Int. J. Morphol.,
40(4):915-919, 2022.

Histochemical Assessment of Mucin-Secreting Cells in the Stomach of Domestic Rabbit

Evaluación Histoquímica de Células Secretoras de Mucina en el Estómago de Conejo Doméstico

Mircea Cipou[1]; Aurel Damian[1]; Vasile Rus[1]; Cristian Martonos[1]; Ioana A. Ratiu[2]; Viorel Miclaus[1] & Adrian F. Gal[1]

CIPOU, M.; DAMIAN, A.; RUS, V.; MARTONOS, C.; RATIU, I. A.; MICLAUS, V. & GAL, A. F. Histochemical assessment of mucin-secreting cells in the stomach of domestic rabbit. *Int. J. Morphol., 40(4)*:915-919, 2022.

SUMMARY: The mucous substances of the stomach in mammals are important not only for the protection of the gastric epithelium from the acid environment and grinding actions, but it facilitates some other functions of the stomach such as antibacterial, antimetastatic, and immunological roles. The goal of the study is to highlight the distribution of mucin-secreting cells in the gastric mucosa in domestic rabbits, including the type of mucus synthesized. The gastric samples collected from ten individual rabbits were fixed in 10 % buffered formalin and underwent later standard paraffin tissue sample processing, which included dehydration, clarification, and embedding in paraffin. The tissue sections were eventually stained histochemically by PAS reaction and by Alcian blue method (pH 2.5) for neutral and acidic mucins detection, respectively. The quantification of mucins in the cytoplasm of mucus-secreting cells was performed by grading the gastric tissue samples from negative (-) to intensely positive (++). The mucus elaboration was observed in all the regions of the stomach (i.e., cardial, fundic, and pyloric regions), but only for the neutral mucin. The acidic mucin synthesis occurred only in the secretory units of the gastric glands from the cardial region in the stomach. Pyloric glands synthesized the largest amounts of neutral mucins, followed by moderate amounts elaborated by cardial glands, while the fundic region does not synthesize it at all. The description of new microscopic features of the stomach in rabbits is fundamental not only for comprehending species-related physiological features but gastric pathological processes.

KEY WORDS: Stomach; Mucins; Gastric mucosa; Rabbit.

INTRODUCTION

In the stomach of mammals are present cells that secrete mucin that is made up of carbohydrates. These mucous substances are very important for the proper functioning of the stomach which facilitates selective impermeability to hydrochloric acid, lubrication of the lumen, and prevention of tissue edema. Additionally, they also have antibacterial, antimetastatic, and immunological roles. In humans, the mucins synthesized by the gastric cells are large glycoproteins with side carbohydrate chains and a terminal group of sialic acid (Prasanna *et al*., 2015). Their distribution and activity in the stomach differ from one species to another, the secretion being adapted quantitatively and qualitatively to the volume and composition of the food ingested (Gal & Miclaus, 2013; Liebich, 2019).

When the distribution of mucin-secreting cells in the stomach is inappropriate or their functionality is impaired, functional deficiencies may occur, such as intestinal metaplasia of the gastric mucosa, or gastric erosions/ulcers (Corfield *et al*., 2000; Linden *et al*., 2008). The administration of nonsteroidal anti-inflammatory drugs may predispose to gastric ulcerative lesions along with inhibition of prostaglandin metabolism (Aughey & Frye, 2001). Alteration of mucin secretion can be identified by histochemical reactions, a fact that can provide usefull evidence about the state of the gastric mucosa in various pathological conditions (Prasanna, 2016). The main mucins synthesized by the glandular cells of the gastric mucosa are neutral mucins and acidic mucins, which can be identified by PAS reaction for neutral mucins (Wulff, 2004; Layton & Brancoft, 2013) and by alcian blue stain for acidic mucins (Kiernan, 1999). The aim of the study is the identification and distribution of mucin-secreting cells in the gastric mucosa in domestic rabbits, including the type of mucus synthesized in the stomach.

[1] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, Calea Manastur 3-5, 400372, Cluj-Napoca, Romania,
[2] First Decembre Square nr 10, Oradea, 410073, Bihor, University of Oradea, Faculty of Medicine and Pharmacy, Romania.

Received: 2022-05-29 Accepted: 2022-06-13

MATERIAL AND METHOD

The biological material used in this study was represented by tissue samples harvested from ten common breed domestic rabbits which were slaughtered for meat consumption in a slaughterhouse. The gastric fragments were collected from the three gastric zones: cardial, fundic, and pyloric regions. The samples were fixed in 10 % buffered formalin for three days, dehydrated in three baths of ethylic alcohol in ascending concentration, an hour each, and clarified in three baths of 1-Butanol (an hour each bath) and embedded later in paraffin. Eventually, the samples were cut at a thickness of 5 micrometers using a rotative Leica rotary microtome (RM2125, Germany) and displayed on the histological slides for further histochemical staining. Quantification of neutral mucins was made using the PAS reaction, and by Alcian blue staining at pH 2.5 for detection of acidic mucins (Kiernan,1999; Suvarna *et al*, 2018). The obtained slides were assessed with the aid of an Olympus BX 41 (Olympus, Japan) microscope that was connected to an Olympus E-330 digital camera (Olympus, Japan) for image capture and analysis. Quantification of mucin was performed by assessing the intensity of the histochemical reaction from the cytoplasm of the mucus-secreting cells and scoring as follows: negative reaction (-), faintly positive (+), and intensely positive (++).

RESULTS

In the cardial region of the rabbit stomach, the mucosa contains PAS-positive cells, both in the covering epithelium (+), including in the epithelium lining the gastric pits (++), and in the secretory units of the gastric glands (+). Consequently, most of the cells of the covering and glandular epithelium from the cardia region of the stomach in rabbits synthesize significant quantities of neutral mucins (Fig. 1A). As regards the acidic mucin production in the cardia region, the alcian-blue stain was positive (++) only in the lining epithelium of the secretory units of the gastric mucosa, and absent (-) in the covering epithelium of the mucosa and the lining epithelium of the gastric pits (Fig. 1B; Table I).

Regarding the distribution of mucins in the fundic region of the stomach, neutral mucins elaboration is restricted to the covering epithelium (++) and the epithelium lining the gastric pits (+), but not in the epithelium belonging to the secretory units of the gastric glands from this region (-) (Fig. 1C). Interestingly, no acidic mucins (-) can be detected in the cells belonging to the mucosa from the fundic region (Fig. 1D; Table I).

In the pyloric region, the only mucus synthesized is the neutral one (Fig. 1E), a fact suggested by an intense PAS-positive reaction (++) in both covering mucosal epithelium and deep glandular epithelium (including in the cells from the gastric pits). As suggested, no acidic mucin is elaborated by the cells of the mucosa from the pyloric region (Fig. 1F; Table I).

Morphologically, the mucus synthesizing cells display a different morphology depending on their location in the gastric mucosa. The cells from the covering epithelium have a columnar appearance and a large mucous droplet in the apical pole, and a central or subcentral nuclear location (Figs. 1A,C,E). In the region of the gastric pits, the mucous cells are low columnar to cuboidal, the mucus being localized still in the apical pole providing a fine foamy appearance, whereas the nucleus is spherical and pushed towards the basal pole (Figs. 1A,B). However, the mucous cells from the secretory units of the gastric glands are cuboidal, with a foamy aspect of the cytoplasm and a basally located nucleus with an irregular outline (Figs. 1A,B,E).

DISCUSSION

Most of the surface areas of the stomach and the epithelium lining the gastric pits are covered with mucous cells. These cells synthesize a thick and persistent mucus that is important for protecting the gastric epithelium from the acid environment and grinding action that is present in the lumen. Once the mucus-secreting cells are damaged, the gastric ulcer is a frequent outcome (Herdt, 2020). Regarding the mucins, the main building structure in mucus are mucins that are large glycosylated proteins. The mucins include more

Tabla I. The distribution of mucus-secreting cells in the gastric mucosa in rabbits.

Gastric zone	Covering epithelium		Epithelium of the gastric pits		Glandular epithelium	
	PAS stain	Alcian blue stain	PAS stain	Alcian blue stain	PAS stain	Alcian blue stain
Cardial zone	+	-	++	-	+	++
Fundic zone	++	-	+	-	-	-
Pyloric zone	++	-	++	-	++	-

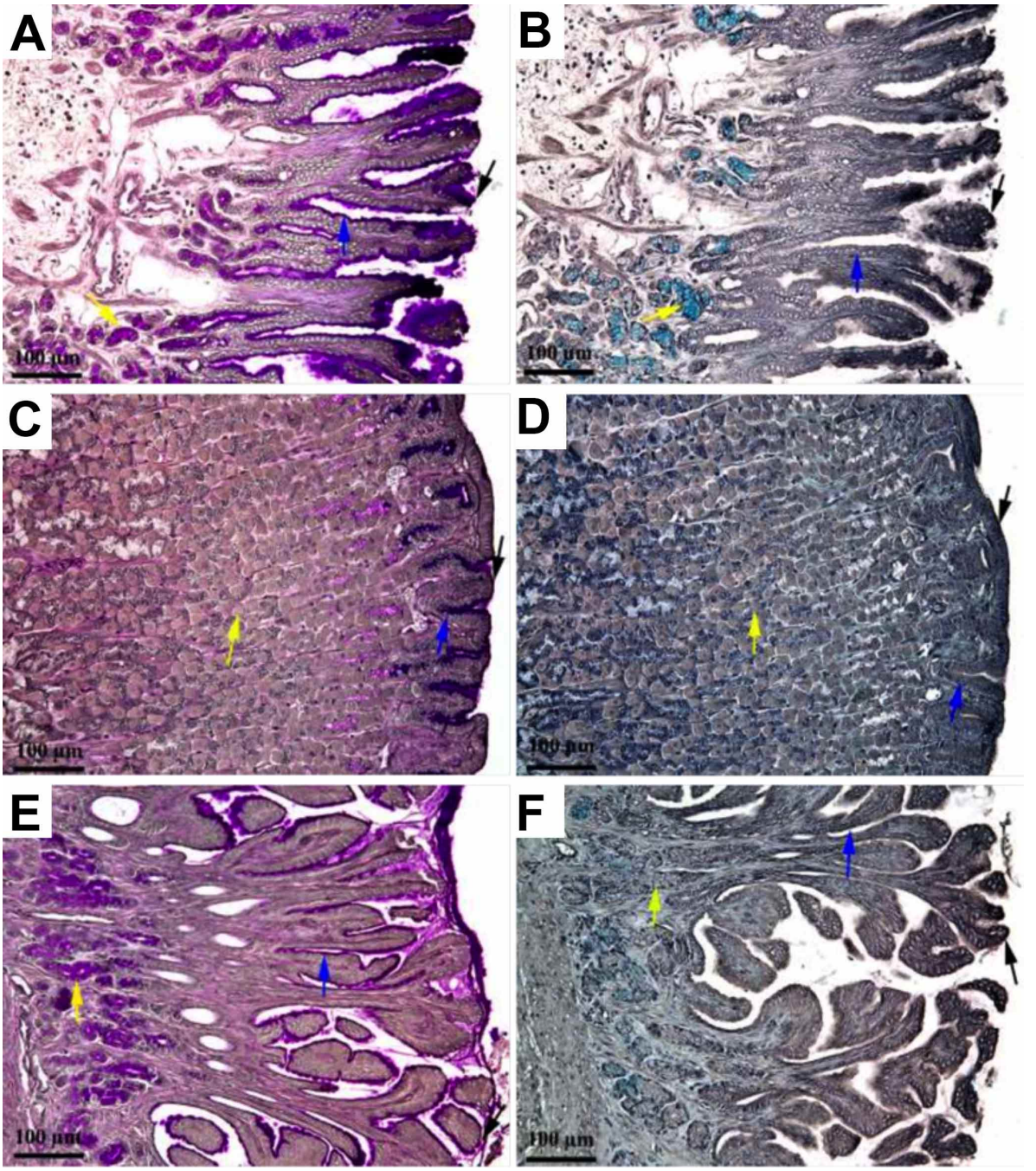

Fig. 1 Histochemical staining of the mucosa of the stomach in rabbits. (A) cardial region (PAS stain) – columnar PAS-positive cells in the covering epithelium of the mucosa (black arrow), along with PAS-positive cells in the in the epithelium lining the gastric pits (blue arrow), and prominent mucous content in the cytoplasm of the cells from cardial gastric glands (yellow arrow); (B) cardial region (alcian-blue stain) – alcian blue negative cells in the surface epithelium (black arrow) and lining epithelium of the gastric pits (blue arrow) and large amounts of acidic mucin in the multi vacuolated cells from the piloric glands (yellow arrow); (C) fundic region (PAS stain) – neutral mucins are only synthesized by the cells from the covering epithelium (black arrow) and by the epithelium belonging to the gastric pits (blue arrow), whereas no mucin were identified in the glandular epithelium of the fundic glands (yellow arrow); (D) fundic region (alcian-blue stain) – no acidic mucins can be identified in the covering epithelium (black arrow), gastric pits (blue arrow) and fundic glands (yellow arrow); (E) pyloric region (PAS stain) – significant quantities of neutral mucins are detected in the covering (black arrow) and glandular epithelium (gastric pits - blue arrow and piloric glands - yellow arrow); (F) pyloric region (alcian-blue stain) – similarly with the fundic region of the stomach, no mucins can be visualized throughout the mucosal epithelium (covering epithelium - black arrow, gastric pits - blue arrow and pyloric glands - yellow arrow).

than 80 % carbohydrate and are concentrated in mucin domains that are built on a protein core, which is rich in proline, serine, and threonine amino acids (also called PTS sequences) (Hattrup & Gendler, 2008). With reference to the gastric mucus, in humans was described as a two-layered mucus system, made of an internal layer that is an attached mucus, and an external layer that is a loose and not attached mucus. Both mucus layers include MUC5AC mucin produced by the superficial gastric epithelium (Johansson *et al*., 2013). Additionally, the gastric glands elaborate the gel-forming mucin MUC6 (Bartman *et al*., 1998). The internal mucous layer, manufactured based on MUC5AC, acts as a diffusion barrier for hydrochloric acid (Johansson *et al*., 2013). The superficial epithelial cells secrete bicarbonate, generating a pH gradient from an acidic in the lumen of the stomach to neutral pH at the cell surface (Allen & Flemström, 2005). Hydrochloric acid is produced and secreted in the gastric glands together with MUC6 and pepsin, and they can cross the superficial internal mucous layer through some "temporary canals" that are subsequently immediately closed (Johansson *et al*., 2000). It is not very clear how the gastric glands can tolerate the very high proton concentration (pH 1–2), but it seems MUC6 is very important in this sense. The contribution of MUC6 to the formation of the internal gastric mucus layer is still controversial (Phillipson *et al*., 2008).

In veterinary medicine, due to a large variety of feeding behavior, the information regarding the mucin-producing cells, including the type of mucus secreted in the stomach, is scarce. Our study showed that the distribution of mucus synthesis and the type of elaborated mucus in the mucosa of the stomach in rabbits have some questioning peculiarities. The mucus elaboration was observed in all the regions of the stomach (i.e., cardial, fundic, and pyloric regions), but only for the neutral mucin (except for the secretory units of the gastric glands from the fundic region in which no mucins were detected). As concerns the acidic mucin synthesis in the stomach of rabbits, it is restricted only to the secretory units of the gastric glands from the cardial region (++ alcian-blue stain). No other regions of the stomach do synthesize acidic mucus type. Regarding the neutral mucin secretion throughout the gastric mucosa, the covering epithelium and the epithelium lining the gastric pits from all the three regions of the stomach in rabbits synthesize significant amounts of neutral mucins (+ to ++ PAS-positive reaction). As regards the neutral mucin secretion derived from secretory units of the gastric glands, in rabbits the pyloric glands are the most active (++ PAS-positive reaction), followed by moderate amounts of mucins elaborated by cardial glands (++ PAS-positive reaction), whereas the fundic region does not synthesize it at all.

Similar information suggesting the distribution of mucus synthesis in the gastric mucosa have been presented in other reports on several species such as guinea pigs (Chende *et al*, 2020), chinchillas (Ghiurco *et al*., 2021), dogs (Bacha & Bacha, 2006), pigs (Eurell & Frappier, 2006), and humans (Raica *et al*., 2004). Accordingly, in guinea pigs, the cardial glands of the stomach contained some PAS-positive and alcian blue positive cells, suggesting that this region of the stomach synthesizes low amounts of neutral and acidic mucins (Chende *et al*., 2020), an aspect observed in chinchillas too (Ghiurco *et al*., 2021). In humans, the cardial glands are synthesizing only neutral mucins (Raica *et al*., 2004). Reports regarding the mucus synthesis in the fundic region of the stomach have been described in dogs (i.e., neutral PAS-positive mucins; Bacha & Bacha, 2006), but not in humans (Raica *et al*., 2004). However, neutral PAS-positive mucins were detected in the pyloric glands in dogs and pigs (Eurell & Frappier, 2006; Bacha & Bacha, 2012), whereas significant amounts of both neutral and acidic mucins were identified in guinea pigs (Chende *et al*., 2020). In chinchillas, the gastric pyloric glands synthesize small quantities of neutral and acid mucins (Ghiurco *et al*., 2021). Overall, piloric glands from the stomach synthesize large quantities of mucins in goats, pigs, and dogs (Augley & Frye, 2001; Bacha & Bacha, 2006), and moderate amounts in guinea pigs and humans (Raica *et al*., 2004; Chende *et al*., 2020).

CONCLUSIONS

The microscopic features of the gastric mucosa in domestic rabbits with reference to the distribution of mucus synthesis showed significant quantities of neutral mucins and small amounts of acidic mucins secreted by the stomach mucosa. While the neutral mucins were identified in all the regions of the stomach (i.e., cardial, fundic, and pyloric regions), the acidic mucins were synthesized only by the secretory units of the gastric glands from the cardial region. As a pet, farm, or laboratory animal used for many biological studies, rabbits should be a priority for researchers to establish their welfare needs based on species-related morphophysiological features, which can help future research. Given that, understanding the microanatomy of rabbits is crucial for comprehending species-related physiological and pathological processes.

CIPOU, M.; DAMIAN, A.; RUS, V.; MARTONOS, C.; RATIU, I. A.; MICLAUS, V. & GAL, A. F. Evaluación histoquímica de células secretoras de mucina en el estómago de conejo doméstico. *Int. J. Morphol., 40(4)*:915-919, 2022.

RESUMEN: Las sustancias mucosas del estómago en los mamíferos son importantes no solo para la protección del epitelio

gástrico del ambiente ácido y las acciones de trituración, sino que facilitan además otras funciones del estómago, como son las funciones antibacterianas, antimetastásicas e inmunológicas. El objetivo del estudio fue resaltar la distribución de las células secretoras de mucina en la mucosa gástrica de conejos domésticos, incluido el tipo de moco sintetizado. Las muestras gástricas recolectadas de diez conejos se fijaron en formalina tamponada al 10 % y se sometieron a un procesamiento que incluyó deshidratación, clarificación e inclusión en parafina. Las secciones de tejido finalmente se tiñeron histoquímicamente mediante la reacción de PAS y el método del azul de Alcian (pH 2,5) para la detección de mucinas neutras y ácidas, respectivamente. La cuantificación de mucinas en el citoplasma de las células secretoras de moco se realizó clasificando las muestras de tejido gástrico desde negativas (-) hasta intensamente positivas (++). La elaboración de moco se observó en todas las regiones del estómago (es decir, cardias, fúndica y pilórica), pero solo para la mucina neutra. La síntesis de mucina ácida ocurrió solo en las unidades secretoras de las glándulas gástricas de la región correspondiente al cardias del estómago. Las glándulas pilóricas sintetizaron la mayor cantidad de mucinas neutras, seguidas de cantidades moderadas elaboradas por las glándulas cardiales, mientras que la región fúndica no las sintetizó en absoluto. La descripción de nuevas características microscópicas del estómago en conejos es fundamental no solo para comprender las características fisiológicas relacionadas con las especies sino también para entender los procesos patológicos gástricos.

PALABRAS CLAVE: Estómago; Mucinas; Mucosa gástrica; Conejo.

REFERENCES

Allen, A. & Flemström, G. Gastroduodenal mucus bicarbonate barrier: protection against acid and pepsin. *Am. J. Physiol. Cell Physiol., 288(1)*:C1-19, 2005.

Aughey, E. & Frye F. L. *Comparative Veterinary Histology with Clinical Correlates*. Iowa, Iowa State University Press, 2001. pp.105-12.

Bacha, W. J. & Bacha, L. M. *Atlas Color de Histología Veterinaria*. 3th ed. Hoboken, Blackwell Publishing, 2012. pp.156-60.

Bacha, W. J. & Bacha, L. M. *Color Atlas of Veterinary Histology*. Iowa, Blackwell Publishing, 2006. pp.109-13, 2006.

Bartman, A. E.; Buisine, M. P.; Aubert, J. P.; Niehans, G. A.; Toribara, N. W.; Kim, Y. S. & Ho, S. B. The MUC6 secretory mucin gene is expressed in a wide variety of epithelial tissues. *J. Pathol., 186(4)*:398-405, 1998.

Chende, A.; Rus, V.; Martonos, C.; Pivariu, D.; Damian, A.; MiclauS, V. & Gal, A. Histochemical assessment of mucin-secreting cells from the gastric mucosa of guinea pigs. *Bull. UASVM Vet. Med., 77(2)*:115-22, 2020.

Corfield, A. P.; Myerscough, N.; Longman, R.; Sylvester, P.; Arul, S. & Pignatelli, M. Mucins and mucosal protection in the gastrointestinal tract: new prospects for mucins in the pathology of gastrointestinal disease. *Gut, 47(4)*:589-94, 2000.

Eurell, J. N. & Frappier, B. L. *Dellman's Textbook of Veterinary Histology*. 6th ed. Hoboken, Blackwell, 2006. pp.186-189.

Gal, A. F. & MiclauS, V. *Histology.* Cluj-Napoca, Risoprint, 2013. pp.234-8.

Ghiurco, I. F.; Gal, A. F.; Rus, V.; Martonos, C.; Matei, M. C.; MiclauS, V. & Damian, A. Distribution and activity of mucin-secreting cells in the stomach of Chinchilla. A Histochemical study. *Rev. Rom. Vet. Med., 31(2)*:18-23, 2021.

Hattrup, C. L. & Gendler, S. J. Structure and function of the cell surface (tethered) mucins. *Ann. Rev. Physiol., 70*:431-57, 2008.

Herdt, T. H. *Secretions of the Gastrointestinal Tract*. In: Klein, B. G. (Ed.). *Cunningham's Textbook of Veterinary Physiology*. 6th ed. Amsterdam, Elsevier, 2020, 307-315.

Johansson, M. E. V.; Sjövall, H. & Hansson, G. C. The gastrointestinal mucus system in health and disease. *Nat. Rev.. Gastroenterol. Hepatol., 10(6)*:352-61, 2013.

Johansson, M.; Synnerstad, I. & Holm, L. Acid transport through channels in the mucous layer of rat stomach. *Gastroenterology, 119*:1297-304, 2000.

Kiernan, J. A. *Histological and Histochemical Methods: Theory and Practice.* 3th ed. Oxford, Butterworth Heinemann, 1999. pp.170-196.

Layton, C. & Brancoft, J. D. *Carbohidrates*. In: Suvarna, S. K.; Layton, C. & Bancroft, J. D. Bancroft's Theory and Practice of Histological Techniques. 7th ed. Amsterdam, Elsevier, Churchill Livingstone, 2013. pp.221-40.

Liebich, H. G. *Veterinary Histology of Domestic Mammals and Birds*. 5th ed. Minnesota, 5M Publishing, 2019. pp.198-212.

Linden, S. K.; Sutton, P.; Karlsson, N. G.; Korolik, V. & McGuckin, M. A. Mucins in the mucosal barrier to infection. *Mucosal Immunol., 1(3)*:183-7, 2008.

Phillipson, M.; Johansson, M. E. V.; Henriksnäs, J.; Petersson, J.; Gendler, S. J.; Sandler, S.; Persson, A. E. G.; Hansson, G. C. & Holm, L. The gastric mucus layers: constituents and regulation of accumulation. *Am. J. Physiol. Gastrointest. Liver Physiol., 295(4)*:G806-12, 2008.

Prasanna, L. C. Analysis of the distribution of mucins in adult human gastric mucosa and its functional significance. *JCDR, 10(2)*:AC01-4, 2016.

Prasanna, L. C.; Thomas, H. T.; D'Souza, A. S.; Bhat, K. M. R.; Thomas, H. T.; D'Souza, A. S. & Bhat, K. M. R. The histochemical assessment of sulpho-, sialo-, and neutral- mucosubstances in fetal gastric mucosa. *Int. J. Res. Med. Sci., 3(1)*:235-8, 2015.

Raica, M.; Mederle, O.; Caruntu, I. D.; Pintea, A. & Chindris, A. M. *Practical and Theoretical Histology.* Timisoara, Brumar, 2004. pp.257-66.

Suvarna, K.; Layton, C. & Bancroft, J. D. *Bancroft's Theory and Practice of Histological Techniques.* 8th ed. Amsterdam, Elsevier, 2018. pp.176-197.

Wulff, S. *Guide to Special Stains*. Philadelphia, DakoCytomation, 2004. pp.24-44.

Corresponding author:
Ioana Adela RaTiu
MD, Ph.D., Lecturer
Discipline of Nephrology
Department of Medical Sciences
University of Oradea
Oradea
ROMANIA

E-mail: ratiu_ioana@yahoo.com

https://orcid.org/0000-0003-0879-1903