28. T.S. of Corpus of drifted <u>H</u>. toli showing degeneration
- Fig. 28. T.S. of Corpus of drifted <u>H. toli</u> showing degeneration of muscle. HE. X 400.
  Fig. 29. T.S. of Pyloric stomach of immature <u>H. ilisha</u> showing
- normal epithelium. HE. X 63.

stained and possessed spindle shaped nuclei. Many blood vessels were present.

The compact circular muscle layer was deeply stained with eosin (Fig. 32). The serosa was thin and well vasculorised. <u>Pyloric stomach of maturing H. ilisha captured from sea before</u> <u>migration</u>:

The histological structure was similar to immature <u>H. ilisha</u>.

# Pyloric stomach of migrating H. ilisha captured from river:

In the epithelium normal villi had disappeared leaving stomach folds with irregular surface (Fig. 33).

Glands of pyloric stomach were shrunk and lost at many places (Fig. 33). The cells possessed eosinophilic granules and deeply stained nuclei. Cells of the neck showed shrunken appearance. The nuclei were shrunk and deeply stained. These cells were wanting at some places due to disintegration. Cells of the crypt were cuboidal with granules and centrally placed nuclei.

The subepithelial connective tissue was faintly stained. It showed several lacunae. The muscle fibres seemed to have separated. Some muscle cells were lost (Fig. 34). Nuclei were large and faintly stained and were more or less empty.

The serosa layer was thin and vascularised. <u>Pyloric stomach of spent H. ilisha captured fron river</u>:

The histological details observed were similar to that of mature <u>H</u>. <u>ilisha</u> captured from river.



## Pyloric stomach of immature H. toli captured from sea:

The histological structure was similar to that of the immature <u>H. ilisha</u> captured from river.

#### Pyloric stomach of mature H. toli captured from sea:

The epithelium was broken at a few places. The cells were low columnar and possessed basal nuclei. The latter were moderately stained with haematoxylin.

Gland cells were with plenty of granules and deeply stained basal nuclei. Neck cells showed deeply stained basal nuclei. Some glands were shrunk and neck cells were not clearly seen so as to reveal the histological details.

Subepithelial connective tissue showed some dacunae but otherwise the structure was normal.

Circular muscles showed separation of a few fibres here and there, with spindle shaped faintly stained nuclei containing little of chromatin.

Serosa was with connective tissue fibres and was vascularised. The thickness was more than that of immature <u>H</u>. <u>toli</u>. It was broken in some regions.

## Pyloric stomach of spent H. toli captured from sea:

No major histological differences were noted from the details described in the case of mature <u>H</u>. <u>toli</u>.

## Pyloric stomach of drifted H. toli captured from river:

Marked destruction and shrinkage were prominent in the epithelial and subepithelial tissue, including the glands. Epithelium was tikally lost in the part of the stomach examined.

Thus the subepithelial tissue with glands was exposed to the lumen.

Glands were completely shrunk. Neck cells could not be differentiated. Nuclei of shrunk gland cells showed varied conditions viz. some were deeply stained, some were curdled and some were pycnotic and devoid of chromatin (Fig. 35).

The subepithelial tissue stained faintly. It showed many lacunae. Nuclei were curdled or irregular in shape.

The circular muscle layer showed many lacunae. Some fibres were detached and broken also. Nuclei were enlarged, some showing a curdled condition. Some were empty without chromatin.

The serosa was thick and separated from the circular muscle. It was found to be discontinous and the intact regions showed lacunae. Some of the nuclei had enlarged and were empty. Others were normal and deeply stained.

#### INTESTINE

The wall of the intestine consists of (i) the innermost epithelium (ii) the subepithelial connective tissue- the region between the epithelium and stratum granulosum (iii) stratum granulosum (iv) stratum compactum (v) circular muscle (vi) longitudianl muscle layer of smooth muscle and (vii) serosa. Internally the intestine shows numerous tall villi (Fig. 36). Intestine of fingerling of H. ilisha captured from river:

The epithelial cells were tall and slender with oval, deeply stained nuclei. Mucus or goblet cells were also present. Subepithelial tissue was much less in comparisom to the other



Fig. 36

- Fig. 34. T.S. of pyloric stomach of mature <u>H</u>. <u>ilisha</u> showing alterations of muscle layer. <u>HE</u>. X 400.
  Fig. 35. T.S. of pyloric stomach of drifted <u>H</u>. <u>toli</u> showing shrunk, broken glands and eosinophilic mass in subepithelial connective tissue. <u>HE</u>. X 400.
  Fig. 36. T.S. of intestine of immature <u>H</u>. <u>ilisha</u> showing normal villi. <u>HE</u>. X 63.

layers. Stratum granulosum and stratum compactum were not present. Circular and longitudinal muscle layers were compact showing spindle shaped nuclei.Serosa was very thin and vascularised.

## Intestine of immature H. ilisha captured from sea:

The prominent top plate was followed by tall, slender cells with little granules and deeply stained, basally situated oval nuclei (Fig. 37). Goblet cells were observed interspersed, very often showing the discharge of the secretion. The basement membrane on which the epithelial cells were situated appeared normal.

Subepithelial connective tissue appeared normal with their deeply stained oval nuclei.

Cells of the stratum granulosum were observed forming a continous layer. These cells were stained deeply with eosin and azocarmine. These cells, somewhat oval in shape contained numerous granules in their cytoplasm. However, a few cells appeared empty due to the discharge of granules. The nuclei were excentric and faintly stained. Very often extrution of granules from these cells were observed.

The layer of stratum compactum was of considerable thickness. It showed many lacunae and deeply stained cytoplasm. It stained blue with Azan stain and faint red with ecsin.

Circular muscle layer was generally compact, staining more deeply with eosin than the layer of stratum compactum. The spindle shaped nuclei were deeply stained.

Longitudinal muscle layer also was found to be compact and deeply stained with moderately stained nuclei. Blood vessels were seen in this region. A very thin serosa showed deeply stained nuclei.

Intestine of maturing H. ilisha captured from sea prior to migration:

Tall, slender cells with moderately stained oval nuclei and goblet cells formed the epithelium. Prominent top plate was present covering the epithelium. Basement membrane supporting the cells was clearly visible.

The subepithelial connective tissue was somewhat shrunk and well vascularised. Nuclei were oblong and faintly stained. In comparison to stratum compactum, this region was stained faintly.

The stratum granulosum was represented by scattered cells, full of granules, out side the subepithelial connective tissue. The cells were less in number than those found in immature <u>H</u>. <u>ilisha</u>.

Stratum compactum showed loss of some region and empty spaces, Blood capillaries and wandering blood cells were also seen in this region.

. The circular muscle layer showed a wavy appearance, some fibres were separated from each other causing lacunae. Irregular shaped nuclei stained faintly. The longitudinal muscle layer showed presence of blood capillaries and blood cells. At many places fibres were loosely arranged and some were lost.

The serosa was formed of a thin layer with blood vessels.

## Intestine of migrating mature H. ilisaha captured from river:

In most cases the intestine was found contracted. The long, tall villi showed prominent goblet cells, the latter being present in large numbers in comparison to the intestine of <u>H</u>. <u>ilisha</u> captured from sea. Rarely the intestinal lumen contained mud.

The epithelium possessed a prominent top plate. Tall slender epithelial cells contained basally situated, oval nuclei, the latter being faintly stained with very few chromatin granules. Numerous small and large goblet cells were observed in the epithelium. The basal membrane was thick and deeply stained.

The subepithelial connective tissue layer contained many blood vessels. The region towards the epithelium was compact than the one towards the stratum granulosum.

Cells of the stratum granulosum, less in number comparatively, were observed scattered above the layer of stratum compactum. Rarely they were in groups. The cells contained excentric nuclei finely dusted with chromatin material and were filled with granules. Extrusion of granules could be seen in very few cells.

Deeply stained stratum compactum appeared to have increased considerably in thickness. Faintly stained, irregular shaped nuclei were observed scattered in this region.

The circular muscle layer contained a few lacunae. The nuclei were spindle shaped, empty and faintly stained.

Longitudinal muscle showed empty spaces in some regions. Some fibres were lost. Nuclei were faintly stained. The serosa formed of a thin layer covering the longitudinal muscle layer. <u>Intestine of spent H. ilisha captured from river</u>:

Marked degenerative changes such as loss of villi, separation of the epithelium from subepithelial connective tissue, presence of many lacunae in the latter etc. could be noticed (Fig. 38).

The top plate appeared to have increased in size (Fig. 39). Epithelial cells of the intact villi possessed enlarged oval nuclei which were faintly stained and devoid of chromatin. Goblet cells were fewer in number than those in spawning <u>H</u>. <u>ilisha</u> but more than the immature <u>H</u>. <u>ilisha</u> from sea.

Deeply stained nuclei of subepithelial connective tissue were somewhat enlarged. The presence of lacunae indicated loss of some regions.

The stratum granulosum layer was not observed as such. Very few scattered, degranulated cells were observed in a few cases.

The stratum compactum appeared to have increased in thickness. Partial loss was noted in this region. The staining affinity was uneven. Nuclei were few, curdled and faintly stained.

The circular muscle layer was loose, with the fibres separated forming lacunae. The entire region was faintly stained with irregular, curdled and faintly stained nuclei.

\_ 49



Fig. 37



Fig. 38



Fig. 39

- Fig. 37. T.S. of intestine of immature <u>H. ilisha</u> showing epithelium with top plate (arrow). HE. X 400
  Fig. 38. T.S. of intestine of spent <u>H. ilisha</u> showing marked alterations in epithelium (arrow) and subepithelial connective tissue. HE. X 63.
  Fig. 39. T.S. of intestine of spent <u>H. ilisha</u> showing thickened top plate (arrow). HE. X 400.

The longitudinal muscle layer showed lacunae and 68 occasional partial loss too (Fig. 40). Faintly stained in 8 gular nuclei were observed.

Intestine of immature H. toli captured from sea:

The epithelium was covered by a prominent topplate. The tall slender epithelial cells possessed basally situated oblong nuclei and showed the presence of granules. The nucleus contained finely granular chromatin and a nucleolus. Many mucus secreting cells were present in the epithelium.

Subepithelial connective tissue was compact with deeply stained nuclei.

The histological structure of the remaining layers was similar to that of immature <u>H</u>. <u>ilisha</u> except that the cells of the stratum granulosumshowed scanty granules. Intestine of mature H. toli collected from sea:

The epithelium was made up of tall slender cells containing oval nuclei. A great number of goblet cells than in immature <u>H. toli</u> were present, but these cells were mostly in the exhaust phase. The epithelium was found broken at some places and frequently were separated from the layers below.

The subepithelial connective tissue gave a loose shrunk appearence and was faintly stained. Many lacunae were present. Deeply staining nuclei with no definite shape were prominent.

Cells of the stratum granulosum were found in groups. These cells were mostly granulated and with excentric nuclei.

The stratum compactum was faintly stained with eosin. It showed many lacunae with faintly stained curdled nuclei containing scanty fine chromatin.

Circular muscles were faintly stained and contained empty spaces between the fibres. The nuclei were spindle shaped and deeply stained.

The longitudinal muscle layer showed some shrunken regions. In such regions, the nuclei were deeply stained and appeared to have shifted to one side of the fibres. In normal regions the nuclei possessed little chromatin.

The serosa showed spindle shaped, deeply staining nuclei. In some places it was not continous. Intestine of spent H. toli captured from sea:

The structure showed many similarities to that of spent <u>H. 11isha</u> captured from river. However, in the following details it differed from spent <u>H. 11isha</u>. The goblet cells were lesser in number. The circular muscle layer was stained deeply. The nuclei also were deeply stained. The longitudinal muscle layer contained a few lacunae and the nuclei were deeply stained. Intestine of drifted mature H. toli captured from river:

In many regions the entire villi along with the subepithelial connective tissue and other layers except the outermost layer were found destroyed. The histological organization of the villi and of the other layers was completely lost (Fig. 41). Tremendous shrinkage, loss of tissue etc. were observed in almost all the samples examined.

Muscle layers also showed prominent alterations like separation and loss of fibres.



Fig. 40



Fig. 41

Fig. 40. T.S. of intestine of spent <u>H</u>. <u>ilisha</u> showing muscle degeneration. HE. X 400.
Fig. 41. T.S. of intestine of drifted <u>H</u>. <u>toli</u> showing marked destructive alterations in almost all layers. HE. X 100.

### DISCUSSION

The general histological features of the alimentary canal of <u>H</u>. <u>ilisha</u> and <u>H</u>. <u>toli</u> were found to be more or less similar to those of other teleosts studied by earlier investigators. However, a few remarkable differences were observed.

In general the epithelial layer of oesophagus is composed of stratified epithelium. But in both the species of <u>Hilsa</u> studied the epithelium was was formed of columnar cells. In <u>Hilsa</u> species the muscle layers consisted of a layer of inner longitudinal muscles in the subepithelial connective tissue and an outer layer of circular muscles. Both these muscle layers were composed of striated fibres. The above arrangement of muscle layer is common to many fishes and was reported by several workers (Dawes, 1929; Blake, 1936; Vanajakshi, 1938; Nagar and Khan, 1957; Ishida and Sato, 1960; Hale, 1965). It is also known that the inner longitudinal muscle layer may not be represented in certain teleosts (Greene, 1912; Blake, 1930; Weinreb and Bilstad, 1955).

The musculature of the corpus region showed certain characteristic features. In all the fishes studied the longitudinal muscle layer occurs outer to the circular muscle layer and both are composed of unstriated fibres. The corpus musculature is the direct continuation of the musculature of the oesophagus. A shift in the position of the above muscle layers in corpus has been clearly shown in the catfish, <u>Parasilurus</u> <u>asotus</u> (Ishida and Sato, 1960). In the <u>Hilsa</u> species studied

here it is remarkable that throughout the length of the corpus the arrangement of the muscle layer was as in the oesophagus, i.e. the circular muscle layer was outer to the longitudinal muscle layer. Moreover, both the muscle layers were composed of striated muscle fibres. It may be mentioned here that a continuation of the striated muscles of oesophagus into corpus for a very short distance, but not the entire length of corpus, has been noted in certain fishes (Blake, 1930, 1936; Vanajakshi, 1938; Hale, 1965).

55

Gastric glands are not known to occur in the pyloric stomach. However, in the <u>Hilsa</u> species the occurrence of gastric glands in the pyloric stomach has been noted. Such a condition has been reported only in two fishes by Nagar and Khan (1957) and Prakash (1961). The stomach musculature of the <u>Hilsa</u> species exclusively consisted of a single layer of circular smooth muscles, the outer longitudinal layer being absent. No record of such a condition in any of the teleosts is available.

It has been observed that migratory <u>H</u>. <u>ilisha</u> stores more lipids than non-migratory <u>H</u>. <u>toli</u> (Chapter,4). Storage of fat in the corpus was noted only in <u>H</u>. <u>ilisha</u>. The spaces occupied by the longitudinal muscle bundles in the subepithelial connective tissue in the corpus of immature <u>H</u>. <u>ilisha</u> are filled with fat loaded cells in the mature <u>H</u>.<u>ilisha</u>. Possibly the muscle cells are converted into fat cells. In maturing <u>H</u>. <u>ilisha</u> captured from sea prior to migration, transitional conditions showing both muscles and fat cells have been observed. It may be mentioned here that the lateral muscle fibres of the same fish, <u>H</u>. <u>ilisha</u> have been observed storing fat within the fibres during migration (Joseph, 1967). Baily (1952, cf. Vague & Fenasse, 1965) also has reported similar storage of fat within the fibres in the salmon and herring. No storage of fat in the  $(f_{i}, 2H, h)$ corpus of non-migratory <u>H</u>. <u>toli</u> could be noticed. Thus the corpus of <u>H</u>. <u>ilisha</u> stores fat for energy expenditure during migration and starvation.

From the present study it is evident that sexually mature H. ilisha shows marked atrophy and degenerative alterations in the various parts of the alimentary canal. The stomach epithelium shows atrophy, varied degrees of lesions and ulcerations. Such alterations are most marked in spent H. ilisha. Due to starvation the stomach was tightly contracted, leaving practically no lumen within. Compared to mature H. ilisha, H. toli of the same stage of maturity showed very less alterations, mostly due to shrinkage. The epithelium showed normal structure with occasional lesions. Muscle in some cases, however, showed fragmentation and vacuolization. The stomachs were not in contracted condition. Even empty stomach in a few cases were flacid, suggesting that the fish is not starving. In drifted H. toli, however, marked, abrupt destruction of various parts have been observed. The epithelium of stomach showed lesions and ulceration. In one case perforation of the stomach wall would have been complete but for a few external muscle fibres. Intact regions of the epithelium showed normal cells.

The alterations in the alimentary canals is related to the changes in the secretory cycle of the adrenal gland. In mature H. ilisha adrenal is in an active state showing hyperplasia and it can be assumed that more corticosteroids are produced. In spent H. ilisha adrenal showed atrophy indicating the absence of corticosteroid secretion. In drifted H. toli, the adrenal showed marked destruction. Thus in this case also no corticosteroids are available. In mature H. toli also a mild hyperplasia has been observed (Desai, 1967). The alterations observed in the alimentary canal of migratory H. ilisha and drifted H. toli may be attributed to the functional inactivity due to starvation and the activity of corticosteroids. Studies on rainbow trout, steelhead trout, kokanee salmon and Pacific salmon by several workers (Robertson and Wexler, 1960, 1962; Robertson et al., 1961a, b, 1963) have clearly indicated that histological alterations in the tissues and organs are due to hyperadrenocorticism accompanied with starvation. Marked rise in 17-hydrocorticosteroid was observed during spawning. Effect of this steroid is more marked when the animal is fasting as shown by observations on migratory and non-migratory rainbow trout (Robertson et al., 1961).

Bonta (1965) has reviewed the effects of corticoids on the induction of ulcers. It has been shown that the absence or overactivity of corticoids can induce ulcers and lesions. The importance of normal pituitary-adrenal endocrine system function in maintaining normal healthy condition has been shown.

It can be concluded that ulcerations and lesions which was found to be more prominent in spent <u>H</u>. <u>11isha</u> may be caused by the following factors: (1) stress due to spawning and change in the external medium (2) adrenal hormone system showing the least activity as evident by degeneration of adrenal glands (Desai, 1967) and (3) starvation. The interactions of these three factors in addition to aging would in general govern the alterations observed in the alimentary canal. In drifted <u>H</u>. <u>toli</u> the factors (1) and (2) mentioned above alone might have been effective in causing lesions, ulcers etc.

	INSTESTINE	lls tall. Mucus cells . S.G. S.C. not noticed. Lar comtact. Serosa rascularized.	<pre>nt top plate. Tall epi. cells with s. Many Tablet cells ging. S.G. cells and winn planty of and winn disonarged tent. S.C. of much ss and sinwed many . Circular musclas and normal. Serosa in and normal.</pre>	ne contracted. Long, tall ontained Gobiet cells. ium was with prominent te. Numerous Gobiet cells s. S.E.C.T. was with s. S.E.C.T. was with s. than cells of S.C. Cells th granules. Compactum stained and appeared to creased in thickness. r muscles were with few . Longitudinal muscles lacunae.	degeneration. Loss of Villi ion of epi. from S.E.C.T. te increase in size. Fred cells. Many lacunae in S.E. conticed in size partially creased in size partially ircular muscles losse shrun incular muscle losse Same no longi. muscle layer.
		Ep1. ce present chin &	Promina Slender Granule drachar compact the compact thickne lacunae compact thin th	Intesti Villi c Epithel Epithel top-pla nontroed numere le vere vi densely have circulan Circulan showed	Marked separat fop pla Goblet S.G. no S.G. 1n Lost. 1 Lost. 1 Canary 1 ced 1 L
-	PYLORIC	Tall cuboidal calls of Epithelium. Giand Cells with plen'y of g"anules. Circular muscles and other layers normal.	Fairtly stained epi. cells with granules. Glands deeply stained. Neck cells faintly stained. S.E.C.f. appeared compact and well vascularized. Muscle layer and serosa normal and vascularized respectively.	Epi. Normal Villi dis- appeared. Glands shrunk with eosinophilic cyto- plasmand granules Neck cells also shrunk. Cells of crypt cubidal. S.F.C.T. faintly stained and with many lacunae Muscle fibres appear to have lost. Serosa Was thin & vascularized.	Similar changes as noticed in migrating, mature <u>H. illsha</u> captured from river.
MIGRATORY H. ILISHA	CO oPUS(CARDIAC) STOMACH	Epithellum(Epi.) closely packed columnar cells. dlands granular, 3tratum compactum(SC, ar. St stun Granulosum(S0) not found. Mt.scle layerless thicker than inner layers.	Tall columnar cpl. cells. Compact calls of Gastric glands with Zymogen granules. Neck cells faintly stained. Connective tissue Septe clear. 5.k.C.T. with pleny of blood vessels and normal. S.d. of densely granula- ted cells with well differentia- ted supporting tissue. S.G. of hyaline tissue circular Muscles normal. Serosa thin with connective tissue.	Contracted. Lumen much reduced Epi. broken. Glands shurnk. Neck cells and Gland cells were de- granulated. Crypts shrunk. At several places glands & S.E.C.T. were lost, many lacunae. S.G. & S.C. could not be differen- tiated as they exhibited eosiono- philic band. Many lacunae in circular muscle. Faintly stained region showed loss of fibrils shrinkage eite. Serosa not conti- nuous broken at many places.	Similar histological changes as noticed in mature,migratio H. <u>1119ha</u> . In addition, loss of neck cells, loss of gland cells, complete loss of glands, dissolu- tion of glands elc.
	OESOPHAGUS	Large mucus secreting cells. All larers n. r.al.	Epi. of tall columnar cells and mucus secreting cells - large S.E.f.t.compact and well vascularized. Muscle layers and Serosa normal.	All layers showed degenerative changes. Epi. lost at some places. S.w.C.T. also was affected. Presence of fat cells. Clrcular muscles were loose and irregularly arranged. Many lacunae in muscu- laris, serosa separa- ted from muscla at several places.	Similar to migrating <u>H.ilisha</u> . Ept. colls were separated at some places. Mucus cells plenty. S.E.C.T. loosely arranged and with many lacunae. All other layer showed degenerative chan entres similar to <u>H.ilisha</u>
	PHARYNX	Gompacily arranged. All layers normal. Nujus sucreting cells discharging mucus.	Thickness of all layers increased. Tall mucus secret- frg cells. Ept. cells uriformaly a 2.2.4. Mary taste buds. Sub- epithelial connec- tive tissue (S.E.C.T.) with many blood vessels. Compactly arranged.	Epi, ruptured at few places. Mucus cells increased. S.e.C.T. loose and separated from epi. at some places, well vascularised. Longi, muscles wavy and with many lacunae Fibres broken at many places.	Similar histological changes as noticed in mature migrating H. <u>1115ha</u> captured from river,
	SPECIES	Fingerling of H.115ha certure from river	Immature II. 111518 from sea.	Meture migrating H. <u>113ha</u> captured. from river.	Spent <u>H. 111sha</u> captured from river.

96		( ) <sup>pr</sup>	epi, cells secreting Remaining fimmature	ere et cells was B.c.r. B.c.r. a g. Jup: a g. Jup: stained. k.	1. acunae, ue re- hi bi ted	g with t all loss of genera- lmost lmost
		INTESTINE	Prominant to plate. Tall with granules. Many mucus cells. S.E.G.T. compact. laye's similar to thue c <u>H</u> . <u>ilisha</u> .	Tall slender epi, cells w with top-plate. Many Gohl in 'exhaust phase'. Epi. Bave loose and shrunk app gave loose and shrunk app Many lacunas. S.G. were lis and were granulated cells Circular muscles faintly Longi. muscles were shrun Serosa not continous.	Goblet cells fewer. Long muscle layer showed few L except this, all the dege tive changes shown by the H. <u>illsha</u> (spent) were exh here.	At many places villi alon S.E.C.T. were lost. Almos layers destroyed prially Tremendous shrinkage and tissue. Marked, severe de tion and destruction of a all the regions noticed.
		PYJORIC	Similar to immature <u>H</u> . <u>111sha</u> .	Ept. broken at few places. Low columnar cells. Gland cells with plenty of granules. Some glands sh.unk and some neck cells also shrunk. 5.E.C.T. showed shrunk. 5.E.C.T. showed some lacunge otherwise normal. Circular muscles showed separation of few fibres. Serosa well vascularized and broken at some places.	Similar to mature <u>H. toli</u> captured from Sea.	Marked destruction and shrinkage noticed in Epithellum totally lost, Glands completely shrunk, Neck cells could not be differentiated. S.E.C.T. stalned faintly. Circu- lar muscles with many lacunae. Fibres broken, Serosa thick & separated.
TTETE TT	NON-MIGRATORY HILSA TOLI	STOMACH CORPUS (CARDIAC)	More or less simi'ar to immature H. ilisha. Str. comp. 75; absent. S.G. one to three layer in thickness cells were full of granules.	Ept. cells trumpet shapked & with scanty granules. Gastric glands were normal. few broken and lost, Debris of broken cells noticed. Mg cells did shou little destructive changes. S.E.C.T. shouedanges ob- served in migrating mature I. <u>115ha</u> . Longi, muscles exhi- bited vacuolization. S.G. became thick and cells shrunk and de- generative changes. Circular muscles and Serosa showed changes similar to mature migrating <i>L</i> . <u>115ha</u> .	Trumpet shaped epithelial cells were intact. Most of gland cells normal. At few places glands were lost. Neck cells were also lost. Remaining layer as noticed in mature <u>H. 1115ha</u> .	Epi. tissue, crypts, glands were destroyed at many places. S.E.C.T. also showed marked, severe destru- ction. S.G. cells structure could not be visualized. Muscle layers and Serosa also exhibited loss of fibres, fragmentation etc.
}		SUDPHAGUS	Histological observations as observed in immature त. रेज्येऽप्रे.	Tall mucus cells- many empty. S.E.C.T. lorsely emrty. S.E.C.T. lorsely larized. Fat cells ware present. Longi, muscies "ere loose. Both muscies layers showed very less alterations when compared with mature, migrating, H. ilishe.	Similar to mature H. toli.	Major portion of epi. destroyed along with S.E.C.T. cells shrunk. Marked destruction and degeneration in all layers. Different affinities for eosin were exhibited by muscle layers. Va.uol1- zation prominant. Jerosa also destroyed at few
, i		PHARYNX	Histological observa- tions as found in . immature <u>H. 1115ha</u> .	Cells of epi, layer normal except lesions at few places. Numerous m: us secreting cells m: its secreting cells arranged with lacunge. Many emptrad, storing- cells observed. % few portions showed loss of fibrils-muscles. Unlike mature migrating H.	Similar to mature H, <u>toll.</u>	Epithelium detached. S.E.C.T. showed pro- nounced degenerative changes. Muscle layer loose and with many lacunae. Loss of many ruscle fibres noticed. Presence of many blood cells in empty spaces in muscle layers formed.
		SPECIES	Immature Filss toll captured from ses.	Mature Hilsa toli from see.	Spent Hilsa toll captured from sea.	Drifted Hills captured from river.