CHAPTER 2

HISTOLOGICAL CHANGES IN THE RED AND WHITE MUSCLES OF <u>HILSA ILISHA</u> AND <u>HILSA TOLI</u> DURING DIFFERENT STAGES OF LIFE CYCLE.

Histological studies by various investigators have revealed degenerative changes in the various organs and tissues of the salmon and rainbow trout during migration. The pre-migratory storage of fat in the muscular tissue of <u>Salmo salar</u> was considered by Miescher (1880) to be a stage of fatty degeneration. Studies made on the king salmon, by Greene (1913) did not show any fatty degeneration in the lateral muscle, but the messeter muscle showed degenerative changes. In the Pacific salmon obvious degenerative changes such as fungus infection, focal necrosis of the skin and loss of muscular strength and balance, which are initiated in many fishes at the time of sexual maturity, progress following spawning, affecting all the fishes and death ensues within a short period (Robertson and Wexler, 1960). Histological studies of various organs and tissues of the Pacific salmon at successive stages of sexual maturation from sea to the spawning grounds (Robertson and Wexler, 1960) and of senile castrated kokanee salmon, <u>Oncorhynchus nerka kennerlyii</u> (Robertson and Wexler, 1962) have shown degenerative and atrophic changes in the pituitary, stomach, intestine, liver, kidney, spleen, thymus, thyroid, cardiovascular system and muscles while the adrenocortical tissue and pancreas showed hyperplasia and hypertrophy. While spawning salmon showed degenerative changes only in the messeter muscle, the senile castrated kokanee salmon showed degenerative changes in the messeter as well as in the lateral muscle.

In the previous chapter it was pointed out that the distance covered by *H. ilisha* in its ascent up the river is short when compared to the extensive track covered by the spawning salmon. According to Robertson *et al.*(1961) the influence of the distance of migration as a factor promoting degeneration can be eliminated, since the spawning steelhead trout covering only 5 miles upstream showed as much degenerative changes as did those secured 160 miles from the sea. The present study was undertaken with a view to obtain information regarding the changes that take place in the lateral muscle of a migratory fish (*Hilsa ilisha*) in comparison with the lateral muscle of a closely related but non-migratory form (*Hilsa toli*).

MATERIALS AND METHODS

Hilsa ilisha (Gonadal stage, III & IV) and *Hilsa toli* (immature, mature and spent) were caught from the sea during different months of the year. The mature forms of *H. ilisha* (Gonadal stage, V & VI) were obtained from the estuarine zone - Bhadbhut, and the freshwater zone - Makthampore and Zadeswar, at the time of migration into River Narbada. Juveniles and spent forms of *H. ilisha* were collected from the freshwater zone. The non-migratory *H. toli* (all stages of maturity) were also caught from the river, but only at the time of highest high tide in the year.

Stages of the gonads were determined using Gokhale's (1953) and Bower's (1954) classification of gonads.

Fishes were sacrificed immediately after collection from the nets and pieces of red and white lateral muscles from the middle and tail regions were fixed in the various fixatives as required. Bouin's and Zenker's fixed materials were washed thoroughly in water, upgraded in alcohol, cleared in xylene and embedded in paraffin. Sections of 5 - 10µ thickness were cut and stained with Haematoxylin - Eosin for general histological observations, and with Heidenhain's Azan stain (Gurr, 1956) for the study of the connective tissue.

For staining lipids, the tissues were fixed in Baker's (1946) calcium formol for 24 hours, washed in running tap water for the same time, passed through different gelatin grades and embedded in 20% gelatin. Sections cut at 20µ thickness on a freezing microtome were stained with Sudan Black B in 70% alcohol (Pearse, 1960) and Nile blue sulphate (Gurr, 1956; Pearse, 1960).

Sections stained with Sudan Black B and mounted in glycerine jelly were used for measuring the fibre diameter. The diameter of fibres was measured by using an ocular eyepiece and a micrometer slide.

OBSERVATIONS

The lateral muscle of *H. ilisha* and *H. toli* consists of a strip of well developed red muscle along the lateral line region composed of narrow, fat-loaded fibres while the epaxial and hypaxial musculature consists of only the broad, white fibres containing very little fat. In the red muscle region the fibre diameter varied between 15μ to 50μ and accordingly they may be classified into R_1 (15 - 30μ) and R_2 (35 - 50μ) types. In the juveniles only R_1 type fibres were observed. In the white muscle the fibres showed wide variations in diameter (25μ - 160μ). Accordingly they may be classified into W_1 (25 - 45μ), W_2 (50 - 95μ) and W_3 (100 - 160μ) types. In the juveniles only W_1 and W_2 types were present, while W_3 type was found to be absent. In both the types of fibres the nuclei occupied a peripheral position(Figs. 1 & 2).

Juveniles of H. ilisha collected from the river:

The fasciculi in both the red and white muscle regions showed a compact arrangement with little interfascicular connective tissue (Fig. 3 in chapter 3).

Immature H. ilisha collected from the sea:

Immature *H. ilisha* collected from the sea differed from juveniles in the greater development of inter-fascicular connective tissue laden with fat cells in both the red and white muscle regions. However, the amount of connective tissue varied in individual fishes. Degenerative changes in the fibres were noticed for the first time in this phase in a few of the fishes examined. Many fibres at the periphery of the fasciculi were seen to have undergone various stages of degeneration as manifested by the presence of ruptured fragments of fibres in the red muscle region(Fig. 3). At some places few fasciculi were

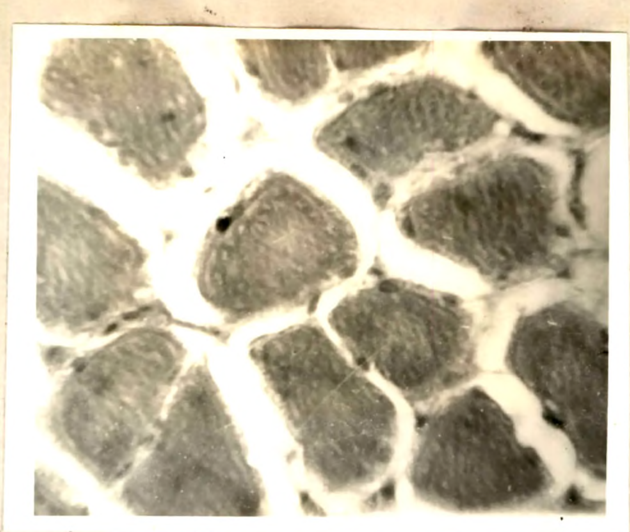

Fig. 1

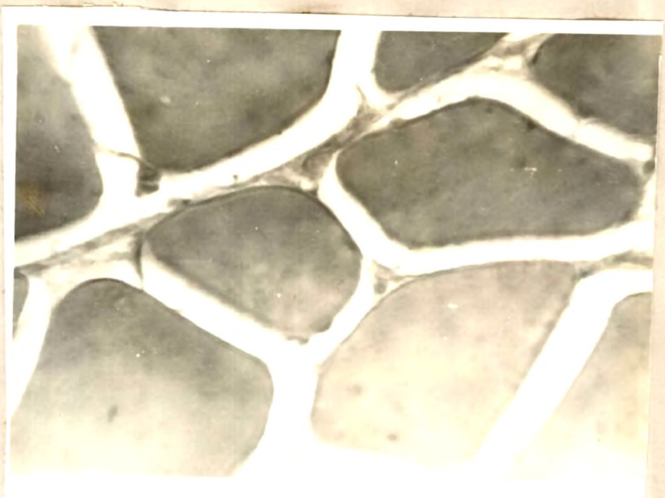

Fig. 2

Figs. 1 & 2. Transverse section (T.S.) of normal red and white fibres of immature H. ilisha stained with Haematoxylin and Eosin. 360 X.

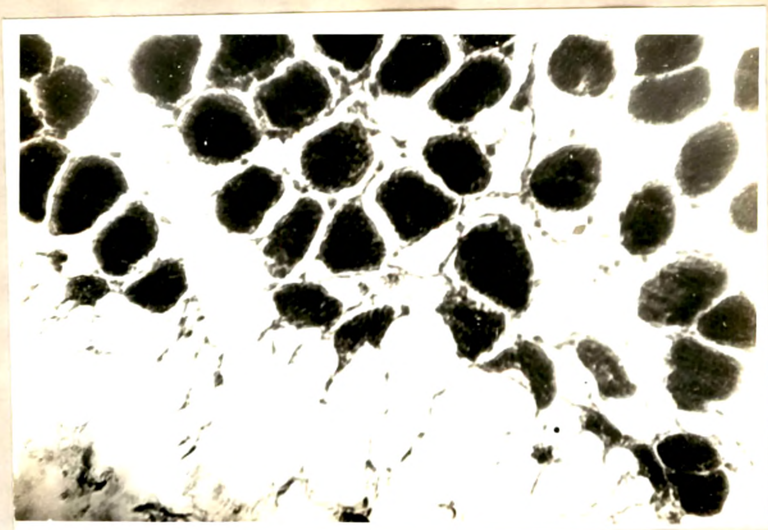

Fig. 3. T. S. showing fibre degeneration at the periphery of fasciculus of immature H. ilisha. Heidenhain's Azan stain. 225 X.

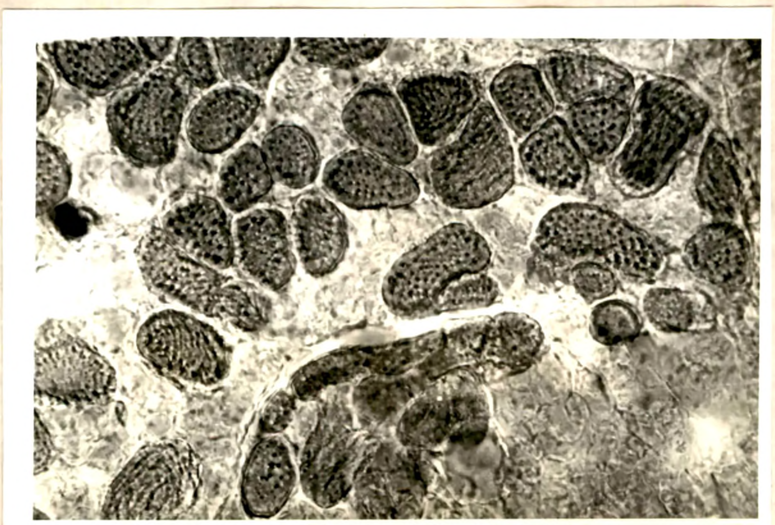

Fig. 4. Same as Fig. 3., but stained with Nile blue sulphate. 225 X.

seen to be cut through by connective tissue with fat cells in between. Groups of muscle fibres cut off from the main fasciculi and surrounded by connective tissue could be seen at other sites (Figs. 4 &5). Such isolated groups of fibres showed variability in the number of fibres constituting them, the diameter of the fibres (55µ - 10µ) and in the loss of myofibrils. In sections stained with Nile blue sulphate a clear contrast between muscle fibres and fat cells could be obtained since the former stained blue and the latter pink. Some of the fibres were seen to be stained partly blue and partly pink and gave the impression that the degenerating muscle fibres were being converted into fat cells. Clumps and chains of nuclei were present in the strands of connective tissue which appeared to replace the sites formerly occupied by muscle fibres.

Occasionally some of the red fibres were seen to have one or two vacuoles in the centre and with Haematoxylin - Eosin staining these were found to be devoid of any substance. In sections stained for fat they were seen to be filled with neutral fat (Fig. 6). Except for the presence of vacuoles, these fibres appeared normal in the arrangement and distribution of nuclei and in other structural details with regard to the remaining perpheral band of protoplasm.

In the white muscle region, but for the presence of better developed interfascicular connective tissue, laden with fat cells as mentioned earlier, most of the fishes examined

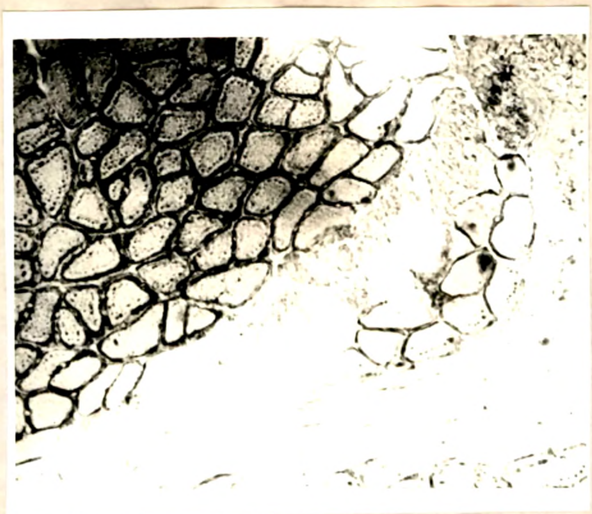

Fig. 5. Red fibres (T.S.) of immature H. ilisha collected from the sea, stained with Nile blue sulphate showing the formation of isolated groups of fibres by the infiltration of fat laden connective tissue. 225 X.

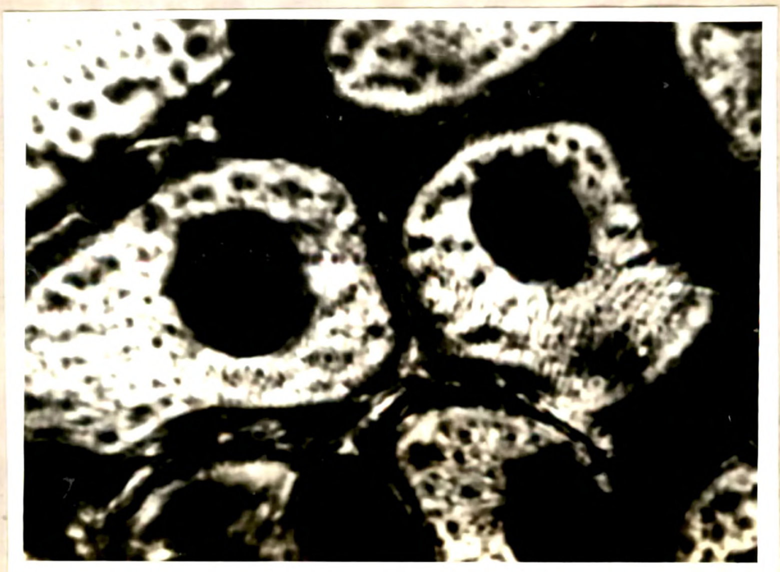

Fig. 6. Red fibres (T.S.) of immature H. ilisha stained with Sudan Black B showing vacuoles filled with fat. 1,134 X.

did not differ from juveniles in the arrangement, structure and diameter of muscle fibres. In the case of those fishes which showed degenerative changes in the red muscle region, necrosis and fragmentation of sarcoplasm were observed in occasional white fibres also.

<u>Mature H. ilisha collected from the freshwater zone</u> :

Mature fishes collected from the river showed a more compact arrangement of the muscle fibres unlike the immature ones from sea. An apparent reduction in the interfascicular connective tissue and fat cells was noted. In the red muscle of some of these fishes the degree of degenerative changes was similar to that observed in some of the immature fishes from sea, whereas it was more pronounced in other fishes. At the periphery of the fasciculi the number of fibres showing various stages of degeneration and the groups of muscle fibres cut off from the main fasciculi and surrounded by connective tissue were more numerous than in the sea samples. Eventhough an overall reduction in the interfascicular connective tissue and fat cells was noticed, at the sites where the fibres appeared to be undergoing degeneration, a number of fat cells were present (Fig. 21 in chapter 3).

Red fibres with vacuoles filled with neutral fat were quite numerous and found to be aggregated at the margin of the fasciculi (Fig. 7,8). While some of the fibres contained one or more (maximum 3) small or moderately sized vacuoles, in others the vacuole was so large that it occupied practically the whole area within the intact sarcolemma (Fig. 9). In many fibres the myofibrils were mainly distributed around the

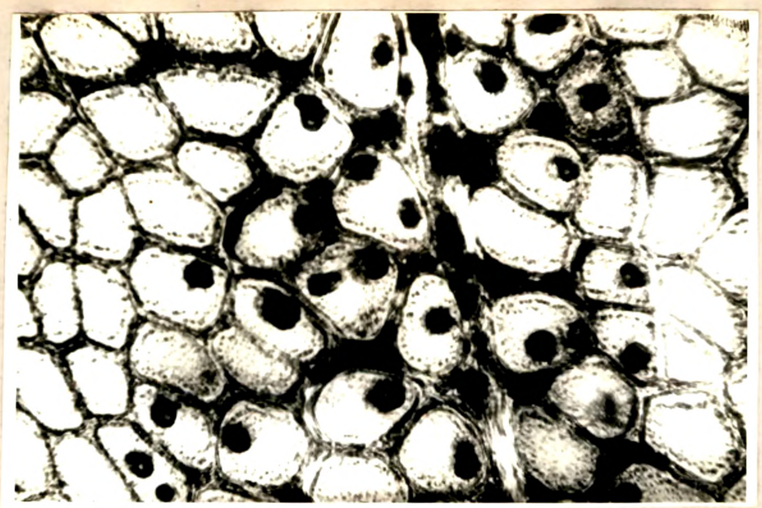

Fig. 7. Same as in Fig. 6, but that of mature H. ilisha from river. Note the abundance of fibres with vacuoles filled with fat at the periphery of fasciculus. 225 X.

Fig. 8

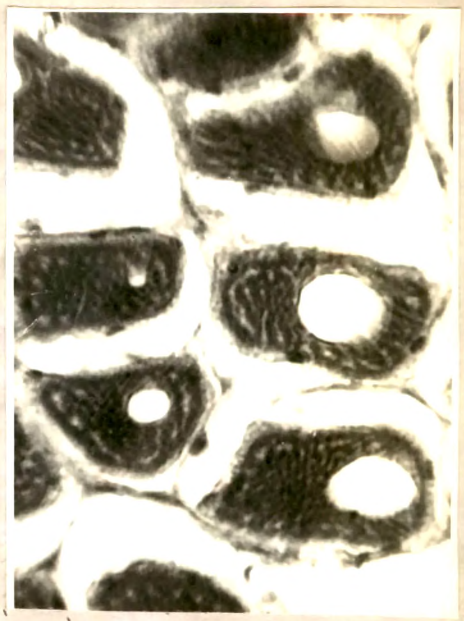

Fig. 9

Fig. 8. Same as Fig.7, but stained with Nile blue sulphate. The fat present inside the vacules showed positive staining. 567 X.

Fig. 9. Same as Fig. 7, but stained with Haematoxylin and Eosin. Note the different stages in vacuole formation. 567 X.

periphery, while the central portion was clear. Invasion of the connective tissue into many fibres was also observed.

In the region of the white muscle, many fibres showing various stages of vacuolization and fibril loss, and at places sarcolemmal tubes filled with masses of fragmented sarcoplasm were noted (Figs. 10,11). Unlike in the red muscle, in the white muscle, fat cells were not seen to replace the degenerated and perished fibres. Except for the above mentioned changes and the reduction in the intermuscular connective tissue and fat, the mature fishes do not differ from the immature ones from the sea in relation to the arrangement and structure of the white fibres.

Spent H. ilisha from the freshwater zone :

In the red muscle region the degenerative changes were more pronounced than in the mature fishes. The fasciculi showed various degrees of connective tissue infiltration and and in some of them the infiltration extended into the whole fasciculus (Figs.12,13,14,15). The presence of widely separated groups of muscle fibres showing variations in number, diameter and degree of degenerative changes in the connective tissue mass clearly indicates that muscle fibres are replaced by fat cells and connective tissue. Apart from the presence of fibres having vacuoles filled with fat and fibres with peripherally arranged myofibrils as in the mature fishes, many fibres also displayed necrosis and complete loss of myofibrils at some places. The nuclei of degenerating fibres were increased in number and were larger and more varied in shape than in the normal. In many

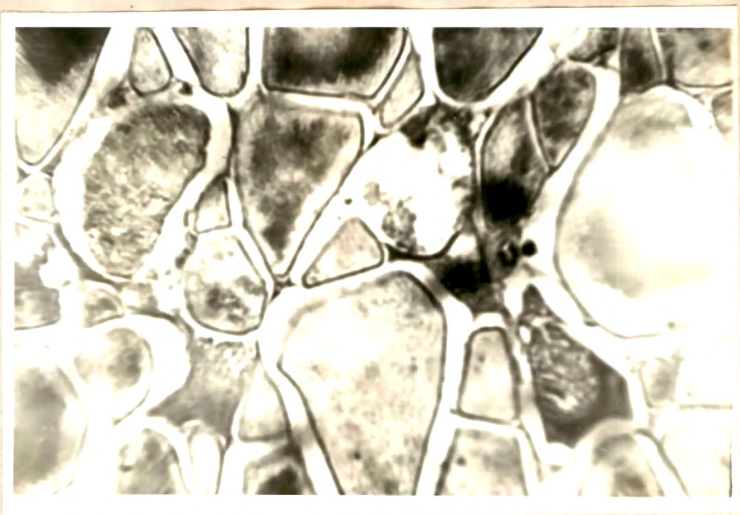

Fig. 10

Fig. 11

Figs 10 & 11. White fibres (T.S.) of mature H. ilisha collected from river stained with Haematoxylin - Eosin and Sudan Black B respectively. Note the degenerative changes shown by some of the fibres. 225 X.

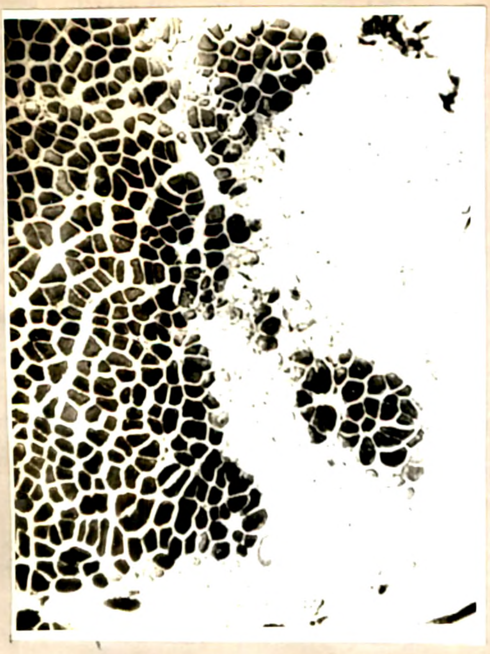

Fig. 12

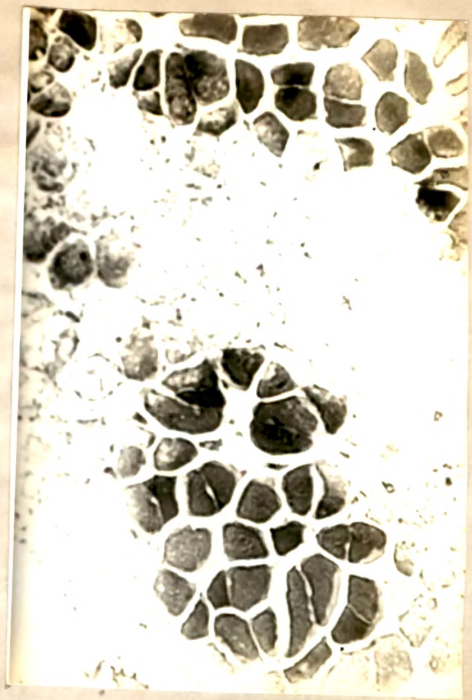

Fig. 13

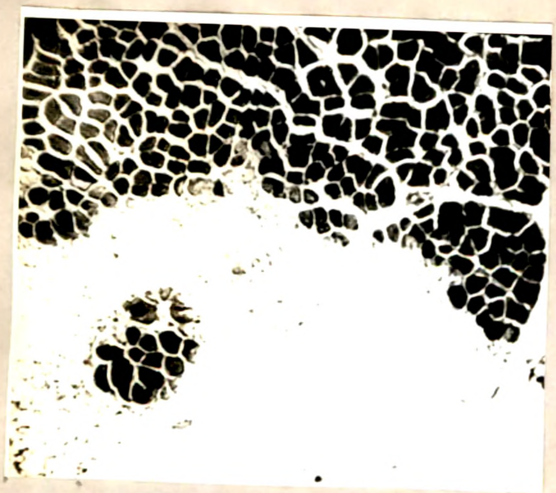

Fig. 14

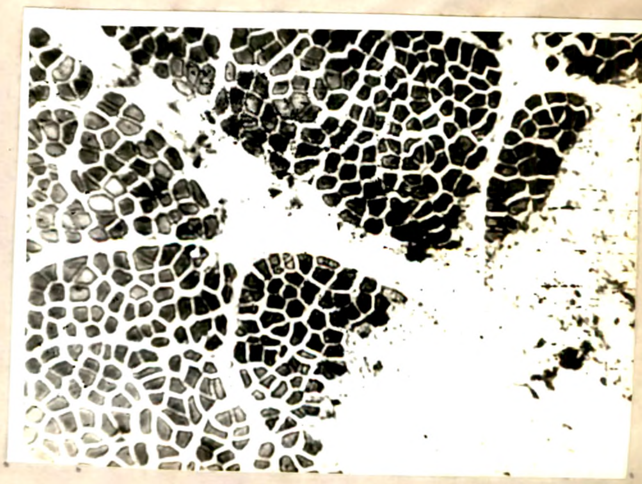

Fig. 15

Figs. 12 - 15. Red muscle (T.S.) of spent H. ilisha collected from river, showing different stages in degeneration of fibres and infiltration of connective tissue. Haematoxylin-Eosin.

Figs 12,14 & 15 - 53 X; Fig. 13 - 144 X.

fibres the nuclei were centrally located and not peripheral as in the normal fibres (Fig. 16). The completely degenerated fibres were represented by scattered darkly stained nuclei which lie in the connective tissue with or without any visible remnants of the sarcolemma (Fig. 17).

In the white muscle region, fibres showing various degenerative changes such as necrosis, fibril loss and sarcolemmal tubes being filled with fragmented sarcoplasm, were comparatively more numerous than in the mature fishes. Occasionally certain fibres showed one or more vacuoles (Fig. 18) filled with a watery substance, the nature of which is not known. These stained negative for fat.

H. ilisha collected from the estuarine zone :

In both the muscle regions of *H. ilisha* from the mouth of the river, all the changes seen in mature fishes from the spawning grounds, but to a lesser degree, could be observed.

Changes in H. toli :

Some of the maturing and spent *H. toli* from the sea and all the *H. toli* of all the stages of maturity collected from the river showed the same type of degenerative changes in the red muscle as noted in immature *H. ilisha*, but to a lesser degree. In mature *H. toli* from sea except for the presence of degenerative changes in some of the red fibres at the periphery of the fasciculi, no other histological changes as observed for mature *H. ilisha* could be noticed. The inter-

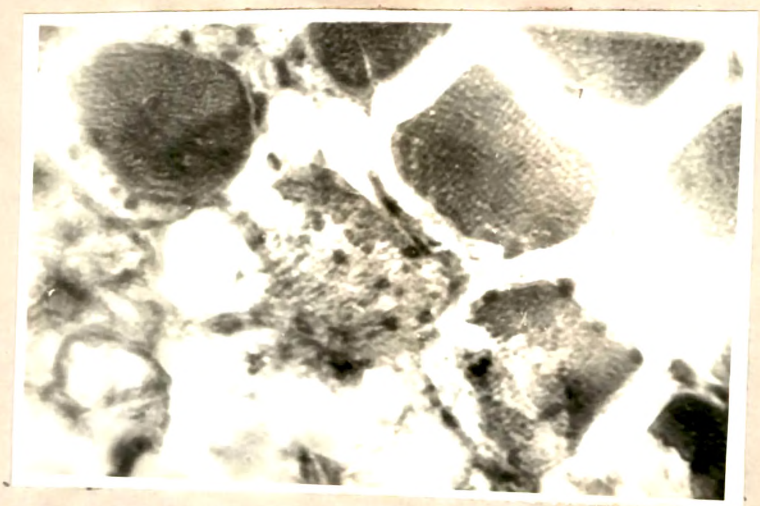

Fig. 16. Red fibres (T.S.) of spent H. ilisha, collected from river, stained with Haematoxylin - Eosin. Note the numerous centrally located nuclei in the degenerating fibre. 567 X.

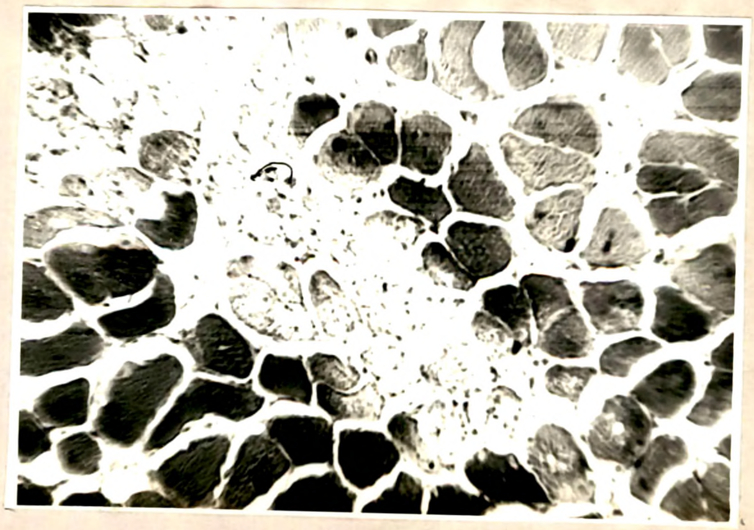

Fig. 17. A stage in degeneration of red muscle (T.S.) in spent H. ilisha. Note the scattered darkly stained nuclei in the connective tissue with or without the remnants of sarcolemma. Haematoxylin - Eosin. 144 X.

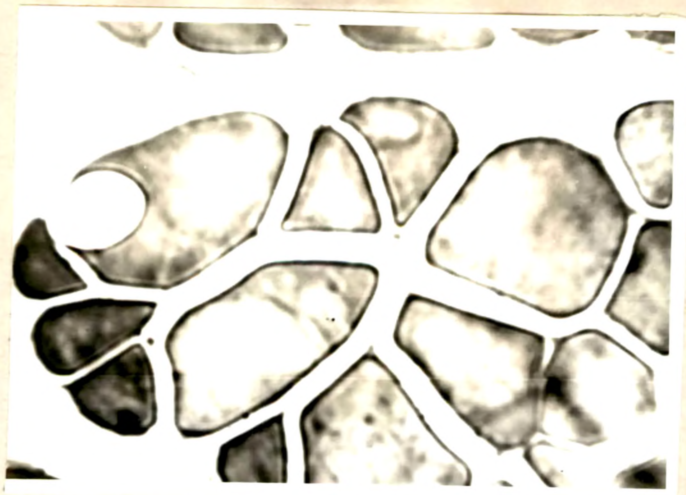

Fig. 18. White muscle (T.S.) of spent H. ilisha collected from river stained with Haematoxylin - Eosin. Note the presence of a vacuole in one of the fibres. 225 X.

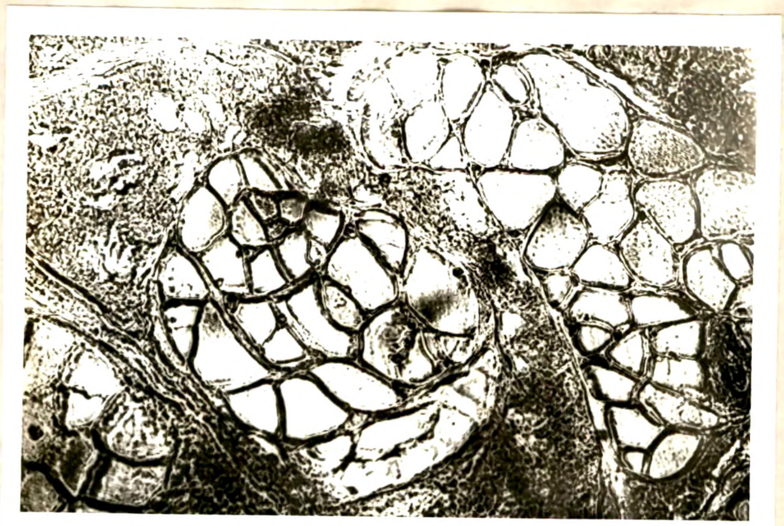

Fig. 19. Red muscle (T.S.) of H. toli collected from river stained with Nile blue sulphate showing degenerative changes in the fibres. 225 X.

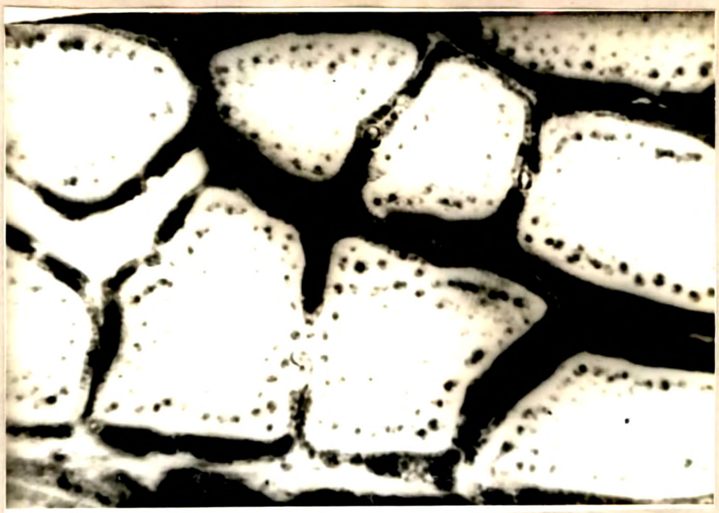

Fig. 20. T. S. of red fibres showing longitudinal division. 567 X. Sudan Black B stained.

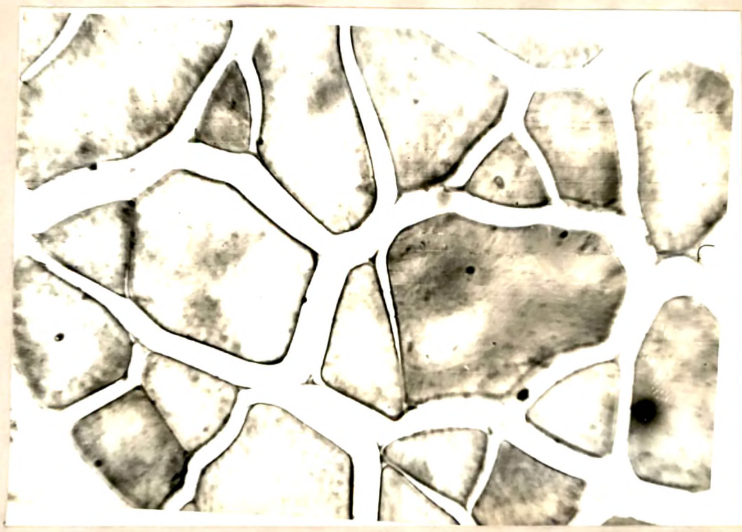

Fig. 21. White fibres (T.S.) showing stages of regeneration of fibres by longitudinal division. Haematoxylin - Eosin. 225 X.

muscular connective tissue and fat cells were much less when compared to immature H. toli from sea and the fishes collected from the river. Fibres with vacuoles filled with fat were observed in a few of the immature fishes from the sea while in all the other fishes examined, such fibres were not observed.

In the white muscle region degenerative changes as observed in mature H. ilisha were noted in a slight degree in some of the spent fishes from the sea and to the same extent in all the fishes collected from the river.

Regeneration by longitudinal division of fibres was seen to be a common feature in all the stages of maturity and was more pronounced in fishes where degenerative changes were more. The red fibres were found to get enlarged prior to division. They lost their circular outline and this was followed by division through the centre dividing the fibre into two more or less equal halves (Fig. 20). In the white fibres, which are characterised by a wide variation in size, some of the fibres appeared to be larger than the normal (150μ - 160 μ). One to many small fibres were seen adjacent to such large fibres either closely adhering to them or detached, thereby suggesting that the small fibres had originally been a part of the large fibre and were subsequently cut off to form new fibres (Fig. 21).

DISCUSSION

The results obtained in the present investigation show conspicuous degenerative changes in the lateral muscle of

H. ilisha during their spawning migration. It was found that the degeneration started while in the sea and progressed as the fishes migrated into the river. While most of the fat cells occurring in the inter-fascicular connective tissue disappeared after the spawning migration, fat cells were plenty in the regions where the degenerative changes were niticed. It appears, therefore, that the degenerating muscle fibres are replaced by fat cells. The changes observed in the lateral muscle of *H. ilisha* seem to bear resemblance to the histo-pathological changes that occur in the various types of muscular dystrophies in man and animals. That in the regeneration that follows destructive diseases of muscle, the adult muscle fibres can form new fibres by longitudinal division has been shown (Adams *et al.*, 1954). The presence of the degenerating fibres observed might have induced the normal fibres to divide in order to replace the degenerated cells. Eventhough degenerative changes have been reported in the messeter muscle of the spawning salmon (Greene, 1913; Robertson and Wexler, 1960), no deteriorative changes have been observed by them in the lateral muscle. However, it should be mentioned that the presence in the lateral muscle, of fibres with a fat droplet occupying the central portion has been reported by Greene (1913), though he did not consider this to be a sign of degeneration. It may also be mentioned that McBride *et al.* (1965) have reported extensive degeneration of myofibrils in the lateral muscle of actively feeding spent sockeye salmon, *Oncorhynchus nerka*.

It may be noted that such degenerative changes seen in the lateral muscle of *H. ilisha* appear to be concerned with migration. But such changes though in a much less pronounced level were observed in the non-migratory fish, *H. toli* also. According to Robertson and Wexler (1962) conditions such as stress due to long migration and the physiological adjustments involved in the transition from salt- to freshwater appear to bear no relationship to the initiation of the series of progressive degenerative changes which follow the spawning act.

In contrast to the Pacific salmon, all of which die after the first spawning and the rainbow trout, many of which die after the first spawning and only a few survive for a second spawning, *H. ilisha* is known to be capable of undertaking many spawning migrations during its life span. Evidence for ageing as a possible cause for the degenerative changes is available from the fact that the senile castrated kokanee salmon which had survived from one to about four years beyond the normal life span showed alterations in the lateral muscle (Robertson and Wexler, 1962) which resemble those seen in *H. ilisha*. Ageing mammalian musculature is also known to undergo similar changes. Changes such as increase in the number of sarcolemmal nuclei, atrophy of muscle fibres, great diversity of the size of muscle fibres, proliferation of the elements of connective tissue and storage of varying amounts of fat have been reported by Stolk (1960, 1962) in middle aged and older Siamese fighting fish, *Betta splendens*. Based on the presence of certain enzymes which hydrolyze many high energy phosphates and phosphorylated co-enzymes in the proliferating connective tissue, Stolk (1960) came to the conclusion that these enzymes play some part in the genesis of muscular dystrophy and the fundamental defect may not be in the muscle fibres as such, but in the connective tissue which supports them. In the light of these observations there is a possibility that deteriorative alterations noted in _H. ilisha_ and _H. toli_ represent an acceleration of the ageing process. If ageing may be considered as a cause for the degenerative changes, it would account for the appearance of degenerative changes in some of the maturing fishes from the sea (which may be recovering spent fishes) and the considerable variation in the degree of degenerative changes shown by individual mature and spent fishes from the spawning grounds. It may also be mentioned that the changes in the lateral muscle occur in conjunction with the degenerative changes in various other organs and tissues in _H. ilisha_ and _H. toli_ (unpublished observations from this laboratory) as in the case in ageing.

Several investigators have shown the presence of hyperplastic adrenocortical tissue and high levels of 17-hydroxy cotricosteroids (17-OHCS), hydrocortisone and cortisone in the blood with sexual maturation and spawning in the Pacific salmon(Robertson and Wexler, 1959; Hane and Robertson, 1959; Philipps _et al._, 1959; Robertson _et al._, 1961 b; Idler _et al._, 1959, 1961; Schmidt and Idler, 1962). In mature and spent _H. ilisha_ also a higher level of adrenal hormones should be expected since the adrenal tissue showed hyperplasia and hypertrophy as in the case of the Pacific salmon (unpublished observations from this laboratory). A higher level of adrenal hormones have also been shown to induce degenerative changes in the skeletal muscle of various animals. On comparing the histological alterations that take place in the spawning salmon with that of Cushing's syndrome, experimental hyperadrenocorticism and ageing in mammals, Robertson _et al._ (1961) suggested that many of the tissue changes found in the migrating salmon could be ascribed to the effects of hyperactivity of the adrenal tissue. Robertson _et al._ (1963) also showed that degenerative changes occur in various organs and tissues including the lateral muscle of the immature rainbow trout, on experimental hydrocortisone treatment. In rabbit skeletal muscle, Ellis (1956) observed swelling, vacuolization, transformation of fibrils into granules and necrosis after cortisone treatment. After 21 days of cortisone treatment 40 - 80% of the muscle fibres showed various phases of degeneration and regeneration. When injections of cortisone were discontinued complete healing occurred. Perkoff (1959) showed the presence of vacuoles in fibres of quardriceps femoris and psoas muscles of patients after prolonged administration of cortisone or prednisone. Starvation and temperature are known to enhance the catabolic effects of corticoids. Robertson _et al._(1963) suggested that the absence of degeneration in the skeletal muscle of the salmon in contrast to its occurrence in hydrocortisone treated immature rainbow trout may be explained atleast in part by the protein sparing action of the large store of fat which the salmon accumulates in its muscle and liver before beginning the prolonged fast during the spawning migration. But the large storage of fat as well as degenerative changes observed in the lateral muscle of H. ilisha in the present investigation contradict the above mentioned hypothesis.

Apart from the adrenal gland, other endocrine glands have also a role in inducing the degenerative changes in the muscle. Burrows (as cited by Robertson and Wexler, 1962 a) showed that injections of large doses of pituitary extract (6 - 8 pituitaries) induced the development of all the characteristic changes seen in the spent salmon under natural spawning conditions in blueback salmon. The changes included deterioration of the skin, with focal necrosis and fungus infection, loss of muscular power and balance, and death before full gonadal development. In human hyperthyroidism, infiltration of fat between muscle fibres and atrophy of the fibres have been observed by various workers (Adams et al., 1954). It may be mentioned here that in H. ilisha as in salmon, the pituitary and thyroid were found to exhibit markedly increased activity in the maturing fishes from sea and river mouth. In the spent fishes from the spawning grounds while the pituitary and thyroid showed degeneration, the adrenal and pancreas showed hyperplasia and hypertrophy (unpublished observations from this laboratory).

H. ilisha is subjected to starvation during its migratory ascent and the question may be raised whether starvation has any role in bringing about the degenerative changes. Babinski and Onanoff (cited by Widdowson and Dickerson, 1960) have reported that the muscles in the body of an animal probably undergo some degree of atrophy during undernutrition, but the order in which the individual muscles develop and the extent to which they are used probably accounts for the fact that some muscles atrophy more than others. Prolonged starvation has been shown to induce degenerative changes in the lateral muscle of the rainbow trout (Robertson et al., 1963). Following forced feeding in Oncorhynchus nerka there was a return to green colouration and this change was coupled with an icreased colouration of the muscle, greatly increased vigour and marked increase in weight thereby suggesting that the reversible changes may be more directly connected with starvation than with sexual maturation (McBride et al., 1963). The changes noticed in the present investigation bear resemblance to the histopathological changes that occur in Vitamin-E deficiency in various farm and laboratory animals (West, 1963). Absence of Vitamin-E intake due to starvation may cause its deficiency during migration and thus induce degenerative changes in the muscle.

Fibres showing single or multiple vacuoles observed in the present study, except for the contents of the vacuoles, bear resemblance to the fibres in Periodic paralysis, central core disease and glycogen storage disease in man. With Haematoxylin - Eosin staining, while the fibres in the central core disease stained more deeply in the core region than in the non-core region and showed a closer packing of myofibrils in the core region than in the non-core region (Engel et al., 1961), the vacuoles of the fibres in Hilsa were seen to be without any substance. The presence of great variation in the size of the vacuoles in the individual muscle fibres, i.e. from 1 - 3 small vacuoles, to one and rarely two moderately sized vacuoles and in occasional fibres the whole sarcoplasm inside the swollen intact sarcolemma replaced by a big vacuole, suggests the possibility that small vacuoles are formed first which by coalescence leads to the formation of bigger vacuoles and finally destroys the fibres. In Periodic paralysis electron microscopic studies by Shy et al. (1961) and Pearson (1964) have shown that the sarcoplasmic reticulum irregularly dilates, sometimes over several sarcomeres in length and some of the dilations apparently initiate the vacuole formation. Progressive and more permanent cystic dilation and coalescence of smaller vacuoles lead to the formation of bigger vacuoles. Based on the recognised ability of exercise followed by rest or administered glucose, insulin and adrenalin, to initiate an attack of periodic paralysis and the presence of glycogen or other carbohydrate complexes in the watery vacuoles, it has been suggested that the handling of carbohydrate within the muscle fibre may be at fault (Shy et al. 1961; Pearson, 1964).

In contrast to the presence of glycogen or carbohydrate complexes in the periodic paralysis, fat fills the vacuoles of the fibres in Hilsa. Similar types of fibres have been noted in salmon (Greene, 1913) and in the muscles of dystrophic chicken (Julian and Asmundson, 1963). Epinephrine, growth hormone and cortisone stimulate lypolysis in the adipose tissue, increasing their output of FFA into the blood stream. Insulin is known to foster triglyceride synthesis in adipose tissue thereby suppressing the release of FFA and thus reducing the absolute extraction of FFA by muscle and other tissues. In the red muscle fibres, which utilize fat as the major fuel for energy, a defective handling of fat metabolism by the disturbance in hormonal milieu, may be responsible for the accumulation of fat in the fibres. Since fat is insoluble in the cell water, it could cause electrolyte fluxes between the extracellular and intracellular fluid phases and thus an osmotic imbalance.

It may be concluded that the degenerative changes that are brought about during migration are not due to the direct effect of any one of the factors alone, but may be attributable to an interplay of all the factors discussed above. The reproductive stress initiates the changes by the stimulation of the endocrine glands, which in turn are influenced by factors such as ageing, starvation, stress from migration and physiological adjustments required in the transition from salt- to freshwater. In mature and spent *H. ilisha* and *H. toli* from river more factors come into play and hence greater degenerative changes, while the less marked changes in *H. toli* from sea may be attributable to the lesser number of factors which come into play.