CHAPTER IV

HISTOLOGICAL STUDIES OF THE PITUITARY GLAND OF THE MIGRATORY(ANADROMOUS) *HILSA ILISHA* (HAM.) AND NON-MIGRATORY *HILSA TOLI* (CUV. & VAL.) DURING MIGRATION, SEXUAL MATURATION AND DURING DIFFERENT PHASES OF THE LIFE-CYCLE

INTRODUCTION

Exhaustive literature can be referred for the physiological, histological, cytological and experimental studies of the pituitary gland of fishes (Pickford and Atz., 1957; Pickford, 1959; Bern and Nandi, 1964). Though many lacunae continue to persist, viz. the role and significance of proadenohypophysis or pars follicularis, site and presence of follicle-stimulating-like hormone, FSH; site of lactogenic hormone, LH; etc. It is now an establish fact that the pituitary gland or pituitary complex (Dodd, 1963a) of fish perform functions similar to those of the pituitary gland of higher vertebrates. The terminology followed in the running chapter was framed by Pickford and Atz (1957).

Few workers have suggested the proximal proadenohypophysis as the site of adrenocorticotropic hormone, ACTH secreting cells (Pickford, 1957; Olivereau, 1965). While few workers do insist on inclusion of mesoadenohypophysis (Chavin, 1958; in discussion). Whatever the site may be for ACTH, but it is definite that the pituitary gland of fishes have ACTH secretion which regulates cortical tissues situated in the head kidney (Pickford, 1957, 1959) and ACTH is obtained in pure form from the pituitary gland of fishes (Rinfret and Hane, 1955).

Thyroid stimulating hormone or TSH is also believed to be present. It is suggested that A.F. positive basophils of proximal part of proadenohypophysis appear to be source of TSH (Fontaine and Fontaine, 1962; Olivereau, 1954;Pickford, 1957; Olivereau and Herlant, 1960). According to other school of workers, TSH secreting cells are present in mesoadenohypophysis (Pickford, 1957, 1959). Recently the experimental work on Lepidiostus pituitary by Sage (1965) has supported the hypothesis of presence of thyrotropes in proximal proadenohypophysis. It is confirmed, after carrying out several experimental works, that thyrotropes are present in fish pituitary (Sokol, 1955; Barrington and Matty, 1954; Olivereau, 1960, 1963b; Olivereau and Ball, 1964).

Liu and Tan (1963) have differentiated thyrotropes and gonadotropes in Silver carp, Hypophthalmichthys molitrix, on basis of PAS reaction. Experimental work of Baker (1964) on early stages of teleost, *Herichthys cyanoguttatus*, states that few A.F. positive cells in juveniles are including thyrotropes also. Some workers could not differentiate thyrotropes from gonadotropes by histological techniques (Sokol, 1961; Robertson and Wexler, 1962a,b).

It was possible to isolate lactogenic hormone-like and follicle stimulating hormone-like activities from the extracts of the pituitary gland of fishes (Ramaswami and Sundarraj, 1958). In support to this, if we consider, it was possible to demonstrate histologically the site of FSH and LH in fish pituitary gland by Olivereau and Herlant (1960), though many workers failed to show (Matty and Matty, 1960; Sundarraj, 1959). Schmidt *et al*. (1965) affirmed that pituitary gland of mature fish secretes hormones which can stimulate gonads of immature fish, by stimulating the gonads of immature, *Salmo gairdnerii* with the extracts obtained from gonads of the mature Pacific salmon(Onchorhyncus). Many workers have demonstrated the increase in activities of basophils at the time of sexual maturation (Barr and Hobson, 1964; Robertson and Wexler, 1962a,b; Sokol, 1961; Liu and Tan, 1963; Olivereau, 1954). Experimental work has confirmed the presence of gonadotropes in fish pituitary and supported the work of above mentioned authors (Sokol, 1955).

There remains little doubt about the presence of growth hormone or somatotropic hormone (STH) in the pituitary gland of fishes and it is presumed that it is secreted by acidophils of mesoadenohypophysis (Matty and Matty, 1959; Olivereau and Ball, 1964). Follenius and Porte (1961a,b) supporting above mentioned view, stated that, they are (acidophils-probable source of the STH) present in the onset of the development. Recent work of Sage (1965) also suggested that the site of STH is in proximal part of mesoadenohypophysis (pars distalis). Enhanced secretory activities of the acidophil during growth and sexual maturation is also observed by Sokol (1961), Robertson and Wexler (1962a,b). Little doubt now remains about the STH production by acidophils of pituitary of fish. One school of thought has put forward, an osmo-regulatory role for growth hormone (Pickford, Robertson and Sawyer, 1965), in fish, though, Burden (1956) could not support it, but work of Smith (1956) supported the view stating, growth hormone increases salinity tolerance in Salmo trutta.

The role of pars follicularis, (Proadenohypophysis) is not yet fully established. Olivereau (1954) observed noticeable changes and increase in holocrine secretions in the proadenohypophysis of <u>Salmo salar</u> at the time of its entry into freshwater on the onset of spawning migration and decrease in activity during spawning. She also noted the transport of colloid of the follicles into the blood-stream. Recently, the presence of a group of cells, 'Erythrosinophil Cells', is noted in the rostral part of proadenohypophysis (Pars distalis) by several workers (Ball and Pickford, 1964, Ball and Olivereau, 1964) and are termed as 'Eta Cells'(Olivereau and Ball, 1964; Ball, 1965a,b). These 'Eta Cells' are believed to produce mammalian Prolactin-like hormone (paralactin). Eta cells were inactive in the normal pituitary of <u>Poecilia latipinna</u>, but were active when <u>Poecilia latipinna</u> was transferred to frewsh water (Ball and Olivereau, 1964; Olivereau and Ball, 1964) hence these authors believed that prolactin-like hormone is essential for the survival in the fresh water (Ball, 1965). The experimental work done on euryhaline Killifish, <u>Fundulus heteroclitus</u>, also suggest that a hormone resembling mammalian prolactin is essential for the survival of the fish in hypotonic external media (Pickford, Robertson and Sawyer;, 1965). On the other hand the pigeon-crop assay method for the test of prolactin-like hormone in fishes Salmo gairdnerii, Anoplopoma fimbria, Hydrolagus colliei, Squalus acanthias and several other fishes, has failed to yield positive results (Nicoll and Bern, 1964).

In a view to study the histological changes occuring in the pituitary gland, in correlation with migration, from hypertonic media (Sea) to hypotonic media (river), in correlation with the spawning, and ageing,the present work on the pituitary glands of migratory (anadromous) Hilsa ilisha and of nonmigratory Hilsa toli was undertaken. In addition to above, the study (histological) of the pituitary gland of nonmigratory H. toli, drifted into the estuary was also carried out, with a view that this may throw light on some unopened and unexplored fields. Later may be considered as an experimental work done completely under natural conditions.

In collection of all these fishes, enough care was taken to discard wriggled fish and to sacrifice fish immediately.

MATERIALS AND METHODS

The live fish was removed immediately from the net and the head was cut off. Upper roof of the skull was removed and brain was exposed to the fixatives. Within few minutes, the entire brain, alongwith the pituitary gland was fixed in the following fixatives.

(1) Bouin's fluid

(2) Zenker's formol

(3) Formol sublimate

(4) 10% Neutralized formalin

(5) Calcium formol

(6) Formal saline.

The tissue was fixed for 3 to 8 days and after dehydration, 4 μ to 5 μ paraffin sections were taken, and were stained by following staining procedures.

(1) Haematoxyline Eosin

(2) Heidanhain's Azan

(3) Masson's Trichrome (Masson's)

(4) Aldehyde Fuchsin (A.F.

(5) Paget's Aldehyde-Thionin-PAS. Mixture (Stahl, Leray, 1964)

Bouin's was found to be the best of all the fixatives.

RESULTS

Before studying the changes occurring in the different cell types, it is extremely needed to be aquainted with the terminology and different zones of the pituitary glands of

H. ilisha and *H. toli*. There is not much difference in the structure and cell types of the pituitary glands of *H. ilisha* and *H. toli*. The terminology adopted and suggested by Pickford & Atz (1957) is used throughout in the running chapter. To have an idea about the terminology used by other workers, the following details are given.

	Details		Terminology used
1.	Anterior lobe or Pars anterior or Anterior glandular region	Homologus to pars distalis of mammals or rostral pars distalis	Proadenohypophysis (Pickford & Atz 1957)
2.	Dorsal lobe or mid-glandular region	Homologus to proximal pars distalis of mammals	Mesoadenohypophysis (Pickford & Atz 1957)
3.	Ventral lobe or Posterior glandular region or Pars intermedia		Metaadenohypophysis (Pickford & Atz 1957)
4.	Neural lobe or neurointermediate lobe.		Neural lobe.

The above mentioned different zones are shown clearly in Figs. 1,6, 10,15,24 and 28.

Proadenohypophysis:

It is the anterior most part of the gland occupying one third part of the pituitary gland. It had extremely striking follicular structure which gives very sharp demarkation from the other zones of the gland. Recently, few workers have suggested 'Paralactin hormone' (similar to prolactin of mammals) secreting role for the follicles of this zone (Ball and Olivereau, 1964; Ball and Pickford, 1964).

In fresh tissue, this region exhibits milky white colour. The follicles were composed of cuboidial or tall columnar cells arranged compactly on the thick membrane surrouding the lumen of the follicles. The lumen contained colloid. The cells were acidophlic, and stain orange with Azan, Pinkish purple with Masson's, magneta or faint green with Aldehyde Fuchsin (A.F.) and yellow with Aldehyde-Thionin-PAS staining procedure. The colloid of the follicles stained light purple with A.F.; bluish with Azan, green with Masson's, and with Aldehyde-Thionin-PAS staining. Many sinusoids and blood vessels were observed.

Mesoadenohypophysis:

It occupies the region below cavity of third ventricle and approximately middle region of the pituitary gland. This region differes from proadenohypophysis in having diversely staining cells and complete absence of follicular structures, when the pituitary gland of sexually matured *H. ilisha* or *H. toli* was examined, this zone appears to occupy more than

1/2 portion of the entire gland. Two distinct types of cells could be identified. Acidophils, which stain red with Azan's, magneta to reddish with Gomori's Aldehyde Fuchsin (A.F.), and Masson's, yellow tih Paget's Aldehyde-Thionin-PAS mixture. They dominated till the maturity was attained. Basophils, which stained blue with Mallory's, green with Masson's, purple to deep purple with A.F., and bluish with Paget's A.F. thionin-PAS mixture. They were more in number in sexually matured animal and show intense granulation. Third type of cells, chromophobes, which were paler in colour were seen more in number as the maturity proceeded, i.e. in spent (Stage VI-VII of maturity), their cytoplasm as well as nuclei were devoid of granules and chromatin respectively. It is not known, from which type of cells, acidophils or basophils, they derived.

Metaadenohypophysis:

This region was also invaded by the branches of neural lobe and most of the neural lobe branches were found in this zone. It consisted of two types of cells, acidophils and basophils. Basophils dominated in this zone as sexual maturity proceeded. The cells were arranged in the form of columns and columns were always with thin basement membrane. This membrane was found prominently in mature and spent fishes, whereas it was rarely present in the fingerlings. Very few basophils were present in this zone of fingerling and they were compactly arranged in small groups. The nuclei were oval and comparatively larger in size and were placed centrally. This zone could not exhibit any cellular changes alongwith maturity.

Neural Lobe:

The region consisted of nervous tissue and blood vessels. It was devoid of vividly staining different types of cells. It was observed in the form of a branching tree. The branches penetrated deeply in almost all the different regions of the pituitary gland. The glial tissues,connective tissues and blood vessels were major components of this zone. The nuclei of the nervous tissues were perfectly round or oval and were filled densely with chromatin material. Masson's stain gave good results; it also indicated scanty of cytoplasm. This region brought neurosecretory material (neurohypophyseal hormone) from the neurosecretory cells i.e. Nuclei Preopticus and Nuclei lateralis tuberis. Neural lobe did show changes which could be correlated with ageing, and migration. In few cases colloid like materials were observed in neural lobe. Occassionally cyst formation and presence of lacunae could also be demonstrated histologically.

THE PITUITARY GLAND OF FINGERLING OF H. ILISHA CAPTURED FROM RIVER NARBADA:

The entire gland is 'dot' like, but exhibited well-organised microscopic structure (Fig.1)

Proadenohypophysis:

As stated before, this zone differed from all the other zones in possessing follicular structure. There were small follicles filled with ribbonshaped and homogeneous colloid. This colloid stained blue with Azan, green with Masson's, and light purple with A.F. The follicles were occupying roughly 1/4th part of the entire gland (Fig.1). The thick basement membrane surrounding each follicle was prominent. The cells were somewhat cuboidial in shape and were compactly arranged together. They stained brilliant red with Azan's, magneta with A.F., and dullrose with Masson's trichrome. Their oval,distinct, shaped nuclei were situated away from lumen and were dusted with finely granular chromatin material. Few granules in their cytoplasm, were also concentrated away from the lumina (Fig.2).

Mesoadenohypophysis:

The acidophils were dominating in this zone. They were arranged in the form of tall columnar cells on the basement membrane, later stains blue with Azan's. In most of the cells nuclei were placed centrally,whereas in few cells nuclei were very near the basement membrane. Nuclei of acidophils were oval and were laden with chromatin materials. Moderate amount of granules were observed in these cells. They also stained orange with A.F. staining; and dull rose with Masson's, red with Azan's (Fig.3).

Basophils were few in groups of four to six cells, their nuclei were of fairly good in size and were observed in centre of the cells. Nuclei were round and were dusted with finely granular chromatin material (Fig. 3).

Metaadenohypophysis:

The acidophils dominated in this zone also. They exhibited similar staining characteristics to acidophils of mesoadenohypophysis. They were arranged in columns and were columnar in shape, Their cytological characteristics were similar to acidophils of mesoadenohypophysis (Fig. 4). Basophils exhibited similar histological and cytological characteristics to those of mesoadenohypophysis, but in this region they were arranged at the extreme edge of metaadenohypophysis (Fig. 4).

Neural lobe:

This region penetrated deeply in almost all the region. It branched thickly in meso and metaadenohypophysis. The glial tissues were compactly placed and at very rare regions they were observed shrunk. There was complete absence of any colloidial material, cysts and lacunae. The nuclei in this zone were perfectly oval and were filled with chromatin material. The prominent blood supply was noticed in this region. This region showed very moderate amount of neurosecretory material when stained with A.F. staining, and it was in the form of colloid and granules (Fig. 5).

THE PITUITARY GLAND OF IMMATURE HILSA ILISHA CAPTURED FROM THE SEA:

When compared with the pituitary gland of fingerling (Fig. 6), remarkable increase in size of all the regions was noticed. In addition to this, different cell types also appeared, especially in mesoadenohypophysis.

Proadenohypophysis:

The tall columnar cells surrounding the lumen of the follicles were loosely arranged occassionally. The well defined basement membrane was observed to be thicker than that of noticed in this region of the fingerlings. The cells appeared to be inactive and loosely arranged cells were observed shrunk. Their nuclei were of irregular shape. Chromatin material was densely filled. Some follicles did exhibit solidly packed, tall columnar cells with round nuclei. The nuclear diameter of the follicles varied from 4.8 μ to 4.9 μ. Their cell height was from 16.34 μ to 16.64 μ. Nuclei were away from the lumen. The granules away from the lumen were also observed in the cytoplasm. These follicles - 'Erythrosinophilic follicles' were noted to occupy roughly more than 1/3rd region of the entire pituitary gland. The colloid of these follicles was comparatively more and was observed to be completely filling the lumen in few follicles. This colloid stained similar to the colloid of follicles of the pituitary gland of fingerlings (as in Fig. 13).

Mesoadenohypophysis:

This region occupies about 2/3rd of the entire pituitary gland. Two types of cells could be distinguished easily. The presence of many sinusoids, blood vessels and capillaries indicated prominent blood supply. Acidophils had increased tremendously in volume and in number too. The nuclei of acidophils were filled with densely filled granular chromatin and were with prominent nucleolei.

The nuclei were generally situated in centre of the cells. Acidophils showed strong affinity towards the stains and their many granules were seen clearly with Masson's and Azan's stain (Fig.7). Basophils, which stained blue with Azan , bluish purple with Paget's A.F. Thionin-PAS staining, green with Masson's and deep purple with A.F., showed accumulation of granules in their cytoplasm. These granules were beautifully observed with Azan. The nuclei were round and with finely granular chromatin material (Fig. 8).

Basophils were more in number and exhibited secretory activity when compared with those of fingerling.

Metaadenohypophysis:

The cells are densely packed instead of loosely arranged columns. Acidophils and basophils both appeared. All the acidophils cells stained dull orange with Masson's, redish with Azan's. They appeared inactive as they were seen shrunk and compressed. Their nuclei were of fairly large size comparatively and were densely laden with the chromatin material; little cytoplasm was also noted in few acidophils (as in Fig. 7). Very few basophils were observed with round, large sized nuclei, with chromatin and nucleolus (as in Fig. 8).

In most of the acidophils and basophils granules in moderate amount was noticed, but granules in acidophils were more (Fig.7). This region was profusely supplied with large sized blood vessels.

Neural lobe:

It had enormously expanded and invaded profusely all the regions of the pituitary gland. The glial tissues were compactly placed. The nuclei of this zone were big and oval frequently elongated nuclei were also observed. There were many blood vessels and capillaries observed throughout this region (Fig.9). In the region of mesoadenohypophysis, the neural lobe exhibited colloid like structure bounded by definate cell-layers (Fig.9). Few neurosecretory granules were observed scattered throughout the zone (Fig.9), the neural lobe penetrating meta-adenohypophysis, was totally devoid of neurosecretory granules; while the mesoadenohypophyseal neural-lobe contained few granules and granules were often seen surrounding blood vessels (Fig.9).

On the whole, pituitary gland of immature *H. ilisha* exhibited more acidophils and basophils, presence of NSM in neural lobe, than the pituitary gland of fingerling of *H. ilisha* captured from river.

PITUITARY GLAND OF SEXUALLY MATURE *H. ILISHA*
CAPTURED FROM SEA PRIOR TO MIGRATION:

The noticeable increase in size is worthnoting (Fig. 10).

Proadenohypophysis:

The tall columnar cells (acidophilic) are compactly placed with each other on the thick basement membrane surrounding the lumen. The nuclei were oval and filled with chromatin material due to which sharp visibility to nucleolus was not imparted. The granules in the cytoplasm and nuclei were situated away from the lumen of the follicles. The colloid filled the all follicles (Fig.11). The nuclear diameter varied from 4.8 μ to 4.9 μ and cell height of the follicular cells-erythrosinophilic follicular cells - varied from 16.8 μ 16.9 μ. Entire proadenohypophysis roughly occupied 1/3rd of the entire gland (Fig.10). Follicles had increased their lumina. When compared with sexually immature *H.ilisha* the proadenohypophysis appeared to be increased in size.

Mesoadenohypophysis:

Both the cell types had increased in number and in volume along with the advancement of the sexual maturity. In the central zone of the mesoadeno. basophils dominated, whereas in the dorsal zone - below third ventricle cavity. Acidophils suppressed over basophils.

Basophils:

These cells were arranged in columns and were bounded by thin, delicate basement membrane, which stained blue with Azan and green with A.F. Their granules were sharply distinguished by Azan and were observed in plenty. The nuclei were perfectly round in shape and were centrally located in the cytoplasm of basophils (Fig. 12). Masson's technique was best for the demonstration of nuclei, with which nuclei exhibited finely granular chromatin and a nucleolus. These cell groups were separated by basement membrane, some basophils exhibited different staining intensity with Azan. Frequently few of them were observed with little granules.

Acidophils:

These cells stained wonderfully with Azan. The granules were plenty in the cytoplasm, whereas nuclei were small and were situated near the basement membrane. Acidophils stained dullred or orange with Masson's,yellow with Paget's aldehyde thionin-PAS mixture and greenish to orange with A.F. They exhibited coarse granules in plenty of numbers. They did not show any change in staining reaction, when stained with Azan's.

Chromophobes:

These nongranular cells appeared in very few numbers in mesoadenohypophysis. They stained dull yellow with A.F., their nuclei and cytoplasm both were observed to be empty. They were easily distinguished by A.F. staining.

Ghost cells were observed in the regions of neural lobe boarding mesoadeno. in very few numbers.

Neural lobe:

It penetrated deeply by its branches in all the regions of pituitary gland. It appeared as a compact mass but at few places in mesoadenohypophysis, it did exhibited loose and shrunk structure. The presence of plenty of blood vessels and capillaries was noted. There were neither colloidial substances nor cysts were seen. The neurosecretory material, A.F. +ve, (NSM) was observed in the form of granules in moderate quantity throughout the branches of neural lobe into mesoadenohypophysis and proadenohypophysis. Frequently these granules were observed surrounding the blood vessels in the neural lobe (Fig.13). The nuclei of neural lobe were of fairly big oval form with finely granular chromatin. These nuclei were sharply noted by Masson's. Ghost cells were few and they exhibited irregular shaped nuclei and were devoid of any content in the mesoadeno. region (Fig.13).

Metaadenohypophysis:

This region is composed of two distinct cell types, acidophils and basophils. The branches of neural lobe is of fairly good size in this region and contains little NSM and many blood vessels. Acidophils were arranged in columns, which were bound by delicate basement membrane, which was distinctly stained blue with mallory and green with A.F. They were loosely arranged and their nuclei were filled with chromatin densely due to which nucleolei were not visible (Fig. 14).

Basophils were arranged in groups at the edge; they were full of granules (as in Fig. 12).

PITUITARY GLAND OF MATURE(STAGE V AND VI OF MATURITY) HILSA ILISHA CAPTURED FROM RIVER DURING MIGRATION:

The tremendous increase in size including all the different regions, increase in number and secretory activities of both acidophils and basophils, remarkable expansion in the lumina of follicles of proadenohypophysis and increase in secretory activities of follicular cells of it, discharge of NSM in noticeable quantity from neurosecretory cells into neural lobe and presence of chromophobes and ghost cells etc. were remarkable changes observed in the pituitary gland of migrating mature H. ilisha (Figs. 15 and 16).

Proadenohypophysis:

The region roughly occupied half of the entire gland (Fig. 17). Few follicles were observed as broken and almost all follicles were devoid of colloid. Small follicles with small lumina were fewer in number (Fig.17). The basement membrane and the layer of the follicular cells facing lumina had increased in thickness. These cells stained bright red with Azan , magneta with Masson's, grey with A.F. and bright yellow with Paget's A.F.-Thionin-PAS mixture. Their nuclei were round, large and mostly devoid of chromatin, in few cells finely divided, granular chromatin were observed in the nuclei alongwith well defined nucleolus (Fig.17). All these nuclei were towards the lumina. Plenty of granules in these cells were observed concentrating near the lumen of the follicles. Nuclear diameter varied from 6.7 μ to 6.8 μ and cell height from 22.4 μ to 22.5 μ.

NSM,(A.F. positive) purple, Paget's A.F.-Thionin-PAS-positive, green, was noticed in the region among follicles and in the follicular cells too. The appearance of NSM in the cells could be observed in successive stages. Its entry started with the basement membrane, then to the base of the follicle cells(Fig.18), then in the cytoplasm of the follicles followed by the upper region around the nuclei of the follicles(Fig.19).

Mesoadenohypophysis:

In the central zone of the mesoadenohypophysis basophils dominated. They were loaded with plenty of granules throughout their cytoplasm. Their nuclear structures were also not visible very often. Few of them showed more degranulation, which accompanied by empty places in the cytoplasm. In these cells, nuclei were small, round and centrally located. Nuclei were laden with finely granular chromatin and a prominent nucleolus in each nucleus (Fig. 20). Few cells turned into chromaphobes which were devoid of granules, their nuclei were of irregular shape and without chromatin material. They stained dull yellow with A.F. The granules of basophils were beautifully stained by Azan. Basophils of dorsal region - below cavity of third ventricle - were fully loaded with granules and there was not a basophil which was showing sign of degranulation (Fig.20). The nuclei were oval,small and stained red with Azan. The membrane on which these cells were situated appeared thicker in comparison with that of fingerling and immature Hilsa ilisha. It stained blue with Azan's, green with Masson's and A.F.

Acidophils of central mesoadenohypophysis were tall columnar cells with fairly large sized oval nuclei. They were situated on the thick basement membrane in the form of columns. Plenty of the granules were noticed in their cytoplasm. Nuclei were filled with granular chromatin and were with on small nucleolus in each cell. Nuclei were situated near the membrane (Fig.21). Acidophils dominating in the dorsal zone of mesoadenohypophysis. Acidophils never exhibited degranulation or any change in staining affinity.

The remarkable blood supply in this region was noticed (Fig.21).

Metaadenohypophysis:

Intense blood supply was noticed by the presence of many large sized blood vessels in this region. In comparison with increase in size of other regions of the pituitary, this zone had also increased. Basophils, which present the similar staining affinity as shown by that of mesoadeno., but in this zone none of them exhibited degranulation, all were fully loaded with granules. They were situated at the edge of metaadenohypophysis forming a rim like structure. Their nuclei were oval and fairly large in size (Fig. 22).

Acidophils bounded by thin delicate membrane were arranged in the form of columns, and maintained the structures and cytological pecularities of acidophils of mesoadenohypophysis. Their nuclei were abnormally enlarged and little chromatin were noticed in it (Fig.23).

NSM was observed densely filling neural lobe here and was concentrated around the blood vessels (Fig.21). In short, acidophils of metaadenohypophysis showed striking changes (Fig. 21).

Neural lobe:

It had also increased in size and deep penetration in all regions was maintained. All the branches of all the regions were fully loaded with NSM (A.F. positive, Paget's A.F.-Thionin-PAS mixture +ve) that cytological study of neural lobe was not possible. The region in dorsal part of mesoadeno. was comparatively with less amount of NSM, whereas the branches of metaadeno. were fully loaded with NSM which concentrated heavily around the blood vessels (Figs. 21, 23).

PITUITARY GLAND OF SPENT HILSA ILISHA(STAGE VII & VIII OF MATURITY) ON RETURN MIGRATION TO SEA, CAPTURED FROM RIVER:

It is evident from the photograph that the gland had reduced in size and is about half of the pituitary gland of mature, migrating and non-migratory *H. ilisha* and *H. toli*, respectively (Fig.24). The presence of many chromophobes, many ghost cells, degranulation of basophils, presence of scanty NSM in neural lobe shrinkage in and presence of many blood vessels in neural lobe etc., are worthnoting pecularities observed in this gland.

Proadenohypophysis:

It occupied roughly more than 1/2 region of the entire gland. New smaller follicles appeared to have developed which were filled with colloid (green with Masson's, light purple with A.F., blue with Azan). Larger follicles were noted as broken (Fig. 25). It was interesting to note their opening into the exterior of the gland. This was observed in few samples of mature migrating *H. ilisha* also. At some places entire portion was destroyed.

The cells of smaller follicles were closely packed and as in the larger follicles, nuclei occupied polar portions alongwith plenty of granules. The cells were tall and columnar and were situated on comparitively thicker basement membrane. In larger follicles few cells were shrunk and were pale yellow coloured (A.F. stain). Few were lost also leaving empty space in the follicular epithelium (Fig. 25). Many blood vessels were noticed among the follicles. Nuclear diameter varied from 6.7 μ to 6.9 μ and cell height 21.2 μ to 21.3 μ.

Mesoadenohypophysis:

The first striking feature was the presence of plenty of chromophobes in central region of mesoadeno. and degranulation of many basophils. Polygonal shaped basophils showed wonderfully the degranulation by A.F. and Azan's staining. The nuclei of these cells were placed at one end of the cell (Fig. 26).

Many of them appeared to have shrunk. Many basophils with very few A.F. positive granules were also noticed. The basophils-degranulated completely or more or less completely degranulated, were also observed boardering pars nervosa where many ghost cells were also noticed(Fig.28). It is probable that these basophils may also get themselves converted into ghost cells and slowly enter in the pars nervosa or neural lobe region. Many basophils exhibited pycnosis and cytolysis also (Fig.28). Basophils situated in the dorsal region were not much more affected. They had maintained their entity of mature H. ilisha.

Acidophils also exhibited similar degenerative changes as shown by basophils. The increase in thickness of basement membrane, degranulation, pycnosis, cytolysis and complete degranulation etc., degenerative characteristics were noticed in acidophils of central zone of mesoadeno. (Fig. 27), but acidophils of the dorsal region of mesoadeno. did not exhibit similar degenerative changes.

Chromophobes appeared in central zone of mesoadeno. They stained pale yellow or dull yellow with A.F. and were devoid of granules. Their nuclei were noticed in good numbers in the region where neural lobe came in contact with mesoadeno. region. These cells had very irregular shaped nuclei and wavy cell boundaries. Cytoplasm was devoid of any granules (Figs. 26 & 27).

Many small and large sized blood vessels appeared in mesoadenohypophysis. Very frequently empty spaces were also noted, this may be due to loss of acidophils or basophils. Sometimes, loose, shrunk cell without definite cell boundaries was seen in empty space, and frequently only scattered nuclei were observed (Figs. 26 and 27).

Metaadenohypophysis:

The striking changes as shown by mesoadeno. were not exhibited by metaadenohypophysis. Basophils were fully loaded with A.F., positive purple granules and were arranged in groups of six to eight. Their small, centrally placed nuclei were laden with chromatin and nucleolus. As the region in ventral side is studied, basophils were observed in the form of a rim only.

Acidophils did show changes. Their nuclei were abnormally enlarged that little cytoplasm was observed in some cells. Chromatin material was seen in the form of fine granules and nucleolus was prominant. Basement membrane, on which acidophils were arranged in the form of columns had increased notably in size, which stained green with Masson's.

Neural lobe:

Pars nervosa or neural lobe also showed noticeable changes. Neural lobe was shrunk and loose. The appearance of many blood vessels was prominent feature. The nuclei were oval and enlarged. There were no chromatin material in nuclei (Fig. 28). Many ghost cells were observed in the region which came in contact with cells of meso and metaadenohypophysis. At some places empty spaces were also observed, this might have been caused by loss of glial and connective tissues. The compactness was not maintained (Fig. 28). At few places, colloid like material surrounded by follicle cells was noticed, which obtained faint orange with Masson's trichrome.

On the whole, neural lobe was devoid of NSM except in few sections, neural lobe of metaadenohypophysis showed NSM especially around the blood vessels.

PITUITARY GLAND OF NONMIGRATORY IMMATURE(STAGE III OF MATURITY) HILSA TOLI CAPTURED FROM THE SEA:

The entire gland is small in size but it showed all the zones clearly and it reasembled in all respects to the pituitary gland of H. ilisha.

Proadenohypophysis:

Unlike the region of H.ilisha, it was very small and it occupied not even 1/4th of the entire gland. Small follicles with small lumina full of the colloid were observed. The cells were inactive as observed in immature H. ilisha. Nuclear diameter varied from 4.5 µ to 4.7 µ, cell height from 15.3 µ to 15.4 µ. Basophils were dusted with A.F. granules and were arranged in columns in compact fashion. Their oval nuclei occupied central position in the cells.

Acidophils were arranged on thick membrane and were with plenty of the granules. Their nuclei were situated near the basement membrane and were with finely granular chromatin and a prominent nucleolus in each cell. Many blood vessels were noted. Except above mentioned differences the other characteristics were found similar to the pituitary gland of immature Hilsa ilisha.

Mesoadenohypophysis:

Many basophils were noted in the central region of mesoadeno. and they were dominating. They did show many A.F. positive granules, but vacuoles were also alongwith the granules. They were polygonal in shape with centrally placed oval nuclei filled with granular chromatin. Few of basophils appeared shrunk also. Their basement membrane was delicate. Acidophils were plenty and were dusted with plenty of granules they were smaller in size than basophils. They were arranged in columns on delicate basement membrane towards which their nuclei were located. The nucleus may be observed in close contact with the cells, which was dusted with fine granular chromatin and a nucleolus. Many blood vessels were also observed in this region.

Metaadenohypophysis:

Basophils, fewer in number were seen arranged in the form of a thin, narrow ribbon at the edge of meta-adeno. They were fully loaded with A.F. positive granules. Their oval or elongated centrally placed nuclei were with finely granular material. Acidophils were observed in columns bounded by delicate thin basement membrane which stained orange with A.F. stain. Their small nuclei were dusted with finely granular chromatin and were generally situated near the basement membrane. The granules in their cytoplasm were not sharply visible. This region was observed to have maximum blood supply.

Neural lobe:

This region was compact and was supplied with many blood vessels. Colloidial matter was often observed. The fairly large sized round nuclei were observed with finely granular chromatin. Very little quantity of NSM was observed in the neural lobe.

In other respects pituitary gland resembled the cytological and histological study of pituitary gland of sexually immature migratory *H. ilisha* captured from the sea.

PITUITARY GLAND OF MATURE (STAGE V-VI OF MATURITY) *HILSA TOLI* CAPTURED FROM SEA:

The gland had increased tremendously in size (Fig. 30).

Proadenohypophysis:

It occupied roughly less than 1/4th of the entire gland, unlike *H. ilisha* (mature). In some, lumina had increased noticeably and large follicles were observed to be devoid of colloid. Smaller follicles contained colloid. Other cytological characters were similar to those observed in *H. ilisha* (mature). In later cells of the follicles were destroyed whereas in *H. toli* destruction was not observed.

NSM was also observed as noticed in proadeno. of mature migrating H. ilisha captured from river.

Mesoadenohypophysis:

Basophils of dorsal region appeared fully laden with the granules. Their nuclei were placed at one end of the cells, in many basophils, nuclear structure was not possible to study due to loaded granules. The basement membrane was thick and stained blue with Azan's and green with A.F. Central region exhibited many degranulated basophils, with enlarged nuclei devoid of chromatin. Some basophils due to heavy loss of granules were observed as empty cells with empty nuclei.

Many chromophobes gave appearance.

Acidophils of the dorsal region appeared as compact mass of columnar cells situated on thin delicate basement membrane. They showed plenty of granules in them and their nuclei were oval and laden with fine granular chromatin material. A prominent nucleolus was also noticed in each nucleus. Acidophils of central zone of mesoadeno. were degranulated. They were with large sized nulceus, which had little chromatin and nucleolus in each cell.

Many chromophobes had appeared. Many ghost cells were also observed, as observed in mature migrating *H. ilisha.* Much of blood supply was noticed.

Metaadenohypophysis:

The acidophils dominated in this region and were found in groups bound by delicate membrane, later stained blue with Azan's. Some cells of this group had destroyed leaving empty spaces behind. They stained redish with Azan. They exhibited cytological and histological characteristics similar to acidophils of migrating mature *H. ilisha*. Basophils were fully granulated.

Neural lobe:

The region was shrunk and was with many empty spaces. Blood supply had remarkably increased and the neural lobe in metaadeno. was fully laden with NSM. Many lacunae were observed. The fibres were wavy in shape and coarse.

In other all characters and pecularities, pituitary gland of mature *H. toli* resembled pituitary gland of migrating mature *H. toli* (Fig. 29).

PITUITARY GLAND OF SPENT *HILSA TOLI* CAPTURED FROM THE SEA (STAGE VII & VIII OF MATURITY):

The gland had maintained the size of gland of mature *H. toli* unlike gland of spent *H. ilisha*. Cellular disintegration and destruction was more pronounced than migratory H. ilisha. Many of cells were observed destroyed leaving empty spaces behind. The debris of destroyed cells were frequently observed in these empty spaces.

Proadenohypophysis:

Many of the follicles were destroyed. Separated cells were observed wandering in the empty spaces made by destroyed cells. In few follicles, inside the lumina, instead of colloid, disintegrated cells were also observed. Follicular epithelial cells were loosely arranged. Probably due to shrinkage and loos of cells many empty spaces were also observed in follicular lining. Many blood vessels were observed. The region occupied roughly less than 1/4th of the entire gland.

Mesoadenohypophysis:

The changes can be compared with that of observed in spent H. ilisha. Presence of many chromophobes in the central region of mesoadeno., degranulation in most of the basophils, cytolysis and final dissolution of basophils, pycnosis, etc. changes were noticed. Other changes similar to those of spent H. ilisha were observed.

Acidophils also exhibited similar degenerative changes as were noticed in acidophils of spent H. ilisha.

Chromophobes were many in numbers and were noticed in central zone of mesoadeno. Probably they had derived from degranulated basophils.

Presence of 'Ghost cells' was similar to that of noticed in mesoadeno. of spent H. ilisha.

PITUITARY GLAND OF MATURE (STAGE V-VI OF MATURITY) HILSA TOLI DRIFTED INTO THE ESTUARY OF RIVER NARBADA ON THE HIGHEST HIGH TIDE DAY OF THE YEAR:

The severe, intense and pronounced destructive and degenerative changes observed in the pituitary might be due to sudden drifting into the estury. Many cells of different regions were destroyed leaving empty spaces behind. Empty spaces were occassionally filled with the debris of the dead and destroyed cells (Fig.31).

Proadenohypophysis:

This region occupies more than 1/2 of the entire pituitary gland (Fig. 31). Follicular region had increased tremendously and this expansion and enlargement resembled the proadeno. of migratory mature and spent H. ilisha. The

H. toli of the same stage of maturity captured from the sea did not show such enlargement, hence, this drastic change may be due to sudden change in medium and hormone (probably paralactine) responsible for osmoregulation might have been needed more.

Most of the follicles were broken resulting into a long entire follicle, which was with light green positive colloid. In small follicles colloid was dense; small follicles appeared many in number, probably they have developed later on. At some places, follicular epithelium was noticed broken at the peripheri (Figs. 32 and 33).

The epithelial cells appeared to have shrunk and compressed. There were many empty spaces observed among them. In some follicles cells were noticed to have destroyed and lost. Remnants of destroyed cells were visible wandering in empty spaces. At the extreme tip of the proadenohypophysis, the cellular organisation was lost. Few follicles, after destruction had formed a loop which was noticed in the lumen. The nuclei of intact follicular cells were oval much enlarged, and devoid of chromatin. Many nuclei were pycnotic too. Plenty of the granules were also observed near the follicular lumen (Figs. 33 and 34). Many blood vessels were noticed.

Nuclear diameter varied from 8.0 μ to 8.1 μ and cell height from 21.5 μ to 21 6 μ.

Mesoadenohypophysis:

The destructive and degenerative changes were noticed in this region also. Both the cell types, acidophils and basophils, were affected. Shrinkage, loss of entire cells, loss of cytoplasm, loss of nuclei, extreme and abnormal enlargement of nuclei, rupture of cell membrane and flowing out of cytoplasm, complete loss of cytoplasm and nuclei were among the features observed (Fig. 35). In few intact cells - basophils - granules were observed in plenty, and their nuclei were fully laden with finely granular chromatin. Intact acidophils exhibited peak of secretory activity (Fig. 36). In short intact acidophils and basophils exhibited all cytological and histological features shown by the cells of mature *H. toli* and mature *H. ilisha*. Many blood vessels were noted in addition to many wandering blood cells throughout the region of the pituitary gland.

Metaadenohypophysis:

Similar degenerative and destructive changes as noticed in mesoadeno. were observed. Many blood vessels were visible in this zone.

Neural lobe:

The neural lobe was provided with extensive blood vessels throughout the entire region. At many places the region was lost or destroyed completely, leaving empty spaces behind. The glial tissues and connective tissues were shrunk and were wavy in shape. At many places fibres had shown appearance of coild wires. Many 'ghost cells' were observed near the region of meso and metaadenohypophysis (Fig. 37). Nuclei of neural lobe were abnormally enlarged and were devoid of chromatin materials. Many big lacunae were also noticed throughout the neural lobe. The loss of region was much pronounced here than observed in other fishes (Fig. 38).

PITUITARY GLAND OF SPENT (STAGE VII-VIII OF MATURITY) HILSA TOLI DRIFTED IN THE ESTURY ON THE HIGHEST HIGH TIDE DAY OF THE YEAR:

The pronounced degenerative changes observed in the pituitary gland of mature *H. toli* captured from river were noticed with more severe intensity (Fig. 32).

Proadenohypophysis:

This region comprised more than 1/2 of the entire gland and when compared with the spent *Hilsa toli* (Stage VII-VIII of maturity) captured from sea, it had enlarged more than double size of the region of later. The lumina of the follicles had enlarged tremendously, due to which, unusual enlargement of the proadeno. was visible. The extremely enlarged follicles contained little colloid in them in the form of ribbons. It appeared that the formation of large colloid was at the cost of the breaking of small follicles. The follicles at the tip of proadeno. were broken. Nuclei of the follicles were round and enlarged in their diameter varied from 8.04 µ to 8.1 µ . Follicular cell height varied from 21.6 µ to 21.7 µ. Plenty of granules were observed in their cytoplasm which were towards the lumina (Fig. 39). NSM was also noticed in the follicular cells and in the basement membrane.

Mesoadenohypophysis:

The degenerative changes noticed in mature drifted *H. toli* were noted and at many instances they were noticed with extreme severeness. Much of blood vessels were seen.

Metaadenohypophysis:

The degenerative changes were more pronounced that observed in mature drifted *H. toli*. It was interesting to note complete loss of all basophils in this region. Acidophils cells were at many places not so much affected but at the several places they were lost and shrunk leaving empty places.

HISTOLOGICAL DETAILS

Species	Stage of Gonads	Media Na	K	Ca	Chlorides	No.of follicles measured
		River:				
(I) H.ilisha	I	1.74	0.102	.0991	1.9	32
		1.75	0.127	.0981	2.051	
		1.52	0.102	.0.995	1.748	
		Sea:				
(II) H.ilisha	III	610.4	14.32	22.46	646.0	55
		608.2	14.32	22.46	650.5	
		608.2	13.82	21.96	658.5	
		592.6	13.82	22.46	668.5	
(III) H. ilisha	IV	**Sea:**				64
(IV) H. ilisha	VI-V	**River:** Same as in (I)				68
(V) H. ilisha	VII-VIII	**River:** Same as in (I)				68
(VI) H. toli	VI-VII	**River:** Estuary				61
H. toli	III	**Sea:** Same as in (II)				62
H. toli	VI & VII	Same as in (II)				64
H. toli	VII-VIII	Same as in (II)				66

In short all the degenerative changes, noticed in mature H. toli, drifted in estury, were observed more pronounced in spent H. toli, drifted into the estury.

Neural lobe:

The degenerative changes observed in mature H. toli drifted into the estury continued to exhibit with much prominancy (Fig. 38). It was noted that presence of plenty of NSM in neural lobe may be due to more demand for neurohypophyseal hormones for osmoregulation as neural lobe of spent H. ilisha and spent H. toli never showed plenty of NSM but instead scanty of NSM was noticed in them.

DISCUSSION

The cell height and nuclear diameter of the cells of the proadeno.of migratory H. ilisha exhibited considerable increase during the maturity. The enhanced secretory activity, accompanied with loss of colloid from the follicles, migration of nuclei towards the lumina, in the mature and spent H. ilisha, suggests that the release of the colloid from the follicles may play some role in the maturation process of H. ilisha.

Similarly, the follicular cells of the nonmigratory H. toli showed increase in the secretory activity accompanied with increase in the cell height, increase in the nuclear diameter, abundance of granules in the cytoplasm of the follicular cells, enlargement of the lumina and release of the colloid during maturity.

It may be noted that new small follicles with colloid appeared in the migratory, spent H. ilisha and in nonmigratory marine, spent H. toli. These may take over the function of colloid, during the subsequent stages, since the old follicles have degenerated.

Olivereau (1954) and Robertson and Wexler(1962a,b) have also recorded enhanced secretory activity of the follicles of the proadenohypophysis during growth and maturity in fishes.

Recently several workers have put forward a hypothesis, based on the experimental works, that the cells of the rostral pars distalis or the follicles of the pars distalis (our proadenohypophysis) secrete a hormone - Paralactine - similar to prolactine of the mammals. In a teleost Poecila latipinna, these follicles - Erythrosinophilic follicles - or Erythrosinophils, were inactive in sea water, but became active, when the fish was transferred to freshwater. The paralactine secreted by the follicles was considered to be essential for survival in the fresh water (Ball et al.,1965; Olivereau and Ball, 1964; Ball and Olivereau,1964; Ball and Pickford, 1964). Hisaw (1963,p.121) had stated that FSH, LH and probably LTH or prolactine are secreted by all the species of the vertebrates. Ball (1965) noted a striking difference in the Erythrosinophil cells of marine, Killifish, *Fundulus heteroclitus*, when the fish was kept in fresh water. Ball and Pickford (1964) also reported hypertrophy in Erythrosinophil cell types of Killifish kept for eight months in fresh water or in diluted sea water (24%). The authors considered that the hormone secreted by Erythrosinophil cell types, probably, was responsible for the prolonged survival of Killifish in hypotonic medium. Pickford *et al.* (1965) made a detailed study of the effect of several mammalian hormones on the fresh water tolerance of the marine fish *Fundulus heteroclitus* and found that prolactine had a positive influence in increasing the tolerance of the fish. However, Nicoll and Bern (1964) could not demonstrate the occurrence of a prolactine hormone in the pituitaries of several fishes by Pigeon-crop assay method. This may be due to hormonal specificity.

It is clear from Table I, that, when *H. ilisha* enters in the hypotonic medium - river waters - the follicles exhibit enhanced secretory activity. This enhancement was considerably more than was observed in mature H. ilisha captured from the sea prior to the migration (roughly of the same stage of the maturity of migrating H. ilisha). It may be noted that the proadenohypophysis occupies roughly, more than half of the entire region of the pituitary in migratory H.ilisha, whereas in the nonmigratory H. toli same region occupies less than half of the entire region of the pituitary gland. These observations laid support to the hypothesis that a hormone prolactine-like, paralactin, produced by the Erythrosinophils follicles, may be for the adjustment in the fresh water. H. toli also recorded enhanced secretory activity of the follicles of the proadenohypophysis during maturation (Table I), when the cells of the follicles of the proadenohypophysis of mature and spent H. toli drifted into the estury are compared with those of mature and spent H. toli captured from the sea. Striking histological changes are evident. In drifted H. toli, the follicles showed destruction, shrinkage and loss of cells, loss of cytoplasm from the cells etc. destruction and degenerative changes. The derangement of the follicular cells might have been caused by the inability of these cells to cope up with the functional demands, probably the increased requirement for a prolactin like hormone produced by them for the survival in the fresh water.

The NSM (A.F. +ve, Paget's A.F.-Thionin-PAS +ve material) was observed in the cells of the follicles of the proadenohypophysis of the mature and spent H. ilisha, and mature and spent H. toli and in drifted matured and spent H. toli (Figs. 18,19,31,32 and 37). The successive entry of the NSM into the cells of the follicle from the basement membrane and neural lobe region can be noticed from the above mentioned photographs. Da Lage (1955, 1958a,b) has described net like terminations of nerve fibres, on the basophils of mesoadeno. in Hippocampus and he has suggested, that NSM may act as direct mediator in neuroglandular synapse. In the present case, as the NSM is present only in the follicles of the mature and spent stages of both the species studies, it may be presumed that NSM may act as a direct mediator for the stimulation of the cells of the follicles for the synthesis and discharge of the hormone.

The A.F. +ve, few basophils observed in the mesoadeno. and in the metaadeno. of the fingerling may be considered as thyrotropes. Thyroid follicles of the fingerling of H. ilisha were in active state of secretion (Chapter-X) and gonads of fingerling were infantile. The basophils of the mesoadeno. of both H. ilisha and

H. toli, exhibited a secretion cycle which can be correlated with maturity. Hence these A.F. +ve basophils of mesoadeno. of both *H. ilisha* and *H. toli* can considered as Gonadotropes. The A.F. +ve basophils situated in the extreme dorsal region of the mesoadeno. of mature and spent *H. ilisha* did not show secretion cycle in relation with maturity as they were granulated even in spent *H. ilisha*, whereas basophils of central region of mesoadeno. of spent *H. ilisha* were degranulated. It noted (Chapter V) that small few thyroid follicles appeared in the spent *H. ilisha*. It is quiet probable that granulated, A.F. +ve basophils probably the thyrotropes might be responsible for the appearance of few small follicles in the spent *H. ilisha*. Several workers have confirmed the presence of thyrotropes and gonadotropes experimentally, in the pituitary of fish (Olivereau and Ball, 1964; Olivereau, 1963a,b; Sokol, 1955; for review; Pickford and Atz, 1957; Bern and Nandi, 1964). Baker (1964) after treating juveniles of the Herichthys, noted few hypertrophied A.F. +ve cells in mesoadeno. He, then, named them as thyrotropes. Stahl (1963) stated that, only experimental studies (Gonadectomy or introduction of radioactive iodine in the body of fish) or a study of functioning hypophysis, during biological cycle, can permit the separation of gonadotropes from thyrotropes. Our study on the pituitary gland of *H. ilisha* is based on the entire life cycle of *H. ilisha*. It should be noted that the A.F. +ve cells of mesoadeno. of the fingerling can be called as thyrotropes (as the A.F. +ve cells of metaadeno. remains unchanged throughout the life cycle of *H. ilisha*). The basophils of central region of the mesoadeno. exhibited a secretion cycle which can be correlated with maturity, in mature and spent *H. ilisha*, hence they should be named as gonadotropes, whereas granulated basophils, situated in the dorsal region of the mesoadeno.of the spent *H.ilisha* can be termed as thyrotropes as mentioned above.

The A.F. +ve basophils of the metaadenohypophysis did not exhibit secretion cycle. They were granulated in fingerling of *H. ilisha*,in immature *H. ilisha*, in mature and spent *H. ilisha* also. Their function remains still obscure.

The basophils of mesoadeno. of *H. toli* also exhibits a secretion cycle which can be correlated with the gonad cycle. This observation is similar to *H. ilisha*.

The period of active growth of fishes is also correlated with secretion cycle of the acidophils - especially of the mesoadeno. (Follenius & Porte, 1960 1960a, b;

Olivereau and Fontaine, 1961; Olivereau and Ridgway, 1962; Pickford and Atz, 1957 - for review;pp.404-420; Robertson and Wexler, 1962a,b). The predominance of the acidophils in the mesoadeno. of fingerling of H. ilisha and in immature H. ilisha and H. toli suggest that these acidophils may be responsible for the growth of the fishes. Their increased, secretory activity may be correlated with the growth of the fishes. In spent of both, H. ilisha and H. toli, the acidophils degranulate. This suggests that as the growth is ceased, acidophils degranulate.

The degenerative changes viz. pycnosis, cytolysis, loss of cells, flowing out of cytoplasm from the cells, increase in connective tissue, loss of certain regions in neural lobe or neurohypophysis, appearance of the 'Ghost cells' in the neural lobe, appearance of the chromophobes etc. which were mild in mature, migrating H.ilisha and were much pronounced in spent H. ilisha and in marine spent H. toli, were similar to the degenerative changes noticed by Robertson and Wexler in Rainbow trout (Salmo gairdnerii) and in Pacific Salmon (Genus Onchorhyncus). These authors suggested that these changes were as result of ageing and stress experienced due to (A) nonfeeding during migration and (B) stress due to maturity. *Hilsa ilisha* also faces stress caused by change in external medium, which should be considered as one of the factors bringing about the observed changes. The above mentioned observations are supported by the occurrence of much severe degenerative changes in drifted mature and spent *H. toli*. When the factors, ageing stress due to maturity and nonfeeding are eliminated and the pituitaries of mature and spent *H. toli* are compared with that of drifted mature and spent *H. toli*, it becomes evident that change in media plays an important role in degeneration of the pituitary gland. Hence the change in medium should be considered as a prime factor in bringing about the observed changes.

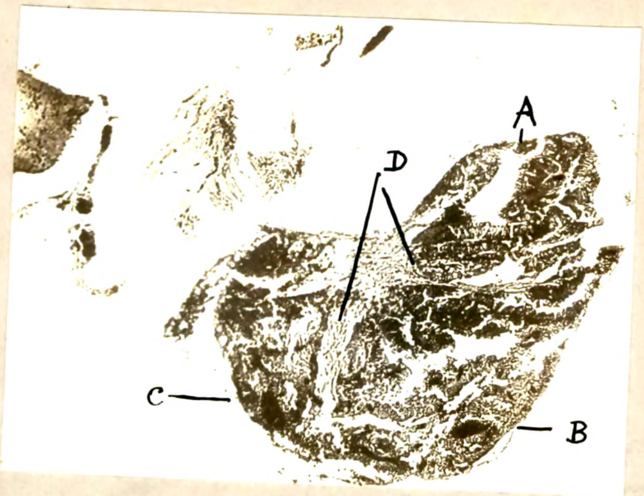


Fig. 1

Pituitary gland of the fingerling of H. ilisha.
Azan, x 160.

A - Proadenohypophysis
B - Mesoadenohypophysis
C - Metaadenohypophysis
D - Neural Lobe

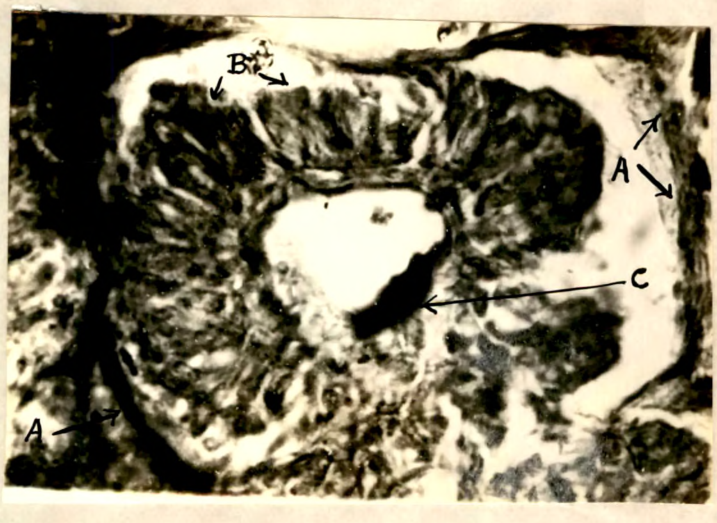
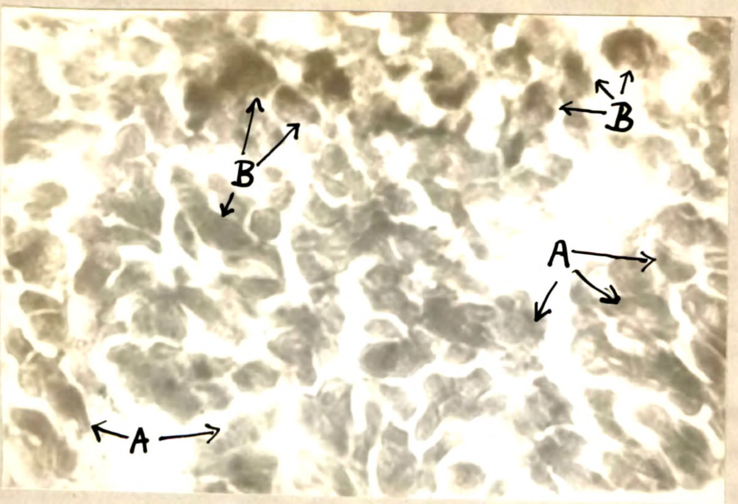


Fig. 2

The Proadenohypophysis of the fingerling of H. ilisha.
Azan, x1000.

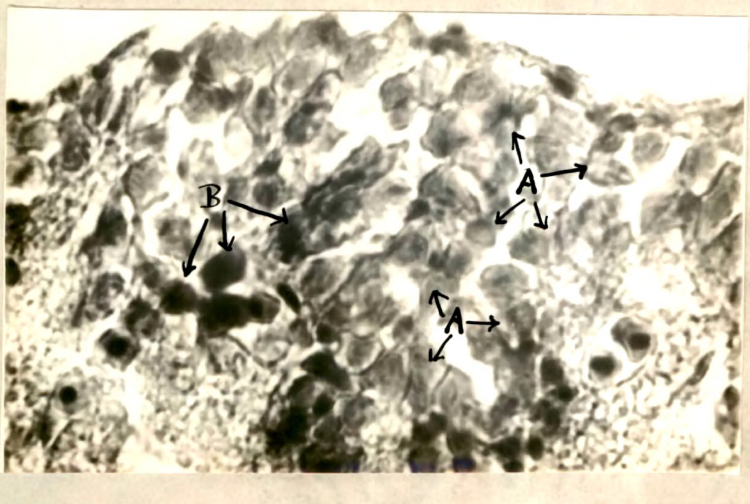
A - Basement membrane
B - Cells of the follicle
C - Colloid



Acidophils(A) and basophils(B) of the mesoadenohypophysis of the fingerling of <u>H</u>. <u>ilisha</u>.

Aldehyde Fuchsin, x1000

Fig.3



Acidophils(A) and basophils(B) of the metaadenohypophysis of the fingerling of <u>H</u>. <u>ilisha</u>.

Aldehyde Fuchsin, x1000

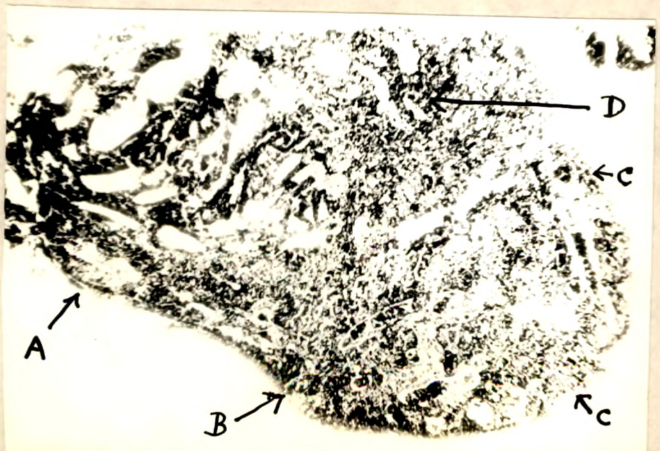
Fig.4

Neural lobe of the pituitary of the fingerling of the H. ilisha.

Aldehyde Fuchsin, x1000

N - Nuclei
B - Blood vessel
C - Neurosecretory material

Fig. 5

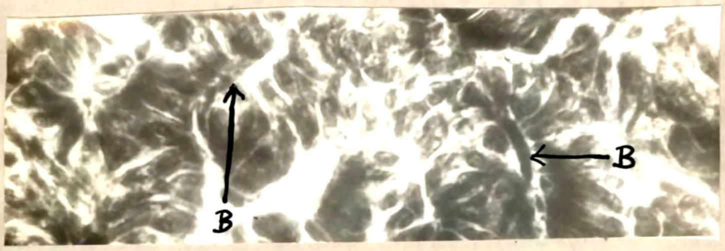


Pituitary gland of the immature H. ilisha.

Azan, x25

A - Proadenohypophysis
B - Mesoadenohypophysis
C - Metaadenohypophysis
D - Neural Lobe

Fig. 6

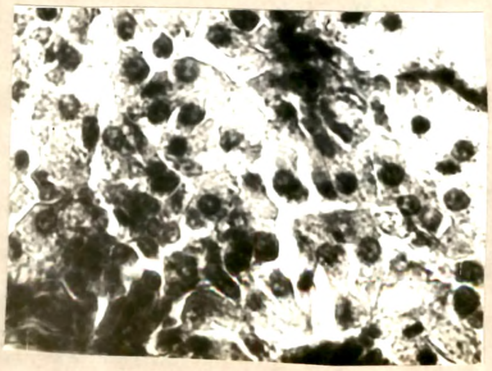


Acidophils of the meso-adenohypophysis of the non-migratory immature <u>H</u>. <u>ilisha.</u>
Azan, x630.

B - Basement membrane

(Note the abundance of the granules)

Fig. 7



Basophils of the meso-adenohypophysis of the non-migratory,immature, <u>H</u>. <u>ilisha.</u>
Azan, x630.

(Note the moderate amount of the granules)

Fig.8

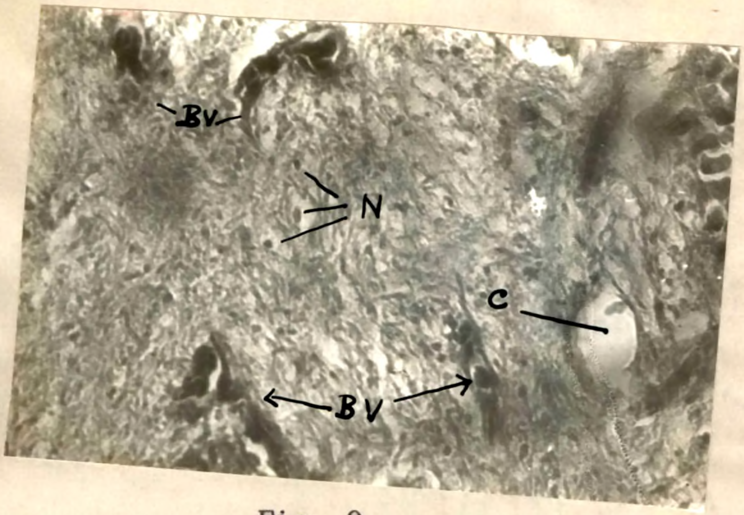


Fig. 9

Neural Lobe of the immature *H. ilisha*.

Aldehyde Fuchsin, x400.

N - Nuclei
B - Blood vessel
C - Colloid

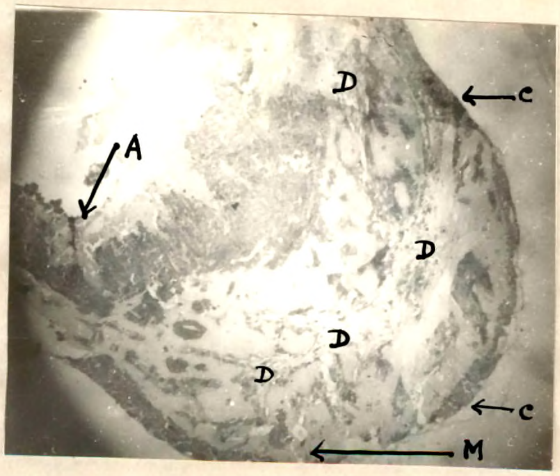
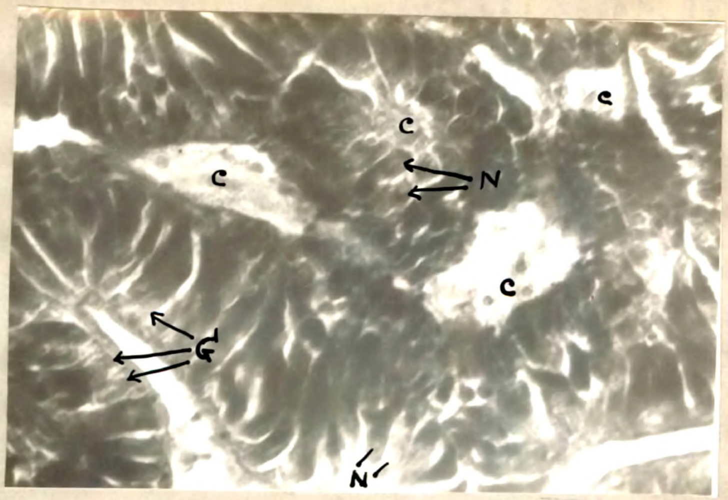


Fig. 10

Pituitary gland of the mature *H. ilisha* captured from the sea prior to migration.

Azan, x25.

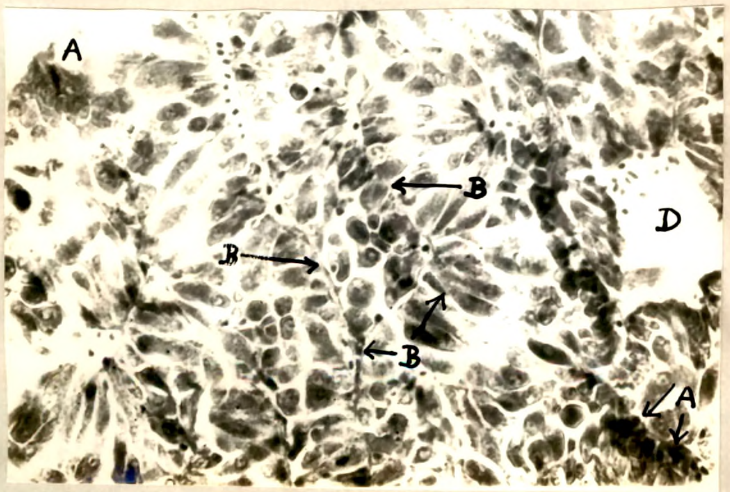
A - Proadenohypophysis
B - Mesoadenohypophysis
D - Neural Lobe
C - Metaadenohypophysis



Proadenohypophysis of the mature H. ilisha captured from the sea.
Azan, x400.

C - Colloid
N - Nucleus of the columnar cells of the follicle
G - Granules

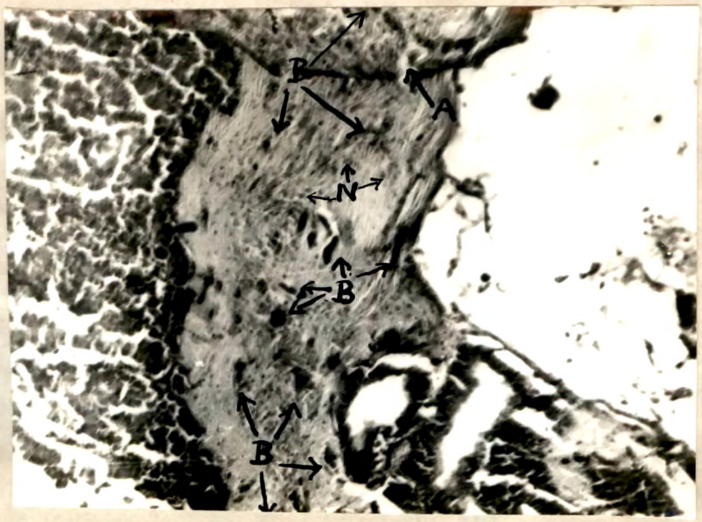
Fig. 11



Basophils of the meso-adenohypophysis of the mature, H.ilisha captured from the sea.
Azan, x 400.

D - Blood vessel
C - Basophils with few granules
A - Acidophils
B - Basement membrane
(Note moderate amount of granules in basophils)

Fig. 12

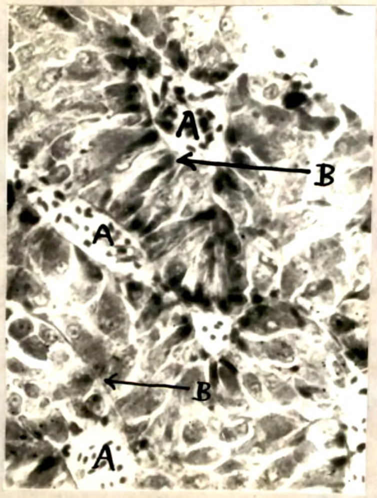


Neural lobe of the mature H. ilisha captured from the sea.
Aldehyde Fuchsin, x400.

N - Nucleus
A - Blood vessel
B - Neurosecretory material

(Note somewhat loose structure)

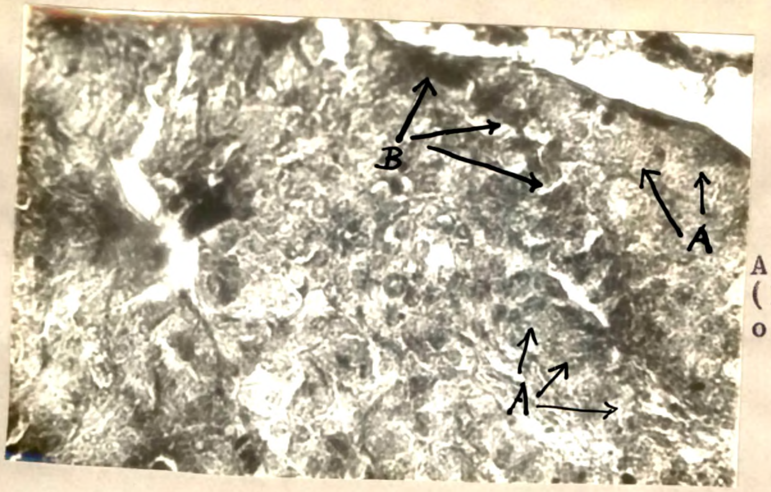
Fig. 13



Acidophils of the meta-adenohypophysis of the mature H. ilisha captured from the sea.
Azan, x400.

A - Blood vessel
B - Basement membrane.

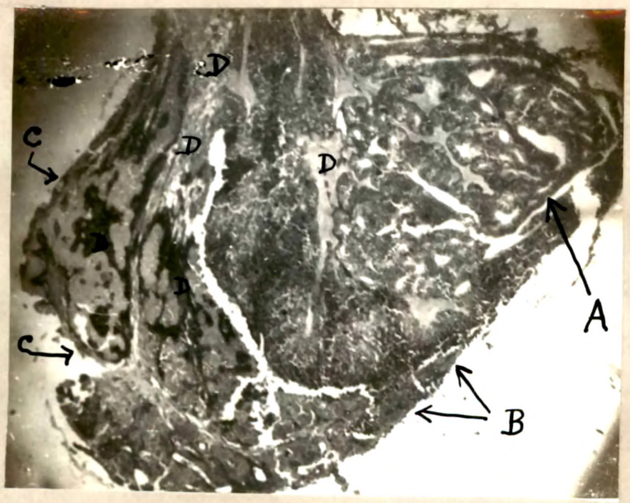
Fig. 14



Acidophils(A) and Basophils (B) of the metaadenohypophysis of mature H. ilisha.

Azan, x630.

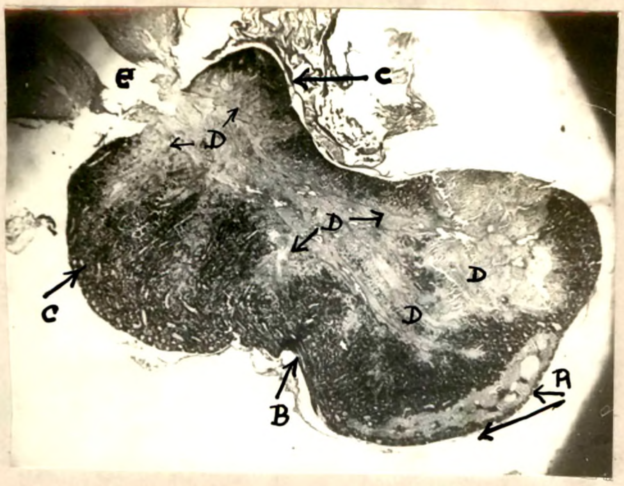
Fig.14-A



Pituitary gland of migrating, mature H. ilisha captured from the river.
Aldehyde Fuchsin, x25.

A - Proadenohypophysis
B - Mesoadenohypophysis
C - Metaadenohypophysis
D - Neural lobe
NS - Neurosecretory material
(Note enlarged follicles and enlarged size of the entire gland, compare with Figs. 1 & 6)

Fig. 15

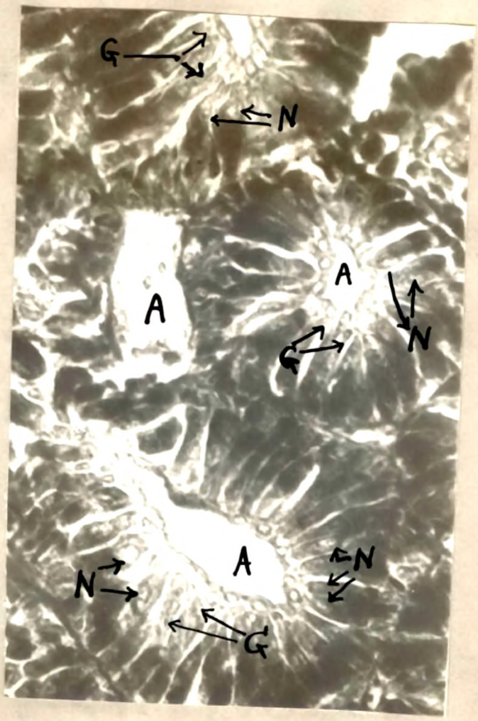


T.S. of the pituitary gland of migrating, mature H.ilisha captured from the river.
Aldehyde Fuchsin, x25.

(Legend as in Fig. 15)

E - Cavity of the third ventricle.

Fig. 16



Proadenohypophysis of the migrating, mature H.ilisha captured from the river.
Azan, x 400.

A - Lumina of follicles
G - Granules
N - Nucleus of the follicular cell

(Note the absence of colloid in lumina, Nuclei of the follicles towards lumen and plenty of the granules towards the lumen. Compare with Figs.2, 11).

Fig. 17

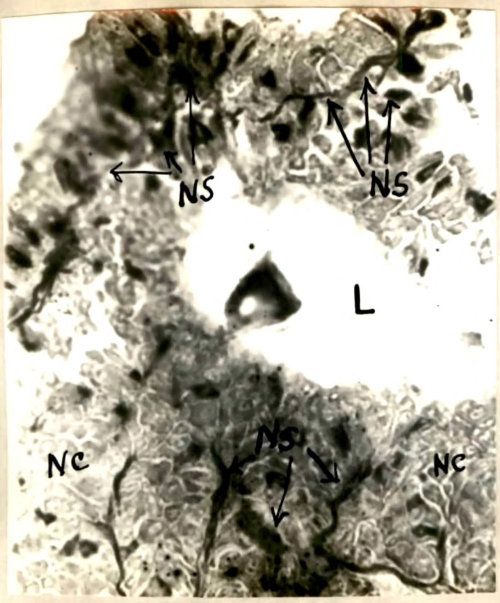


Fig.18

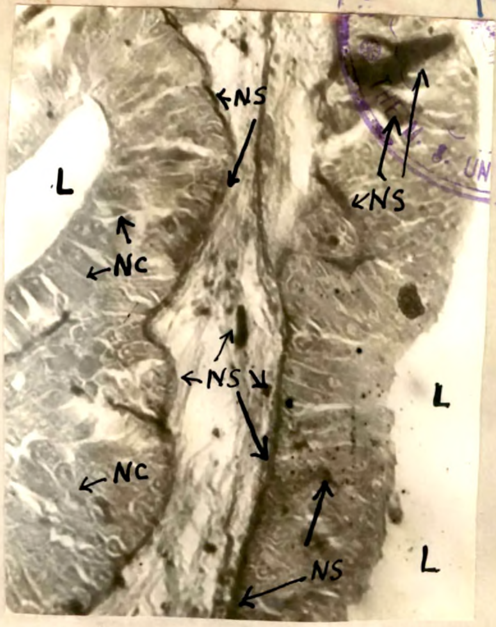
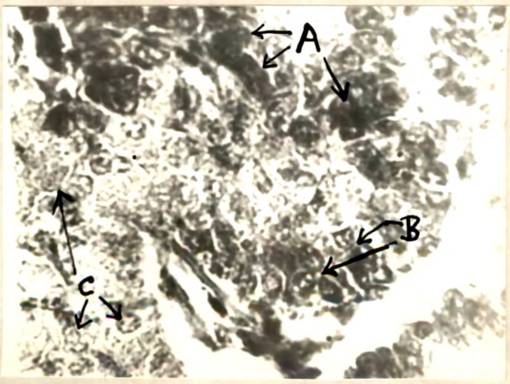


Fig.19

Follicles of the Proadenohypophysis of migrating, mature H.ilisha captured from the river.

Aldehyde Fuchsin, x400.

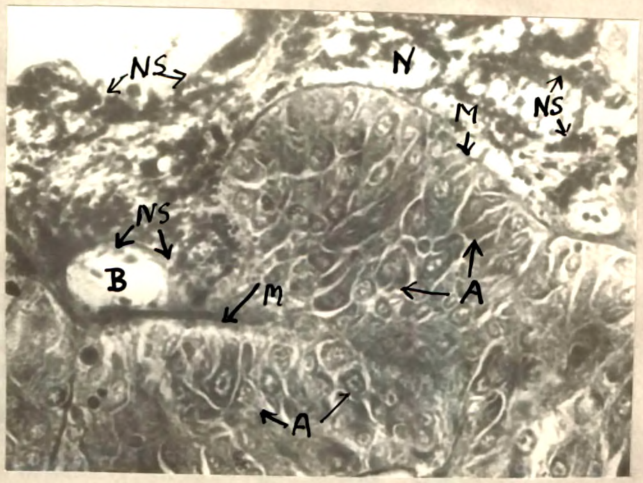
L - Lumen of the follicle
NC- Cells of the follicle
NS -Neurosecretory material
NE- Neural lobe region



Basophils of the meso-adenohypophysis of the migrating, mature H.ilisha captured from the river.
Aldehyde Fuchsin, x400.

A - Heavily granulated cells
B - Partially degranulated cells
C - Chromophobes.

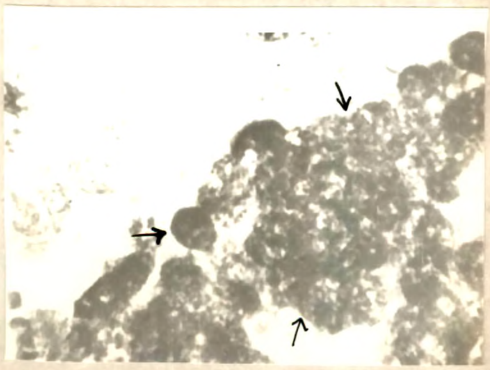
Fig. 20



Acidophils of the meso-adenohypophysis of the migrating, mature H. ilisha.
Aldehyde Fuchsin, x630.

A - Acidophils
B - Blood vessel
NS - Neurosecretory material
N - Part of the neural lobe
M - Basement membrane of the acidophils
(Note neural lobe filled with NSM and presence of the NSM around the blood vessel)

Fig. 21



Heavily granulated basophils of the metaadenohypophysis of the migrating, mature H. ilisha.
Aldehyde Fuchsin, x630.

(Compare with Figs. 8, 12 and 20)

Fig. 22

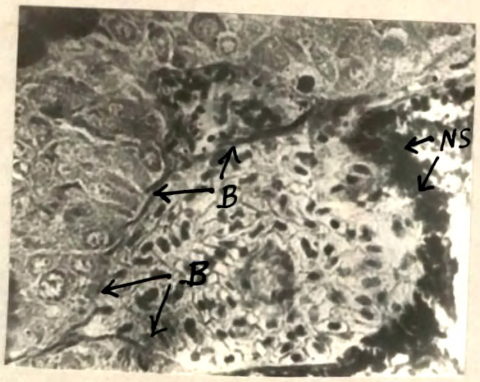


Fig. 23

Acidophils of the meta-adenohypophysis of the migrating, mature *H. ilisha*. Aldehyde Fuchsin, x630.

B - Basement membrane of acidophils
NS- Neurosecretory material around the blood vessel.

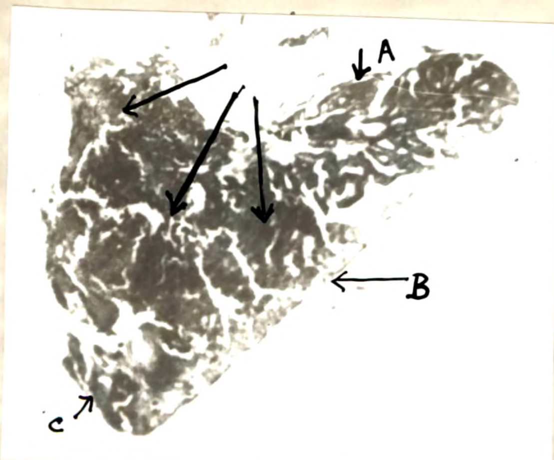
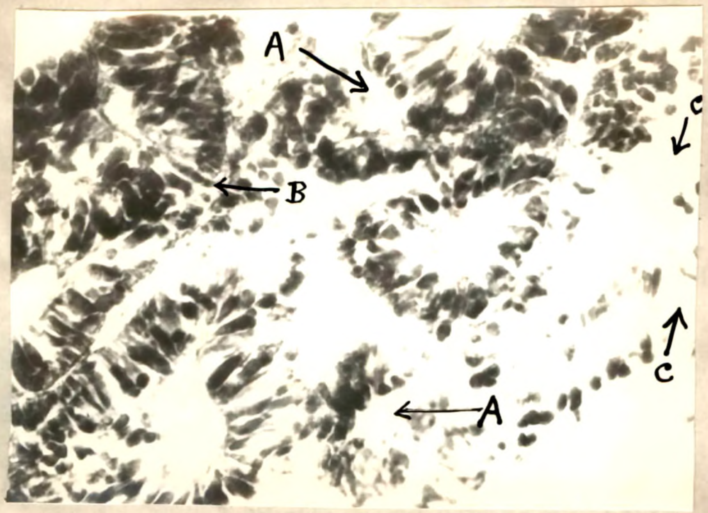


Fig. 24

Pituitary gland of the migrating, spent, *H. ilisha*. Azan, x25.

A - Proadenohypophysis
B - Mesoadenohypophysis
C - Metaadenohypophysis

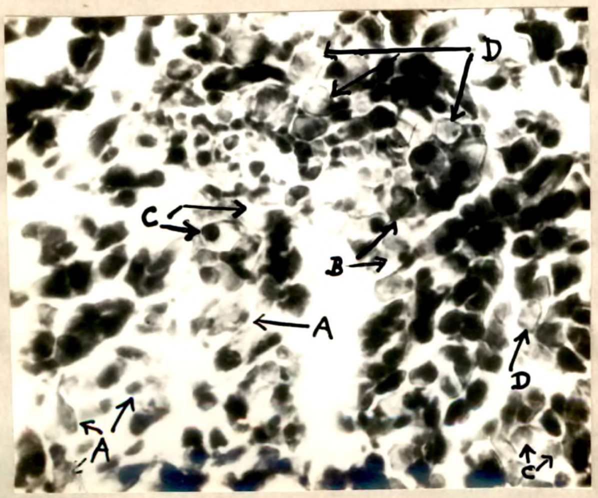
(Arrow indicates neural lobe. Note the loose and shrunk appearance. Compare with Figs.10,15,16 & 28).



Follicles in the pro-adenohypophysis of the pituitary gland of the spent H. ilisha.
Azan, x400.

A - Broken follicle
B - Thick basement membrane
C - Broken follicle with opening into the exterior

Fig. 25

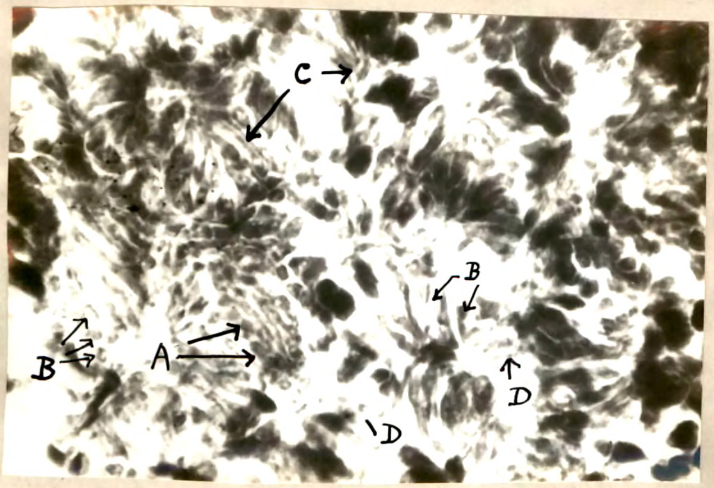


Basophils of the meso-adenohypophysis of the spent H. ilisha.
Azan, x630.

A - Shrunk basophil
B - Cell with few granules
C - Completely degranulated basophil
D - Chromophobes

(Note the pycnosis & cytolysis and compare with Figs.8,12 and 20).

Fig. 26

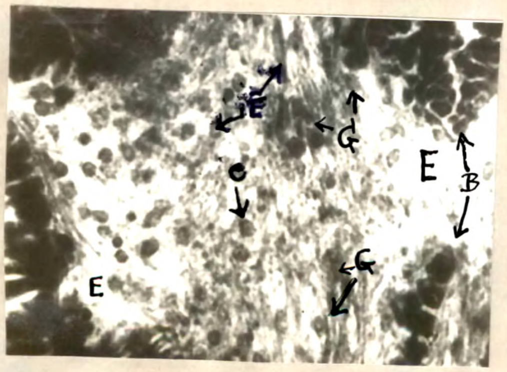


Acidophils of the meso-adenohypophysis of the pituitary gland of the spent H. ilisha.
Azan, x630.

A - Shrunk Acidophil
B - Cell showing degranulation
C - Thick basement membrane
D - Chromophobe

(Note pycnosis & cytolysis. Compare with Figs.7 & 21)

Fig. 27



A region of the Neural lobe of the pituitary gland of the spent H. ilisha.
Aldehyde Fuchsin, x630.

B - Basophils
G - Ghost cells
E - Empty spaces in neural lobe region

Fig.28

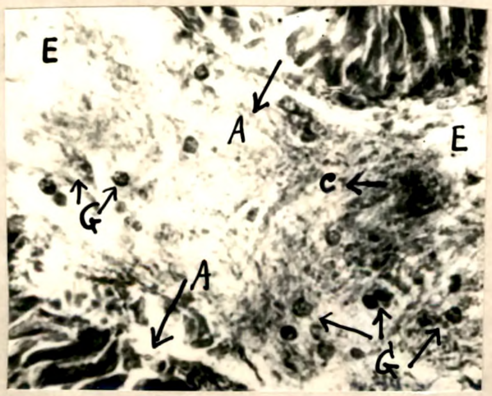


Fig. 29

Neural lobe of the pituitary gland of the non-migratory, spent H. toli.
Aldehyde Fuchsin, x630.

A - Acidophils
G - Ghost Cells
E - Empty spaces in neural lobe

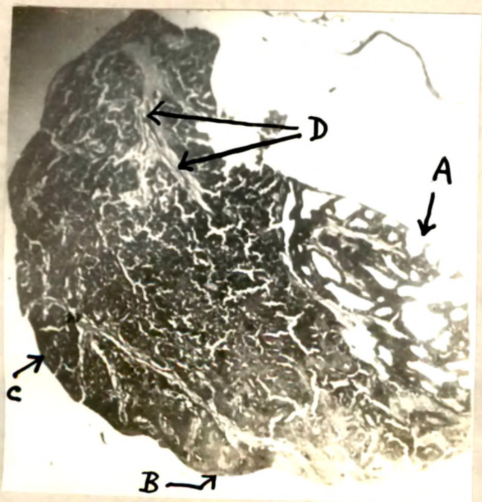


Fig.30

Pituitary gland of the non-migratory, mature H. toli.
Aldehyde Fuchsin, x25.
(Compare with Figs.6,10 and 29)

A - Proadenohypophysis
B - Mesoadenohypophysis
C - Metaadenohypophysis
D - Neural lobe
(Note NSM in the neural lobe)

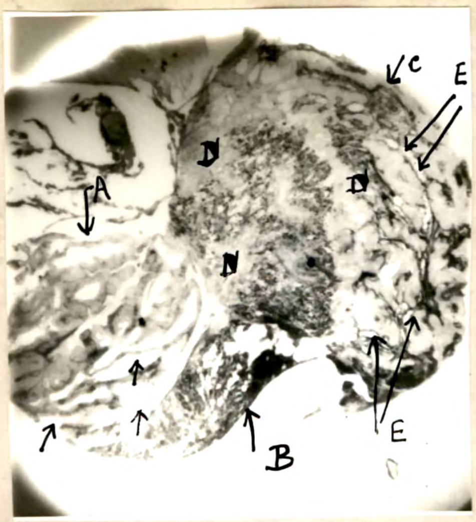


Fig. 31

Pituitary gland of the non-migratory, mature, drifted H. toli captured from the estury on the highest high tide day.
Aldehyde Fuchsin, x25.

(Legend same as in Fig.28. Compare with Figs.6,10,28 and 29. Note the enlargement of Proadenohypophysis (P) and loss of certain regions as indicated by arrows).

Fig. 32

Pituitary gland of the drifted non-migratory, spent H. toli.
Aldehyde Fuchsin, x25.

A - Proadenohypophysis
B - Mesoadenohypophysis
C - Metaadenohypophysis
D - Neural lobe

(Note destroyed follicles of proadeno., cytolysis etc. degenerative changes in all regions. Compare with Fig. 24).

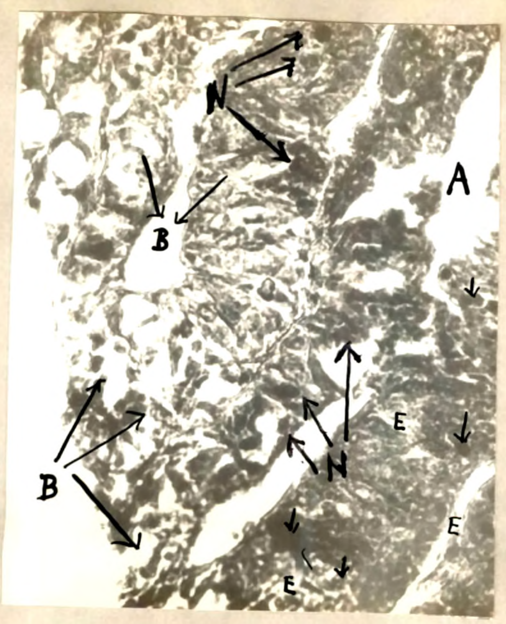
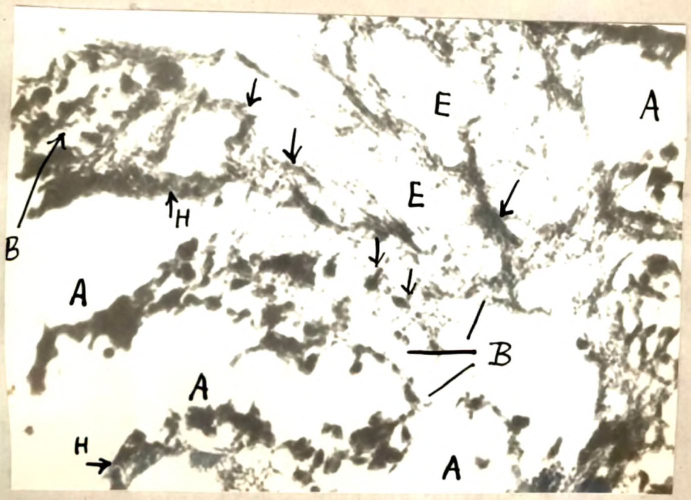


Fig. 33

Proadenohypophysis of drifted non-migratory, spent H. toli.
Aldehyde Fuchsin, x630.

A - Broken lumen of the follicle.
B - Shrunk & destroyed follicular cells
C - Empty spaces in neural lobe
N - NS granules

(Compare with Figs.2,11, 17,18,19 and 25)

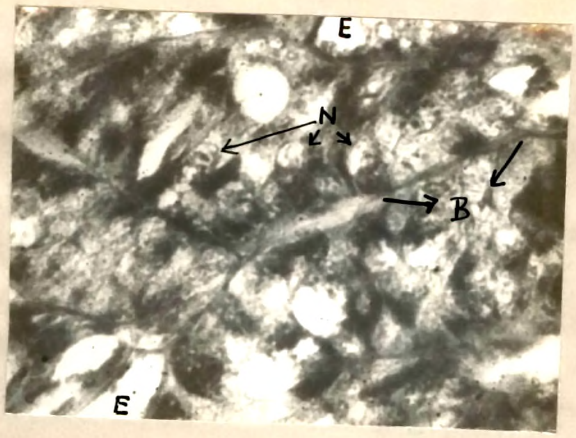


Proadenohypophysis of the drifted non-migratory, spent H. toli.
Aldehyde Fuchsin, x630.

A - Broken lumen
B - Shrunk & destroyed follicular cells
C - Empty spaces in the neural lobe
NS granules indicated by arrows
H - Healthy, intact cells

(Compare with Figs.2,11, 17,18,19,25 and 31)

Fig. 34

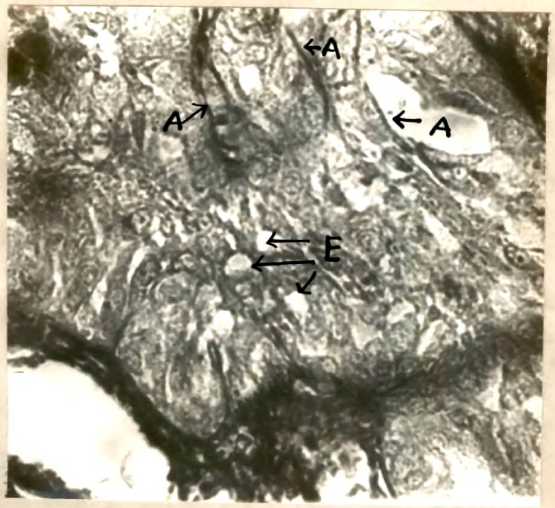


Basophils of the meso-adenohypophysis of the mature, drifted non-migratory H. toli.
Aldehyde Fuchsin, x630.

E - Empty spaces
B - Membrane
N - Pycnotic nuclei

(Note the degenerative changes, compare with Figs.8,12,20 and 26)

Fig. 35



Acidophils of the meso-adenohypophysis of the nonmigratory, drifted, mature H. toli.
Aldehyde Fuchsin, x630

A- Thick basement membrane
E- Empty spaces

(Note cytolysis, pycnotic nuclei. Compare with Figs.7,14,21 and 27)

Fig. 36

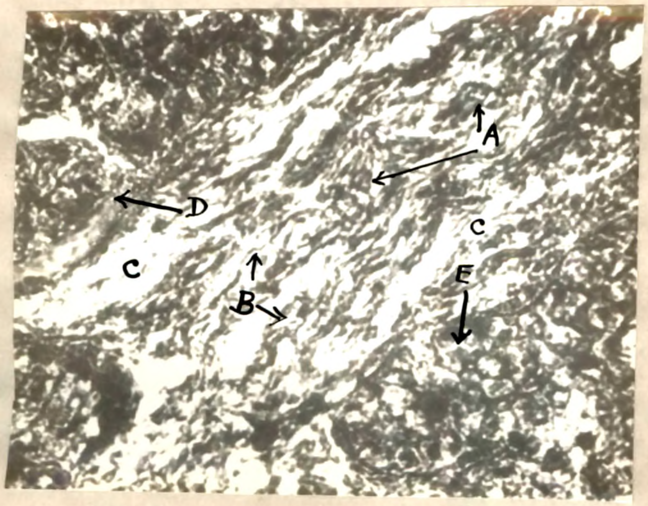


Fig. 37

Part of the neural lobe of nonmigratory, mature, drifted, H. toli.
Masson's Trichrome, x40

A - Ghost cells
B - Wavy nervous tissue
C - Lacunae
D - Acidophils, E - Basophils

(Note shrunk tissue, presence of lacunae and compare with Figs.5,9,13,18,19 and 28)

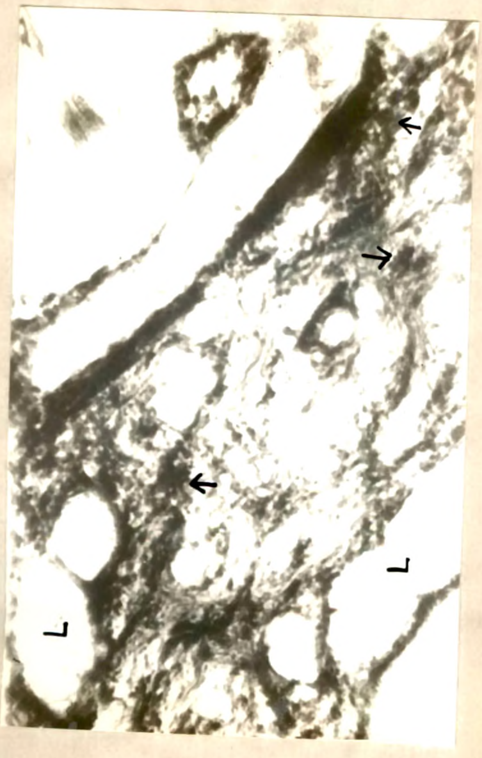
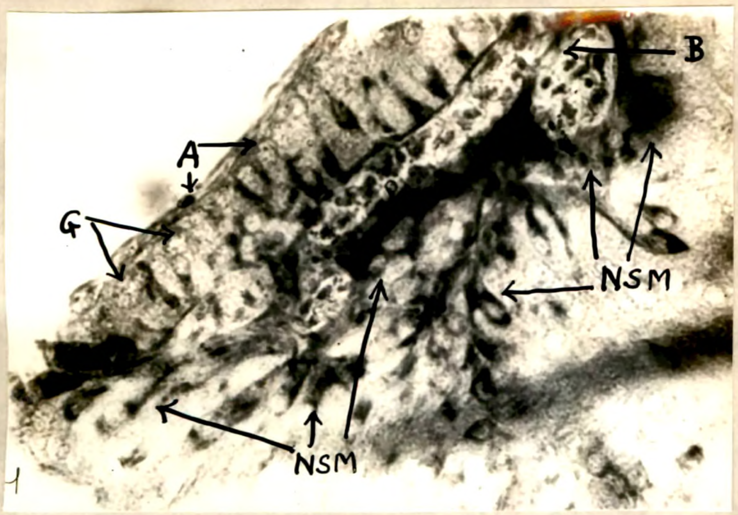


Fig. 38

Part of the neural lobe of the pituitary gland of the nonmigratory, drifted, spent H. toli.
Aldehyde Fuchsin, x630.

Arrows indicate neuro-secretory material.

L - Lacunae
B - Blood vessel
(Note presence of large lacunae, ample of neuro-secretory material and loose fibres. Compare with Figs.5,9,13,18,19 and 28)

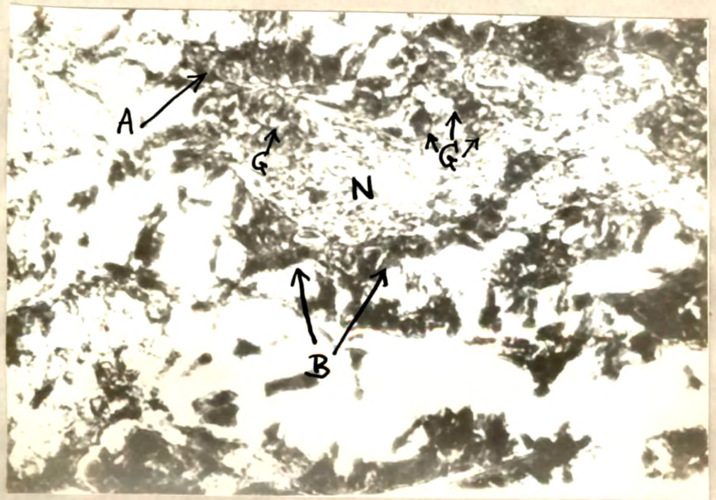


Part of the proadeno-hypophysis of drifted non-migratory spent H. toli, with neurosecretory material (NSM)
Aldehyde Fuchsin, x630

A - Cells of the proadeno. follicles
B - Blood vessel
G - Granules in the cells

Note NSM around Blood vessel and in the cells.

Fig. 39



Part of mesoadeno. of drifted, mature H.toli.
Masson's Trichrome, x400

N - Part of the neural lobe
G - Ghost cells
A - Destroyed & shrunk basophil.
B - Destroyed & shrunk Acidophil

(Note loose and shrunk neural lobe and extreme destruction of acidophils and basophils. Compare with Figs.12,20,21,26 and 27)

Fig. 40

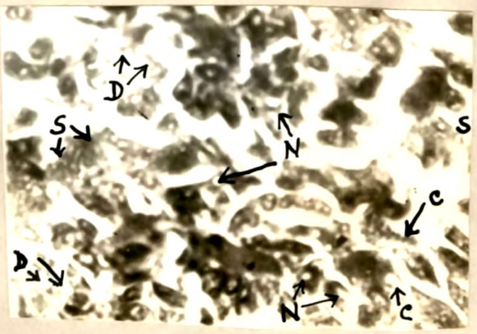


Fig. 41

The degeneration and destruction of the acidophils and basophils of metaadenohypophysis of drifted, spent H.toli.
Masson's Trichrome, x 400

Note pycnotic nuclei (N), destroyed cells (D), shrunk cells (S) and loss of cytoplasm from the cells (C). Compare with Figs. 14,22, 23)