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Original Article

A comparative study of the histoarchitecture of endocrine pancreas in *Labeo bata* (Hamilton, 1822), *Sperata aor* (Hamilton, 1822) and *Chitala chitala* (Hamilton, 1822)

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Abstract: The disposition and cellular organization of the endocrine pancreas were studied in three species of freshwater teleosts viz., *Labeo bata* (Hamilton, 1822), *Sperata aor* (Hamilton, 1822) and *Chitala chitala* (Hamilton, 1822) using histological techniques. In *L. bata,* the endocrine pancreas tissues were mainly distributed in the adipose tissue among the intestinal coils and adjacent to extrahepatic bile duct, while in *S. aor* and *C. chitala,* the endocrine pancreas predominantly attached with wall of the stomach along with exocrine pancreatic part. Histological analysis demonstrated that the endocrine components of all the three species were enclosed in a thin capsule provided with different cells, interspersed with blood sinuses. The cytoarchitectural analysis showed that in *L. bata*, β cells were usually arranged in groups while α cells were often interspersed with blood vessels. In *S. aor* and *C. chitala*, the rounded or oval α cells were usually arranged either in groups or scattered to the islets periphery and β cells which were densely granulated and typically stained with Aldehyde fuchsin (AF), Romies azan (RA) and Mallory's triple (MT) were observed in the central areas of the islets and intercalated with blood vessels. The δ like cells were founded at a low frequency and intermingled with β cells and exhibited moderate cytoplasmic granules in *L. bata*, *S. aor* and *C. chitala.* Despite being the subject of extreme controversy, the nature and function of different islet cells were discussed.

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Introduction

The endocrine pancreas present in different teleosts plays a momentous role in fish physiology. In fish, it exhibits various degrees of morphological, anatomical and cytological variations (Lazarow, 1963; Kudo and Takahashi, 1973; Falkmer and Ostberg, 1977). Morphocytologically, the patterns of endocrine pancreas in fishes exhibits numerous species variations and quite often consists of several prominent knots of tissue, more or less segregated from the acinar parenchyma known as principal islets (Brinn and Epple, 1972). A compact terapod-like organization of the pancreas with islets distributed throughout the exocrine parenchyma has been observed in *Clarias batrachus* (Khanna and Mehrotra, 1968), *Mystus seenghala* (Khanna and Gill, 1973) and *Conger japonicas* (Kobayashi and Takahashi, 1974).

Tinctorial criteria, following granule staining and optical microscopic examination have usually been used for the identification of cells. Location of B and D cells tend to occupy the central part of the islet whereas the A and F cells are more numerous at the periphery. In *Cottus scorpius* and *Channa punctatus*, the B and D cells generally occupy central part of the islet whereas α and granular cells are mainly located at the periphery (Falkmer, 1961; Khanna and Singh, 1971). In the goldfish *Carassius carassius*, B cells are usually arranged in groups or in cords which run irregularly or anastomose with each other to form a complicated network. The A and C cells are intermingled with these B cells cords of groups and

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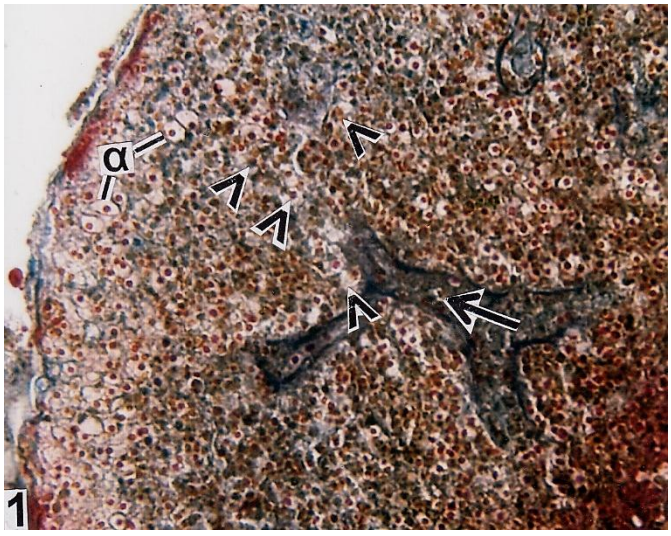

Figure 1. Endocrine pancreas in *L. bata* showing α cells at the periphery and β cells (arrow heads) in the central region. Arrow indicates blood vessel (MT, 100X).

A cells often occur in small patches of few cells (Kobayashi and Takahashi, 1970). As regards the physiological role of the islet cells, ultimate proof have been obtained for the production of insulin by β cells in coho salmon, *Oncorhynchus kisutch* (Plisetskaya et al., 1985). The nature and function of A cell has been settled in favour of their being the source of glucagon in *C. carassius* (Kudo and Takahashi, 1973). Sayrafi et al. (2011) differentiated α and β cells by haematoxylin and eosin staining in the islets of Langerhans of *Pangasius sanitwongsi*. The aim of the present work is to characterize the cellular details and functional aspects of the endocrine pancreas in *Labeo bata*, *Sperata aor* and *Chitala chitala* having different feeding habits, by histological analysis.

Materials and Methods

Healthy fishes of *L. bata* (16.02±1.89 cm in total length; n=16), *S. aor* (31.35±2.43 cm in total length; n=12) and *C. chitala* (46.38±3.17 cm in total length; n=14) were collected from local freshwater fish farm of Burdwan (23.2333°N, 87.8667°E), West Bengal, India during February-August, 2015. Fishes were killed with an overdose of anesthetic (0.03% MS 222; Tricaine methone-sulphonate, Sigma Chemical Co.) following the guidelines given by the Institutional Ethical Committee.

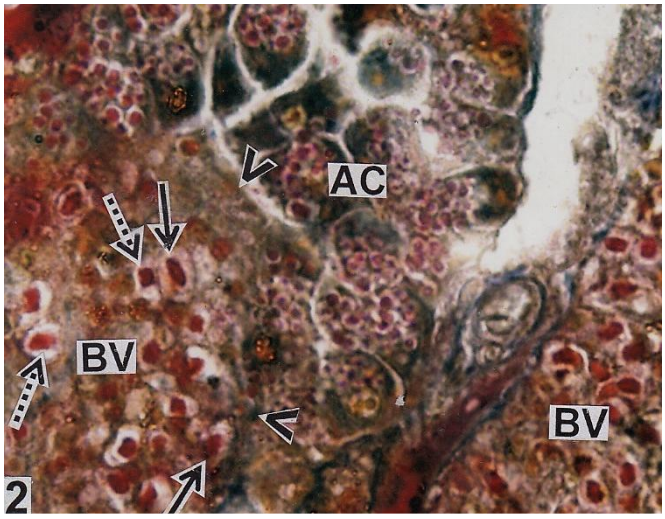

Figure 2. Acinar cells (AC) separated from islets of Langerhans by connective tissue septa (arrow heads) in *L. bata*. Note homogenous cytoplasm and deep nucleus in β cells (solid arrows) and deep nucleus in α cells (broken arrows). BV indicated blood vessels (MT, 1000X).

For histological studies, the fishes were dissected and small pieces of the pancreas of *S. aor* and *C. chitala* and pancreatic tissues associated with adipose tissues among the intestinal coils of *L. bata* were removed and fixed in aqueous Bouin's fluid for 18 hrs. The tissues were then placed in 70% ethanol and subsequently dehydrated through the ascending series of ethanol, followed by acetone and cleared with xylene. Tissues were embedded in paraffin wax (56-58°C). Serial sections were cut at 4 µm thickness. Deparaffinized sections were brought to distilled water through descending series of ethanol and were stained with Delafield's Haematoxylin-Eosin (HE), Mallory's triple (MT) (Mallory, 1936), Aldehyde fuchsin (AF) (Halami, 1952), and Romies azan (RA) stains. Sections were dehydrated through ascending series of ethanol, cleared in xylene, mounted in DPX, observed and photographed under LEICA EC3 compound microscope.

Results

The endocrine pancreas in *L. bata* (herbivore), *S. aor* (carnivore) and *C. chitala* (highly carnivore) exhibit a great deal of variations in relation to their occurrence as well as morpho-histological peculiarities. The endocrine pancreas in *L. bata* is associated with the adipose tissue located in between

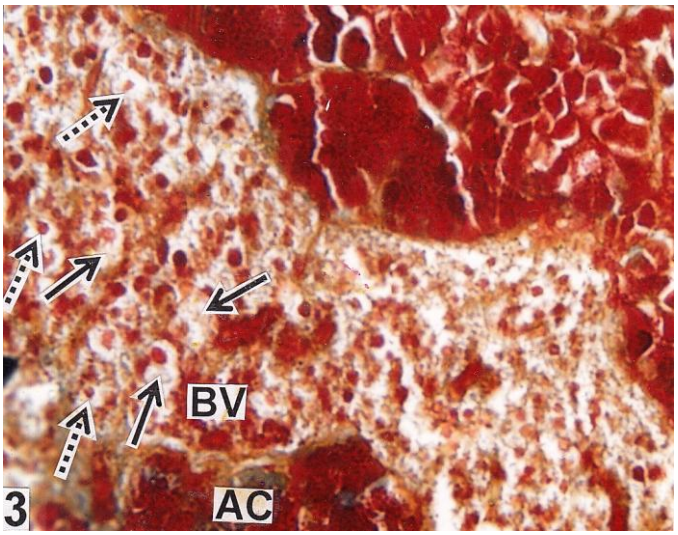

Figure 3. Showing α cells at the periphery (broken arrows) and inner β cells (solid arrows) adjacent to blood vessels (BV) and acinar cells (AC) in *L. bata* (RA, 400X).

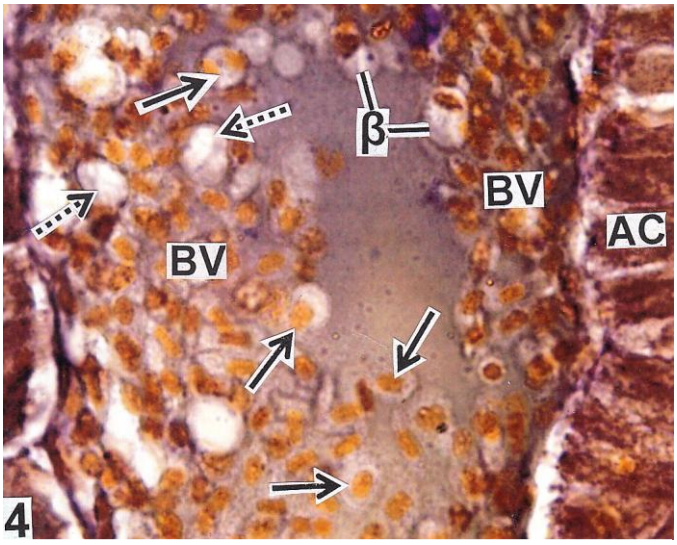

Figure 4. Endocrine pancreas in *L. bata* showing AF positive β cells, α cells (solid arrows) and vacuolated δ cells (broken arrows) encircling blood vessels (BV) (AF, 1000X).

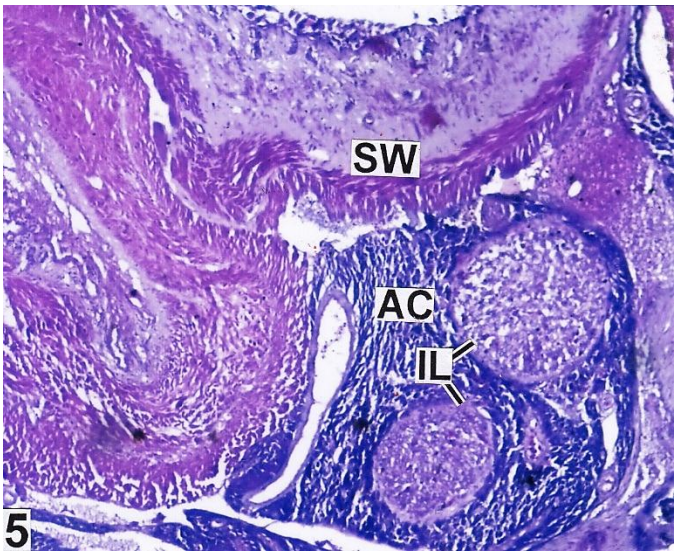

Figure 5. Comparatively light stained islet of Langerhans (IL) attached to the wall of the stomach (SW) and encircled by acinar cells (AC) in *S. aor* (HE, 100X).

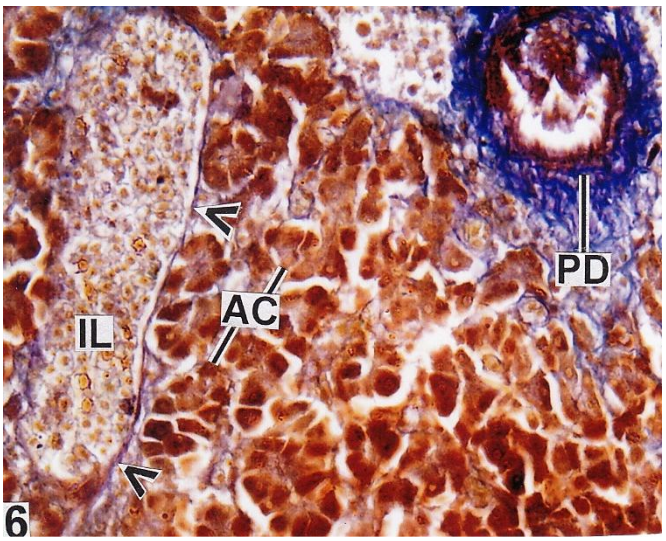

Figure 6. Scattered elongated islet of Langerhans (IL) positive to AF in between acinar cells (AC) and pancreatic duct (PD) in *S. aor*. Arrow heads indicate septa in between AC and IL (AF,

coils of the intestine around the blood vessels and the bile duct within the mesentry. However, in *S. aor* and *C. chitala*, the pancreas is found to occur as a well-developed compact organ situated in between the lobes of liver and seen to span from the border of the stomach to the anterior part of the intestine. The intrahepatic pancreatic tissue of hepatopancreas in *L. bata* is usually devoid of islets of Langerhans.

Different cell types could be identified in the pancreatic islets, based mainly upon differences in the density of the cytoplasmic ground substance, in the shape and secretory granules. In *L. bata*, the pancreatic islet cells are separated from exocrine part by a thin capsule of connective tissue mainly of collagenous fibres. The distribution of α cells is restricted to the islet periphery facing towards acinar cells (Figs. 1, 2). The islet of α cells contained sparse cytoplasm and densely stained nucleus (Fig. 2). In the central portion of the islets the AF positive β cells exhibit rounded moderately dense cytoplasmic mass and direct contact with blood cells (Figs. 3, 4). Distinct granules of β cells are not seen in Mallory's triple stain since the granules are soluble in ethanol. In *S. aor*, the pancreas is relatively more organized

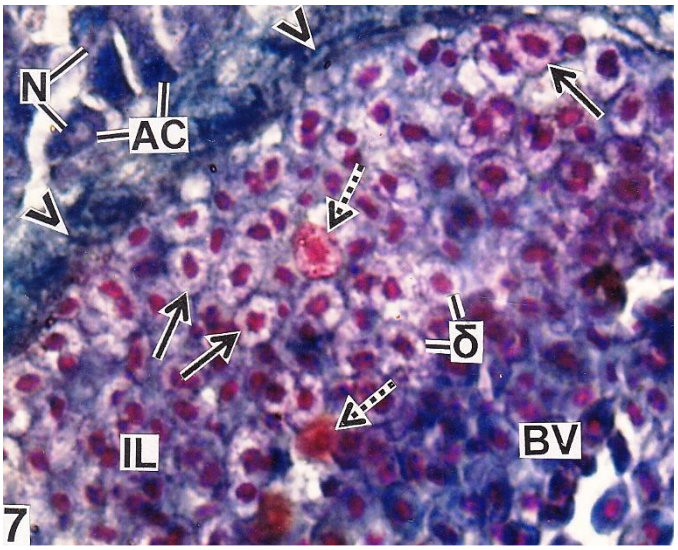

Figure 7. Islet of Langerhans (IL) separated from acinar cells (AC) by septa (arrow heads) in *S. aor*. Note peripheral α cells (solid arrows), intense red coloured β cells (broken arrows) and innermost δ cells adjacent to blood vessels (BV) (RA, 1000X).

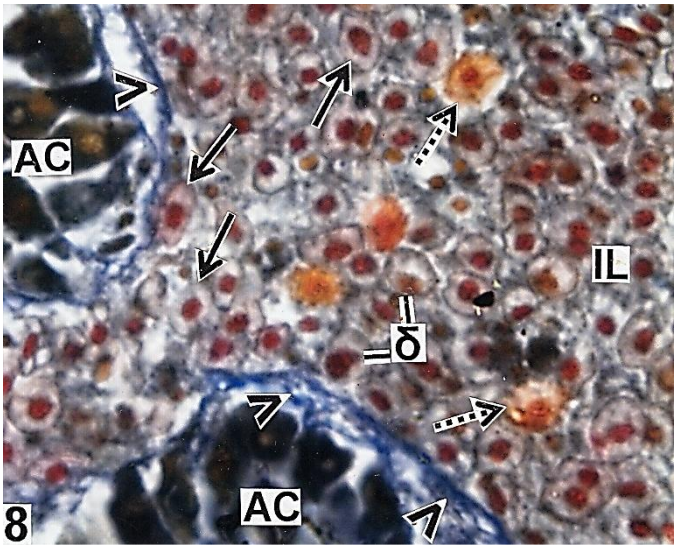

Figure 8. Islet of Langerhans (IL) in *S. aor* showing β cells with prominent nucleus and cytoplasm mass (broken arrows), α cells (solid arrows) and innermost δ cells in between acinar cells (AC). Arrow heads indicate septa (MT, 1000).

in its structural architecture and patches of endocrine islets are surrounded by acinar cells (Figs. 5, 6). The rounded α cells are positive to Romies azan stain provided with conspicuous dense nucleus and homogenous cytoplasm which are situated at the surface of islets below the septa (Fig. 8) adjacent to blood vessels. The β cells are comparatively larger in size and ovoid in shape and clustered in the central portion of islets and are stained with Romies azan and Mallory's triple stain (Figs. 7, 8). However, δ

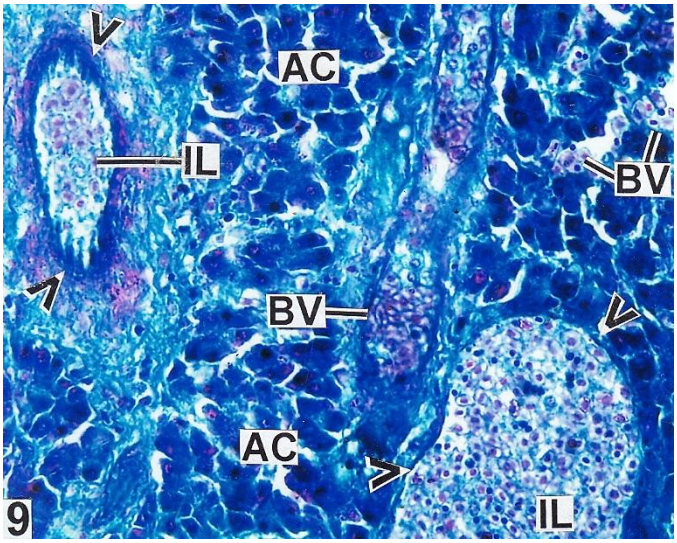

Figure 9. Light stained small elongated or large oval islet Langerhans (IL) in *C. chitala* in between acinar cells (AC) and blood vessels (BV). Arrow heads indicate septa (RA, 150X).

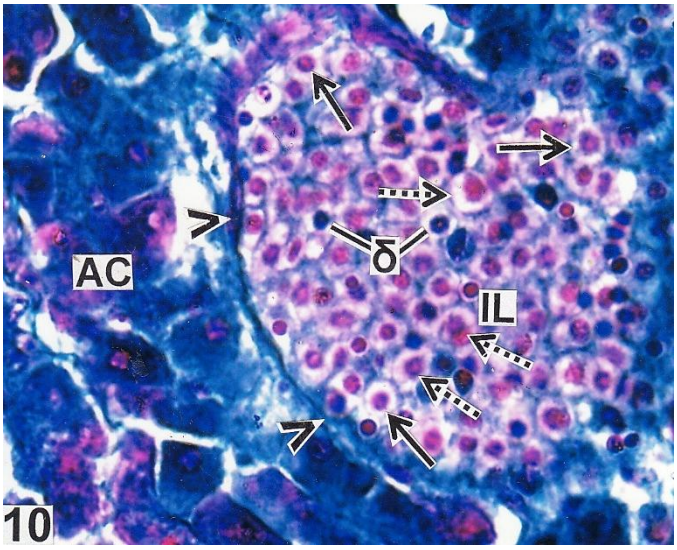

Figure 10. Higher magnification of islet Langerhans (IL) in *C. chitala* showing α cells at the periphery (solid arrows), β cells (broken arrows) and δ cells in central position. Arrow heads indicate septa in between IL and acinar cells (AC) (RA, 1000X).

cells are solitary and few in number, provided with densely stained nuclei and scantly cytoplasm (Figs. 7, 8).

The small and large islet of Langerhans in *C. chitala* scattered as irregular elongated or oval masses of comparatively pale staining cells adjacent to rich blood vessels and acinar cells (Figs. 9, 10). α cells are at the periphery of the islet but occasional isolated cells may be found in its interior. β cells are numerous in the centre of the islets positive to AF having prominent nucleus and homogenous

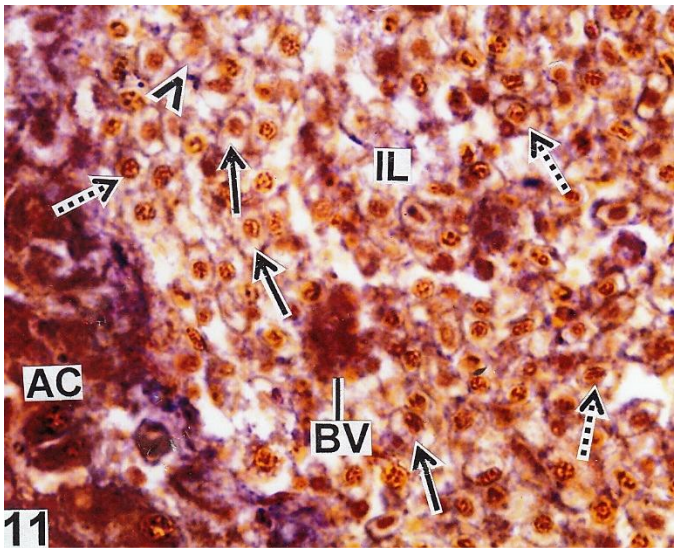

Figure 11. AF positive β cells (solid arrows), α cells (broken arrows) and fairly stained δ cells (arrow head) in the islet of Langerhans (IL) of *C. chitala* interspersed with blood vessels (BV). AC indicates acinar cells (AF, 600X).

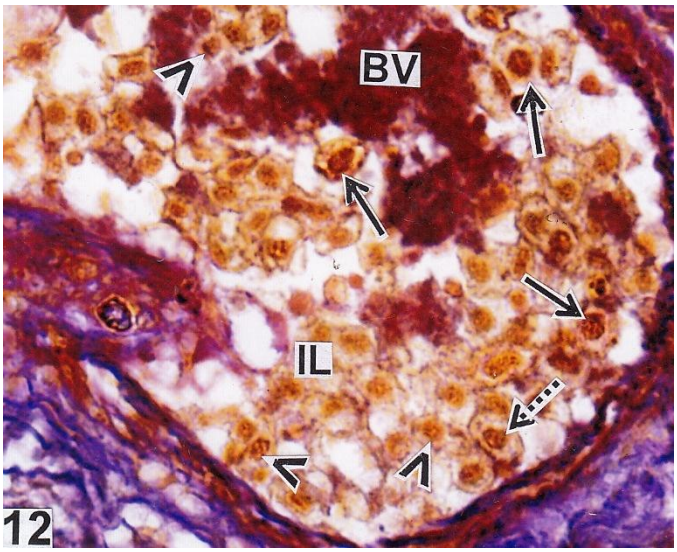

Figure 12. Higher magnification of AF positive β cells (solid arrows) adjacent to blood vessels (BV) in *C. chitala*. Note the presence of α cells (arrow heads) and solitary δ cell (broken arrow) (AF, 1000X).

cytoplasm (Figs. 11, 12). Occasional isolated comparatively larger blue coloured agranular δ cells are scattered in between β cells (Fig. 10).

Discussion

In the present observation, the islet of Langerhans are dispersed throughout the pancreas in *L. bata*, *S. aor* and *C. chitala*. In all the fish species, the islet of Langerhans are enclosed in a thin capsule and consists of comparatively lightly stained fusiform cells with distinct nuclei and interspersed with blood sinuses. The endocrine portion of the *L. bata* is mainly distributed around intestinal bulb, intestine and bile duct. These descriptions resemble those given for islet aggregates in Salmoniformes (Wang et al., 1986). In *S. aor* and *C. chitala*, a number of lightly staining islet of Langerhans of different sizes and shapes occur in between exocrine acinar cells and around the pancreatic duct. Youson and Al-Mahrouki (1999) considered the islet of Langerhans as principal islets of various sizes in fishes. They further opined that the intrahepatic pancreatic tissue is usually devoid of islets.

In the present investigation in all the three fish species, the α cells are the most dominant cell types and most common on the periphery that appeared ovoid structure having oval nuclei and intense acidophilic cytoplasm. β cells are large in size, oval or rounded in shape with a granular or homogenous cytoplasm. On contrary, δ cells are solitary and few in number. Shyamsundari et al. (2006) reported that ovoid islets of Langerhans are usually with a central cluster of β cells and the α cells in the periphery in lizard fish *Saurida tumbill*. Mokhtar (2015) opined that the endocrine part of *Ctenopharyngodon idella*, the ovoid α cells are dominate, β cells are polyhedral and they grouped in small clusters while δ cells are small, fusiform and argyrophilic cells. Boquist and Patent (1971) by ultrastructural analysis noticed that in the islet of the teleost *Scorpaena scropha* the α cells are mostly abundant in the periphery of the islets and contained numerous secretory granules with electron dense core while β cells are central in position having medium sized rounded moderately dense secretory granules. In the present study, it has been observed that α, β and δ cells have contacts with the blood capillaries and sometimes the secretory contents of the cells are observed into the blood vessels. Therefore, it may be conjectured that after being secreted from the endocrine cells the hormones are carried out into the capillaries and thereby promoting movement of glucose. Iaglov (1978) emphasized that in *C. carassius*, *Cyprinus carpio*, *Tinca tinca* and *Silurus glanis* α, β and δ cells

are shoot shaped and all have contacts with the capillaries and the hormones from the endocrine cells is carried out via emiocytosis into the blood vessels. In the present investigation, in all the three fish species the most important hormones secreted by the islet of Langerhans are insulin from β cell and glucagon from α cells. Both play a role in proper metabolism of sugars and starches in the fish body. Insulin promotes the movement of glucose and other nutrients out of the blood and into cells. When blood glucose rises, insulin released from the β cells causes glucose to enter body cells to be used for energy. Also, it sometimes stimulates conversion of glucose to glycogen in the liver. Another hormone, glucagon from α cells promotes the movement of glucose into the blood when glucose levels are below normal. It causes the breakdown of stored liver glycogen to glucose, so that the sugar content of blood leaving the liver rises (Sorokin et al., 1982; Plisetskaya et al., 1985; 1986). However, immunological analysis to endocrine cells of the aforesaid three species under study is further needed for detailed cellular structure and functions.

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چکیده فارسی

مطالعه مقایسه‌ای ساختار بافت‌شناسی غده‌ی درون ریز پانکراس در ماهیان *Labeo bata* (Hamilton, 1822)، *Sperata aor* (Hamilton, 1822) و *Chitala chitala* (Hamilton, 1822)

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[3]گروه جانورشناسی، کالج دختران آسانسول، آسانسول، بوردوان ۷۱۳۳۰۴، بنگال غربی، هندوستان.

چکیده:

وضعیت و سازمان سلولی غده‌ی درون‌ریز پانکراس در سه گونه ماهی استخوانی آب‌شیرین شامل *Labeo bata* (Hamilton, 1822)، *Sperata aor* (Hamilton, 1822) و *Chitala chitala* (Hamilton, 1822) با استفاده از تکنیک‌های بافت‌شناسی مورد مطالعه قرار گرفت. در گونه *L. bata* بافت‌های غده پانکراس عمدتاً در بافت چربی بین توده روده‌ای نزدیک مجرای صفراوی واقع در بالای پانکراس پراکنده شده بودند، در حالی‌که در دو گونه *S. aor* و *C. chitala*، غده پانکراس به‌طور غالب چسبیده به دیواره معده همراه با بخش برون‌ریز پانکراس بود. تحلیل بافت‌شناسی تائید کرد که اجزای درون ریز هر سه گونه مورد مطالعه توسط یک کپسول نازک حاوی سلول‌های مختلف پراکنده شده در بین سینوس‌های خونی احاطه می‌شدند. تحلیل ساختار سلولی نشان داد که در گونه *L. bata*، سلول‌های β معمولاً به‌صورت تجمعی آرایش داشتند در حالی‌که سلول‌های α اغلب بین رگ‌های خونی واقع شده بودند. در گونه‌های *S. aor* و *C. chitala* سلول‌های گرد و بیضی شکل α معمولاً به‌صورت تجمعی یا پراکنده در اطراف جزایر لانگرهانس آرایش داشتند و سلول‌های β دانه‌دانه مانند متراکم که به‌طور معمول با رنگ‌های آلدئید فوشین، رومیز آزان و تریپل مالوری رنگ آمیزی می‌شوند، در ناحیه میانی جزایر لانگرهانس و در اتصال با رگ‌های خونی مشاهده شدند. سلول‌های شبیه δ نیز به‌تعداد اندک و آمیخته با سلول‌های β سیتوپلاسمی یافت شدند و گرانول‌های سیتوپلاسمی میانه را در گونه‌های *S. aor* و *C. chitala* نشان دادند. در ضمن در این مقاله به جای تاکید بیشتر بر تفاوت‌ها، طبیعت و عملکرد سلول‌های مختلف جزایر لانگرهانس مورد بحث قرار گرفت.

کلمات کلیدی: ساختار بافتی، غده‌ی درون ریز پانکراس، *Labeo bata*، *Sperata aor*، *Chitala chitala*.