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A Screening of Mucin-Producing Cells and Structures, from Digestive System, in Rats

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RESEARCH ARTICLE

Abstract

A screening and a morphological analysis of mucin-producing cells and structures, from the digestive tract, in rats, will further allow to quantify their local and systemic response. 10 Wistar rats were selected for this study. Sections of the sublingual gland, Weber's gland, stomach, jejunum and colon were taken. The samples were stained by the Trichrome Goldner technique. Based on the histological examination, both morphometric and statistical analysis were performed using specific software's (ToupView Soft and GraphPad Prism Software). The histological examination showed normal characteristics for the analyzed cells and structures. The test for the normal distribution of the data revealed that they are normally distributed and statistical comparison between cell surfaces suggested a significant difference between means with $p<0.0001$. Moreover, statistically significant differences were observed between the areas of Weber gland acini and those of sublingual gland acini ($p<0.05$). Based on our results we can conclude that the observed differences, regarding the cellular surfaces, are in accordance with the physiological characteristics of each analyzed structure.

Keywords: histological examination; morphological analysis; mucin-producing cells; statistical analysis.

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INTRODUCTION

The digestive tract is one of the main ways for pathogens to enter the body, and protective mechanisms are needed to prevent this from happening. The mucus layer lining the digestive tract has a key role in maintaining homeostasis. The integrity of the physical barrier is possible because digestive enzymes are not able to damage the glycosides bonds of mucins (Hansson, 2020). Mucins are high molecular weight glycoproteins produced by different secretory cell types, depending on the organ, with structural and functional roles within the mucus layer (Dharmani et al., 2008; Kim and Ho, 2010). These, through their hydrophilic properties, represent the basic structural component of mucus, giving it its physical characteristics (Dharmani et al., 2008). In the oral cavity, in addition to the mucous membrane that lines it, there are also major and minor salivary glands, whose secretion, including certain mucins, forms saliva (Amano et al., 2012). Both humans and rodents (mice and rats) have three pairs of major salivary glands: parotid, submandibular, and sublingual (Amano et al., 2012) and another three pairs of minor salivary glands: Blandin-Nuhn, von Ebner, and Weber (Nagato et al., 1997). The secretory component of these structures is organized in the form of acini, the cells that make up the adenoma having a serous, mucous or mixed production (Amano et al., 2012). The rodent esophagus is devoid of mucous secretory

structures, the mucins and mucus at this level originating from the oral cavity (Kinoshita et al., 1999).

In the stomach there are cells whose functions include the secretion of mucins: surface mucous cells and mucous neck cells (Helander, 1981; Laine et al., 2008). Some researchers (Wattel et al., 1997a,b) have reported the presence of a third type of mucous cells, the intermediate ones, whose secretory granules represent a mixture between the secretions of the other two cell types.

At the level of both small and large intestinal, the main structures involved in the secretion of mucins are the goblet cells (Paone, 2020). At the level of these segments, especially in the colon, there is another type of mucin-producing cells called deep crypt secretory cells, which have physiological characteristics different from goblet cells (Altmann, 1983; Chende et al., 2022).

Thus, it is important to maintain the structure of the mucus layer in the segments of the digestive tract, but at the same time the integrity of the epithelia must be preserved, because in addition to the secretion of mucins, the previously mentioned cells also have the role of secreting other molecules involved in immunity processes and body recovery (Kim and Ho, 2010).

Based on these considerations, it is desirable to know as many morphofunctional aspects of these mucin secretory structures as possible, further allowing to quantify their local and systemic response.

MATERIALS AND METHODS

Biological material

10 Wistar rats (5 females and 5 male) aged 7–8 weeks and body weight 160 g ± 32 g were selected for this study. They were purchased from the Biobase of the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca. The animals were housed in the institutional Biobase of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, under conditions adapted to their physiological needs and in accordance with the legislation in force: controlled microclimate with a constant temperature of 23°C and alternating cycles of 12 h light-dark, water and food *ad libitum* and appropriate animal hygiene conditions. The animals were acclimatized for a period of 6 days, before performing any intervention.

Bioethics Commission approval

The experiment received the favorable opinion of the USAMV Cluj-Napoca Bioethics Commission, no. 248 of 29.03.2021 and project authorization no. 258 of 13.05.2021 from the National Sanitary Veterinary and Food Safety Authority (ANSVSA).

Histological examination

In order to perform the screening of mucin-producing cells and their morphological analysis, portions of the sublingual gland, Weber's gland, stomach, jejunum and colon, with dimensions ≤5 mm, were taken. After harvesting the tissues, they were kept for 24 hours in containers with 10% formalin solution. The next steps involved shaping, dehydration by successive immersion in 96° alcohols (absolute and butyl) and embedding the preparations in paraffin. The paraffin blocks in which the tissues were embedded were sectioned at a thickness of 5 µm with the Leica RM 2125 microtome, and the obtained samples were spread on pre-degreased slides. For the morphological examination, all the samples of major and minor salivary glands, stomach, small and large intestine were stained by the Trichrome Goldner (TG) technique and examined with an Olympus BX41 microscope connected to an Olympus E-330 digital camera (Olympus, Japan). The images taken were processed using Adobe Photoshop 2021 version 22.0.1.

Morphometric analysis

The measurements of the surfaces of the mucin-producing elements were made using the ToupView (Amscope) Soft program.

Statistical analysis

As part of the experiment in order to perform the screening of the mucin-producing structures, the data obtained were statistically analyzed using the GraphPad Prism Software program, version 8.0.1. The D'Agostino & Pearson test and the Kolmogorov-Smirnov test were performed for the normal distribution of the data. The statistical comparison between the cell surfaces was performed with the help of One-way ANOVA and Tukey's post hoc test, the $p \leq 0.05$ value being considered statistically significant. The T-test was also performed, which statistically compared the sizes of the acinar surfaces. In addition, the descriptive statistics for all the analyzed structures were also made.

RESULTS AND DISCUSSIONS

Morphological analysis of mucin-producing structures

Table 1 shows the results of the descriptive statistics for the surfaces (μm^2) of all cell types with a role in the

synthesis and secretion of mucins, evaluated in this study. The results of the test for the normal distribution of the data revealed that they are normally distributed (D'Agostino & Pearson test – alpha≤0.05; Kolmogorov-Smirnov test – *p*>0.1000), therefore no correction was applied to the data.

Tabel 1. Descriptive statistics of surface areas (μm^2) of mucin-producing cells

	Weber's gland	Sublingual gland	Stomach	Goblet cells small intestine	DCS small intestine	Goblet cells large intestine	DCS large intestine
Number of values	19	19	19	19	19	19	19
Minimum	805.3	495.5	279.1	448.2	538	557.4	721.8
Maximum	1685	881.3	558.5	887.8	1049	1075	1585
Range	879.7	385.8	279.4	439.6	510.8	517.6	863.1
Mean	1212	682.9	373.5	618.4	780.3	765.5	1020
Std. dev.	225.8	106.7	79.09	125.6	177.1	155.1	217.3
Std. error of mean	51.8	24.47	18.14	28.82	40.64	35.59	49.84
Coefficient of variation	18.64%	15.62%	21.18%	20.32%	22.70%	20.26%	21.30%

Oral cavity

Among the major salivary glands in rodents, only the sublingual has a mixed secretion, while the parotid and submandibular glands contain only acinar cells with serous production. The sublingual glands represent a paired organ located in the anterior part of the neck, between the submandibular lymph node and the sternum. Microscopically, these glands are composed of small acini, with a central arrangement of mucous cells, organized in the form of tubulo-acinar structures and a peripheral distribution of cells with serous secretion, in the shape of demi-lunes (Amano et al., 2012). These aspects can be observed in our histological samples as well (Figure 1).

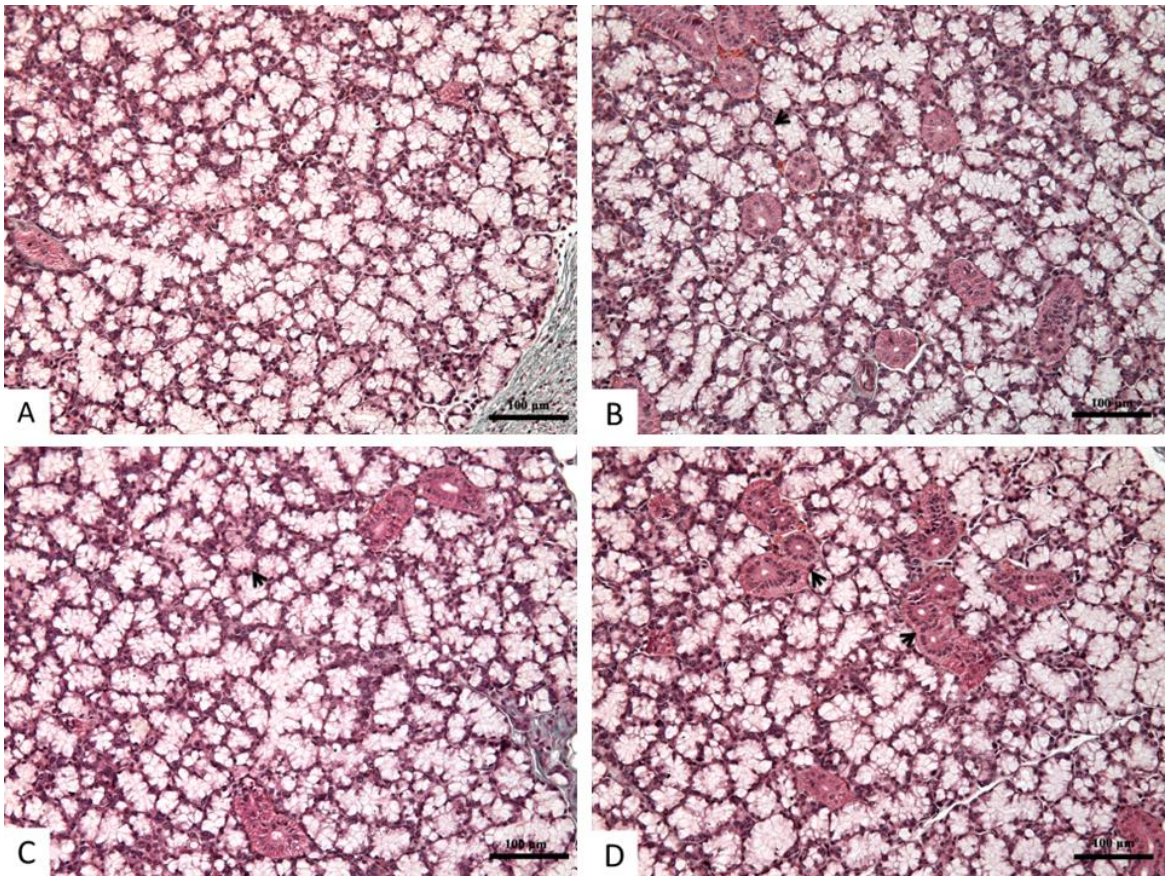

Figure 1. The microscopic features of sublingual gland (Wistar rats, Trichrome Goldner staining, 20X): A – mucous part of the gland; B –mucin-producing cells (black arrow); C –mucous acini (black arrow); D – intra-lobular excretory duct (black arrow)

Weber's gland, located at the base of the tongue, belongs to the category of minor salivary glands, in rats it is a gland with mixed secretion. Histologically, Weber's gland has a tubulo-acinar appearance, classic for gland types with both serous and mucous secretion. The serous secretory component is arranged in a demilune shape, while the mucous cells are pyramidal or columnar in shape and intermixed with the serous cells. Cell types are differentiated both morphologically and by the microscopic appearance of the secretory granules they contain (Nagato et al., 1997). The micro anatomical aspects mentioned in the literature mentioned above, were also identified in the histological preparations made by us (Figure 2).

The major secretory component being the mucous one, these salivary glands are of interest for the present study.

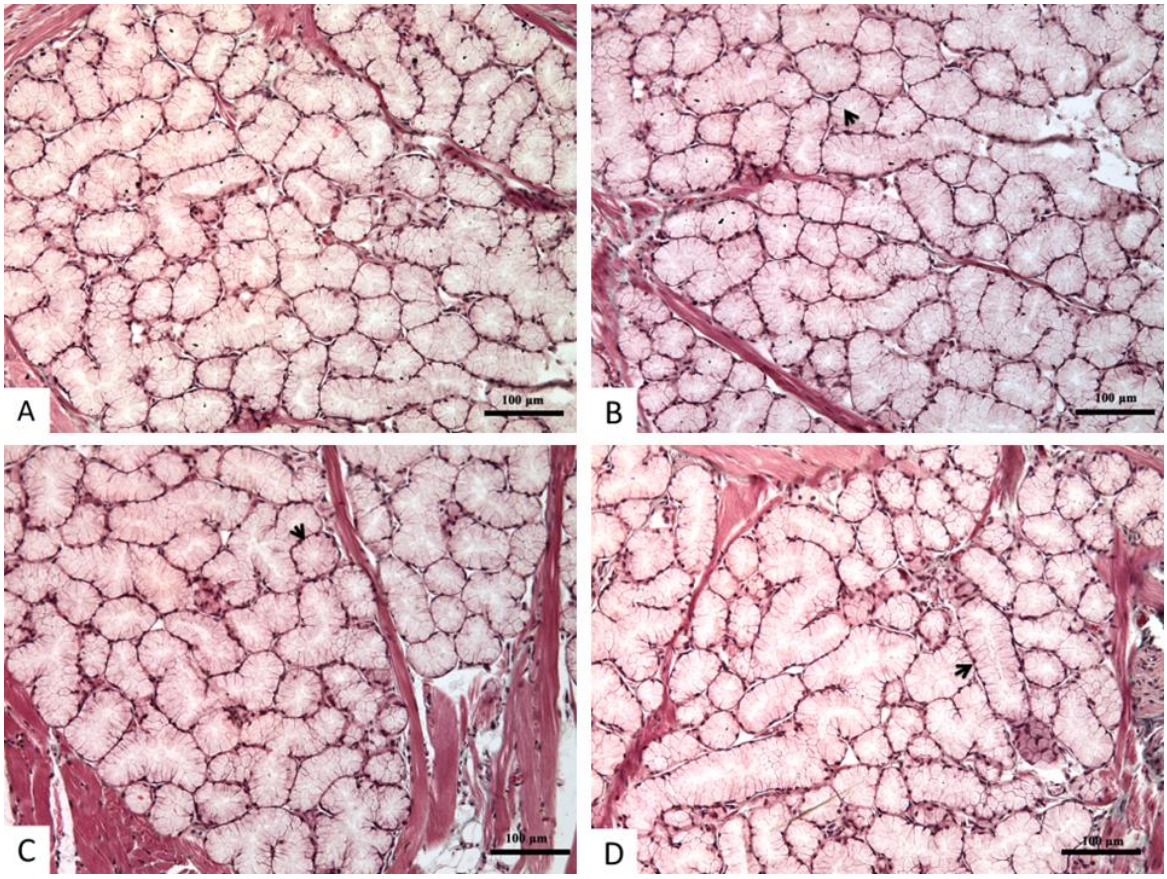

Figure 2. The microscopic features of Weber's gland (Wistar rats, Trichrome Goldner staining, 20X): A – mucous part of the gland; B –mucin-producing cells (black arrow); C –mucous acini (black arrow); D – collector duct (black arrow)

In the case of these two organs, that have the secretory component organized in acinar form, the surfaces of the mucous acini were also analyzed (Table 2) and compared with each other using the T test.

Tabel 2. Descriptive statistics of surfaces (µm^2) of mucous acini

	Weber's gland	**Sublingual gland**
Number of values	30	30
Minimum	5677	3738
Maximum	81484	10772
Range	75807	7035
Mean	11160	6166
Std. deviation	13380	1774
Std. error of mean	2443	323,9
Coefficient of variation	119.9%	28.78%

T-test results suggest that there are statistically significant differences between the areas of Weber's gland acini and those of sublingual gland (p<0.05). Also, the value of the coefficient of variability (CV%) indicates that at the level of the Weber's gland, the acinar surface shows a high polymorphism (CV% = 119.9%), while the differences in the surface of the acini in the sublingual gland are moderate (CV % = 28.78%). These results are confirmed by the F-test (which compares the variance): p<0.0001**.**

Esophagus

The esophagus of some rodents, including rats, is known to lack submucosal glands or mucin-secreting cells. The protection of this organ is conferred by the mucins from the oral cavity. Salivary glands secrete these glycoproteins, which end up making up the mucus layer that lines the esophagus, maintaining the integrity of this structure (Kinoshita et al., 1999). Taking into account the mentioned aspects, this segment is not a point of interest for the conducted study.

Stomach

In the gastric mucosa are found multiple cell types, some of them with role in the production of the mucins: the surface mucous cells, the mucous neck cells and the intermediate ones (Helander, 1981; Wattel et al., 1997a,b; Laine et al., 2008).

The surface ones are columnar cells, becoming cuboidal towards the isthmus area. In general, the cells in the upper segment of the crypts secrete the greatest amount of mucins, which is why they are easier to identify at this level. The secretory granules are mainly found at the level of the supranuclear cytoplasm, being so compact that they deform each other. The second mucin-producing cell type is mainly located in the neck and isthmus area. Their shape is cuboidal, sometimes round or pear-shaped, with a dilated region at the apical pole. As in the surface cells, the mucous granules are located mostly in the supranuclear cytoplasm, having different densities but being less dense and larger than the others (Helander, 1981). A third type of mucin-secreting cells with mixed granule characteristics between those of surface cells and those of the neck region has also been described (Wattel et al., 1977a, b). The described aspects can be observed in the histological preparations made by the authors (Figure 3).

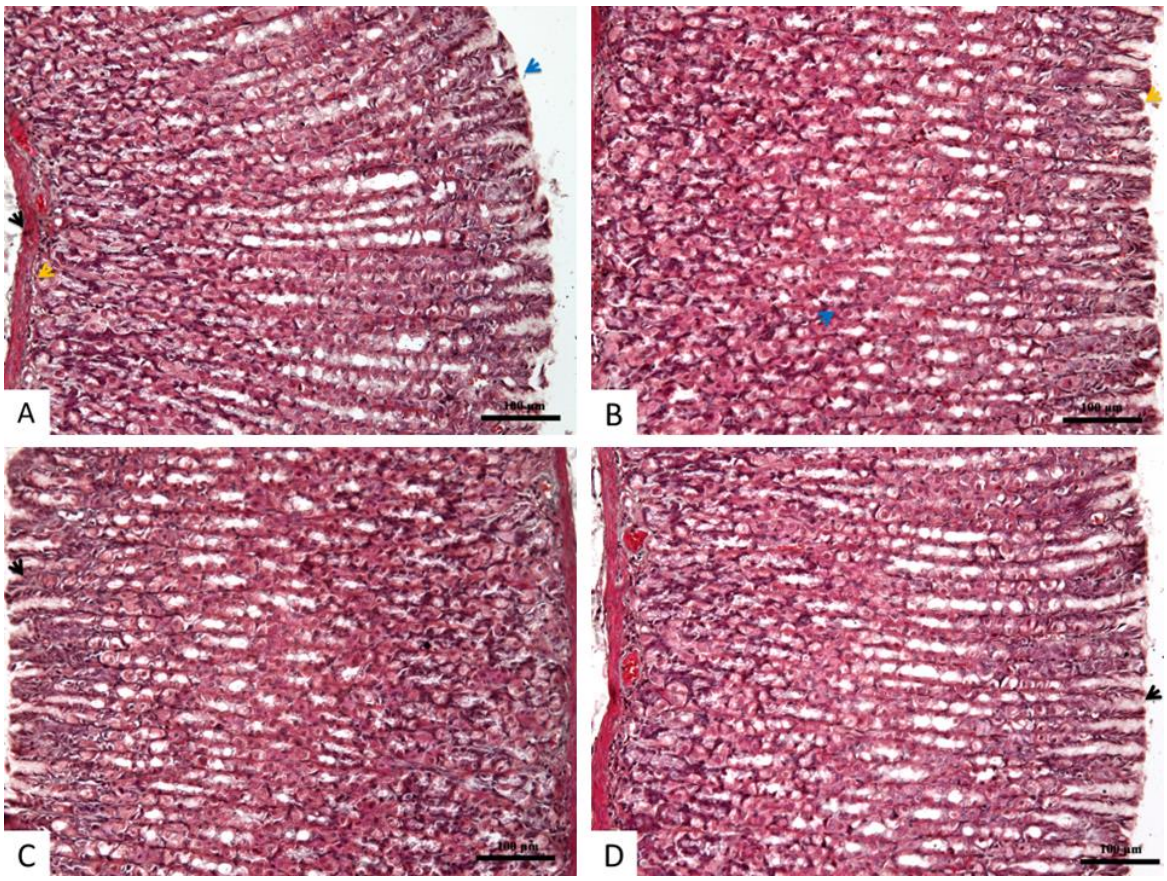

Figure 3. The microscopic features of stomach (Wistar rats, Trichrome Goldner staining, 20X): A – the appearance of the epithelium (blue arrow), lamina propria (orange arrow) and muscularis mucosae (black arrow); B – the surface epithelium (orange arrow) and the glandular epithelium (blue arrow); C – intestinal crypts (black arrow); D – cytoplasm with foamy appearance (black arrow)

The histological preparations made were also analyzed, selecting a number of 19 mucin-producing cells from the digestive mucosa in order to perform morphometry. The minimum area of these cells was 279.1 μm^2, while the maximum 558.5 μm^2 and mean 373.5 $\mu m^2 \pm 79.09 \mu m^2$, resulting in a coefficient of variation of 21.18%. It supports the morphological uniformity of the cells involved in the secretion of mucins from the gastric mucosa.

Small and large intestine

The mucosa of the intestine is made up of surface and glandular epithelium, chorion and muscularis mucosae, at the level of intestinal epithelium being found several cell types, including cells with a role in the synthesis and secretion of mucins (Miclăuș et al., 2017). Among these cells we mention: goblet cells and non-goblet cells, also called deep crypt secretory cells (DCSc) (Altmann, 1983; Chende et al., 2022).

Goblet cells have the shape of a calyx (cup), with the apical pole more dilated and the basal one narrower, their cytoplasm being full of mucin granules, the main function being the synthesis and secretion of mucus (Birchenough et al., 2015). They are located at the level of the surface epithelium, both in the small and in the large intestine, their percentage among all cell types increasing from one intestinal segment to another, being the highest in the distal colon (Kim and Ho, 2010).

The terminology of non-goblet cells is controversial, the most used variant in the literature being deep crypt secretory cells (DCSc) (Altmann, 1983; Chende et al., 2022). Initially these DCSc were identified, in certain species including rats, in the large intestine, especially in the colon (Altmann, 1983). Recent studies have also reported their presence in the small intestine of guinea pigs (*Cavia porcellus*), specifically in the jejunum and ileum. These cells are larger in size than the goblet cells, have a pyramidal shape, with the base at the level of the basement membrane, and in the usual staining (TG) it is observed that they have multi-vacuolate cytoplasm, full of mucus, and their nucleus is oval, pushed basally and parallel to the cell membrane (Chende et al., 2022).

The different cell types and normal characteristics of the small intestine mucosa were also observed in our histological preparations (Figure 4 and Figure 5).

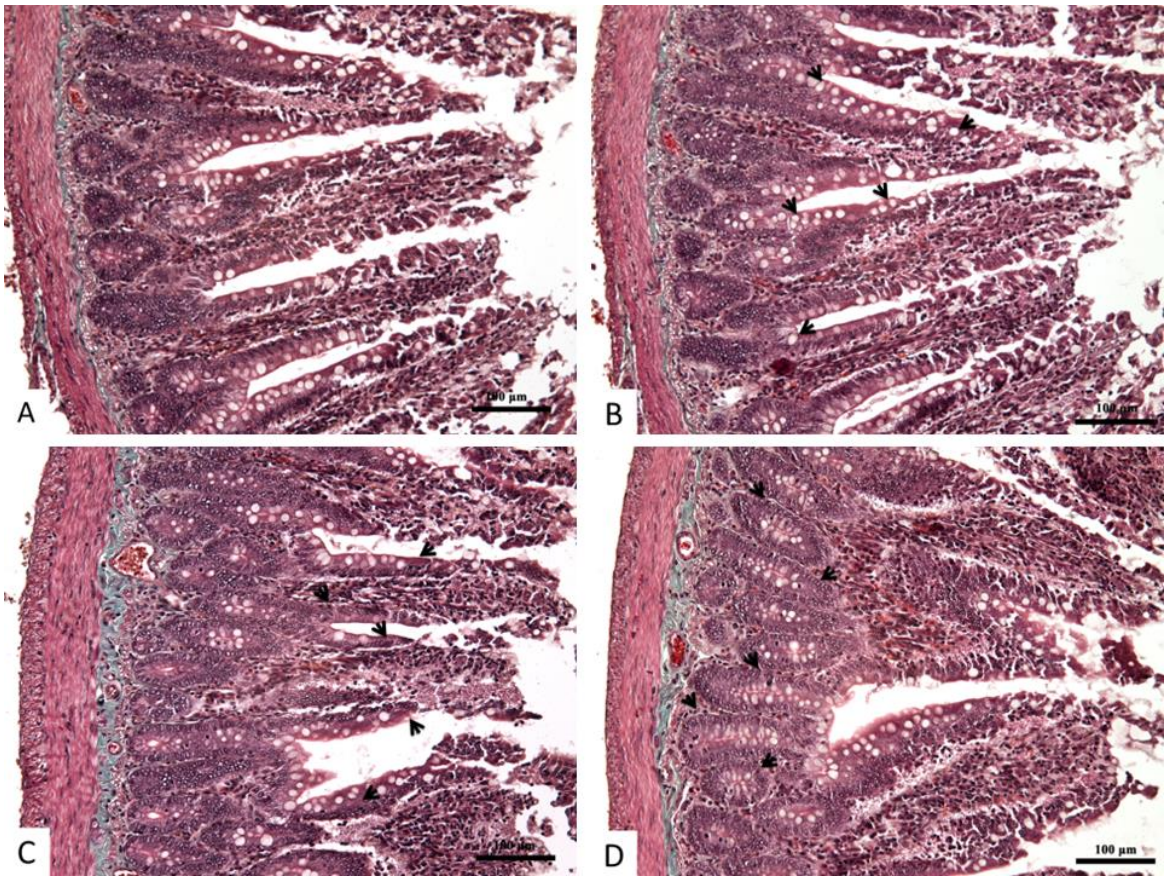

Figure 4. The microscopic features of small intestine (jejunum) (Wistar rats, Trichrome Goldner staining, 20X): A – the normal appearance, without cellular infiltrate, of the intestinal villi; B – goblet cells (black arrow); C – enterocytes (black arrow); D – intestinal glands (black arrow)

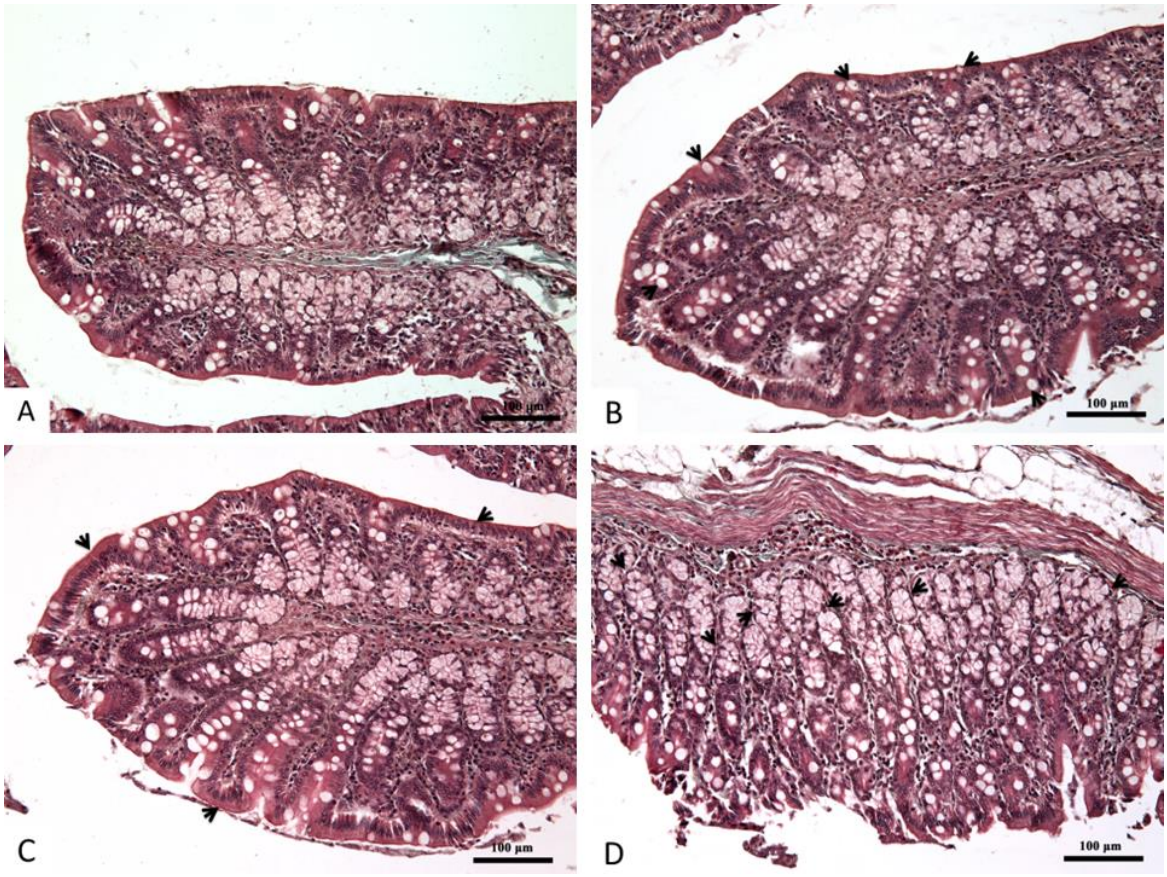

Figure 5. The microscopic features of large intestine (colon) (Wistar rats, Trichrome Goldner staining, 20X): A – the normal appearance, without inflammatory cellular infiltrate, of the intestinal crypts; B – goblet cells (black arrow); C – surface columnar epithelium (black arrow); D – intestinal glands (black arrow)

Regarding the number of goblet cells on a given surface, an average of 40.25 goblet cells/ 281971.5517 µm^2 was identified in the small intestine and an average of 83 goblet cells/611594.2 µm^2 in the large intestine. The morphometric analysis of both goblet cells and DCSc showed that the average size of DCSc is larger than that of goblet cells (Table 1), results consistent with the information mentioned in other studies (Chende et al., 2022).

The coefficients of variability calculated for these cell types, both in the small intestine and in the large intestine, revealed a moderate polymorphism in the case of cells belonging to the same type. In the study conducted by Chende et al. (2022) the same physiological characteristic was observed in guinea pigs.

Comparative analysis of mucin-producing structures

Statistical comparison between cell surfaces was performed using One way ANOVA test and Tukey's post hoc test. The first one suggested a significant difference between means (p<0.0001), while the statistical analysis carried out, through Tukey's post hoc test, gives us the comparative table of the surfaces (µm^2) of mucin-producing cells (Table 3; Figure 6).

Tabel 3. Statistical differences between mean surfaces (µm^2) of mucin-producing cells (Tukey's post hoc test)

	Number of cells	Mean (µm^2) Std. deviation (µm^2)
Weber's gland	19	1212 ± 225.8 ^{b,c,d,e,f,g}
Sublingual gland	19	682.9 ± 106.7 ^{a,c,g}
Stomach	19	373.5 ± 79.09 ^{a,b,d,e,f,g}
Goblet cells small intestine	19	618.4 ± 125.6 ^{a,c,e,g}
DCSc small intestine	19	780.3 ± 177.1 ^{a,c,d,g}
Goblet cells large intestine	19	765.5 ± 155.1 ^{a,c,g}
DCSc large intestine	19	1020 ± 217.3 ^{a,b,d,e,f}

Note: values represent the mean ± standard deviation of 19 measurements of mucin-producing cells. $^{a-g}$ means marked with letters per column indicate statistical differences $p \leq 0,05$

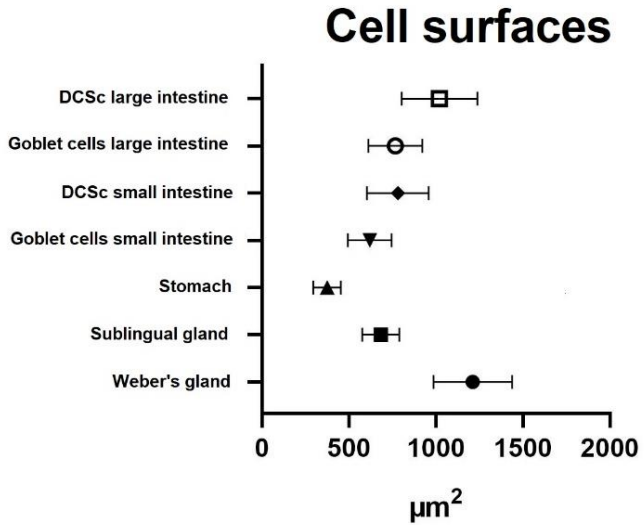

Figure 6. Surface areas of mucin-producing cells (µm^2)

The obtained results reveal a significantly larger surface of the mucous cells of the Weber's gland, compared to all cell types investigated. At the same time, the average surface area of mucin-producing cells in the stomach is significantly smaller. In both small and large intestine, the average surface of the goblet cells is significantly smaller compared to the DCSc in the same segment. We specify the fact that the surface area of DCSc in the large intestine is significantly higher than those in the small intestine. We believe that the observed differences are due to the different functionality of the analyzed segments and the fact that at each level there are also specific mechanisms involved in local immunity.

CONCLUSIONS

The mucus layer is not the only protective barrier at the level of these structures, each organ having multiple types of defense. Our findings lead us to draw the conclusion that the observed variations in cellular surfaces are consistent with the physiological features of each examined structure. The sizes of mucin-producing cells are related to the need for the existence of the mucus layer.

Author Contributions: I.I., L.C.S. Conceived and designed the analysis; I.I., M.-C. M.L., V.R., L.C.S. Collected the data; I.I., A.-F. G., M.-C. M.L., V.R., C.R. Contributed data or analysis tools; I.I., M.-C. M.L. Performed the analysis; I.I. Wrote the paper.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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