ISSN: 1220-3173; E-ISSN: 2457-7618

DISTRIBUTION AND ACTIVITY OF MUCIN-SECRETING CELLS IN THE STOMACH OF CHINCHILLA. HISTOCHEMICAL STUDY

DISTRIBUȚIA ȘI ACTIVITATEA CELULELOR SECRETOARE DE MUCINE DIN STOMACUL DE CINCILA. STUDIU HISTOCHIMIC

I.F. GIURCO[1)], A.F. GAL[1)], V. RUS[1)], C. MARTONOS[1)], Maria-Cătălina MATEI[1)], V. MICLĂUȘ[1)], A. DAMIAN[1)]

ABSTRACT | REZUMAT

The type of food consumed by each species of mammal requires morphological and functional adaptation from the organs involved in the digestion of food. The stomach of the chinchilla must provide an environment suitable for the processing of the feed consumed. A basic component of gastric secretions is mucins. The aim of this study was the histochemical investigation of mucin-secreting cells in the chinchilla stomach, in number, disposition, and secretory activity. Stomach fragments from three regions were collected from five fresh chinchilla carcasses: cardiac, fundic, and pyloric. The harvested pieces were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 5 µm. The sections were processed by the PAS reaction to highlight neutral mucins and staining with Alcian blue to highlight acidic mucins. It has been found that all the cells that make up the surface epithelium and the ones that line the gastric crypts synthesize large amounts of neutral mucins and are not involved at all in the synthesis of acidic mucins. The fundic glands do not synthesize mucins, and the cardiac and pyloric ones synthesize both neutral and acidic mucins, but in negligible quantities. In conclusion, the chinchilla stomach synthesizes large amounts of neutral mucins and very small amounts, practically negligible, of acidic mucins.

Keywords: Chinchilla, stomach, neutral mucins, acidic mucins

Tipul de alimente consumat de fiecare specie de mamifere necesită adaptare morfologică și funcțională din partea organelor care participă la digestia alimentelor. Stomacul de cincila trebuie să asigure un mediu adecvat prelucrării furajelor consumate. O componentă de bază a secrețiilor gastrice este reprezentată de mucine. Scopul acestui studiu a fost investigarea histochimică a celulelor secretoare de mucine din stomacul de cincila, ca număr, dispunere și activitate secretorie. Preparatele histologice au fost prelevate de la cinci cadavre proaspete de cincila, din trei regiuni ale stomacului: cardia, regiunea fundică și cea pilorică. Piesele recoltate au fost fixate în formol 10% tamponat, incluse în parafină, secționate la 5 µm. Secțiunile au fost prelucrate histochimic prin reacția PAS pentru evidențierea mucinelor neutre, respectiv cu albastru alcian, pentru evidențierea mucinelor acide. S-a constatat că toate celulele care alcătuiesc epiteliul de suprafață al mucoasei gastrice și cel care tapetează criptele gastrice sintetizează cantități mari de mucine neutre și nu sunt implicate deloc în sinteza de mucine acide. Glandele fundice nu sintetizează mucine, iar cele cardiale și pilorice sintetizează atât mucine neutre cât și acide, dar în cantități mici. În concluzie, stomacul de cincila sintetizează cantități mari de mucine neutre și cantități foarte mici, practic neglijabile, de mucosubstanțe acide.

Cuvinte cheie: cincila, stomac, mucine neutre, mucine acide

The identification of substances in a tissue or organ can be done either by chemical or histochemical methods. The difference is given by the fact that chemical methods involve obtaining a homogenate from which the average concentration of the monitored substance is determined, and the histochemical investigation identifies which components (cells) of the analyzed tissue participate in the synthesis of the investigated substances and which do not.

1) University of Agricultural Sciences and Veterinary Medicine Faculty of Veterinary Medicine, Cluj-Napoca, Romania
*) *Corresponding author: catalina.matei@usamvcluj.ro*

The histochemical investigation highlights the number of secretory cells, the intensity of secretion, and the topographic distribution of cells involved in the secretion of a substance, from the structure of the tissue under investigation. In other words, the examination of histochemical preparations is a combination of a chemical and a morphological analysis that allows the specific identification of some substances, as well as the location of the researched substances at cellular and tissue level. The results can be used to establish correlations between the chemical composition of any tissue or cell and their histophysiology (3, 10).

There are mucin-secreting glandular cells in the

stomach, which, eliminated in the lumen of the organ, ensure the protection of the mucosa against the aggression of hydrochloric acid and the action of enzymes on the cells on the mucosal surface (3). The mucins synthesized by some gastric cells are first of all neutral mucins, and secondly acidic mucins. Neutral mucins can be highlighted histochemically by the PAS (Periodic-Acid-Schiff) reaction, the technique first used to highlight mucin in 1946 by McManus. It has subsequently been shown that the technique can also be used for other types of carbohydrate-containing molecules (7, 8, 15). Acidic mucins can be highlighted due to their affinity for some basic dyes, the best known of which is Alcian blue (7, 10).

The present study aimed to highlight by histochemical reactions the neutral and acidic mucin secretory cells in the stomach of Chinchilla, concerning the number of cells, their distribution in the components of the gastric mucosa, and the intensity of secretion for each cell type.

MATERIALS AND METHODS

The biological material used in this study was represented by 5 fresh Chinchilla corpses, coming from a private breeder from Bistrița-Năsăud County (Romania) and slaughtered by the owner to harvest the furs. Stomach fragments were harvested from three regions: cardial, fundic, and pyloric. The harvested pieces were fixed in 10% buffered formalin for 7 days, then dehydrated in successive baths of ethyl alcohol in increasing concentration (70°, 96° and absolut), clarified in three baths of 1-Butanol and embedded in paraffin at 56°C by going through three bathrooms of 1 hour each. After pouring into paraffin blocks, sections with a thickness of 5 µm were made, which were subjected to processing for the histochemical highlighting of neutral and acidic mucins. To quantify neutral mucins, sections were processed by PAS reaction (7, 8, 15), and acidic mucins were highlighted by staining with Alcian blue (7, 10). The examination of the obtained histochemical preparations was done with an Olympus BX 41 microscope, equipped with a digital camera for capturing images, model Olympus E-330. The histochemical reaction in histological slides was assessed by grading from 0 (negative) to two pluses (i.e., + - faintly positive; ++ - intensely positive) that suggest the quantity of mucins in intestinal glandular cells.

RESULTS AND DISCUSSIONS

All cells lining the gastric mucosa and crypts in the cardiac region of the chinchilla stomach show an intense PAS-positive (++) reaction, with the specification that the positive PAS substances are concentrated in the apical half of the cells (i.e. apical poles). The intensity of the reaction shows that the cells on the surface of the gastric mucosa synthesize large amounts of neutral mucins. As for the cells in the walls of the cardial glands, the situation is different from the cells on the surface of the gastric mucosa. Most cells in the walls of the cardial glands are negative PAS (-) and only a small part of the cells arranged in the deep third of the glands are positive PAS (+). Moreover, the intensity of the PAS reaction in cardial gland cells is moderate, being well below that of surface cells and crypts (Fig. 1 A). Cells lining the gastric mucosa on the surface and in the crypts show a positive PAS reaction (++) in the fundic region of the Chinchilla's stomach. It should be noted that the location of PAS-positive substances is also in the apical pole of the cells and the intensity of the reaction is comparable to that shown in cells in the cardial region. Regarding the cells in the walls of the fundic glands, the situation is different in the sense that they are all PAS negative, which shows that they do not synthesize neutral mucins (Fig. 1 B).

The situation of the cells on the surface of the gastric mucosa and those lining the crypts is also preserved in the pyloric region in the sense that all cells are PAS positive (++) of intensity comparable to that of the cells on the surface of the other two regions described above. Regarding the cells of the pyloric glands, they are mostly PAS negative (-), and only in the deep area of the glands are identified isolated cells or small groups of weakly PAS-positive cells (+; Fig. 1 C).

Regarding the reaction with Alcian blue, the cells on the surface of the mucosa and in the crypts of the stomach of Chinchilla are negative (-) in all three regions of the stomach, which proves that they do not secrete acidic mucins. Regarding the gastric glands, the situation is different depending on the gastric region. Thus, in the cardia region only in a small number of cells in the deep portion of the glands, a moderately positive reaction to alcian-blue (+) is identified. In this situation, we can say that the cardiac glands in the chinchilla stomach synthesize a negligible amount of acidic mucins (Fig. 1 D). The glands in the fundic region do not contain alcian-positive (-) cells, which shows that acidic mucins are not synthesized in this region of the chinchilla stomach (Fig. 1 E). Some of the glands in the pyloric region of the Chinchilla's stomach contain groups of Alcian-positive cells whose intensity is very discrete (+), which shows that the mucosa in the pyloric region synthesizes small amounts of acidic mucins (Fig. 1 F).

Mucins are carbohydrates with a complex structure present in the epithelial cells that line the gastrointestinal tract (GIT). The normal distribution of these mucins in different parts of GIT, as well as their alteration in various inflammatory diseases, benign and malignant tumor lesions, has aroused particular interest in the field of mucin histochemistry (12).

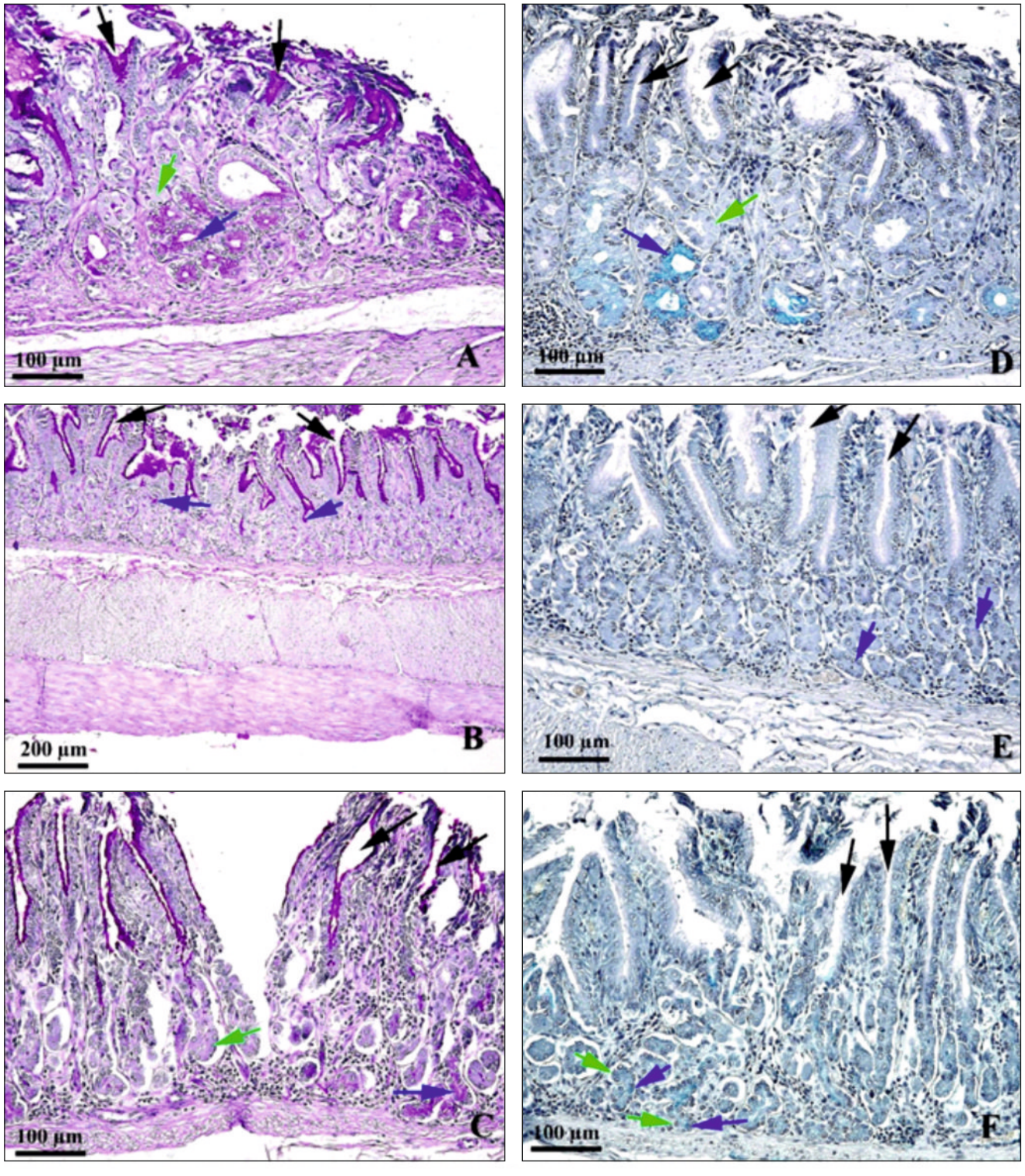

Fig. 1. A. Stomach, cardial region (PAS stain): black arrow – gastric crypts with PAS++ cells; blue arrow – glandular PAS+ cells; green arrow - glandular PAS- cells. **B**. Stomach, fundic area (PAS stain): black arrow – gastric crypts with PAS++ cells; blue arrow - glands with PAS- cells. **C**. Stomach, pyloric area (PAS stain): black arrow – gastric crypts with PAS++ cells; blue arrow - glandular PAS+ cells; green arrow - glandular PAS- cells. **D**. Stomach, pyloric area (Alcian Blue stain): black arrow – gastric crypts; blue arrow - glandular Alcian++ cells; green arrow - glandular Alcian- cells. **E**. Stomach, fundus area (Alcian blue stain): black arrow – gastric crypts; blue arrow – glandular Alcian- cells. **F**. Stomach, pyloric area (Alcian blue stain): black arrow – gastric crypts; blue arrow - glandular Alcian+ cells; green arrow – glandular Alcian- cells.

The epithelium of the human gastric mucosa produces different types of mucins. Stomach mucins are large glycoproteins with carbohydrate side chains, N-acetyl derivatives of hexosamine and terminal group of sialic acid (11). Mucins perform significant functions such as selective impermeability to hydrochloric acid and bile salts, antibacterial effect, lumen lubrication, adjusts epithelial dehydration, prevents tissue edema, have an antimetastatic and immunological role. Any change in mucin production, either quantitative or biochemical, may have major implications for the etiology of intestinal metaplasia of the gastric mucosa and gastric erosions or ulcers (6, 9). In order to detect altered mucin secretions, histochemical labeling methods can be used, which can confirm the diagnosis and prognosis of gastric pathology (12).

In the present study, positive PAS cells of comparable intensity were identified in all areas of the stomach, respectively both in the cells in the surface epithelium of the gastric mucosa and in those lining the gastric crypts. In their case, only the apical half of the cells show an intense PAS-positive reaction, because at this level the mucus synthesized by these cells accumulates. The intensity of the reaction shows that these cells synthesize large amounts of neutral mucus. The situation is totally different in the gastric glands where there are differences from one region of the stomach to another. In the cardial region, PAS-positive cells were identified only in some glands, but with a multifocal distribution. It should be noted that the number of positive PAS cells in this region is small and the intensity of the reaction is low to moderate. These aspects show that the glands in the cardia region synthesize neutral mucins in small amounts. The glands in the fundic region of the Chinchilla's stomach do not contain PAS-positive cells, which shows that acidic mucins are not synthesized here. Only some of the glands in the pyloric region of the chinchilla stomach have a small number of PAS-positive cells, but the intensity of the reaction is low. The glands in the pyloric region of the Chinchilla's stomach synthesize neutral mucins in very small amounts.

The cells on the surface of the mucosa, as well as those lining the gastric crypts, do not synthesize acidic mucins (negative reaction to Alcian blue), an aspect found in all three regions of the chinchilla stomach. Regarding the glands, those in the fundic region do not have alcian-positive cells. Regarding the glandular epithelium of the chinchilla's stomach mucosa, a moderate secretion of acidic mucins (weak alcian-positive reaction) was identified only in the glands of the cardia and pyloric region, an aspect observed in small groups of cells located in the deep area of the glands.

As a result, the histochemical investigations we performed on the chinchilla stomach showed that the gastric mucosa synthesizes very large amounts of neutral mucins and very small amounts of acidic mucins. Similar results on the predominant synthesis of neutral mucins have been found in other mammalian species, including humans (13). Our results are similar to those in the literature and in terms of histochemical behavior of cells in the walls of the fundic glands, which are negative on both the reaction of PAS and Alcian blue. The absence of the two histochemical reactions is due to the fact that the fundic glands have walls formed by parietal cells and main cells, which synthesize the precursors of hydrochloric acid and pepsinogen (2).

The situation is different in the cardial area, both in terms of the number of cells positive for the two histochemical reactions and the intensity of the reaction. According to the information in the literature, the cells of the gastric cardial glands are synthesizing mucins in humans (13), stating that it is neutral mucus (4). A similar situation is reported in other mammals, noting that the mucous glands in the cardia region also contain rare parietal cells (2). In the species we studied, the basic secretion of the cells of the cardiac glands is not the mucous one, as indicated in other mammalian species. The glands in the Chinchilla pyloric region also do not produce significant amounts of neutral mucins, an aspect compared to other species (4).

The small number of positive Alcian cells in the cardia and pyloric regions of the Chinchilla's stomach is also different from other mammalian species. The relatively large differences between one species and another between the type and intensity of the secretion of the gastric cardial glands and especially the pylorus suggest that their acidic mucous secretion does not have the same functional significance in all mammalian species. According to data from the literature, the pyloric glands synthesize large amounts of mucins in goats, pigs, dogs (1, 2), average mucin amounts in humans (4, 13) and low amounts in guinea pigs (5). Among the mentioned species, the situation of guinea pigs is the closest to that of Chinchilla, but also between these two species, there are differences, in the sense that Chinchilla has the lowest mucous secretions of the pyloric glands.

CONCLUSION

The cells of the surface epithelium of the gastric mucosa and those lining the gastric crypts show an intense positive PAS reaction in all areas of the stomach and a negative alcian reaction, which suggests that they synthesize only neutral mucins. The cells of the gastric fundic glands are negative for the two histochemical reactions, while the epithelium of the cardial and pyloric glands contains small groups of PAS-positive and alcian-positive cells, which suggests a reduced secretion of mucins at this level. As a gene-

ral aspect, the gastric mucosa of chinchilla synthesizes large amounts of neutral mucins and small amounts of acidic mucins, which represent the morphophysiological features of the studied species.

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