African Journal of Biotechnology Vol. 9(42), pp. 7209-7215, 18 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.934
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Morphological study of pancreatic duct in red jungle fowl

Khalid K. Kadhim[1], A. B. Z. Zuki[1]*, M. M. Noordin[2] and S. M. A. Babjee[2] and Zamri-Saad, M[2]

[1]Department of Veterinary Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor.
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor.

Accepted 3 September, 2010

Morphological and histochemical study of the pancreas and pancreatic ducts of ten adult red jungle fowl (*Gallus gallus spadiceus*) were carried out by means of light microscopy. The bulk of the pancreas consists of a dorsal, ventral, third and small splenic lobe. Three pancreatic ducts were recognized as they join the proximal end of the ascending duodenum. The exocrine units were composed of main pancreatic tissues, while the endocrine units were observed frequently in the third and splenic lobes. A single layer of pyramidal cells with acidophilic zymogen granules and small centro-acinar cells were formed in the exocrine acinus. The intercalated duct was lined by flattened epithelium which changed to cuboidal in the intralobular duct, while the interlobular and main pancreatic ducts were lined by simple columnar epithelium. Three types of cells were detected within the surface epithelium: principal, light and basal cells. These cells possess short luminal projections with a fuzzy surface coat. Apical neutral and sulfomucin reaction indicated involvement of these epithelial with secretion. Neither goblet cells nor ductal glands were found in the pancreatic ducts. Secretion of both neutral and sulfated materials by the epithelial lining the pancreatic ducts, suggesting that they are acting not only to facilitate the transport of the pancreatic juice, but also as a protective barrier to protect the gland from autodigestion.

Key words: Red jungle, morphological study, pancreatic duct, histochemical, mucin.

INTRODUCTION

The red jungle fowl (RJF; *Gallus gallus*) is a tropical member of the pheasant family. *G. gallus spadiceus* is the only subspecie that inhabits Peninsular Malaysia (Nishida et al., 1992; Lee and Amin-Babjee, 1993). According to the Handbook of Avian Anatomy: Nomina Anatomica Avium (Baumel et al., 1993), the pancreas of the birds is considered to have four lobes: ventral, dorsal, third and splenic; with three ducts: ventral, dorsal and third, as described in chicken and quail. In the duck, the bulk of the pancreas consists of a dorsal, ventral and a small splenic lobe with three pancreatic ducts (Liu et al., 1998). The morphologic studies of the pancreas is mainly focused on acinar and islet cells, with less attention given to the ductal epithelium. However, Yoshizawa (1978), Madden and Sarras (1989) and Motta et al. (1997) have described the pancreatic duct for several mammalian species, while histo-chemical analysis of the pancreatic ductal cells in mammalian species were studied by McMinn and Kugler (1961) and Kendrey and Roe (1969). Pancreatic ducts from the acini to the point where it empties its contents into the duodenum are arranged in the following order: intercalated ducts (the smallest in size), intralobular ducts, interlobular ducts and pancreatic ducts (main ducts) (Baumel, 1993). The pancreatic ductal epitheliums have revealed a number of specialized cell types that are different according to the species (Weyrauch and Schnorr, 1976). The role of the ductal epithelium in electrolytes transport, macromolecular

*Corresponding author. E-mail: zuki@vet.upm.edu.my. Fax: 603-89468333.

Abbreviations: PAS, Periodic acid-schiff; **HE,** haematoxylin and eosin stain.

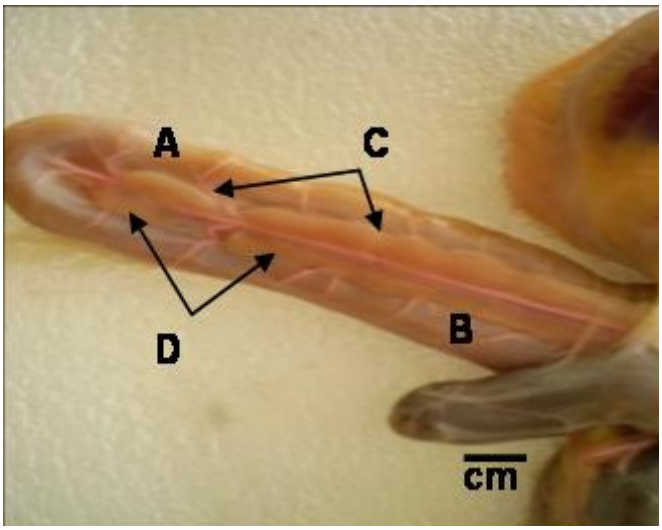

Figure 1. The pancreas fills the gap between A, descending and B, ascending duodenum; C, dorsal lobe. D, ventral lobe.

secretion and the pathologic conditions of the pancreas, opens questions as to the cell types involved and the organelles related to these processes (Madden and Sarras, 1989).

There is little information available concerning the morphology of the pancreatic duct of birds in general and fowl in particular. Therefore, this study focuses on the histological and histochemical features of the pancreatic ductal system in the red jungle fowl.

MATERIALS AND METHODS

Animals

In the present study, ten adult male RJF were used. Animals were obtained from the stock reared at the University Putra Malaysia (UPM) farm. The birds were reared in small cages (five birds in each cage) with feed and water provided for *ad libitum* consumption. The RJF descended from stock that agree with wild RJF, and differ from domestic chickens, in all eight characters that sensitively differentiate between most wild RJF and domestic chickens (Jackson and Diamond, 1996).

Collection of tissue samples

All birds were euthanized by intravenous (cutaneous ulnar vein) administration of sodium pentobarbitone (80 mg/kg) (Mitchell and Smith, 1991). A mid-ventral line incision was made to expose the digestive organs. Collected specimens included the whole pancreas and the main pancreatic ducts. These organs were washed with saline solution to remove blood and any other adhering debris. The main pancreatic ducts were viewed *in situ* by Nikon stereomicroscope image analysis (SMZ1500, with an attached DS-F1 digital camera).

The isolated main pancreatic ducts were embedded vertically in paraffin wax. Serial sections of 3 µm thick were cut. The histological and histochemical methods of staining were employed as follows: Harris Haematoxylin and Eosin (HE) and Masson trichrome stain (Masson, 1929); Modified aldehyde fuchsin method for elastic fibers (Bancroft and Gamble, 2002). Meanwhile, the characteristics of mucins secreted by the epithelial mucous cells were obtained using a series of histochemical tests. The periodic acid-schiff (PAS) (McManus, 1946) was performed for the presence of mucin. The acid mucins was by alcian blue (AB) pH 1.0 and 2.5 (Bancroft and Gamble, 2002) for the strong and weak acid mucins, and the AB pH 2.5 in combination with the PAS technique for neutral and acid mucins (Bancroft and Gamble, 2002); the combined aldehyde fuchsin-alcian blue pH 2.5 method was used to differentiate between sulphated and carboxylated mucins (Spicer and Myer, 1960).

All stained slides were viewed under an Olympus image analysis (BX 51 TF, with attached CC 12 camera).

RESULTS

Macroscopic findings

Grossly, the pancreas appeared as a pale pinkish and ribbon-like organ, located between the ascending and descending limbs of the duodenum and enclosed with the pancreatico-duodenal ligament. The bulk of the pancreas consists of a dorsal, ventral and small thin lobe that extends toward the spleen and thus called the splenic lobe. However, the subdivision of the ventral lobe was found as the third lobe which is the delicate parenchymal bridges connecting the dorsal and ventral lobes. The dorsal and ventral lobes of the pancreas completely fill the gap between the duodenal limbs (Figure 1). There were three pancreatic ducts recognized as they join the proximal end of the ascending duodenal limb, two of which arise from the ventral lobe and its subdivision (third lobe) as the ventral and third pancreatic ducts, respectively, and one from the dorsal lobe as a dorsal pancreatic duct. These ducts empty into the duodenum closely with both *ductus hepatoentericus* and *ductus cysticoentericus* from the liver and gall bladder, respectively (Figure 2).

Microscopic findings

The pancreas is covered by a thin layer of loose connective tissue capsule, from which septa extend to divide the gland into lobules. Blood and lymph vessels, nerves and excretory ducts run within the connective tissue septa. The gland was mainly composed of exocrine units. These glandular units were formed by pyramidal cells disposed in a single layer and showing a polarized cytoplasm with acidophilic zymogenic granules and small centroacinar cells without granules were observed in the lumen of the acinus (Figure 6). The islets of Langerhans were not numerous and were irregular in shape; they were observed frequently in the third lobe, however, in the splenic lobe, the islets of Langerhans appeared more than that in the other lobes.

There were no differences in the histological features of the ducts of the pancreatic lobes; however, the duct of the third lobe was smaller in diameter than those of the

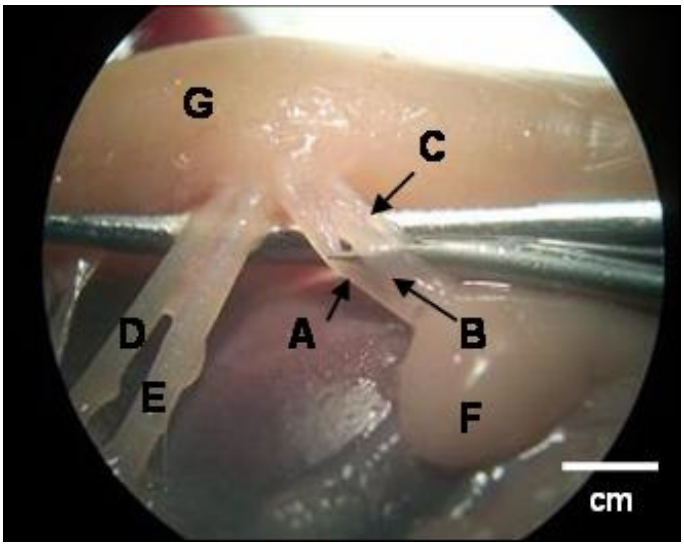

Figure 2. Pancreatic ducts. A, Dorsal pancreatic duct; B, ventral pancreatic duct; C, third pancreatic duct; D, *ductus cysticoentericus;* E, *ductus hepatoenterrici*; F, pancreas; G, duodenum. Bar, 20 mm.

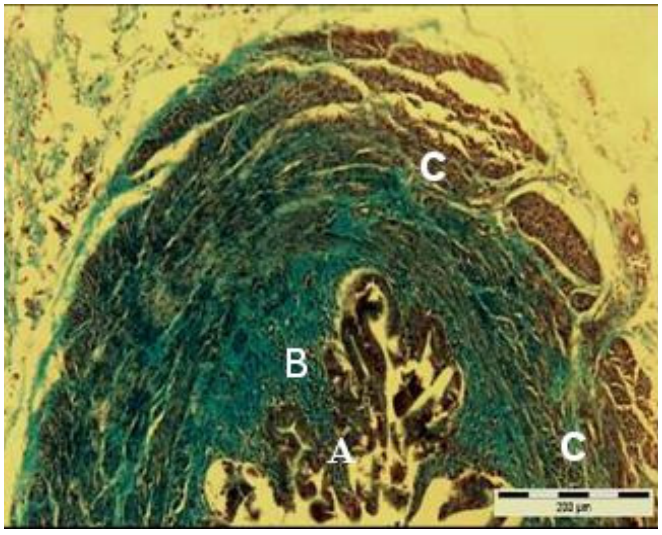

Figure 3. Pancreatic duct. A, Mucosal folds; B, connective tissues of lamina propria; C, muscular layers. Masson's Trichrome stains.

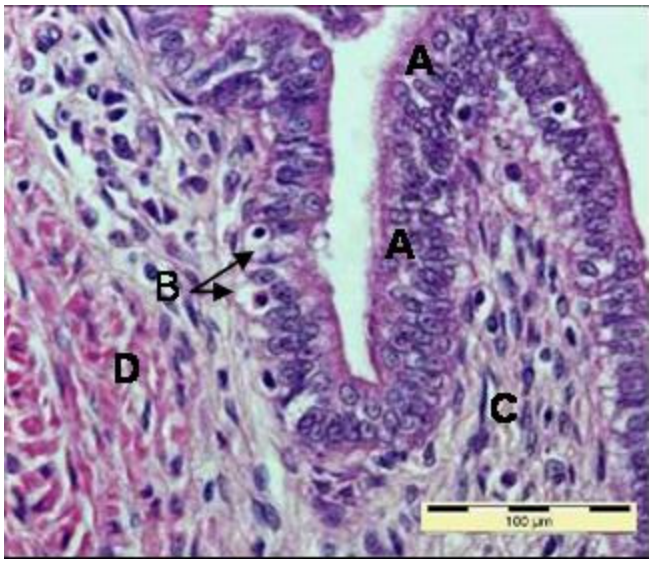

Figure 4. Epithelial cells lining the large pancreatic duct. A, Principle cells; B, light cells; C, *lamina propria*; D, muscular layers. HE.

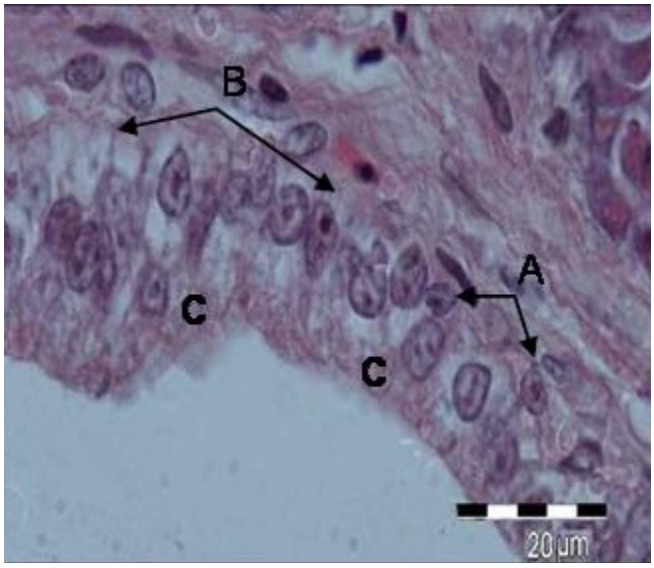

Figure 5. Epithelial cells lining the interlobular duct. A, Basal cell; B, basement membrane; C, principle cells. HE.

dorsal or ventral lobes. All pancreatic ducts and the interlobular ducts were lined by simple columnar epithelium, with folds, while the ducts lumen was filled with secretory material. At least three types of cells were recognized lining the epithelium: the dark principal, light and the basal cells (Figures 4 and 5). The principal cells make up majority of the ductal cells. These cells increased gradually from flattened or low cuboidal in the intercalated duct (Figure 7) to cuboidal in the intralobular duct, while they became high columnar cells in the interlobular and main ducts. The luminal surface of these cells had short apical plasma membrane projections (Figure 11). The light cell was rarely seen between the principal cells, but appeared more frequently in the main and interlobular ducts, and seemed absent in other segments. These cells had light cytoplasm and large rounded nuclei (Figure 4). The third type was the basal cell; these cells were few in number with relatively small dark nuclei and were seen between the epithelial cells and the basement membrane of the large ducts (Figure 5). The ductal epithelium had apical secretory granules, which were clearly observed even after treating sections with modified

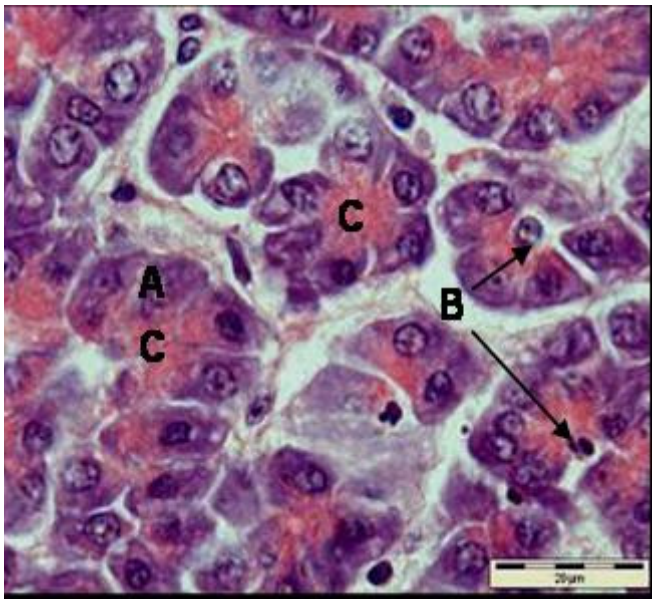

Figure 6. Exocrine pancreatic units. A, Acinar cells (pyramidal); B, centro-acinar cell; C, acidophilic zymogenic granules. HE.

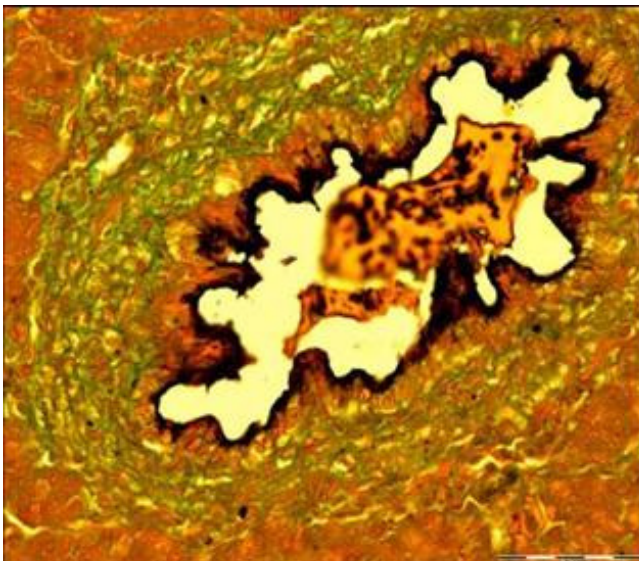

Figure 8. Intralobular duct show luminal surface with mucin reaction. Aldehyde fuchsin stain.

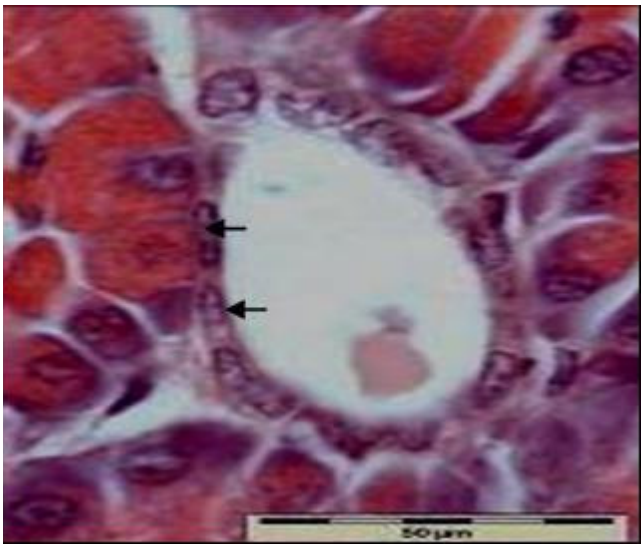

Figure 7. Intercalated duct. Note the flat epithelial cells lining the duct (arrows). HE.

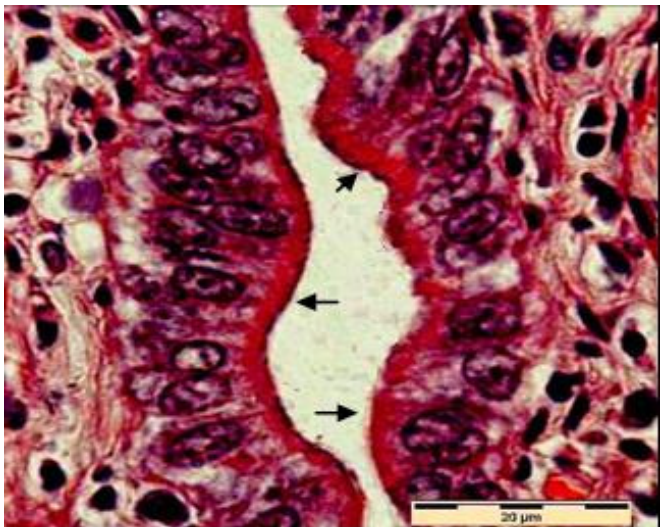

Figure 9. Pancreatic duct, note the PAS positive reaction (arrows) of the luminal surface of the epithelial lining. PAS stain.

aldehyde fuchsin stain for elastic fibers (Figure 8). However, PAS appeared positive (Figure 9). While these components showed an alcianophilic reaction (blue color) with alcian blue pH 2.5 stain (Figure 10), in the same time, this reaction was weak at pH 1 (Figure 11). When the sections were stained with alcian-PAS method, both secretory granules and apical surface coats of luminal border of these cells were stained with purple color (Figure 12). However, after being treated with the combined aldehyde fuchsin-alcian blue pH 2.5 method, these materials showed a deep purple color (Figure 13).

Mostly, the ducts lumens were filled with eosinophilic secretions. The epithelium lining the intercalated ducts and intralobuler ducts were surrounded by connective tissue fibers with few elastic fibers. In addition to this, the smooth muscle fibers were surrounding both the main pancreatic duct and interlobular ducts (Figure 3), which was thicker in the main duct with outer circular and inner longitudinal arrangements, and connective tissue fibers (collagen fibers with few elastic fibers) were observed in between these muscle bundles. Externally to these smooth muscle fibers, there were connective tissues of the tunica adventitia.

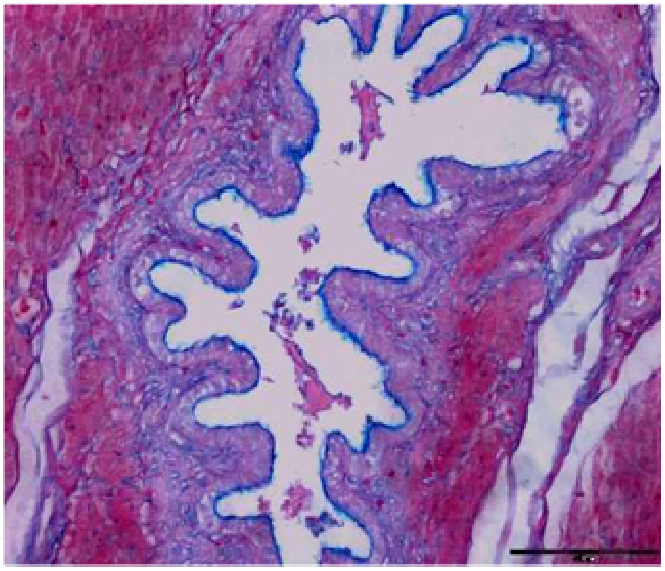

Figure 10. Interlobular duct show luminal mucin reaction with alcian blue stain (pH 2.5). Bar, 100 µm.

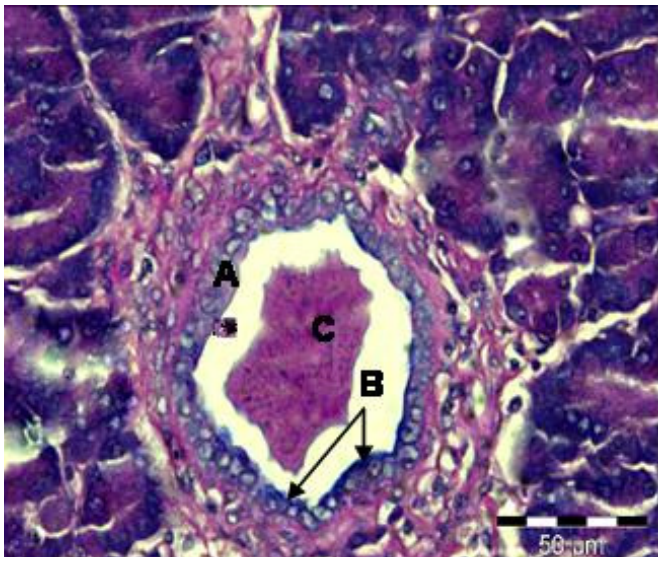

Figure 12. Intralobular duct. A, Epithelial cells (simple cuboidal). B, luminal mucin reaction (purpul) for neutral and acid mucins. C, acini secretion. Alcian-PAS method.

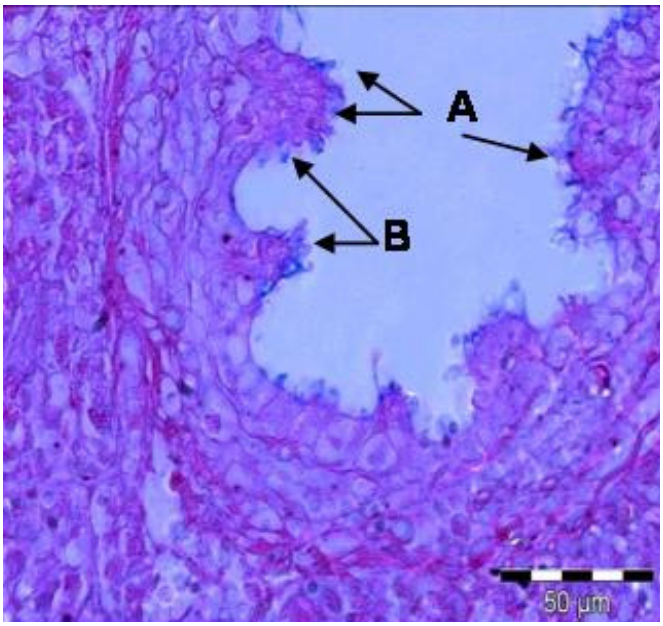

Figure 11. Intralobular duct. A, Luminal mucin reaction (weak); B, apical projection of the epithelial cell. Alcian blue stain (pH 1).

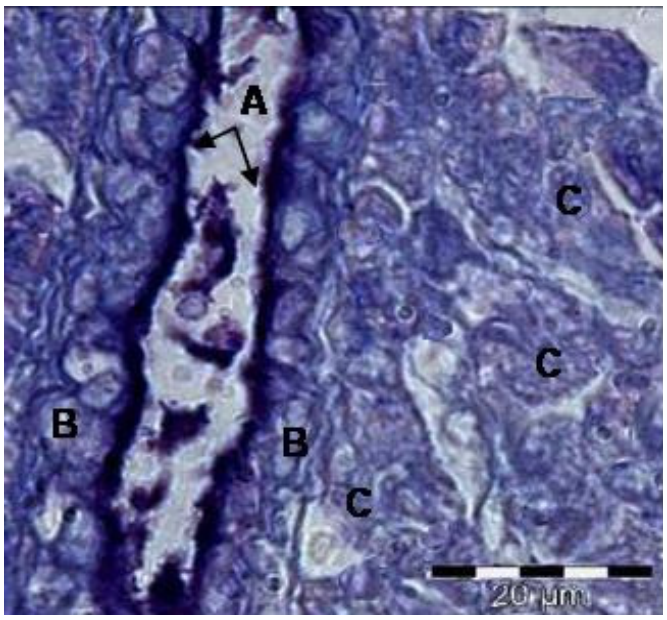

Figure 13. Intralobular duct (longitudinal section). A, Luminal mucin reaction (purple) for sulfated mucin; B, epithelial cells; C, pancreatic acini. Aldehyde fuchsin-alcian blue method.

DISCUSSION

In the present work, the pancreas fills the gap between the duodenal limbs, while in the duck and goose, the pancreas is too short to reach the end of these limbs (Getty, 1975; Nickel et al., 1977). The shortest pancreas is found in bustards, which consist of two lobes (Bailey et al., 1997). According to suggestions by Gussekloo (2006) on chicken and other birds that feed on grains and seed, they need more enzymatic activity to compensate for their lack of teeth and hydrolytic enzymes in their saliva. Our data agreed with Nickel et al. (1977) who reported that there are three efferent pancreatic ducts in the fowl and pigeon, two of which arise from the ventral and one from the dorsal lobe. These ducts enter the proximal loop of the ascending duodenum; there are only two ducts in

goose (Gulmez, 2003) and duck, in addition to the first pancreatic duct from the dorsal pancreatic lobe, which enters the duodenum loop between its descending and ascending limb (Liu, et al. 1998).

The results of the histological study of the pancreas showed no difference with the findings of Hodges (1974) or Baumel (1993) when they described the pancreatic exocrine and endocrine tissues. The exocrine glands consisted of tall columnar epithelial tissues that had acidophilic zymogen granules on their apical surface. Furthermore, there was agreement with the report of Mikami and Ono (1962) that the third lobe has more endocrine islets than the dorsal and ventral lobes, although the splenic lobe appeared to be greater than the other lobe according to our results. The exocrine tissue of the pancreas was similar to that described by Kendrey and Roe (1969) that with PAS-stained sectionsm the cytoplasm of the acinar cells contains red granules. PAS-positive material is also seen in the lumen of acini.

Regarding the epithelial cells lining the pancreatic ducts, our results are in line with Madden and Sarras (1989), who showed that in addition to the principal epithelial cells, there were light and basal cells in the main and the interlobular ducts of rat. In our findings, short projections in the luminal surface of the cells lining the ducts were clearly observed. These apical projections were previously described in rat by Ekholm et al. (1962) and Motta et al. (1997), who suggested that they reflected secretory activity by the ductal epithelial cells, while Yoshizawa (1978) described the luminal border as a fuzzy surface possessing many short micro-villi. Similar structures have been observed in the epithelial cells of the choroid plexus and have been shown to be involved in secretory processes (Gudeman et al., 1987). On the other hand, these apical plasma membrane projections do not appear to be fixation artifact because they were confined to the principal and light cells and were not associated with the other types like the basal or the acinar cells. However, these luminal projections could facilitate the propulsion of the secretory products (Motta and Fumagall, 1974). In our experiment, the light cells, which were mostly observed in the main and interlobular ducts, appeared to be different from the principal cells by their lightly staining cytoplasm. The degenerative appearance of some light cells could be fixation artifact (Madden and Sarras, 1989) or reflect the actual cell degeneration or apoptosis. However, it seems unlikely that the morphology of light cells resulted from poor fixation because all other epithelial cells were well preserved.

Gulmez (2003) reported the presence of basophilic staining on the apical surface of the goose pancreatic ducts starting from the interlobular ducts to the pancreatic ducts. In our results, the reaction of the ductal epithelium that was observed after alcian-PAS stain, indicated that their luminal borders and the supra-nuclear regions (secretory granules) of the lining of the epithelium contained both neutral and acid mucins. However, the intensity of the alcian blue at different pH indicated mostly, the presence of weak types of acid mucin, while the reaction with aldehyde fuchsin-alcian blue pH 2.5 method, showed sulfated mucins. These facts agreed with the findings of Bock (1978) and Geleff and Bock (1984), who stated that the secretion of these epithelia in the mammals and the human pancreas were shown to represent neutral and sulfated mucin. McMinn and Kugler (1961) and Madden and Sarras (1989) showed the presence of goblet cells as the sole source of duct secretion in rats. There was another source of secretion, the extra-epithelial ductular glands of the pancreatic duct, which are found in most mammals (Bock, 1978) and in geese (Gulmez, 2003). Whereas the goblet cells are absent in the main ducts of cats (Geleff and Bock, 1984), dogs and humans (McMinn and Kugler, 1961). This is in contrast with our study, where no ductular glands were found in the wall of the pancreatic ducts, and the goblet cells seem absent in the epithelial lining of the pancreatic ducts. However, the goblet cells make up not more than 2% of the ductal cell population in mammalian species (Madden and Sarras, 1989). Actually, there is a little information concerning the presence of mucin in the pancreatic duct of birds, particularly fowl. In mammals, Zharkov et al. (1994) stated that the features of the mucous of the epithelium of the pancreatic duct are found to be different depending on the type of digestion. Furthermore, the secretion contains neutral mucin in guinea pigs, while all herbivorous animals have more protection due to a high concentration of acid mucin (Zharkov et al., 1994). McMinn and Kugler (1961) suggested the functions of mucin as: warding off possible injury to the mucosa caused by ascending duodenal contents. However, this explanation does not justify the existence of these mucin materials in the smallest ducts, because we believe that it is difficult if not impossible, to reach the duodenal contents of these areas within the gland. However, the proposal on the protection of tissues from the same gland secretions is more acceptable. While some mucins aid bacterial growth, others may inhibit the proliferation of microorganisms, and this is a possible additional form of protection. Furthermore, the apical sulfomucin droplets suggest that they are acting not only as a protective barrier, but also facilitate the transport of the pancreatic juice (Yoshizawa, 1978).

REFERENCES

Bailey TA, Mensah-Brown EP, Samour JH, Naldo J, Lawrence P, Garner A (1997). Comparative morphology of the alimentary tract and its glandular derivatives of captive bustards. J. Anat. 191: 387-398.

Bancroft JD, Gamble M (2002). Theory and practice of histological techniques.5th ed. Churchill Livingstone, Philadelphia.

Baumel JJ (1993). Handbook of Avian Anatomy: Nomina Anatomica Avium. 2nd Ed. Cambridge, MA, USA: Nuttall Ornithological Club.

Bock P (1978). Pancreatic duct glands. I. Staining reaction of acid glycoprotein secret. Acta Histochemica. 61: 118-126.

Ekholm R, Zelander T, Edlund Y (1962). The ultrastructural organization of the rat exocrine pancreas. 11. Centroacinar cells, intercalary and intralobular ducts. J. Ulttrastruct Res. 7: 73-83.

Geleff S, Bock P (1984). Pancreatic duct glands. II. Lectin binding affinities of ductular epithelium, ductular glands, and brunner glands. Histochemistry, 80: 31-38.
Getty R (1975). Sisson and Grossman's the Anatomy of the Domestic Animals. Saunders, Vol. 2.5th edn, W. B Saunders Company, Philadelphia, USA. pp. 1874-1875.
Gudeman DM, Nelson SR, Merisko EM (1987). Protein secretion by choroid plexus: isolated apical fragments synthesize protein *in vitro*. Tissue Cell, 19: 1-9.
Gulmez N (2003). Are glands present in goose pancreatic ducts? A light microscope study. J Pancreas. 4(3): 125-128.
Gussekloo SWS (2006). Feeding structures in birds. In, feeding in domestic vertebrates: from structure to behaviour. Edn V. Bels. Wallingford, UK, Combridge.
Hodges RD (1974). The histology of the fowl. Academic Press. London, pp. 35-88.
Jackson S, Diamond J (1996). Metabolic and digestive responses to artificial selection in chickens. Evolution, 50: 1638-1650.
Kendrey G, Roe FJC (1969). Histopathological changes in the pancreas of laboratory rats. Lab. Anim. 3: 207-220.
Lee CC, Amin-Babjee SM (1993). New host records of parasites in the Malayan red jungle fowl, *Gallus gallus spadiceus*. Pertanika J. Trop. Agric. Sci. 16: 107-110.
Liu JW, Evans H, Larsen P, Pan D, Xu SZ, Dong HC, Deng XB, WAN B, GR T (1998). Gross anatomy of the pancreatic lobes and ducts in six breeds of domestic ducks and six species of wild ducks in china. Anat. Histol. Embryol. 27: 413-417.
Madden ME, Sarras MP (1989). The Pancreatic Ductal System of the Rat: Cell Diversity, Ultrastructure, and Innervation. Pancreas, 4: 472-485.
Masson PJ (1929). Connective tissue stains. In theory and practice of histological techniques. Edn V (Eds). Bancroft JD, and Gamble M, 2002. Churchill Livingstone. New York. p. 153.
McManus JFA (1946). Mucin. In theory and practice of histological techniques. Edn V. (Eds) Bancroft JD, Gamble M 2002. Churchill Livingstone. New York. p. 175.
McMinn RMH, Kugler JH (1961). The glands of the bile and pancreatic ducts Autoradiographic and histochemical studies. J. Anat. 95: 1-11.
Mikami SI, Ono K (1962). Glucagons deficiency induced by extirpatation of alpha islets of the fowl pancreas. Endocrinology, 71: 464-473.
Mitchell MA, Smith MW (1991). The effects of genetic selection for increased growth rate on mucosal and muscle weights in the different regions of the small intestine of the domestic fowl (*Gallus domesticus*). Comp. Biochem. Physiol. 99: 251-258.
Motta P, Macchiarelli G, Nottola SA (1997). Histology of the Exocrine Pancreas. Micros. Res. Tech. 37: 384-398.
Motta PM, Fumagalli G (1974). Scanning electron microscopy demonstration of cilia in rat intrahepatic bile ducts. Z. Anat. Entwickl-gesch. 145: 223-226.
Nickel R, Shummer A, Seiferle E (1977). Anatomy of the domestic birds. Verlag Paul Parey. Berlin. Hamburg.
Nishida T, Hayashi Y, Shotake T, Maeda Y, Yamamoto Y, Kurosawa Y, Douge K, Hongo A (1992). Morphological identification and ecology of the red jungle fowl in Nepal. Anim. Sci. Technol. Jpn. 63: 256-269.
Spicer SS, Meyer DR (1960). Demonstration of mucins. In Theory and practice of histological techniques. Edn V (Eds). Bancroft JD, Gamble M 2002. Churchill Livingstone. New York. p. 187.
Weyrauch KD, Schnorr B (1976). Die feinstruktur des epithel des ductus pancreaticus major des schafes. Acta Anatomica, 96: 232-247.
Yoshizawa S (1978). Studies on pancreatic duct system. I) The Fine Structure of the Major Pancreatic Ducts of Normal and Chronic Pancreas Injury Dogs. Gastroenterol. Jpn. 13: 213-223.
Zharkov VP, Yarygin VN, Dolzhikov AA (1994). Structural basis for an epithelial barrier in the main pancreatic duct of some mammals. Bull. Exp. Biol. Med. 118: 1211-1213.