CHAPTER 7

HISTOLOGICAL CHANGES IN THE SPLEEN OF *HILSA ILISHA* AND *HILSA TOLI* DURING DIFFERENT PHASES OF LIFE CYCLE

It is well established that glucocorticoids enhance the destruction of lymphocytes from spleen and lymph organs . It also holds good in fishes and in lower vertebrates. Several workers have studied experimentally the effect of increase in glucocorticoids on lymphocytes destruction in spleen.(Robertson and Wexler, 1960; Bern, 1963; Rasquin, 1951). There remains little doubt that glucocorticoids cause increase in destruction of lymphocytes from spleen, increase stroma and fibrous tissue in spleen and also may enhance the production of pigmented phagocytes in spleen.

Histological changes in the corticosteroid secreting cells-interrenals have been observed in the migratory *H. ilisha* and non-migratory H. toli during maturity and spawning (Desai, 1967). To confirm indirectly the variations in corticosteroid level and to study the effect of changes in corticosteroids on spleen, a histological study of spleen during different phases of life cycle was undertaken.

MATERIALS AND METHODS

The live fishes were removed from the net and immediately sacrificed by decapitation. The isolated spleen was fixed in Bouin's fixative. Wax sections of 5 μ thickness were cut. They

were stained with haematoxylin-eosin and Heidenhain azan stain.

RESULTS

The results obtained in H. ilisha and H. toli are presented in a summarized form in Table I. The state of the adrenocortical tissue during the different phases of life cycle (from Desai, 1967) are also presented for correlation.

Spleen of fingerling of H. ilisha:

The entire tissue was compact. The lymphocytes were closely packed. Many blood vessels with RBC were seen. The muscular capsule was thin. No stroma was seen in the spleen.

Spleen of immature migratory H. ilisha from sea:

The entire spleen was filled with densely packed lymphocytes. Many large blood vessels were observed with RBC inside. The large, yellowish pigmented cells were found in big groups scattered throughout the spleen and occupying the major portion of the spleen. There was little or no stroma in the spleen (Fig. 1).

Spleen of mature migratory H. ilisha captured from river:

The spleen showed empty spaces throughout the gland and the lymphocytes were less numerous than in the spleen of immature H. ilisha. Large groups of phagocytes were observed. The connective tissue stroma had markedly increased (Fig. 2). The stroma stained blue eith Heidenhain azan stain. Connective tissue also had increased in thickness. The brown pigmented cells had decreased in number and few such groups were seen in mature spawning fish. In some samples very few pigmented

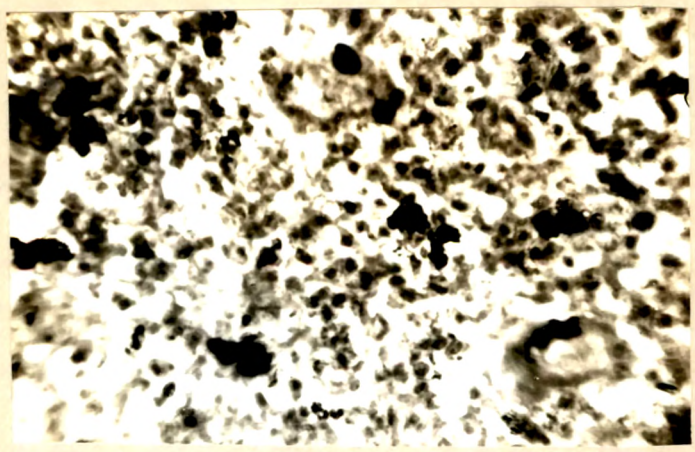

Fig. 1

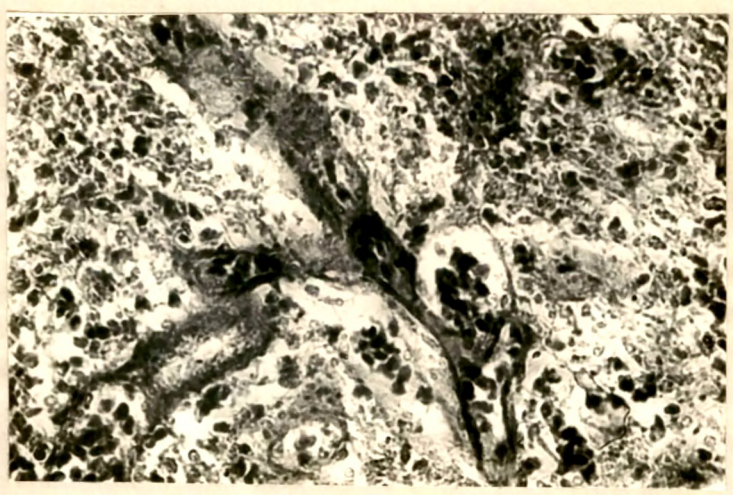

Fig. 2

Fig.1 T.S. of spleen of immature H.ilisha showing normal structure. HE. X630

Fig.2 T.S. of spleen of mature H.ilisha. Note increased connective tissue septa. HE. X630

cells were observed. In short, a remarkable reduction in the number of lymphocytes and a significant increase in stroma and connective tissue were seen in the spleen of spawning migratory H. ilisha.

Spleen of spent migratory H. ilisha captured from river:

The changes observed in the migratory fish were at its climax in this stage. Loosening of splenic substance (pulp) was noticed. Lymphocytes were very few in number. Fibrous tissue had markedly increased. Occasionally the thick capsule layer penetrated into the pulp of the spleen forming thick trabeculae like structures. These fibrous projections were thick and some times reached deep upto the centre of the spleen pulp. The brown pigmented cells were very few and were found scattered throughout the spleen pulp.

Spleen of immature, mature and spent non-migratory H. toli captured from sea:

The changes observed in the spleen at various stages were similar to the changes observed in migratory H. ilisha. as it attained maturity and migration proceeded. The common features were depletion in number of lymphocytes, increase in connective tissue and increase in the thickness of the capsule as the sexual maturity is attained.

Spleen of mature non-migratory H. toli drifted into fresh water, collected from Bhadbhoot:

The capsule wall surrounding spleen appeared to be

shrunk and muscle fibres of the capsule wall were wavy. Remarkable reduction in the number of lymphocytes was observed. The connective tissue stroma had increased considerably but was found to be loose. Numerous empty spaces in the pulp were noted. Many blood vessels showed progressive arteriosclerosis. There were a few pigmented cells scattered in the pulp of the spleen. Enlargement of trabeculae-like structures was also found.

DISCUSSION

It is now established that active secretions of glucocorticoids from adrenal cortex causes splenic atrophy and reduction in number of lymphocytes in lower vertebrates and fishes, as in mammals.

From the table it is seen that when migratory H. ilisha ascends the river during migration, the hyperplasia in adrenocortical tissue is observed. It may be presumed here on the basis of work done by several workers (Idler, Ronald and Schmidt, 1959; Robertson and Wexler, 1959; Robertson et al., 1961a) that corticosteroid level must have also been increased which may have caused the observed changes in the spleen. The pronounced hyperplasia of adrenocortical tissue in migratory H. ilisha was not observed in non-migratory H. toli captured from sea. However, a certain degree of hyperplasia and degeneration occurs in H. toli from sea due to stress of maturity. Also observed was simultaneous reduction in the number of lymphocytes and increase in stromal elements. Increase in phagocytes in mature fish were observed. Bern (1963) has observed such increase in

phagocytes after treatment with corticoids in the spleen of Tilapia mossambica.

The most pronounced changes shown by the spleen of H. toli drifted into the estuary may be the result of stress caused by sudden changes in ion concentration of water. The hyperplastic, degenerative changes observed simultaneously in adrenocortical tissue supports this observation. The stress due to sudden change in salinity, stress due to maturity and starvation might have forced adrenocortical tissue to secret more corticoids. This might have caused the splenic destruction and degeneration.

Table. I. Showing the state of the spleen and the adrenocortical tissue during different stages of maturity of Hilsa ilisha. and Hilsa toli.

Species	State of maturity	Spleen	Adrenocortical tissue
HILSA ILISHA	Fingerling.	Entire tissue was compact. Lymphocytes densely packed. Many blood vessels seen. Capsule covering spleen consisted of thin layer of muscle fibres. No connective tissue stroma observed in pulp.	Very small and few cells in head kidney. Nuclei small, perfectly round with fine granular chromatin. Few granules seen in the cytoplasm of the cells.
	III Immature	Solidly packed with lymphocytes. Many large blood vessels seen. In few pigmented cells were found in large clusters. Stroma was little.	Number of groups had increased. 1 to 3 cells thick with round nuclei filled with finely granular chromatin and prominent nucleolus. Cells showed mitosis and moderately distributed granules.
	V - VI Mature	Showed empty spaces. Lymphocytes were less in number then found in immature H. ilisha from sea. The stroma had increased, occupying considerable area. Brown pigmented cells were less and only few small groups found scattered. Capsule had increased in size.	Hyperplasia was noticed. 2-3 layers thick cells had increased in number and found not only round veins but scattered in haemopoetic tissue of the head kidney. Increase in size and volume of cells also noticed. Nuclear diameter had increased. Many nuclei showed pycnosis. Many granules of big size noticed in the cytoplasm.
	VII Spent	Lymphocytes rare. Tremendous increa ase in connective tissue stroma. Capsule showed increase in thickness. Trabeculae like projections from capsule project into the pulp. Brown pigmented cells, few and scattered.	Cells loosely arranged, small groups were separated by sinusoids. Most of the cells lost affinity for stain. Few granules were seen.Vacuolization was prominent feature. Degenerative changes such as shrinkage,of cells, pycnosis of nuclei and vacuolization were seen.

Table I (Continued)

Species	Stage of maturity.	Spleen	Adrenocortical tissue
	II-III Immature	Densely packed lymphocytes.Many blood vessels seen. Pigmented cells in large clusters. Little stroma seen.	1-3 cells thick with round nuclei filled with finely granular chromatin and a prominent nucleolus.Granules of small size were seen distributed in cytoplasm.
	V-VI	Spleen showed few empty space. Lymphocytes were less numerous than in the spleen of immature stage. Connective tissue stroma had increase considerably. Brown pigmented cells found scattered but very less.	Little hyperplasia observed. No. of cells in each group had multiplied and few more groups were found around veins. Small groups seen in haemopoetic tissues. In comparison with H.ilisha hyperplasia is less More big granules were seen in cytoplasm of the cells. Few nuclei pycnotic. Cells were enlarged in volume.
HILSA TOLI	VII Spent	Little loosening of pulp accompanied by remarkable increase in stroma and trabeculae like projections deep into the splenic substance. Reduction in number of lymphocytes. Brown pigmented cells very few.	Cells were packed, few specimen showed rarely few cells destroyed. The marked a and severe degenerative changes as observed in spent migratory H.ilisha were not observed. Vacuolization occasionally observed. Granules sparse, staining affinity lost.
	VI	The degenerative changes exhibited by spent fishes of both H. ilisha and H. toli were observed at climax. The entire pulp appeared to be shrunk. Tremendously thick stroma was observed. Trabeculae-like projections were very thick in size. Lymphocytes very few.	Complete degenerative and destructive changes. Many cells broken, nuclei of few intact cells enlarged and pycnotic. Vacuolization at climaz. Granules densely packed, big in size, were seen in few intact cells.