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Full Length Research Paper

Comparative histological features of the pancreas in fruit-eating bat (*Eidolon-helvum)* and pangolin (*Manis tricuspis)*

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Mammals have varying histological features in their gastrointestinal tract to enable them cope with their dietary preferences. Fruit-eating bat (*Eidolon helvum*) is a frugivore while Pangolin *(Manis tricuspis*) is an insectivore, pancreases of the two animals were investigated histologically to know if the histological features of pancreas could have any implication on their dietary preferences. The animals were sacrificed by cervical dislocation and the pancreases of the two mammals were harvested. The pancreases were divided into three regions of head, body and tail and fixed in 10% formol saline for histological analysis. The sections were stained with routine Haematoxylin and Eosin and Halmi's modified Gomori Aldehyde Fuchsin method. The results revealed that the pancreas of the bat consisted of acini as well as islet of Langerhans but that of Pangolin contained only acini with no stainable islet of Langerhans. Despite being a mammal, pangolin still retains some primitive features like third tronchater and convex head. Absence of islet of Langerhans in the pancreas may be one of its primitive features and the islet of Langerhans may be located in other part of gastrointestinal tract higher up than the location of pancreas. Also, it is possible that pangolin may metabolize glucose via another pathway aside insulin/glucagon mechanism which may not yet be elucidated.

Key words: Pancreas, food preference, histological investigation, pangolin, bat.

INTRODUCTION

Pancreas is an organ found in every mammal. It is divid-ed into head, body and tail macroscopically. Histologi-cally, it is designated as a tubulo-alveolar gland, which is divided into lobes and lobules by extensions from the thin connective tissue capsule (Thurlo, 1937). It consists of three main structural components: the acini, the system of ducts and the islet of Langerhans.

The acini consist of a single row of pyramidal epithelial cells resting on a basement membrane and converging toward a central lumen in which the centro-acinar cell may be seen (Heath et al., 1999).

The ductular system of the pancreas which may be included in the centro-acinar cells, comprises the main excretory duct, the interlobular and the intralobular or intercalated ducts (Bertelli and Bendayan, 1997).

Islets of Langerhans consists of A and B (Bloom, 1931) as well as D cells (Thomas, 1982). Mankowski discover-ed a cell type in the pancreas which he called Mankowski cells (McMary, 1954). Ferner (1952) discovered X cells which was said to be the same as Mankowski cells (Berelli and Bendayan, 1997).

Pancreas was reported to develop from duodenum as a mass of undifferentiated cells (Berelli and Bendayan, 1997). Islet cells could originate from primitive cords (Hard, 1944). However Pearse (1972) postulated the

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Table 1. Findings with routine Haematoxylin and Eosin staining method (Luna 1968).

S/N	Pancreas	Bat (H)	Bat (B)	Bat (T)	Pangolin(H)	Pangolin(B)	Pangolin(T)
1.	ACINI	Present	Present	Present	Present	Present	Present
2.	MAIN EXCRETORY	Present	Present	Present	Present	Present	Present
3.	INTERLOBAR DUCT	Present	Present	Present	Present	Present	Present
4.	INTERCALATED DUCT	Present	Present	Present	Present	Present	Present
5.	ARTERY	Present	Present	Present	Present	Present	Present
6.	ATERIOLE	Present	Present	Present	Present	Present	Present
7.	VEIN	Present	Present	Present	Present	Present	Present
8.	VENULE	Present	Present	Present	Present	Present	Present
9.	ISLET OF LANGERHANS	Present	Present	Present	?	?	?

Keys: H - Head of pancreas; B - Body of pancreas; T - Tail of pancreas; ? - There was no islets observed.

Table 2. Findings with Gomori Aldehyde Fuchsin staining method.

S/N	Pancreas	Bat (H)	Bat (B)	Bat (T)	Pangolin(H)	Pangolin(B)	Pangolin(T)
1.	ISLET OF LANGERHANS	Present	Present	Present	Absent	Absent	Absent
2.	Cells	Present	Present	Present	Absent	Absent	Absent
3.	cells	Present	Present	Present	Absent	Absent	Absent

Keys: H - Head of pancreas; B - Body of pancreas; T - Tail of pancreas.

APUD theory by which is meant amine precursor uptake decarbohylase theory. It was believed that various pancreatic islet cells have a common origin i.e neural crest. It is known clinically that tumors of the pituitary, parathyroid, adrenal medulla and pancreas occur together as multiple adenoma syndromes. The basis of which has been APUD theory. The aim of this research is to comparatively examine the pancreases of the two mammals in view of their different food preferences. This will add to the known biology as well as the adaptability of the pancreases to their respective diets.

MATERIALS AND METHODS

Care of animals

We studied the pancreas sections from eight fruit-eating bats and eight pangolins of both sexes. The bats were harvested from their roosting colony in the Obafemi Awolowo University Campus Ile-Ife, Nigeria while the pangolins were procured and brought to the Animal Holdings of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife. The care and handling of the animals conform to the rules and guidelines of the animal right committee of Obafemi Awolowo University, Ile-Ife, Nigeria.

Sacrifice of animals and excision of pancreas

The Pancreases were harvested following the sacrifice of the animals by cervical dislocation and divided into head, body and tail.

Histological procedure

The excised tissues were fixed in 10% formol saline and processed for light microscopic study. These include dehydration through graded ethanol, clearing in xylene, infilteration in paraffin wax for 2 h at 56^{o}C and embedding of the tissues in paraffin wax for 48 h. Sections were then obtained on a rotary microtome at 5 µm thickness. The sections were finally subjected to Haematoxylin and Eosin stain (H and E) and Halmin's modified Gomori Aldehyde Fuchsin staining procedures.

RESULTS

Routine haematoxylin and eosin straining – (Luna, 1968)

Pangolin Pancreas (Table 1-2 and Figures 1-3)

i) Head has acini, main excretory duct as well as interlobar duct, intercalated duct, also artery, arteriole, vein and venule are present but islet of Langerhans was not observed.

ii). Body contains acini, main excretory duct, Interlobar duct, intercalated duct, artery, as well as arteriole, vein and venule are present but islet of Langerhans was not observed.

iii). Tail has acini, main excretory duct, interlobar duct, intercalated duct, artery, as well as arteriole, vein and venule are present but islet of Langerhans was not observed.

Figure 1. Head of Pancreas of Pangolin using H and E. A : Interlobar Duct; B : Acini X100.

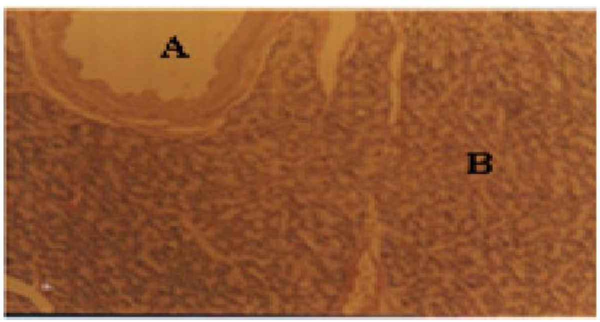

Figure 2. Body of Pancreas of Pangolin using H and E. A: Interlobar Duct; B: Acini X100.

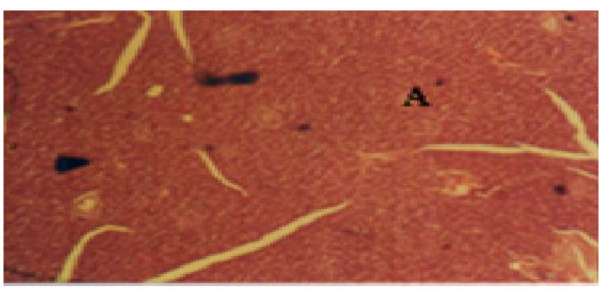

Figure 3. Tail of Pancreas of Pangolin. using H and E. A: Acini. X100.

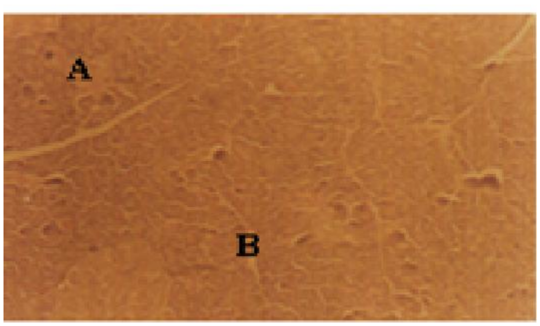

Figure 4. Head of Pancreas of Bat Using H and E. A: Islet of Langerhans; B: Acini X100.

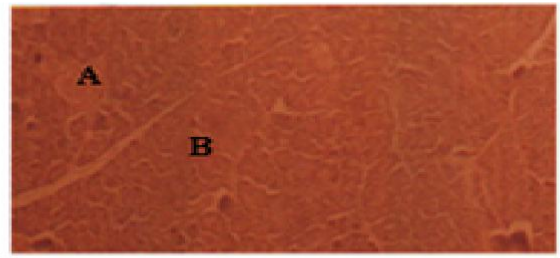

Figure 5. Body of Pancreas of Bat using H and E. A: Islet of Langerhans; B: Acini X100.

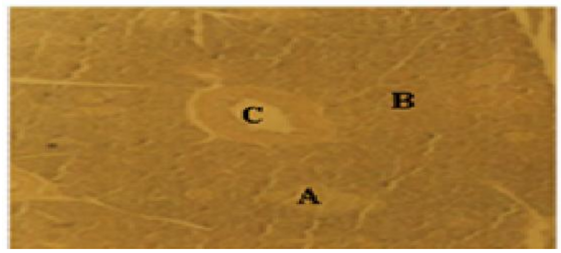

Figure 6. Tail of Pancreas of Bat using H and E A: Islet of Langerhans. B: Acini; C: Interlobar Duct X100.

Bat pancreas (Figures 4-6)

(i). Head has acini, main excretory duct as well as inter-lobar duct, intercalated duct; also artery, arteriole, vein, venule and islet of Langerhans are present.
ii). Body consists of acini, main excretory duct, and inter-lobar duct, also present within it are intercalated duct, artery, arteriole, vein as well as venule and Islet of Langerhans.
iii). Tail contains acini, main excretory duct, interlobar duct with intercalated duct, artery, and arteriole while vein, venule and Islet of Langerhans are parts of its features.

Modified Gomori Aldehyde Fuchsin method (Halmi, 1952)

Pangolin Pancreas (Figures 7-9)

i) Head did not have Islet of Langerans, alpha cells and beta cells.
ii) Body had no Islet of Langerhans, alpha cells and beta cells.
iii) Tail had none of Islet of Langerhans, alpha cells nor beta cells.

Bat pancreas (Figures 10-12)

i) Head contains Islet of Langerhans, alpha cells and beta cells.

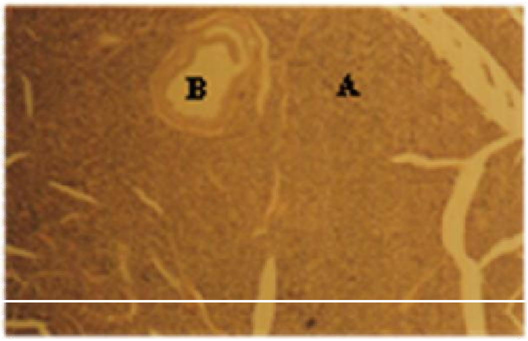

Figure 7. Head of Pancreas of Pangolin using Modified Gomori Aldehyde Fuchsin method. A: Acini; B: Interlobar Duct X100.

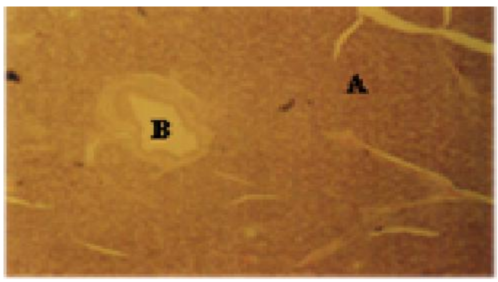

Figure 8. Body of Pancreas of Pangolin using modified Gomori Aldehydefuchsin method. A: Acini; B:Interlobar Duct X100.

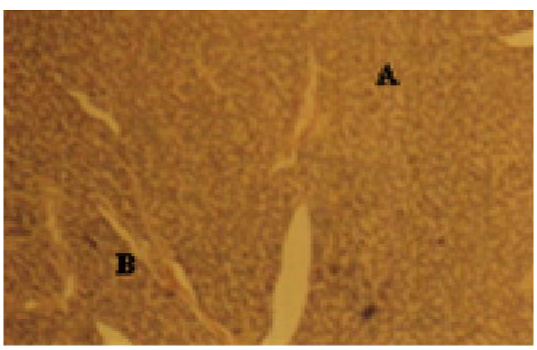

Figure 9. Tail of Pancreas of Pangolin using modified Gomori Aldehyde Fuchsin method. A: Acini; B: Interlobular Duct X100.

ii) Body has Islet of Langerhans, alpha cells and beta cells.
iii) Tail consists of Islet of Langerhans, alpha cells and beta cells.

DISCUSSION

In this investigation, following haematoxylin and eosin

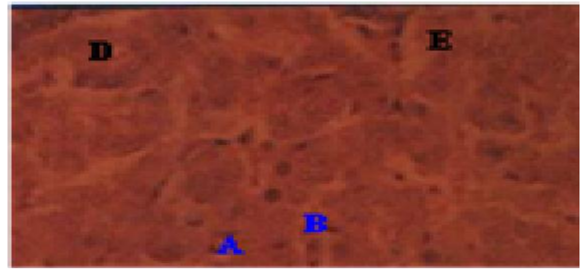

Figure 10. Head of Pancreas of Bat using Gomori Aldehyde Fuchsin method. D: Islet of Langerhans E: Acini; B: Beta cell; A: Alpha cell X400.

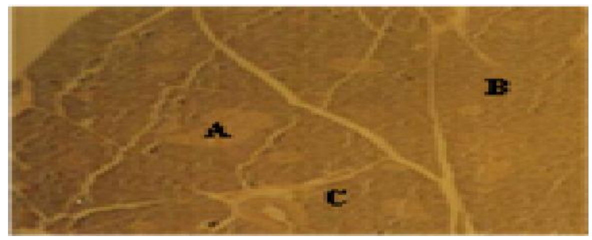

Figure 11. Body of Pancreas of Bat using modified Gomori Aldehyde Fuchsin method. A: Islet of Langerhans; B: Acini C: Interlobar Duct X100.

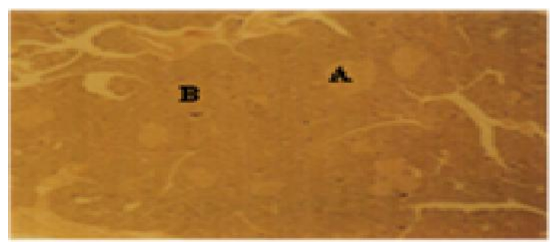

Figure 12. Tail of Pancreas of Bat using modified Gomori Aldehyde Fuchsin Method. A: Islet of Langerhans; B: Acini X100.

(Luna, 1968) staining the bat pancreas exhibited features comparable with that of other mammals (Thomas, 1982), but there was no stainable evidence for the presence of islet of Langerhans in the pangolin pancreas (Figures 1-3). This was also confirmed with the modified Gomori Aldehyde Fuchsin (Halmi, 1952) confirming our earlier observation in the Luna's method (Figures 7 - 9). Other pancreatic features as reported earlier (Jorge and Hector, 1998) confirmed that the head, body and tail of pancreas of bat consist of acini and islet of Langerhans which is in agreement with present work (Figures 4-6; 10-12).

The microanatomical features of the head, body and tail of pancreas of pangolin, an insectivore showed the presence of acini with no stainable islet of Langerhans by both Haemotoxylin and Eosin and modified Gomori Aldehyde Fuchsin method (Halmi, 1952) . Whereas, the previous report on the pancreases of snakes and lizards which are insectivores showed that they consist of acini and islet of Langerhans (Moscona, 1990) . In the advanced snakes like varasus, the islet cells are found within the spleen (Moscona, 1990).

Also, the islet cells in the pancreas of pangolin may not be in clusters or encapsulated. They may be scattered like erythropoietin producing cells in human kidney (de Bruijin et al., 2007) . The erythropoietin producing cells in human are in form of intra-renal scattered cells (de Bruijin et al., 2007). The acini may also be separated entirely from the islet of Langerhans as in the advanced snake like Varasus where islet cells are found within the spleen (Moscona, 1990). Also in the adrenal gland of fish the adrenal medulla is entirely separated from the adrenal cortex (Chandrasekar et al., 2007). In the adrenal gland of reptiles e.g alligator, the adrenal medulla is also separated from adrenal cortex (Morici et al., 1997). The two parts are not the same entity in Man.

In the evolution trend, the adrenal glands of fish (Chandrasekar et al., 2007) and reptile (Morici et al., 1997) do not have cortex and medulla as the same entity. Since the pangolin has some primitive features like third trochanter and convex head, (Decher, 2006), the separation of islet cells from the acini may also be one of its primitive features.

Pearse (1972) postulated that the islet cells might originate from the neural crest. It is possible that during the migration from the neural crest, the cells could migrate to any organ. Also, chromaffin cells which are derivatives of neural crest cells are distributed at various areas in the gastro- intestinal tract. It is possible that, islet of Langerhans may also be located at other parts of gastro-intestinal tract higher than the location of pancreas in pangolin. This may be another feature of primitivity of pangolin. Moreover, as islet cells are found in the spleen of Varasus (Moscona, 1990), the islet cells in the pangolin also could have migrated to any organ other than pancreas.

Islets of Langerhans constitute the endocrine portion of the vertebrate pancreas on which beta and alpha cells are usually located. Beta cells are involved in the production, storage and secretion of insulin and alpha cells are for the production, storage and secretion of glucagon. Insulin and glucagon are concerned with the regulation of carbohydrate metabolism.

Gomori reported that aldehyde fuchsin stained the granules of pancreatic islet beta cells selectively and without needing permanganate pretreatment (Halmi, 1952). Others adopted permanganate oxidation because it makes stained faster though much less selective (Mowry and Kent, 1988).

The unstainability of the islet of Langerhans using the methods reported may suggest that the pangolin may not regulate carbohydrate metabolism via insulin/glucagon mechanism. Insulin causes most of the body's cells to take up glucose from the blood (including liver, muscle, and fat tissue cells), storing it as glycogen in the liver and muscle, and stops use of fat as an energy source. When it is absent (or low) glucose is not taken up by most body cells and the body begins to use fat as an energy source. Glucagon is an important hormone involved in carbohydrate metabolism. Produced by the pancreas, it is released when the glucose level in the blood is low (hypoglycemia), causing the liver to convert stored glycogen into glucose and release it into the bloodstream. The action of glucagon is thus opposite to that of insulin. It may be possible that carbohydrate metabolism in pangolin is mediated via other mechanism not yet elaborated.

Also, islet cells may be in other organs of pangolin other than pancreas as seen in Varasus where islet cells are in spleen (Moscona, 1990). Other organs like spleen, liver or kidney and other parts of gastro-intestinal tract will have to be investigated to find out if islets of Langerhans are located there. It can be inferred from the present study that there are some microanatomical features in the pancreases of the two mammals adopted to cope with their food preferences. However, more research is still needed to corroborate this investigation.

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