

## Some Histochemical Properties of the Ceruminous Glands in the Meatus Acusticus Externus in Cats and Dogs

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**Abstract:** This study was conducted to determine some histochemical properties of the ceruminous glands located in the meatus acusticus externus in cats and dogs. Ten cats and 10 dogs of various breeds were used as the study materials.

To determine the properties of the secretions of the ceruminous glands in the meatus acusticus externus of cats and dogs, the periodic acid Schiff (PAS) stain was used for neutral mucosubstances and glycogen, while the Alcian blue stain (pH 2.5) was applied for the presence of acidic mucosubstances. The diastase reaction was applied to confirm the presence of glycogen.

In dogs, positive staining was observed for glycogen and neutral mucosubstances, whereas, in cats, negative reactions were observed. The positive reactions observed in dogs were found not to originate from glycogen by the negative diastase reactions observed.

The Alcian blue stain (pH 2.5) for acidic mucosubstances gave positive reactions for cats and negative reactions for dogs.

**Key Words:** Ceruminous gland, histochemistry, meatus acusticus externus, cat and dog

### Kedi ve Köpeklerde Dış Kulak Yolundaki Serumen Bezlerin Bazı Histokimyasal Özellikleri

**Özet:** Bu çalışma dış kulak yolundaki seruminöz bezlerin bazı histokimyasal özelliklerini ortaya koymak amacıyla yapıldı. Çalışma materyali olarak çeşitli ırklardan 10 adet kedi ve 10 adet köpek kullanıldı.

Kedi ve köpeklerde dış kulak yolundaki seruminöz bezlerin salgı özelliklerini belirlemek amacıyla; nötral mukosubstans ve glikojen için periyodik asit Schiff (PAS), asit mukosubstans için Alcian blue (pH 2,5) boyamaları yapıldı. Glikojen varlığı için diastase reaksiyonu uygulandı.

Glikojen ve nötral mukosubstans için köpeklerde pozitif, kedilerde negatif reaksiyon gözlemlendi. Köpeklerdeki pozitif reaksiyonun glikojenden kaynaklanmadığı, diastazın negatif reaksiyon vermesi ile belirlendi.

Asidik mukosubstans için Alcian blue (pH 2,5) kedilerde pozitif, köpeklerde negatif reaksiyon gözlemlendi.

**Anahtar Sözcükler:** Serumen bez, histokimya, dış kulak yolu, kedi ve köpek.

### Introduction

The meatus acusticus externus is a canal that extends from the external auditory canal to the tympanic membrane. The external one-third of this canal is composed of cartilage with the temporal bone making up the roof of the remaining part. The ceruminous glands are located in the submucosa of the cartilaginous part. The ceruminous glands are tubular glands with wide folding lumen. These glands secrete the cerumen (waxy material). The cerumen is a semisolid brownish secretion

composed of fats and earwax. Sparsely distributed hair in the duct and the cerumen serve a protective role (1).

In the auditory canal of healthy dogs, ceruminous glands are located in the deeper layers of the dermis. In cats, the upper one-third of the horizontal canal have increased numbers of ceruminous glands. The hair is either scanty or absent in this region (2-5).

Cerumen serves as an important barrier to infections by microorganisms and also protects the skin against

injury (6). Human cerumen exhibits both bactericidal and antifungal activities (7,8). However, cerumen has also been reported to facilitate the growth of the *Malassezia pachydermatis* fungus (9).

Accumulation of cerumen in the meatus acusticus externus predisposes the external ear to inflammation (10). Secretions of the ceruminous glands prevent the skin of the ear from drying and irritations (11).

In cats, the ceruminous glands occur as numerous, folded and wide tubular glands in the meatus acusticus externus. These glands are surrounded by myoepithelial cells (12).

In dogs, apocrine glands are found under the adipose glands deep inside the dermis at varying diameters. They are also surrounded by myoepithelial cells (4,5,13).

Normally, cerumen occurs as a sticky material in the meatus acusticus externus. It is composed of a combination of desquamated cornified flat epithelial cells, fat and the fatty secretions of the ceruminous glands. For this reason, they are composed of a mixture of hydrophobic fatty components of proteins, lipids, amino acids and mineral ions. The substance cerumen, due to its high fat content, does not stain. In contrast, the desquamated cornified epithelial cells stain and hence are identifiable (3,4,14,15).

In a study conducted on human ceruminous glands, the presence of neutral mucosubstances in the cytoplasm of the cells were identified. Also, in the apical halves of the cells acidic mucosubstances were observed (16).

In the ceruminous glands of apocrine nature located in the meatus acusticus externus in cats acidic mucous substances are found in the apical parts of the glandular cells and lumen of the glands (12).

The diastase resistant PAS positive granules of the apocrine glands found in the meatus acusticus externus are located near the apical borders of the glandular cells in cats (12).

The various sizes of diastase resistant PAS positive staining material have been observed in the glandular cells adjacent to the luminal border of the apocrine glands in dogs. This material has also been observed in the gland lumen. Also, an acidic mucosubstance showing a positive reaction with Alcian blue has been observed (13).

The ceruminous glands, which are modified apocrine glands, are tiny. They are located deep in the dermis

beneath the adipose glands (4,5). The upper one-third of the meatus acusticus externus contains a large number of these glands (3). Hair follicles are smaller and fewer than those of normal skin (4,5).

This study was conducted to determine the histochemical properties of the ceruminous glands located in the meatus acusticus externus in cats and dogs.

## Materials and Methods

The materials were obtained from 10 cats and 10 dogs of different breeds brought to the Ankara University Faculty of Veterinary Medicine with no ear disease.

The specimens were taken from the cartilaginous layer of the meatus acusticus externus of both ears from each animal. The tissue specimens were fixed in 10% Neutral Formalin solution and after passing through graded alcohols and xylols they were embedded in paraplast. Six-micron sections from the blocks were subjected to the processes as mentioned below.

- \* Mallory's triple staining technique modified by Crossman for general histological examinations (17),
- \* The periodic acid Schiff (PAS) reaction for identification of glycogen and neutral mucosubstances in secretory epithelial cells (17),
- \* The diastase/periodic acid Schiff (PAS) reaction for the enzymatic confirmation of glycogen (17),
- \* The Alcian Blue method (pH 2.5) for the determination of acidic mucosubstances (18),
- \* Mallory's phosphotungstic acid hematoxylin method for the identification of myoepithelial cells (19).

## Results

The ceruminous glands were located in the deeper layers of the mucosa just beneath the adipose glands in cats and dogs. These glands were identified by their characteristic folded tubular structures. In cats, secretions from the cells forming the ceruminous glands did not stain by the triple staining method, whereas those in dogs were observed to stain. Also, these glands showed mucous and serous glandular properties in cats and dogs, respectively (Figures 1, 2).

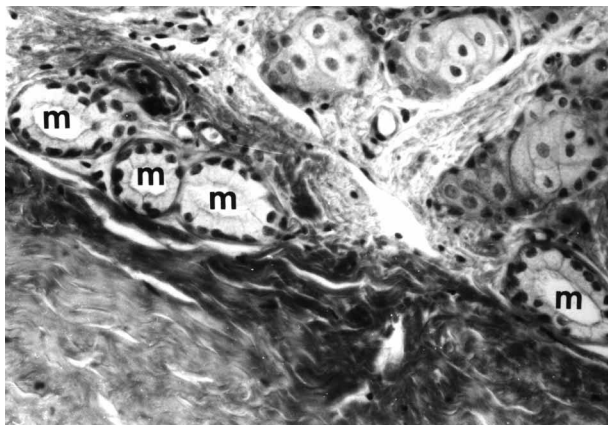


Figure 1. Mucous properties of the ceruminous glands in the cat (m). Triple x 420.

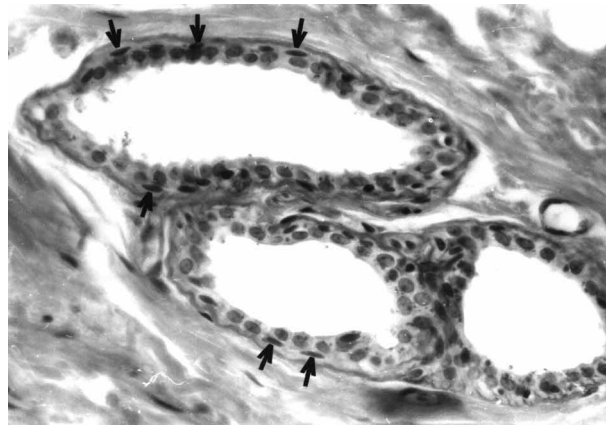


Figure 3. Myoepithelial cells in the ceruminous glands in the dog (arrows). Mallory's phosphotungstic acid hematoxylin x 680.

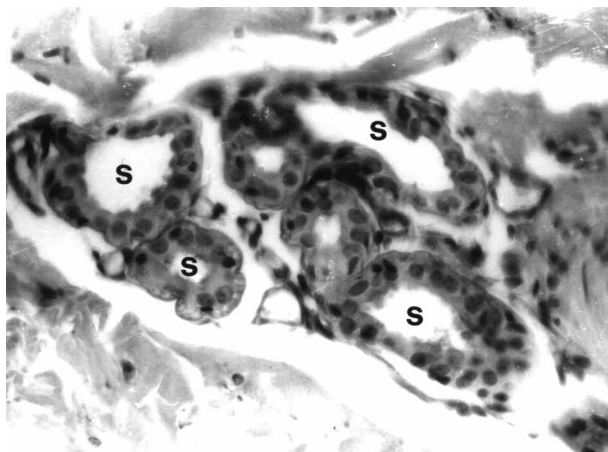


Figure 2. Serous properties of the ceruminous glands in the dog (s). Triple x 540.

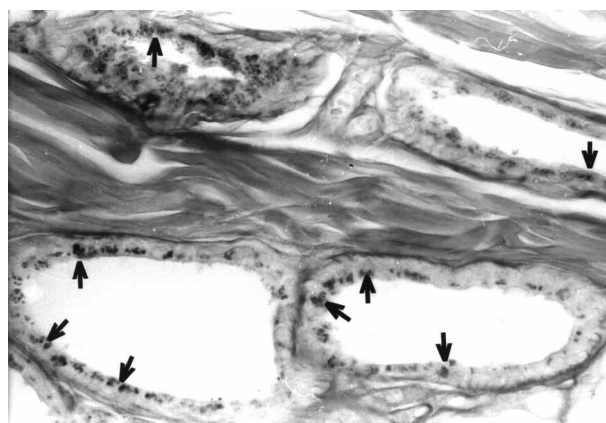


Figure 4. PAS positive ceruminous glands in the dog (arrows). PAS x 490.

In the serial sections examined it was observed that numerous myoepithelial cells surrounded the seruminous glands in the dogs (Figure 3) while only a few could be seen in the cats.

A positive reaction was observed with the periodic acid Schiff (PAS) method for the presence of glycogen and neutral mucosubstances in the dogs (Figure 4). This positive granulation was encountered in the apical aspects of the cells. There was no change in positive reaction in staining by PAS method in sections that the glycogen digested by diastase. It was therefore suggested that the PAS positive granules could have originated from the neutral mucosubstances.

A negative reaction was observed in the PAS method applied to determine glycogen and neutral mucosubstances in the cats (Figure 5).

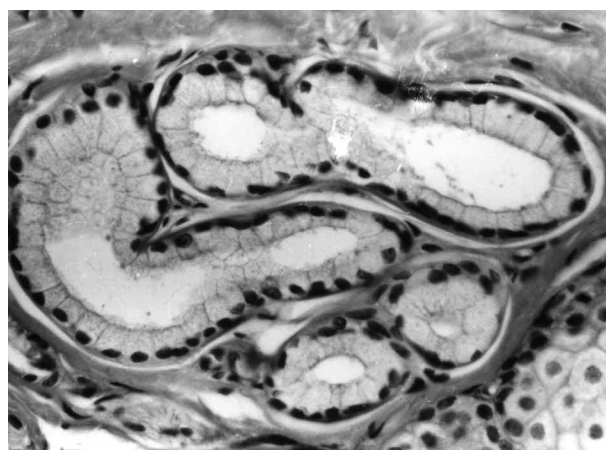


Figure 5. PAS negative ceruminous glands in the cat. PAS x 500.

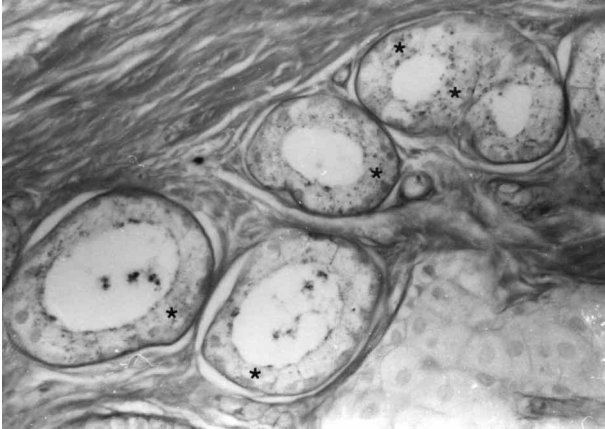


Figure 6. Alcian blue positive ceruminous glands in the cat (asterisks). Alcian blue x 470.

The cells of the dogs gave a negative reaction while those of the cats gave a positive one in the Alcian blue (pH 2.5) staining method to determine acidic mucosubstances (Figure 6).

## Discussion

A few studies have been conducted on the ceruminous glands found in the meatus acusticus externus in cats and dogs (12,13).

The ceruminous glands in the meatus acusticus externus have been defined as enlarged tubular glands that exhibit a folded tortuous course in cats and dogs (1,12). The ceruminous glands in the meatus acusticus externus of cats and dogs were observed to have the tortuous and enlarged tubular structures in this study as well. Also, a few hair follicles were observed as reported in the literature (1,12).

The ceruminous glands were observed to contain acidic mucosubstances in the apical aspects of the

secretory epithelial cells and in the lumen of the glands in cats (12). The ceruminous glands in dogs were observed to give positive reactions to stains for acidic mucosubstances (13). In the ceruminous glands of humans acidic mucosubstances have been identified in the apical halves of the glandular epithelial cells (16). However, in this study, the cells of the cats gave a positive reaction whereas those of the dogs gave a negative one for acidic mucosubstances (pH 2.5).

The ceruminous glands in the meatus acusticus externus were reported to give positive reactions for neutral mucosubstances in a study conducted on cats (12). Positive reactions were also observed in dogs (13). In this study, the cells of the dogs were observed to give a positive reaction while those of the cats gave a negative one for neutral mucosubstances.

In this study, to determine whether the PAS positive appearance observed in the cats and dogs was due to the presence of glycogen or not a negative reaction with diastase was observed suggesting the absence of glycogen (12,13). The failure to elicit a positive reaction with diastase by the positively stained neutral mucosubstances observed in the dogs led to the conclusion that the positivity did not originate from glycogen, a view shared by the above investigators.

In conclusion, therefore, it was suggested that the secretions from the ceruminous glands of normal appearance in the meatus acusticus externus in cats are mucous while those in dogs are serous in character. Depending on the nature of the secretions from the ceruminous glands located in the meatus acusticus externus of cats and dogs, cerumen was concluded to be capable of creating suitable media for external ear diseases.

## References

1. Junqueira, L.C., Carneiro, J., Kelley, R.O.: Basic Histology 9th ed. Appleton & Lange Stanford, Connecticut., 1998; 358-360.
2. Logas, D.B.: Diseases of the ear canal. Vet. Clin. North Am. Small Anim. Pract., 1994; 24: 905-919.
3. Augus, J.R.: Diseases of the ear canal. In the Complete Manual of Ear Care. Lawrenceville, New Jersey, Veterinary Learning Systems. Inc., 1986.
4. Griffin, C.: Histopathology of the external ear. In the Complete Manual of Ear Care. Lawrenceville, New Jersey, Veterinary Learning Systems, Inc., 1986.
5. Van der Gaag, I.: The pathology of the external ear canal in dogs and cats. Vet. Q., 1986; 8: 307-317.
6. Huang, H.P., Fixter, M.L., Little, C.J.L.: Lipid content of cerumen from normal dogs and otitic canine ears. Vet. Rec., 1994; 134: 380-381.
7. Chai, T.J., Chai, T.C.: Bactericidal activity of cerumen. Antimicrob. Agents Chemother., 1980; 18: 638-641.
8. Megarry, S., Pett, A., Scarlett, A., Teh, W., Zeigler, E., Canter, R.J.: The activity against yeast of human cerumen. J. Laryngol. Otol., 1988; 102: 671-672.



9. Gabal, M.A.: Preliminary studies on the mechanism of infection and characterization of *Malassezia pachydermatis* in association with canine otitis externa. *Mycopathologia*, 1988; 104: 93-98.
10. August, J.R.: Otitis externa, a disease of multifactorial etiology. *Vet. Clin. North Am. Small Anim. Pract.*, 1988; 18: 731.
11. Bergman, R.A., Afifi, A.K., Heidger, P.M.: *Atlas of Microscopic Anatomy. Section 7- Integument*, Iowa. 1999.
12. Fernando, S.D.A.: Microscopic Anatomy and Histochemistry of glands in the external auditory meatus of the cat (*Felis domesticus*). *Am. J. Vet. Res.*, 1965; 26: 1157-1161.
13. Fernando, S.D.A.: A Histological and histochemical study of the glands of the external auditory canal of the dog. *Res. Vet. Sci.*, 1966; 7: 116-119.
14. Woody, B.J., Fox, S.M.: Otitis externa: Seeing past the signs to discover the underlying course. *Vet. Med.*, 1986; 81: 616-624.
15. Johnson, A., Hawke, M.: The non-auditory physiology of the external ear canal. In John AF Santos-Sacchi J(eds): *Physiology of the Ear*. New York, Raven Press., 1988; 41.
16. Sirigu, P., Cossu, M., Puxeddu, P., Marchisia, A.M., Perra, M.T.: Human ceruminous glands: a histochemical study. *Basic Appl. Histochem.*, 1983; 27: 257-265.
17. Denk, H., Künzele, H., Plenk, H., Rüschoff, J., Seller, W.: *Romeis Mikroskopische Technik. 17., neubearbeitete Auflage*. Urban und Schwarzenberg, München-Wien. Baltimore. 1989; 439-450.
18. Culling, C.F.A., Allison, R.T., Barr, W.D.: *Cellular Pathology Technique. 4th ed.*, Butterworths, London. 1985; 214-255.
19. Bancroft, J.D., Gamble, M.: *Theory and Practice of Histological Techniques. 5th ed.* Churchill Livingstone. Toronto, 2002; 133-135.