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Research article

Mucous cell distribution and mucus production during early growth periods of the giant African snail (*Achatina fulica*)

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Abstract

The giant African snail (*Achatina fulica*) secretes mucus for crawling and protection from moisture loss and pathogenic infections. The snail mucus has been popularly used as a bioactive reagent in medical and cosmeceutical products. This study observed the distribution of pedal mucous cells of *A. fulica* and the snail growth effect (1–3 mth) on pedal mucus production and the types of mucous cells. Foot tissues were processed using a paraffin technique and stained with Masson's trichrome, periodic acid Schiff, and periodic acid Schiff-Alcian Blue at pH 2.5. Visualization under a light microscope showed that the pedal mucus of *A. fulica* was mainly produced from two types of mucous glands: 1) tubular mucous gland cells located on the dorsal part of foot that produced acid mucopolysaccharide mucus; and 2) round mucous gland cells located on the ventral part of foot that produced acid and neutral mucopolysaccharide mucus. A positive correlation between the acid mucin level and snail age was only observed on the dorsal area. The level of neutral mucin from both sides was highest after 2 mth. The average length of the mucous cells on the dorsal surface significantly increased during growth. This study revealed the change and distribution of the mucous cells in early *A. fulica* development to inform further applications in snail bioactive compounds.

Introduction

The giant African snail (*Achatina fulica*) is a gastropod pest that damages agricultural crops and ornamental plants (Thiengo et al., 2007). Pesticides have been heavily used to control the population of *A. fulica*; however, the over use of pesticides can be toxic to other organisms and environments (Roda et al., 2016). Gastropods secrete mucus for skin hydration, locomotion, reproduction, adhesion, osmoregulation and bacterial protection (Iguchi et al., 1982; Davies and Hawkins, 1998; Smith, 2010). Gastropod mucus has several bioactive molecules valuable for medical and cosmetic applications (Iguchi et al., 1982). Jeong et al. (2001) reported several bioactive molecules such as achatinin, acharan sulfate and lectin in the mucus of *A. fulica*.

Studies have shown that the foot tissues of snails can secrete different types of mucus. Trail mucus secreted from the ventral gland of *Helix pomatia* and *H. aspersa* is responsible for locomotion (Pawlicki et al., 2004). This mucus consists of acidic glycoproteins and glycoproteins (Campion, 1961; Smith and Morin, 2002). The dorsal mucus or adhesive mucus plays a role as lubricant to reduce the friction between the soft foot skin and the hard-external shell (Herfs, 1921; Kilias, 1985; Pawlicki et al., 2004). The adhesive mucus is used for attachment to substratum and forming an epiphragm (Greistorfer et al., 2017). This type of mucus consists of roughly 3% protein and 1% carbohydrate (Smith and Morin, 2002). The structure

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and type of mucous cells were studied in *Incilaria fruhstorferi* (Yamaguchi et al., 2000), *H. aspersa* (Campion, 1961), and *H. pomatia* (Greistorfer et al., 2017). Yamaguchi et al. (2000) found two types of mucous cells (round and tubular mucous cells) in the land slug *I. frushstorferi*. Granules within the round mucous cells were proteinaceous while the tubular mucous cells had more acidic mucopolysaccharides. Campion (1961) showed that the foot of *H. aspersa* had two types of the mucous glands on the dorsal surface (types A and B), where type A glands positively reacted with Alcian Blue and weakly with periodic acid Schiff (PAS), while type B glands were positively stained with Alcian Blue and PAS. Types C and D glands were located on the ventral surface of *H. aspersa* foot and both were positively stained for Alcian Blue and PAS. Another study in the genus *Helix* by Greistorfer et al. (2017) reported that the dorsal surface of *H. pomatia* foot consisted of four types of mucus glands (H1d, H2d/H2d* and H3d). H1d and H2d/H2d* produced acid glycoproteins, while H3d positively reacted with calcium and lipid staining. The ventral surface of the foot had two types of mucus glands (H1v and H2v) that produced acid glycoproteins and lipids. However, the microanatomical structure and distribution of different mucous cell types in *A. fulica* remain poorly understood.

This study aimed to investigate the microanatomical structure of the pedal mucus glands of *A. fulica.* Localization and distribution of different mucous cell types in the dorsal and ventral areas of *A. fulica* at age 1–3 mth were compared. This study provided useful information applicable to mucus production in the later growth stage of *A. fulica* for cosmetics and medical research.

Materials and Methods

Animal rearing and sample collection

The newly-hatched *Achatina fulica* snails (age 1 d) were reared in a glass chamber covered with plastic net and placed in an environmental chamber at 25 ± 2°C and fed with mixed fresh vegetables at 50 mg/animal/d. Three samples were collected every month during their early-growing period (age 1–3 mth), and fixed with 10% neutral buffered formalin solution for 8 hr. The samples ($n = 9$) were washed with running tap water for 3 hr and preserved in 70% ethanol for histological study.

Histological study of A. fulica pedal tissues

All samples were decalcified by soaking in 10% aqueous formic acid for 15–20 min and then washed with running tap water for 6 hr. The tissues were then processed using a standard paraffin technique and cut into slices 5 µm thick before staining with Masson's trichrome using Aniline Blue (for collagen fibres), periodic acid-Schiff-haematoxylin (PAS-H; for neutral mucopolysaccharides and glycoproteins), and periodic acid-Schiff-Alcian Blue at pH 2.5 (PAS-AB; for acid mucopolysaccharides and glycoproteins). Tissue sections were observed under a light microscope (Olympus BX51) and photographed (Olympus DP70, digital camera system).

Image and statistical analysis

Images (taken using microscopy) from the stained tissue sections (three slides for each sample and a minimum of 20 images per slide) were analyzed using the imageJ software (NIH, version 1.4.3.1) according to the color deconvolution and quantification method of Ruifrok and Johnston (2001). The quantification of the mucous cell length and area was carried out according to Schneider et al. (2012) using the imageJ tools. Differences in the area of mucous cells from age 1 mth to 3 mth and between the dorsal and ventral pedal areas were statistically compared using analysis of variance following by Tukey's Hornest Significant Difference test. The significance levels were considered at $p < 0.05$ and $p < 0.01$ for all comparisons.

Results

The comparative microanatomical structure of the pedal mucus production areas in *A. fulica* at age 1–3 mth showed that the mucous cells were mainly located in the connective tissue structure of the snail foot. Based on a cross section of the foot tissue structure of *A. fulica* (Fig. 1A), the core pedal area was mainly composed of connective tissue (collagen and muscular fibers) which were stained as blue and red using Masson's trichrome staining (Fig. 1D−1E) surrounded by a simple, columnar, epithelial lining on both the dorsal and ventral surfaces (Fig. 1B−1C). Different mucous cell types of *A. fulica* were found underneath the epithelial surface and could be divided into two major types: tubular mucous cells and round mucous cells. The dorsal pedal area had more tubular mucous cells (cylindrical shape containing numerous large mucous granules) than round mucous cells (spherical shape containing small mucous granules) as shown in Fig. 1A–1E). Noticeably, some of the tubular mucous cells had open ends penetrating out through the epithelial intercellular junctions. The ventral pedal area had only round mucous cells. The nucleus of the tubular mucous cells was located at the basal end, while the nucleus of the round mucous cells was located in the middle of the cells. The tubular mucous cells were stained positive using PAS–AB indicating that the cells produced acid mucopolysaccharide or acid mucin. The round mucous cells were stained positive using PAS-H and PAS-AB stain which showed the synthesis of neutral and acid mucopolysaccharides.

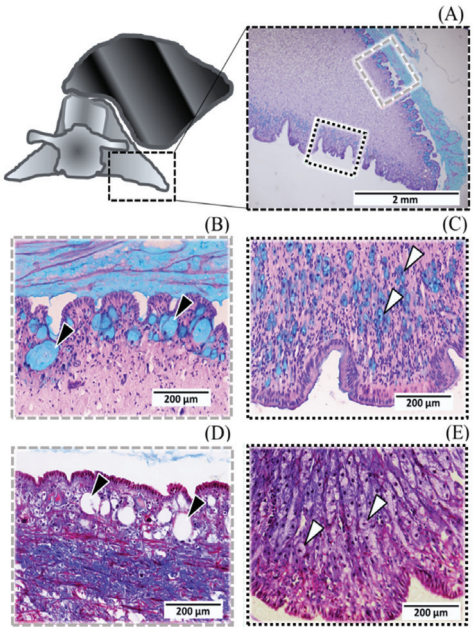

Fig. 1 Histochemistry study of the structure of mucous cells on the dorsal and ventral surfaces: (A) orientation and section plane of *Achatina fulica* foot tissue structure (gray large and black dotted dashed line boxes indicate dorsal and ventral areas, respectively); (B) periodic acid Schiff-Alcian Blue (PAS-AB) staining on dorsal surface; (C) PAS-AB staining on ventral surface; (D); Masson's trichrome staining on dorsal surface; (E) Masson's trichrome staining on ventral surface, where black arrowheads = tubular mucous cells; white arrowheads = round mucous cells.

Comparison of the mucopolysaccharide or mucin production at different ages of *A. fulica* showed that the level of acid mucin (positively stained with AB) on the dorsal pedal area was significantly higher than that on the ventral area. At age 1 mth, the snails had a higher level of acid mucin on the ventral surface than on the dorsal surface. In contrast, at age 2–3 mth, the snail had a higher level of acid mucin on the dorsal surface than on the ventral surface (Fig. 3). Image analysis of the mucous cells quantitatively confirmed that the acid mucin level on the dorsal pedal area increased in correlation with early snail age. The acid mucin level on the ventral pedal area, in contrast, decreased from 1 mth to 3 mth. Snails aged 1–2 mth had similar levels of neutral mucin on both the dorsal and ventral pedal areas (Fig. 2G–2L). Snails aged 3 mth had a significantly higher level of neutral mucin on the dorsal area than on the ventral area (Fig 4; Table 2). The average length of the tubular mucous cells on the dorsal area was significantly higher than that of the ventral area for all snail ages (Fig. 5; Table 3). On the other hand, the average length of the round mucous cells on the ventral surface significantly decreased during early growth.

Discussion

Microanatomical studies on the mucous cell types and localization of the different mucins of *Achatina fulica* have not previously been reported. The current study indicated that the dynamic change in the mucus level during early snail growth could be useful in determining mucus production through the later growth stages and allow the precise induction of snail mucus contents at the industrial farm scale. Different mucin types play different roles in the snail survival process, including locomotion, reproduction, adhesion, hibernation and protection (Denny, 1980). Knowing the localization of different mucin types could help in the development of new mucus collection methods to obtain particular contents or mucus types for specific usage in cosmetic and medical applications. The distribution identified of the two different types of mucous cells of *A. fulica* was similar to the findings of Yamaguchi et al. (2000) for the land slug *Incilaria fruhstorferi*. However, the current findings strongly indicated that the mucous cells of *A. fulica* lining underneath the pedal epithelial layer were quite different from those reported for *I. fruhstorferi*, whose mucous cells are located on the epithelial layer of their body surface skin, whereas the mucous cells of *A. fulica* were located deeper underneath the epithelial layer (in the connective tissue area) and some of the open ends penetrated in between the epithelial intercellular junctions. The microanatomical structures of the mucous cells in *A. fulica* were similar to the description by Yamaguchi et al. (2000) in the land slug, perhaps through their evolutionary relatedness. The round mucus cells were more abundant on the ventral area than the dorsal area and they had an oval nucleus located at the center of the cells. The tubular mucous cells were located in the muscular layer with a long secretory process extending to the surface of the epidermis. These cells had a nucleus located in the basal area of the cells.

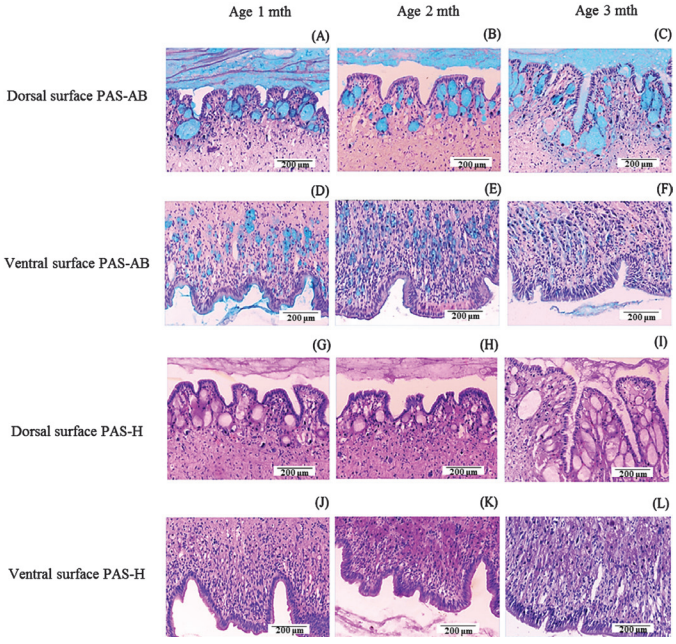

Fig. 2 Type of mucous cells on dorsal and ventral surfaces using periodic acid-Schiff-haematoxylin (PAS-H) staining on the dorsal surface (A–C) and ventral surface (G–I); and periodic acid-Schiff-Alcian Blue (PAS-AB) staining on the dorsal surface (D–F) and ventral surface (J–L).

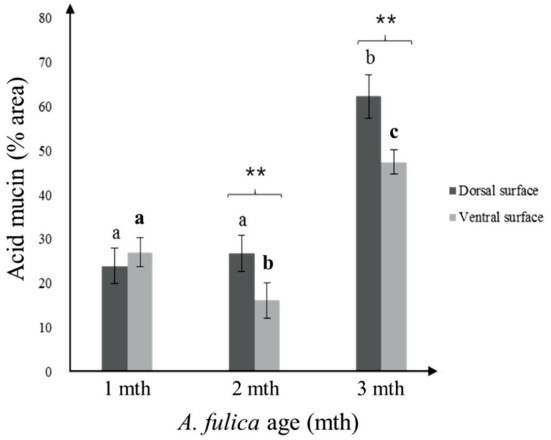

Fig. 3 Comparison of area of acid mucin production on dorsal and ventral surfaces, where different lowercase letters for same text style (normal and bold) bars indicate significant difference at $p < 0.01$; ** = highly significant ($p < 0.01$); error bars represent ± SD of the mean; and additional comparison pairs shown in Table 1.

Table 1 P-value from Tukey's Hornest Significant Difference analysis for comparison of acid mucin productivity on dorsal (D) and ventral (V) surfaces

Age–Surface	1 mth–D	2 mth–D	3 mth–D	1 mth–V	2 mth–V	3 mth–V
1 mth–D	–	0.808	0.000	0.749	0.084	0.000
2 mth–D	0.808	–	0.000	1.000	0.004	0.000
3 mth–D	0.000	0.000	–	0.000	0.000	0.000
1 mth–V	0.749	1.000	0.000	–	0.003	0.000
2 mth–V	0.084	0.004	0.000	0.003	–	0.000
3 mth–V	0.000	0.000	0.000	0.000	0.000	–

Differences at $p < 0.05$ were considered significant and at $p < 0.01$ for highly significant.

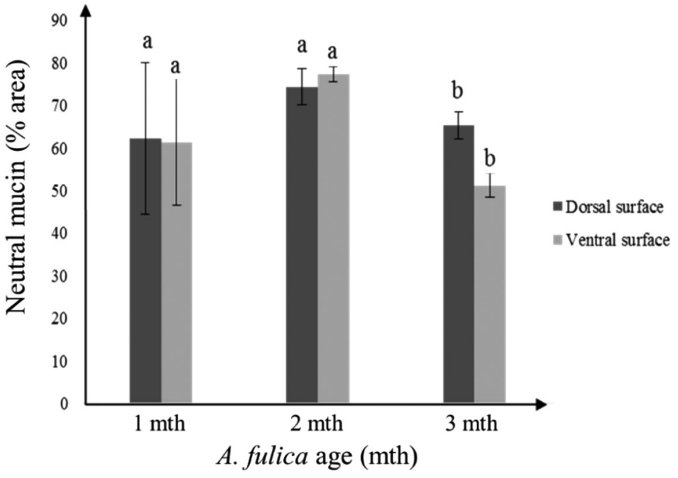

Fig. 4 Comparison area of neutral mucin production on dorsal and ventral surfaces, where different lowercase letters for same text style (normal and bold) bars indicate significant difference at $p < 0.01$; ** = highly significant ($p < 0.01$); error bars represent ± SD of the mean; and additional comparison pairs shown in Table 2.

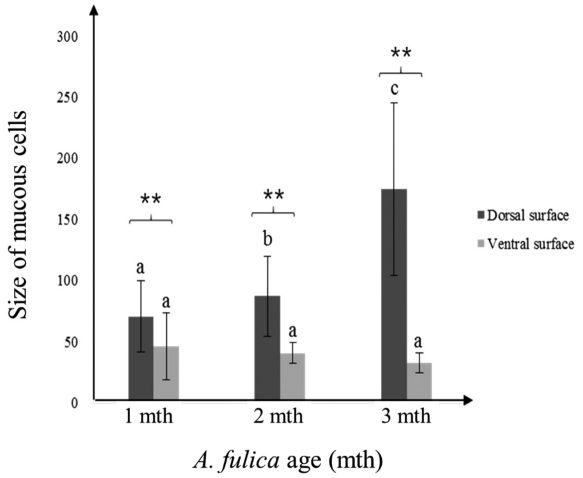

Fig. 5 Size comparison of mucous cells on dorsal and ventral surfaces, where different lowercase letters for same colored bars indicate significant difference at $p < 0.01$; ** = highly significant ($p < 0.01$); error bars represent ± SD of the mean; and additional comparison pairs shown in Table 3.

Table 2 P-value from Tukey's Hornest Significant Difference analysis for comparison of neutral mucin productivity on dorsal (D) and ventral (V) surfaces

Age–Surface	1 mth–D	2 mth–D	3 mth–D	1 mth–V	2 mth–V	3 mth–V
1 mth–D	–	0.354	0.999	1.000	0.141	0.300
2 mth–D	0.354	–	0.574	0.261	0.995	0.003
3 mth–D	0.999	0.574	–	0.993	0.278	0.154
1 mth–V	1.000	0.261	0.993	–	0.095	0.399
2 mth–V	0.142	0.995	0.278	0.095	–	0.001
3 mth–V	0.300	0.003	0.154	0.399	0.001	–

Differences at $p < 0.05$ were considered significant and at $p < 0.01$ for highly significant.

Table 3 P-value from Tukey's Hornest Significant Difference analysis for comparison of length of mucous cells on dorsal (D) and ventral (V) surfaces

Age–Surface	1 mth–D	2 mth –D	3 mth –D	1 mth –V	2 mth –V	3 mth –V
1 mth –D	–	0.207	0.000	0.010	0.001	0.000
2 mth –D	0.207	–	0.000	0.000	0.000	0.000
3 mth –D	0.000	0.000	–	0.000	0.000	0.000
1 mth –V	0.010	0.000	0.000	–	0.975	0.421
2 mth –V	0.001	0.000	0.000	0.975	–	0.875
3 mth –V	0.000	0.000	0.000	0.421	0.873	–

Differences at $p < 0.05$ were considered significant and at $p < 0.01$ for highly significant.

Greistorfer et al. (2017) reported four types of glandular structure in the mucous cells of *Helix pomatia*. The dorsal gland types contained: 1) H1d (positive to AB at pH 1.0, 2.5 and negative to PAS); 2) H2d (positive to AB at pH 1.0, 2.5 and PAS); 3) H2d* (stain reaction similar to H2d); and 4) H3d (negative staining for AB and PAS). The ventral surface had two types of the mucous glands: H1V and H2V, both of which had a positive reaction to PAS and AB at pH 1.0 and 2.5. The current results were similar to Greistorfer et al. (2017). The tubular mucous cells of *A. fulica* were similar to H1d because they were positive to AB staining at pH 2.5, but negative to PAS staining. The round mucous cells on the ventral pedal area were alike to the H1V and H2V types due to their strong reaction with PAS and AB (pH 2.5) staining.

Jeong et al. (2001) identified acharan sulfate (a glycosaminoglycan [GAG] secreted from the body skin of the giant African snail). The snail GAG was inside the granules secreted to the surface as mucous materials (Jeong et al., 2001). The GAG-containing mucus of *A. fulica* was positively stained using Alcian Blue. Proteoglycans and GAGs can be stained by a number of dyes, such as Alcian Blue, Azure A and Toluidine Blue (Cowman et al., 1984; Rice et al., 1987). Therefore, the GAG-containing mucus could be visualized using the blue stain of A Blue (Jeong et al., 2001). The current findings showed that the acid mucin-producing cells could be found on both dorsal and ventral pedal areas indicating GAGs distribution on both pedal surfaces. In general, GAGs can be usually found in the extracellular matrix of invertebrate and vertebrate tissue (Jeong et al., 2001). Previous reports mentioned the protein binding property of GAGs and their important roles in cellular response in development and homeostasis (Capila and Linhardt, 2002). For example, heparin (Lever and Page, 2002) and heparan sulfate (Sasisekharan et al., 2002) have important roles in regulating biological processes and molecules through their interactions with many proteins, including growth factors, adhesion

molecules, cytokines and extracellular matrix proteins. The biological roles of acharan sulfate (a type of acid mucin or GAG) in the mucus secreted to the outer surface of *A. fulica* might involve: 1) the binding, uptake, and transport of divalent cations; 2) antidesiccation; 3) snail mobility; or 4) antibiotic or antipredator roles (Jeong et al., 2001). Further investigation on the mucin-related proteins in the *A. fulica* mucus collected from both pedal surfaces might reveal their specific molecular functions.

The ventral glands or ventral mucous cells play crucial roles in the locomotion of the snail, the so-called trail mucus (Pawlicki et al., 2004) which consists of acidic glycoproteins (Campion, 1961; Greistorfer et al., 2017). The dorsal or adhesive mucus is used for attachment to the substratum and also to form an epiphragm during snail hibernation (Campion, 1961; Pawlicki et al., 2004). The adhesive mucus has intriguing properties for developing drug delivery vehicles and biomedical adhesives (Petka et al., 1998; Miyata et al., 1999; Wang et al., 1999; Peppas et al., 2000; Nowak et al., 2002). The current finding showed that neutral mucin was produced mainly on the ventral pedal areas and decreased as the snails grew. This suggested that the trail mucus should be collected from the ventral side of the snail foot. A higher level of acid mucin was observed when the snails grew because their body surface expanded with more mucous cells producing more of this mucus to avoid dehydration. Even though there have been no previous reports on the dynamic change in mucin production by snails during growth, other environmental factors might affect the change in the mucin contents in some tissues such as the extracellular matrix of cerebral tissue reported by Londhe and Kamble (2014). The change in the acid mucin levels in *A. fulica* was reported to be caused by parasite infection, as *A. fulica* has been widely known to be an intermediate host for *Angiostrogylus cantonensis* (Prociv et al., 2000) and some species of *Strongyloides*. It has been claimed that the high expression of acharan sulfate (a heparin-related GAG) was positively correlated with the parasitic contamination rate (Vieira et al., 2004). Londhe and Kamble (2014) used a histochemical study to investigate the cerebral neurons of the freshwater snail *Bellamya bengalensis* after intoxication with mercuric chloride ($HgCl_2$) and zinc chloride ($ZnCl_2$). The neutral mucin decreased significantly but the acidic mucopolysaccharide significantly increased after exposure to $HgCl_2$ and $ZnCl_2$. Kamble and Londhe (2012) reported that the neutral mucin decreased with an increase in acidic mucosubstances in the extracellular matrix of the cerebral neurons in the terrestrial slug *Semperula maculata*. The change in mucin types could indicate the health stages of snails being farm-reared if they were parasite-infected or had accumulated metal compounds. Therefore, monitoring mucous changes during snail growth could provide useful information on their health and readiness for mucus collection. However, infection and contamination were less likely to occur in the current experiments since the snails were hatched and reared under carefully controlled conditions.

In conclusion, this study revealed the change and distribution of the mucous cells during early growth of the snails and used microscopy to investigate the histological structures of the mucus glands as well as determining some of the biochemical properties of the *A. fulica* mucus for further applications of snail bioactive compounds in medical and cosmetic products.

Acknowledgments

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References

Capila, I., Linhardt, R.J. 2002. Heparin-protein interactions. GDCh. 41: 390−412.

Campion, M. 1961. The structure and function of the cutaneous glands in *Helix aspersa*. J. Cell Sci. 102: 195–216.

Cowman, M.K., Slahetka, M.F., Hittner, D.M., Kim, J., Forino, M. Gadelrab, G. 1984. Polyacrylamide-gel electrophoresis and alcian blue staining of sulphated glycosaminoglycan oligosaccharides. Biochem. J. 221: 707–716.

Davies, M.S., Hawkins, S.J. 1998. Mucus from marine molluscs. In: Blaxter, J.H.S. Southward A.J., Tyler, P.A. (Eds.). Advances in Marine Biology, Vol. 34. Academic Press/Elsvier Science, London, pp. 1–71.

Denny, M. 1980. The role of gastropod pedal mucus in locomotion. Nature. 285: 160–161.

Greistorfer, S., Klepal, W., Cyran, N., Gugumuck, A., Rudoll, L., Suppan, J., von Byern, J. 2017. Snail mucus-glandular origin and composition in *Helix pomatia*. Zoology (Jena). 122: 126–138.

Herfs, A. 1921. Die Haut der Schnecken in ihrer Abhängigkeit von der Lebensweise. Naturwissenschaftl. Wochenschrift. 20: 601–609. [in German]

Iguchi, S.M.M., Aikawa, T., Matsumoto, J.J. 1982. Antibacterial activity of snail mucus mucin. Comp. Biochem. Physiol. A Physiol. 72: 571–574.

Jeong, J., Toida, T., Muneta, Y., Kosiishi, I., Imanari, T., Linhardt, R.J., Choi, H.S., Wu, S.J., Kim, Y.S. 2001. Localization and characterization of acharan sulfate in the body of the giant african snail *Achatina fulica*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 130: 513–519.

Kamble, N.A., Londhe, S.R. 2012. Epileptic activity in neuronal cells, induced by mercuric chloride and cadmium chloride in terrestrial slug *Semperula maculata*: Fine structure investigated by histology and histochemistry. Toxicol. Environ. Chem. 94: 109–120.

Kilias, R. 1985. Die Weinbergschnecke. A. Ziemsen Verlag, Wittenberg, Germany. [in German]

Lever, R., Page, C.P. 2002. Novel drug development opportunities for heparin. Nat. Rev. Drug Discov. 1: 140–148.

Londhe, S., Kamble, N. 2014. Mucin reactivity after acute exposure to mercury and zinc chloride in neurocytes of freshwater snail *Bellamya bengalensis*: A histochemical study. Adv. Inorg. Chem. 2014: 1−11.

Miyata, T., Asami, N., Uragami, T. 1999. A reversibly antigen-responsive hydrogel. Nature. 399: 766–769.

Peppas, N.A., Huang, Y., Torres-Lugo, M., Ward, J.H., Zhang, J. 2000. Physicochemical foundations and structural design of hydrogels in medicine and biology. Annu. Rev. Biomed. Eng. 2: 9–29.

Nowak, A.P., Breedveld, V., Pakstis, L., Ozbas, B., Pine, D.J., Pochan, D., Deming, T.J. 2002. Rapidly recovering hydrogel scaffolds from self-assembling diblock copolypeptide amphiphiles. Nature. 417: 424–428.

Pawlicki, J.M., Pease, L.B., Pierce, C.M., Startz, T.P., Zhang, Y., Smith, A.M. 2004. The effect of molluscan glue proteins on gel mechanics. J. Exp. Biol. 207: 1127–1135.

Petka, W.A., Harden, J.L., McGrath, K.P., Wirtz, D., Tirrell, D.A. 1998. Reversible hydrogels from self-assembling artificial proteins. Science. 281: 389–392.

Prociv, P., Spratt, D.M., Carlisle, M.S. 2000. Neuro-angiostrongyliasis: Unresolved issues. Int. J. Parasitol. 30: 1295–1303.

Rice, K.G., Rottink, M.K., Linhardt, R.J. 1987. Fractionation of heparin–derived oligosaccharides by gradient polyacrylamide-gel electrophoresis. Biochem. J. 244: 515–522.

Roda, A., Nachman, G., Weihman, S., Yong, C.M., Zimmerman, F. 2016. Reproductive ecology of the giant African snail in south Florida: implications for eradication programs. PLOS ONE 11: e0165408, doi. org/10.1371/journal.pone.0165408

Ruifrok, A.C., Johnston, D.A. 2001. Quantification of histochemical staining by color deconvolution. Anal. Quant. Cytol. Histol. 23: 291–299.

Sasisekharan, R., Venkataraman, G. 2000. Heperin and heperan sulfate: Biosynthesis, structure and function. Curr. Opin. Chem. Biol. 4: 626−631.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W. 2012. NIH image to ImageJ: 25 years of ImageJ analysis. Nat. Methods. 9: 671–675.

Smith, A.M. 2010. Gastropod secretory glands and adhesive gels. In: von Byern, J., Grunwald, I. (Eds.). Biological Adhesive Systems: From Nature to Technical and Medical Application. Springer, Vienna, Austria. pp. 41–51.

Smith, A.M., Morin, M.C. 2002. Biochemical differences between trail mucus and adhesive mucus from marsh periwinkle snails. Biol. Bull. 203: 338–346.

Thiengo, S.C., Faraco, F.A., Salgado, N.C., Cowie, R.H., Fernandez, M.A. 2007. Rapid spread of an invasive snail in South America: the giant African snail, *Achatina fulica*, in Brasil. Biol. Invasions. 9: 693–702.

Vieira, T.C.R.G., Costa-Filho, A., Salgado, N.C., Allodi, S., Valente, A.P., Nasciutti, L.E. Silva, L.-C.F. 2004. Acharan sulfate, the new glycosaminoglycan from *Achatina fulica* bowdich 1822. Eur. J. Biochem. 271: 845–854.

Wang, C., Stewart, R.J., KopeČek, J. 1999. Hybrid hydrogels assembled from synthetic polymers and coiled-coil protein domains. Nature. 397: 417–420.

Yamaguchi, K., Seo, N., Furuta, E. 2000. Histochemical and ultrastructural analyses of the epithelial cells of the body surface skin from the terrestrial slug, *Incilaria fruhstorferi*. Zool. Sci. 17: 1137–1146.