Comparative features of the mucous glands in the gastroduodenal junction in guinea pigs and chinchillas: microanatomy and histochemistry

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Abstract

Background: Mucous glands from the gastroduodenal junction are briefly represented by glandular cells in the stomach and by Brunner's glands in the duodenum. The aim of the study was to describe the main histological/histochemical features of the gastroduodenal junction in guinea pigs and chinchillas.

Results: The material was represented by tissue samples collected from the stomach and duodenum processed by paraffin technique. Histological sections were stained by Goldner's trichrome stain, while mucous content was identified by Periodic acid–Schiff (PAS) stain (for neutral mucosubstances) and Alcian blue stain (for acid mucosubstances). In guinea pig, the pyloric glands were positive for both mucus types, whereas in chinchillas a low amount of mucin was identified. The Bruner glands, in guinea pigs displayed a weak reaction for the both mucin types. In chinchillas, the Bruner glands showed a significantly higher amount of PAS and Alcian blue-positives mucosubstances types. As observed, a higher amount of neutral mucus was identified in the pyloric glands in guinea pigs comparing to chinchillas. Contrariwise, chinchillas displayed a suggestively higher amount of mucus in Bruner glands comparing to guinea pigs.

Conclusions: Concluding, this is the first report describing the comparative features of the mucous glands in the two rodents, with detailed histological and histochemical features.

Background

The gastroduodenal junction is the borderline between the pyloric stomach and the duodenum of the small intestine. The pyloric sphincter controls the passage of the gastric content in the duodenum. The stomach and the duodenum are made of the four layers specific for the gastrointestinal tract. However, mucous glands from the gastroduodenal junction are briefly represented by glandular cells in the pyloric region of the stomach and by Brunner's glands in the duodenum. Pyloric glands are situated in the mucosa whereas the duodenal glands are placed in the submucosa [1].

Developmental studies have proved that Brunner's glands can derive from undifferentiated gastric epithelium and/or intestinal epithelium in the proximal duodenum [2]. Accordingly, some authors [2] observed that, the mucous surface cells of the stomach, pyloric glands, duodenal goblet cells, and Brunner's glands secretory epithelium had diverse lectin-binding patterns. The lectin-binding profiles of the secretory epithelium of Brunner's glands bear a resemblance to that of pyloric glands (more closely than that of mucous surface cells of the stomach and duodenal goblet cells). Mucins derived from pyloric glands and Brunner's glands showed a greater terminal carbohydrate residue variety than in the case of gastric mucous surface cells or duodenal goblet cells. The lectin-binding profile claims for the evolution of analogous mucins from the epithelia of pyloric glands and Brunner's glands. The higher diversity of carbohydrate residues in mucins elaborated by Brunner's glands suggests that such mucus type is more adaptable. This could be the reason why Brunner's glands undergo more frequently metaplasia (comparing to goblet cells) in the mucosa adjacent to chronic intestinal ulcers [1, 3, 4].

Brunner's glands in mammals are limited to the submucosa of the proximal duodenum. In most species, they begin at the gastrointestinal junction and extend for variable distances in the initial potion of the small intestine. The ducts of Brunner's glands drain either directly in the intestinal lumen or merge with overlying Lieberkühn glands, dependent on the species. Secretion of the Brunner's glands is mostly mucous, which forms a slippery, viscoelastic gel that lubricates and protects the mucosal lining of the proximal intestinal tract [5, 6].

Mucin glycoproteins produced by Brunner's glands consist of mainly O-linked oligosaccharides attached to the central protein core of the glycoprotein molecule. In addition, a low amount of bicarbonate and a variety of additional factors (epidermal growth factor, trefoil peptides, bactericidal factors, proteinase inhibitors, and surface-active lipids) were identified within the secretory product of Brunner's glands. All the above-mentioned factors embedded in the mucous layer, protect against the degradation of the protective barrier (i.e. mucus) and underlying intestinal mucosa by gastric acid, pancreatic enzymes, and other surface-active agents linked with this region. Additionally, several other factors produced by Brunner's glands provide active and passive immunological protection, promote cellular proliferation and differentiation, contribute to elevate the pH of the intestinal luminal contents by promoting secretion of the intestinal mucosa, including pancreatic secretion and gall bladder contraction [5, 6].

The aim of the study was to assess the main histological and histochemical features of the gastroduodenal junction, with emphasis on the pyloric and Brunner's glands in guinea pig and chinchilla.

Results

In guinea pigs, the gastric pits of the pyloric glands are significantly longer comparing to the ones of the glands located in the fundic region. The gastric pits of the pyloric glands occupy about one-third from the thickness of the mucosa in this region (Fig. 1a). Concerning the duodenum, the number of goblet cells is lower in guinea pigs compared to chinchillas. In guinea pig, the mucosa is clearly delineated from the submucosa by *muscularis mucosae* that is continuous in this species (Fig. 1b). The Lieberkühn glands are placed with the secretory unit against the *muscularis mucosae,* whereas the Bruner glands are entirely located in the submucosa.

In chinchillas, the gastric pits of the pyloric glands are much longer comparing to the ones in guinea pigs, occupying more than a half from the thickness of the mucosa (Fig. 1c). Regarding the duodenum in chinchillas, a poor delimitation of the mucosa from the submucosa can be observed. The ill-defined bordering of the mucosa is the consequence of a discontinuous *muscularis mucosae* (Fig. 1d), which is suggested only by isolated smooth myofibers. Therefore, the duodenal mucosa and submucosa appear to form a merged layer. In this situation, the Lieberkühn glands are no longer clearly delineated from the Bruner glands situated bellow the first.

Distribution of the mucous content in the pyloric and Brunner's glands varied in the two analyzed species. A higher amount of mucus was identified in the pyloric glands in guinea pigs comparing to the ones in chinchillas. In guinea pigs, the pyloric glands were intensely PAS positive (i.e., high content in neutral

mucin; Fig. 2a), being identified a moderate to low amount of acid mucosubstances (discrete Alcian-blue reaction; Fig. 2bB). In chinchillas, however, the reaction was discrete for the both PAS (Fig. 2c) and Alcian blue reactions (Fig. 2d), suggesting a low amount of mucin in the pyloric glands.

Regarding the duodenal Bruner glands, in guinea pigs the glands displayed a weak reaction for the both neutral mucins (PAS stain; Fig. 3a) and acid mucosubstances (Alcian blue stain; Fig. 3b). As a comparison, in chinchillas the duodenal (Bruner) glands showed a significantly higher amount of PAS-positive neutral mucin (Fig. 3c) and a very intense reaction for acid mucosubstances (Fig. 3d).

Discussion

As observed in the presented results, in guinea pigs the mucous pyloric glands display a high activity by synthetizing neutral, but also acid mucosubstances. Oppositely, the secretory activity of the mucous cells in the Bruner glands is modest.

In the case of chinchillas, the situation is reversed, that is a negligible secretion of mucosubstances in the pyloric glands, and a significantly higher mucous content in the Bruner glands (i.e., for the both categories of mucosubstances). In other words, there appears to be a functional bond between the two types of glands (pyloric and Bruner glands). It seems one completes the secretion of the other. In this sense, if the amount of mucus synthesized by the cells in the pyloric area is high, it will be supplemented with small quantities from the Bruner glands, and vice versa. In the case of the two rodents described, the pyloric glands are more active in guinea pigs, whereas the Bruner glands are significantly more dynamic in chinchillas.

Some comparative data regarding the mucins secreted by the Brunner's glands and the duodenal goblet cells in the chinchilla, guinea-pig and the house mouse were provided by some authors [7, 8]. In the guinea-pig, the goblet cells from the duodenum display a mucous content with sialic acid and N-acetylgalactosamine subterminal to sulfated groups. As a comparison, in the same species, the Brunner's glands produce class-III stable sulfosialomucins. However, the Brunner's glands of the mouse produce class-III stable neutral mucins that are binding to the same lectins as the ones observed in the guinea-pig, not including those specific to sialic acid. In contrast to Brunner's glands, in the mouse, the duodenal goblet cells do not have stable class-III mucins and present tiny amounts of sialic acid [7, 9].

The species analyzed in this study (i.e., guinea-pig and chinchilla) are frequently used as a comparative model in a series of experiments. Among the main topics assessed worldwide is the infection with *Helicobacter pylori* and *H. spp.*,, including the efficacy of some new therapies. The distribution efficiency of the antibiotic agents through the mucous layer of the stomach, where *H. pylori* resides, has been unexpectedly little studied. Since *H. pylori* is becoming more refractory to standard antibiotics, there is a necessity to point out the essential mechanisms that may interfere with the delivery of the antibiotic in the stomach, so as to help the design of some new therapeutic agents. The gastric mucous layer, whose purpose is to protect the gastric mucosal epithelium from injurious agents/substances, may also act as obstacle to antibiotic penetration. Accordingly, some mucolytic agents were utilized with good

effects to improve the penetration rates throughout the gastric mucous layer. In view of that, a better understanding of the main peculiarities of the digestive tract in the species used as a comparative model is more than welcome [10, 11].

Conclusions

Concluding,a higher amount of mucus (mainly PAS positive neutral mucin) was identified in the pyloric glands in guinea pigs comparing to chinchillas. Contrariwise, chinchillas displayed a suggestively higher amount of mucus (including the acid mucosubstances and PAS-positive neutral mucin) in duodenal Bruner glands comparing to guinea pigs. Adjustment of the gastrointestinal microenvironment, inhibition of disorders and preservation of local microflora can enlighten the presented results and the differences noticed between the two rodents. Finally, this is the first report describing the comparative features of the mucous glands in the two rodents, with detailed histological and histochemical features. Additional insights regarding the role of Brunner's glands in the mammalian gastrointestinal tract should be achieved from a basic comprehension of their pathobiology.

Methods

The material was represented by dead adult guinea pigs (Cavia porcellus; n = 5) and chinchillas (Chinchilla lanigera; n = 5) that were brought for complete necropsy survey to the Department of Pathology (Faculty of Veterinary Medicine Cluj-Napoca, Romania). In all cases, the gastric and duodenal tissue samples were harvested from subjects that died by lobar pneumonia (in both species). The gastric and intestinal tissue samples collected were fixed in neutral 10% buffered formalin. Tissues were subsequently embedded in paraffin wax, sectioned at 5 μm, and stained by Goldner's trichrome method. Additionally, the mucous content of the glands located in the gastroduodenal junction was identified by standard PAS for neutral mucosubstances [11] and by Alcian blue (pH = 2.5) stain for acid mucosubstances [12]. The microscopic samples were assessed using an Olympus BX 41 microscope (objectives lens specifications: magnification—10X; N. A. - 0.4; aberration corrections - UPlan SApo; magnification—20X; N. A. - 0.75; aberration corrections - UPlan SApo). The microphotographs were acquired by Olympus photo camera (model no. E 330, 7.4 MPxl) and finely adjusted in Adobe Phothoshop (CS2 v.9.0).

Abbreviations

PAS: Periodic acid–Schiff; *H. spp.*:: *Helicobacter species; H.: Helicobacter;* N. A.: Numerical Aperture; UPlan SApo: super apochromat objectives compensate for both spherical and chromatic aberrations; MPxl: Mega Pixel.

Declarations

Authors' contribution

All authors contributed to the initiation, design, conception, analysis and interpretation of data execution, including writing of this study. Accordingly, GAF and MV participated in the initiation, conception, and interpretation of the data. GAF and RV performed the tissue samples harvesting, processing and AS performed the elaboration of chemicals required for staining. Finally, all authors revised critically the paper.

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Availability of data and materials

The datasets used and / or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This article does not contain any studies with live animals performed by any of the authors. The dead animals were brought by the owners for complete necropsy and we obtained the written informed consent to use the animals in our study.

Consent for publication

All data was anonymised for the purposes of analysis and publication. Owners were asked to sign a consent form for use the animals in research and/education.

Competing interests

The authors declare that they have no competing interests.

References

1. Sorenson RL, Brelje TC. The Atlas of Human Histology: A Guide to Microscopic Structure of Cells, Tissues and Organs. 3rd ed. Minnesota: University of Minnesota Bookstore; 2014.

2. Schumacher U, Duku M, Katoh M, Jörns J, Krause WJ. Histochemical similarities of mucins produced by Brunner's glands and pyloric glands: A comparative study. Anat Rec A Discov Mol Cell Evol Biol. 2004;278(2):540-50.
3. Ahnen DJ, Poulsom R, Stamp GW, Elia G, Pike C, Jeffery R, Longcroft J, Rio MC, Chambon P, Wright NA. The ulceration-associated cell lineage (UACL) reiterates the Brunner's glands differentiation programme but acquires the proliferative organisation of the gastric glands. J Pathol. 1994;173:317–326.
4. Kushima R, Manabe R, Hattori T, Borchard F. Histogenesis of gastric foveolar metaplasia following duodenal ulcer: a definite reparative lineage of Brunner's gland. Histopathology. 1999;35:38 – 43.
5. Krause WJ. Brunner's glands: a structural, histochemical and pathological profile. Prog Histochem Cytochem. 2000;35(4):259-367.
6. Akiba Y, Guth PH, Engel E, Nastaskin I, Kaunitz JD. Dynamic regulation of mucus gel thickness in rat duodenum. Am J Physiol Gastrointest Liver Physiol. 2000;279(2)Ş G437-G447.
7. Scillitani G, Mentino D. Comparative glycopattern analysis of mucins in the Brunner's glands of the guinea-pig and the house mouse (Rodentia). Acta Histochem. 2015;117(7):612-623.
8. Rus V, Ruxanda F, Gal AF, Damian A, Ghirco F, Miclăuș V. The Glandular Activity from Gastroduodenal Junction in Chinchilla and Guinea Pig. Histological and Histochemical Study. In: Stănică F, Cazimir I, Chervase D, editors. Proceedings of the 10th Meeting of the Young Generation of Veterinary Anatomists – YGVA. Bucureşti: Ex Terra Aurum Publishing House; 2019. Poster 31: p. 123-124.
9. Holmén Larsson JM, Thomsson KA, Rodríguez-Piñeiro AM, Karlsson H, Hansson GC. Studies of mucus in mouse stomach, small intestine, and colon III. Gastrointestinal Muc5ac and Muc2 mucin O-glycan patterns reveal a regiospecific distribution. Am J Physiol Gastrointest Liver Physiol. 2013;305(5):G357–G363.
10. Akamatsu T, Gotoh A, Shimizu T, Maeyama H, Fujimori K, Shimodaira K, Kiyosawa K, Kawakami Y, Ota H, Katsuyama T. () Combination effect of pronase for eradication therapy against Helicobacter pylori. Gastroenterology. 1997;112(4S):A54-A54.
11. Dağ S, Sözmen M, Cihan M, Tunca R, Kurt B, Devrim AK, Özen H. Gastric helicobacter like organisms in stray cats: identification, prevalence, and pathologic association. Pak Vet J. 2016;36(2):199- 203.
12. Kiernan JA. Histological & histochemical methods: theory & practice. 2nd ed. Toronto: Pergamon Press; 1990.

Figures

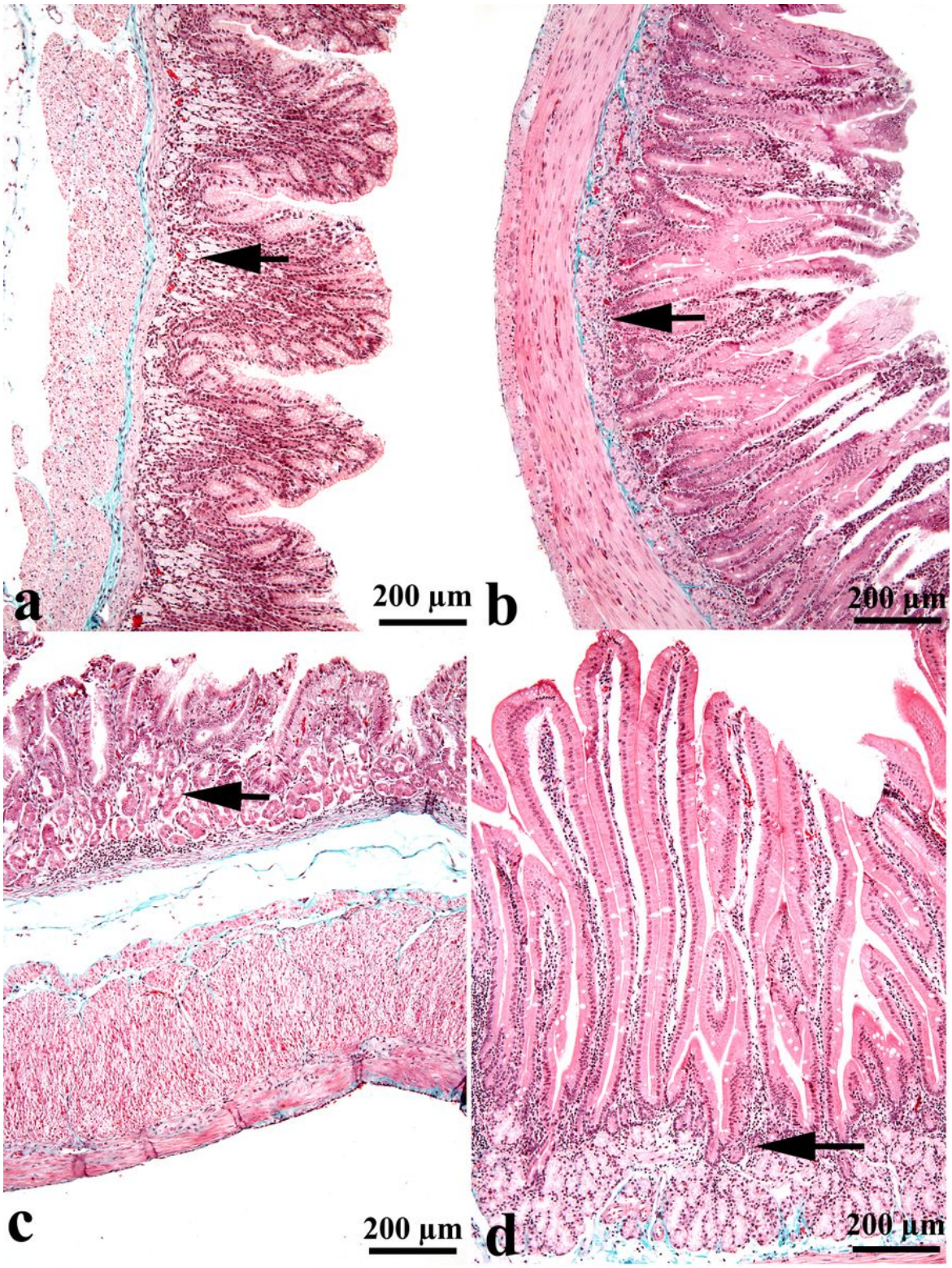

Figure 1

a. Histological features of the gastric wall in the pyloric region in guinea pigs (pyloric glands – arrow); b. General aspect of the duodenum in guinea pigs; a clear delineation of the mucosa from the submucosa can be observed (arrow); c. Microanatomy of the stomach in the pyloric region in chinchillas (pyloric glands – arrow); d. Structure of the duodenum in chinchillas: a poor delimitation of the mucosa from the

submucosa can be observed, due to a discontinuous muscularis mucosae (arrow). Goldner's trichrome stain.

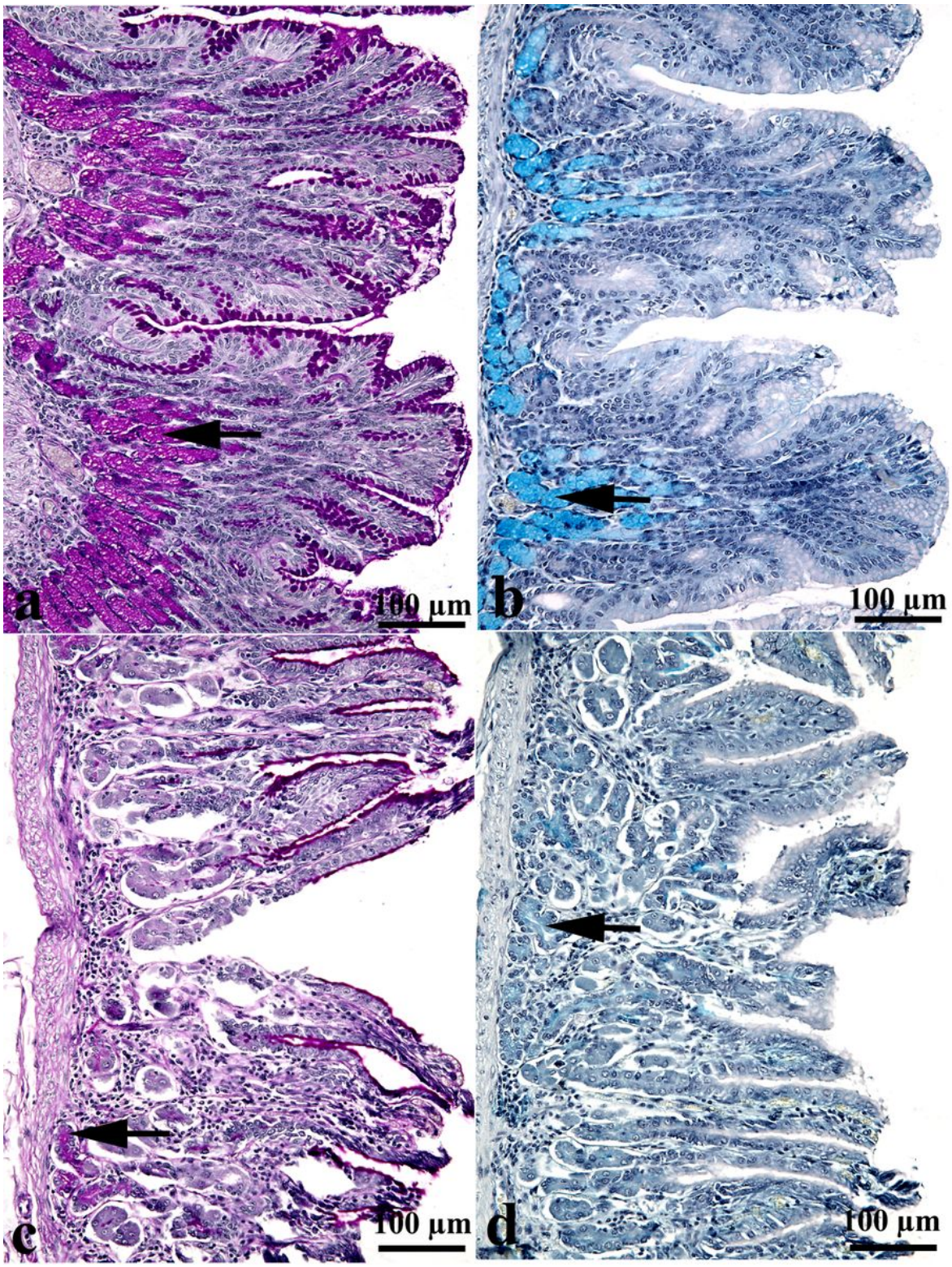

Figure 2

Histochemical features of the gastric glands in the pyloric region in guinea pigs (a, b) and chinchillas (c, d). a. PAS stain: high amount of PAS positive mucous material (neutral mucin) in the pyloric glands

(arrow). b. Alcian blue stain: moderate amounts of acid mucin (arrow). Low amounts of PAS-positive (c) and Alcian blue-positive (d) mucin types (arrows).

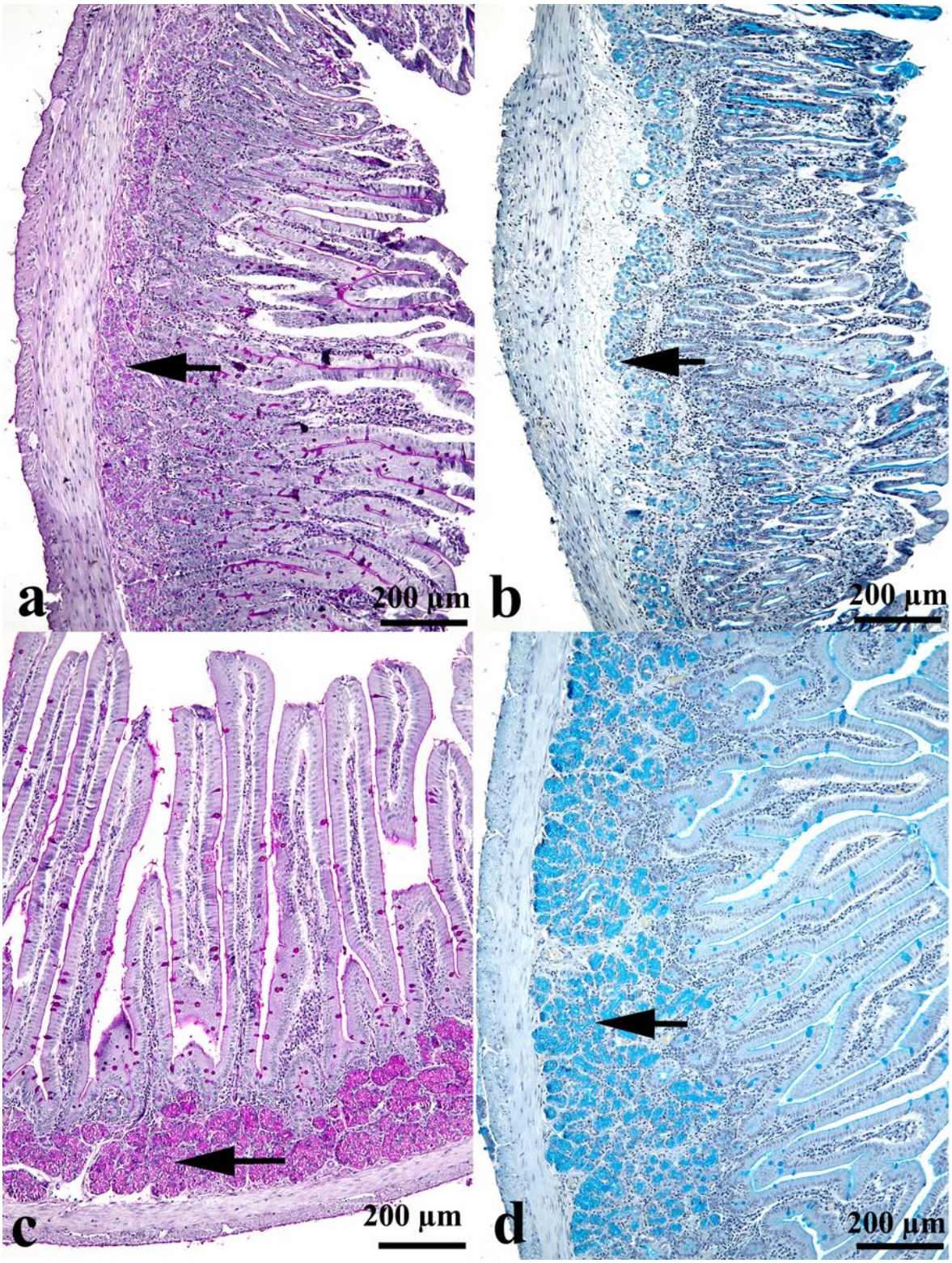

Figure 3

Histochemical features of the duodenal glands in guinea pigs (a, b): a weak reaction (arrows) for the both neutral (a; PAS stain) and acid mucins (b; Alcian blue stain). Histochemical features of the duodenal

glands in chinchillas: c. a high amount of neutral mucin (arrow; PAS stain). d. intense reaction for acid mucin (arrow; Alcian blue stain).