CHAPTER 6

HISTOLOGICAL CHANGES IN THE PANCREAS OF H. ILISHA AND H. TOLI DURING DIFFERENT PHASES OF LIFE CYCLE

Histological and physiological studies on the pancreatic islets of certain fishes have been made, mostly in the last decade, because the endocrine tissue is found separate and concentrated in a macrospic structure- the so called principal islet. Various studies have shown the presence of granular cells like mammalian beta and alpha cells together with agranular cells (Grosso, 1950; Falkmer, 1961; Hellerstorm et al., 1964; Sivadas, 1964; Falkmer et al., 1964, '65; Bencosme et al., 1965).

The fish, H. ilisha, do not feed during migration and spawning. Remarkable changes in the lipid content of liver (Chapter 4), subcutaneous fat depot and muscles (Joseph, 1967) have been observed. Since pancreas plays an important role in lipid and carbohydrate metabolism, in view of the above mentioned parallel observations, it seemed relevent to undertake a comparative study of the histological changes occuring in the pancreas of migratory H. ilisha and non-migratory H. toli during different phases of life cycle.

MATERIALS AND METHODS

Live fishes were decapitated immediately after removal from net. Small pieces of the tissue were fixed in Bouin's fluid.

Paraffin sections of 4 μ thickness were cut. The following staining methods were used 1- Aldehyde fuchsin counterstained with Jane E. Cason's stain,2- Heidenhain Azan using iron-alum as mordant, 3- Chrome-Haematoxylin counterstained with Phloxin and 4- Haematoxylin-eosin.

RESULTS

Pancreas of H. ilisha and H. toli is a diffuse gland present in fragments situated between pyloric caecae. The islets of Langerhans are found in exocrine tissue.

Beta cells with granules were stained clearly with aldehyde fuchsin and chrome-haematoxylin. However, when beta cells were totally degranulated, these procedures did not help much to differentiate beta cells from alpha cells.

Using Heidenhain's Azan stain it was possible to distinguish the two types of cells, the beta and alpha. Beta cells showed red nuclei with bluish cytoplasm when in degranulated state. The granules of beta cells were stained red. Alpha cells stained ornage yellow with nuclei also more or less of the same colour.

Alpha cells stained reddish after staining the islets with aldehyde fuchsin and chrome-haematoxylin-phloxin staining procedures.

Certain islets did not show staining affinity towards any of the conventional stains described above. The nuclei stained faintly.

Pancreas of fingerling of H. ilisha captured from river:

Exocrine cells were polyhedral and full of zymogen granules. These cells showed central nuclei containing little, finely granular chromatin and a prominent central nucleolus. Fatty tissue surrounding pancreas was absent.

Islets were small, showing some beta cells degranulated. Some islets exclusively contained either alpha or beta cells. Islet cells were deeply stained with Azan procedure, indicating active phase (Fig. 1).

Pancreas of immature H. ilisha captured from sea:

Exocrine cells were compactly placed and were full of granules. Very few cells were devoid of granules. Prominent round nucleus, with a nucleolus, was centrally placed. Numerous large granules were observed extruding out, the cells were probably discharging.

The islets were small, compact, surrounded by exocrine tissue (Fig. 2). Two types of islet cells could be easily distinguished when stained with aldehyde fuchsin and chrome-haematoxylin. Nucleus of beta cells was not clearly visible with these procedures of staining. However, with azan stain beta cells showed oval, centrally placed nuclei and large red granules. Yellow-orange alpha cells showed central, rounded large nucleus.

Beta cells were very few in comparison to alpha cells and were heavily granulated. Beta cells were situated on the periphery of islets and 2-3 cells in the centre of the islet also. The remaining central area was completely filled with alpha cells (Fig. 3). Few islets showed such types of cells. Remaining

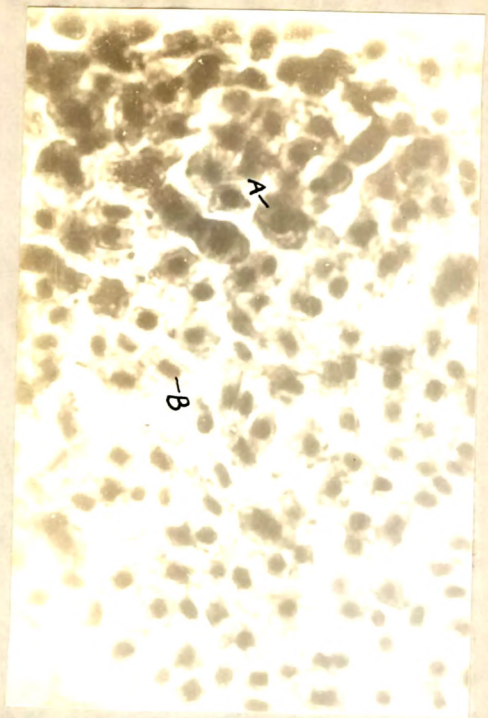

Fig. 1

Fig. 2

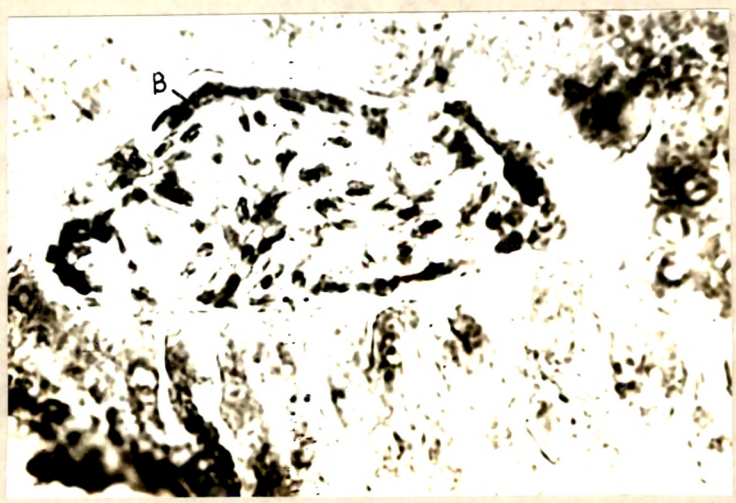

Fig. 3

Fig. 1. Pancreatic islet of fingerling of H. ilisha showing alpha (A) and beta (B) cells. Azan. X 63.
Fig. 2. Pancreas of immature H. ilisha containing small islets of Langerhans (I). Azan. X 63.
Fig. 3. Islet of immature H. ilisha showing deeply stained beta (B) cells, mostly at the periphery. Aldehyde fuchsin. X 1000.

islets showed no affinity towards any stain.

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

Exocrine cells were polyhedral with large deeply stained granules. The basally situated round nuclei showed prominent nucleoli. In many cells granules were found near the periphery. The number and size of islets had increased.

Beta cells were with bluish cytoplasm containing large but few red granules. The nuclei were faintly stained. These cells were aldehyde fuchsin and chrome-haematoxylin positive, with granules. The granules were less than those present in immature fish as revealed by aldehyde fuchsin staining.

Alpha cells were orange coloured with little granules and oval nuclei. They appear reddish after chrome-haematoxylin-phloxin staining. The number of alpha cells was more than beta cells (Fig. 4). Islets containing colourless cells, showing no affinity towards any stain were also present.

Pancreas of mature migrating H. ilisha captured from river:

Exocrine cells, most of which were devoid of granules or contained scanty granules showed central nuclei with nucleoli.

The islets showed hypertrophy and hyperplasia (Fig. 5). Beta cells had increased in number, being more than the alpha cells, but were degranulated. Some islets showed only beta cells. These cells were spindle shaped with central, oval nuclei. Alpha cells were orange yellow and larger than the beta cells.

These cells also were mostly degranulated.

Some islets were broken and islet cells were destroyed. Remaining cells of such islet were degranulated (Fig. 6). Blood vessels also might have been broken as wandering blood cells were observed. The exocrine cells were loosely arranged in comparison with those of immature H. ilisha from sea.

Pancreas of spent H. ilisha captured from river:

The histological structure was similar to that observed in the case of mature migrating river H. ilisha showing destruction of islets and exocrine tissue (Fig. 7). In addition, the pancreas of spent H. ilisha showed small islets with compactly situated cells. These could be newly formed islets (Fig. 8).

Pancreas of immature H. toli captured from sea:

The details observed in immature H. toli were similar to those observed in immature H. ilisha from sea.

Pancreas of mature H. toli captured from sea:

The exocrine cells were compactly arranged, with basal round nuclei containing chromatin. These cells showed scanty granules.

The islets showed mild hypertrophy (Fig. 9) than the hypertrophy observed in the case of mature H. ilisha captured from river. The islets were compactly arranged. Beta and alpha cells were more or less equal in number. Beta cells were more degranulated than alpha cells. However, no degeneration of beta cells or islets were observed. The structure and staining affinities of beta and alpha cells were similar as observed in other cases.

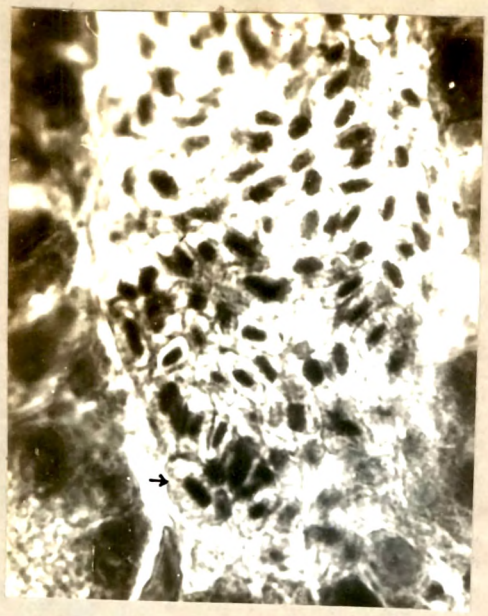

Fig. 4

Fig. 5

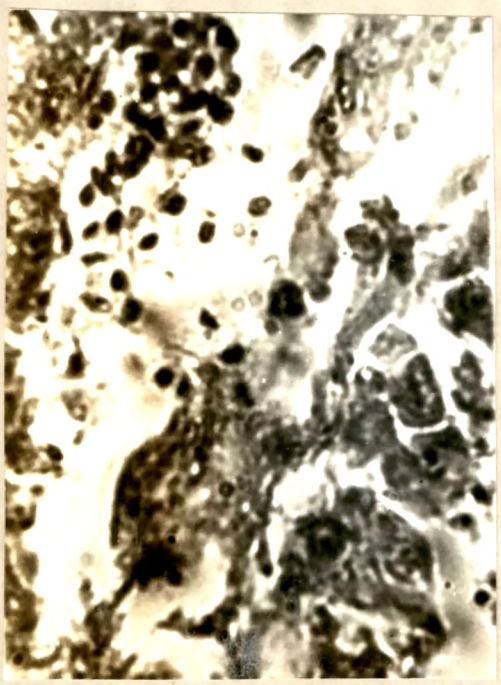

Fig. 6

Fig. 7

Fig. 4. Islet of maturing H. ilisha prior to migration showing showing large beta cells (arrow). Azan. X 1000.
Fig. 5. Pancreas of spawning H. ilisha showing hypertrophied islets (arrow) surrounded by exocrine tissue. Aldehyde-fuchsin. X 63.
Fig. 6. The broken islet surrounded by loosely arranged exocrine cells of spawning H. ilisha. Islet showing remaining degranulated cells. Azan. X 1000.
Fig. 7. Pancreas of spent H. ilisha showing partial destruction of islets and exocrine tissue. (Arrows) Azan. X 63.

Pancreas of spent H. toli captured from sea:

The pancreatic structure was more or less same as observed in the case of mature H. toli from sea.

Pancreas of drifted H. toli captured from river:

Marked destruction of the pancreatic tissue was the most prominent feature. Most of the exocrine tissue was found destroyed. Cells were lost. Very often blood cells were found wandering in this tissue. Intact cells showed central round nuclei with chromatin. These cells were devoid of granules. Many islets were destroyed and cells were lost leaving empty space (Fig. 10). Other remaining islets were shrunk. Wherever islet tissue was found, beta cells were degranulated and alpha cells were with scanty granules.

DISCUSSION

In addition to the two types of cells distinguished using the conventional staining procedures, groups of cells were observed which showed no affinity towards any of the stains used. These cells might be agranular cells, as observed by Falkmer et al. (1964), representing the young, immature form of other islet cells.

Exocrine tissue was in active state, showing cells full of granules, in juvenile, immature and maturing H. ilisha prior to migration. In migrating and spawning H. ilisha captured from river, these cells were mostly degranulated, indicating 'exhaust' phase. Exocrine cells in mature and spent H. toli, however, showed

Fig. 8

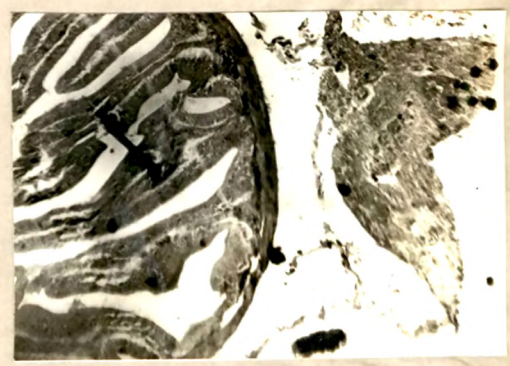

Fig. 9

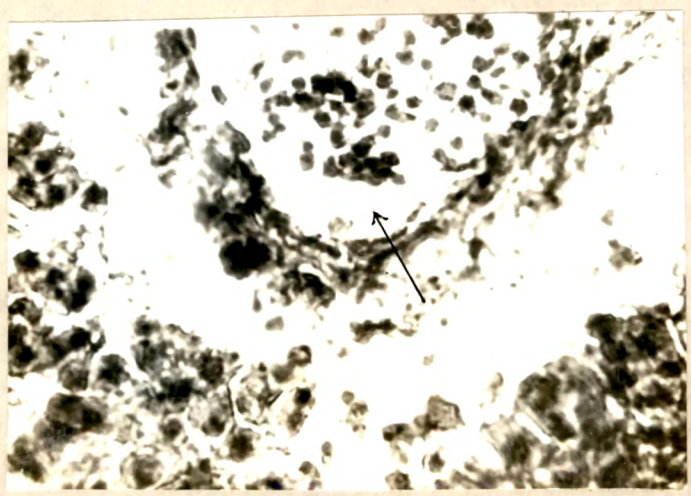

Fig. 10

Fig. 8. Pancreas of spent H. ilisha showing newly formed small islets (arrow). Azan. X 63.

Fig. 9. Pancreas of mature H. toli showing mild hypertrophy of islets. Azan. X 63.

Fig. 10. Pancreas of drifted H. toli showing destruction of islets. (arrow) and surrounding exocrine tissue. Azan. X 63.

scanty granules. General degranulation in H. ilisha might be due to non-feeding during migration and spawning, accompanied by hyperadrenocorticism, as observed by Desai (1967). Exocrine cells of hyrdrocortisone treated immature rainbow trout (Salmo gairdnerii) showed degranulation (Robertson et al., 1963). On the other hand, exocrine cells in spawning Pacific salmon were well granulated (Robertson and Wexler, 1960).

The islets in mature H. toli, migrating and spawning H. ilisha and also in maturing H. ilisha captured from sea prior to migration showed hyperplasia and hypertrophy. Compared to the immature H. ilisha in which the islets were small and few in number, the islets in mature H. ilisha were relatively large and more in number. These hypertrophy and hyperplasia might be due to hyperadrenocorticism. Interrenal tissue shows hyperplasia in maturing H. ilisha prior to migration, migrating ans spawning H. ilisha and also mild hyperplasia in mature H. toli (Desai, 1967). Maturation is not the cause for such alterations (Robertson and Wexler, 1962). That adrenocorticism induces hypertrophy and hyperplasia has been shown by Robertson et al. (1963).

In mature H. ilisha the beta cells had increased in number than the alpha cells. Some islets showed exclusively beta cells. These cells were found to be degranulated. Some islets showed degeneration and destruction of cells. Beta cells in mature H. toli had not increased in number when compared to the alpha cells. These cells showed scanty granules. These alterations in the number of beta cells as well as granulations might be due to hyperadrenocorticism. As mentioned earlier the interrenal

gland is more active in mature fish. Similar observations of degranulation and degerneration of beta cells have been made in some of the steel-head trout examined by Robertson and Wexler (1961). Hydrocortisone injection in immature rainbow trout also resulted in degranulation of beta cells. In the Pacific salmon, on the other hand, beta cells were typically well granulated (Robertson and Wexler, 1960).

It has been shown that teleost pituitaries produce the growth hormone (cf. Falkmer and Matty, 1966). Pituitary is in active state in immature (stage II and III) and mature spawning H. ilisha. Interrenal tissue is also active as mentioned earlier. Thus the growth hormone (GH), ACTH and corticosteroids are active in the mature fish and it is known that the action of the above hormones are accentuated during starvation. Action of GH and ACTH-adrenal cortex system on carbohydrate and lipid metabolism has been reviewed by Weil (1965). Based on the conclusions arrived at by Weil (1965) it is reasonable to presume as follows: GH is a lipolytic hormone, initially it will increase glucose production and uptake. GH and epinephrine-non-epinephrine also mobilize lipid from fat depot (Chapter 4). This will alter the carbohydrate cycle in the animal and effect a depression of glucose uptake by the tissues. Glucocorticoids also inhibits glucose uptake and utilization. Thus GH and glucocorticoids are two principal hormones inducing inhibition of glucose uptake, the effect being further accentuated by starvation. This condition is in fact observed in the case of H. ilisha during migration and spawning. Simultaneously

this will result in hyperglycemia. Rise in blood glucose value would cause the activation of the islet cells with increased insulin production as evident by degranulation and hyperplasia in maturing H. ilisha prior to migration. This is essential to overcome the hyperglycemia and resistance to glucose uptake. Total exhaution of beta cells, resulting in degeneration also, has been observed in migrating and spawning H. ilisha. This must be the result of prolonged GH action as evident by a very active pituitary in spawning fish too (Desai, 1967). Falkmer and Matty (1966) carried out experiments to find out the role of pituitary in blood sugar regulation in the marine teleost, Cottus scorpius. They failed to arrive at any conclusive evidence for the presence or absence of any diabetogenic principle because the experiments were performed on sick fish and secondly they could study only short time effects.

In mature H. toli the beta cells showed scanty granules and no degeneration. These suggest the low demand for insulin, probably due to mild hyperglycemia which in turn must have been induced by mild hyperadrenocorticism as compared to mature H.ilisha.

In spent H. ilisha the presence of new small islets with compact cells suggests the regeneration of islets. Thus it might be presumed that the degeneration of islets was due to excessive demand of insulin which results in exhaustion of cell functions, but these alterations are reversible.

The role of alpha cells is to produce glucagon. The latter has been considered to be a mild adipokinetic agent like epinephrine. Glucogon along with other lipolytic agents must be

helping mobilization of lipids, when the gonads are developing. These cells are active in maturing H. ilisha prior to migration and are degranulated in mature spawning H. ilisha, suggesting the exhaustion of alpha cells. In mature H. toli alpha cells showed scanty granules indicating less demand for glucagon as compared to H. ilisha of the same stage. As discussed in chapter on liver lipids it is seen that H. toli requires less mobilization of lipids than is observed in H. ilisha.

The marked destruction of pancreas in drifted H. toli captured from river could be attributed to stress caused by the abrupt change in external medium.