

A Gross and Histomorphologic Study of Chick Pancreas

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ABSTRACT

Background: Pancreas is a very vital organ because it suffers from two main diseases: diabetes mellitus and pancreatic cancer. The avian pancreas shares some similarities with mammals. By having sound knowledge of the anatomy of chick pancreas, different studies can be performed to know the pathogenesis and treatment of these diseases. Therefore, the present research was conducted to study the gross anatomy of pancreas and histo-morphological distribution of cells responsible for release of glucagon & insulin.

Methodology:

This study was conducted on 28 newly hatched chicks. The chicks were dissected on hatching. The pancreata of chicks were dissected out. The gross features of pancreas were recorded in terms of appearance, color, shape and number of lobes along with their orientation. For general histology, sections were stained with haematoxylin and eosin (H & E), and for pattern of distribution of cells by aldehyde fuchsin and orange G-light green as described by Halmi.

Results:

This study showed that chick pancreas is composed of four distinct morphological lobes. The glandular tissue was subdivided into exocrine part and endocrine part similar to human. The pattern of distribution of cells by aldehyde fuchsin and orange G-light green method showed that the exocrine part formed acini and endocrine part formed islets that were further subdivided into alpha, beta and mixed types. The distribution of islets in different pancreatic lobes was not uniform. The alpha islets were found almost exclusively in the 'splenic' and 'third' lobes whereas beta islets were evenly distributed throughout the pancreas.

Conclusion:

The chick pancreas showed many similarities with the pancreas of mammals so it can be used as an effective model for different studies in medical field.

INTRODUCTION

Herophilus (335–280 BC) was a Greek surgeon and anatomist who first identified the pancreas.¹ Later, after a few hundred years, Ruphos was another Greek anatomist who gave the pancreas its name. The word "pancreas" is derived from the Greek 'pan, πᾶν ("all", "whole"), and 'kreas' κρέας ("flesh") – perhaps because of its fleshy texture.

The pancreas is a large dual-function gland, having both exocrine and endocrine components. It plays an important role in the digestive and endocrine systems of vertebrates. The main bulk of the gland is formed by exocrine component. It secretes an alkaline fluid into the duodenum through the pancreatic ducts which is rich in enzymes. The endocrine part which although forms very little part of the

gland, but secretes several imperative proteinaceous hormones including insulin, glucagon and somatostatin into the circulation.

The whole avian pancreas is approximately 0.1% of the total body weight but only 1-2% of pancreatic tissue is the endocrine pancreas, the other 98% being the exocrine pancreas.² Pancreas of chicken resembles that of mammals regarding cell cytology.³

In human medicine, the pancreas is a very vital organ because many diseases of pancreas impair patients' quality of life and increase the mortality. These include diabetes mellitus, chronic pancreatitis and pancreatic cancer.⁴ The avian pancreas shares some similarities with mammals.³ By having sound knowledge of the anatomy of chick pancreas, different studies can be performed on the pancreas of chick

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In human medicine, the pancreas is a very vital organ because many diseases of pancreas impair patients' quality of life and increase the mortality. These include diabetes mellitus, chronic pancreatitis and pancreatic cancer.⁴ The avian pancreas shares some similarities with mammals.³ By having sound knowledge of the anatomy of chick pancreas, different studies can be performed on the pancreas of chick to know the pathogenesis and treatment of these diseases.

MATERIALS AND METHODS

The study was conducted on 28 newly hatched chicks. The weights of newly hatched chicks were 38.92 ± 0.049 g. All the newly hatched chicks were euthanized by exposing them to chloroform in large closed glass jar. Prior to dissection, chicks were soaked thoroughly with water, to avoid the mess of flying feathers and interference during dissection. To locate the pancreas, a ventral midline incision was given on the skin, extending from neck to cloaca (Fig. 1).



Figure 1: Line of incision to dissect out the pancreas. Chick was soaked with water; a ventral midline incision was given on the skin (dotted line) to expose the underlying structures.

The duodenal loop was found vertically placed in abdominal cavity. It enclosed the pancreas (Fig.2).

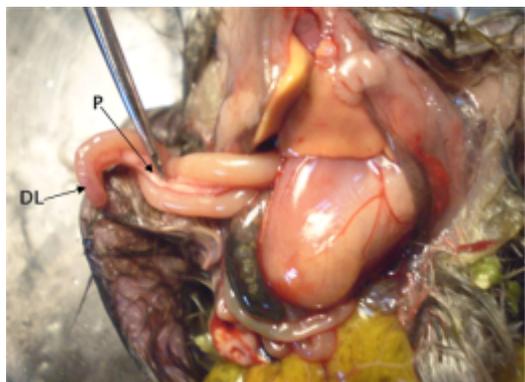


Figure 2: Dorsal view of Duodenal loop (DL) showing two lobes of pancreas (P). Pancreas was dissected out in total along with the duodenal loop for fixation.

The gross features of pancreas were recorded in terms of appearance, color, shape, and number of lobes along with their orientation. For histological studies, the pancreas was removed in total along with the duodenal loop and immersed in formol saline for 24-36 hours. The fixed pancreata were trimmed out of duodenal loop and then processed for paraffin embedding. After making paraffin blocks, serial transverse sections of pancreas at right angle to its long axis, were cut at $7\mu\text{m}$ thickness by rotary microtome to study its histology.

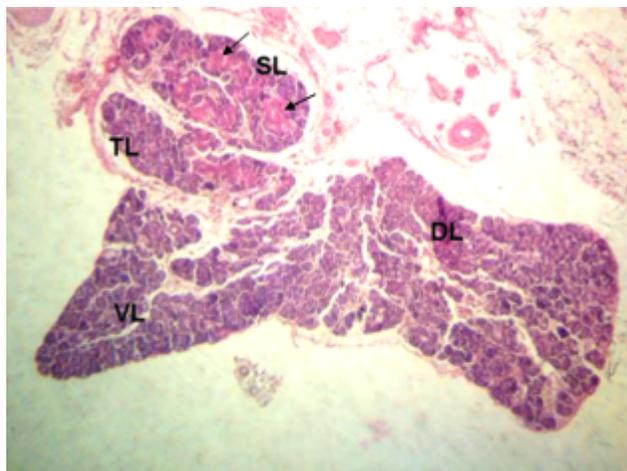
Serial sections were divided in sets of seven sections each. Each set contained sections, numbered from 1 to 7. Section 1 and 2 were sampled on a separate glass slide and stained by haematoxylin and eosin (H & E) for general histology. Sections from 3 to 7 were sampled on a separate glass slide and stained by aldehyde fuchsin and orange G-light green as described by Halmi (1952)⁵ for demonstration of alpha/A, beta/B and delta/D cells distinctly. By this method A cells would be stained yellow, B cells purple and D cells would neither be stained orange nor purple. These sections were observed under 40X objective magnification, because at this magnification all islets per section can be observed easily.

The pattern of distribution of each type of cells was studied per section of pancreas lobe wise and recorded.

RESULT

On gross examination, the chick pancreas was found to be a discrete lobular structure located in duodenal loop in right side of abdominal cavity. It was soft, pink, elongated and tongue shaped gland having well defined clefts and morphological folds. Four morphologically distinct lobes were identified. Three lobes were large whereas fourth lobe extended as a small strip from one of the large lobes upto the hilum of spleen. The first lobe was situated towards the ascending duodenum ventral to duodenal loop. The second lobe was situated towards the descending duodenum, dorsal to duodenal loop. The third lobe extended along the dorsal surface of the ascending duodenum to the spleen. The fourth smaller lobe extended dorsal to the spleen from the cranial part of the third lobe. Each large lobe had a discrete pancreatic duct which entered the duodenum at the distal part of the ascending part of the duodenum.

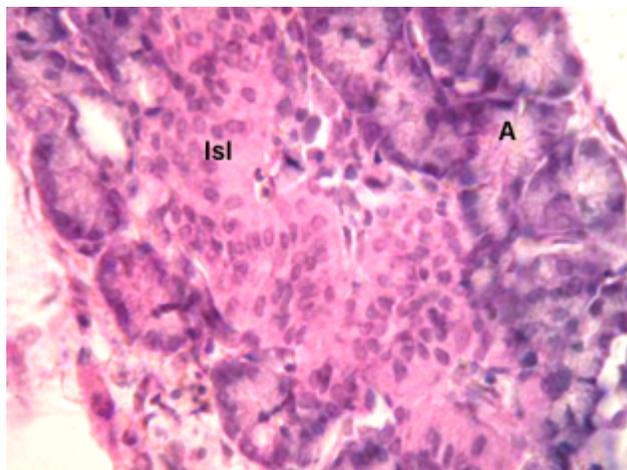
In H & E stained sections, it was observed that whole gland is enveloped in a thin connective tissue capsule (Fig. 3).



Haematoxylin and Eosin stain

Figure 3: Transverse section of pancreas of the newly hatched chick showing four lobes: dorsal lobe (DL), ventral lobe (VL), third lobe (TL) and splenic lobe (SL). Arrows are pointing to the Islets of Langerhans in splenic lobe and basal part of third lobe.

There were two quite different types of glandular tissue. The exocrine pancreas had glandular structure. The glands are branched acinar and arranged in the form of lobules. Their secretory cells were pyramidal in shape with basal nuclei and contain many secretory (zymogen) granules (Fig. 4).



Haematoxylin and Eosin stain

Figure 4: Transverse section of the pancreas of chick showing an Islets of Langerhans (Isl) surrounded by pancreatic acinar (A) cells. The islet cells form cords.

An extremely thin layer of loose connective tissue surrounded the acini, which becomes more defined around the larger ducts. Centrally within the connective tissue among lobes, the pancreatic arteries and veins coursed throughout the pancreas up to the apex of the

duodenal loop. The endocrine cells were mainly grouped into the pancreatic Islets of Langerhans, recognized as pale staining areas. The endocrine cells of islets were arranged in clumps and cords between which were found capillary networks and a very little amount of connective tissue fibers. A thin connective tissue capsule separated these areas from the exocrine serous acini. In special stained sections by Halmi, there were three principal types of endocrine cell distinguished histochemically. One type of cells was recognized as cells with rounded nuclei and orange/yellow stained cytoplasm. These were labeled as 'A' cells. The second type of cells was identified as cells with rounded nuclei and pink staining cytoplasm containing discrete purple stained granules of insulin. These were labeled as 'B' cells. Third type of cells was with elongated nuclei and their cytoplasm neither stained orange nor had purple granules. These were D cells. Likewise, three types of islets were identified. One type of islets that was comparatively of larger size was orange stained. These were labeled as alpha islets (Fig. 5).

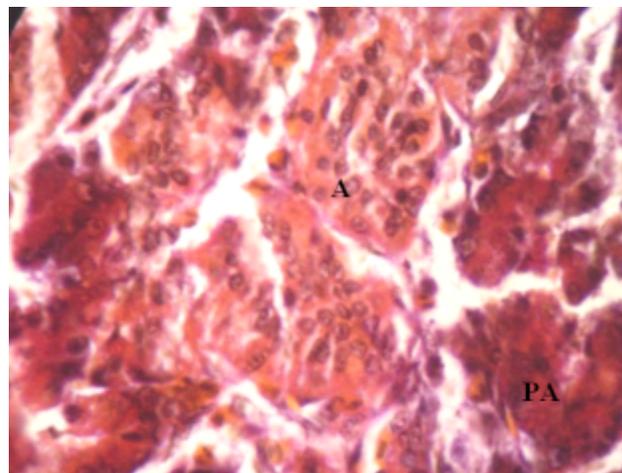
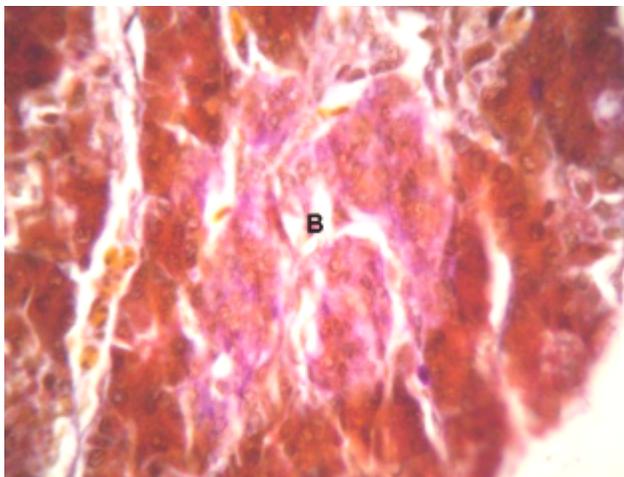


Figure 5: Transverse section of splenic lobe of pancreas of chick showing central part occupied by alpha islet (A), surrounded by pancreatic acinar cells (PA).

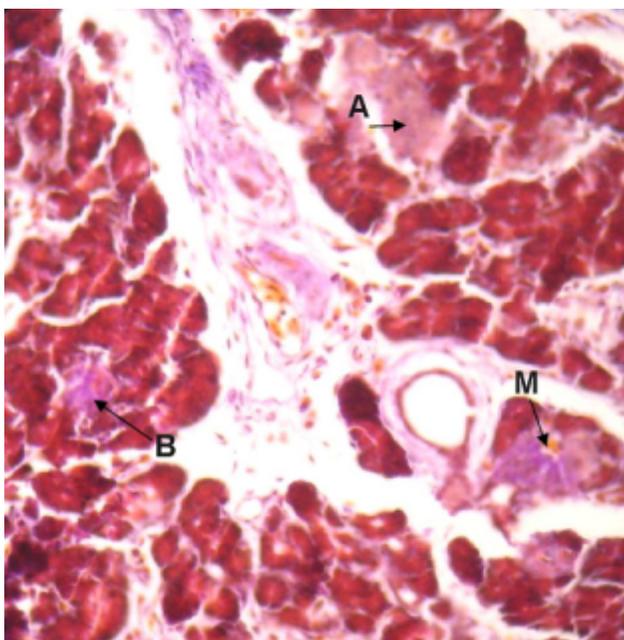
Second type of islet of small size was purple stained. These were labeled as beta islets (Fig. 6).



Aldehyde fuchsin and Orange G-light green

Figure 6: Transverse section of splenic lobe of pancreas of chick showing beta islet (B) surrounded by pancreatic acinar cells.

The third type of islets which was of medium size had almost 50% orange stained cells and 50% purple stained cells. These were labeled as mixed islets (Fig. 7).

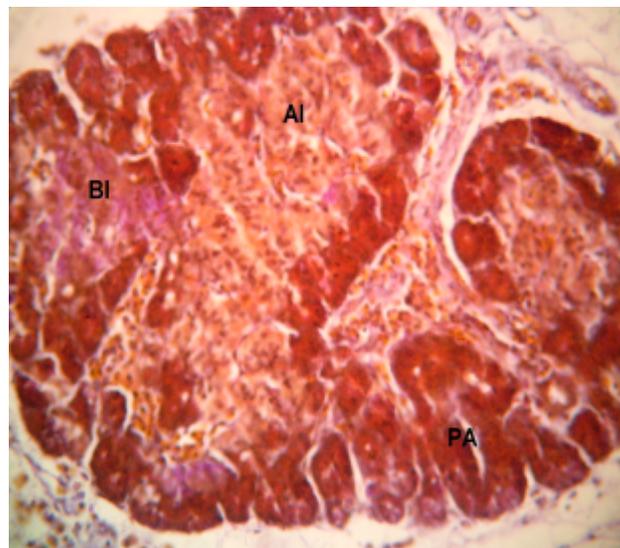


Aldehyde fuchsin and Orange G-light green

Figure 7: Transverse section of pancreas of the chick showing three types of islets: alpha islets (A) orange stained cells, beta islets (B) purple stained cells, mixed islets (M) having both types of cells surrounded by pancreatic acinar cells.

A cells (glucagon secreting) were the predominant cell type found in alpha islets with some B and D cells, whereas the

beta islets have B (insulin) cells as the predominant cells with occasional D and sometimes A cells. The distribution of islets in different pancreatic lobes was not uniform. The alpha islets were found almost exclusively in the 'splenic' and 'third' lobes. The alpha islets of splenic lobe were conspicuously larger, irregular in shape, ramified complicatedly or fused with each other, so appeared to have belt-like distribution, and continued in third lobe. These were surrounded by many beta islets of small size (Fig. 8).



Aldehyde fuchsin and Orange G-light green

Figure 8: Transverse section of splenic lobe of pancreas of chick showing central part occupied by large alpha islets (AI) surrounded by pancreatic acinar cells (PA), beta islets (BI) at the periphery of alpha islets and arrows pointing to small beta islets.

Those in the third lobe were small and restricted to its basal part. Beta islets were spherical or oval in shape, relatively fewer, smaller and were distributed throughout the pancreas. However, those found in splenic lobe were rather large. In all the lobes of pancreas, B cells were smaller in size except in the splenic lobe where B cells are comparatively larger in the beta islets. A special feature that was found was that A cells were in larger proportion than other two cell types.

DISCUSSION

Avian small intestine consists of three parts; duodenum, jejunum and ileum, that are not always clearly recognizable. Duodenum is the first part of small intestine and it forms a loop called 'ansa duodeni', with descending part called 'pars descendens' and an ascending part called 'pars ascendens'. Ansa duodeni points caudally and is located at the ventral side of the body cavity. It encloses the pancreas.⁶ In all birds the placement of avian pancreas in the abdominal cavity is on the front of right side.⁷ This is in accordance with location of pancreas in our study. Regarding the pancreatic lobes and their location, a previous study has shown that the pancreas of domestic fowl generally consists of four discrete lobes. According to this study, three lobes were of large size and they named them dorsal, ventral and third lobes whereas the fourth lobe was of smaller size named as splenic lobe. It extends towards the hilum of spleen as a small protrusion from the 'third' lobe. 'Ventral lobe' lies ventrally along the pars ascendens. 'Dorsal lobe' lies dorsally along the pars descendens. Third lobe lies dorsally along the pars ascendens.⁸ The number and arrangement of the lobes of pancreas found in the current study agrees with the results of previous studies. However, some authors believed that ventral lobe has a longitudinal cleft running throughout its length giving the appearance of two separate lobes. The central portion of this, closest to dorsal lobe, is sometimes confusingly referred to as the third lobe.²

Dorsal, ventral and third lobes have the exocrine pancreatic ducts, whereas the splenic lobe apparently does not have an exocrine duct.⁹ The ducts of these lobes open at duodenal papilla with the hepatic ducts. According to most researchers, this point of entrance of these ducts signifies the site of junction of duodenum with jejunum.⁹ These findings are similar to our study.

Previous studies have shown that the avian pancreas is invested by thin loose collagen capsule. Although the pancreas is lobular in appearance, very little connective tissue is present in the form of septa within the parenchymal tissue.^{3,9} Pancreatic artery supplies blood to pancreas and pancreatic veins take the blood away. Both lie centrally within the connective tissue. These course the entire length of the pancreas caudally, to the apex of the duodenal loop. These findings correlate with our findings. In addition, it has also been described in a previous study that the pancreaticoduodenal vein also drain pancreas, which then empties into the gastroduodenal vein, which in

turn joins the hepatic portal vein near the hilus of liver.⁹

Previous studies related to histology of pancreas have shown that within the pancreas, its exocrine and endocrine components are arranged separately.¹⁰ The cells of exocrine component are arranged in the form of closely packed tubuloacinar glands (acini), that ultimately drain into ducts that are highly branched.⁹ The epithelial cells are columnar to truncated pyramids, radially oriented about the gland circumference. The acinar cells have basal nuclei, reticular endothelium, Golgi complex, and zymogen granules filled with digestive enzymes. Due to presence of basal rough endoplasmic reticulum, the pyramidal acinar cells appeared bizonal structures; apical acidophilic cytoplasm and basal basophilic cytoplasm.¹¹ The cells of endocrine component are arranged in the form of cellular clusters and cords which can only be seen microscopically. These are named as the 'Islets of Langerhans', after their discoverer. The cells in the islets are richly supplied by a network of capillaries. These islets are of various sizes and scattered throughout the exocrine tissue. Under light microscope these appear as lightly staining clusters of cells surrounded by darker staining acini. Different types of cells can be differentiated within the islets. These are A cells capable of secreting glucagon, B cells secreting insulin and D cells secreting somatostatin.^{2,3,6,8,9} We also found in our study that exocrine and endocrine part of pancreas have the same histologic features as described in previous studies. However, one of the studies also shown an additional type of cells named PP or 'F' cells. These are endocrinal cells and secrete avian pancreatic polypeptide (APP). These cells spread throughout the exocrine pancreas and do not contribute in the formation of islets. The avian pancreatic polypeptide was first discovered in the domestic fowl by Kimmell, Pollock and Hazelwood in 1968, and was subsequently found in mammals also.²

There is a slight difference in histologic structure of the pancreatic islets of chicken and mammals. In chick, on the basis of predominance of cellular type in each islet, these are named as alpha (or dark islet), beta (or light islet) and mixed type islets.¹² The predominant cell type found in alpha islet is A cells (glucagon secreting) with some B and D cells, whereas the beta islets have B (insulin) cells as the predominant cells with occasional D and sometimes A cells.^{2,3,6,8,9} These findings are exactly in line with the finding in our study.

Different studies in the past have shown that in chicken and galliform birds the distribution of islets among

different pancreatic lobes is not uniform. The alpha islets are found almost exclusively in the 'splenic' and 'third' lobes whereas the beta islets are distributed throughout the pancreas. The alpha islets of splenic lobe are conspicuously large, irregular in shape, ramify complicatedly or fuse with each other so appear to have belt-like distribution and continue in third lobe. These are surrounded by many beta islets of small size. Those in the third lobe are small and restricted to its basal part. Beta islets are relatively small and spherical or oval in shape, those found in splenic lobe are rather large, but small and few in other lobes. In addition, the size of B cells that are present in the beta islets of splenic lobe is also very large as compared with the B cells of other lobes. This results in a 10-40 times increase in concentration of insulin in the splenic lobe than in other lobes. A special feature of avian species is that A cells are found in larger proportion than other two cellular types.⁸ These results are consistent with the findings in our study.

CONCLUSION

The chick pancreas showed many similarities with the pancreas of mammals so it can be used as an effective model for different studies in medical field.

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