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Histomorphology of the Brunner's Glands in the Angora Rabbit

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Abstract: The study was aimed to demonstrate the distribution, morphological and histochemical properties of Brunner's glands in the small intestine of the Angora rabbit. The duodenum of 10 healthy animals of both sexes constituted the material of the study. The glands were composed of acini densely packed within the submucosa. The Brunner's glands contained two types of cells, namely, serous and mucous cells. Histochemical examination revealed that the mucous glands and secretory ducts did not react with the Periodic Acid-Schiff (PAS) stain, while serous glands were weakly PAS-positive. Furthermore, mucous glands reacted positively with alcian blue pH 2.5. When applied the combined aldehyde fuchsin-alcian blue pH 2.5 staining procedure, mucous glands were determined to be aldehyde fuchsin (-) and alcian blue (+). These results showed that while a limited amount of neutral carbohydrates was secreted in serous glands, the secretion of the ducts and mucous cells of the duodenal glands in the Angora rabbit was composed of acidic carbohydrates with this acidity being due to the presence of carboxyl groups. Males and females did not differ in the histochemical staining properties of the duodenal secretion. Electron microscopic examination revealed the cytoplasm of mucous gland cells to be filled with electron light secretion granules. Fewer electron dense granules were determined to be present among these electron light granules. The electron dense granules were found within the apical cytoplasm of serous glands.

Key words: Angora rabbit, Brunner's glands, histochemistry, small intestine, ultrastructure, animals

INTRODUCTION

Duodenal glands, also known as Brunner's glands, exist in all mammalian species. These glands, which in general produce a mucous secretion are located in the submucosa of the proximal duodenum (Ainsworth *et al.*, 1995; Krause, 2000; Takehana *et al.*, 1991b, 2000; Verdiglione *et al.*, 2002).

Brunner's glands were determined to be distributed in an area starting from the gastrointestinal junction in the majority of the species studied (Alogninouwa *et al.*, 1996; Krause, 1981, 2000; Takehana *et al.*, 2000; Verdiglione *et al.*, 2002). However, how far Brunner's glands extend distally along the intestinal tract is variable and species-dependent (Krause, 1981, 2000; Takehana *et al.*, 2000; Verdiglione *et al.*, 2002). Brunner's glands have been studied in many species and noteworthy differences have been determined to exist in the morphology and topographic distribution of the glands as well as in the type of complex carbohydrates of

the glandular secretion (Krause, 1981, 2000; Pfeiffer and Dabareiner, 1992; Takehana *et al.*, 2000; Verdiglione *et al.*, 2002). In studies conducted using different techniques, depending on species, Brunner's glands were reported to contain neutral or acidic mucin glycoproteins or the combination of both types of mucin (Crescenzi *et al.*, 1988; Krause, 2000; Takehana *et al.*, 1989, 1991a, 2000; Verdiglione *et al.*, 2002). Generally, the duodenal glands are believed to protect the duodenal mucosa from the gastric hydrochloric acid.

Brunner's glands of the Angora rabbit, which is a species endemic to Turkey have not been studied previously. The aim is to demonstrate the distribution, morphological and histochemical properties of duodenal glands in this species.

MATERIALS AND METHODS

Tissue samples taken from different regions of the duodenum (proximal, mid-and distal) of 10 healthy adult

male and female Angora rabbits, which were obtained from private breeders constituted the material of the study. The rabbits were euthanized with intravenous sodium pentobarbital injection. The small intestines, which were removed from the body of the animals by the immersion method following the dissection of the abdominal cavity were examined both histochemically and electron microscopically.

Part of the tissue samples taken were first fixed in 10% neutral buffered formalin and then subjected to routine tissue processing for light microscopy before being embedded in paraplast. The resultant blocks were cut into sections 5 μ m thick and these sections were stained with Crossmon's modification of Mallory's triple stain for general histological examination, Periodic Acid-Schiff (PAS) for neutral mucosubstance, alcian blue pH 2.5 for acidic mucosubstance and PAS/Ab pH 2.5 for the combined assessment of neutral and acidic mucosubstances (Culling *et al.*, 1985). The aldehyde fuchsin-alcian blue method (Bancroft and Gamble, 2002) was employed for the demonstration of acidic mucosubstance containing sulphate ($-\text{SO}_4$) and carboxyl ($-\text{COOH}$) groups.

Specimens taken for electron microscopy were first subjected to pre-fixation in glutaraldehyde paraformaldehyde (pH 7.4) for 24 h in accordance with the method described by Karnovsky (1965). They were rinsed twice, firstly for 3 h in a cacodylate buffer and then for a second time in a 1% osmic acid solution for 2 h. After being stained with 0.5% uranyl acetate, the tissue samples were dehydrated through an alcohol series cleared in propylene oxide and were embedded in araldite M. Semi-thin sections were stained with toluidine blue for light microscopic examination. Sections with a thickness of 30-40 nm were prepared and contrasted according to the method of Veneable and Coggeshall (1965) and were then examined under a Carl Zeiss EM 9 S-2 model transmission electron microscope (Zeiss Oberkochen, Germany).

The animals were included in the study after the approval of the local ethics committee.

RESULTS AND DISCUSSION

Cells, which compose the Brunner's glands, vary with species. These glands were reported to be composed of two types of cells, serous and mucous cells in the rabbit (Takehana *et al.*, 1989, 1991b) and horse (Oduor-Okelo, 1976; Pfeiffer and Dabareiner, 1992; Takehana *et al.*, 1989, 1991b), while they were demonstrated to be composed of only mucous cells in other species (Krause, 1981, 2000). In the Angora rabbit,

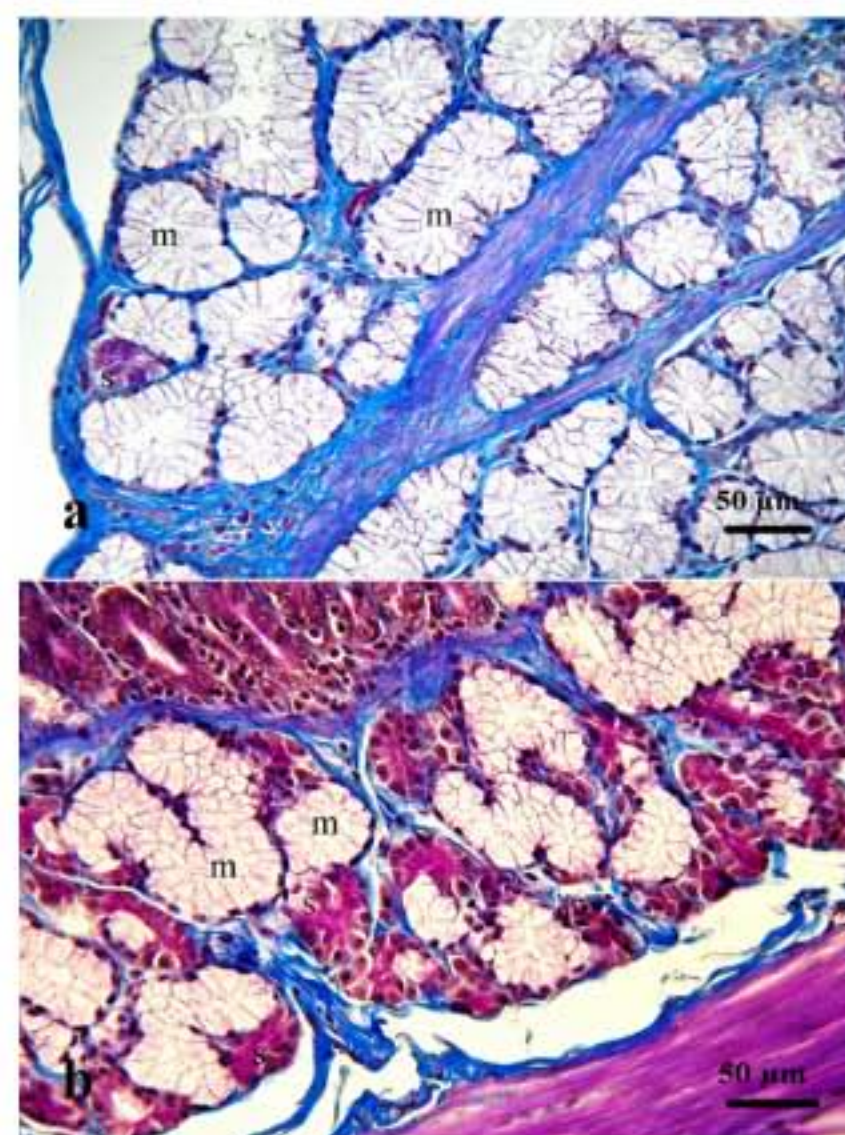


Fig. 1: (a) Serous (s) and mucous (m) glands in the proximal duodenum, triple. Bar: 50 μ m (b): Serous (s) and mucous (m) glands in the distal duodenum, triple. Bar: 50 μ m

there were two different types of cells, serous and mucous cells (Fig. 1a). The glands were composed of acini densely packed within the submucosa.

In most species, Brunner's glands are distributed in an area starting from the gastrointestinal junction and extending to varying distances in the proximal small intestine (Alogninouwa *et al.*, 1996; Krause, 2000; Takehana *et al.*, 2000; Verdiglione *et al.*, 2002), while in humans they extend almost to the level of the papilla of Vater (Treasure, 1978). In rats, the area extends one halfway down to the entry of the bile duct (Treasure, 1978). In several mammals, Brunner's glands are located within the first few mm of the proximal duodenum, just distal to the pyloric sphincter (Krause, 2000). In horses, Brunner's glands occupy a very large area and extend approximately 6 m caudal to the pylorus. They are known to exist also in the jejunum in pigs and large herbivores (Verdiglione *et al.*, 2002). In the Angora rabbit, the distribution of Brunner's glands was determined to start from the pyloroduodenal junction and to extend near the jejunum. In the pony (Takehana *et al.*, 1991b) mucous glands were reported to be present along the duodenum, while serous glands were determined to be located in the upper part of the duodenum within the region extending

to approximately 10 cm caudal to the pylorus. Although, Brunner's glands were observed to be present in all three regions of the duodenum (proximal, mid-and distal), the number of these glands was greater in the proximal region in the Angora rabbit. While, the majority of the glands in the proximal duodenum were composed of mucous cells (Fig. 1a), the number of serous glands increased evidently in the other regions of the duodenum (Fig. 1b).

In studies carried out in the guinea pig, gerbil, hamster (Sheahan and Jervis, 1976) horse and pony (Takehana *et al.*, 1989, 1991b), domestic pig (Takehana and Abe, 1986), sheep (Carvalho *et al.*, 1988), bovine (Takehana *et al.*, 1991a), goat (Ohwada and Suzuki, 1992), American bison, mountain goat (Krause, 1981) and mouse (Obuoforibo, 1975), the majority of Brunner's glands were reported to produce neutral mucin, while acidic mucosubstances were determined to constitute a smaller percentage of the total glandular secretion. In human (Crescenzi *et al.*, 1988) and cat, dog and rat (Sheahan and Jervis, 1976), the glandular secretion is composed of neutral mucin. Acidic mucins are the primary secretory product of Brunner's glands in only a few species (e.g., camel, koala, echidna) (Krause, 2000; Takehana *et al.*, 2000). Oduor-Okelo (1976) has demonstrated the presence of acidic groups in the mucosubstances secreted by the horse's duodenal glands. The Brunner's glands of some ruminants including the American bison (Krause, 1981) and Holstein cow (Takehana *et al.*, 1991a) are characterized by an unusual feature. While, the cells in the central region of the lobules produce neutral mucin, acidic mucins were determined to be present in only the peripheral cells of the glands. It has been reported that in the domestic rabbit and American (Cottontail) rabbit (*Sylvilagus floridanus*) the mucous cells in the acini of Brunner's glands contained neutral, carboxylic and sulpho acidic mucin, while serous cells contained neutral mucin (Krause, 2000). In the Angora rabbit, while the secretion granules in mucous cells and the excretory ducts of Brunner's glands did not react with PAS, they reacted positively with alcian blue pH 2.5 (Fig. 2a). Cells pertaining to serous glands were weakly PAS (+) (Fig. 2b). Furthermore, PAS (+) cells, resembling goblet cells in their morphology were determined to exist among mucous cells (Fig. 2c). When employed combined aldehyde fuchsin-alcian blue pH 2.5 staining, mucous cells were determined to be alcian blue (+) (Fig. 2d). Thus, it was determined that while a limited amount of neutral carbohydrates was secreted in serous glands, the secretion of the ducts and mucous cells of duodenal glands in the Angora rabbit contained acidic carbohydrates with this acidity being due to the presence of carboxyl groups.

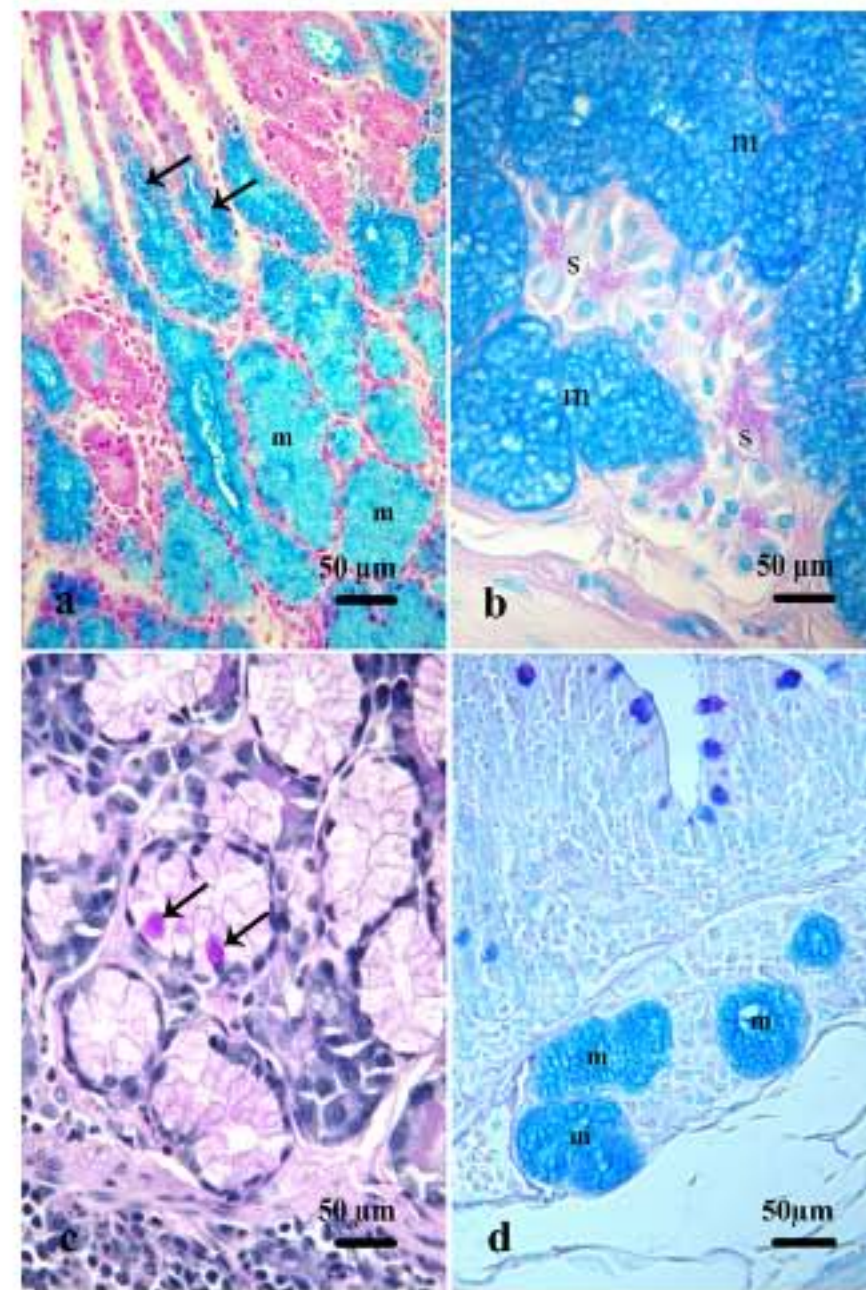


Fig. 2: (a) Mucous glands (m), excretory ducts (arrows), PAS/Ab. Bar: 50 µm (b).Weakly PAS (+) serous glands (s), mucous glands (m), PAS/Ab. Bar: 50 µm (c). PAS (+) cells in the mucous acinus (arrows), PAS. Bar: 50 µm (d). Alcian blue (+) mucous glands (m), aldehyde fuchsin-alcian blue (pH 2.5). Bar: 50 µm

The electron microscopic structure of the secretion granules was in compliance with light microscopic findings. The cytoplasm of the epithelial cells of mucous glands was filled with electron light secretion granules.

However, among these electron light granules, a fewer number of electron dense granules were also present (Fig. 3a). The electron dense granules were found within the apical cytoplasm of serous glands (Fig. 3b).

Shackleford and Wilborn (1978), in a histochemical study carried out in the duodenal glands of hamsters, determined that the amount of acidic mucins varied with sex. These researchers reported that the duodenal glands of male hamsters contained twice as much as acidic mucosubstances of that of the glands of female hamsters. Sexual differences have also been clearly demonstrated by PAS reaction. The PAS reaction is reported to be stronger in females. Krause (1981) in a study carried out

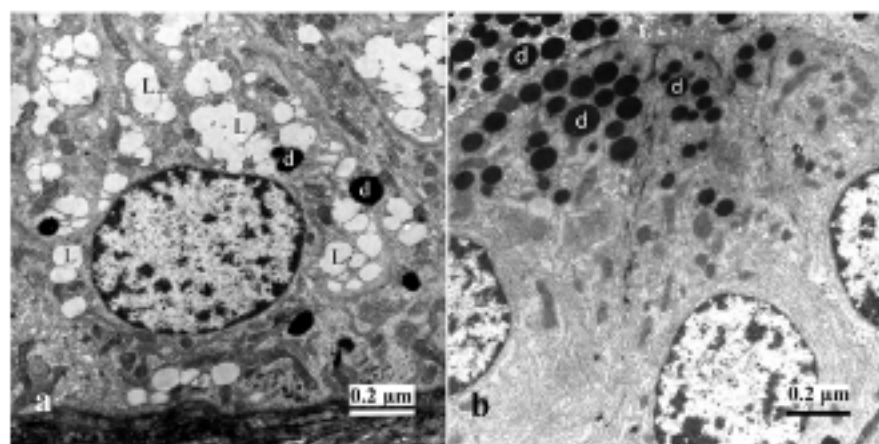


Fig. 3: (a) The mucous acinus cell, electron light granules (L), electron dense granules (d) Bar: 0.2 µm (b). The serous acinus, electron dense granules (d). Bar: 0.2 µm

the in the bighorn sheep, elk, pronghorn and white-tailed deer reported no difference between the two sexes in alcian blue staining. Similarly in the study, no difference was observed between sexes for histochemical staining in the Angora rabbit.

CONCLUSION

In the study, it was demonstrated that the duodenal glands were composed of serous and mucous acini in the Angora rabbit. While serous acini contained neutral mucin, mucous acini different from those of the domestic rabbit and American (Cottontail) rabbit, contained only acidic mucosubstance and this acidity was determined to be due to the presence of carboxyl groups.

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